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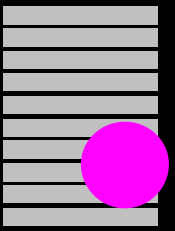

LABORATORY GUIDE

2004

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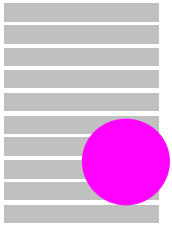

LABORATORY GUIDE

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PREFACE

This practical, user friendly medical laboratory guide does not provide detailed information for individual tests, all of which can be found in standard manuals and textbooks. Rather, it is intended to serve as a pocket book for physicians at all levels, medical students, nurses and other health workers who use the medical laboratory. In most part an attempt is made to provide light but meaningful reading with practical tips and explanatory notes, hoping to promote evidence-based medical laboratory practice. Nevertheless, most up to date information in laboratory medicine will be found in this guide. For instance, TMA (tissue microarray) technique in surgical pathology is alluded to, and which is gaining grounds during the last few years in large immunohistochemistry departments. Short accounts on Transfusion Medicine and Parasitology are presented. However, DNA chip technology, a key development for future analysis of genome characteristics in individuals, remains in the research phase at the moment and is outside the scope of this lab guide. Also, topics like adult postmortems and laboratory management fall outside the interest of targeted readership of this guide and were left out. It is hoped that the 7 core chapters in this guide with several other sections will provide a handy guide in emergency as well as cold clinical situations. All color captions were taken from material that belongs to the house. In any subsequent edition of this guide, it is envisaged that some of its material will be omitted, others may be combined or altogether new topics will be introduced, albeit replacement of some of the tests or modifications in certain modes of laboratory practice.

Yahia F. Dajani February 2004

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CHAPTER 1

QUALITY CONTROL

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Definitions

Quality control (QC) is closely dictated by quality management in the medical laboratory. Quality control is therefore a continuous process. Its central role evolves around finding problems in an organized way and within a sound setting, while recording all events during laboratory operations. In this way, problems can be checked and improvisations are made to circumvent trouble in advance and improve performance. Again, all procedures and checks with resultant data are regularly recorded, subject to assessment of precision, efficiency, quality of performance, productivity and financial ratings.

Quality Assurance/ Quality Control is the practice of encompassing all procedures aimed at attaining and maintaining a specific quality or product. In the medical laboratory, it starts from the time of ordering a laboratory investigation (even before if possible, such as education of laboratory users), through determination, interpretation and reporting as well as applicability to clinical care.

Sensitivity

Sensitivity means the probability of the test being positive in a group of patients with the disease. It is expressed as percentage.

Calculation:

$$\textit{Sensitivity}\ (\%) = \frac{(\#\ \text{of true positives})}{(\#\ \text{tested with disease})}\ \text{x}100$$

$$= \frac{(\#\ \text{of true positives})}{(\#\ \text{true positives} + \#\ \text{false negatives})}\ \text{x}100$$

True positives= # of patients with disease and correctly classified by the test.

False negatives= # of patients with the disease misclassified by the test or alternatively # of normal tests in diseased persons.

Specificity

Specificity means negativity in health and indicates the probability of the test being negative when the disease is not present. It is expressed as percentage.

Calculation:

Specificity (%)=(# of true negatives) divided by (# tested without disease) x100

o*r* (# of true negatives) divided by (# true negatives+ # false positives) x 100

True negatives = # of patients without the disease correctly classified by the test.

False positives= # of patients without the disease misclassified by the test. Or alternatively # of abnormal test results in healthy persons.

Therefore, the larger the percentage of false positives the less the specificity.

Predictive value of a positive test

Indicates probability that the disease is present when the test or procedure indicates disease. It equates with number of positive results expressed as a fraction of all positive results, both true and false.

$$\text{Predictive value of a test} = \frac{\text{\# true positives}}{\text{\# True pos+\# False pos}} \times 100$$

Therefore, the larger the percentage of false positives, the less the predictive value of a test.

Coefficient of variation (C.V.)

C.V. represents relative standard variation. The C.V. provides a measure of relative variability and is calculated as follows:

$$\text{C.V.} = \frac{s}{\bar{x}} \times 100$$

Where s= standard deviation, $\bar{x}$ = mean value,
C. V. = coefficient of variation

Prevalence

Refers to occurrence of disease in a population.

Calculation

# of total with disease divided by total # tested.

Incidence

Refers to number of new cases introduced in a population during a defined period of time: usually expressed in absolute figures per 100, 000 population, or population group, per annum.

Correlation coefficient

Commonly represented by C.C. and ranges from +1 to 0 to -1, where +1 equates with perfect positive correlation, 0 equates with no correlation and -1 equates with perfect negative correlation.

Exercise: Which of the following correlation coefficients shows the strongest relationship between 2 variables: +0.93, +0.82, +1.20, -0.95, 0.00? Answer = +0.93

Analytical bias

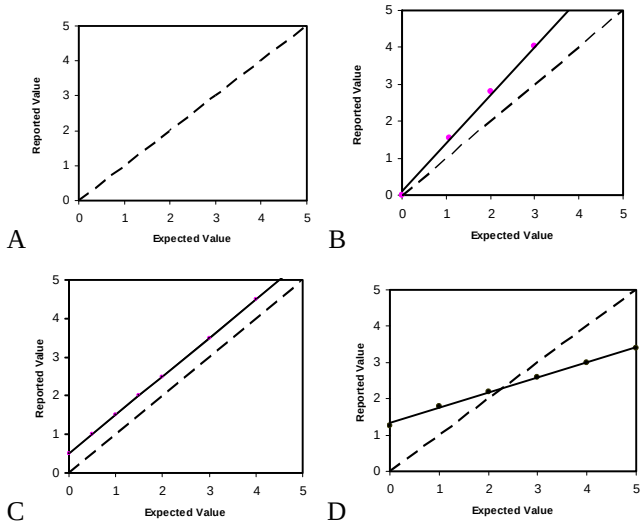
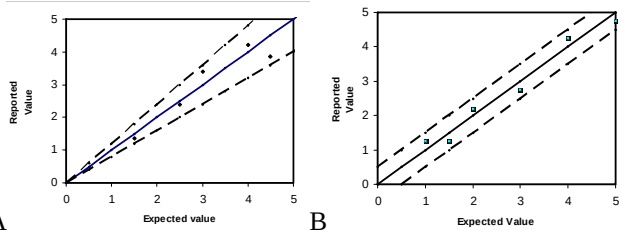


Figure 1. A-D : Operational charts showing an ideal operational line (A) and possible pattern for analytical lines.

All test results are subject to variation, inaccuracy or bias. Reported laboratory values, however, should fall along a slope 1.00 as shown in the operational charts in Figure 1. It may be laborious to ensure an ideal operational line of slope 1.00; the laboratory's customary operational line should at least be ensured in a reproducible manner.

Random Analytical Variability



A B

Figure 2. A&B: Pattern of distribution of reported values in relation to the mean, illustrating random variability.

In order to manage imprecise laboratory results, an operational limiting line is drawn which limits the variability of a test result in relation to a mean analyte concentration. Causes for such variability include volume variation by pipetting of sample aliquot or turbidity variation of various specimens (Figure 2).

Errors

Laboratory tests are subject to errors. Analytical errors are usually repetitive. For example, a wrongly calibrated pipette produces mistaken results in a consistent way. Mislabeling is another source of error, usually due to human carelessness. Such errors are avoidable by rigorous control of procedure, staff and equipment.

Accuracy

Accuracy is the extent to which the mean measurement is close to the true value.

The more reproducible the value of a reference sample the more accurate the test result. Automated enzymatic methods have enabled high degrees of accuracy for laboratory tests. A sufficient accuracy for clinical use should be allowed for.

Precision

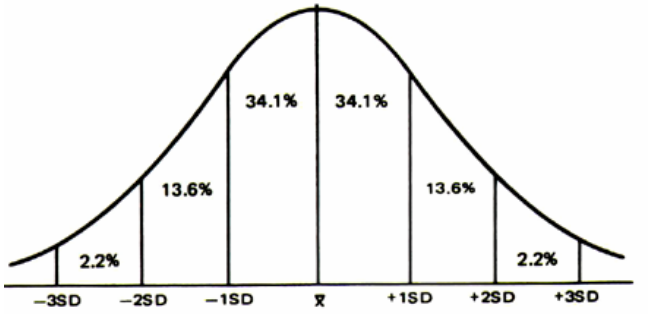


Figure 3. Gaussian curve showing graphical distribution of test result on repeated occasions.

Normal (or Gaussian) curve depicts a graphical distribution of test results performed multiple times; the graph is usually symmetrical or bell shaped (Figure 3). Below the symmetric line, all tests results are plotted, and percentage distribution of test results can be estimated per SD (standard deviation), for example, 68% to fall within one standard deviation and 95. 5% within 2 SD.

Precision is a measure of random variability. Most use 95% confidence limit in expressing precision, namely mean+/-2SD.

Standard Procedures in Quality Control (QC)

Intra- & Interlaboratory Control Programs

A laboratory may compare its current analyses with its own previous results (intra-laboratory) or, alternatively, with those of other laboratories (inter-laboratory) comparison programs. Intra-laboratory assessment can be variously employed, such as by use of sequential or randomized duplicate specimens and so on. In several external or commercial inter-laboratory programs, results from multiple laboratories are analyzed and conclusion of laboratory performance for each individual test included is calculated in relation to the mean value, and a score or evaluation is given for individual participating laboratory and overall for each test included.

Most QC materials are lyophilized products from large pools of serum which are prepared at levels related to clinical use. Such controls are available for all routine chemistry tests and can be purchased from several companies.

Levey-Jennings (L-J) Control Chart

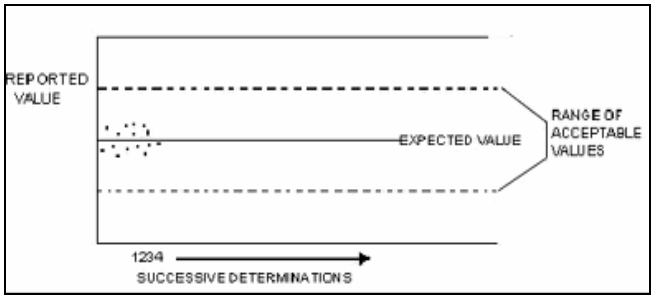


Figure 4. Levey-Jennings (L-J) Control Chart: values between broken lines fall within +/-1 S.D, Values between solid lines fall withen +/-2 S.D.

We plot the *control results* on the ordinate (Y axis) against *time* on the abscissa (X-axis). Results should fall within limits frequently set at maximum of +/-2 SD (Figure 4).

Any error in control results are classified as random or systematic. Random errors show wider range of scatter. Systematic error is evidenced by drift or shift to one side or the other from the central line. Use of two or more known materials can aid in determining if error is constant, proportional or mixed.

Cumulative Sum Technique (CUSUM)

Cumulative sum is done by calculating the actual difference between the individual values and the expected mean value, then add the sum of those differences to determine the cumulative effect for all control values obtained. CUSUM values can be plotted versus time and be used in addition to

L-J control chart. Random CUSUM error is indicated by a horizontal line while systematic CUSUM error is represented by a slope, slope angle getting steeper with increasing CUSUM error. CUSUM technique is more refined than L-J charts in denoting systematic error.

Quality Assurance

The 10-step process guide of QA (Quality Assurance) for medical laboratories is popular and has been advocated by the 'Joint Commission on Accreditation of Healthcare Organizations'.

1. *Assign responsibility* of QA to designated personnel and management team.
2. *Delineate scope of care* which embraces actual areas of operation for QA.
3. *Identify Important Aspects of Care* High volume e.g biochemical screens/ Critically ill e.g. blood gases/ Cumbersome e.g. 24-hr urine estimations/ Expensive e.g. rare tests/ Invasive e.g. bone marrow or fine needle aspiration biopsies.
4. *Identify indicators* as to which activity the observations belong e.g. for clinical use, QC, research studies or administrative, such as by incident reports.
5. *Establish Thresholds for Evaluations* such as threshold of turnaround time for test priorities which vary as to whether they are routine, urgent or stat order.
6. *Collect and Organize Data* which may be retrospective : of previous records/ reports or concurrent: of ongoing observations. Data include any proficiency testing results, performance appraisals, medical charts, laboratory logs, QC charts or QA programs data.
7. *Evaluate Care* Actual performance is compared to the desired indicator and threshold. Inevitable incidences

should be indicated, such as obligatory factors stalling laboratory work such as by war or power failure.

8. *Take Action to Solve Problems* Should identify trouble caused by staff quality, poor motivation, substandard equipment or insufficient personnel.
9. *Assess the Actions and Document Improvement* By monthly QA and so on.
10. *Communicate Relevant Information* Added to implementation and documentation, QA programs and problems to be disseminated among staff and any personnel concerned, both inside and outside the laboratory.

CHAPTER 2

LABORATORY PANELS OF SOME IMPORTANT CLINICAL SITUATIONS

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ABORTION (RECURRENT)

Laboratory tests: CBC, ANA, Anti-DNA, Lupus anticoagulants, Cardiolipin antibodies, Phospholipid antibodies, Protein C, Protein S, Antithrombin III, Activated protein C gene (Leiden Factor V). Prothrombin gene, Methyltetrahydrophosphate reductase (MTHR or Homocysteine) gene, Thyroid function tests, Fasting blood sugar, TORCH IgM & IgGs, Cytogenetic studies of both husband and wife.

Etiology: Spontaneous abortion is the most common complication of pregnancy. Of all clinically recognizable pregnancies, 15% end in spontaneous abortion within the first trimester. In approximately two-thirds of cases, specific etiology can be ascertained. The underlying causation may be genetic, hormonal, infectious, anatomical, auto-immune or systemic disorder. In the remaining cases, no obvious cause can be found, but an underlying defect in maternal immuno-modulation and fetal protection is thought to cause disturbance of the feto-placental unit in utero, leading to fetal loss. This latter mechanism underlies the rationale of transfusing husband's white blood cells into the wife during early pregnancy.

Methodical approach to evaluating a case of recurrent abortion:

(a) Uterine evaluation: Ultra-sound, Hysterosalpingogram.
(b) Genetic evaluation: Chromosomal analysis of husband and wife, Family studies when indicated.

(c) Microbiological evaluation: Cervical cultures (aerobic), Endometrial cultures (aerobic and anerobic), *Chlamydia, Mycoplasma and Ureaplasma*, TORCH.
(d) Endocrine evaluation: 2-hour postprandial blood sugar level, Thyroid function tests, serum Prolactin - early morning and mid follicular phase. Luteal phase evaluation by measuring serum Progesterone levels and Endometrial biopsy around menses time.
(e) ANA, VDRL, Lupus anticoagulats, Thrombophilia screen as detailed above.

ACUTE ABDOMINAL PAIN

Laboratory tests: CBC, Electrolytes, Urea, Creatinine, Urine analysis, Amylase in serum & urine, serum Bilirubin, serum Glucose, urine Porphobilinogen for acute intermittent porphyria, Gram stain for vaginal secretions, Hb electrophoresis in blacks if sickle status unknown.

Causes of acute abdominal pain according to age

*Infants***:** Intussusception, volvulus, Hirschprung's disease, bacterial enterocolitis, strangulated hernia, trauma through child abuse, Meckel's diverticulitis, pneumonitis, pyelonephritis, mesenteric cysts, testicular torsion, pancreatitis.

*3-11 years***:** Appendicitis, trauma, Meckel's diverticulitis, pneumonia, enterocolitis caused by *Salmonella, Shigella, Campylobacter, Yersinia*, Crohn's disease, pancreatitis, infected mesenteric cyst, ruptured tumors, pyelonephritis.

*Adolescents***:** Appendicitis, pelvic inflammatory disease, Crohn's disease, enterocolitis, peptic ulcer disease, chole-

cystitis, pneumonia, trauma, ectopic pregnancy, hematocolpus, psychosomatic recurrent abdominal pain.

Adults: Appendicitis, peptic ulcer disease, cholecystitis, diverticulitis, foreign body perforation, perforated GI malignancy, trauma, ectopic pregnancy, pelvic inflammatory disease, intestinal obstruction, ischemic bowel necrosis, ruptured aneurysm, familial Mediterranean fever.

ACUTE CHOLECYSTITIS

Laboratory tests: CBC which shows elevated White blood cells, serum Bilirubin, Alkaline phosphatase and AST (GOT) which are slightly elevated, while serum Amylase and Lipase are elevated in presence of pancreatitis. Bilirubin >3 suggests presence of stones. AST elevation suggests ascending cholangitis.

Confirmatory tests

(1) Plain X-ray of abdomen, right upper quadrant: for stones (20% radiopaque)
(2) Ultrasonography: shows bile duct size, hepatic ducts and pancreas; better than oral cholecystogram. Does not show cystic duct obstruction.
(3) Oral cholecystogram: Complements ultrasonography.
(4) Cholescintigraphy: uses i.v. Tc99m diisopropyl iminodiacetic acid. In acute cholecystitis, common bile duct is visualized but not gall bladder. Test unreliable with prolonged fasting, liver disease and high hyperbilirubinemia.

ACUTE GLOMERULONEPHRITIS (GN)

Tests in Acute Glomerulonephritis (General and disease specific)

Urinalysis: Proteinuria (0.5-3g/day), hematuria, red cell casts.

ASO: elevated in poststreptococcal GN cases (70%); membranoproliferative GN (20%) not specific for streptococcal infection; normal in early penicillin-treated phase.

Serum complement: Low serum complement in acute GN is explained as due to excessive consumption consequent upon formation of immune complexes in kidneys and other organs at a rate exceeding production of complement components. Alternatively, antibodies form against complement components, for example in membrano-proliferative GN.

Creatinine and creatinine clearance

ANA

Anti-DNA

Anti-GBM (anti-glomerular membrane)

ANCA (Anti-neutrophil cytoplasmic antibodies)

Cryoglobulins

Bence-Jones proteins

Urine protein electrophoresis

Hepatitis viral tests

VDRL

Renal biopsy is indicated in cases where it can make a difference to management.

Types of Glomerulonephritis

(1) With low serum complement level (% of positive cases between brackets)

(a) Renal disease	Acute post-streptococcal GN (90%) Membranoproliferative GN: Types I & II (80%) Renal atheroembolization
(b) Systemic disease	SLE (focal 75% diffuse 90%) SBE (subacute bacterial endocarditis) (90%) "Shunt" nephritis (90%) Cryoglobulinemia (85%)

(2) With normal serum complement level

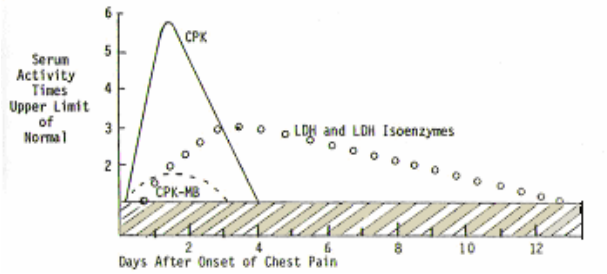
(a) Renal disease	IgA nephropathy (Berger's disease) Idiopathic RPGN* Antiglomerular basement membrane disease (Goodpasture's) Immune complex disease (membranous GN) Minimal change disease (Negative immune deposit findings)
(b) Systemic disease	Polyarteritis nodosa

* RPGN (Rapidly progressive GN) refers to rapidly declining renal function over weeks or months associated with crescent formation.

Hypersensitivity vasculitis
Wegener's granulomatosis
Henoch-Schonlein purpura
Goodpasture's syndrome
Visceral abscess

ACUTE MYOCARDIAL INFARCTION

Laboratory tests: CPK, CPK Isoenzymes, LDH, LDH Isoenzymes, AST (SGOT), Troponin T & I



Serum activityof CPK and LDL after myocardial infarction

Changes in serum enzymes following acute myocardial infarction:

Enzyme hrs	First rise hrs	Maximum level days	Return to normal
CPK	2-12	18-36	3-4
CPK-MB	2-12	12-36	2-3
LDH	8-12	36-72	8-12
LDH-1 & 2	8-12	36-72	10-15

CK-MK tends to peak at 12 hours with small or reperfused infarcts and at 16-24 hours with large or nonreperfused infarcts.

SGOT begins to rise 4-6 hours after acute myocardial infarction, reaches a maximum after 1-2 days and remains elevated for 4-6 days.

CK-MB is highly sensitive and specific for acute myocardial infarction when compared to ECG, CPK and LDH:

Diagnostic parameter	% False (-)	% Sensitivity	% False (+)	% Specificity
ECG	34	66	0	100
Total CPK	2	98	15	85
LDH1>LDH2	10	90	5	95
CPK-MB	0	100	1	99

ACUTE RENAL FAILURE

Laboratory tests:

(1) Urine: Volume/ Sediment/ S.G./ Sodium/ Osmolality.
(2) Serum BUN/ creatinine ratio.
(3) Urine to plasma ratio of (a) osmolality (b) creatinine (c) urea.
(4) Excretion fraction of filtered sodium (EFFS).

Above tests aid in differentiating pre-renal, renal and post-renal oliguria.

Brief notes:

Oliguria is a cardinal sign of acute renal failure.

Urinary sediment in renal state consists of renal tubular cells, casts: granular, hyaline, cellular and occasionally red or white cells. Urine S.G. is high in renal state and low in pre- and post-renal states.

Urine sodium: pre-renal <20mmol/L renal >30-40 post-renal not useful

BUN/ creatinine ratio = >10 in pre-renal, 10/1 in renal and 5/1 in post-renal state.

Urine/ plasma osmolality >1.1 in pre-renal,<1.1 in renal and nonuseful in post-renal state.

Urine osmolality: pre-renal >500moSm/kg renal <350 in post-renal not useful.

Urine/ Plasma creatinine: pre-renal >40 renal (acute tubular necrosis <20, GN >40) Post-renal <20

Urine/ Plasma urea: pre-renal >8 renal (ATN<3 GN>8) post-renal <3

EFFS: pre-renal <1% renal (ATN>2-3% GN<1%) post-renal >2%

ALLERGY TESTS

Food Allergens

1. Carrots
2. Onions
3. Soya beans
4. Tomatoes
5. Bananas
6. Oranges

7. Egg yolk
8. Egg white
9. Cow's milk
10. Wheat flour
11. Sesame
12. Rice
13. Baker's yeast
14. Maize meal
15. Peanuts
16. Nut mix (Walnuts, Hazelnuts, Almonds)
17. Crab
18. Cod
19. Chicken
20. Lamb/Mutton

Pediatric Panel

1. *Dermatophagoides pteronyssinus*
2. *Dermatophagoides farinae*
3. Birch
4. Mixed grasses
5. Cat
6. Dog
7. Altemaria altemata
8. Milk
9. α-lactalbumin
10. β-lactoglobulin
11. Casein
12. Egg white
13. Egg yolk
14. Bovine serum albumin
15. Soya beans
16. Carrots
17. Potatoes

18. Wheat flour
19. Hazelnut
20. Peanuts

Respiratory allergens:

1. *Dermatophagoides pteronyssinus*
2. *Dermatophagoides farinae*
3. Cedar
4. Mosquito
5. Olive
6. Oak
7. Ambrosia, mugwort-leaved
8. Orach
9. Plantain, English
10. Bermuda grass
11. Grass pollens
12. Cat
13. Dog
14. Horse
15. Cockroach
16. *Altemaria altemata /tenius*
17. *Aspergillus fumigatus*
18. *Candida albicans*
19. *Cladosporium herbarum*
20. *Penicillium notatum*

AMNIOCENTESIS

Investigations:

(1) *Karyotyping* (Chromosomal analysis) for numerical (e.g. Down's syndrome which is trisomy 21 in 90% of cases) and structural chromosomal aberrations.

(2) *FISH* (Fluorescent in-situ hybridization) for numerical and structural chromosomal aberrations if urgency in results and time is crucial, since it is a costly test. This technique has become well established in PGD (pre-implantation genetic diagnosis).

(3) *DNA analysis* using amniocytes for specific genetic disorders such as thalessemia, muscular dystrophy, cystic fibrosis, hemophilia and any known or mapped genetic disorder.

(4) *Biochemical assays* on amniotic fluid, such as alpha-fetoprotein, amino acids when specified and bilirubin for evaluation of hemolytic disease of the newborn.

Indications for genetic amniocentesis:

Maternal age >35 years (risk of Down's almost doubles) … Previous child with chromosomal anomaly … Previous child with neural tube defect … Elevated serum alpha-fetoprotein screen … Previous child/ known carrier of a heritable metabolic disorder … Family history of sex-linked recessive disorder.

Sex-linked conditions: Relatively common Color blindness, Hemophilia A & B, Muscular dystrophy, Duchenne type (1:36,000), Glucose-6-Phosphate Dehydrogenase Deficiency. Rare Fabry's disease, Lesch-Nyhan syndrome, Diabetes insipidus, Ectodermal dysplasia of anhydrotic

type, Ocular albinism, X-linked icthyosis, Agammaglobulinemia or Bruton's disease, Wiskott-Aldrich immunodeficiency type syndrome

ANEMIA

It is important not to transfuse a patient with blood for anemia before finding the type of anemia at hand. In cases of emergency where the Hb (hemoglobin) is below 5g/dl, adequate history taking, physical examination and blood samples should be taken for a full blood count beside any other relevant tests such as serum levels of ferritin and transferrin, vitamin B12 and folate, bone marrow and so on.

Full blood count includes Hb, PCV, MCHC, MCH, MCV, RBC, White blood cell count and differential, Platelet count, Reticulocyte count, ESR and blood film. An abnormal blood film should always be read by a qualified person.

The full blood count should give us a directive as to what type of anemia we are dealing with. This has to be considered in the context of the presenting symptoms. The following short note is only intended to act as a guide, while readers are referred to standard textbooks for an adequate account on anemia.

Note: A hypochromic, microcytic anemia with a decreased MCHC should lead us to investigate for iron deficiency, namely, by ordering serum ferritin and transferrin. The earliest change in iron deficiency is a fall in MCV. Fe deficiency is not a final diagnosis and the underlying cause should be determined, such as excessive menstrual or hidden intestinal bleeding, in the chronic cases of which the

reticulocyte count may not be elevated. In acute hemorrhage or hemolysis, reticulocyte count is elevated, usually rising over 4%. In addition, MCH is useful in distinguishing iron deficiency from thalassemia trait, in the latter case of which the MCH does not usually fall below 23pg in anemia of moderate severity. On the other hand, a macrocytic anemia with an MCV beyond 100 should be followed up with serum vitamin B12 and folate levels, and as the need may arise, bone marrow examination. If megaloblastic anemia or vitamin B12 deficiency is established, then the cause should be determined. It is of interest to note that in our communities, vitamin B12 anemia is rare except in persons of Caucasian stock (Gerkas), in whom the disorder is not uncommonly seen. Gastric atrophy or Crohn's disease are two examples which can cause vitamin B12 deficiency. Alternatively, a normochromic, normocytic anemia should be investigated in term of presence of a chronic disorder such as infection, systemic or collagen disease; combined iron and vitamin deficiency occasionally accounts for this type of anemia. In brief, a CBC is the first laboratory step in the hematologic investigation of a patient. The Hb level should never be assessed by skin or mucosal color; it is only estimated by the Hb level determined in the laboratory. Nowadays, this is easy, and rather than ordering Hb and PCV, doctors are encouraged to order CBC as routine, in which case they will not miss invaluable and basic hematologic information about their patients.

Blood film. The blood film is routinely screened, every time, for red cells, white cells and platelets. In occasional cases, parasites may be requested and careful search should be made in those cases, e.g. screening for malaria parasite.

Changes in red cells

Anisocytosis = variation in red cell size

Poikilocytes = variation in red cell shape

Hypochromic = Increased central pallor to > $1/3^{rd}$ of red cell diameter

Normochromic = Central pallor < $1/3^{rd}$ of red cell diameter

Dichromatic = Normochromic and hypochromic red cells present

Dimorphic = 2 red cell forms e.g. micro- and macro-cytes present.

Macrocytic = macrocytes, red cells increased in size, may be ovoid, present

Target = pale ring = in iron deficiency, thalassemia, posts-plenectomy, etc.

Schistocytes = fragmented red cells seen in D.I.C. and other disorders

Tear-drop = or tennis bat cells are seen in myelosclerosis

Crenated = seen in uremia; may be a dehydration artifact

Stippled = fine stippling occur in hemolytic anemia & lead poisoning

Intracellular inclusions = by vital stain, seen in certain hemolytic anemias

Other = elliptocytes are overdiagnosed; elliptocytosis is very rare. Hereditary spherocytoris is more commonly seen.

Types of anemia

Anemias may be divided into 3 classes:

1. Microcytic hypochromic MCV < 80 fl MCHC <32 often < 30 g/dl.
2. Normocytic normochromic MCV & MCHC normal.
3. Macrocytic MCV > 100.

Causes of microcytic hypochromic anemia:

Iron deficiency, Thalassemia syndrome, Sideroblastic (hereditary or acquired), Pyridoxine-responsive, Lead poisoning, Chronic infection and Debilitated states.

Iron deficiency, a worldwide major health problem, may be nutritional, due to blood loss, absorptive such as achlorhydria or malabsorption or due to increased demand such as in pregnancy.

Causes of normocytic normochromic anemia:

Sudden massive blood loss, hemolytic anemias (immune, microangiopathic, D.I.C., hemolytic uremia syndrome, hemoglobinopathies (see Table 7), unstable hemoglobins, spherocytosis, elliptocytosis, stomatocytosis both latter very rare, pyruvate kinase deficiency, glucose phosphate deficiency), productive (pure red cell aplasia, drugs, chronic disease such as infection, renal disease, liver disease and hypothyroidism).

In hemolytic anemia the reticulocyte count is elevated, while in marrow failure it is suppressed.

Causes of macrocytic anemia:

Megaloblastic due to vitamin B12 or folate deficiency, some hemolytic anemias, liver disease, hypothyroidism, normal newborn, sometimes in marrow aplasia and myelofibrosis.

Note that some drugs like co-trimoxazole produce reversible megaloblastic change.

White blood cells

With anemia, it may be normal, increased or decreased.

Leukocytosis = neutrophilic/ lymphocytic/ monocytic/ eosinophilic/ pantype; mild/ moderate/ severe seen in inflammation, etc. vs leukemic proliferation: leukocyte alkaline phosphatase.

Leukopenia = decreased, of protean etiology: specific type or pan drug induced, caused by viral infection, constitutional etc., agranulocytosis = failure of neutrophil production (See Table 26).

Anemia may occur with normal white blood cells, as part of chronic systemic disorder, iron deficiency, hemolytic anemia etc. or may occur with leukocytosis in association with inflammation, hemorrhage, or myeloid neoplasia, or with leukopenia as part of pancytopenia or bilinear deficiency due to myelodysplasia or other cause.

A selection of hematologic disorders is presented in captions 12-20.

Platelets

With anemia it may be normal, increased or decreased.

Anemia and thrombocytopenia or thrombocytosis have protean causes, such as hypersplenism, neoplasia, drugs, infection etc.

ANTENATAL

Screening laboratory tests

1st Trimester

CBC, Urine analysis, Blood grouping and Rhesus typing, Serum ferritin,

Rubella IgG, HBsAg, VDRL

2nd Trimester

CBC, Urine analysis, Random blood sugar

AFP at 15-19 weeks with Triple test if suspected

Genetic counseling, level II Ultrasound and Amniocentesis in >35 year-old

TSH or any other tests as indicated.

APA (Anti Phospholipid Antibody) Syndrome

Recurent thrombotic episodes may result in various injuries to body systems as well as reccurent abortion with fetal loss. This phenomenon is named APA or Hughes' syndrome.

Tests

Antithrombin III, lupus anticoagulant, anti-cardiolipin/ phospholipid antibodies, proteins C and S, Factor II, homocysteine. Genetic testing includes probing for mutations in Factors II (Prothrombin), V (Leiden) and MTHFR (homocysteine): will identify heterozygous and homozygous as well as multiple-factor status. These tests are readily available, simple and precise with a fairly short turnaround time.

APA syndrome results from genetic or acquired factors. Genetic conditions involve resistance to prothrombin or Factor V (Leiden factor) or to the MTHFR (Homocysteine) factor or, alternatively, Antithrombin III deficiency. Acquired causes include SLE (systemic lupus erythematosus), malignancy, thrombocythemia and polycythemia rubra vera.

Thrombotic episodes may affect venous or arterial vessels with concomitant cardiac and cerebrovascular effects. Unusual sites may also be involved such as retinal, renal or hepatic vessels. Cardiac manifestations include myocardial infarction and thrombotic events, while cerebrovascular effect includes stroke, transient ischemic attacks and so on, while body organ affections such as renal and bone lesions are varied. Thrombocytopenia is sometimes seen. Dermal lesions include ulcers, gangrene and so on. Recurrent abortion (more than 2 consecutive events) is a well known complication due to placental insufficiency (See Abortion recurrent panel).

APPENDICITIS

Laboratory tests:

White cell count >11x10^9 per liter

Differential white cell count >75% neutrophils

CRP elevated above 6 units

Tests to exclude URINARY TRACT INFECTION & ECTOPIC PREGNANCY.

If white cell count, neutrophilic percentage and CRP are all within normal limits, the patient is unlikely to have acute appendicitis. However, this is not a reliable method and diagnosis of appendicitis should rely on adequate history, physical examination and a high suspicion index, with close watch if necessary in doubtful cases. Missing a case may be disastrous with subsequent rupture, peritonitis and periappendicular abscess formation.

Differential diagnosis of acute appendicitis

In men, mesenteric adenitis, gastroenteritis and abdominal pain of unknown cause.

Favoring appendicitis: Shifting pain with localizing tenderness in right iliac fossa, rebound tenderness, WCC >11,000 with neutrophils >75%.

In ovulating women, PID (pelvic inflammatory disease) can masquerade as appendicitis. Favoring PID against appendicitis: No anorexia, nausea or vomiting, duration of pain exceeding 2 days, onset within 7 days of menstruation, history of sex transmitted disease, abdominal tenderness out-

side right lower quadrant, cervical motion tenterness and bilateral adnexal tenderness.

In infants and young children, recognition of appendicitis is more difficult and complications such as perforation are more frequently seen than in adults.

AZOOSPERMIA

About 15% of males are subfertile. Of those, about 20% have azoospermia.* Azoospermia is either obstructive, whereby sperm production is present in the testis but discharge is hampered by a lesion of the epididymis or vas deferens, such as congenital absence or atresia of the vas deferens, sometimes associated with a forme fruste of mucoviscidosis, while acquired causes include obliteration of luminal pathways such as occurs with infection of the epididymes and vasa deferentia. The majority of cases of azoospermia are of unknown cause. In recent years a meaningful classification of azoospermia has been used.

Dajani Classification System For Azoospermia:

A1 Many sperm with tail 'obstructive azoospermia'
A2 Sperm in reduced amount 'non-obstructive azoospermia'
B Germ cells but no sperm present'spermatid maturation arrest'
C Sertoli cell only pattern No germ cells at all detected.

* Dajani, YF, Kilani Z. Role of fine needle aspiration in the diagnosis of azoospermia. *International Journal of Andrology* 1998; 21: 295-300.

D Sclerotic tubules with remnant Sertoli or adventitial cells only. Does not mean Klinefelter's syndrome as sometimes quoted

Tests in azoospermia

Clinical history. Cryptorchidism of varying degrees is not uncommonly seen, both unilateral and bilateral, resulting in adverse effects on sperm production. Nowadays, we see more frequently salvaged cases by orchidopexy at early age. The corrective operation is recommended as early as possible in life and certainly by the age of one year, since testicular damage has been demonstrated even at this early stage of life in cryptorchid testes. About 3.2% of newborns have incompletely descended testes but most are corrected spontaneously by the age of 3 months.

The Consulting Medical Laboratories have since many years devised a systematic way of taking essential data in the history and physical examination, which is illustrated in the standard form of reporting semen analysis (see Form 4).

Physical examination. General physique, body hair distribution, position and volume of each testis as well as scrotal status and check for presence of varicocele or hydrocele. Normal testicular volume should exceed 15 ml and both testes should lie loose within the scrotum. Effect of detrusor muscle contraction may lead to tensely held testes on palpation. Retractile testes in one or both sides are not uncommon, especially in men with azoopermia.

Serum FSH level is believed to be the most important routine hormone to be tested in this condition. Level above 10miu/ ml usually reflects a degree of Sertoli cell damage. Although inhibin may be useful, its levels are inversely

related to FSH levels and is not a necessary test but may be used as confirmatory when this test becomes more readily available and cheap. LH, Prolactin and other hormones may be relevant in particular cases but are not required as routine.

Semen analysis. (See Form 4) Absence of sperm in two or more semen analyses, at least one month apart, is a prerequisite for the diagnosis of azoospermia and would be required before considering invasive procedures. Occasionally, azoospermia is intermittent, probably related to partial obstruction, but in such cases sperm counts in semen when present are very low.

Fine needle aspiration of testes. (See Form 5) This is performed by a special technique that was devised by Dr Yahia Dajani, whereby scouting and mapping of sperm within the testes is enabled with no damage sustained to testicular tissue. The 'A-D Grading' classification system avoids ambiguity and enables referenced sites for sperm extraction by testicular aspiration or tissue extraction at marked out sites for the purposes of ICSI (intracytoplasmic sperm insemination), whereby a single sperm can be inoculated into one egg. Scounting method has been very successful for the past few years with pioneering work in this field achieved at the Farah Hospital Assisted Fertility Unit, Amman, while other units elsewhere have followed suit. Fine needle aspiration has been proved superior to open biopsy for detection of sperm production in the testes.

BONE DISEASE

Laboratory tests for bone pathology

Alkaline phosphatase reflects osteoblastic activity. Elevated in Paget's disease, hyperparathyroidism (primary or secondary to renal disease, osteomalacia or malabsorption), Rickets, Hyperthyroidism, healing fractures and osteoblast active tumors in bone.

Hydroxyproline is increased whenever there is a rise in reabsorption of bone collagen. Conditions associated with elevated serum and urinary hydroxyproline include Osteoporosis, Osteomalacia/ Rickets, Hyperparathyroidism, Paget's disease, Rheumatoid arthritis, Hyperthyroidism, Multiple myeloma, Scleroderma, Marfan's syndrome, metastatic tumor to bone.[*]

Serum calcium may be raised in many of the conditions listed above.

Serum phosphate whereby hypophosphatemia is an important diagnostic clue to parathyroid overactivity.

Vitamin D

Parathormone

[*] Common tumors travelling to bone: prostate/ breast/ kidney/ thyroid/ lung.

BREAST CANCER

(See Tumor Markers Panel page 81)

After histological diagnosis of a breast cancer, certain tests can be performed which bear on management and prognosis. The tests are carried out on blood and breast tumor tissue. Controversial tests will be mentioned but well established tests will be stressed. It is important to note that breast cancer markers in blood are not used for screening or diagnosis, while data derived from such blood tests should be used in the light of the general picture with a follow up of those blood results for adequate interpretation.

Blood tests

CBC and General health screen

Specific: CEA, CA15.3 (see tumor markers)

Tests on tumor tissue (tested material should be representative and controlled)
(1) Estrogen & Progesterone receptors
(2) HER-2/neu oncoprotein expression

Others:
(3) Ploidy (DNA histogram) (4) Cathepsin D (5) p53 (6) pS2 protein (7) EGFR (8) nm23 (9) Cyclin E (10) p27

Estrogen & Progesterone Receptors

This is routinely done, together with HER-2/ neu, on paraffin sections of representative parts of viable looking tumor tissue. Paraffin blocks should be preferably prepared within 24-48 hours of postfixation in formalin after taking the tissue biopsy. Paraffin

blocks of previously processed frozen sections are unsuitable for the above tests. And although tests can be done on frozen tissue sections, it has proved more practicable to apply the technique on paraffin embedded tissue. Moreover, the technique can be equally applied on fine needle aspiration material, but adequacy of sampling is best achieved in the routine aforementioned method.

See captions of ER, PR and HER-2/ neu (Figures).

Interpretation of ER/PR

Every time we use control material.

Several methods of assessing ER/PR breast cancer reactions have been described. In the Consulting Medical Laboratories, for each of ER or PR, we follow a simple semi-quantitative system which comprises percentage and intensity of reaction of tumor cells, each of which is graded 0-3, according to strength of the reaction in the respective parameter of percentage and intensity, respectively. Resultant estimates are then summed up into either negative, or alternatively, weak, moderate or strongly positive reaction.

HER-2/neu

As with ER/PR, use of control material is a must and we test for this marker by immunohistochemical method (IHC) on paraffin embedded tissue. IHC is easier to perform with a rapid turn-around time and a relatively less cost, while results correlate with FISH (Fluorescent In Situ Hybridization) methodology.

HER-2/neu protein is a normal component of a variety of epithelial cells and is over-expressed by breast cancer cells. It is controlled by a 17.q21 mutational oncogene on cell membrane receptors affecting growth and proliferation of tumor cells.

It correlates with poor prognosis in LN positive cases and is overexpressed in 25% of breast cancer cases and in 30% with metastatic disease.

HER-2/neu Protein over-expression can be graded into 0-3 according to intensity of membrane staining. Cytoplasmic staining should be considered nonspecific. Since IHC results of HER-2 neu appear to be extremely variable, standardization of results is recommended. However, selection of cases among breast cancers which are candidates for HER-2 neu testing would eliminate much unnecessary work. In fact, it has been found that low grade ductal carcinomas of the breast (Grade I out of III) show no amplification of HER-2 neu in literally 100% of cases, while grades II and III correlate with HER-2 neu expression. In addition, special histologic types of breast cancer, such as tubular carcinoma, colloid carcinoma and lobular carcinoma also predict non-amplification of HER-2 neu.

As to scoring IHC HER-2 neu results, the FDA approved system is recommended. Only membrane staining is considered with intensity assessed as follows: Score 3+ (strong positive) if >10% of tumor cells show strong membrane staining and Score 2+ (weakly positive) if >10% of tumor cells show weak to moderate and complete membrane staining. Score 1+ or 0 (negative) results when <10% of tumor cells were reactive.

Treatment of HER-2/neu positive cases is by Herceptin which, alone produces a 14% response versus 29% by chemotherapy alone, while a combination of both produces a 44% response rate. Herceptin exerts adverse effects on the heart and bone marrow.

BRCA I 17q21 BRCA II 13q12-13 seen in ½ of hereditary breast cancer and in 100% of hereditary breast-ovarian cancer.

CARDIAC RISK (Coronary Artery Disease)

Laboratory tests: Cholesterol Total, HDL, LDL, Total/HDL Ratio, Triglycerides, FBS, CRP sensitive method. *(Fasting specimen is preferable)*

Other tests: Genetic testing for Factors II, V and homocysteine.

Total Cholesterol Levels: Moderate & High Risk Requiring Treatment

Age Group (years)	Moderate Risk (mg/dl)	High Risk (mg/dl)
20-29	>200	>220
30-39	>220	>240
>40	>240	>260

Therefore, it is desirable to seek a total cholesterol level below 200mg/dl while 200-240 is borderline and above that is high.

Total/HDL Ratio and Cardiac Risk

Risk	Percentile	Ratio (Men)	Ratio (Women)
Lowest	<25	<3.8	<2.8
Low	26-50	3.9-4.7	3.0-3.6
Moderate	51-75	4.8-5.9	3.7-4.6
High	76-89	6.0-6.9	4.7-5.6
Highest	>90	>7	>5.7

HDL seems to have a very strong relationship to coronary artery disease. Major causes of reduced HDL include cigarette smoking, obesity, lack of exercise, androgenic or related steroids, B-adrenergic blocking agents, hyper-

triglyceridemia and genetic factors. Measurement of HDL subtypes do not add to evaluation of risk.

LDL >170mg/dl is associated with increased myocardial risk irrespective of total cholesterol level.

Triglycerides role as independent risk factor has not been established. However, reports suggesting a link with coronary artery disease have been published.

C-Reactive protein level Serum <6.0 mg/l

Increased in acute phase reaction. A nonspecific test.

CRP (Sensitive method) Serum <5.0 mg/l

This is a newly developed and highly sensitive method for measurement of CRP that is associated with the metabolic syndrome. The latter identifies a population at high risk of developing cardiovascular events. Serial levels are of value in assessing risk for coronary heart disease. The metabolic syndrome is recognized by presence of 3 of the following characterstics: body obesity, hypertriglyceridemia, low HDL, hypertension and abnormal glucose. Increased levels of CRP occurs in association with all these parameters. CRP is a new and important prognostic factor for cardiovascular disease. However, CRP is a classical test for reaction to acute inflammation, whether infection, collagen disease, colitis or any systemic type of inflammation.

A pilot study of total cholesterol levels in normal Jordanian population was done at the Consulting Medical Laboratories and reported in 1991 (Barakat HF, Sarrif AM & Dajani YF. Reference ranges of total cholesterol in Jordanians. *Bulletin of the Consulting Medical Laboratories* 1991; 9 (4): 1-2). The following table enlists the reference ranges in

this study, which were similar to those reported by the Physicians Health Study (Stampfer MJ, Sacks FM, Salvini S *et al.* A prospective study of cholesterol, apolipoproteins and the risk of myocardial infarction. *New England Journal of Medicine* 1991; 325 (6): 373-381) and close to the Framingham Study (Gordon DJ, Probstfield JL. Garrison RJ et al. High-density lipoprotein cholesterol and cardiovascular disease: four prospective American studies. *Circulation* 1987; 79: 8-15).

Reference Ranges of Total Serum Cholesterol in 2476 Jordanians (mg/dl)

Age Group (yrs)	Number	Percent	Mean Value (+/- S.D)	
			Male	Female
<20	124	4.5	165 (43)	163 (35)
20-24	106	3.8	179 (38)	179 (35)
25-29	238	8.7	197 (37)	189 (39)
30-34	257	9.4	205 (44)	186 (35)
35-39	476	17.3	207 (36)	203 (38)
40-44	478	17.3	212 (36)	211 (40)
45-49	402	14.6	213 (37)	216 (38)
50-54	246	9.0	213 (37)	217 (46)
55-59	225	8.2	214 (38)	214 (43)
>59	194	7.1	215 (41)	211 (35)

COAGULATION

Common screening coagulation tests

PT (Prothrombin time), *PTT* (Partial thromboplastin time), *TT* (Thrombin time) *Activated clotting time, BT* (Bleeding time), *Pl Ct* (Platelet count), *Hess's* or Rumpel Leede (modified Hess's) tourniquet test.

Note*:* Clotting time, synonym Lee White clotting time, has run into disrepute because of lack of sensitivity and reproducibility and it is not recommended as a test for coagulation disorder or heparin control.

Second line coagulation tests

After initial screening tests and taking the clinical picture into consideration, the following tests may be ordered:

(1) Lupus anticoagulant, Protein C, Protein S, Antithrombin III, Phospholipid antibodies.
(2) Fibrinogen (serial), FDPs (Fibrinogen degradation products), Plasminogen, Clot lysis time, Euglobulin lysis time.
(3) Clot retraction time, Platelet aggregation, Platelet adhesiveness.
(4) Factor VIII, Factor IX & other factor assays, Clot urea solubility for Factor XIII.

Coagulation profiles

Preoperative: PT, PTT, Pl Ct, BT.

Abnormal Bleeding Panel: PT, PTT, Pl Ct, BT, TT, Fibrinogen, Hess's test, Specific factor assay.

Easy Bruisability Panel: PT, PTT, Pl Ct, BT, Hess's test, Blood film.

Fibrinolysis Panel: Euglobulin lysis time, TT, FDPs, Lupus anticoagulant.

D.I.C. (Disseminated intravascular coagulation): PT, PTT, Fibrinogen, Pl Ct, FDPs, Lupus anticoagulant, TT, Euglobulin lysis time, Blood film for schistocytes.

Thrombotic tendency: PTT, Antithrombin III, Lupus anticoagulant, Proteins C&S.

Von Willebrand's disease: PTT, Factor VIII assay and related antigen, BT, Pl Ct, Platelet aggregation.

Qualitative platelet disorders: Pl Ct, Platelet Factor 3, BT, Hess's test, Platelet function tests: adhesiveness, aggregation and clot retraction time

Note*:* In general, bleeding in coagulation (clotting) disorders is in the form of joint, soft tissue and organ bleeding. In platelet or capillary dysfunction, bleeding is in the form of purpura and ecchymoses or from mucosal surfaces. However, the pattern does not always obey the rule. For example, hematuria due to anticoagulant therapy is caused by hemorrhages into the pelvicalyceal lining (Dajani YF. *New England Journal of Medicine* 1977; 297:222).

COMA

Causes of coma

Central nervous system: Intracranial disease, injury, infection, subdural hematoma.

Metabolic: Diabetes hyperosmolar or ketoacidotic coma, uremia, hepatic/ anoxic encephalopathy, hypothermia, hypothyroidism.

Electrolyte/acid-base imbalance: hyponatremia, hypernatremia, hypercalcemia, hypo-/ hyperkalemia, hypomagnesemia, respiratory acidosis/ alkalosis.

Drugs and toxic agents: Sedatives such as alcohol, tricyclics and opiates. Metabolic acidosis due to salicylates, methanol, paraldehyde etc.

Enzyme inhibitors such as heavy metals, organic phosphates and cyanide.

Laboratory tests for comatose patients

CBC, Glucose, Calcium, Magnesium, AST, Bilirubin total and direct, Sodium, Potassium, Chloride, Bicarbonate, arterial blood Gases pH, pCO2 and pO2, Acetone, CPK, Urea, Creatinine, Osmolality. Urine analysis, Drug screen (see toxicology page 74).

Also, X-ray of chest and any injuries, CT/MRI for brain lesion or hydrocephalus.

CONNECTIVE TISSUE DISEASE

Laboratory tests:
(1) *Non-immunological*
(2) *Immunological*

Non-immunological lab tests: ESR & CRP: may be raised as markers of inflammation, CBC: white cell count is vari-

able, sometimes shows lymphocytosis, red blood cells show normochromic normocytic anemia, Direct Coombs test may be positive, Renal function tests: may be abnormal. VDRL may be positive

Immunological tests:
(1) ANA (antinuclear antibodies) & antiDNA or specific antinuclear antibodies
(2) Complement profile
(3) Immune complexes (see special section page 49)
(4) Rheumatoid factor
(5) Cryoglobulins.

Connective tissue disease

R.A. (Rheumatoid arthritis) and related syndromes,* SLE (Systemic lupus erythematosus), Scleroderma and PSS (Progressive systemic sclerosis), CREST syndrome (Calcinosis cutis, Raynaud's phenomenon, Esophageal motility disorder, Sclerodactyly and Telengiectasia), Polymyositis and Mixed connective tissue disease.

Rheumatoid arthritis

There may be normocytic and normochromic anemia, lymphocytosis in 25% of patients, raised ESR & CRP according to disease activity, positive rheumatoid factor in 85% of patients with active disease, positive ANA in 20-70% and histone antibodies in 20% of patients. Synovial fluid may

* Felty's syndrome, Sjogren's syndrome, Still's disease, Reiter's syndrome, Ankylosing spondylitis.

be turbid, white cell count 5-20 10^3/ml with neutrophilic response in 2/3rd of patients.

Felty's syndrome = R.A. + Splenomegaly + High WBC + High RF titer

Sjogren's syndrome 90% women, usually middle aged. = Keratoconjunctivitis sicca + pharyngitis sicca + enlarged parotids.

Dense lymphocytic infiltrate in salivary, lacrimal and other glands.

Autoantibodies: 90% RF, 70% ANA, 60-70% anti-salivary (diagnostic), 60-70% SS-A/Ro, 60% SS-B/La.

Still's disease (juvenile rheumatoid arthritis) associated with HLA-B27. As in adults. May be systemic, pauci- or poly-articular.

Reiter's syndrome = a *Triad of* Arthritis, Urethritis, Conjunctivitis. Usually RF and ANA negative. Associated with HLA-B27.

Ankylosing spondylitis (Marie-Strumpell) associated with HLA-B27 Usually male = Erosive sacro-iliitis progressing to ankylosis and immobilization. Small peripheral joints may be involved.

Behçet disease Associated with HLA-B51 and shows increased risk in Chinese and Japanese.

SLE

***Immunological tests in SLE*:**

1) ANA. Immunofluorescent rim or ring pattern specific; ELISA test is reported to be reliable and reproducible test. ANA is present in 99% of SLE patients.
2) Anti-DNA (native, double stranded) high levels in 40-60% with active disease, other diseases may show low levels, and correlates with lupus nephritis.
3) Anti-Smith (Sm) antibodies are almost specific for SLE, found in 20-30% of patients. Associated with vasculitis.
4) Complement profile C1, C4, C2, C3 all depressed.
5) Immune complexes see special section.
6) Others. LE cell phenomenon (Caption 23 – of historical interest), Rheumatoid factor (positive in 30%), VDRL (positive in 10-20%), Anti-platelet, Anti-thyroglobulin and Anti-cytoplasmic (ribosomal) antibodies.

Note: Some drugs can induce SLE like illness, which include *hydralazine* used in treating hypertension, *procainamide* used in treating cardiac arrhythmias, *isoniazid*, *practolol*, *hydantoins*, *chlorpromazine*, *D-penicillamine*, *nitrofurantoin*.

In the drug induced form, antibodies to DNA and Sm are absent but ANA is positive and is usually reversible.

Criteria for the diagnosis of SLE

4 of the following 11 criteria should be present to diagnose SLE:sensitivity 96% and specificity 96% (*Journal of the American Medical Association* 1982, 48:622):

1. Malar rash. 2. Discoid lupus. 3. Photosensitivity. 4. Oral ulcers. 5. Arthritis. 6.Proteinuria >0.5g/day, or Cellular casts. 7. Seizures or psychosis. 8. Pleuritis or pericarditis. 9. Hemolytic anemia/ leukopenia/ lymphopenia/ thrombocytopenia. 10. Anti-DNA or SM positive or VDRL positive. 11. Positive ANA.

Scleroderma & PSS

ANA positive in 80% of patients at low titer.

Nucleolar RNA in 40-50% of patients.

Scl-70 antibodies in 20-40% of patients and is relatively specific.

Rheumatoid factor in 25% of patients.

Cryoglobulin and immune complexes may be positive.

Polymyositis

Laboratory diagnosis is based on 3 tests:

(1) Elevated serum CPK (2) EMG (3) Muscle biopsy: perivascular inflammation.

Immunological tests: ANA, Anti-PM-1 found in 50% of polymyositis and 10% of dermatomyositis patients at low titers, Rheumatoid factor occasionally positive.

Mixed connective tissue disease

A mixed clinical picture with symptoms of SLE, scleroderma and polymyositis. Have unusually high titers of of RNP antibody, positive in 95-100% of patients. Other antinuclear antibodies are absent. About 2/3rd have features

of inflammatory myositis with muscle pain, weakness and tenderness with abnormal EMG. Rheumatoid factor is positive in 50% of patients.

DEPRESSION MARKERS

Laboratory tests

1) *Dexamethasone suppression test* distinguishes endogenous from nonendogenous depression. It should not be used for screening, but only for doubtful cases while some consider its use only for confirming the diagnosis.
   This test is started on day 1 by taking basal cortisol level at 11pm, after which 1mg dexamethasone is given within the following hour. On day 2, serum cortisol is taken 16 and 24 hours (4 and 11pm) after giving dexamethasone. In endogenous depression, serum cortisol level fails to stay suppressed for 24 hours post-dexamethasone. When results of the test show delay in normalization, there is a high risk for early relapse. *False positive* result may be caused by several disorders such as major physical illness, pregnancy, unstable diabetes, starvation, alcoholism and several drugs such as phenytoin, barbiturates, carbmazepine and others.
2) *TRH (Thyrotrophin-releasing hormone) stimulation test* is used to distinguish unipolar from multipolar depression. In this test, TRH is injected intravenously and blood samples are collected at 15-minute intervals for one hour. In 77% of unipolar depressives there is

blunting of response to TRH compared to only 17% of bipolar depressives.

3) *Prolactin response to TRH* (Judd LL *et al. Archives of General Psychiatry* 1982; 39: 1413-1416.
4) *3-Methoxy-4 Hydroxyphenylglycol (MHPG) in urine* (Schildkraut *et al. Psychopharmacology Bulletin* 1981; 17: 90-91.
5) *Platelet monoamineoxidase (MAO) activity* (Weinshilboum RM. *Mayo Clinic Proceedings* 1983; 58: 319-330.
6) *Clonidine release of growth hormone* Depressed patients show a blunted growth hormone response to clonidine due to alpha-2 adrenoceptor sub- sensitivity.

Diseases associated with depression include hyper- and hypo-thyroidism, hyperparathyroidism, Cushing's syndrome, Acute intermittent porphyria, Vitamin B12 deficiency.

DIABETES MELLITUS

Diagnosis of diabetes mellitus

One of the following criteria can lead to a diagnosis of diabetes mellitus:

1. Casual* plasma glucose level 200 mg/dl (11.1 mmol/l) or more
   +
   Symptoms† of diabetes mellitus

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* *Casual*= any time of the day regardless of the last meal.
† *Symptoms*= polyuria, polydypsia, weight loss.

OR

2. FPG (Fasting* plasma glucose)126 mg/dl (7.0 mmol/l) or more

OR

3. 2-hour plasma glucose 200 mg/dl (11.1 mmol/l) or more during a glucose tolerance test as described by WHO, using a glucose load equivalent to 75g anhydrous glucose dissolved in water.

In the GTT (glucose tolerance test), *diabetes mellitus* is diagnosed by an oral glucose load of 75g glucose in adults or 1.75 g/kg body weight in children. FPG exceeds 140 mg/dl (7.8 mmol/l) and plasma glucose level exceeds 200 mg/dl (11.1 mmol/l) at 1 and 2 hours, respectively. *Impaired glucose tolerance* in GTT is defined by a FPG exceeding 140 mg/dl (7.8 mmol/l) and a 1-hour level between 140 - 200 mg/dl (7.8-11.1 mmol/l). In *gestational diabetes mellitus*, screening can be made by measuring plasma glucose level 1-hour after 50g oral glucose; if it exceeds 140 mg/dl (7.8 mmol/l), definitive diagnosis can be made by giving 100g oral glucose and finding FPG exceeding 105 mg/dl (5.8 mmol/l) and plasma glucose level exceeding 190 mg/dl (10.5 mmol/l), 165 mg/dl (9.2 mmol/l) and 145 mg/dl (8.1 mmol/l) at 1, 2 and 3 hours, respectively.

Diabetic Ketoacidosis

DKA (diabetic ketoacidosis) is potentially lethal that may occur at any age and in previously unrecognized diabetics as well as in insulin dependent diabetics. It may be precipitated by pneumonia, pyelonephritis, septicemia, alcoholism,

* *Fasting*= no caloric intake for at least 8 hours.

gastroenteritis, myocardial infarction, pancreatitis and cerebrovascular disease.

Laboratory tests: An average concentration of 500 mg/dl *plasma glucose* is found with 3-4+ glucose and 2-4+ ketones in the *urine*, increased plasma osmolality exceeding 295 mOsmol/l, marked suppression of blood *pH, pCO2, HCO3* and *base excess*, *K* exceeding 5 mmol/l, *Na* below 135 mmol/l and an increased *anion gap* – well above 15 mmol/l, *lactic acid* level above 1.66 mmol/l and increased *urea* level.

Many other tests should be performed as deemed relevant. These include vital signs (B.P., pulse and respiration), body weight for monitoring hydration status, blood and urine cultures, calcium and magnesium, Chest X-ray and CBC.

Treatment is essentially based on correction of fluid and electrolyte disturbances, administration of Na, K and glucose as well as insulin to restore regulation of metabolism e.g. of ketones. Any other treatment may be given as indicated.

In an average weight man of 70 Kg, a deficit of 5-7 liters of water will be present in DKA, 00-450 mmol of Na, 200-400 mmol of K. In children, dehydration should be judged by clinical signs and weight. Insulin is usually given at 0.1 units/Kg/hr; in children under 5 years of age, insulin dose is 0.06-0.08 units/Kg/hr. Insulin treatment is continued until ketosis subsides.

Complications of DKA include shock, acquired respiratory distress syndrome, infection, arterial thrombosis and cerebral edema. The latter is very serious and usually fatal and, if not, causes permanent brain damage.

ENDOCRINE EMERGENCIES

Emergencies can occur in *diabetes mellitus, thyroid, adrenal, pituitary and parathyroid disorders*.

Diabetes mellitus

Emergencies:

1) *Ketoacidosis* Serum glucose 300-800mg/dl, ketonuria, pH <7.1, HCO3 <10, pCO2 low.
2) *Hyperosmolar coma* Blood glucose >900mg/dl, Plasma osmolality >350mosmol/kg.
3) *Lactic acidosis* Plasma lactate very high, Increased anion gap, pH <7.0.
4) *Hypoglycemia* Low blood glucose.

Thyroid gland

Emergencies:

1) *Myxedema coma* Low T4, high TSH, high cholesterol, low Na (dilutional).
2) *Thyroid crisis* High T4, Low TSH.

Adrenal gland

Emergencies:

1) Addison's crisis Low plasma cortisol, High plasma ACTH, Low Na, High K.
2) Acute hypercortisolism Hypokalemic alkalosis, elevated plasma cortisol.
3) Pheochromocytoma Phentolamine test positive. High catecholamines, metanephrine and VMA.

Pituitary – hypothalamus

Emergencies:

1) *Hypopituitary coma* T4, Cortisol, TSH and ACTH all decreased, Low blood glucose and sodium.
2) *Diabetes insipidus* Increased plasma and decreased urine osmolality, Large urine volume.
3) *Inappropriate ADH secretion* Low serum sodium (dilutional), Osmolality decreased in plasma, increased in urine.

Parathyroid gland

Emergencies:

1) *Hypocalcemia* Serum calcium <7mg/dl.
2) *Hypercalcemia* Serum calcium >13mg/dl.

FAT MALABSORPTION

Laboratory tests:

Fecal fat (72 hour excretion) >7g fat / 24 hours positive for steatorrhea.

Microscopic fat in stools Sudan III or Oil Red O positive globules. Sensitivity of this test is 72% and specificity 95%.

Serum immunoreactive trypsin decreased in pancreatic insufficiency with steatorrhea but normal if either of the two factors is absent.

Serum carotene decreased in malabsorption and other states such as liver disease, high fever, infections, stress etc. It is

increased in diabetes mellitus, myxedema, chronic renal disease and high dietary intake.

D-xylose absorption test which assesses proximal small intestinal function and can be used to differentiate malabsorption of pancreatic insufficiency from other causes, in the former case xylose absorption is found normal. D-xylose is not metabolized and is excreted unchanged by the kidney.

Sweat electrolytes test for cystic fibrosis, which is identified by a clinical triad of pancreatic insufficiency, chronic and suppurative lung infections and failure to thrive. A special machine is available to measure sweat sodium chloride level.

Intestinal biopsy which reveals villous atrophy in malabsorption.

Trypsin in stools a test for pancreatic insufficiency.

Glucose tolerance test for pancreatic insufficiency

Fat soluble vitamins A, D & K as well as *folic acid* and *protein* may be decreased.

Diseases causing malabsorption

Pancreatic insufficiency in chronic pancreatitis, cystic fibrosis and carcinoma.

Impaired intestinal absorption in celiac disease, tropical sprue, lymphoma, other rare causes.

Bile secretion deficiency in extra- and intra-hepatic bile obstruction.

Surgical loss of pancreatic, gastric or intestinal tissue bulk.

GASTROINTESTINAL HEMORRHAGE

Laboratory Tests:

Stool for blood, CBC, Gastric aspirate for blood, Urine analysis, Urea, Na, K, Cl, HCO3, Coagulation screen: PT, PTT, Fibrinogen, Platelet Ct, Liver function tests, Serum albumen, Amylase in serum & urine, Ammonia, X-rays of chest & abdomen, ECG in older persons to exclude a silent infarct in severe cases.

Clinical Procedures

Endoscopy upper & lower, Barium enema for diverticulosis, Selective angiography, Other specialized procedures where indicated.

Causes of GI bleeding

Upper Peptic ulcer, Esophageal varices, Gastritis, Stress ulceration, Mallory-Weiss syndrome, Cancer, Gatro-esophageal reflux ulceration, Drugs e.g. aspirin, steroids, iron, alcohol.

Lower Diverticular disease, Vascular, Colorectal cancer, Enterocolitis, Colonic polyps, Intussusception, Volvulus, Inflammatory bowel disease.

Neonates: Anorectal fissure, Hemorrhagic disease of newborn, Infectious diarrhea, volvulus, Vascular malformation.

Infants (up to 2 years): Infectious diarrhea, Anal fissure, Intussusception, Meckel's diverticulum, Peptic ulcer, volvulus.

Children 2-12 years: Infectious diarrhea, Juvenile polyp, Peptic ulcer, Esophageal varices, Inflammatory bowel disease, Meckel's diverticulum, Intestinal duplication, Intussusception, Vascular malformation.

Adolescents: Peptic ulcer, Inflammatory bowel disease, Esophageal varices.

GENERAL HEALTH LABORATORY SCREEN

A general health laboratory screen can never be complete without a full physical examination. The following list has been recommended by the Consulting Medical Laboratories for well over 20 years. This test panel is recommended in men and women over 50 years, but may be done in those over 40 years or perhaps in younger persons as may seem suitable. Additional tests may be designed for each individual case.

Recommended Tests List

Urine Analysis

Stool Analysis including an occult blood test

CBC and ESR

Blood grouping and Rhesus typing

Fasting blood sugar

Serum cholesterol: Total, HDL & LDL Cholesterol, Total/HDL ratio, CRP sensitive method

Triglycerides

Urea, Creatinine, Na, K, Cl, Uric acid

Ca, PO4

ALT (GOT), AST (GPT), GGT, Alkaline phophatase, Bilirubin total & direct

Serum protein total & albumen

TSH in women and PSA in men

GENETIC TESTS

A. Hematological disorders

Genetic tests for thromboembolism:
a1. Mthfr gene, homocysteine mutation
a2. Factor II, prothrombin mutation
a3. Factor V, leiden factor

Genetic tests for coagulation:
a4. Hemophilia A
a5. Hemophilia B

Genetic testing for HBS/thalassemia
a6. Beta-globin gene mutations/hemoglobinopathies

B. Genetic tests for inborn errors of metabolism:
b1. Cystic fibrosis
b2 Hereditary haemochromatosis
b3. Alpha-1 antitrypsin deficiency
b4. Osteoporosis
b5. Adrenogenital syndrome

b6. Angiotensin converting enzyme
b7. Familial thyroid hormone resistance
b8. Maturity Onset Diabetes Mellitus in Young I,II,III
b9. Cystinuria

C. Gene tests for abnormal lipid metabolism:

c1. Apolipoprotein AI/ CIII, APO AI/CIII gene
c2. Apolipoprotein B, APO B gene
c3. Apolipoprotein E, APO E gene
c4. Familial hypercholesterolemia, LDL receptors gene
c5. Sandhoff's disease (hexosaminidase decifiency)

D. Gene testing for azoospermia:

d1. Azoospermia factor

E. Gene tests for neoplasia:

e1. MEN (multiple endocrine neoplasia) type 1
e2. MEN type II
e3. All (acute lymphoblastic leukemia)
e4. Pre B-cell ALL
e5. CML (chronic myeloid leukemia)
e6. Follicular lymphoma
e7. Familial nonpolyposis colon cancer

F. Gene tests for neuromuscular disorders:

f1. Muscle dystrophy
f2. Mitochondrial encephalopathy, MELAS
f3. Myoclonal epilepsy, MERRF gene
f4. Myotonic dystrophy, DMK gene
f5. Fragile-X syndrome
f6. Spinocerebellar ataxia
f7. Amyloidotic polyneuropathy
f8. Huntington's disease

`f9. Spinal muscular atrophy
f10. Friedrich's ataxia
f11. HMSN (Charrot-Marie-Tooth disease)

K. Gene tests for kiney disorders:
k1. Autosomal dominant polycystic kidney disease
k2. Autosomal recessive polycystic kidney disease
k3. Autosomal dominant medullary cystic kidney disease

L. Familial mediterranean fever

HEAVY METAL SCREEN IN URINE

The screen is performed by atomic absorption. The normal is a negative result.

Heavy metals screened for in urine (the more commonly requested are underlined):

Antimony, arsenic, bismuth, boron, cadmium, cobalt, copper, lead, mercury, and zinc.

HEPATITIS, CHRONIC

Laboratory tests: See Table 11.

General: CBC, Liver function tests, Urine routine, others as indicated

Viral tests: HBV profile, HCV, CMV, EBV

Autoimmune tests: ANA, Anti dsDNA, AMA, ASMA, ENA

Metabolic tests: Ceruloplasmin, urinary Copper, Alpha-1 antitrypsin, Serum ferritin and Transferrin.

A liver biopsy is often required for a definitive management.

Hepatitis A does not cause chronic hepatitis or cirrhosis. Usually chronic hepatitis is caused by HBV, HCV or autoimmune diseases and rarely due to other causes. Hepatocellular carcinoma is a recognized complication of viral induced chronic liver disease.

HYPERTENSION

Laboratory tests:

CBC for *anemia* or *polycythemia*, Electrolytes for presence of *hypokalemia* or *alkalemia* due to corticosteroid excess, *acidosis* due to renal insufficiency, as would also be reflected by serum Creatinine level, Glucose for diabetes mellitus, Calcium for parathyroid disease, TSH, T4, T3 for thyroid disease.

Other tests which pose other cardiovascular risks include Cholesterol profile (Cholesterol total, HDL, LDL, Triglycerides) and Uric acid. Urinalysis may show glucose in diabetes, blood or protein in hypertensive nephropathy.

Tests in secondary hypertension should be designed according to the suspected cause. *Renovascular hypertension*: IVP, Suppressed or Stimulated Plasma Renin Activity (PRA), *Primary aldosteronism:* Potassium in serum and urine, PRA, PRA/Aldosterone ratio, *Pheochromocytoma*: Urinary metanephrine, epinephrine, norepinephrine and

VMA, *Cushing's syndrome*: Dexamethasone suppression test, Serum cortisol, Urinary free cortisol, *Coarctation of aorta*: Chest X-ray, B/P in upper and lower extremities.

Causes of hypertension

In a community-based background, over 90% of cases of hypertension were reported as to be either of the essential type (in 2/3rd of patients with elevated blood pressure, borderline in about 15% or of no diagnostic significance in 10%). On the other hand, secondary hypertension accounted for less than 10% of cases (Ferguson RK. *Annals of Internal Medicine* 1975; 82: 761). Secondary hypertension may be caused by renal disease (renal artery stenosis, chronic renal disease, polycystic kidney disease), oral contraceptives or endocrine disorders (primary hyperaldosteronism, 17-hydroxylase deficiency).

Curable causes of hypertension include renovascular disease, primary aldosteronism, pheochromocytoma, coarctation of aorta, unilateral renal parenchymal disease, Cushing's syndrome, adrenogenital syndrome (17- and 11-hydroxylase deficiency), oral contraceptives, licorice, 'sous' drink commonly taken during the fasting month of Ramadan and renin-secreting neoplasms.

IMMUNE COMPLEX PROFILE

Immune complexes are run on serum from patients with diseases associated with circulating immune complexes, the latter combinations of antigen-antibody-complement units. Several different disorders are associated with immune

complexes, with deposition in various body tissues. As a result, vasculitis and glomerulonephritis may ensue.

Tests: (1) C1q binding Assay (2) Raji cell assay (3) Conglutinin assay.

Note: CH50, C3 and C4 are all decreased.

Raji test detects C3 associated complexes. Multiple samplings showing rising level of immune complexes are far more significant than a single high measurement.

Diseases associated with immune complexes:

Connective tissue disorders: Rheumatoid arthritis; SLE; Sjogren's syndrome; Mixed cryoglobulinemia; Mixed connective tissue disease; Necrotizing vasculitis; Relapsing polychondritis.

Glomerulonephritis (GN): Rapidly progressive GN; SLE; Acute GN.

Infectious disease: Bacterial endocarditis; Systemic gonococcal infection; Schistosomiasis; Malaria; Leprosy; Viral hepatitis; Dengue fever.

Neoplasia: Hodgkin lymphoma; Leukemia; Solid tumors; Burkitt's lymphoma; Melanocarcinoma.

Others: Chronic active hepatitis; Primary biliary cirrhosis; Fibrosing alveolitis.

INBORN ERRORS OF METABOLISM

Screening for inborn errors of metabolism requires testing urine and blood.

Tests are performed according to clinical suspicion in some situations, such as for lactase deficiency, or as a routine screening test for all newborns, such as for phenyl ketonuria and hypothyroidism. See also Tables 14, 17 & 18.

Laboratory evaluation of inborn error of metabolism.

Blood: *Sugar level, Na, K, Cl, HCO3, pH, Lactate, Pyruvate.*

Plasma: *Ketones, Ammonia, Amino acids, Organic acids.*

Urine: *Metabolic screening tests, Amino acids, Organic acids, Ketones.*

Urine screening tests for inborn error of metabolism: 3 screening tests may be used:
(1) Ferric chloride test.
(2) DNPH (2,4-Dinitrophenyl hydrazine) test.
(3) Nitroprusside reducing substances test.

Ferric chloride test: positive in several amino acid and other metabolic disorders

Ferric chloride test: **Disorders, Abnormal products in urine versus color change**

Disorder	Product color change
Phenylketonuria Phenylpyruvic acid	Blue +/- green fades to yellow
Tyrosinemia p-Hydroxyphenylpyruvic acid	Green fades in seconds
Alkaptonuria Homogentisic acid	Blue/ Green fades fast
Diabetics, Alcoholics, Starved Acetoacetic acid	Red/ Red brown
Histidinemia Imidazole pyruvic acid	Green/ Blue green
Maple syrup disease: Alpha-ketoisovaleric acid	Blue
Alpha-ketoisocaproic acid	ditto
Alpha-keto-beta-methyl valeric acid	Ditto
Abnormal product: Bilirubin	Blue green
Pyruvic acid	Gold yellow/ green
Alpha-ketobutyric acid	Purple fades to red brown
Xanthurenic acid	Deep green changes to brown
Ortho-hydroxyphenyl acetic acid	Mauve
Ortho-hydrophenyl pyruvic acid	Red
Drug: Salicylates	Purple stable
Aminosalicylic acid	Red brown
Phenothiazines	Purple pink
Antipyrines & acetophenetidines	Red
Phenol derivatives	Violet
Cyanates	Red

DNPH test: Yellow precipitate forms in presence of ketones, aldehydes and keto acids. The DNPH is positive in the following disorders:

Disorder	Urine product
PKU (Phenylketonuria)	Phenyl pyruvic acid
Glycogen storage disease 1/ 3/ 5/ 6	Acetone
Histidinemia	Imidazole pyruvic acid
Maple syrup disease	Keto acids
Ketonuria from any cause	Ditto
Methionine malabsorption	Alpha ketopyruvic acid

Nitroprusside test: for cystine and homocystine, which are reduced by cyanide to cysteine and homocysteine, respectively, then react with nitroprusside to produce a red-purple color.

A practical approach is presented in the clinical assessment of a newborn with suspected metabolic disease:

Initial assessment. Look for the following clinical parameters: Poor feeding / Vomiting / Lethargy / Convulsions and Coma not responding to parenteral glucose or calcium.

Differential Diagnosis. (1) Metabolic disorder or (2) Infection

(1) Metabolic disorder: Do *Plasma ammonia*:

If high:	If normal:
Do *blood pH & CO2*:	Do *blood pH & CO2*:
If normal__ urea cycle defects	If normal __ aminoacidopathies/ galactosemia
If acidosis __ organic acidemias	If acidosis __ organic acidemias

JOINT FLUID (ARTHRITIC)

Laboratory tests: The tests are divided into synovial fluid and blood analyses:

Synovial fluid analyses

ALWAYS: (a) Physical examination: volume, appearance (b) wet preparation (direct smear) (c) crystals under the polarized light * (d) white cell count.

USUALLY: Differential white cell count.

WHEN INDICATED: (a) Gram stain (b) Culture.

In gout, crystals are seen in about 90% of patients during acute arthritic attacks and in 75% between attacks. Urate crystals appear as birefringent rods and needles giving a yellow color when parallel to the axis of the red compensator and blue when perpendicular to the axis. The pyrophosphate crystals of pseudogout have the opposite orientation.

Blood analyses

(a) CBC (b) ESR (c) Serum uric acid (d) Tests for rheumatic diseases: Serum protein electrophoresis, Immunoglobulin levels IgM, IgG & IgA, Rheumatoid factor, ANA, Serum complements, ASO and sterptozyme tests.

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* *Crystals* In pseudogout (chondrocalcinosis), under the polarized light, calcium pyrophosphate crystals appear as rods, rectangles or rhomboids which are weakly birefringent. These crystals are deposited in articular cartilage.

Note. It is usually a waste of time testing in joint fluid for red cells, protein, sugar, enzymes, rheumatoid factor or ANA, mucin clot, complement or immune complexes.

In investigating joint fluid, we want to know if this is inflammatory or noninflammatory arthritis and identify septic arthritis. The following table shows guidelines in making these distinctions:

Test	Noninflammatory	Inflammatory	Septic
Clarity	Transparent	Translucent/ opaque	Opaque
Color	Straw, yellow	Yellow/ yellow-green	Variable
WCC/ml	<2000	<100,000	>100,000
Neutrophils	<50%	50-90%	>95%
Diagnosis	Osteoarthritis	Rheumatoid arthritis	Sepsis*

* Other possible diagnosis: Reiter's disease, Partially treated septic joint, Fungal/ viral infection, Gout/ pseudogout.

Joint diseases and useful serum tests:

Osteoarthritis, traumatic arthritis and pseudogout: None

Gout: serum uric acid

Rheumic fever: ASO and streptozyme

Bacterial arthritis: blood culture

Tuberculous arthritis: (T.B. skin test)

Rheumatoid arthritis: rheumatoid factor, ANA, (see connective tissue panel)

Lupus erythematosus: ANA, anti-DNAds, anti-smooth muscle antibody, serum complement.

MULTIPLE SCLEROSIS

Laboratory tests:

In active MS (multiple sclerosis):

1) *CSF gamma globulin* IgG index is elevated in 75% of MS patients. Other diseases causing increased CSF IgG are encephalitis, meningitis, neurosyphilis, arachnoiditis and some intracranial neoplasms.
2) *Oligoclonal banding in CSF* Normal immunoglobulin migrates on agarose as a diffuse band. Abnormal immunoglobulin migrates as discrete sharp or oligoclonal bands. This is especially useful when CSF electrophoresis is run with serum, whereby oligoclonal banding is found in CSF but not in serum as evidence of MS, seen in 80% of patients. However, oligoclonal banding is also found in other diseases such as inflammatory neuropathy, neurosyphilis, cryptococcal meningitis, chronic rubella panencephalitis, subacute sclerosing panencephalitis and HIV infection.
3) *CSF-myelin basic protein* High levels are seen in acute MS but is absent in inactive phase or chronically active disease. It may be detected in patients with traumatic brain damage or postneurosurgery, myelin disorders such as transverse myelitis, hereditary/metachromatic leukodystrophy, peripheral neuropathy and in neurologic diseases such as encephalitis, CNS-SLE, subarachnoid hemorrhage.

Diagnostic criteria for the diagnosis of MS

Definite MS is indicated by presence of all the criteria below.

Probable MS if all criteria are present except only one objective abnormality present despite 2 episodes *or* one symptomatic episode has occurred.

At risk for MS if all six criteria are fulfilled except one symptomatic episode and corresponding signs detected on examination.

1. (a) Two or more separate episodes of worsening function in different sites within the central nervous system, each episode lasting at least 24hours with a minimum interval of one month.

   Or,

   (b) Gradual progression of illness for at least 6 months with elevated CSF IgG accompanied by two or more oligoclonal bands.
2. Objective CNS abnormalities on physical examination.
3. CNS involvement to reflect major impact on white matter. Long tracts usually include pyramidal pathways, cerebellar pathways, medial longitudinal fasciculus, optic nerve and posterior columns.
4. Two or more areas of the CNS should by implicated.
5. Age of onset between 15-60 years.
6. MRI should either show 4 lesions of white matter or 3 if one is paraventricular.

PANCREATIC INSUFFICIENCY

Laboratory tests:

Requested for pancreatic insufficiency include:
(1) *Serum trypsin*.
(2) *Bentiromide stimulation test.*
(3) *Abdominal radiograph* (for calcification).
(4) *72-hour fecal fat.*
(5) *Fecal fat globules.*
(6) *Stimulation tests:*
    (a) *Secretin.*
    (b) *Secretin-cholecystokinin.*
    (c) *Fatty meal*.

Bentiromide test:

Bentiromide is a simple outpatient procedure used to assess pancreatic function in children and adults. After an overnight fast, oral Bentiromide taken will be cleaved by pancreatic chymotrypsin to produce PABA (Para-aminobenzoic acid). PABA is absorbed in the intestine, conjugated in the liver and excreted in the urine; urinary PABA is estimated in a 6-hour urine collection. Sensitivity of this test is 86-100% but this is reduced in mild degrees of pancreatic insufficiency. False positive results are seen with emesis, gastric retention and mucosal atrophy, gastro-intestinal disease, renal impairment and severe liver disease. Side effects include gastro-intestinal symptoms, headache and transient elevation of liver enzymes. The test is disturbed by drugs like acetaminophen, phenacetin, benzocaine, lidocaine, chloramphenicol, sulfonamides and thiazides.

Fatty meal, Secretin and Secretin-cholecystokinin Stimulation tests

These tests require intubation and duodenal aspiration. Secretin/ Secretin-cholecystokinin are administered, then volume and electrolyte content of duodenal aspirate measured. Alternatively, Fatty meat is ingested followed by analysis of blood lipids (Triglycerides and Chylomicrons), which avoids intubation.

PITUITARY

Anterior pituitary

Tests for work-up of possible anterior pituitary tumor: *(1) Prolactin (PRL) (2) Growth Hormone (GH) (3) Adrenocorticotropin (ACTH) (4) Follicle Stimulating Hormone (FSH) (5) Luteinizing Hormone (LH) (6) Thyroid Stimulating Hormone (TSH) (7) Total Thyroxine T4, T3 (8) Cortisol (9) Estradiol*

Stimulation tests are used to detect pituitary insufficiency that often accompanies pituitary tumor. For example, ACTH secretion can be tested by insulin induced hypoglycemia or, alternatively, by corticotrophin releasing hormone. TSH secretion may be assessed by the TRH (thyrotropin releasing hormone) test, and so on for other anterior pituitary hormones.

Pituitary adenoma

May be detected by elevated hormone level in relation to the adenoma cell type. 40% of pituitary adenomas are

prolactinomas, which usually manifest in men with subfertility and decreased libido while in women they present with galactorrhea, amenorrhea and mastodynia.

Posterior pituitary

The main posterior pituitary disorders are *diabetes insipidus* (DI) and *Inappropriate antidiuretic hormone* (ADH) *secretion*.

Diabetes insipidus

Causes: Idiopathic (30%) Traumatic (30%) Neoplastic (30%). Others (10%): Vascular, Infectious and Infiltrative lesions.

Laboratory findings: elevated plasma ADH level, hypernatremia, increased plasma osmolality, decreased urine sodium concentration and increased urine volume.

Inappropriate ADH syndrome

Causes: Small cell carcinoma, Thymic/pancreatic tumors, Lung T.B., Meningitis, Brain abscess, Post CNS surgery, Stroke, CNS tumors, Vincristine therapy.

Laboratory findings: Elevated plasma ADH level, hyponatremia, reduced osmolality, renal sodium loss, low BUN, low uric acid.

Extracellular hyponatremia (serum Na falls < 120mmol/l) with consequent brain edema leads to anorexia, nausea and vomiting, which may be very serious.

PREMARITAL

Engaged couples often seek advice on laboratory tests prior to marriage, with intent to minimize risks of inherited disease in the children. Of course, there is no test panel which can rule out the possibility of transmitting an inherited disorder from parent to offspring, but careful family history taking and a screen for common disorders in a given community will minimize risks of transmitting an inherited disorder, but will never exclude it completely. In addition, we cannot foresee a neo-mutational event after fertilization of an ovum.

In our region, the following should be routinely performed when premarital tests are sought:

(1) Taking adequate history from the couple, including sexual history, any family illnesses and causes of death in higher generations.
(2) Physical examination, including external sexual organs.

Laboratory tests

Routine

Both: CBC (including MCV), Urine analysis, Blood grouping

Recommended

Men: Hb electrophoresis if MCV is decreased. Semen analysis, HBsAg, HIV.

Women: Hb electrophoresis if MCV is decreased. Rubella IgG, HBsAg, HIV.

Specific tests are indicated according to the clinical situation.

PREOPERATIVE

Routine testing for all elective surgery patients is unproductive and not indicated. For detailed account, refer to McPherson DS. *Medical Clinics of North America* 1993; 77: 289-308.

Although conjectural, urine analysis, CBC and fasting blood sugar are done as a preoperative routine in some hospitals. For ENT surgery, CBC, blood grouping, PT and PTT are done as a routine.

The best policy is to design any preoperative tests in relation to the history and physical signs in individual cases.

For major procedures, especially in orthopedics and neurosurgery, work-up for thrombosis risk would be advisable (see Thrombosis panel page 68).

PROSTATE

A. Prostatitis B. Prostate Specific Antigen

A. Prostatitis – Periurethritis Workup

Recommended *CML* (Cons Med Labs) *procedure*
1- *Resting* urine samplings (#1 & 2)
2- *Prostatic massage* expressed secretion
3- *Post-massage* urine samplings (#3 & 4)

Urine Samplings
#1 reflects urethral wall status
#2 urine status in bladder
#3 for presence of peri-urethral infection

#4 urine status in bladder

Prostatic Secretion

Direct Smear: *Trichomonas* Gram Stain Bacteria & *Neisseria* PAS stain *Candida*

Culture swabs (n= 2): Bacterial, Fungal.

Droplets/Swabs for other studies such as molecular testing for *Chlamydia* etc.

Interpretation: In prostatitis-periurethritis, relatively elevated leukocytic counts are seen in urine samples # 1 and 3 as well as in expressed prostatic fluid with neutrophilia. Bacterial isolation is only possible in a small proportion of cases, while Chlamydia or ureaplasma can be identified in a significant number, although a negative result does not exclude infection.

Note: In men and women, the best sample to retrieve chlamydial infection in suspected cases for DNA testing is in the first drop urine.

B. PSA (Prostate Specific Antigen)

PSA (1) Total (2)Free (3) Free to Total PSA Ratio

Reference value <4 ng/ml At 12-month screen, PSA rise not to exceed 0.75 ng/ml.

PSA is a glycoprotein that is primarily produced in the prostate gland. Also, it has been demonstrated in small amounts in apocrine breast cancer, apocrine sweat glands and neoplasms, male and female peri-urethral and anal glands.

PSA exists in serum in several molecular forms. The 2 major forms are (1) *free* PSA and (2) *complexed PSA*. Most of the complexed PSA is in combination with serine protease inhibitors, mainly ACT (alpha-antichymotrypsin). Prostate cancer cells release substantial PSA direct into the circulation in its active form with greater affinity for ACT and a resultant higher proportion of complexed against free form.

PSA is not specific for prostate cancer. PSA is elevated by urine retention, infection, benign prostatic hyperplasia, for 24 hours after per rectal examination (albeit 1st hour window), ejaculation, cycling, cystoscopy, transrectal ultrasound and prostate biopsy. Therefore, detection of prostate cancer pivots on the triad of *digital rectal examination*, *trans-rectal ultra-sonography* and *PSA*. Ultrasound guided sextant needle biopsies are taken for histological evidence in suspected cases. PSA is currently the marker of choice for prostatic carcinoma and remains invaluable for the follow up of prostatic cancer patients as well as post-prostatectomy monitoring. After prostatectomy, PSA baseline should be made no earlier than 6 weeks postoperatively. During follow up, rise in PSA level precedes clinically confirmed recurrent disease.

PSA is the most useful tumor marker for diagnosis and follow up in prostatic cancer.

But In about 40% of intra-capsular prostatic cancers, PSA level is <4µg/l.

PSA is better than rectal digital examination in detecting prostate cancer, 2.2 versus 1.3% in one study.Alternatively, PSA is rarely above 10 ng/ml in untreated prostatic cancer with abnormal bone scan.

The *Free/ Total PSA Ratio* appears to be lower in prostatic cancer compared to benign hypertrophy. In patients with prostatic disease and PSA levels of 2-10 ng/ml, the *Free/ Total PSA Ratio* gives a significant improvement in prediction of cancer over total PSA alone.

Probability of Prostate Cancer Based on Test Results

PSA Ratio (%)	Probability of Cancer (%)
0 – 10	56
10 – 15	28
15 – 20	20
20 – 25	16
> 25	8

PULMONARY EMBOLISM

Investigations:

Pulmonary angiogram is the definitive and reference method for PE.

Lung scan (Ventilation and Perfusion), though false positive cases occur but negative result excludes PE. An alternative and diagnostic test is the CT Angiogram (CTA) where available. However, CTA is limited to patients with a normal serum creatinine level.

Tests:

Arterial blood gases- pO2 always below 80.

Chest X-ray- can be normal; may show pleural effusion etc.

ECG: abnormal in 80% of massive PE.

Noninvasive leg study: impedance or duplex ultrasound.

Incidental: Elevated fibrin degradation products.

Comment: VTE (venous thrombo-embolism) and PE (pulmonary embolism) constitute the main medical complication following major surgery, with a risk of VTE reaching 15-30% and even greater after some procedures, such as major orthopedic surgery. DVT also develops after long haul flights, with a risk that may be as high as 10%. The problem is that PE and DVT are often asymptomatic. Treatment is by anticoagulants (heparin and coumarins) and thrombolytic agents (streptokinase or urokinase). Selective inhibitors of activated Factor X (Xa) may be considered.

We believe that whenever a major surgical operation is contemplated, thrombosis risk should be assessed by doing antithrombin III, Factors II, V and THFR genetic factors. Any expense in this direction, at least in those who can afford it, should be worthwhile.

SARCOIDOSIS

Laboratory tests:

Serum ACE (Angiotensin-converting enzyme) sensitivity 60-80% but is also elevated in several disorders such as lymphoma, HIV/AIDS, primary biliary cirrhosis, fibrosing alveolitis, asbestosis, silicosis, leprosy, miliary T.B., etc.

Serum lysozyme activity, sensitivity 40-50% (normal 3-12.8 mg/l). Other than sarcoid, conditions with increased serum lysozyme include monocytic leukemia, Crohn's disease, renal disease, T.B., megaloblastic anemia.

Other tests

Gallium citrate GA-67 lung scan high sensitivity but low specificity.

Bronchoalveolar lavage shows increased neutrophils above 10% of white cells.

Kveim test. Sensivity 97%. False positive test occurs in Crohn's disease, EBV infection, CLL and nonspecific lymphadenopathy. A test not commonly used.

Biopsy of involved tissue shows noncaseating granuloma.

SKELETAL MUSCLE

For Genetics tests see Genetics chapter.

Laboratory tests:

1. *CPK* (creatine phosphokinase) is found in high concentration in skeletal and cardiac muscle. Thus it is elevated whenever we have muscle injury.
2. *Aldolase* is specific for skeletal muscle.
3. *Myoglobin urine* is especially useful in massive necrosis of skeletal muscle when the patient is at risk for renal tubular necrosis or acute renal failure (crush syndrome).

Causes of elevated serum CPK

Rhabdomyolysis, polymyositis, mixed connective tissue disease, muscle dystrophy e.g. Duchenne type, female carriers of muscle dystrophy, congenital myopathies, metabolic myopathies such as hypo-thyroidism, alcoholism,

pentazocine and opiate intoxication, malignant hyperthermia. Also, infections such as influenza B, protozoan and parasitic muscle infections.

THROMBOSIS

The aim is to pick out those at risk of sustaining thrombotic events. Especially sought in cases of hypercoagulable state and recurrent abortion.

Tests: Antithrombin III, lupus anticoagulant, anti-cardiolipin/ phospholipid antibodies, proteins C and S, Factor II, homocysteine.

Mutations for Factors II (Prothrombin), V (Leiden) and TMHFR (homocysteine) will identify heterozygous and homozygous as well as multiple-factor status.

These tests are readily available, simple and precise with a fairly short turnaround time.

For individual cases, it is best to consult an experienced specialist in this field.

THYROID

Ultra-Sensitive TSH, Free T4, Free T3

Hyperthyroidism

Hypothyroidism

Thyroid Nodule

Thyroid Antibodies and Thyroiditis

Thyroglobulin as Tumor Marker

Thyroid Testing in Non-Thyroidal Illness & Elderly

TRH Stimulation Test, TSH Receptor Antibodies

Thyroid tests

T4 and T3 are bound to thyroid binding globulin and, to a small extent, to albumen and prealbumen. Only 0.05 to 0.25% of total thyroid hormones remain free, T3 more than T4 because the latter binds more strongly to thyroglobulin. Thyroid hormones are subject to a negative feedback with TSH produced by the anterior pituitary gland.

TSH test at the Consulting Medical Laboratories is currently by the ultra-sensitive method and the detection limit or analytical sensitivity (concentration 2 SD above the response to zero dose) is about 0.002 mU/L with a range of 0.002-75 mU/L. This is far more sensitive than the previously sensitive TSH test by IRMA (Immunoradiometric assay with a detection limit of 0.013 mU/L).

Free T4 test method is also measured by the most highly sensitive methodologies. Its detection limit is 0.18 ng/dL with a working range 0.18-6ng/dL.

Free T3 test method is also measured by most highly sensitive methods with a detection limit of 0.400 pmol/L and a working range of 0.400-50.00 pmol/L.

For reference values of thyroid hormones, see Tests List page (87).

Diagnosis of primary thyroid disease, such as hyper- or hypo- thyroidism is relatively easy, while secondary thyroid

disorder due to pituitary disease is usually more elaborate and requires more specific investigations.

In *hyperthyroidism*, Free T4 & Free T3 are elevated and TSH is suppressed. The routine TSH currently used in our laboratories is highly sensitive so that it is also very useful as a screening test for hyperthyroidism. It is also sensitive enough to circumvent a need for the TRH simulation test, where in hyperthyroidism no elevation of TSH levels is seen.

Follow up of thyroid function after therapy is best done by monitoring TSH level.

In *primary hypothyroidism*, Free T4 and /or Free T3 are suppressed while TSH is elevated, sometimes markedly so. In our laboratories, TSH is used routinely as part of the general health laboratory screen in women and in men below 50 years old. Also, all newborns should be tested for congenital hypothyroidism, a fully treatable disorder. In hypothyroidism, thyroid antibodies may be associated with thyroiditis, especially Hashimoto's thyroiditis. In secondary hypothyroidism, in addition to Free T4 and Free T3, TSH is low. The TRH stimulation test distinguishes between pituitary and hypothalamic failure. In the former no rise in TSH occurs in response to TRH while in hypothalamic failure TSH level rises after TRH injection.

In *Hashimoto's thyroiditis,* hypothyroidism is detected in less than half the patients in the early stages. However, high titers of thyroglobulin antibodies and antithyroid peroxidase antibodies are frequently found. In *Riedel's thyroiditis*, hypothyroidism is present and thyroid antibodies are found. In *granulomatous thyroiditis (De Quervain's)*, at least 50% show hyperthyroidism with thyroid antibodies present.

Thyroglobulin & Thyroid Peroxidase autoantibodies

Used in the diagnosis and classification of inflammatory and autoimmune thyroid disorders, namely, Hashimoto's thyroiditis, atrophic thyroiditis and 70-90% of Graves disease. Greatly elevated levels are especially seen in Hashimoto's thyroiditis.

Thyroglobulin (Tg) and Thyroid peroxidase (TPO) autoantibodies may detect 100% of Hashimoto's disease and 85% of Graves' disease. However, some patients with biopsy proven autoimmune thyroiditis have been reported to lack those autoantibodies. Tg and TPO autoantibodies are not specific for autoimmune thyroid disease, since they are found in other disorders such as Sjogren's syndrome, SLE, rheumatoid arthritis, juvenile thyroiditis, thyroid adenoma and carcinoma.

Tg and TPO autoantibodies are found in 8 & 15% of healthy controls, respectively, with relative increase in the elderly. Age standardized reference values cut down false positive rates for Tg and TPO antibody tests. In addition to the above two, *TSH Receptor autoantibodies* commonly develop in autoimmune disease.

Thyroid nodule has been widely investigated for many years. FNA (Fine needle aspiration) has become a standard initial test in most patients. In addition, TSH, Free T4 and Free T3 are run to exclude thyrotoxicosis and calcitonin in cases suspected of having medullary carcinoma of thyroid gland. FNA gives a good lead as to whether we are dealing with a follicular lesion, papillary carcinoma or a hyperplastic colloid nodule.

Thyroglobulin (Tg) is synthesized and stored in the thyroid gland, where T4 (thyroxine) and T3 (Triiodothyronine) are released into the blood stream. Colloid within gland acini stores thyroid hormones in the form of iodinated thyroglobulin molecules. A small fraction of thyroglobulin escapses into the circulation. Factors influencing serum Tg levels include total thyroid tissue mass, any thyroid lesion and thyrotropin receptor stimulation. In Graves' disease, thyroid receptor autoantibodies induce excessive production of thyroid hormones and higher Tg blood levels.

Tg levels reflect amount of functional thyroid tissue. It is absent in neonatal congenital hypothyroidism. Its major use is in the follow up of thyroid cancer. Tg estimation is not used in the diagnosis of thyroid cancer and cannot distinguish between thyroid adenoma or carcinoma. After total thyroidectomy, any elevation of Tg level indicates residual tumor. Tg levels are increased in subacute thyroiditis but not in thyrotoxicosis factitia, both conditions of which show rise in thyroid hormones.

In the *elderly,* T3 levels tend to decline and approximately 2% may have low TSH. However, many of these patients remain euthyroid. Care should be taken in interpretation of thyroid function tests in the elderly, since atypical presentations are not uncommonly seen in hypothyroidism and hyperthyroidism.

In *non-thyroidal illness,* thyroid tests may be altered, so that definitive diagnosis of thyroid disease should be made with care in critically sick persons, especially in the elderly. Therefore, any abnormality of TSH, Free T4 or Free T3 does not necessarily mean thyroid disease, and many are

probably euthyroid. Follow up testing is the best action and will often reveal a return to normal range.

Thyrotropin Release Hormone (TRH) Stimulation Test TRH stimulation test is used to:

(1) *differentiate* pituitary versus hypothalamic causation of hypothyroidism: In pituitary lesions there is failure of TSH response that is seen in hypothalamic lesions.
(2) *settle* equivocal thyroid screening test results in diagnosing hyperthyroidism: In hyperthyroidism, there is blunting or absence of TSH response to TRH due to feedback inhibition of pituitary by excess thyroid hormones.
(3) *aid* in the diagnosis of certain psychiatric disorders: 25% of depressed patients show blunted response to TRH. Mechanism is unknown.

TSH Receptor Antibodies help in the diagnosis of Graves' disease and in the early detection of hyperthyroidism relapse after therapy. TSH Receptor antibodies are elevated in 85% of cases of Graves' disease irrespective whether treated or not, and in 5-10% of Hashimoto's disease. High levels of TSH Receptor antibodies are particularly associated with a subset of Graves' disease patients who present pretibial dermatopathy or severe exophalmus.

TORCH

TORCH tests are used to detect antibodies to *Toxoplasma, Rubella, Cytomegalovirus and Herpes simplex.* The test panel is ordered to detect infection in early pregnancy, fetal malformation and sequelae such as recurrent abortions, stillbirths and congenital anomalies.

Congenital infection may lead to (1) small for gestation age (2) purpura (3) jaundice (4) anemia (5) chorioretinitis (6) cataracts (7) pneumonitis (8) micro-phthalmia (9) hepatosplenomegaly (10) suspicious rash (11) maternal infection.

Significant IgM level or a rising IgG antibody indicates active infection. Mere elevation of IgG antibody indicates immunity.

Other infecting organisms: Syphilis, HIV, Parvovirus B19, Varicella zoster, Enterovirus.

TOXICOLOGY

Initial toxicology testing recommended: salicylates, alcohol, barbiturate, acetaminophen, iron, volatile screen (methanol, ethylene glycol, isopropanol), carbon monoxide, methemoglobin, opiates, benzodiazepines, cocaine, PCP, tricyclic antidepressants, the latter suggested by a QRS duration >100msec on ECG.

Comment Other drugs may be screened for as the need or history may call for.

In certain cases, samples should be referred to the forensic laboratory, especially where foul play is suspected.

Moreover, testing may be required prior to or during employment, such as for air pilots, as well as for other administrative purposes. For guidelines to meet those requirements, you are referred to Gerson B, Hahn R. *Clinical Laboratory Medicine* 1990; 10: 517-529.

TUMOR MARKERS

Definition

Tumor markers are flags which mark out specific information related to tumors cells.

Historical background

1845	B-J Protein	Multiple myeloma
1932	ACTH	Cushing syndrome
1932	PAP	Cancer of Prostate
1959	R.I.A*	Reference methodology
1960	Ph' t(9q+;22q-)	Chronic myeloid leukemia
1963	AFP	Liver cancer, etc.
1965	CEA	Colon cancer etc.
1969	Oncogenes	Genetic determinant factors of neoplasia
1975	Monoclonal	Ab Refined methodology in diagnosis and treatment
1981	Ca 125	Cancer of ovary
1994	p53	Suppression gene in many cancers

Types of usage

Screening

Monitoring treatment

Diagnosis

Etiology-Prevention

* = Radioimmunoassay

Tumor markers for screening

PSA

See Prostate Panel page 62 and below.

Calcitonin see page 92.

Tumor markers for monitoring treatment

CEA

See page 83.

Ca15.3

See page 82.

Ca 125

See page 86.

AFP

See page 94. Reference range < 25 ng/ml

Elevated in hepato-cellular carcinoma with a sensitivity of 75-90%. Used in the diagnosis and follow-up of hepato-cellular carcinoma. In pregnancy it is used to screen for fetal anomalies in blood and amniotic fluid. It is greatly increased in yolk sac tumor of the testis and ovary. It is also increased in other tumors such as embryonal carcinoma, dysgerminoma, clear cell carcinoma, immature teratoma, polyembryoma or Sertoli-Leydig cell tumor of ovary or testis.

β-HCG

See page 99.

Elevated markedly in gestational trophoblastic tumors hydatidiform mole and choriocarcinoma. It is also elevated in tumors containing syncytiotrophoblastic cells such as embryonal carcinoma, dysgerminoma, endometrioid carcinoma, immature teratoma, polyembryoma of ovary or testis as well as in ectopic production by ovarian and Fallopian tube carcinoma.

Following complete removal of hydatidiform mole, serum B-HCG slowly diminishes to disappear after 14-15 weeks (3-4 months).

Ca 19.9

Reference range < 70 u/ml

Elevated in carcinoma of pancreas with a sensitivity of 80%. Used in the follow-up of pancreatic cancer. Also increased in carcinoma of colon, stomach, liver, gall bladder and benign gastro-intestinal disease.

Tumor markers for diagnosis

Some markers are used in the diagnosis of some tumors. Such markers may be detected in *(1) serum (2) urine (3) cells (4) tissue (5) intranuclear components such as chromosome or DNA aberrations.*

(1) Serum markers

Multiple myeloma: Immunoglobulins IgG, IgM, IgA kappa & lambda chains.

Hepatoma/ Yolk sac tumor: AFP.

Small Cell Lung Carcinoma: NSE, Ectopic ACTH.

Trophoblastic tumor: Beta-HCG.

MEN (Multiple Endocrine Neoplasia) Syndromes: Hormonal products of:

I parathyroid, pancreatic islets, pituitary, adrenal cortex, thyroid.

II medullary carcinoma thyroid, pheochromocytoma, parathyroid.

III medullary carcinoma thyroid, pheochromocytoma, mucosal neuromas. See 5.30.

(2) Urine markers

Multiple myeloma:	Bence-Jones proteins
Carcinoid syndrome:	5-HIAA (hydroxyindoleacetic acid)
Pheochromocytoma/Neuroblastoma:	Metanephrines/ VMA
Hyperparathyroidism:	Hyperphosphaturia
Cushing's syndrome:	17-hydroxy & oxo steroids/ Free cortisol

(3) Cellular markers

Immunophenotyping by flow cytometry, which outlines pattern of immunophenotype. Such technique is essential in typing and subtyping as well as follow-up of leukemias,

lymphoid malignancies etc. Flow cytometry panels also aid in delineating malign from benign lymphocytic cell proliferation in blood, bone marrow and other body tissues.

(4) Tissue markers

Pathology laboratories routinely employ immunohistochemical methods in the diagnosis of tumors in general and specific disorders such as in kidney or skin.

Examples:

Reactive versus malignant lymphocytic cell infiltration of body tissues. Identification of tumor cell type, e.g, melanoma, sarcoma, carcinoma etc.

Prognostic and therapeutic evaluation such as ER, PR and HER-2/ neu status in breast cancer.

As adjunct investigation, e.g. FISH in detecting specific mutaions or alterations. MRD (multiple drug resistance) gene rearrangement.

(5) Intranuclear markers

(a) Cytogenetic markers Certain chromosomal aberrations are associated with certain tumor types:

CML (90%)/ ALL (10%): Ph' del 22: t(9;;22) (q34;q11). Reciprocal translocation paternal-maternal abl=bcr chimeric oncogene product. See 5.40 & 42.

AML: *M2* t(21;8): AML with maturation: blasts 30-90%
M3 t(15;17): APL (Acute Promyelocytic Leukemia)
M4 inversion 16: AMML (Acute Myelomonocytic Leukemia)

Burkitt's lymphoma: t(8;14) (q24; q32)

Follicular lymphoma: t(14;18) (q23;q21) bcl-2 gene. See 5.43
Small lymphotic lymphoma t(9;14) (q13; q32)
B cell lymphoma t(14q32), t(2p12), t(22q11)
Anaplactic large cell lymphoma t(2;5) (p23; q35)
T cell lymphoma t(7q34), t(14q11), t(7p15)
Mantle cell lymphoma: t(11;14) (q13; q32) bcl-1 gene
Ewing sarcoma: t(1;22) (q24;12)
Desmoplastic small round cell tumor t(11;22) (p13; q12)
Alveolar rhabdomyosarcoma t(2;13) (q35; q14)

(b) Genetic markers

Certain genetic markers are associated with certain tumors:

Adenomatous Polyposis Coli	70% APC gene 5q21 mendelian dominant 30% new mutation
Hereditary Nonpolyposis Colon Cancer	MSH 2, MLH 1 3p21.3 in 80% of HNPCC Accounts for 1-5% of colon cancer cases
Breast-Ovarian Familial Cancer	BRCA I 17q21 BRCA II 13q12-13 Found in 50% of hereditary breast cancer Found in 100% of hereditary breast-ovary Ca
Retinoblastoma	RB 1 13Q14 Found in 40% of retinoblastomas
Wilms' Tumor	11p13 In 1% hereditary reactivation of IGF2 *

* IGF2= Insulin Reactivation Factor 2

Comment

Presence of a genetic marker in a tumor does not mean it is inherited. In one example, *RB1* gene mutation found in lung cancer but no lung cancer in individuals inheriting RB1 gene. In another example *p53* mutation is often seen in sporadic cancers which are not inherited.

Tumor markers as etiologic-preventive markers

Certain genetic markers are either of etiologic or preventive significance. Such markers may be identified in several types of tumor:

Liver cancer: Marker: HBsAg: Line of action: Vaccination; HCV interferon.

Cervical cancer: HPV: Line of action: Surgical procedure/ Follow up Pap smears.

MEN II: Marker: RET oncogene. Line of action: Genetic counselling + screen tests.

FAP (Familial adenomatous polyposis): Marker: APC gene. Action: Genetic counselling + surveillance.

Breast cancer: Marker: BRCA I, II. Action: Genetic counselling + surveillance.

Nasopharyngeal carcinoma/ Burkitt's: Marker: EBV. Action: Uncertain.

TUMOR MARKERS BY SYSTEM

The following will be individually discussed: Breast cancer, gynecologic cancer, prostate cancer, colorectal cancer, neu-

roendocrine tumors, germ cell tumors, monoclonal gammopathies, lung cancer.

Breast cancer

Breast is site for the most common cancer in women worldwide. Tumor markers have not proven useful as screening tests but provide useful data in management. Prognosis in breast cancer depends on tumor size, pathologic classification, lymph node status and tumor stage. Ancillary investigations include tumor marker assessment, the following which proved of use in management:

1- Estrogen & Progesterone receptors (ER & PR).

2- CA 15.3.

3- CEA.

4- BR 27.29 (CA 27.29).

5- HER-2/neu (c-erb B2) oncoprotein.

CA 15.3

Reference range < 25 u/ml. A high molecular weight mucin type glycoprotein.

CA 15.3 should be assayed on fresh serum or, alternatively, stored under -20°C on short term and –70°C on long term basis.

Useful in follow-up of breast cancer: elevated in 1/3 of breast cancer patients and 80% of breast cancer patients with metastases.

Serial levels may indicate progression or regression of disease and are best done along with CA 27.29 levels. CA 15.3 is superior as a tool in the follow up of breast cancer. Sensitivity of detecting clinical progression or regression is increased by using CEA in addition to CA 15.3. However currently it is recommended to use both CA 15.3 and CA 27.29 in monitoring breast cancer patients.

Increased levels of CA 15.3 are also seen in cancers of lung, liver, pancreas, ovary and benign breast disease.

CA 27.29

CA 27.29 should be performed on fresh serum or, alternatively, stored under –20°C on short term and –70°C on long term basis.

As with CA 15.3, this other mucin type tumor marker lacks specificity and sensitivity required for use in routine screening for breast cancer. However, CA 27.29 is more sensitive but less specific marker than CA 15.3 in monitoring breast cancer disease and is best used in follow up of advanced breast cancer.

CEA

See page 90.

Estrogen & progesterone receptors. Caption 22.

ER and PR receptors are routinely recommended on primary lesions (see captions). They are done on paraffin sections of representative parts of viable looking tumor tissue. Paraffin blocks should be preferably prepared within 24-48 hours of fixation in formalin after taking the tissue biopsy.

Paraffin blocks of previously processed frozen sections are unsuitable for the above tests. And although tests can be done on frozen tissue sections, it has proved more practicable to apply the technique on paraffin embedded tissue. Moreover, the technique can be equally applied on fine needle aspiration material, but adequacy of sampling is best achieved in the routine aforementioned method.

In addition assay of HER-2/neu is now mandatory in patients with metastatic breast cancer before using the monoclonal antibody trastuzumab (Herceptin).

ER and PR act as predictive indicators of response to endocrine therapy, such as tamoxifen and medroxyprogesterone acetate. They are also prognostic factors to assess the clinical progress of breast cancer patients, since ER positive cases have a better prognosis. Reporting on ER and PR may be designed to portray intensity and proportion of staining tumor cells, bearing in mind that any reporting of ER PR results should be made with the full understanding of the treating physician.

Interpretation of ER/PR

Every time we use control material.

Several methods of assessing ER/PR breast cancer reactions have been described. In the Consulting Medical Laboratories, for each of ER or PR, we follow a simple semi-quantitative system which comprises percentage and intensity of reaction, each of which is graded 0-3, according to strength of the reaction in the respective parameter of percentage and intensity, respectively. Resultant estimates are then summed up into either negative, or alternatively, weak, moderate or strongly positive reaction.

HER-2/neu. Caption 22.

The c-erb B2 or HER-2/neu oncogene is amplified in human breast cancer and correlates with shorter survival rate (see captions).

As with ER/PR, use of control material is a must and we test for this marker by immuno-histochemical method (IHC) on paraffin embedded tissue. IHC is easier to perform with a rapid turn-around time and a relatively less cost, while results correlate with FISH (Fluorescent In Situ Hybridization) methodology.

HER-2/neu protein is a normal component of a variety of epithelial cells and is over-expressed by breast cancer cells. It is controlled by a mutational oncogene on cell membrane receptors affecting growth and proliferation of tumor cells.

It correlates with poor prognosis in lymph node positive cases and is over-expressed in 25% of breast cancer cases and in 30% with metastatic disease.

HER-2/neu Protein over-expression can be graded into 0-3 according to intensity of membrane staining. Cytoplasmic staining should be considered nonspecific.

HER-2/neu amplification in breast cancer indicates Herceptin (trastuzumab) therapy in metastatic disease. Treatment by Herceptin alone produces a 14% response versus 29% by chemotherapy alone, while a combination of both produces a 44% response rate.

Other cell markers can be used (see page 27). Newer tests may add to evaluation of tumor profile. For example, Cyclin E overexpression and low p27 expression were re-

ported to correlate with poorer prognosis, but such tests have not been shown to be independent prognostic markers.

Gynecologic cancers

Ovarian Cancer, Cervical Cancer, Endometrial Cancer.

Ovarian cancer

Majority of ovarian cancers are epithelial tumors while due to lack of early symptoms, about 70% present with advanced disease and an overall 5 year survival of about 30%. In those diagnosed at early stage 90% 5 year survival may be achieved.

Ca 125

Monoclonal antibody with a reference range < 35 u/ml.

Not useful for screening due to lack of sensitivity for early disease. However, it is increased in serous carcinoma of the ovary and doubling of serum levels or significantly (>95 u/ml) elevated level in a postmenopausal woman with a pelvic mass should lead to laparotomy. Following surgery, 3-month monitoring of serum level is advised. Level should be determined during primary therapy. An increase >35 u/ml or doubling should prompt further investigations including laparoscopy, and in patients on chemotherapy indicates treatment failure. However, serum Ca 125 levels can remain unmoved despite progress of metastatic disease.

Pre-operative level correlates with survival and recurrence rates. In women with a strong family history or BRCA1 and BRCA2 mutation, Ca 125 may be recommended 6-monthly with transvaginal sonography annually.

Ca 125 is also elevated in endometrial, Fallopian tube, cervix, pancreas, colon, breast and lung adenocarcinoma, early pregnancy, endometriosis, benign ovarian cysts and pelvic inflammatory disease.

Cervical cancer

Cervical cancer ranks second to breast cancer as a cause of mortality worldwide. Early diagnosis ensures 5 year survival >90% compared to 70% overall survival.

SCCA (Squamous Cell Carcinoma Antigen) is the marker of choice in squamous cell carcinoma of the cervix.

Reference range <2 ug/l. Elevated levels at time of diagnosis may be seen in Stage IB or IIA cervical cancer. It may be used with caution to monitor disease recurrence. Two consecutive rising values indicate recurrence or disease progression. However, clinical value of this test is still debated.

SCCA is not a specific marker of cervical cancer. It is also elevated in some cases of cancer of skin, lung, head and neck, esophagus, bladder, penis and anus, as well as in benign skin disease, lung diseases and renal failure.

Endometrial cancer

No tumor marker exists for endometrial cancer. For monitoring, the only available marker is Ca 125 found elevated in about 60% of patients with recurrent disease. However, this is of questionable value for clinical purposes.

Prostate cancer

PSA (see page 205)

Total PSA

Reference range < 4 ng/ml.

If there is an increase of 0.75 ng/ml per year, investigate for cancer.

The most important marker in evaluation of prostatic cancer, both for diagnosis and management. About one quarter of patients with prostate cancer have normal PSA level, while in one-third of patients with cancer of prostate confined to the prostate gland, serum level of PSA is below 4 ng/ml.

The positive predictive value* of PSA is only 30%.

PSA, however, is the best screening test for prostatic cancer. Elevated levels should be accompanied by the Free PSA level with calculation of the PSA Ratio (by dividing Free over Total PSA levels).

PSA should not be used as an only diagnostic tool of prostate cancer but should be used as an adjunct to digital rectal examination (PR) and if tumor suspected transrectal sextant prostatic biopsy.

Men with strong family history should begin screening at the age of 40 years. Over-diagnosis should be avoided to circumvent over-treatment. To date, conflicting data are reported with regard to a positive impact of prostate cancer testing on reduced prostate cancer mortality. However, some reports relate early detection to improvement in dis-

* No. of positive tests in patients with the disease divided by total No. of positive tests: total= true positives+ false positives.

ease-specific survival and decline in the rate of advanced disease with corresponding improvement in survival. The latter may also be related to change in strategy with use of more radical treatment in recent years.

PSA plays a vital role in follow up of prostate cancer patients. Following radical prostatectomy, levels fall to almost undetectable levels. A rising PSA level after radical prostatectomy indicates disease recurrence. However, not all cases of biochemical recurrence will develop metastatic disease. Determining factors include speed of biochemical recurrence, Gleason score and PSA doubling time.

Elevated in cancer of prostate, also in benign prostatic disease, infection, urine retention, biopsy, PR at 1-24 hours.

Free PSA

The higher the ratio of (Free: Total PSA) the less the risk for cancer. At a PSA ratio of <0.05, the risk of cancer is high. At PSA ratio of 0.10 to 0.20, risk is low. 0.20-0.30 favors benign. > 0.30, benign.

Colorectal cancer

Colorectal cancer is one of the major cancers worldwide.

Early detection concerns average-risk and high-risk individuals. Most colorectal cancers are from the average-risk group. For average-risk individuals, annual fecal occult blood test (FOBT) is recommended, with optional colonoscopy every 5 to 10 years. For high-risk individuals, such as those with inflammatory bowel disease, familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer or a family history of cancer, colorectal screening should be

commenced earlier, for example, at the age of 40 years. Removal of polyps provide an effective preventive measure in many of those cases which leads to a decrease in disease-specific mortality. Public awareness is essential in bringing susceptible persons to screening in the early stage of the disease.

CEA

Normal <2.5ng/ml. Smokers <4ng/ml. Sensitivity 80%. Specificity 70%

The marker of choice in follow up of colorectal cancer patients, while stressing that it has no role in early detection or diagnosis of colorectal cancer. However, initial CEA level has been reported as a prognostic factor in the perioperative period, and may be considered along with pathological staging and surgical treatment plan. CEA plays a limited role in indicating disease recurrence that can precede clinical symptoms and signs by several months. Detection of early recurrence is achieved by CEA along with intensive follow-up which has been reported to improve 5 year survival. CEA also reflects disease activity and can be used to monitor response to chemotherapy in metastatic disease.

Elevated CEA levels correlate with well differentiated histology and liver metastases and shows a 65% sensitivity for recurrence. High levels persist until 6 weeks after successful surgery.

High levels of CEA may be seen in conditions unrelated to recurrence. It is elevated in cancer of breast, pancreas, lung, stomach, ovary, pulmonary emphysema, acute ulcerative

colitis, alcoholic cirrhosis, cholecystitis, rectal polyps and benign breast disease.

Neuroendocrine tumors

Neuroendocrine tumors originate from cells derived from the neural crest. They include pheochromocytoma, medullary carcinoma of thyroid gland, islet cell carcinoma, carcinoid of the gut and lungs, as well as Merkel cell tumor.

Neuroendocrine tumors may be secretory or nonsecretory, so that absence of a marker does not exclude disease. It is important to circumvent misinterpretation by meticulous specimen collection according to required conditions and considering any iatrogenic interferences.

Pheochromocytoma

Lab Tests:

1- Plasma and Urine Catecholamines.
2- Epinephrine, Norepinephrine and Dopamine.
3- VMA (Vanillylmandelic acid), HVA (Homovanillic acid).

Catecholamines

24-hour urine specimen is required and sample acidity not to fall below pH2 by adding not more than 6ml of 6N HCl for 1-2 liters of urine with adjustment in pediatric samples. For plasma, heparinized tubes are required with collection in ice. Storage should be at –70°C. Drugs such as Methyldopa, Monoamine oxidase inhibitors, isopreterenol, isoetharine and labetalol interfere with estimation.

Reference ranges: Measurements in µg/24h

Age (years)	Norepinephrine	Epinephrine	Dopamine
0-1	0-10	0-2.5	0-85
1-2	1-17	0-3.5	10-140
2-4	4-29	0-6	40-260
4-7	8-45	0.2-10	65-400
7-10	13-65	0.5-14	65-400
>10	15-80	0.5-20	65-400

VMA and VHA

24-hour collection of urine is required. Random urine samplings may be used and ratio of VMA or HVA to urinary creatinine reported.

Reference Ranges for VMA and HVA by Age: Units in *µg/mg creatinine*

Age	VMA	HVA
1 day	<17	<42
1 year	<13	<33
2 years	<11	<26
5 years	<8	<17
10 years	<6	<10
18 years and adult	1.08-4.23	0.5-4.2

Iatrogenic agents influencing VMA and VHA determinations: Amphetamines and amphetamine-like compounds, Appetite suppressants, Bromocriptine, Buspirone, Caffeine, Carbidopa-levodpa (Sinemet), Clonidine, Dexamethasone, Diuretics (with Na depletion), Methyldopa, MAO inhibitors, Nose drops, Propafenone, Tricyclics, Vasodilators.

Medullary thyroid carcinoma

Laboratory Test:

Calcitonin. Caption 24.

Basal levels= 5-19 pg/ml

Produced by parafollicular C-cells of the thyroid gland and accounts for about 10% of all thyroid cancers. Calcitonin plays a central role in calcium metabolism in balance with parathyroid hormone.

Calcitonin is the only biochemical marker that can be used to detect preclinical tumor development in occult C-cell carcinoma or suspected familial medullary carcinoma of thyroid gland.

Calcitonin level correlates with tumor mass. After total thyroidectomy, calcitonin levels should be used to detect disease recurrence.

In normal individuals provocative pentagastrin test is used to demonstrate a fall to at least 4-fold below the upper normal limit of calcitonin level with normalization after 30 minutes.

Islet cell carcinoma

Laboratory Test: Insulin (non-specific)

Insulin levels are routinely measured and have to be interpreted in the light of the clinical background.

Carcinoid tumor

Laboratory Test: 5-HIAA (Urinary 5-Hydroxyindoleacetic acid)

5-HIAA

Reference range= 3-15 mg/dl

5-HIAA is the major metabolite of serotonin and is measured in a 24-hour urine sample. Patients should abstain from taking foods rich in serotonin and indoles as well as medications.

5-HIAA is found elevated especially in carcinoid syndrome of the gut with liver metastases and carcinoid of the lung.

Foods and medicines that interfere with Urine 5-HIAA measurement:

Foods

Avocados, Bananas, Coffee, Eggplant, Plums, Pineapple, Tomatoes, Walnuts.

Medicines

Acetaminophen, Acetanilid, Aspirin, Caffeine, Chlorpromazine, Corticotropin, Coumarin, Diazepam (Valium), Dihydroxyphenylacetic acid, Ephedrine, Ethanol, Fluorouracil, Gentisic acid, Glycerol, Guaiacolate, Homogentisic acid, Hydrazide derivatives, Imipramine, Isocargoxazid (Marplan), Levodopa, Ketoacids, Melphalan, MAO inhibitors, Mephenesin, Methamphetamine, Methenamine, Methyldopa, Phenothiazine, Perchlorperazine, Phenacetin, Phenmetrazine, Phenobarbitol, Phentolamine, Promazine,

Promethazine, Naproxen, Nicotine, Rauwolfia, Reserpine, Robaxin, Thorazine.

Germ cell tumors

Non-seminomas or germ cell tumors of ovary or testis include single type or any combination of teratoma, embryonal cell carcinoma, choriocarcinoma and yolk sac tumor.

For testicular or ovarian germ cell tumors, relevant biochemical markers include AFP, B-HCG and LDH. Production of AFP is limited to yolk sac tumor or a yolk sac tumor component in a mixed tumor. Monitoring of AFP provides a true reflection of any yolk sac tumor presence and evaluation of treatment response.

β-HCG, on the other hand, provides a tool for diagnosing or evaluating activity of a choriocarcinoma component, since it is produced by syncytiotrophoblastic cells. Presence of choriocarcinoma element can be presumed in a germ cell tumor in presence of high β-HCG level even if histology cannot reveal a trophoblastic cell component in a tumor.

Routine evaluation of a testicular or ovarian mass includes CT scan of chest, abdomen and pelvis as well as measurement of AFP, β-HCG and LDH.

LDH or the isoenzyme LDH-1 is a prognostic factor in advanced germ cell tumors. It is elevated in 60% of advanced germ cell tumors and 80% of seminomas with metastases. The level is governed by tumor load, growth rate and ratio of proliferative to apoptotic index.

PLAP (Placental Alkaline Phosphatase) can be measured in serum and is normally expressed by placental sycytiotrophoblastic cells, normal testicular, cervical, lung and

thymic tissue, as well as in malignant tumors such as germ cell tumors, seminomas and lung tumors.

Metastatic Germ Cell Tumors: Serum Markers in Prognosis

Prognosis	Tumor marker Concentration		
	AFP (ng/ml)	HCG (u/l)	LDH (Multiple of Upper Normal Limit)
Good	<1000	<5000	<1.5
Intermediate	1000-10,000	5000-50,000	>1.5 – 10X
Poor	>10,000	>50,000	>10

After treatment, AFP and β-HCG are used for monitoring purposes and normalization within one week suggests disease eradication. Monthly review of AFP, β-HCG and LDH is recommended. A slow marker decline, even after chemotherapy, is usually followed by relapse. The large majority of those having persistently elevated levels indicates residual disease and are candidates for chemotherapy trial.

Monoclonal gammopathies

Monoclonal gammopathies constitute a group of proliferative disorders distinguished by production of M (monoclonal type) protein. The lesion may be due to a systemic infiltration of bone marrow and perhaps other tissues by plasma cells or immunocytes, or may be due to a localized solitary or multiple tumors in bone or extra-skeletal site(s). Almost invariably, every single case shows one single clone of immunocytes which produce a single type of M protein. Every M protein possesses two heavy polypectide chains of the same class of immuno-globulin (namely:

gamma in IgG, alpha in IgA, mu in IgM, delta in IgD and epsilon in IgE), as well as only one type of light polypeptide chains, namely, either kappa or lambda type.

Tests:
1-Protein Electrophoresis.
2-Immunofixation.
3-Immunoglobulin levels.
4- Cryoglobulins.

Protein electrophoresis is performed to detect multiple myeloma, Waldestrom's gammaglobulinemia or primary systemic amyloidosis. (See graphs in Tests list page 79).

A monoclonal (M-spike) band is detected on EP (electrophoresis) and light chains may be detected in the urine. EP is a screening test for diagnosis and is useful in follow up of patients on treatment for multiple myeloma or Waldestrom's macroglobulinemia (IgM gammopathy). Therefore, EP is used in monitoring response to therapy.

Immunofixation (imunoelectrophoresis) to confirm presence of monoclonal versus polyclonal gammopathy or to detect a small M protein in patients with primary systemic amyloidosis or in follow up of patients treated for solitary plasmacytoma. Immunofixation is also useful in detecting biclonal and triclonal gammopathies. Caption 25.

For quantitation of *immunoglobulins,* nephlometry or densitometry detects individual immunoglobulin class levels and should continue with the same technique in follow up assessment.

Cryoglobulins are proteins which precipitate on cooling and dissolve on heating. Type I are monoclonal IgM, IgG, IgA or rarely light chain, while Type II are mixture of two or

more immunoglobulins. Type III are polyclonal with no M protein detectable. Specimen should be collected at 37°C and allowed to clot and then centrifuged at 37°C. Serum is kept in fridge or ice bath and read at 24 hours. If no precipitate occurs, specimen is kept at 0°C for 7 days and any precipitate is washed, and immunofixation is performed to determine the type of immunoglobulin in the cryoprecipitate.

Lung cancer

A poor prognosis in this lesion is accompanied by a dismal response to therapy in recurrent disease. Primary malignant lung tumors are divided into 4 major histologic types: squamous cell carcinoma, adenocarcinoma, large cell carcinoma and SCLC (Small Cell Lung Carcinoma). The latter accounts for about 25% of all the major types and differs from the other 3 in its clinical and biologic behaviour. However, many lung cancers have more than one histologic type so that SCLC and NSCLC (Non Small Cell Lung Cancer) represent heterogeneous entities with great overlap between the major histological types in a single tumor.

Tumor markers:
1- NSE (Neuron Specific Enolase).
2- CEA.
3- SCCA (Squamous Cell Carcinoma Antigen).
4- CYFRA 21-1.
5- ProGRP (Pro Gastrin Releasing Peptide).

Although the above tumor markers correlate with tumor burden, no consistent relationship is seen. They should not be used for screening purposes since they lack the required criteria in terms of sensitivity and specificity.

NSE

This glycolytic enzyme is produced in the central and peripheral neurons and malignant neuroectodermal tumors such as SCLC, neuroblastomas and carcinoid tumors.

Although tumor markers cannot replace histological results, presence of NSE may be helpful to establish a diagnosis of SCLC in lung tumor tissue or in the serum of patients with no clear histological evidence to support such a diagnosis. Moreover, serum NSE levels may be ordered as baseline before starting therapy for SCLC, since serial levels can be used to weigh response to chemotherapy. It should be noted that a transient rise of NSE level is seen 24-72 after commencing chemotherapy and is believed a good sign of response (tumor lysis syndrome). In cases with pre-treatment high levels, acute drop occurs within a week and certainly by the end of the first treatment cycle; failure of therapy is indicated by rising levels.

SCCA and CYFRA 21-1

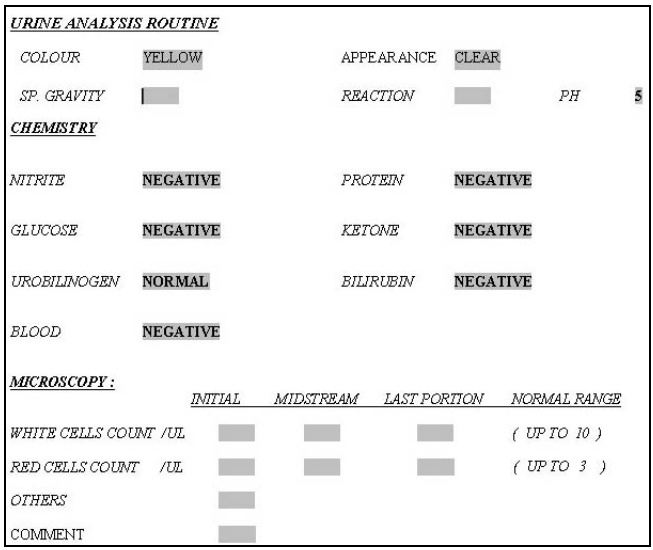
SCCA is related to squamous cell carcinoma type. A high serum level provides a strong suspicion of NSCLC of squamous cell type. However, CYFRA 21-1, directed at cytokeratin 19 fragment, is more sensitive than SCCA and correlates with all rather than one major type of lung cancer. In general, high tumor marker levels indicate advanced disease while low levels do not exclude poor outcome. Pre-treatment measurement may be helpful and those cases with raised marker level are candidates for monitoring. Post surgical levels may indicate residual tumor mass.

Evidence for the usefulness of tumor markers in SCLC and NSCLC is controversial. Underlying problems in reported

studies include heterogeneity of tumor histology and stage, methods of study and interpretation difficulties.

Since recurrence rate in lung cancer is 70-90%, postoperative baseline level is essential for interpreting follow-up levels of tumor markers, since a confirmed increase indicates recurrent disease. Follow up of asymptomatic patients after treating primary lung cancer is of controversial value. Depending on histology, CYFRA 21-1, SCCA, CEA and / or NSE may be ordered in lung cancer patients prior to treatment. Careful attention should be made in handling specimens and serum should be separated from the blood clot within the first hour of collecting the sample. Samples may be stored at 4°C on short term and –70°C on long term basis.

URINE ANALYSIS



URINE ANALYSIS ROUTINE

COLOUR	YELLOW	APPEARANCE	CLEAR		
SP. GRAVITY		*REACTION*		*PH*	5

CHEMISTRY

NITRITE	NEGATIVE	*PROTEIN*	NEGATIVE
GLUCOSE	NEGATIVE	*KETONE*	NEGATIVE
UROBILINOGEN	NORMAL	*BILIRUBIN*	NEGATIVE
BLOOD	NEGATIVE		

MICROSCOPY :

	INITIAL	*MIDSTREAM*	*LAST PORTION*	*NORMAL RANGE*
WHITE CELLS COUNT /UL				*(UP TO 10)*
RED CELLS COUNT /UL				*(UP TO 3)*
OTHERS				
COMMENT				

The most important single laboratory test. It is essential for every medical facility and is routinely employed in the following situations: The 'physical' (for medical insurance, immigration visa etc.), in any 'antenatal routine', at the 'beside', the Doctor's office and, of course, in the 'medical laboratory'.

Routine Urinalysis consists of 4 parts, namely, Physical examination, Chemical examination, Microscopic examination and Culture & sensitivity.

CML Urinalysis Form #2.

Method of 2 consecutive portions collection:

Container #1 = first spurt of urine - washes out the urethral wall. Container #2 = rest of the urine - urinary bladder urine.

Advantages:

a. Avoids repeats for peri-urethral discharge contamination.
b. Picks out urethritis/peri-urethritis in men.
c. Provides a midstream equivalent sample.
d. Avoids futile urine cultures. In males and females, any method of collection to be preceded by cleansing.

1. *Urinalysis- Physical examination (*Color, Specific gravity, Quantity, Macrodeposits)

Color

Cloudy = blood

Red = blood/ beetroot/ sweets (aniline dyes)

Orange = bile/ urobilin/ rhubarb/ cascara (constipation)

Pink = dindevan

Frothy = protein

Specific gravity

(Refractometer: uses a single drop of urine)

SG of pure water is 1000.

Normal SG= 1001-1040 (50-1300 mOs/Kg).

At 1010 (280 mOs/Kg), urine is isotonic with plasma (chronic renal failure).

Most concentrated sample is fasting / early morning after waking up.

Quantity

Normal diet (80g protein,50-100 meqt Na): 1-2 liters/ 24 h.

Large volume e.g. 10 liters: diabetes insipidus. Nocturnal frequency: diabetes mellitus / UTI / chronic renal failure.

Low volume <400ml (Oliguria): Dehydration/ Congestive heart failure/ Acute tubular necrosis.

Polyuria

Diabetes mellitus/ insipidus, chronic renal failure, diuretics, hypercalcemia, 'infection', "anxiety".

Oliguria

Oliguria is a cardinal sign of acute renal failure.

Tests to differentiate pre-renal, renal and post-renal oliguria

(1) Urine: Volume/ Sediment/ S.G./ Sodium/ Osmolality.
(2) Urine to plasma ratio of (a) osmolality (b)creatinine (c) urea
(3) Excretion fraction of filtered sodium (EFFS)

Note that in oliguria, relevant urinary tests are of paramount importance.

1) Urinary sediment in renal state consists of renal tubular cells, casts: granular, hyaline, cellular and occa-

sionally red or white cells. Urine S.G. is high in renal state and low in pre- and post-renal states.

2) Urine sodium: pre-renal <20mmol/L renal >30-40 post-renal not useful
3) BUN/ creatinine ratio= >10 in pre-renal, 10/1 in renal and 5/1 in post-renal state.
4) Urine/ plasma osmolality >1.1 in pre-renal,<1.1 in renal and nonuseful in post-renal state.
5) Urine osmolality: pre-renal >500mOsm/kg renal <350 in post-renal not useful.
6) Urine/ Plasma creatinine: pre-renal >40 renal (acute tubular necrosis <20, GN >40) Post-renal <20.
7) Urine/ Plasma urea: pre-renal >8 renal (ATN<3 GN>8) post-renal <3.
8) EFFS: pre-renal <1% renal (ATN>2-3% GN<1%) post-renal >2%.

2. *Urinalysis-Chemical examination* (Sugar, Ketones, Protein, Bile, Urobilinogen)

Stick test is simple, accurate, can be performed at the bed side, with a wide range of 3-10 parameters to choice and takes one minute only.

Glycosuria

Causes

Diabetes mellitus Endocrinopathies: Cushing's etc. Pancreatic disease.

Hypothalamic dysfunction. Severe trauma. Drug induced: Cortisone etc.

Decreased renal threshold as in Pregnancy & Older persons.

Nonglucose sugars in urine: Galactose, Fructose produce glycosuria.

Blood sugar level is used for diagnosis/management of diabetes mellitus. Urine sugar incidental: not to be relied upon.

Ketone bodies (hydroxybutyric acid, aceto-acetic acid and acetone)

Seen in *Starvation* – physiological and *Diabetes mellitus*- pathological

Proteinuria

Albumen Albustix detects 20mg/100ml

24-hour total protein Normal up to 133 mg/24hours; <1g/24hours: Postural, Emotional, Exercise, excess Heat/Cold, CHF; <4g/24hours: Pyelitis, Renal stones, Diabetes, Heavy metals, Myeloma; >4g/24hours: Nephrotic syndrome, Amyloidosis, Chronic glomerulitis.

Microalbuminuria (< 20 mg/24h) for early detection of diabetic nephropathy.

Protein electrophoresis in urine Normal: 25% albumen 75% α-1&2 globulin. In postural proteinuria: normal distribution. In most diseases: 90% of protein is albumen. If globulin > albumen: suspect IgA nephropathy (Berger's disease).

Immunoelectrophoresis Separation of immunoglobulins. Smaller mol wt molecules with minimal glomerular damage. Larger mol wt molecules in severe nephropathies. Molecular weights: Albumen 69,000 β globulin 90,000.

Globulins α-1 200,000 β-2 300,000 (Glucose= 180 Urea= 60 Uric acid= 168).

B-J protein: L-chain Ig Boiling test: coagulum forms at 45-55°C and dissolves at 95°C.

Amino-acid analysis by Gas-liquid/HPLC chromatography, or qualitative (thin-layer) cystine, methionine, isoleucine, tyrosine, Ph alanine etc.

Bile

Bile In *obstructive jaundice* and *cholestatic hepatitis*

Urobilinogen A screening test for *hemolytic anemia*

3. *Urinalysis - Microscopic examination* (WCC, RCC, Deposits, Crystals). Caption 26.

Urine Cell Counts: Normal range for white (up to 5-10) and red cells(0-2).

Increased WCC (white cell count): Many causes: First rule out contamination.

(Examination of MSU alone misses cases of urethritis).

White cells: pyuria = inflammation: bacterial or nonbacterial.

Red cells: hematuria: gross or microscopic.

Deposits: Formed elements in urine Cells/ Casts/ Crystals.

Cells= White cells (inflammation), *Red* cells (hematuria), *Epithelial* cells (different).

Casts= *Granular* (degenerate cells); *Hyaline* (protein); *Fatty*: derived from distal tubules *Red cell* (sign of glomerulonephritis) *White cell* (infection).

Crystals= Oxalate, Uric acid, Phosphates (abnormally present in fresh urine)

(See captions).

Hematuria

Gross hematuria main causes: *tumor/ coagulopathy/ injury.*

Microscopic hematuria caused by:

Stone disease or crystalluria –common in young adults (Ramadan).

Tumors kidneys, bladder.

Infection cystitis or prostatitis, pyelitis.

Bleeding disorder ITP, anticoagulant therapy (side effect).

Acute glomerulonephritis.

4. *Urinalysis: Bacteriological examination* (Gram stain, Culture & Sensitivity)

Gram stain: for bacteria etc.

Culture: Most common organism *E. coli,* also *Enterobacteria.*

Sensitivity pattern discs: for suitable drugs - commonly available.

SPECIAL URINE TESTS

Many tests are performed in the urine, data for many of which will be found in the relevant sections. Examples include Pregnancy testing, Catecholamines, VMA, Oxalate, Urea/ Creatinine, Electrolytes, Drugs, Water Concentration test, Clearance of creatinine, xylose, aniline and other agents etc.

Method of collection

We found that routine urine analysis is best done by taking two consecutive portions each time, container #1 of the first spurt or void at urination, representing the first portion of urine which washes out the urethral wall; container #2 includes the rest of the urine voided and represents urinary bladder urine. In females, collection of urine specimen in a single container would be often interfered with by a slight to moderately elevated white cell count caused by contaminated peri-urethral secretion or discharge. Despite instructions on adequate cleansing before giving the urine sample, we find this 2-specimen method the most satisfactory in ruling out contamination by peri-urethral material. In men, it is a very useful way of marking out urethritis/peri-urethritis from urinary tract infection. Although the two-specimen method may be more demanding than asking the patient to void the first urine spurt out into the toilet and then collect the 'midstream' sample, we find the 2-specimen method a far more practical and rewarding way. It avoids having to repeat urine samples and doing unnecessary urine cultures.

CHAPTER 3

LABORATORY TESTS LIST [*]
WITH BRIEF COMMENTS

5-Nucleotidase

Serum 2.0-10.0 u/l.

Increased in hepatobiliary disorders, including hepatic carcinoma, cirrhosis, biliary obstruction; also in 3rd trimester pregnancy.

5-Hydroxyindoleacetic Acid (HIAA)

24-hour urine 1.0-5.0 mg/24h.

Serotonin is synthesized from tryptophan by enterochromaffin cells in the gut and bronchi, metabolized in the liver to 5-HIAA and eventually ends up in the urine. *Increased* in carcinoid tumor, celiac disease and Whipple's disease. *Avoid food products like bananas, tomatoes, plums, eggplant, pineapple and walnut; also avoid drugs like acetaminophen (3 days before starting urine collection).*

17-Hydroxycorticosteroids

24-hour urine Male: 10-30 µmol/24h, Female: 5-25 µmol/24h; Male: 3-10 mg/24h, Female: 2-8 mg/24h.

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[*] Alphabetical order. See also Serology and Parasitology in Chapter 4 and Pathology-Cytology Chapter 6.

Replaced by plasma cortisol, urinary free cortisol and other intermediate steroids. *Increased* in Cushing's syndrome, congenital adrenogenital syndrome and hirsutism. *Decreased* in Addison's disease, liver disease, hypopituitarism and hypothyroidism.

17-Hydroxyprogesterone

Serum Male: 1.5-7.5, Follicular phase: 0.6-2.4, Luteal phase: 2.7-9.2, Children: 0.6-1.6 nmol/l.

17-hydroxyprogesterone is a steroid precursor; accumulates as a result of 21-hydroxylase deficiency; used in the diagnosis of adrenogenital syndrome and late-onset form with polycystic ovaries.

17-Ketosteroids

24-hour urine Male: 20-70 µmol/24h, Female: 20-60 µmol/24h; Male: 6-20 mg/24h, Female: 6-17 mg/24h.

Replaced by plasma cortisol, urinary free cortisol and other intermediate steroids. *Increased* in Cushing's syndrome, congenital adrenogenital syndrome and hirsutism. *Decreased* in Addison's disease, liver disease, hypopituitarism and hypothyroidism.

25-Hydroxy-Vitamin D3

Serum 20-100 nmol/l.

Decreased in osteomalacia, malabsorption and cirrhosis of liver.

Acetone

Serum or urine Expressed in terms of dilutions ½, ¼, ⅛, Negative.

Increased in metabolic acidosis. See also ketones, *urine* and *serum*.

Acetylcholine Receptor Antibodies

Serum <0.25 nmol/l.

Autoantibodies against acetylcholine receptors in the thymus (myasthenia gravis) leading to muscular weakness.

Acetylsalicylic Acid

Serum Theraputic level: <20, toxic level: >30, lethal level: >60 mg/dl.

Useful for cases suspected of drug overdose.

Acid Phosphatase Prostatic

Serum <1.0 u/l.

Increased in carcinoma of prostate. Replaced by PSA total & free.

Acid Phosphatase Total

Serum <4.0 u/l.

Increased in carcinoma of prostate. Replaced by PSA total & free.

Activated Protein C Resistance Factor V (Factor V Leiden)

EDTA whole blood Absence of mutations. See 5.14

Activated Protein C Resistance Factor (Factor V Leiden) is an autosomal recessive, associated with thromboembolic disorders. Screening is of importance in females who wish to use contraceptive pills as it increases the risk of thrombosis in healthy carriers (heterozygous for the mutation). Useful in those with a positive family history of thromboembolic phenomena, and in candidates for sugery.

Adeno Virus Antibodies (IgG,IgA, IgM)

Serum <10 liter.

Present in patients exposed to adenovirus infection.

Adrenocorticotrophic Hormone (ACTH or Corticotropin)

Plasma <10 pmol/l.

ACTH is a protein hormone secreted from the anterior pituitary gland. Its main function is the regulation of cortisol. *Increased* in adrenocortical insufficiency and ectopic tumor producing ACTH for example, small cell carcinoma of the lung. *Decreased* in Cushing syndrome and pituitary insufficiency.

AGS (Adrenogenital Syndrome) 21-Hydroxylase Deficiency Gene See 5.24

EDTA whole blood Absence of mutations.

AGS is caused by genetic mutations in the 21-steroid-hydroxylase enzyme gene found on the short arm of chromosome 6. It is autosomal recessive and manifests in different clinical forms. In severe forms AGS causes premature virilization of the female fetus in-utero. Late onset, non classical form of the disease presents after puberty, adoles-

cents and young men are usually asymptomatic, while girls show signs of premature puberty, acne, seborrhea, hirsutism, tall stature, accelerated bone aging and clitoris hypertrophy.

Alanine Aminotransferase (ALT) SGPT

Serum <35 u/l.

Increased in liver disease, congestive heart failure, infectious mononucleosis, acute myocardial infarction, renal infarcts, skeletal muscle disease, acute pancreatitis drug toxicity and heparin therapy.

Albumin, CSF

CSF <45 mg/dl.

Increased in bacterial meningitis, multiple sclerosis, Guillain-Barre syndrome and polyneuritis.

Albumin, Serum

Serum 35-55g/l.

Albumin is synthesized by the liver. *Decreased* in starvation, liver disease, malabsorption, nephrotic syndrome, enteric loss, severe burns and hemodilution.

Albumin, Urine

Urine <150 mg/24h.

Increased in nephrotic syndrome, eclampsia, glomerulonephritis and polycystic kidney disease.

Albumin, Urine Microalbuminuria

Urine <20 mg/24h. See 2.109 & p 64

Microalbuminuria assessment is of special value in detecting early diabetic nephropathy and enables reversal of progression of renal damage by adequate control of carbohydrate metabolism.

Alcohol-Ethanol

Serum mg/dl. Normally not detected.

Useful in detecting alcohol intoxicated individuals.

Aldolase

Serum Adults: 2.0-8.0, Children: 2.0-16.0 u/ml.

Aldolase is an enzyme present in high concentration in muscles. *Increased* in progressive muscular dystrophy, myositis, liver damage and miscellaneous conditions.

Aldosterone

Serum Standing: 110-860, Recumbent: 28-444 pmol/l.

Increased in hypertension of primary aldosteronism. Useful in distinguishing primary (renin elevated) from secondary (renin normal) aldosteronism.

Alkaline Phosphatase

Serum Adults: <115, children: <500 u/l.

Increased in extrahepatic biliary obstruction and various liver and bone conditions. *Elevation* characteristic of cholestasis in liver disorders and osteoblastic activity in bone disorders.

Allergen Specific IGE (RAST)

Serum See report for normal and 2.12

Useful in the diagnosis of allergy caused by different allergens. *Different types of panels are available.*

α-1 Antitrypsin

Serum 0.7-2.0 g/l.

Deficiency associated with pulmonary emphysema and liver cirrhosis. Early detection allows replacement therapy in young adults.

α 1 Antitrypsin Deficiency AAT-Gene

EDTA whole blood Absence of mutations. See 5.22

α 1 antitrypsin is an acute-phase protein that inhibits protease enzymes. It is synthesized mianly in hepatocytes, alveolar macrophages and in blood monocytes. Gene carrier deficiency presents with lung and liver problems. ATT deficiency of childhood involves predominantly liver and to a lesser extent lungs and kidneys. ATT in adulthood is dominated by lung emphysema. Determination of Z and S mutations on chromosome number 14 is done by PCR and DNA sequencing.

α Thalassemia Genetic Testing

EDTA whole blood See report for normal.

For the detection of the most common mutations in the α thalassemia gene.

Alkaline Phosphatase Isoenzymes

Serum u/l. See report for normal. Adult: Total: <116, Bone <57 Liver <60 u/l. *Do not freeze sample.*

Useful in differentiating source of elevated alkaline phosphatase, namely, liver or bone.

α-Fetoprotein, Amniotic Fluid

Amniotic fluid ng/ml. Normal value is related to period of gestation. See Table 9.

Increased in pregnancies with neural tube defects.

α-Fetoprotein, Pleural Effusion

Pleural effusate <10.0 ng/ml.

Increased in primary and secondary malignant tumors of liver.

α-Fetoprotein, Serum

Serum For pregnant women: related to period of gestation; see Table 9. All other patients: <10.0 ng/ml.

Useful in diagnosis and follow up of hepatocellular carcinoma, some testicular and ovarian tumors; differentiation of neonatal hepatitis versus biliary atresia and detection of fetal abnormalities.

Amebic Antibodies

Serum Titer <100.

Present in patients infected with ameba.

Amino Acid Chromatography

Serum or urine Qualitative. Normal pattern. See Table 17.

Detects abnormal amino acids in patients with inborn errors of metabolism.

Amino Acid, Quantitative

Plasma, urine mg/dl or mg/24h for normal see report.

Quantitates specific amino acid levels for the detection of inborn errors of amino acid metabolism.

Ammonia

Plasma 12-47 µmol/l.

Increased in severe liver disease, Reye's syndrome, GI bleeding, shock, valproic acid and urinary tract anomalies.

Amylase, Serum

Serum <95 u/l.

Increased in acute pancreatitis, acute cholecystitis, and diseases of the salivary glands, gut disorders, renal failure, tubal pathology and malignancy.

Amylase, Urine

24-hour urine <500 u/l.

Increased in acute pancreatitis, acute cholecystitis, and diseases of the salivary glands, gut disorders, renal failure, tubal pathology and malignancy.

Amylase Clearance

Serum and urine <5.0%.

Useful in the diagnosis of macroamylasemia.

Amyloidotic Polyneuropathy

5-10 ml EDTA blood See 5.52.

A heterogeneous group of autosomal dominant disorders resulting in sensiromotor polyneuropathy, digestive autonomic disturbances and cardiomyopathy.

Androstenedione

Serum Male: 0.5-10.5, Female: 3.0-10.5 nmol/l.

Increased in Stein-Leventhal (polycystic ovaries) syndrome and acne.

Angiotensin-Converting Enzyme (ACE)

Serum 18-55 u/l. See 2.70.

Increased in sarcoidosis, lung disease & infections, primary biliary cirrhosis and lymphoma.

ACE Gene

EDTA whole blood See report for normal. See 5.26.

Three genotypes of ACE gene are present: 1L1, 1LD, DLD. The most important is the DLD type that carries higher risk for developing myocardial infarction.

Anti-Adrenal Antibodies

Serum Titer. Negative.

Increased in Addison's disease and autoimmune disorders.

Anti Candida Species *(albicans, gabarates, krusel, tropicales, parapsilosis)* Antibodies

Serum Titer<100.

For the diagnosis of candida infections.

Anti-Cardiac Muscle Antibodies

Serum Titer <10.

Auto antibodies against cardiac muscle fibers; are present in a number of auto-immune diseases.

Anti-Cardiolipin Antibodies, IgM & IgG

Serum IgM up to 11 mpl/ml and IgG up to 23 gpl/ml. See 2.21.

Increased in some cases of thrombosis, recurrent abortion, systemic lupus erythematosus and thrombocytopenia.

Anti-Centromeres Antibodies

Serum Titer<40.

Found in 50% of CREST syndrome, systemic lupus erythematosus and primary biliary cirrhosis.

Anti-Diuretic Hormone

Serum and plasma <2 if serum osmolality <280, 2.0-12.0 pg/ml if serum osmolality > 285 mosm/kg.

Decreased in diabetes insipidus, congestive heart failure and nephrotic syndrome.

Anti-dsDNA Antibodies

Serum Titer <10.

Positive in 90% of SLE patients and in connective tissue disease. Most useful in predicting and monitoring therapy of SLE patients.

Anti-Endomesial Antibodies

Serum Titer <10.

Useful in diagnosis of GSE (gluten-sensitive enteropathy) or GSE associated with dermatitis herpetiformis.

Anti Gangliosides Antibodies (GM1, GM2, GM3, GD1A, GD1B, GT1B, GQ1B)

Serum Titer <50.

Associated with the following diseases: multifocal motor neuropathy (GM1), Guillain-Barre syndrome (GMI, GD1, GT1), sensory neuropathy (GD1), Miller-Fisher syndrome (GQ1).

Anti-Gliadin Antibodies

Serum Titers IgA: <10, IgG: <10, IgM: <10.

Useful in screening for celiac disease. *Increased* in 25% of dermatitis herpetiformis.

Anti-GBM (Glomerular Basement Membrane Antibodies)

Serum Titer <10.

Increased in Goodpasture's syndrome or anti-GBM antibody-induced glomerulonephritis and some SLE patients.

Anti-Histones Antibodies

Serum Units <10.

Increased in 60% of systemic lupus erythematosus, rheumatoid arthritis and 95% of drug induced lupus erythematosus.

Anti-Intrinsic Factor Antibodies

Serum Negative.

Increased in 70% of pernicious anemia.

Anti-Islet Cell Antibodies

Serum Titer <10.

Found in 80 % of patients with newly diagnosed insulin dependent diabetes and in 3-4% of their relatives; also in 0.5% of clinically normal subjects.

Anti-Liver Kidney Microsomal Antibodies

Serum Titer <10.

Increased in chronic hepatitis and cirrhosis.

Anti Listeria Antibodies

Serum Titer <100.

Increased in cases infected with *Listeria monocytogenes.*

Anti-Mitochondrial Antibodies

Serum Titer <10.

Increased in biliary cirrhosis, liver cirrhosis and chronic active hepatitis.

Anti-Mitochondrial Antibodies, M2, M4, M9 Profile

Serum Titer <100.

Sub group of AMA that shows specificity for primary biliary cirrhosis, especially M2 antibodies (96%).

Anti-*Mycoplasma genitalium* Antibodies

Serum Titer <100.

Increased with cases infected with *Mycoplasma genitalium.* Urogenital tract is the primary infection site where it may cause nongonococcal urethritis.

Anti-*Mycoplasma hominis* Antibodies

Serum Titer <100.

Increased in cases infected with *Mycoplasma hominis.*

Anti-Neuronal Antigens Antibodies (Anti GAD, Anti MAG, Anti Ri, Anti Hu, Anti Yo)

Serum Titer <10.

Antibodies against myelin and myelin associated glycoprotien (MAG) are present in multiple sclerosis patients and sometimes in Guillian-Barre patients. Other neuronal antibodies are present in various disorders such as disorders of the cerebellum (Anti Yo, Hu, Ri).

ANCA (Anti-Neutrophil Cytoplasmic Antibodies)

Serum Titer <20.

Useful in diagnosis and management of Wegener's granulomatosis (>90%) and some types of glomeru-

lonephritis. c-ANCA: cytoplasmic granular pattern. P-ANCA: perinuclear pattern.

Anti-Nuclear Antibodies

Serum Titer <40.

Increased in 98% of SLE patients, connective tissue disease, liver and renal diseases, and in many other disorders; occasionally in normal persons.

Anti-Oxidant Status, Total

Serum >1.3 mmol/l.

Anti-oxidants work against harmful free radicals which have been implicated in the development of many chronic diseases.

Anti-Parietal Cell Antibodies

Serum Titer <10.

Increased in 90% of pernicious anemia.

Anti-Phospholipid Antibodies, IgM, IgG

Serum IgM up to 11 mpl/ml, IgG up to 23 gpl/ml. See 2.21.

A sub-population of anti-cardiolipin antibodies with lupus anticoagulant activity.

Anti-Pneumococcus Antibodies

Serum <20 mg/l.

To assess immune status of individuals suspected of having immune deficiency pre and post immunization with pneumococcal polysaccharide antigens.

Anti-Reticulin Antibodies

Serum Titers IgA: <10, IgG: <10, IgM: <10.

Increased in 40% of adults and 60% of children with celiac disease.

Anti-RNP Antibodies

Serum <20 Units.

Increased in 95% of mixed connective tissue disease and 30-40% of systemic lupus erythematosus.

Anti-Scl-70 Antibodies

Serum <20 Units.

Increased in up to 40% of progressive systemic sclerosis.

Anti-Sjögren Syndrome A Antibodies (SSA)

Serum <20 Units.

Increased in Sjögren's syndrome, systemic lupus erythematosus and progressive systemic sclerosis.

Anti-Sjögren Syndrome B Antibodies (SSB)

Serum <20 Units.

Increased in Sjögren's syndrome, systemic lupus erythematosus and progressive systemic sclerosis.

Anti-Sm Antibodies

Serum <20 Units.

Increased in 30% of systemic lupus erythematosus and mixed connective tissue disease.

Anti-Smooth Muscle Antibodies

Serum Titer <10.

Increased in chronic active hepatitis, biliary and liver cirrhosis and rheumatoid arthritis.

Anti-SLA/LP (Soluble Liver Antigen/ Liver Pancreas Antigen)

Serum Titer <100.

SLA is located in the cytosol of liver cells. These antibodies are present in 30% of patients with autoimmune hepatitis, but their predictive value is almost 100%.

Anti-Sperm Antibodies

Serum or semen Titer <16.

Increased in cases of subfertility, especially idiopathic type. The test is of debatable value.

Anti-ss-DNA Antibodies

Serum <20 Units.

Increased in Felty's syndrome, systemic lupus erythematosus and progressive systemic sclerosis.

Anti-Streptolysin O

Serum <200 Units. See 4.24.

Increased in acute poststreptococcal glomerulonephritis, rheumatic fever and sometimes in rheumatoid arthritis.

Anti-Striated Muscle (Skeletal) Antibodies

Serum Titer <10.

Increased in myasthenia gravis; also in myopathic disorders and apparently healthy individuals in low titers.

Anti-Tetanus Antibodies

Serum <0.03 iu/ml.

To assess immune status of individuals suspected of having immune deficiency pre and post immunization with tetanus toxoid.

Anti-Thrombin III

Plasma 240-300 mg/l. See 7.72.

Decreased in individuals at risk of thrombosis; also in chronic liver disease, nephrotic syndrome, disseminated intervascular coagulation, deep vein thrombosis etc.

Anti-Thyroglobulin Antibodies

Serum Titer <20. See 2.75.

Increased in inflammatory and autoimmune thyroid disorders, especially Hashimoto's thyroiditis. Not specific for thyroid disease and seen in other autoimmune diseases e.g. Sjögren's, SLE and rheumatoid arthritis.

Anti-Thyroid Microsomal Antibodies

Serum Titer <20. See 2.75.

Increased in inflammatory and autoimmune thyroid disorders, especially Hashimoto's thyroiditis. Not specific for thyroid disease and seen in other autoimmune disease e.g. Sjögren's, SLE and rheumatoid arthritis.

Anti-Thyroid Peroxidase Antibodies

Serum <50 iu/ml. See 2.72.

Increased in Graves' disease and Hashimoto's thyroiditis (correlates with active clinical disease).

Anti-TSH Receptor Antibodies

Serum Negative: <4 Positive: >9 Borderline: 4-9 u/l. See 2.73.

Auto antibodies against TSH receptor in the thyroid gland associated with hyperthyroidism.

Anti-*Ureaplasma Urealyticum* Antibodies

Serum Titer <100.

Present in patients exposed to Ureaplasma urealyticum organism.

Apolipoprotein B

Serum 240-300 mg/l.

Increased in chronic ischemic heart disease, arteriosclerosis and liver disease.

Apolipoprrptein A1/C111 Gene, Apolipoprotein B Gene, Apolipoprotein E Gene

EDTA whole blood See 5.30.

For the genetic differentiation of the different types of hyperlipidemia syndromes.

Ascitic Fluid Analysis

Ascitic fluid See Table 13a.

Most common causes: liver disease, malignancy, congestive heart failure, tuberculosis and other causes.

AST (Aspartate Aminotransferase) SGOT

Serum <38 u/l.

Increased in liver disease, acute myocardial infarction, acute myocarditis, cardiomyopathies, congestive heart failure, hemolytic anemia, megaloblastic anemia, skeletal muscle diseases, malignancy, infectious mononucleosis, acute pulmonary infarction and any tissue necrosis.

ADMCKD (Autosomal Dominant Medullary Cystic Kidney Disease) Genetic Testing

EDTA whole blood See 5.58.

Cystic disease of the medulla of the kidney, leading to asymmetric, scarred and shrunken kidney and renal failure usually after the second decade of life (adult onset).

ADPKD (Autosomal Dominant Polycystic Kidney Disease) Genetic Testing.

EDTA whole blood See 5.56.

Characterized by cysts in both cortex and medulla of both kidneys leading to enlargement of the kidney. Symptoms start in the third or fourth decades with flank pain, gross and microscopic hematuria. Hypertension occurs in 75% of patient. Leads to end stage renal failure.

ARPKD (Autosomal Recessive Polycystic Kidney Disease) Genetic Testing

EDTA whole blood See 5.57.

Detection of mutations for ARPKD and prenatal diagnosis is possible. In two forms: infantile and child hood. The infantile form can be recognized at birth by diffuse enlargement of both kidneys, renal failure and maldevelopment of intrahepatic bile ducts. Childhood form consists of medullary ductal ectasia which is usually asymptomatic, congenital hepatic fibrosis and partial hypertension. Renal failure might occur. Both forms are autosomal recessive.

Avian influenza virus

See page …

Azoospermia Factor

2-5 ml EDTA blood. See 5.36.

Detection of microdeletions in Y_q11 region may be responsible for azoospermia.

Basophilic Stippling

EDTA Blood Negative.

Found in thalassemia major and minor, sideroblastic anemia, lead poisoning and vitamin B6 deficiency.

Basophils

EDTA Blood $<0.120\times10^9$/l.

Increased in chronic myelocytic leukemia, autoimmune hemolytic anemia and mildly in polycythemia vera.

Bence-Jones Protein

Urine Negative.

Positive in multiple myeloma and absent in alpha and gamma heavy chain disease.

Benzodiazepine Level

Serum, urine Theraputic level: 20-80 ng/ml serum. Non detectable in urine.

Benzodiazepines are sedative, anticonvulsant and hypotonic drugs useful for the treatment of anxiety and insomnia. Overdose can lead to respiratory arrest.

β 2-Microglobulin, Serum, Urine, CSF

Serum, Urine, CSF 1.0-2.1, <0.3, 0.7-1.8 mg/l.

Serum and urine: increased in sarcoidosis (some cases), malignancies e.g. Hodgkin's disease, CLL, ALL & multiple myeloma, HIV infection, rheumatoid arthritis and chronic renal failure. *CSF: increased* in acute leukemia and lymphoma.

β-HCG (Beta-Human Chorionic Gonadotrophin)

Serum miu/ml. See Table 10 and 2.81 and 99.

Increased in normal and ectopic pregnancies, trophoblastic disease, ovarian and testicular malignancy. This test is used in trisomy 21 risk calculation.

β-HCG Free

Serum Nonpregnant & postmenopausal women & men <0.1 ng/ml

HCG (Human chorionic gonadotropin) is a glycoprotein secreted by placenta and detected in blood and urine of pregnant women. HCG and HCG-like products are also

produced by a variety of tumors. Intact HCG consists of α and β subunits bound to each other, but which also occur in a free or unbound form. HCG α subunit is identical for HCG, FSH, LH and TSH. However, β subunit of HCG and LH are similar and exerts the same biological effects. Free β HCG levels are used in the monitoring of trophoblastic diseases and certain testicular tumors, while ration of free βsubunit to intact HCG can be very high, sometimes with no detectable intact HCG. Free βHCG levels are also used in monitoring outcome of IVF in early pregnancy as well as in prenatal screening for Down syndrome during the first and second trimesters. A combination of maternal age, serum free β HCG and PAPP-A levels added to fetal nuchal translucency substantially potentiates the efficacy of prenatal screening, with detection rates of 85-90% for Down syndrome and a 5% false positive rate.

β Thalassemia Genetic Testing

EDTA whole blood, Chorionic Villus Sampling Absence of mutations. See 5.17.

Many mutations have been described in beta thalassemia, some of which affect the synthesis of beta globin, others with complete absence of beta globin. Double heterozygous or homozygous mutants can have severe clinical manifestations & become transfusion dependent with chronic anemia and extramedullary erythropoiesis leading to hepatosplenomegaly, accompanied by malnutrition and predisposition to infection. The disease manifests between 6 –12 months after birth with a poor life expectancy.

Bicarbonate

Serum 20.0-30.0 mmol/l.

Increased in respiratory dysfunction, Cushing syndrome, hyperaldosteronism and excessive vomiting. *Decreased* in ketoacidosis, lactic acidosis, kidney disease, Addison's disease and diarrhea.

Bilirubin, Total

Serum <1.0 mg/dl, <17.0 µmol/l.

Increased in viral or toxic hepatitis, cholestasis, liver cirrhosis, extrahepatic biliary obstruction, erythroblastosis fetalis, Gilbert disease, Crigler-Najjar disease, thalassemia major, sickle cell anemia, acquired and other hemolytic anemias.

Bilirubin, Direct

Serum <0.25 mg/dl, <4.3 µmol/l.

Increased in extrahepatic biliary obstruction and Gilbert disease.

Bleeding Time

<3.0 minutes.

Increased in thrombocytopenia, impaired platelet function, von Willebrand's disease, Factor VII deficiency and thrombasthenia.

Blood Grouping and Rhesus Typing

2 ml clotted blood. See7.6.

An important identification marker and the most frequent test in blood banking.

Borrelia Antibodies IgG, IgM

Serum Titer <10. See 4.17.

For the diagnosis of *Borrelia burgdorferi* infection (Lyme disease).

Brucella Antibodies IgG, IgM

Serum Titer <160. See 4.4.

For differentiating acute from chronic brucellosis.

C-1 Esterase Inhibitor (Human C-1 Inactivator)

Serum 250-420 mg/l.

Deficiency is the cause of hereditary angioedema Type 1.

C-Peptide

Serum 0.90-4.00 ng/ml, 298-1324 pmol/l.

Useful in evaluating insulin dependent diabetics. *Normal* values indicate production of some insulin. *Low* or *no* detectable values indicate poor or no production of insulin.

CA-125

Serum 1.70-32.0 u/ml. See 2.90.

Useful in follow up of serous carcinoma of ovary. Also *increased* in early pregnancy, endometriosis and other malignancies. Not recommended for screening.

CA-15.3

Serum 7.50-53.0 u/ml. See 2.86.

Useful in follow up of breast cancer, in combination with CEA. Also may *increase* in cancer of lungs, pancreas and ovary; sometimes in benign breast disease.

CA-19.9

Serum <35.0 u/ml. See 2.81.

Useful in follow up of pancreatic cancer, also sometimes in colon cancer. May *elevate* in some stomach and hepatobiliary malignancies.

Calcitonin

Serum <50.0 pg/ml. See 2.97. Caption 24.

Increased in medullary carcinoma of thyroid, multiple endocrine neoplasia and ectopic production by neoplasms.

Calcium, Ionized

Serum 4.49-5.36 mg/dl, 1.12-1.32 mmol/l.

Increased in hyperparathyroidism, metastatic bone lesions, milk-alkali syndrome, multiple myeloma, Paget's disease, sarcoidosis and vitamin D intoxication. *Decreased* in hypoparathyroidism, osteomalacia, malabsorption, pancreatitis, renal failure, rickets and vitamin D deficiency. (ionized calcium is the active portion of total calcium).

Calcium, Total

Serum 8.30-10.50 mg/dl, 2.10-2.60 mmol/l.

Increased in hyperparathyroidism, metastatic bone lesions, milk-alkali syndrome, multiple myeloma, Paget's disease, sarcoidosis and vitamin D intoxication. *Decreased*

in hypoparathyroidism, osteomalacia, malabsorption, pancreatitis, renal failure, rickets and vitamin D deficiency.

Calcium, 24h Urine

24-hour urine <300 mg/24h, <7.5 mmol/24h.

Increased in idiopathic hypercalcuria, milk-alkali syndrome, osteolytic bone lesions, Cushing syndrome, osteoporosis, primary hyperthyroidism, renal tubular acidosis, sarcoidosis and vitamin D intoxication.

Carbamazepine (Tegretol, Carbatol)

Serum 4.00-10.0 µg/ml, 17.0-42.0 µmol/l.

This drug controls certain types of seizures in epilepsy and treats pain caused by trigeminal neuralgia.

CEA (Carcinoembryonic Antigen)

Serum Male smoker: <6.2, & nonsmoker: <3.4, Female smoker <4.5 & nonsmoker: <2.5 ng/ml. See 2.94.

Elevated mainly in cancer of lung, breast and large intestine. Useful in follow-up after therapy or surgery.

CRP (C-Reactive Protein)

Serum <6.0 mg/l

Increased in acute phase reaction. A nonspecific test.

CRP (Sensitive method)

Serum <5.0 mg/l. See 2.49.

This is a newly developed and highly sensitive method for measurement of CRP that is associated with the meta-

bolic syndrome. The latter identifies a population at high risk of developing cardiovascular events. The metabolic syndrome is recognized by presence of 3 of the following characterstics: body obesity, hypertriglyceridemia, low HDL, hypertension and abnormal glucose. Increased levels of CRP are seen in association with all these parameters. CRP is a new and important prognostic factor for cardiovascular disease. Serial levels are of value in assessing risk for coronary heart disease. However, CRP is a classical test for reaction to acute inflammation, whether infection, collagen disease, colitis or any systemic type of inflammation.

Catecholamines

24-hour urine 10.0-270 µg/24h.

Increased in pheochromocytoma, manic depressive disorder, anxiety and stress. *Avoid intake of walnuts, bananas, chocolates, vanilla- containing foods, citrus fruits and certain medications 3 days before and during collection.*

CSF (Cerebrospinal Fluid Analysis)

CSF See Table 12.

CSF Bacterial Antigen Latex Test

CSF Negative.

Useful in detecting main groups of bacteria causing meningitis, namely: *Streptococcus* Group B, *E. Coli, H. influenzae, N. meningitides* and *S. pneumoniae*.

CSF Index (IgG / Albumin)

CSF <0.6.

Increased in intrathecal production of IgG (as in multiple sclerosis).

CSF Oligoclonal Banding

CSF Negative. See page 78.

Present in multiple sclerosis and, chronic immune related disorders and chronic brain infections.

Ceruloplasmin

Serum 0.155-0.592 g/l.

Decreased in hepatolenticular degeneration (Wilson's disease): homozygous 85-95% heterozygous 10-20%, fulminant hepatic, failure, malnutrition, intestinal malabsorption, renal protein loss, hereditary hypoceruloplasminemia, normal neonates, chronic active hepatitis in children (25%).

CH50 (Complement Function)

Fresh serum Titer: 1/40-1/80

Increased in acute phase reaction. Decreased in active phases of immunologic disorders, autoimmune diseases, malignancies, burns, C1q-precipitin syndrome and congenital deficiencies.

Charcot-Marie-Tooth (HMSN) Genetic Testing

5-10 ml EDTA blood See page 52 and 5.55.

For genetic diagnosis of Charcot-Marie-Tooth disease. Hereditary sensory neuropathies types I & II both with mendelian mode of inheritance.

Chlamydia Antibodies, IgG, IgM
Chlamydia Antigen
Chlamydia Antigen, By PCR

Serum, Urine, urethral or *vaginal discharge* Titer <16 for antibodies and negative for antigen tests. See 4.6 and 49.

Different strains of *Chlamydia* cause genital, eye, lymph node and respiratory infections. *Chlamydia* is the most common cause of blindness in the world. A child born to a woman with Chlamydia infection of the cervix may acquire an acute eye or lung infection. Men may develop nonspecific urethritis and orchitis. In women infection may lead to cervicitis, salpingitis and can lead to infertility. Direct laboratory examination for Chlamydia is possible; PCR method is fast, specific and sensitive. IgG and IgM antibodies in blood indicates past and recent infection, respectively.

Chlamydia pneumoniae by PCR

Respiratory secretions & lavages, throat swabs Negative. See also Serology section.

Chlamydia pneumoniae is a major cause of acute respiratory tract disease in humans and has been responsible for both endemic and epidemic pneumonia. PCR is a quick and reliable method for diagnosis.

Chlamydia trachomatis by PCR

First void urine, urethral discharges, cervical scrapings Negative.

Chlamydia trachomatis is one of the most frequent causes of sexually transmitted diseases. *Chlamydia trachomatis* infection may lead to pelvic inflammatory dis-

ease, resulting in tubal infertility, ectopic pregnancy and chronic pelvic pain.

Chloride, Serum

Serum 98-107 mmol/l.

Increased in dehydration, kidney dysfunction, chronic hyperventilation, Cushing syndrome, excess infusion of normal saline, metabolic acidosis, eclampsia and renal tubular acidosis. *Decreased* in Addison disease, chronic respiratory acidosis, congestive heart failure, excessive sweating, overhydration, salt losing nephritis and vomiting.

Chloride, 24h Urine

24-hour urine 110-250 mmol/24 h.

Increased in diabetes mellitus and anterior pituitary hypofunction. *Decreased* in bacterial pneumonia, diarrhea and vomiting.

Chloride, CSF

CSF 120-126 mmol/l.

May *increase* in encephalomyelitis. *Decreased* in bacterial meningitis.

Chloride, Sweat

Sweat 17-80 mmol/l. See 5.20

Increased in cystic fibrosis and intrinsic asthma.

Cholesterol, HDL

Serum Male: 41-59, Female: 48-75 mg/dl; Male: 1.1-1.5, Female: 1.25-2.0 mmol/l. See 2.31.

Referred to as "good" cholesterol; *increases* on exercise. *Increases* also in chronic active hepatitis and alcoholism.

Cholesterol, LDL

Serum <160 mg/dl, <4.16 mmol/l.

Referred to as "bad" cholesterol; correlates with cardiovascular disease.

Cholesterol, Total

Serum 130-200 mg/dl, 3.38-5.2 mmol/l. See 2.31.

Excess serum cholesterol correlates with cardiovascular disease. *Increased* in familial hyperlipidemias, myocardial infarction, atherosclerosis, high cholesterol diet, hypothyroidism, nephrotic syndrome, biliary cirrhosis and uncontrolled diabetes. *Decreased* in hyperthyroidism and liver disease. See also Lab Panels.

Cholesterol, Total/ HDL Ratio

Serum Ratio: 4.5-5.5. See 2.31.

Cholesterol related parameter. It is a risk factor measurement of coronary heart disease. The lower the better.

Chromosomal Analysis

Heparinized blood, bone marrow, amniotic fluid, abortus See 5.5.

For detection of numerical or structural chromosomal abnormalities.

Clostridium difficile

Serum for CFT and ELISA testing. See 4.7.

Clot Retraction Test

Blood Retraction begins after 30-60 minutes.

Poor in Waldenstrom's macroglobulinemia, congenital dysfibrinogenemia, idiopathic & thrombotic thrombocytopenic purpura. It is abnormal with low platlet count, aspirin therapy, polycythemia, Glanzmann's disease and D.I.C.

Clotting Time

Blood 3.0-10.0 minutes.

Increased in hemophilia, factor XI, XII, V, X & II deficiency, congenital afibrinogenemia, Von Willebrand's disease, heat stroke and toxic effect of venom. The test has gone into disfavor because of poor reproducibility.

Cold Agglutinins

Serum Titer<1/16. See 7.4 and 14.

Increased with *Mycoplasma pneumoniae*, infectious mononucleosis, multiple myeloma, scleroderma, cytomegalovirus, hemolytic anemia, cirrhosis and idiopathic cold agglutinin disease. It is also associated with Hodgkin and non Hodgkin lymphoma, CLL, CML, tropical diseases (malaria, tropical eosinophilia, trypanosomiasis), listeriosis, subacute bacterial endocarditis and syphilis.

Complement C3

Serum Male: 0.91-1.56, Female: 0.93-1.49 g/l.

Increased in cancer and ulcerative colitis. *Decreased* in hereditary angioedema, cirrhosis, glomerulonephritis, hepatitis, renal transplant rejection and systemic lupus erythematosus.

Complement C4

Serum Male: 0.13-0.43, Female: 0.15-0.38 g/l.

Increased in cancer and ulcerative colitis. *Decreased* in hereditary angioedema, cirrhosis, glomerulonephritis, hepatitis, renal transplant rejection and systemic lupus erythematosus.

Complete Blood Count (CBC)

EDTA blood See Tables 1a&b.

Coombs' Test, Direct

EDTA blood Negative. See 7.14.

May be *positive* in acquired hemolytic anemia. Positive in erythroblastosis fetalis.

Coombs' Test, Indirect

Serum Negative titer.

See Rhesus antibodies.

Copper, Serum
Copper, 24-hour Urine

Serum, 24-hour urine 70.0-150 µg/dl or 11.0-24.0 µmol/l, <40 µg/24h.

Most useful in the diagnosis of hepatolenticular degeneration (Wilson's disease) where serum copper is *decreased* and 24h urinary copper excretion is *increased*.

Coproporphyrin

24-hour urine <200 µg/24h.

Increased in congenital erythropoietic porphyria, acute intermittent porphyria, porphyria variegata, porphyria cutanea tarda, hereditary coproporphyria, Dubin-Johnson syndrome, Rotor's syndrome, hemolytic anemia and lead poisoning.

Cortisol, Serum

Serum am: 5.0-25 µg/dl or 138-690 nmol/l; pm: 2.0-15 µg/dl or 50-410 nmol/l.

Increased in malignancy of adrenal gland and adrenal cortical hyperfunction Cushing's syndrome, *decreased* in adrenal cortical hypofunction, primary or secondary, and in congenital adrenal hyperplasia.

Cortisol, Free, 24-hour Urine

24-hour urine 10.0-120 µg/24h, 226-3312 nmol/24h.

Increased in malignancy of adrenal gland and adrenal cortical hyperfunction Cushing's syndrome, *decreased* in adrenal cortical hypofunction, primary or secondary, and in congenital adrenal hyperplasia.

C-Peptide

Fasting: Children 0.4-2.3 µg/ml, Adult= 0.5-25 µg/ml Post-prandial: 2.2-6.5ug/ml

C-peptide connects A and B chains of insulin. *Increased* in insulinoma and normal in fictitious hypoglycemia. Useful in the assessment of β-cell function in diabetics and reflects insulin secretion. In insulin-dependent diabetes mellitus (type I), no or very low level of C-peptide is seen. In noninsulin-dependent diabetes mellitus (type II), C-peptide is either normal or elevated.

Creatine Kinase, CK

Serum Male: 35-194, Female: 33-143 u/l.

Increased in progressive muscular dystrophy, myositis, acute myocardial infarction, acute myocarditis, hypothyroidism, crush injury and exercise.

Creatine Kinase-MB (CK-MB)

Serum 0-25 u/l (<5% of CK). See 2.10.

Isoenzyme, specifically *increased* in acute myocardial infarction. *Allows early diagnosis. Do not freeze sample.*

Creatinine

Serum <1.3 mg/dl, <115 umol/l.

Increased in acute and chronic renal failure and conditions leading to reduced renal blood flow.

Creatinine, 24-hour Urine

24-hour urine 1.0-2.0 g/24h, 7.5-17 mmol/24h.

Decreased in acute and chronic renal failure, conditions leading to reduced renal blood flow and in protein malnutrition.

Creatinine Clearance

Serum and 24-hour urine 90-160 ml/min, (depends on body surface area). See Lab Panels.

Decreased in acute and chronic renal failure and conditions leading to reduced renal blood flow.

Cryoglobulins

Serum Negative.

May be *positive* in auto-immune disorders, Waldenstrom's macroglobulinemia, multiple myeloma, lymphoma, chronic lymphocytic leukemia, infectious mononucleosis and leprosy.

Cyclosporin

EDTA plasma predose Weeks post transplant: 1-3: 250-300 3-6: 200-250, 6-11: 150-200, >11: 125-150 ng/ml.

Immunosuppressant drug; helps preventing graft vs. host disease in kidney, liver, bone marrow and heart transplantation.

CFTR- Gene (Cystic Fibrosis Or Mucoviscidosis)

*EDTA blood A*bsence of mutations. See 5.20.

Cystic fibrosis is the most common lethal autosomal recessive disorder in Caucasians with multi-organ disease, found on chromosome 7. Mutations affect the CFTR-protein that regulates transport of chloride through cell

membranes. The respiratory tract is most frequently affected, with majority of patients dying from advanced bronchopulmonary disease. 85 % of patients develop pancreatic insufficiency. 10-20% of newborn CF patients develop meconium ileus. Liver involvement can occur with cirrhosis developing in adulthood. Heterozygous males may be infertile due to obstructive azoospermia, while female patients have a reduced fertilization rate due to secretory malfunction.

Cystinuria Genetic Testing

EDTA whole blood See Genetics Chapter.

For genetic diagnosis of cystinuria; prenatal diagnosis is also possible

CMV (Cytomegalovirus) Antibodies IgM & IgG

Serum Titer <16. See 4.7.

Infection with CMV is very common. 80% of adults have IgG antibodies. Usually CMV produces no symptoms, but serious infections can occur in people with impaired immunity. It is a common complication in post transplantation. In pregnancy, infection may result in fetal death or severe damage to CNS. IgM antibodies indicate a recent infection.

CMV Antigen By Immunofluorescence

EDTA blood Negative.

CMV antigen detection is important in immunocompromised (cancer & AIDS) patients and developing fetus, that may result in either localized or disseminated disease. Also, it has been reported of value in the diagnosis

and monitoring of active CMV infection in solid organ & bone marrow transplant patients. *Early and rapid diagnosis of CMV avoids over-treatment by immunosuppressive drugs and helps in guiding antiviral therapy.*

CMV Antigen By PCR (Early Antigen)

Blood, urine, tissue biopsy, paraffin embedded tissue Negative.

CMV antigen detection is important in immunocompromised (cancer & AIDS) patients and developing fetus, that may result in either localized or disseminated disease. It has been reported of value in diagnosing and monitoring active CMV infection in solid organ & B.M. transplant patients. *Early and rapid diagnosis of CMV avoids over-treatment by immunosuppressive drugs and guides antiviral therapy.*

CMV Antigen By PCR (Late Antigen)

Blood, urine, tissue biopsy, paraffin embedded tissue Negative.

CMV antigen detection is important in immunocompromised (cancer & AIDS) patients and developing fetus, that may result in either localized or disseminated disease. It has been reported of value in diagnosing and monitoring active CMV infection in solid organ & B.M. transplant patients. *Early and rapid diagnosis of CMV avoids over-treatment by immunosuppressive drugs and guides antiviral therapy.*

D-Dimer

Plasma <0.5 Feu.

Increased in deep venous thrombosis. Useful in monitoring post-operative thrombotic risk.

D-Xylose Excretion Test

Serum and 5-hour urine >16%.

This test is performed to help evaluate cases of persistent diarrhea, unexpected weight loss, suspected malnutrition and general weakness. *Decreased* in malabsorption. Also may be *decreased* in Crohn's disease, *Giardia lamblia* infection, hookworm infection, lymphatic obstruction, radiation enteropathy, celiac disease, viral gastroenteritis and Whipple's disease. *If renal insufficiency is suspected, estimation of D-xylose in blood 2 hours after an oral dose is recommended.*

DHEA-S (Dehydroepiandrosterone Sulphate)

Serum Male: 80-560, Female: 35-430 µg/dl; Male: 2.2-15, Female:0.9-11.7 µmol/l.

Useful in evaluating function of adrenal glands. May be *increased* in virilism, hirsutism, acne and Stein-Leventhal syndrome.

Digoxin

Serum 0.8-2.4 ng/ml, 1.0-3.1 nmol/l.

Drug monitoring, 6-10h post dose. Used to treat congestive heart failure and irregular heart beat.

Duchenne Muscular Dystrophy Mutation Detection

EDTA blood See 5.45.

Detection of mutations of Duchenne; also carrier status and prenatal diagnosis possible.

Eosinophils

EDTA Blood <0.400×10^9/l.

Increased in allergic conditions.

EBV (Epstein-Barr Virus) Antibodies

Serum Titer: IgG: <10, IgM: <10. See 4.8.

Increased in infectious mononucleosis. Raised IgM indicates recent infection.

Erythrocytes

EDTA Blood See Table 1a.

ESR (Erythrocyte Sedimentation Rate)

EDTA Blood Male: 1-12, Female: 2-18 mm.

Nonspecific test; *increased* in acute or chronic inflammatory disease and used for monitoring the response of such disease to therapy. Also *increased* in large variety of disorders. *Decreased* in polycythemia vera. Non indicative of malignancy.

Erythropoietin

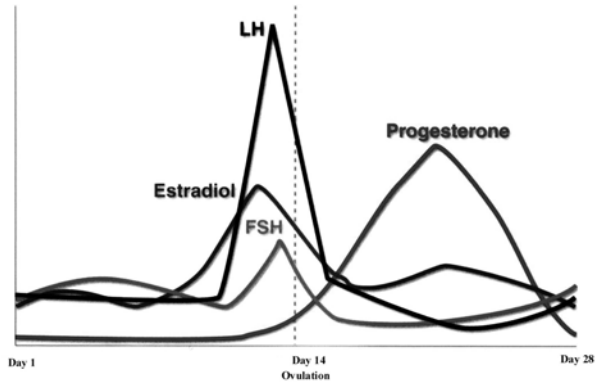
Serum 6.0-10.0 mu/ml.

Increased in lung, kidney and liver tumors, aplastic anemia, pheochromocytoma, acute renal failure and secondary polycythemia. *Decreased* in chronic renal failure, polycythemia vera and Hodgkin lymphoma.

E2 (Estradiol 17-B)

Serum Male: <205, Female: follicular: <976, midcycle: 433-130, luteal: 95-606 pmol/l.

Increased in ovarian tumors and in patients receiving ovarian stimulating drugs. *Decreased* in postmenopausal women and Turner's syndrome. See Graph on Menstrual Hormonal Cycle page 41.



Menstrual Hormonal Cycle

E3 (Free Estriol)

Serum Related to period of gestation. Expressed in ng/ml.

Declining values indicate fetal jeopardy. A useful parameter in trisomy 21 evaluation tests. See Table 15.

Ethanol Gelation Test

Plasma Negative.

May be *positive* in D.I.C. *This test is insensitive and nonspecific.*

Euglobulin Clot Lysis Time

Plasma 2.0-5.0 hours. See 2.34

Increased in alpha 1 antitrypsin deficiency and shock.

ENA (Extractable Nuclear Antigens) Antibodies

Serum <20 units. See 2.36.

Used to identify auto-antibodies in connective tissue disease. See individual antibodies namely: anti-RNP, anti-SCL-70, anti-sjogren, anti-SM and anti-SS-DNA antibodies.

Familial Hypercholesterolemia Genetic Testing

EDTA blood See 5.33.

For detection of mutations in the LDL- receptor gene leading to hypercholesterolemia. Carrier status can also be detected.

FMF (Familial Mediterranean Fever) Genetic Testing

*EDTA blood*Absence of mutations. See 5.60.

FMF gene (MEFV) is recessively inherited on Chromosome 16. FMF presents with recurrent episodes of unprovoked inflammation involving joints, pleural and peritoneal cavities, less frequently skin. Exons 10 & 2 are screened for mutations which represent more than 80% of mutations in the mediterranean region, namely, M694V, V726A, M680I, F479L and E148Q.

Fat, Stool

Stool Negative.

Useful in evaluating fat absorption to indicate liver, gall bladder, pancreatic and intestinal functions.

Ferric Chloride Test

Urine Negative. See 2.55.

This test is a simple screening test for detecting some amino acids in urine of patients with inborn error of metabolism. *Positive in aspirin users.*

Ferritin

Serum: Male: 18.0-370, Female: 10.0-120, Children: 9.0-120 ng/ml.

Increased in thalassemia major, hemochromotosis, acute and chronic myelocytic leukemia, acute lymphocytic leukemia, multiple myeloma, chronic infections, sideroblastic anemia and acute hepatitis. *Decreased* in iron deficiency anemia and celiac disease.

Fibrinogen

Plasma 250-450 mg/dl.

Increased in acute myocardial infarction. *Decreased* in congenital dysfibrinogenemia and afibrinogenemia, hepatic failure, disseminated intravascular coagulopathy and acute hemorrhage.

Flow Cytometry, Immunophenotyping

5-10 ml EDTA blood. See 6.20.

Allows immune typing of cells in blood, bone marrow and lymph nodes.

Folate

Serum 6.8-39 ng/ml, 3.0-17 nmol/l.

Decreased in folic acid deficiency anemia, vitamin B6 deficiency anemia, malabsorption and pregnancy.

FSH (Follicle Stimulating Hormone)

Serum Male: 1.0-12, Female: Follicular: 3.0-20, Midcycle: 9.0-26, Luteal: 1.0-12 miu/ml. See graph on Menstrual Hormonal Cycle page 41.

Increased in menopausal symptoms, testicular failure, Klinefelter and Turner's syndromes. *Decreased* in anterior pituitary hypofunction and Stein-Leventhal syndrome.

Fragile X Syndrome Gene

5-10 ml EDTA blood. See 5.49.

On X chromosome CGG repeats can account for mental retardation of varying severity. Genetic counselling provides informed advice that aids prevention.

Friedrich's Ataxia Mutation Detection

EDTA blood See 5.54.

For the genetic diagnosis of Friedrich's ataxia.

Fructose

Semen 100-500 mg/dl.

Normal values confirm presence of seminal vesicle fluid in semen of azoospermics. *Low* values are found in post seminal vesicle obstruction.

Fungal Examination (Direct KOH Preparation)

Skin scraping Negative. See 4.10.

Helps in detecting hyphi and spores of fungi at site of infection.

Galactose, Blood, Urine

Blood, Urine <4.3 mg/dl, <240 µmol/l. Not detected in urine and expressed in units as in blood.

Increased in galactosemia. If neglected, infants develop fluid imbalance and failure to thrive. It can also result in cataract, jaundice, hepatomegaly, cirrhosis and mental retardation.

GGT (Gamma Glutamyl Transpeptidase)

Serum <54 u/l.

Increased in congestive heart failure, cholestasis, cirrhosis, hepatic ischemia and necrosis, hepatic tumor, hepatitis and hepatotoxic drugs. Very sensitive to alcohol ingestion.

Gastric Secretion Acidity

Gastric fluid See Table 8.

Gastrin

Serum <90 pg/ml.

Increased in Zollinger-Ellison syndrome, cirrhosis, hyperthyroidism, pernicious anemia and G-cell hyperplasia.

G-6-PD (Glucose-6-Phosphate Dehydrogenase)

Blood 4.6-13.5 units/g Hb.

Decreased or *absent* in *G-6-PD deficiency*. This can cause hemolytic episodes triggered by drugs (e.g. aspirin, sulfonamides, nitrofurantoin, phenacetin, primaquine, quinidine, thiazide diuretics and tolbutamide), infections, severe stress, or certain foods such as fava beans.

Glucose, CSF

CSF 50.0-70.0 mg/dl, 2.78-3.9 mmol/l.

See Table 12.

Glucose, Serum, Fasting
Glucose, Serum, 2h Post Prandial
Glucose, Serum, Random

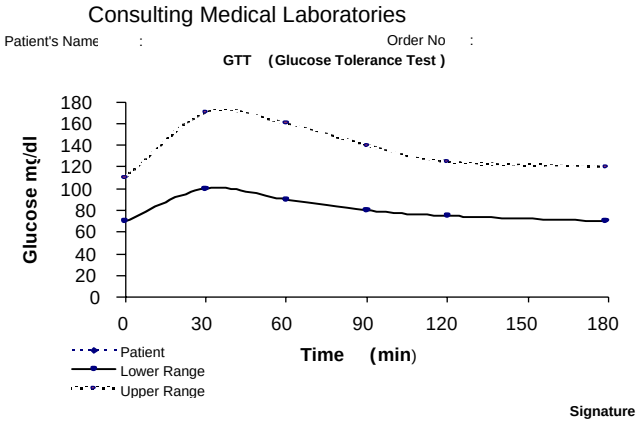
Serum <130 mg/dl, <7.2 mmol/l for post prandial,70-110 mg/dl, 3.9-6.2 mmol/l for fasting, <180 mg/dl, <10.0 mmol/l for random. See 2.42.

Increased in diabetes mellitus, pancreatic cancer, pancreatitis and hyperthyroidism. *Decreased* in insulinoma, hypothyroidism, hypopituitarism, starvation and overdose by hypoglycemic drugs. Glucose measurement is often used to diagnose diabetes or to monitor adequacy of diabetic control.

Glucose Tolerance Test

Serum mg/dl, mmol/l

See also Table 7.



Patient's values plotted and examined in relation to the normal range.

Urine Negative.

Usually glucose appears in urine when values in blood exceed 180 mg/dl (10 mmol/l) or in conditions where renal threshold for glucose is decreased (renal glycosuria).

Gonococcal Antigen Assay

Cervical or urethral swab.

Positive in gonococcal infection and unfit for use as a screening test.

Hb A1C (Glycosylated Hemoglobin)

Blood 4.3-6 %. Good control <7%.

Increased values are directly proportional to degree of diabetic control during preceding 10-12 weeks.

Gram Stain

Fluid or sampling as requested. See 4.69.

Useful in identification of certain bacteria.

GH (Growth Hormone)

Serum Adults: 0.06-5.0 ng/ml or 0.15-13miu/ml, Children: 0.30-5.7 ng/ml or 0.8-15.0 miu/ml.

Increased in acromegaly, gigantism and pituitary tumor. *Decreased* in hypopituitanism disorders and short stature, the latter testing for which may be difficult and requires stimulation tests.

GH Stimulation Test

Serum Rise by >4.0 ng/ml, rise by >10.0 miu/ml.

Failure to respond to growth hormone stimulation may be seen in dwarfism or hypopituitarism.

Haptoglobin

Serum 0.70-3.8 g/l.

Most commonly used to detect hemolytic anemias where values may fall to undetectable levels.

Heavy metals screen

Urine. See page 65.

Helicobacter pylori

Gastric biopsy Negative. Caption 28.

H. pylori can be detected in routine gastric biopsy sections. Associated with gastropathies and gastric neoplasia.

***H. pylori* Antibodies, Total, IgA, IgG, IgM**

Serum Negative, <10 units for IgA, <0.38 units for IgG, <10 units for IgM.

H. pylori is associated with ulcers of the gastric or duodenal mucosa. Total antibodies to *H. pylori* is a screening test. IgM is of little value since it disappears rapidly after 2-3 weeks of infection. IgA is associated with active infection. IgG remains for months even after complete recovery from infection.

***H. pylori* Antigen In Stool**

Stool < 0.190 O.D

> 0.190 indicates presence of *H. pylori*. Positive results strongly correlate with chronic gastritis, ulcer disease of stomach & duodenum. *A simple, non-invasive and inexpensive test.*

Hemoglobin

EDTA Blood g/dl. See Table 3a.

Increased in polycythemia vera, secondary polycythemia, hypoxia related to pulmonary insufficiency and congenital heart disease. *Decreased* in various types of anemia, hemolysis, hemorrhage, lead poisoning, nutritional deficiency of iron, vitamin B12, folate, and vitamin B6,

erythropoietin deficiency secondary to renal disease, malignant disease, hemoglobinopathies and thalassemias.

Hemoglobin, Free, Serum

Serum <6.0 mg/l.

Useful in detecting or monitoring the severity of various kinds of hemolytic anemia. Also *increased* in paroxysmal nocturnal hemoglobinuria.

Hemoglobin, Free, Urine

Urine Not detected. Expressed in mg/l.

Useful in detecting or monitoring severity of various kinds of hemolytic anemia. Also *increased* in paroxysmal noctorial hemoglobinuria.

Hemoglobin Electrophoresis

EDTA Blood Normal pattern. See Table 3.

Hemophilia A, Factor VIII, Gene

EDTA blood Absence of mutations. See 5.15.

Hemophilia A is a relatively common inherited blood coagulation anomaly. It is an X-linked recessive disorder affecting males, 30-40 % of hemophilia A cases are new mutations where no family history exists. Several forms of hemophilia A are usually diagnosed early because of bleeding episodes.

Hemophilia B, Factor IX, Gene

EDTA blood Absence of mutations. See 5.17.

Hemophilia B is the second most common inherited blood coagulation anomaly. Like *hemophilia* A it is an X-linked recessive disorder affecting males. It results from a genetic defect in factor IX-gene.

Hepatitis A Total Ab (Antibodies)

Serum Negative. See Table 11.

Hepatitis A IgM Ab

Serum Negative. See Table 11.

Hepatitis B DNA (by PCR)

Serum Negative. See Table 11.

Hepatitis Bc IgM Ab

Serum Negative. See Table 11.

Hepatitis Bc Total Ab

Serum Negative. See Table 11.

Hepatitis Be Ab

Serum Negative. See Table 11.

Hepatitis Be Ag (Antigen)

Serum Negative. See Table 11.

Hepatitis Bs Ab

Serum <10 iu/ml. See Table 11.

Hepatitis Bs Ag

Serum Negative. See Table 11.

Hepatitis C Ab

Serum Negative. See Table 11.

Hepatitis C RNA (by PCR)
Hepatitis C RNA, Viral Load

Serum for qualitative and *EDTA plasma: in special buffer,* for viral load. Negative or <400 copies/ml.

Used to detect early HCV infection before antibodies appear in serum. Also useful in monitoring viral load in patients under antiviral therapy.

Hepatitis D Ab

Serum Negative. See Table 11.

Hepatitis E Ab

Serum Negative. See Table 11.

Hereditary Hemochromatosis Genetic Testing

EDTA blood Absence of mutations. See 5.21.

Hemochromatosis is a potentially fatal disease found in Chromosome 6, results from excessive deposition of iron in parenchymal cells and leads to damage in liver, heart,

pancreas, endocrine organs, skin and joints. Most common clinical manifestations are hepatomegaly, cirrhosis, cardiomyopathy, diabetes mellitus, hypogonadisim, skin hyperpigmentation & arthropathy.

HLA-B5 (HLA: Human Leukocyte Antigens)

Heparinized whole blood Negative.

HLA-B5 has been associated in some studies with Behcet's disease.

HLA-B27

Heparinized whole blood.

HLA B27 antigen is found in 80-90% of persons with ankylosing spondylitis and Reiter's syndrome. However, it is also present in 5-7% of Caucasians without autoimmune disease.

HLA Typing ABC

Heparinized whole blood. See 7.7.

HLA glycoproteins are present in cell membranes of all nucleated cells, with particularly high concentrations in leukocytes. HLA antigens are the major histocompatibility antigens for tissue recognition. They are especially important in considering any type of tissue transplant, for example, kidney or bone marrow. Many HLA antigens exist, but *some are of special interest because they are more commonly seen in certain autoimmune diseases.*

HLA Typing DR

Heparinized whole blood. See 7.7.

HLA-DR or class II antigens are the major antigen presentation parts of the immune system. They are present on the surface of B lymphocytes, monocytes, various cells of the macrophage lineage and activated T lymphocytes. HLA-DR typing is important in organ transplantation and disease associations.

Homocysteine

Serum 5.0-15.0, in those >60 years: 5.0-20.0 µmol/l.

Elevated total homocysteine is an independent risk factor of cardiovascular disease. *Increased* levels are associated with increased risk of both venous and arterial thrombosis. *Markedly elevated* in infants with homocystinuria (a rare genetic amino acid disorder).

HSV I (Herpes simplex I) Ab IgM & IgG

Serum IgM Titer <10, IgG: Titer <10.

Positive in herpes infection with vesicular lesions at muco-cutaneous junctions of lips/ conjuctivae. IgM Ab indicates a recent infection.

HSV II (Herpes simplex II) Ab IgM & IgG

Serum IgM: <10 units, IgG: <100 units.

Positive in herpes infection which causes genital lesions (worldwide, sex transmitted disease). IgM Ab indicates a recent infection.

B-HCG (Human Chorionic Gonadotropin, Beta Subunit)

Serum miu/ml. See Table 10.

HIV ELISA Screening Test
HIV Western Blot

Serum Negative. See 4.12.

Positive in HIV (Human Immunodeficiency Virus) infection. Positive ELISA test should be repeated, then confirmed by western blot test. Negative results do not rule out a recent infection. Incubation period may reach up to 6 months. *A recent influenza shot can result in false positive HIV ELISA test but not a western blot test.*

HIV Viral Load

EDTA plasma in special buffer <400 Viral RNA copies/ml.

Used to monitor viral level following triple therapy.

Huntington's Disease Genetic Testing

EDTA blood See 5.52.

For genetic diagnosis of Huntington's Disease.

Immunoelectrophoresis, Serum, Urine

Serum or urine See report & Figure.

Detects qualitative abnormalities in immunoglobulins (monoclonal gammopathies). See color caption.

Immunofixation, Serum, Urine

Serum, Urine Normal pattern. See Lab Panels.

The primary use of immunofixation is the identification and monitoring of monoclonal proteins (IgG, IgM, IgA, lambda light chain and kappa light chain) such as are present in multiple myeloma and Waldenstrom's macro-

globulinemia. Immunofixation has also been used to study protein polymorphism (e.g. G-6-PD) and in the genetic typing of alpha-1 antytrypsin.

IgA (Immunoglobulin A)

Serum See Table 5.

Increased in IgA monoclonal gammopathy. *Decreased* in IgA deficiency which causes recurrent chest and gastrointestinal infections.

IgD (Immunoglobulin D)

Serum Male: 9.4-35.8, Female: 9.4-46 mg/l.

Increased in hyper IgD syndrome.

IgE (Immunoglobulin E)

Serum Adult: <120, Children: <12 iu/ml.

Increased in certain allergic diseases and in hyper IgE syndrome.

IgG (Immunoglobulin G)

Serum See Table 5.

Predominant immunoglobulin in serum (75%). *Increased* polyclonally in chronic liver diseases, autoimmune disorders, sarcoidosis and acute infections. *Increased* monoclonally in multiple myeloma and benign monoclonal gammopathies of unknown significance (MGUS). *Decreased* in IgG deficiency which causes recurrent infections. Also in nephrotic syndrome and protein losing enteropathy.

IgM (Immunoglobulin M)

Serum See Table 5.

The earliest immunoglobulin to appear in response to an antigenic stimulus and subsequently falls. *Increased* polyclonally in acute infections, rheumatoid arthritis, chronic liver disease, sarcoidosis, collagen vascular disease and hyper IgM syndrome. *Increased* monoclonally in Waldenstrom's macroglobulinemia, lymphoma, and cold agglutinin disease. *Decreased* in immunodeficiency diseases and causes recurrent pyogenic infections. Also in protein losing enteropathy.

Infectious Mononucleosis Monospot Test

Serum Negative.

Positive in infectious mononucleosis. *This test is not sensitive*. EBV IgM & IgG are more reliable.

CIBD (Inflammatory Bowel Disease) Autoantibody Screen

Serum Titer <10.

Recent studies indicate presence of auto antibodies against various organs (pancreas, intestinal *goblet* cells, granulocytes) in sera of patients (4 out of 5) with chronic inflammatory bowel disease such as Crohns disease and ulcerative colitis.

Insulin

Serum 3.0-35 µiu/ml.

A critical amount of insulin secretory reserve distinguishes between two qualitatively distinct clinical syndromes: true diabetes mellitus and the syndrome of pure

resistance to insulin. *Absent* in severe diabetes mellitus with ketosis and weight loss. In less severe cases insulin is frequently present but only at lower glucose concentration.

Iron

Serum 10.7-28.6 mg/dl, 60-160 mmol/l.

Increased in hemochromatosis, hemolytic anemias, hemosiderosis, thalassemia major & minor, iron & lead poisoning, liver cirrhosis and hepatitis. *Decreased* in iron deficiency anemia, bleeding, malnutrition, malabsorption, and nephrosis.

Iron Binding Capacity, Total

Serum 44-73.4 mg/dl, 250-410 mmol/l.

Increased in iron deficiency anemia of any cause, hemolytic anemia, thalassemia major (Caption 4) & minor, hemochromatosis and chronic active hepatitis. *Decreased* in malignancy, nephrotic syndrome and chronic renal failure.

Kappa & Lambda Light Chains, Serum or Urine

Serum or urine Negative. Caption 25.

See immunofixation, serum or urine

Ketones, Serum
Ketones, Urine-Qualitative

Serum or urine 0.5-3.0 mg/dl or Negative.

Increased in uncontrolled diabetes mellitus, starvation and alcoholic ketoacidosis. *A diet low in carbohydrates can result in increased ketone bodies in serum with spill over into urine.*

Lactate, Plasma

EDTA plasma 5.7-22.0 mg/dl, 0.63-2.44 mmol/l.

Increased in severe oxygen deprivation of tissues, hypoxia, congestive heart failure, hepatic ischemia and pulmonary insufficiency.

Lactate, CSF

CSF 10.8-18.9 mg/dl, 1.2-2.1 mmol/l.

Increased in bacterial meningitis and convulsions.

LDH (Lactate Dehydrogenase) Body Fluids

Serum Transudate: <200 u/l, exudate: >200 u/l.

See 2.10.

LDH Serum

Serum 240-460 u/l.

Increased in hemolytic anemia, liver disease, myocardial infarction, pancreatitis, pulmonary & intestinal infarctions, neoplasia and cerebrovascular accident.

LDH Isoenzyme Serum

Serum LDH-1 17-27%, LDH-2 27-37%, LDH-3 18-25%, LDH-4 18-25%, LDH-5 0-5%. *Do not freeze sample.*

Because LDH is found in many body tissues, total LDH is not a good indicator of heart disease. After a heart attack, the concentration of LDH-1 is generally higher than that of LDH-2 (this is called a "flipped" LDH pattern). *The serum LDH level rises within 24 to 72 hours after a heart attack, peaks at 3 to 4 days and returns to normal in about 14 days.*

Lactose Tolerance Test

Serum Expressed in mg/dl or mmol/l. See 2.55.

Deficiency of small bowel mucosal lactase is associated with intolerance to lactose manifested by diarrhea and other symptoms following ingestion of milk. Lactose tolerance test can evaluate this condition, where in lactase deficiency, lactose tolerance curve appears flat and does not exceed 20mg/dl over the fasting level.

Lead, Blood

Heparinized whole blood <30 µg/dl, <1.45 µmol/l.

Increased in lead poisoning. Lead may be found in paints, batteries, solders and toys. Workers exposed to lead and lead compounds are at risk of lead poisoning.

Lead, Urine

24-hour urine <40 µg/24h, <1.95 µmol/24h.

Legionella pneumophilia Ab IgM & IgG
Legionella pneumophilia Ag

Serum for antibody, *Sputum* for Ag (antigen) Titer<128 for antibody, Negative for Ag. See Microbiology 4.16.

Legionella pneumophilia is the causative agent for legionellosis or legionnaires' disease. At least 18 species are associated with pneumonia, accounting for approximately 1-5% of all cases of pneumonia. Antigen can be detected during the acute stage of the disease from sputum or body fluids. Antibodies can be detected in serum. IgM indicates a recent infection.

Leiden or Factor V

EDTA blood. See 5.14.

A test for those at higher risk for thromboembolism.

Leishmania Smear

Pus from lesion Negative. See 4.80 and Caption 29.

Positive skin lesion in cutaneous leishmaniasis.

Positive bone marrow in visceral leishmaniasis, a disease of the reticulo-endothelial system caused by kinetoplastid protozoa of the genus *Leishmania.*

LAP (Leukocyte Alkaline Phosphatase) Score

Heparinized whole blood Score 20-180.

Increased in leukemoid reaction, infections, multiple myeloma and Hodgkin's disease. *Decreased* in myloid leukemia.

LE Cells (Lupus Erythematosus Cells)

Whole blood Negative. Caption 23.

Positive in lupus erythematosus. The test is specific but not sensitive. It has been replaced by ANA and anti-DNA.

Leukocyte Count & Differential Count

EDTA blood. See Tables 1a & 1b.

Increased or *decreased* components in various conditions.

Leukocyte Surface Markers By Flow Cytometry Or Immuno-Phenotyping

EDTA blood. See 6.20.

Cells have surface antigens that can be detected by specific antibodies. This can be used in fluid or solid phase to immunophenotype various cell types to define their lineage in various disorders, e.g. malignant disease.

LH (Leutenizing Hormone)

Serum Male: 0.8-6.1, Female: Follicular: 1.6-7.9, Midcycle: 13.2-82.7, Luteal: 0.70-9.90 miu/ml. See graph on Menstrual Hormonal Cycle page 41.

LH is secreted by the anterior pituitary gland. In women, LH surge at mid-cycle causes ovulation. In men, LH stimulates production of testosterone in testes. *Increased* in anorchia, hypogonadism, Klinefelter's syndrome, menopause, ovarian failure, polycystic ovary disease, precocious puberty, Turner's syndrome. *Decreased* in hypopituitarism.

Lipase

Serum <190 u/l.

Increased after damage to pancreatic acinar cells such as in pancreatitis, pancreatic cancer, and cholecystitis (secondary effect on the pancreas).

Lipoprotein Electrophoresis

Serum α: 28-40%, pre β: 12-21%, β: 45-56%, chylomicrons: 0-2%.

Limitations of this method and the realization that it is unnecessary for diagnosing most dyslipoproteinemias have considerably limited the use of lipoprotein electrophoresis in routine clinical practice.

Lithium

Serum 0.6-1.2 mmol/l.

An antidepressant agent, whose level is measured for drug monitoring.

Lupus Anticoagulant

Citrated plasma Negative.

Lupus anticoagulant is an immunoglobulin which interferes with blood coagulation and has antithromboplastin activity. *Positive* in 25% of SLE patients. It is also associated with an increased risk of thrombosis and may be related to recurrent spontaneous abortion.

Lyme Disease Test

Serum. ELISA most commonly used. See 4.17.

Lyme disease is caused by *Borrelia burgdorferi.* Culture and PCR are available as a research tool.

Lymphocytotoxicity Test

Donor's heparinized blood + recipient's serum Negative.

Lymphocytotoxic Abs (antibodies) may be present in sera of patients being prepared for renal transplants and such Abs cause acute rejection. Screening for these Abs is essential.

Lymphocyte T-Cell

EDTA blood Constitute >60% of blood lymphocytes.

Magnesium, Serum
Magnesium, Urine

Serum, 24-hour urine 1.8-2.5 mg/dl, 0.80-1.0 mmol/l for serum and 75-150 mg/dl, 30-60 mmol/l for urine.

Magnesium is necessary for biochemical processes that involve transfer of phosphate groups. *Increased* in chronic renal failure, Addison's disease, diabetic acidosis and dehydration. *Decreased* in chronic diarrhea, excessive administration of insulin, hemodialysis, hepatic cirrhosis, pancreatitis, hyperaldosteronism, toxemia of pregnancy and ulcerative colitis.

Malaria Smear

EDTA blood Negative. See Table 4, 4.81 and Caption 30.

Malaria is caused by blood-borne pathogens of the genus *Plasmodium*. Four species can infect humans: *P. vivax, P ovale, P malariae and P falciparum*.

Metanephrines

24-hour urine <1.0 mg/24h, <5.5 µmol/24h.

Increased in pheochromocytoma, manic depressive disorder, anxiety and stress. Avoid intake of walnuts, bananas, chocolates, vanilla- containing foods, citrus fruits and certain medications 3 days before and during collection.

Microalbuminuria

24-hour urine <20 mg/24h. See 2.109.

Microalbuminuria assessment is of special value in detecting early diabetic nephropathy and enables reversal of progression of renal damage by adequate control of carbohydrate metabolism.

Mitochondrial Encephalopathy MELAS tRNA leu UUR Gene

EDTA blood See 5.46.

For detection of MELAS mutations which is an inhereted metabolic disorder belonging to the mitochondrial cytopathy group.

MODY, Maturity Onset Diabetes Mellitus in the Young I, II, III

2-5 ml EDTA blood. See 5.28.

MODY I anomaly is located on chromosome 20, MODY II on chromosome 7 and MODY III on chromosome 12.

Monospot

Serum Negative.

Positive in infectious mononucleosis. The test is not sensitive. EBV IgG & IgM are more reliable.

MTHFR (Methylene Tetrahydrofolate Reductase) Homocysteine Gene

EDTA blood Absence of mutations. See 5.13.

MTHFR gene mutations are associated with increased risk for thrombo-embolism and hypercysteinemia. It is inherited as autosomal recessive disorder on Chromosome 1. The trait predisposes to venous thrombosis; homozygous forms carry 2-3x increased risk.

Mucopolysaccharides (Screening - Qualitative Test)

Urine, Spot urine Negative. See Table 18.

MPSS (mucopolysaccharidoses) are disorders resulting from defects in the stepwise degradation of MPSS. The MPSS are characterized by chronic progressive course, organomegaly, dysostosis multiplex and coarsening of facial features. Profound mental retardation is seen in certain types.

MEN I&II Genetic Tests

2-5 ml EDTA blood. See 5.38.

Screening families for MEN I or II genes are useful in early detection and treatment.

Mycobacterium tuberculosis (BY PCR)

Various specimens, Body fluids and tissues Negative.

PCR is a rapid, sensitive and specific method for detecting *Mycobacterium tuberculosis* in various specimens. Errors due to sampling problems and contamination in the laboratory should be handled with care.

Mycoplasma pneumoniae Ab IgM IgG

Serum <10 units for both IgM & IgG. See 4.18.

Mycoplasma pneumoniae is the main cause of atypical pneumonia. *Increased* IgM indicates a recent infection.

Myoglobin, Serum, Urine

Serum, Urine 0 – 85 ng/ml for serum, Negative for urine.

Myoglobin levels may confirm suspected muscle damage including heart muscle damage. *Increased* in muscular dystrophy, myocardial infarction, rhabdomyolysis, myositis, skeletal muscle trauma and skeletal muscle ischemia.

Myoclonal Epilepsy MERRF TRNA LYS Gene

EDTA blood and may require muscle biopsy for diagnosis. See 5.47.

MERRF (myoclonal epilepsy with ragged red fibers) is an inherited defect in mitochondrial biosynthesis; in 85% of cases mutation is in T-RNA genes. Present with rhythmic myoclonal attacks, ataxia and muscular weakness.

Myotonic Muscular Dystrophy DMK Gene

EDTA blood See 5.48.

The most commonly inherited neuromuscular disease. Autosomal dominant on Chromosome 19. Incidence 1/25000-1/8000. Has a protean clinical picture.

Mycoplasma pneumoniae BY PCR

Nasopharyngeal aspirations or bronchio-alveolar lavage (BAL) Negative.

Mycoplasma pneumoniae is a common cause of a wide range of upper and lower respiratory tract infections, especially in children and in young adults.

NBT (Nitroblue Tetrazolium) Test

EDTA blood Up to 20% if stimulated.

Stimulated NBT test is valuable for detecting intrinsic defects in neutrophil function. Poor or no formation of deposits in neutrophils can be seen in neutrophils with metabolic defect (e.g. chronic granulomatous disease). It is sometimes used to differentiate bacterial from viral infection, whereby NBT test is positive in the former.

Occult Blood, Feces

Stool Negative.

Blood in stools may come from any part of the alimentary tract. Black stools suggest upper GI bleeding, e.g. peptic ulcer or gastritis. Maroon-colored stools suggest middle GI bleeding, e.g. intestinal infection, inflammatory bowel disease, vascular malformation or tumor. Bright red stools suggest lower GI bleed, e.g. hemorrhoids, anal fissure, colorectal polyp or cancer, diverticular disease or inflammatory bowel disease.

Occult Blood, Gastric

Gastric fluid Negative.

Positive in bleeding ulcers of stomach, duodenum or esophagus, erosive esophagitis/ gastritis, esophageal/ gastric tumors, ingested blood.

Occult Blood, Urine (Hemoglobinuria)

Urine Negative.

Erythrocytes in low S.G. urine get hemolyzed, causing hemoglobinuria. *Other causes of hemoglobinuria*: trauma to

red cells (e.g. prosthetic cardiac valves, extensive burns, strenuous exercise, severe trauma to muscle and other vascular tissues), organisms (e.g. *Malaria, Bartonella*, etc.), G-6PD deficiency, unstable Hb diseases, oxidative hemolysis due to drugs and immune mediated hemoglobinuria.

Osmolality, Serum
Osmolality, Urine

Serum, Urine 275-300 mosmol/kg in serum, 500-1200 mosmol/kg in urine.

Osmolality of serum and urine allows calculating the ratio of urine to serum osmolality. This ratio expresses the degree of concentration of glomerular filtrate by the kidney, which, in respect to osmolality, is very close to serum. The ratio is greatly affected by volume of fluid intake. More meaningful data are obtained if fluid intake is restricted. This ratio *falls* in diabetes insipidus, tubular dysfunction and in acute renal failure. See also Lab Panels.

Osmolality Ratio (Urine/ Serum)

Serum & urine Average fluid intake: 1.0-3.0, fluid restriction: 3.0-4.7. See Acute Renal Failure Panel p. 11.

Osmotic Fragility

Heparinized blood 0.42-0.48% NaCl concentration.

This test is performed mainly to detect hereditary spherocytosis and thalassemia. Red cells are more fragile in spherocytosis but more resistant to hemolysis in thalassemia.

Osteocalcin

Serum 1.8-6.6 ng/ml.

An indicator of osteoblastic activity or metabolic turnover of bone. *Increased* in primary hyperparathyroidism, Paget's disease, chronic renal failure and some cases of osteoporesis. *Decreased* in pregnancy and in glucocorticoid treatment.

Osteoporosis Test

Urine Male: 2.3-5.4, Female: 3.0-7.4 nm DPD/ mm CR.

Osteoporosis is the commonest type of metabolic bone disease. Calcium and phosphate present in bones are reabsorbed back into the blood. This results in brittle, fragile bones that are subject to fractures. Causes are multiple and include hormone deficiencies (estrogen in women and androgen in men), corticosteroid excess (Cushing's syndrome), hyperthyroidism, hyperparathyroidism, immobilization, bone malignancies, certain genetic disorders and other causes such as low calcium diet. *Osteoporosis is more frequent in women and those over 60 years old. Premature menopause is a risk factor.*

Osteoporosis, Vitamin D Receptor Gene

EDTA blood See 5.24.

For the detection of vitamin D receptor gene mutations responsible for defective bone formation.

OVU Test

Urine.

Detects day of ovulation (LH surge). Has been replaced by LH level.

Oxalate

24-hour urine Male: 7-44, Female: 4-31 mg/24h; Male: 0.08-0.49, Female: 0.04-0.32 mmol/24h. See 2.110 Figure.

Sparingly soluble, calcium oxalate in the urine is considered a major factor in urolithiasis. *Increased* excretion of oxalate is due to excess ingestion of oxalate-rich foods, formation due to metabolic defects such as primary hyperoxaluria and excessive oxalate absorption in gastro- intestinal disorders, e.g. Inflammatory bowel disease, ileal resection, biliary diversion, pancreatic insufficiency and celiac disease.

PAPP-A (Pregnancy-associated plasma protein)

Serum Risk value (with Free β HCG)

PAPP-A is a placenta derived glycoprotein produced in high concentration by trophoblast during pregnancy, reaching peak levels in maternal blood during the latter part of pregnancy. PAPP-A is reported to be reduced in cases with fetal chromosomal abnormalities and, in conjunction with free beta HCG, can be used to screen dor Down syndrome during 8-14 week, optimally at around 10 weeks gestation.

Note: Hyperlipemia and hyperbilirubinemia bear no effect on precision of the assay, while hemolysis may exert a slight elevation of measured value.

PTH (Parathyroid Hormone)

Serum 13.0-55.0 pg/ml, 1.3-7.6 pmol/l.

PTH is secreted by the parathyroid glands which regulate calcium and phosphate metabolism. Low serum calcium induces PTH secretion whereas increased serum calcium inhibits PTH release. *Increased* in chronic renal failure, hyperparathyroidism, rickets, vitamin D deficiency, osteomalacia and malabsorption syndrome. *Decreased* in hypoparathyroidism, hypomagnesemia, sarcoidosis, autoimmune destruction of parathyroid gland, metastatic bone tumor and vitamin D intoxication.

PTT (Partial Thromboplastin Time) Activated

Citrated plasma Normal: 30-40, therapeutic: 60-70. Therapy ratio: 1.5-2.5 seconds. See 2.33.

PTT is used to assess intrinsic clotting system and the common pathway of clot formation. It evaluates Factors I (fibrinogen), II (prothrombin), V, VIII, IX, XI and XIII. When one of these factors is deficient, the PTT is *prolonged*. Also PTT is prolonged in cirrhosis, D.I.C., malabsorption, vitamin K deficiency, von Willebrand's disease & in patients receiving anticoagulant therapy.

Philadelphia Chromosome T(9;22) CML BCR/ABL Oncogene BY PCR

EDTA blood, bone marrow Absence of mutations. See 2.79 & 83.

CML involves proliferation of pluripotent stem cells, characterized by a cytogenetic Philadelphia chromosome. Hybrid m-RNA is the product of this translocation. By PCR, one in a million copies can give a positive result of this translocation.

Phenobarbital (Luminal, Mysolin)

Serum 15-40 µg/ml, 65-170 µmol/l.

Barbiturates are CNS depressants. Used as anticonvulsants to decrease anxiety or tension before surgery and to treat insomnia on short term basis.

Phenylalanine, Serum

Serum Negative. See 2.55.

Phenylalanine accumulates in the body as a result of phenylalanine hydroxylase deficiency – phenylketonuria; a genetic defect (incidence 1/10000). Injury to brain starts within $2^{nd}/3^{rd}$ week of life, and progresses. Damage is checked if placed on a low-phenylalanine diet *soon after birth*.

Phenylalanine, Urine

Urine Negative.

Phenytoin (Epanutin)

Serum 10-20 µg/ml, 40-79 µg/ml, µmol/l.

Phenytoin is an anticonvulsant which act on the CNS to decrease the frequency and severity of seizures.

Phosphate, Serum

Serum Adults: 2.5-5.0, children: 4.0-7.0 mg/dl; Adults: 0.8-1.6, children: 1.3-2.3 mmol/l.

Phosphate levels are controlled by PTH (1,25-dihydroxy vitamin D) and to a lesser extent by calcitonin. *Increased* in hypoparatyhroidism, hypocalcemia, renal failure, liver disease, bone metastasis and sarcoidosis. *De-*

creased in hyper-parathyroidism, hypercalcemia, diabetic ketosis, rickets and osteomalasia.

Phosphate, Urine

24-hour urine 0.4-1.3 g/24h, 13-42 mmol/24h.

Increased in hyperparathyroidism, vitamin D deficiency rickets, cystinosis and proximal renal tubular acidosis. *Decreased* in hypoparathyroidism and osteomalacia.

Platelet Antibodies

Serum Negative. See 7.8.

Bind to platelets and may result in increased platelet destruction. These antibodies may be associated with idiopathic or thrombotic thrombocytopenic purpura. Antibodies may be either IgM or IgG in type.

Platelet Count

EDTA blood 150-500×10^9/l.

Increased (thrombocytosis) in polycythemia vera, post-splenectomy syndrome, primary thrombocytosis, certain malignances and myeloprolifer active disorders. *Decreased (thrombocytopenia)* in ITP, TTP, leukemia, D.I.C., chemotherapy, hemolytic anemia, hypersplenism, prosthetic heart valve and sequelae of massive blood transfusion.

Pneumocystis carinii by PCR

Sputum, Lung tissue, BAL (bronchio-alveolar lavage), TBB (transbronchial biopsy) or lung biopsy Negative.

PCP (*Pneumocystis carinii* pneumonia) is the most common opportunistic infection among HIV infected indi-

viduals. AIDS patients not receiving anti PCP prophylaxis will ultimately develop PCP. Immune compromized patients are also at risk.

PBG (Porphobilinogen) Qualitative
PBG (Porphobilinogen) Quantitative

Urine, 24-hour urine Negative for spot, <2 mg/24h urine. See Table 14.

Increased levels of PBG may indicate porphyria (several types), hepatitis, hepatic carcinoma and lead poisoning. Several drugs can affect the results including aminosalicylic acid, barbiturates, chloral hydrate, ethyl alcohol, oral contraceptives, procaine and sulphonamides.

Porphyrins Total, Urine Qualitative
Porphyrins Total, Urine Quantitative

Urine, 24-hour urine Negative for spot, <300 mg/24h urine. See Table 14.

Increased levels of PBG may indicate porphyria (several types), hepatitis, hepatic carcinoma and lead poisoning. Several drugs can affect the results including aminosalicylic acid, barbiturates, chloral hydrate, ethyl alcohol, oral contraceptives, procaine and sulphonamides.

Porphyrins Copro, Urine
Porphyrins Uro, Urine

24-hour urine <200 µg/24h for copro-, <40 µg/24h for uroporphyrins. See Table 14.

If urinary total porphyrins are abnormally elevated, it is advisable to determine uro- or copro-porphyrins, or both.

Coproporphyrins are *increased* in porphyria (several types), hepatitis, hepatic carcinoma and lead poisoning.

Potassium, Serum
Potassium, Urine

Serum, 24-hour urine 3.5-5.5 mmol/l; 25-120 mmol/24h.

K (potassium) is the major cation within cells, concerned with maintenance of electric charge on the cell membrane, necessary for neuromuscular communication and nutrients' transportation. Small changes in serum K level can substantially affect neuro-muscular activity, especially cardiac muscle. *Increased* in renal failure, metabolic or respiratory acidosis, hyperkalemic periodic paralysis, crush injury and hemolysis. *Decreased* by diuretics, GI tumors, vomiting, diarrhea and hypokalemic periodic paralysis. *Fruit juices have high potassium content.*

Pregnancy Test

Urine or blood Negative for males and non-pregnant women, Positive in pregnant women. See Table 10.

Progesterone

Serum Males: 0.9-2.9, Females: follicular: 0.8-2.9, preovulatory:1.4-14.8, luteal: 10.5-55.4 nmol/l. See graph on Menstrual Hormonal Cycle page 41.

Progesterone is a steroid hormone synthesized in, and released from, the corpus luteum, placenta and adrenal gland. In women, progesterone prepares the uterus for pregnancy and breast lobules for lactation. After ovulation, progesterone blocks estrogen-induced proliferation of endometrium and stimulates glandular secretion. Serum pro-

gesterone levels start to *rise* with LH surge midway through the menstrual cycle, continue to *rise* for about 6-10 days, then *fall*. Useful in the evaluation of ovulation; a *low* progesterone level in luteal phase suggests anovulation.

Prolactin

Serum Male: 2.5-17, Female: 3.0-20 pg/ml; Male: 110-748, Female: 132-880 pmol/l.

Prolactin is secreted by the anterior pituitary gland. Prolactin stimulates production of milk in females. *Hyperprolactinemia* is seen in pituitary tumor, pregnancy & lactation; also with excitation, breast stimulation and hypothyroidism. *Hyperprolactinemia* may cause impotence in males and benign mastopathy in females.

Prostatic fluid, direct smear, gram stain & culture

Prostatic fluid Negative.

Examination of prostatic secretion is useful to detect parasitic or bacterial infections. Direct smear can detect *Trichomonas vaginalis*, gram stain to detect gram-negative diplococci, culture to detect any specific bacterial infection. Negative ordinary cultures do not rule out nonspecific infection, e.g. C*hlamydia* and/ or *Mycoplasma*.

PSA (Prostatic Specific Antigen) Total
PSA Free
PSA Ratio

Serum Total PSA <4.0 ng/ml; PSA Ratio (Free:Total) >30%: benign, >20%: suggestive of benign prostatic hypertrophy, 10-19%: borderline, <10%: suggestive of malignancy. See 2.67.

PSA is a glycoprotein in the cytoplasm of prostatic epithelial cells. PSA level is greatly *increased* in men with prostatic cancer, but can also be increased in benign prostatic hypertrophy (BPH) and prostatitis. *Free PSA/ total PSA ratio* is useful in differentiating BPH from malignancy.

Protein C

Citrated plasma 1.8-3.2 mg/l, (70%-140% mg/l Inhibition).

Protein C and protein S are involved in the regulation of coagulation. They inactivate factors V and VIII. Deficiency may lead to thrombosis in arteries or veins. *Decreased* by vitamin K antidotes & oral anticoagulants. Protein C or S levels may be difficult to interpret in patients on oral anticoagulants.

Protein S

Citrated plasma 13-21 mg/l, (66%-112% mg/l Inhibition).

See above.

Protein Electrophoresis, CSF (Oligoclonal Bands)

CSF <2 bands.

Oligoclonal bands are defined as two or more discrete bands in the gamma region of CSF that are absent in the concurrently run patient's serum. *Positive* in 83-94% of patients with definite multiple sclerosis. Also seen in nearly all patients with subacute sclerosing panencephalitis and in 25-50% of patients with various viral CNS infections, neurosyphilis, neuroborreliosis, cryptococcal meningitis, Guillain-Barre syndrome, transverse myelitis, meningeal carcinomatosis & few other diseases.

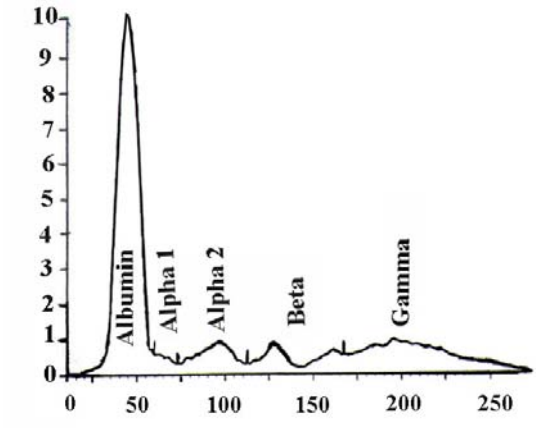
Protein Total, Serum

Serum 65-85 g/l.

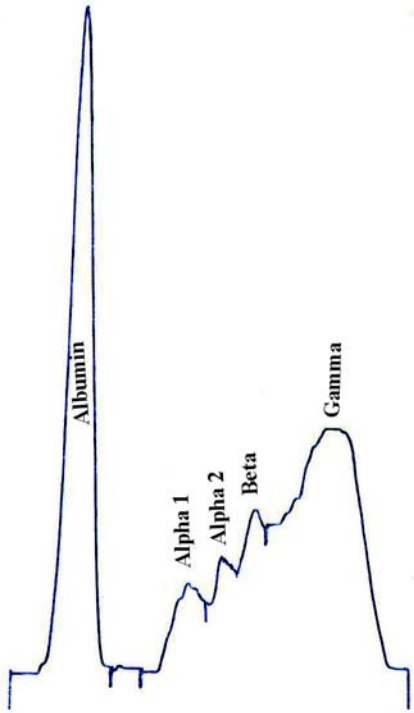
Serum proteins are separated into albumin (see albumin) and globulins. Globulins are roughly divided into α-1, α-2, β, and γ globulins. These can be separated and quantitated by electrophoresis. The α-1 fraction includes α-1 antitrypsin and thyroxine binding globulin. The α-2 fraction contains haptoglobin, ceruloplasmin, HDL and α-2 macroglobulin. α-1 and α-2 globulins are *increased* in acute phase reaction. The β fraction includes transferrin, plasminogen and β-lipoproteins. The γ fraction includes the various types of immunoglobulins.

Protein Electrophoresis, Serum

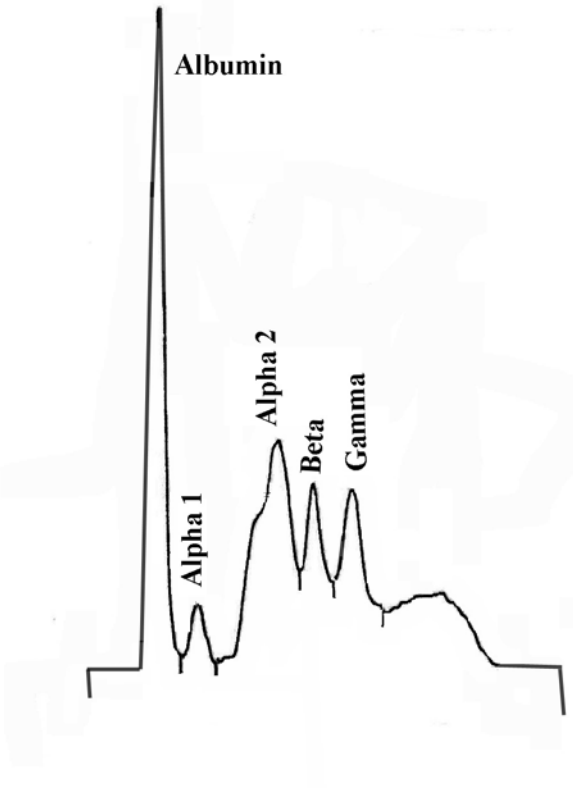
Serum Albumin: 35-55, α-1 globulin: 1-3, α -2 globulin: 6-10, β-globulin: 7-12, γ-globulin: 7-16 g/l. See graphs.



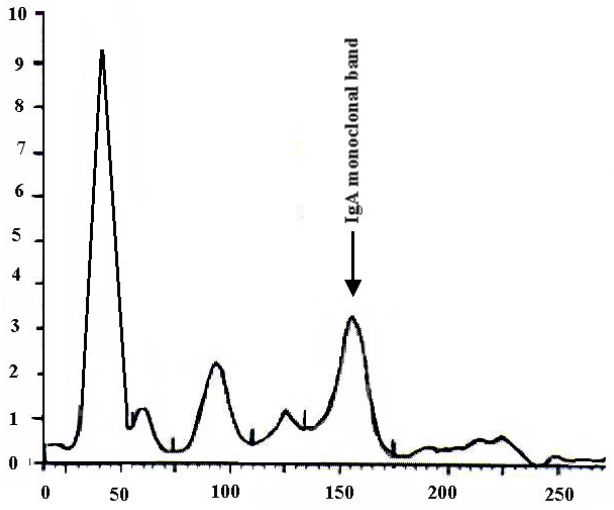
Protein EP(1) Normal pattern



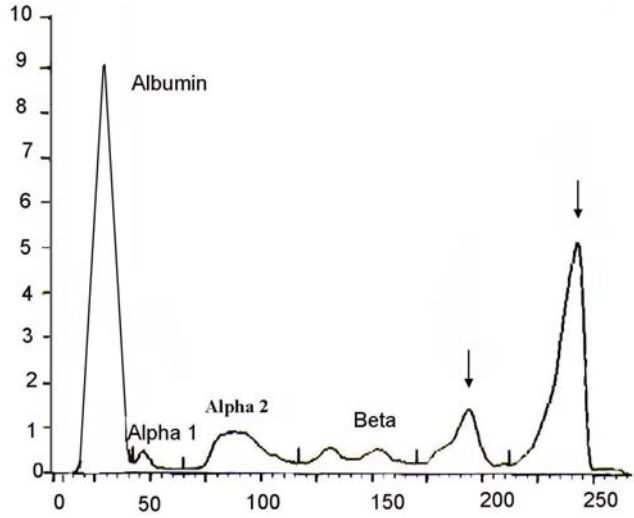
Protein EP(2) Liver cirrhosis pattern



Protein EP(3) Nephrotic syndrome pattern



Protein EP(4) Multiple myeloma with 1 monoclonal band



Protein EP (5) Multiple myeloma with 2 monoclonal bands (arrows).

Protein Total, 24-hour Urine

24-hour urine <0.15 g/l.

Increased amount of urine protein may be found in renal failure, decreased kidney function, nephrotic syndrome, multiple myeloma, acute inflammation, diabetic nephropathy and urinary tract infection.

Protein Electrophoresis, Urine

Morning urine sample.

Increased in conditions as for total protin in urine. Urinary protein electrophoresis is useful for follow up purposes.

Prothrombin Time

Citrated plasma 11-15 seconds, anticoagulated patients: INR 2-4. See 2.33.

Apart from anticoagulant therapy prothrombin time is *prolonged* in the following conditions: liver disease, D.I.C., congenital factor II, V, VII, & X deficiencies, vitamin K deficiency and malabsorption.

Prothrombin or Factor II BY PCR

EDTA blood Absence of mutations. See 2.72 and 5.13.

Factor II mutation is autosomal recessive, detected on Chromosome 11; a risk factor for thrombo-embolism.

Pyruvate

Citrated plasma 0.3-0.7 mg/dl, 0.03-0.08 mmol/l.

Increased levels associated with neuromuscular disorders, hepatic cirrhosis, diabetes mellitus, vitamin deficiencies, uremia and congestive heart failure.

Reducing Substances, Stools

Stools Negative.

Fecal reducing substances may be positive in infants with persistent diarrhea due to disaccharidase deficiency.

Reducing Substances, Urine

Urine Negative. See 2.55.

Apart from glucose, the most important reducing substance which may be present in urine is galactose. Galactose appears in the urine of infants with galactosemia.

Renin: PRA (Plasma Renin Activity)

EDTA plasma Erect: 1.31-3.95, supine: 0.15-2.33 ng/ml per hour.

Renin is an enzyme released by specialized renal cells into blood, in response to sodium depletion and/ or low blood volume. PRA is measured for diagnosing and treating hypertension. In primary hyperaldosteronism PRA is decreased. In secondary hyper- aldosteronism caused by renal disease, PRA is increased.

Reticulocyte Count

EDTA blood Newborn: 3-7, others: 0.5-2.0% of red blood cells.

Increased in all types of hemolytic anemia, some leukemias, after bleeding and post splenectomy. *Decreased* in

bone marrow failure, liver cirrhosis, iron/ vitamin B12/ folate deficiency, radiation therapy and kidney disease with decreased erythropoietin production.

Rh (Rhesus) Antibodies, *or* Indirect Coombs Test

Serum Negative. Expressed as titer. See 7.6.

Maternal antibodies produced against Rh factors account for the majority of severe cases of erythroblastosis fetalis. Statistically, a Rh negative woman has a 1 in 11 chance of developing antibodies during her 2nd pregnancy with a Rh positive fetus.

RF (Rheumatoid Factor), *or* RA Latex
RF (Rheumatoid Factor) IgM

Serum <20 iu/ml for RF, <20 relative units (RU) per ml for RF IgM. See 2.36.

RF is found in patients with rheumatoid arthritis. It is not specific for this disease and may be found in other diseases including SLE, polyarteritis, tuberculosis, leprosy, syphilis, bacterial endocarditis, Waldenstrom's macroglobulinemia, ankylosing spondylitis, juvenile rheumatoid arthritis and psoriatic arthritis. It is occasionally found in apparently normal persons. RF IgM is useful in diagnosing a recent rheumatic attack.

Rocky Mountain Spotted Fever

Serum or *Skin biopsy*. See 4.18.

Caused by *Rickettsia rickettsii* and diagnosed by latex indirect hemagglutination and indirect IF methods. Frozen section of skin biopsy is diagnosed by direct IF method.

Rotavirus, Stools

Stools Negative. See 4.19.

Positive in rotavirus infection causing diarrhea, especially in children.

RPR (Rapid Plasma Reagin) VDRL

Serum Negative. See Serology section.

VDRL is a nonspecific screening test for the detection of syphilis. If positive the disease should be confirmed by TPHA and FTA test. Positive results can also be seen in some autoimmune diseases.

RSV (Respiratory Syncytial Virus)

Serum for ELISA diagnosis. See 4.19.

Also diagnosis may be made on infected cells or fluid by antigen testing a culture on HEp-2 cells.

Rubella Antibodies IgM & IgG

Serum IgM: negative, IgG: <10 units. See 4.20.

Rubella infection in pregnant women, especially during 1st trimester, can injure the fetus, causing cataract, deafness, glaucoma, congenital heart disease, mental retardation and even fetal death. IgM indicates recent infection while IgG indicates prior infection or immunity.

Salicylate

Serum Therapeutic: <20, Toxic: >30, Lethal:>60 mg/dl; Therapeutic: <1.5, Toxic: >2.2, Lethal: >4.3 mmol/l.

Estimates circulating levels of the drug, indicating if toxic levels have been reached.

Sandhoff's Disease

EDTA blood Negative. See 5.34.

For genetic diagnosis of Sandhoff's disease, carrier status and prenatal diagnosis.

SARS

Serum for antibody tests. See 4.21. *Serum, stools, nasal secretions* for RT-PCR.

Schistosomal Antibodies Total

Serum Titer <40. See 4.83.

Presence of these antibodies indicates exposure to schistosomal organism (*mansoni, haematobium, intercalatum*).

Semen Analysis

Semen See Seminal analysis form.

A test of fertility potential in males.

SGOT or AST (Aspartate Aminotransferase)

Serum <38 u/l.

Increased in liver disease, acute myocardial infarction, acute myocarditis, cardiomyopathies, congestive heart failure, hemolytic anemia, megaloblastic anemia, skeletal muscle disease, malignancy, infectious mononucleosis, acute pulmonary infarction and any tissue necrosis.

SGPT or ALT (Alanine Aminotransferase)

Serum <35 u/l.

Increased in liver disease, congestive heart failure, infectious mononucleosis, acute myocardial infarction, renal infarcts, skeletal muscle disease, acute pancreatitis drug toxicity and heparin therapy.

Skeletal Muscle Antibodies

Serum Titer <10.

Autoantibodies against skeletal muscle are present in various auto immune diseases and can cause muscular weakness.

Sickle Cell Screen

EDTA blood Negative.

Positive in sickle cell anemia and sickle cell trait.

Sodium (Na), Serum
Na, Spot urine
Na, 24-hour urine

Serum, Spot urine, 24-hour urine 133-149mmol/l for serum. Varies with diet and expressed in mmol/l for spot urine, 40-220 mmol/24h.

Na is the major anion in extracellular fluid and the major determinant of extracellular osmolality. Na content of blood is resultant upon a balance between dietary intake and renal excretion. Factors affecting Na levels include: *aldosterone* which decreases Na urine loss, *ANP* (atrial natriuretic protein hormone) secreted by the heart increases Na loss, and *water* (controlled by ADH). *Increased* serum Na values may indicate dehydration, diabetes insipidus or extensive thermal burns. *Decreased* serum Na values may indicate ascites, congestive heart failure, diarrhea, excessive

sweating, ketoacidosis, kidney disease, osmotic dilution, peripheral edema, pleural effusion and vomiting.

Spinal Muscular Atrophy Genetic Testing

EDTA blood See 5.53.

Genetic diagnosis of spinal atrophy by mutation analysis.

Spino Cerebellar Ataxia (1,2,3,6,7,8,12,17) Mutation Analysis

EDTA blood See 5.51.

Detection of mutations for spinocerebellar ataxia types.

Stool Analysis

Stools Normal.

Stools examination may detect a parasitic infection including amebiasis. A fresh sample is required to detect ameba trophozoites. Also may detect other abnormalities including blood, pus, fat, reducing substances, mucus etc.

Streptococcal Antibodies

Serum. See page 17 and 4.24.

ASO, Anti-DNAase-B tests.

Synovial Fluid Analysis

Synovial fluid Normal. See Lab Panels.

Tests are performed to identify the cause of swelling in joints and to diagnose certain types of arthritides and in-

flammatory joint disease. Abnormal results may indicate rheumatoid arthritis, septic arthritis, acute or chronic gouty arthritis and inflammatory diseases. Investigation may include physical, chemical, microbiological, serological or cytologic tests.

Syphilis Tests

Serum for RPR, VDRL, TPMA and FTA. See page 96 and 4.26.

Testosterone Free
Testosterone Total

Serum Male: 43-139, >50years: 37-85, Female: up to 10, Children: 0.5-2.5 pmol/l for free testosterone; Males: 8.7-36.5, Females: up to 3.33, Children: up to 1.0 nmol/l for total testosterone.

Testosterone is a steroid secreted by Leydig cells of testes in males and from the adrenal cortex and ovaries in females. Testosterone is necessary for development and maintenance of secondary sex characteristics in males and is stimulated by the pituitary hormone LH. *Increased* in testicular cancer in males and in polycystic ovary disease, virilism and hirsutism in females. *Decreased* in Klinefelter's syndrome, testicular failure and prolactinoma. Free testosterone is the active portion of this hormone.

Theophyline

Serum 10-20 µg/ml, 56-110 µmol/l.

Theophylline is a drug used to treat asthma and bronchitis. It acts by dilating the bronchi. Dose individual.

Thyroid Hormone Receptor β-Gene

2-5 ml EDTA blood. See 5.27

TR β gene is tested for, while TR α gene is lethal in utero. The mutation is sited on chromosome 2. Resistance of target organs leads to elevated T3 and T4 levels without TSH suppression.

Thyroglobulin

Serum 1.7 - 56 ng/ml.

Thyroglobulin is a glycoprotein confined to thyroid gland. It is the source of thyroxine and triiodo-thyronine hormones in the body. Thyroglobulin level in blood is useful in monitoring progress of thyroid carcinoma after therapy.

TSH (Thyroid Stimulating Hormone)

Serum 0.4-5.0 uiu/ml. See 2.72.

TSH is a peptide hormone secreted by the hypophysis. It stimulates synthesis and secretion of T3 and T4 from the thyroid gland. TSH is, in turn, stimulated by TRH, which is released by the hypothalamus. T3 and T4 feedback inhibit release of both TSH and TRH in normal people. *Increased* in congenital (primary) hypothyroidism and secondary hypothyroidism. *Decreased* in hyperthyroidism and hypopituitarism.

T4 Free (Free Thyroxine)
T4 Total (Total Thyroxine)

Serum Free T4: 0.78-1.94 ng/dl, 10-25 pmol/l Total T4: 66.9-160 nmol/l, 5.2-12.5 µg/dl.

T4 is the major hormone controlling the basal metabolic rate. T4 is secreted from thyroid gland. Most of the T4 in blood is bound to proteins and only the free portion of T4 (0.03% of the total T4) is active toward target cells. *Increased* in hyper- thyroid states including Graves' disease, Plummer's disease, thyroid cancer and acute thyroiditis. *Decreased* in hypothyroidism, e.g. Hashimoto's disease, cretinism and myxedema, protein malnutrition and renal failure.

T3 Free (Triiodothyronine Free)

Serum 1.50-4.10 pg/ml, 2.30-6.30 pmol/l.

T3 is measured as part of thyroid function evaluation. Only 0.3% of the T3 is free; the rest is bound to protein. *Increased* in hyperthyroidism, T3 thyrotoxicosis, thyroiditis and thyroid cancer. *Decreased* in hypothyroidism, chronic illness and starvation.

T3 Total (Triiodothyronine Total)

Serum 82-179 ng/dl.

Toxoplasma antibodies IgM & IgG

Serum Titer <16. See 4.28.

Toxoplasma gondii is an obligate intracellular parasite. *Toxoplasma* infection acquired in utero can cause abortion or severe CNS and ocular abnormalities in the fetus or newborn. IgM antibodies suggest a recent infection. The role of *Toxoplasma* has been overplayed in recent years over recurrent abortion.

Transferrin

Serum 2.0-4.0 g/l.

Increased in iron deficiency anemia. *Decreased* in hemolytic anemia, congenital absence of transferrin, chronic inflammation, malnutrition and hepatic disorders.

TPHA (*Treponema pallidum* Hemagglutination)

Serum Negative. Expressed as a titer. See 4.26.

TPHA detects syphilis, an infectious disease caused by the spirochete *Treponema pallidum*, which penetrates broken skin or mucous membranes. Transmission occurs most frequently by sexual contact. Can also infect the fetus via placenta after the 10^{th} week of pregnancy causing abortion, stillbirth or neurological damage to the fetus.

Triglycerides

Serum <165 mg/dl, <1.9 mmol/l. See 2.31

Increased in various types of hyperlipoproteinemia. Also may be *increased* in nephrotic syndrome, coronary artery disease, hypothyroidism, diabetes mellitus and liver disease.

Troponin T

EDTA blood Negative.

Troponin T is released into blood 2-8 hours after myocardial damage. The diagnostic window for Troponin test is thus between 2 hours and 14 days.

Urea

Serum 10-50 mg/dl, 1.6-8.3 mmol/l.

Increased levels are associated with renal disease, dehydration, diabetic coma, hypoadrenal crisis, gastro- intestinal hemorrhage and shock. *Decreased* in some cases of severe liver disease and malnutrition.

Urethral Discharge, Direct Smear, Gram Stain and Culture

Urethral discharge. See 2.66.

Examination of urethral discharge is useful in detecting parasitic or bacterial infections. Direct smear detects cells & *Trichomonas vaginalis*. Gram stain detects gram-negative diplococci. Culture detects specific bacterial infection. In presence of pus, negative cultures do not rule out nonspecific infection such as *Chlamydia* and/ or *Mycoplasma*.

Uric Acid Serum

Serum Male: 3.0-7.0, Female: 2.5-6.5 mg/dl; Male: 0.178-0.42, Female: 0.150-0.38 mmol/l.

Uric acid is the end product of purine metabolism. *Increased* in gout, leukemia, toxemia of pregnancy, severe renal impairment, hypoparathyroidism, polycythemia rubra vera and severe exercise.

Uric Acid Urine

24-hour urine 250-750 mg/24h, 1.5-4.5 mmol/24h.

Uric acid level in urine is measured for the diagnosis or treatment of gout. Some kidney stones are composed of uric acid. Increased level may be found in gout, leukemia, liver disease, polycythemia rubra vera and high purine diet.

Urine Analysis

CML method 2 samplings technique. See 2.105.

Valproic Acid (Depakene, Convulex)

Serum 50-100 µg/ml, 350-700 µmol/l.

Valproic acid is an anticonvulsant that helps to control certain types of seizures associated with epilepsy.

VMA (Vanillylmandelic Acid)

24-hour urine 1.9-9.8mg/24h.

Increased in pheochromocytoma, manic depressive disorder, anxiety and stress. *Avoid intake of walnuts, bananas, chocolates, vanilla- containing foods, citrus fruits and certain medications 3 days before and during the 24h urine collection.*

VZV *(Varicella zoster Virus) IgM & IgG*

Serum Titer <10.

Measurement of IgM and IgG antibodies to VZV is important in determining the immune status to VZV or a recent infection.

VDRL

Serum Negative. See Microbiology 4.26.

VDRL is a nonspecific screening test for detecting syphilis. If positive the disease should be confirmed by TPHA. Positive results can also be seen in some autoimmune diseases and occasionally normal persons.

Vitamin B12

Serum 160-800 pg/ml.

This test is performed mainly to diagnose a deficiency state such as pernicious anemia, also to help determine cause or nervous system disorders. Causes of vitamin B12 deficiency include inadequate intake, malabsorption states, lack of intrinsic factor, hyperthyroidism and pregnancy. *Increased* vitamin B12 levels may be found in excessive intake of vitamin B12, hepatic disease and some myeloproliferative disorders.

Widal Test

Serum Titer <1/80. See 4.21.

This test for typhoid or paratyphoid infections is valid only when a significant rise in titer (>3 fold) is noted after one week of baseline titer. Misinterpretation of results are not infrequently seen. Clinical picture is essential in interpretation of results. See also Serology section.

Xylose Tolerance Test (See D-Xylose Excretion Test)

Serum and 5-hour urine >16%.

This test is performed to help evaluate cases of persistent diarrhea, unexpected weight loss, suspected malnutrition and general weakness. *Decreased* in malabsorption. Also may be *decreased* in Crohn's disease, *Giardia lamblia* infection, hookworm infection, lymphatic obstruction, radiation enteropathy, celiac disease, viral gastroenteritis and Whipple's disease. In renal insufficiency, blood D-xylose at 2 hours post dose is recommended.

Y-Chromosome Deletions

EDTA blood: Absence of mutations. See 5.36.

Many genes responsible for germ cell growth and maturation are carried on Y chromosome (Yq11 region). Mutations found in these region are associated with azoospermia. Screening of loci A, B and C respectively are performed in our laboratories. Test value is controversial.

ZN Stain For AFB (Ziehl-Neelsen Stain For Acid-Fast Bacilli)

Body fluids or tissue samples Negative. See 4.70.

Used to stain and detect *Mycobacterium* species. Sensitivity and specificity are low compared to other methods, e.g. PCR. Culture is the reference method.

Zinc, Semen
Zinc Serum
Zinc Urine

Semen or Serum or 24-hour urine 2-10 mg/dl, 0.3-1.5 mmol/l for semen; 68-107 µg/dl, 10.4-16.4 µmol/l for serum; 150-1200 µg/24h, 2.3-18.4 µmol/24h for 24h urine.

Zinc plays an important role in the proper functioning of the immune system. It is required for enzyme activities necessary for cell division, cell growth and wound healing. Zinc is also involved in carbohydrate metabolism. Deficiency may lead to slow growth, poor appetite, decrease in wound healing, loss of hair, impairment of taste & smell, hypogonadism in males (low semen zinc), more frequent infections and difficulty in adapting vision to the dark. Zinc toxicity is low; 70-100 times the recommended dietary

amount may cause diarrhea, abdominal cramps and vomiting. Excess zinc in the body is excreted in the urine.

CHAPTER 4

CLINICAL MICROBIOLOGY

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SECTION 1.

COLLECTION OF SAMPLES FOR THE MICROBIOLOGY LABORATORY

AEROBIC CULTURES

Blood

Specimen collection

Select a different site for each culture drawn. Vigorously clean the venipuncture site with 70% isopropyl ethyl alcohol. Swab concentrically with 1-2% tincture of iodine for 30 sec. allowing to dry: do not touch the phlebotomy site prior to puncture. Disinfect top of culture bottle with alcohol or iodine; allow to dry. Withdraw blood and quickly mix well to avoid clotting. Add sufficient volume of blood to attain 1: 10 ratio of blood to medium. After phlebotomy, clean the iodine on site with 70% alcohol. Recommended blood volume collected per venipuncture in children is 1-5 ml and in adults 10-30ml, evenly divided into 2 bottles (aerobic & anaerobic). In infants, 0.5-3.0 ml/ blood culture bottle is acceptable. Frequency and numbers of blood cultures depends on the clinical situation: Acute sepsis, meningitis, osteomylitis, arithritis, acute untreated bacterial

pneumonia or pyelonephritis: 2 samples from two 2 sites before treatment.

Suspected endocarditis, continuous bacteremia of low magnitude

Acute: 3 stat samples from different sites before treatment starts.

Subacute: 3 samples on Day 1 (15 mins or more apart). If all are negative take 3 more samples on day 2 as in Day 1.

In endocarditis patients on antimicribial therapy:

1. Draw six samples within 48 hours.
2. Collect samples immediately before next dose of antimicrobial.

Fever of unknown origin (e.g. occult abscess, typhoid fever or brucellosis). Obtain separate samples, initially within 24-36 hours, then two more before *the temperature rises.* (Yield beyond 4 cultures is very minimal).

CSF

Specimen collection

Collection of CSF is made by physicians only, by lumbar puncture or from Ommaya reservoir fluid.

Samplings are placed in 3 sterile, leak proof tubes: one for chemistry, second for hematology and third for microbiology. The latter should be the least turbid whenever present. Bacterial culture requires 1ml and each of fungal or mycobacterial culture needs 2ml sampling.

Brain abscess aspirate or CNS biopsy samples: For anerobic culture, specimen should be handled as a stat specimen. Specimens are kept at room temp. or at 37°C, unless viral studies alone are requested when the sample should be refrigerated or frozen.

Eye Specimens

Specimen collection

Most specimens are collected by an ophthalmologist. Specimens are inoculated onto culture media at the bedside, in the clinic or the physician's office.

A *conjunctival swab* is always taken, as a control, along with any other sampling using invasive procedure.

Types of specimens:

Conjuctival scraping or corneal scraping.

Conjuctival swabs.

Intraocular fluid Organisms sought: Bacterial such as *H influenzae, Staph aureus, Strep pneumoniae, Strep pyogenes, Enterobacteriaceae, Neisseria gonorrheae* or, alternatively, Viral or Chlamydial infection.

Genital

Methods of collection

Urethral or vaginal swab for *Neisseria gonorrhoeae* needs direct inoculation into culture medium. Samples are collected at least one hour after urination. No lubricant is allowed since it kills the gonococci. The best samples in females (and often males) for *Chlamydia* is the first small spurt of a urine sample. A PCR test for *Chlamydia* is accurate and readily available in our laboratories.

Trichomonas vaginalis is best picked in the doctor's office on a direct smear of a vaginal or cervical swab.

Fungi, anerobes, *Chlamydia trachomatis, Treponema pallidum, Haemophilus ducreyi, Listeria and Gardenella vaginalis* need special collection procedures and it is always advised to contact the laboratory asking for the microbiologist when any of those organisms are suspected.

Prostatic massage samplings: A special procedure has been in use for the past 20 years in our laboratories and is recommended:

Step 1

First 2-sample urine test (#1 & 2)

Leave some urine in bladder

#1 reflects urethral wall status

#2 reflects urine in bladder base

Step 2

Prostatic massage expressed secretion

Smears: direct for *Trichomonas*/ gram stain for bacteria and *Neisseria*/ PAS for *Candida* / unstained for procedures such as IF test for *Chlamydia*-now obsolete.

Culture swabs (n= 2): bacterial, fungal.

Droplets/Swabs for other studies such as molecular testing for *Chlamydia*.

Step 3

Second 2-sample urine test (#3&4)

#3 reflects periurethral status for presence of infection (high neutrophil count).

#4 reflects urine status in bladder, having washed out the prostatic secretion.

Lower Respiratory Tract Specimens

Specimen collection

Sputum:

Either expectorated, preferably early morning, or induced after chest tapping, 3 samples are required or until a positive result is obtained.

The following samples are obtained by sterile catheters or special procedures:

Tracheal Aspirates

Tracheostomy & Endotracheal Aspirates

Bronchial Washings

Bronchio-Alveolar Lavage Fluid

Lung Aspirate & Biopsy

Skin and Subcutaneous Tissue Specimens

Specimen collection

For most infections tissue samples, or material aspirated by needle and syringe, would be optimal & preferred to scrapings or swabbing. If swabs are collected they must be received in suitable transport medium such as Stuart's or Amies. Dry swabs are unacceptable. Specimens should be transported promptly.

Preparation: Skin surface should be appropriately disinfected, using 70% alcohol & then 1-2% tincture of iodine or a 10% povidone-iodine solution, prior to specimen collection (this minimizes external contamination). After collection, iodine is removed by 70% alcohol to prevent burns.

Stools Analysis

Method of collection

Stool sample (1-2 g) should be submitted fresh (within 1-2 hours of passage) twice or thrice on separate days to increase probability of pathogen isolation.

Portions for testing should contain pus, blood or mucus whenever present.

A rectal swab is often used in children.

Where inoculation cannot be made promptly, stool transport medium or gram negative broth can be used.

For bacterial culture, 3 samples are recommended.

For fungi, rectal swab can be used.

For worms, swabbing peri-anal area in the morning is used in children and by use of overnight tape.

Selective media are needed for specific pathogens, such as *Campylobacter, Aeramonas, Plesiomonas, Vibrio spp, Yersinia spp, Clostridium difficile, Hemorrhagic E.col, Bacillus cereus, Clostridium perfringens, Staph.aureus, Clostridium botulinum, E.coli* (enterophathogenic, enteroinvasive, enterotoxigenic, adherent).

Entameba histolytica is endemic in the Arab world and is most often picked up in fresh stool samplings containing erythrophagic trophozoites.

Upper Respiratory Tract Specimens

Specimen collection

Throat: (Pharyngeal specimens) Throat swab is taken from the posterior pharynx, tonsils and any inflamed or ulcerated area by sweeping.

Nasal swabs: Insert a sterile swab about 2.5cm into each nose and rotate against nasal mucosa.

Nasopharyngeal swabs: Insert a flexible wire calcium alginate-tipped swab through the nose into the posterior nasopharynx & rotate.

Nasal washings: For viral culture.

Sinus aspirate: is usually taken by ENT specialist. Material is obtained from maxillary, frontal or other sinuses.

Tympanocentesis fluid: (Middle ear infections) Fluid is aspirated via tympanic membrane or through eardrum, and if ruptured, exudate is swabbed using an auditory speculum.

Oral cultures: For detecting yeast or fusospirochetal disease, swab or scrape area of exudation or ulceration, after having rinsed mouth with saline first & wiped any crust mounted on the surface of the lesion.

For *Diphtheria* and *Pertussis*, collect samples from the throat & nasopharynx.

Urine

24-hour Urine Method of collection

At exactly zero hour (say 7 am) bladder is voided into the toilet. Afterward, all urination is collected into the collection bottle, making sure to retain some urine before hour 24 is due (say 7am the following day), at the exact time which the bladder content is collected for the last time into the collection bottle.

Urine routine analysis

Method of collection. See also p. 126.

We found that routine urine analysis is best done by taking two consecutive portions each time, container #1 of the first spurt or void at urination, representing the first portion of urine which washes out the urethral wall; container #2 includes the rest of the urine voided and represents urinary bladder urine. In females, collection of urine specimen in a single container would often be interfered with by a slight to moderately elevated white cell count caused by contaminated peri-urethral secretion or discharge. Despite instructions on adequate cleansing before giving the urine sample, we find this 2-specimen method the most satisfactory in ruling out contamination by peri-urethral material. In men, it is a very useful way of marking out urethritis/ peri-urethritis from urinary tract infection. Although the two-specimen method may be more demanding than asking the patient to void the first urine spurt out into the toilet and then collect the 'midstream' sample, we find the 2-specimen method a far more practical and rewarding way.

It avoids having to repeat urine samples and doing unnecessary urine cultures.

Note: We find it useful to hang on the wall of the toilet (especially female toilet) an illustration as to how to obtain a catchment sample. A 2nd sample or "midstream" urine should be preceded by cleansing the urethral opening or vaginal vestibule area with soapy water and thoroughly dried. Females should hold labia apart during voiding, while males should retract foreskin if uncircumsized.

Advantages of 2 consecutive portions as method of urine collection:

Container #1 = first spurt of urine ---- washes out the urethral wall

Container #2 = rest of the urine ------ urinary bladder urine

1. Avoids repeats for peri-urethral discharge contamination.

2. Picks out urethritis/peri-urethritis in men.

3. Provides a midstream equivalent sample.

4. Avoids futile urine cultures .

In males and females, any method of collection to be preceded by cleansing.

Urine culture is taken from Container #2, equivalent to midstream urine.

ANAEROBIC CULTURES

Anaerobic bacteria are usually isolated from the following sites: abscesses, body fluids such as CSF, pleural fluid, pericardial fluid, synovial fluid, pelvic cavity, amniotic fluid, or tissues, surgical wounds, autopsy material, placenta, sinus tracks, tracheostomy sites, blood, bone marrow, duodenal aspirates, middle ear and mastoid aspirates, eyes, bronchial washings.

Some sites are not candidates for a routine anaerobic culture, such as throat swab, urine, sputum, feces, rectal swabs, vaginal swabs, ulcers and fistulae.

Some anaerobes are killed by exposure to oxygen after only a few seconds.

Care should be made in collecting samples for anaerobic culture, such as by delivery into a protective double lumen catheter. A syringe may be used to collect and transport the specimen by expulsion of air and capping the needle before immediate transport. A special transport medium can also be used.

MYCOBACTERIAL (T.B.) SPECIMENS

Specimens for mycobacteria should be adequate in amount and transported promptly to avoid overgrowth contamination. No preservative or fixative should be used. *Sputum* 5-10ml of early morning sputum is collected in a sterile, wax free container. Specimens are obtained from productive cough on at least 3 consecutive days. Specimens are not to be pooled. For follow up, collection is made on weekly basis for mycobacterial check after initiating therapy.

Bronchoalveolar lavage or Bronchial wash 1ml or more collected in a sterile container. *Gastric lavage* 5-10ml collected in a sterile container first thing in the morning after the patient wakes up to catch sputum resting in the stomach.

Abscesses should be aspirated in a sterile syringe.

Blood is collected in a sterile tube or directly inoculated e.g. into Bactec 13a vial.

Bone marrow Add as much as 5ml or more into SPS collection tube (for children 1.5ml suffices) or the bone marrow aspirate may be directly inoculated onto e.g. Bactec 13a vial.

Body fluid As much as 10-15ml in sterile syringe or container is collected. If specimen is bloody, add to SPS blood collection tubes.

CSF 2ml collected in a sterile container.

Urine As much as 40ml of a first morning urine can be obtained from midstream or catheter specimen and collected in a sterile container.

Stools 1g or more is placed in a sterile, wax free and disposable container.

Tissue biopsy specimen As much as 1g of biopsy tissue is placed in a sterile container; use no fixative and no preservative.

MYCOLOGY (FUNGI)

Successful mycological examination is determined by the following factors:

1. Proper collection procedure. 2. Rapid transport to lab. 3. Prompt processing of samples. 4. Direct screen for Mycobacteria by KOH and Z-N. 5. Incubation at suitable temperatures.

Sputum or Lavage from respiratory tract Handling of fresh samplings is a must: 5-10ml are collected in a sterile container.

Abscesses Aspirate collected into a sterile syringe or a sterile container. Direct expression or by using a sterile scalpel is sometimes done.

Vaginal material is collected on sterile swabs and inserting swabs into a sterile container.

Nails After cleaning with 70% alcohol, scrapings of outer surface are discarded and scrapings of deeper parts are collected in sterile container.

Hair Selected areas are sampled with at least 10 hairs epulated for examination.

Skin and Interspaces Wipe the lesion clean with alcohol then scrape all involved surface by a sterile scalpel.

Urine First morning sample desirable: midstream or catheter specimen is collected in a sterile container.

Body fluids Aseptic method of collecting samples is made into sterile containers.

Blood 8ml is mixed with SPS as anticoagulant.

Bone marrow 3-5ml of bone marrow aspirate is placed with SPS or heparin in a sterile container.

CSF 2ml is collected in a sterile container.

SECTION 2.

BACTERIAL CULTURES

Culture Guidelines

Specimen	Organisms of interest	# of cultures recommended
Sputum	Acid-fast Bacilli	minimum 3 on consecutive mornings
	Bacteria	2 consecutive morning specimens
	Fungi	as acid-fast Bacilli
Blood	Bacteria	3-4 sets of blood culture bottles*
Urine	Bacteria	Two for women and one for men
Stool	Bacteria	3 specimens, one per day
	Ova & Parasites	3 specimens each 2-3 days apart
Wound	Bacteria	1-2 specimens

* each set 2 bottles: 5ml blood/bottle sets taken at hourly intervals; each set drawn from different site.

Cell Cultures for Viruses

In special cases, request may be made for culture of the following viruses:

Adenoviruses, Enteric, Hepatitis B &D, HIV, Influenza A&B, Measles, RSV (Respiratory syncytial virus) and Rotavirus.

Special collection can be arranged with the laboratory.

Brief Notes on some Cultures

Anerobic cultures: Gram stain is helpful as a screen to identify characteristic morphology for any anaerobes present. Examples: *C. perfringens* Gram positive, large rods with blunt ends in suspected gangrene. *Bacteroides or Fusiformes spp* Gram negative bipolar staining rods in abscesses. *Actinomyces* Sulphur granules from cervico-facial lesions.

Blood: Gram stain is routinely made. In suspected bacteremia or septicemia by gram positive cocci such as *Streptococcus viridans* in infective endocarditis, gram negative cocci such as *N. meningitides* or gram negative bacilli such as *Bacteroides fragilis*. Very useful in the diagnosis of *Salmonella* and *Brucella* infections, positive blood culture yield rate being superceded by bone marrow samplings.

CSF Bacterial meningitis is most commonly caused by the following organisms according to age group:

First month of life	*E. coli, Group B Streptococci, L. monocytogenes*
1-3 months age	*E. coli, Group B Streptococci, L. monocytogenes, H. influenzae, S. pneumoniae*
3 months - 18 years	*H. influenzae, N. meningitides, S. pneumonia*
18-50 years	*S. pneumoniae, N. meningitides*
> 50 years	*S. pneumoniae, N. meningitides, L. monocytogenes*
	Gram negative Bacilli

Chlamydia Samples are usually taken from urethra, first drop of urine or eye. Cervical swabs are better substituted by first drop urine sampling, the latter which is adequate for the purpose of isolation or identification of Chlamydia. A special transport medium is required. Culture has been largely replaced by molecular testing which is fast, less fastidious and highly sensitive with great specificity.

Chlamydia are gram negative intracellular bacteria. 3 species are pathogenic in man, namely *C. trachomatis, C. pneumoniae, C. psittaci*. *C. trachomatis* causes urogenital and eye infections. *C. trachomatis* presents with urethritis, cervicitis, epididymitis or prostatitis; it is characterized by recurrence and chronicity. It may account for pelvic inflammatory disease in women. Lymphogranuloma venereum is also caused by *C. trachomatis* in sexually active men and women. Conjunctivitis in the newborn is caused by *C. trachomatis* in 75% of cases. It can also cause pharyngitis, otitis media, pneumonia and infections at other sites. *C. pneumoniae* causes community related pneumonia and is the cause of 5-10% of pneumonias in adults. *C. psittaci* is usually pathogenic in animals but can be transmitted to man causing psittacosis.

Treatment of Chlamydia is by erythromycin, tetracycline and other new antibacterials.

Eye Conjunctivitis is the most frequent mode of eye infection. Infection may be bacterial or viral and has to be differentiated from allergic etiology. Bacterial infection presents with purulent discharge, severely injected conjunctiva; common bacteria responsible include *H. influenzae, S. pneumoniae, Staph. aureus,Staph. epidermidis, N. gonorrheae* and *Chlamydia.* Viral infection presents with watery discharge with moderate injection often accompanied by periauricular lymphadenopathy. Viruses implicated include *Adenovirus, Herpes simplex, Varicella zoster, Coxsackie virus, Epstein-Barr virus* and *Influenza.* Allergic form presents with white ropy discharge, mild injection and eosinophils in the smear.

Other forms of eye infections include corneal ulcers, endophthalmitis, keratoconjunctivitis sicca, blepharitis and lacrimal infections.

Sputum Sputum culture is sought to detect any bacterial, fungal or mycobacterial infection. Gram stain is done routinely and a cytology test may be requested on the same sample. Also, screen for acid fast and fungal stains may be applied in cases with suspected mycobacterial or fungal infection.

Stool Stool culture is usually sought to exclude bacterial cause of diarrhea by *Salmonella, Shigella, Campylobacter jejuni, Yersinia enterocolitica, Clostridium difficile* and *E. coli.*

Dysentery presents with abdominal cramps, frequent motions with blood and mucus. It is usually caused by *Shigella*

but may also be caused by *Salmonella*, *Campylobacter* and *Yersinia*. *Salmonella* enterocolitis can be complicated by pericarditis, arthritis, osteomyelitis, neural and muscular disorders. *Salmonella* and S*higella* are food borne. *Campylobacter* is transmitted directly or indirectly from poultry. *Yersinia* infection is self limited. *Clostridium difficile* infection is associated with antibiotic induced colitis. *E. coli* is the most common cause of traveller's diarrhea. It produces enterotoxins and may cause outbreaks in nurseries. It may be complicated by hemolytic uremic syndrome.

Throat/ Nasopharyngeal Swab for culture should be taken before starting antibiotic therapy.

The most common cause of bacterial pharyngitis is Group A beta hemolytic *Streptococci*, with the potential of developing rheumatic carditis, glomerulonephritis and chorea in the untreated. Presence of the organism is usually confirmed by culture and supportive testing such as by the use of optichin and/ or bacitracin test for *Streptococcus*. Treatment is by penicllin or erythromycin. At the same time, other confirmatory tests may be used for other organisms, such as Factors V and X for *Hemophilus influenzae*.

A special note should be made of *Bordetella pertussis* (whooping cough) and *Corynebacterium diphtheriae* (diphtheria) infections. If either case is suspected, the laboratory should be notified. Immunization has largely eradicated both those infections in immunized communities. However, occasional sporadic cases are seen and should be suspected in non-immunized persons.

For *Bordetella pertussis*, a nasopharyngeal and not throat swab should be taken with direct inoculation on special media. This organism is fastidious, requires long culture

time and sensitivity is decreased by previous antibiotic therapy or immunization. A rare complication is encaphilitis which can be fatal.

For *Corynebacterium diphtheriae*, any exudates should be wiped off and vigorous swabbing be made with direct inoculation on special media in order to obtain the best yield of positive cultures. Despite immunization, immunity may fade out in later years and a booster in adults may be useful. Affected patients may or may not have a diphtheritic membrane.

Urethral/ Gonococcus Specimens are usually obtained from the cervix, vagina, urethra, prostate, throat or joint fluid. *Neisseria gonorrhoeae* is an aerobic oxidase positive gram negative diplococcus. Plating is immediately made on special media. Infection is often first detected in Gram stain as intracellular or extracellular gram negative diplococci. It is a common sex transmitted disease. Diagnosis should be confirmed by serological tests since *Moraxella* and *N. meningitides* are gram negative diplococci and both give a positive oxidase reaction.

Urine A bacterial count of 10^5 or more indicates presence of infection. Lower counts may be regarded significant in symptomatic patients, those who have undergone urological procedure or at follow up of antibiotic treatment for established urinary infection. In suprapubic aspirates, 150 bacteria/ ml or more is a significant count.

Sterile pyuria may be caused by nonbacterial affections such as T.B., Chlamydia etc.

But the most common cause of apparent sterile pyuria in women in practice is that caused by noncompliant method

of urine collection resulting in contamination of urine sample by vaginal discharge.

Sensitivity profile should be set up to suit the clinical situation. Empirical therapy may be started but should be checked by the antibiotic sensitivity pattern of the isolate of interest.

Occasionally in complicated cases with indwelling catheters and so on, fungal culture may be requested and special culture should be set up for the purpose.

Wound Culture is usually requested for aerobic bacteria. For anaerobic bacteria, it is best sampled from deep parts by syringe and follow special collection and inoculation procedures. Surgical wound infection poses a nightmare to hospital theater personnel, since it indicates nosocomial infection and necessitates laborious infection control measures.

Minimum Inhibitory Concentration

MIC (Minimal inhibitory concentration) testing is performed on already isolated bacterium from a patient and is requested within 48 hours of specimen collection to ensure availability, before disposal, of the isolate by the microbiology laboratory.

MIC represents the least concentration of an antibiotic which inhibits growth of the organism of interest, so that no turbidity can be observed. The test takes 15-18 hours. (See Section 4 Microbiology).

MIC enables a better informed selection of an anti-microbe against a given organism. The antimicrobial panel should be chosen to suit the organism in question to the best of

knowledge by the therapeutic team, taking into consideration current resistance patterns or relevant endemic and epidemic data.

SECTION 3.

MICROBIAL DIAGNOSIS

4A. Diagnosis of Bacterial Infections at Different Body Sites

Site of Infection	Specimen to Culture	Potential Bacterial Pathogens
(1) Urinary Tract:-		
* Urinary bladder infection.	Clean-catch and midstream urine	*Enterobacteriacae* *E.coli* *Klebseilla cpp* *Proteus cpp* *Other Member* *Group D Streptococci* *Enterococci) Ps.* Aeruginosa
* Kidney infection.		*Staph.aureus* *Staph.epidermidis* *Staph.saprophiticus*
(2) Respiratory Tract:-		
A * Upper Tract-Nose & Sinuses	Acute - Nasopharyngal Swab - Sinus Washings	*Strep.pneumonia.* *Strep. B-haemolytic* *Group A.Streptococci* *Staph. aureus*
	Chronic Washings Biopsy	*Haemoph.influenzae* *Klebsiella spp Entero-bacter* *Bacteroides spp.* & Other Anaerobes

Site of Infection	Specimen to Culture	Potential Bacterial Pathogens
* Upper Tract- Throat & Pharynx	Swab Posterior Pharynx. Swab Tonsils (Abscess) . Nasopharyngeal Swab	*Strep. ,B-haemolytic Group A Strep.* *Corynebact.diphtheriae* *N.gonorrhoeae* *Bordetella pertussis*
B * Lower Tract- Lungs & Bronchi	- Sputum (Poor Return) - Blood - Bronchoscopy Secretions - Transtracheal Aspirate - Lung Aspirate or Biobsy & other	*Strep pneumoniae* *Haemophilus influenzae* *Staph. aureus* *Klebsiella pneumonia* *Enterobacteria* *Legionella spp.* *Mycobacterium spp.* *Fusobacterium Nuclea-tum.Bacteroides Melaninigenicus &* other *Anaerobic spp.*
(3) Gastrointestinal Tract :-		
	- Stool Specimen - Rectal Swab or Rectal Mucus	*Campylobacter jejuni* & other *Campylobacter spp.* *Salmonella spp.*
	- Blood Culture (Typhoid Fever)	*Shigella spp.* *E.coli* (Toxinogenic strains) *Vibrio cholerae* & other *Vibrio spp.* *Yersinia spp.* *Clostridium difficile*

Site of Infection	Specimen to Culture	Potential Bacterial Pathogens
(4) Wounds :- - Discharge: Serous / Purulent - Abscess: Subcutaneous or Submucous Drainage	-Aspirate of Drainage. -Deep Swab of Purulent -Tissue Biopsy	*Staph. aureus* *Strep. pyogenes.* *Clostridium spp.* *Bacteroides spp.* & other anaerobes *Enterobacteriaceae.* *Pseudomonas aeruginosa.* *Enterococci.*
(5) Meninges :-	-Spinal Fluid (CSF). -Subdural Aspirate. -Blood Culture -Throat or Sputum Culture	*Neisseria meningitides* *Haemophilus. influenzae.* *Strep. pneumonia.* *Strep.,B-Group A & B* (*Group-B* in infants) *Enterobacteriaceae* *Listeria. monocytogenes.*
(6) Genital Tract:- * Male :-	-Urethral Discharge -Prostatic Secretion (Serous or Purulent)	*Neisseria.gonorrhoeae* (*N. meningitidis*) *Haenophilus. ducreyi.* *Treponema pallidum* *Mobiluncus spp.* & other anaerobes.
* Female:-	-Uterine Cervix. -Purulent Vaginal Discharge . -Rectal (Anal Swab) -Urethral Swab -Mucous Membrane Chancre or Chancroid	*Gardnerella vaginalis* Nonbacterial infections *Trichomonas vaginalis* *Candida albicans* *Mycoplasma spp.* *Treponema pallidum spp.* *Herpes simplex*

Site of Infection	Specimen to Culture	Potential Bacterial Pathogens
(7) Bacteremia		
	* Blood: 3 to 4 cultures /day @ 1-hour intervals or more. For endocarditis patients on therapy 6-8 cultures/day predose if possible Alpha-hemolytic Strep. endocarditis	*Streptococcus spp. Group A - all ages*
	* Suspected Sites of Primary Infection:- C.S.F/ Resp Tract Wounds/ Urinary Tract Skin-Umbilicus/ Ear	*Group A.B.D* (Newborns) *Staph. aureus. Strep. pneumoniae. E.Coli. Pseudom. aeruginosa Listeria monocytogenes Haemoph. influenzae. Salmonella typhi Bacillus fragilis & other anaerobes*
(8) Eye - Conjunctiva Serous or Purulent.	-Purulent Discharge. -Lower Cul-De-Sac. -Inner Canthus.	Haemophilus spp. Moraxella spp. Neisseria gonorrhoeae. Staph. aureus. Strep. pnuomoniae. Strep. pyogenes. Pseudomonas aeruginosa

Site of Infection	Specimen to Culture	Potential Bacterial Pathogens
(9) Middle Ear :-		
- Serous or Purulnt Drainage.	* Acute:- -No Culture -Nasopharyngel Swab. -Tympanic Membrane Aspirate.	**Acute :- *Strep.pneumoniae* & other *Streptococci* # *Haemoph. iInfluenzae*
	* Chronic Drainage of External Meatus	** Chronic:- *Pseudom. aeruginosa.* *Proteus spp.* Anaerobic bacteria
(10) Bones And Joints :-		
- Joint Swelling.	-Joint Aspirate. -Synovial Biopsy. -Bone Spicules or Bone Marrow Aspirate	*Staph aureus.* *Haemoph.iInfluenzae.* *Strep.pyogenes* *Neisseria gonorrhoeae* *Strep.pneumoniae.* *Enterobacteriaceae.* *Mycobacteria.*

4B. Diagnosis of Infectious Disease by Direct Examination of Culture Specimens

Specimen	Suspected Disease	Laboratory Procedure	Positive Finding
(1) Throat culture:-			
	Diphtheria.	Gram's stain	Delicate pleomorphic positive bacilli in chinese letter arrangement
		Methylene blue stain:-	Light-blue-staining bacilli with prominent metachromatic granules
	Acute streptococcal pharyngitis.	Direct fluorescent Antibody technique (after 4 to hours incubation in todd hewett broth)	Fluoresent cocci in chains: use positive & negative controls with each stain
(2) Oropharyngel ulcers:-			
	Vincent's disease.	Gram's stain	Presence of gram-negative bacilli & thin. Spiral-shaped bacilli.
(3) Sputum, transtrachealbronchial aspirate/ washing:-			
	Bacterial pneumonia.	Gram's stain	Variety of bacterial bronchial types. *Strep.pneumoniae* with capsules particularly diagnostic
	Tuberculosis.	acid fast stain	acid fast bacilli.

Specimen	Suspected Disease	Laboratory Procedure	Positive Finding
	Pulmonary mycosis.	Gram's stain - Giemsa's stain Gram-Weigert stain	budding yeast pseudo-/ true hyphae fruiting bodies
(4) Cutaneous wounds or purulent discharge from subcutaneous sinuses :-			
	Bacterial cellulitis.	Gram stain	variety of bacterial types suspect anaerobes
	Gas gangrene (myonecrosis).	Gram's stain	gram-positive bacilli. Suspicious for *Clostridium perfringens*. Spores usually not seen.
	Actinomycotic mycetoma.	Direct saline mount Gram's stain or modified acid-fast stain	"sulfur granules" delicate branching gram positive filaments. *Nocardia* spp. may be weakly acid-fast.
	Eumycotic mycetoma.	direct saline mount Gram's stain or lactophenol cotton blue mount	white grayish,or black grains true hyphae with focal swelling or chlamudospores.

Specimen	Suspected Disease	Laboratory Procedure	Positive Finding
(5) Cerebrospinal fluid:-			
	Bacterial meningitis.	Gram's stain	small Gram negative Pleomorphic bacilli *(Haemophilus.spp)* Gram neg *(Neisseria meiningitidis)* Gram pos Diplococci *(Strep. Pneumoniae)*
		Methyline blue stain:	bacterial forms that Stain blue black
		Acrodine orange stain:	bacterial forms that glow brilliant orange under ultraviolet light
		Quelling reaction: (type-specific antisera)	swelling and ground glass bacterial capsule
	Cryptococcal Meningitis.	India ink or nigrocin mount	encapsulated yeast cell with buds attached by thin threads.
	Listeriosis.	Gram's stain: hanging-drop mount	delicate gram-positive Bacilli. Bacteria with tumbling motility.
(6) Urine:			
	Yeast infection.	Gram stain or Giemsa stain	pseudohyphae or budding yeasts.
	Bacterial infection.	Gram's stain	variety of bacterial types.
	Leptospirosis.	Dark-field exam.	loosely coiled motile Spirochetes.

Specimen	Suspected Disease	Laboratory Procedure	Positive Finding
(7) Purulent urethral or cervical discharge :-			
	Gonorrhea.	Gram's stain	intracellular Gram neg Diplococci.
	Chlamydial infection.	Direct fluorescent Antibody stain of smear.	elemental bodies.
(8) Purulent vaginal discharge:-			
	Yeast infection.	Direct mount	psudohyphae /budding yeasts.
	Trichomonas infection.	Direct mount	flagellates with darting motility.
	Gardnerella vaginalis.	Pap stain or Gram	"clue cells" or pH of vaginal secretion >5.5
(9) Penile or vulvar ulcer (chancre):-			
	Primary syphilis.	Dark field mount of Chancre secretions	tightly coiled motile spirochetes.
	Chancroid.	Gram's stain of Ulcer secretion or Aspirate of inguinal Bubo.	intracellular & extra cellular small Gram negative bacilli.
(10) Eye:-			
	Purulent conjuctivitis.	Gram's stain	variety of bacterial spp.
	Trachoma.	Giemsa stain of corneal Scrapings	intracellular perinuclear inclusion clusters.

Specimen	Suspected Disease	Laboratory Procedure	Positive Finding
(11) Feces:-			
	Purulent enterocolitis.	Gram's stain	neutrophils & aggregates of Staphylococci.
	Cholera.	Direct mount of alkaline peptone water enrichment	bacilli with characteristic darting motility, no neutrophils.
	Parasitic disease.	Direct saline or iodine mounts Examine purged specimens	adult parasites or parasite fragments, protozoa or ova.
(12) Skin scrapings, nail fragments, or plucked hairs:-			
	Dermatophytosis.	10% KOH mount	delicate hyphae or clusters of spores.
	Taenia versicolor.	10% KOH mount or lactophenol cotton blue mount.	hyphae and spores resembling spaghetti & meat balls.
(13) Blood:-			
	Relapsing fever (Borrelia).	Wright's or Giemsa stain Darkfield examination	spirochetes with typical morphology.
.	Blood parasites: Malaria, filariasis, trypanosomiasis.	Wright's or Giemsa stain	intracellular parasites
		Direct examination of anticoagulated blood for the presence of microfilaria	(malaria, babesia) extracellular forms trypanosomes or microfilaria.

SECTION 4.

ANTIBACTERIAL THERAPY

We teach our students to abstain from prescribing antibiotics for children with upper respiratory infection, most of which are viral, unless streptococcal or other bacterial infection is suspected. In the latter case, the proper line of action to be taken is pharyngeal culture; this dictum is advocated to prevent extension of resistant strains of bacteria to old and newly introduced antibiotics. Admittedly, in practice we often see such rules broken and antibacterial therapy is empirically served over the counter in our part of the world. Deprecation of this particular malpractice cannot be overemphasized. However, great efforts by medical educationalists, microbiologists and medical practitioners in different specialties to curb indiscriminate use of antibiotics have gone unheeded. It is not surprising, therefore, to see frequently resistant strains of bacterial organisms to some very useful and initially effective drugs. Field studies in the community for surveillance of drug resistance patterns by bacteria are not easy to attain. We often have to rely on hospital based studies as well as those issued from microbiology laboratories serving outpatient clinics. Table 1 serves to portray effects of antibiotic prescribing patterns in a study undertaken on bacterial strains isolated from outpatients versus inpatients in a teaching hospital laboratory.

Table 1. Outpatient versus Inpatient Susceptibility Rates in 2335 *E. Coli* isolates[*] from Urine Cultures at Jordan University Hospital, Amman: 2000-2001.

Antimicrobial Agent	% Susceptibility			
	Out-patient	In-patient	Total	Mic90 (Mg/L)[†]
Ampicillin	12	10	11	>127
Cotrimoxazole	22	24	23	>127
Tetracycline	25	27	26	>31
Augmentin[‡]	45	21	33	--
Nalidixic acid[‡]	68	37	53	>7
Gentamicin[‡]	70	34	52	>15
Norfloxacin[‡]	81	41	61	--
Cefuroxime[‡]	83	47	65	--
Nitrofurantoin[‡]	94	58	76	--

[*] = about 1% of total number of isolates recorded during same period of time.

[†] = MICs of 20 multi-resistant (>3 resistant drugs) *E. coli* strains.

[‡] = p value= < 0.05; significant between total in- and out-patients.

(The above table was provided and permitted to publish by Dr. Asem Shihabi, Professor of Microbiology, Jordan University Hospital, Amman, Jordan).

It is evident that with regard to agents infrequently used at the community level, susceptibility performance in outpatients obviously outranked that seen in hospital patients, while for agents commonly used in the community, for example ampicillin and others, susceptibility was not significantly different.

Uses of antibiotics

Whenever bacterial culture yields a pathogenic organism, one expects to see a variety of antibiotics tested with a report on sensitivity and resistance patterns of that organism. Selection becomes important, with preferences for particular groups of drugs for certain kinds of organisms, based on past experience on efficacy in the clinical situation of interest. Emergence of resistance should be anticipated from time to time, and oral drugs are usually preferred to parenteral in ambulatory patients. Cost and safety always play an important role in determining which drug to prescribe. There are different groups of drugs which are chosen in panels for finding sensitivity-resistance patterns of different bacteria. Dosage is determined by age and severity of the clinical situation as well as the condition of the patient, for example renal function status, and so on. Sensitivity of antibiotics guides their selection in clinical infections, whether by assessing clinical or in vitro response.

The reader is referred to current textbooks and manuals in clinical microbiology for use of antibiotics, as well as the clinical microbiology laboratory of choice.

SECTION 5.

PARASITOLOGY

Infestation implies parasite upon human body while *infection* implies parasite inside human body.

A parasitic organism is one which needs to thrive in part or whole of its life in or on the body of another bigger organism. Parasites damage their host either directly, for example *Entamoeba histolytica* causing colonic ulcers and hepatic abscesses, or indirectly by inducing an abnormal host response, such as through IgE mediated hypersensitivity reaction caused by a ruptured hydatid cyst of liver or lung.

Parasites may invade man through skin (*Schistosoma* – swimmers' itch), digestive system (*Ascaris lumbricoides*) or reproductive system (*Trichomonas vaginalis* – genital pruritis). When larval stage fails to mature and roams among body organs causing pathological lesions, the condition is termed visceral larva migrans. When larvae wander in skin, the condition is termed cutaneous larva migrans.

Direct examination of the parasite is the most reliable approach in diagnosis and in cases of worm infection a mature form is required for precise identification. Serological tests can be used in the diagnosis of certain parasitic infections, such as for *Schistosoma, Trichinosis* and *Toxoplasma*. Serological methods include complement fixation test, immunodiffusion, agglutination, intradermal testing and indirect immunofluorescence antibody and enzyme immunoassay

methods. Serological tests may be used in early stage of infection, where the parasite load is low or in infections of deep body sites. However, serological tests usually cannot distinguish acute from chronic phase of the disease or a past infection. Culture, when possible, remains the mainstay in confirming an active infection.

Entameba histolytica

In endemic regions, *Entamoeba histolytica* is transmitted by contaminated water or uncooked food and commonly causes amebic dysentery, amebic colitis and in a small percentage amebic abscesses or rarely amebic hepatitis. Amebic dysentery presents with mucous diarrhea, with or without blood, alternating with constipation, abdominal cramps and weight loss. Stools contain many eosinophils and frequently Charcot-Leyden crystals. Chronic amebic colitis mimics chronic ulcerative colitis. Amebic colitis is sometimes complicated by amebic liver abscess and occasionally amebic hepatitis. Hematogenous spread rarely leads to abscesses in lung, brain and other body organs. Rectal ameboma can clinically mimic rectal tumor.

Diagnosis: Stool examination detects erythrophagocytic trophozoites or cysts of *E. histolytica*. Liver abscess aspirates reveal amebae facilitated by PAS (Periodic Acid Schiff) stain. *E. histolytica* trophozoites are 10-60 µm for invasive and 15-25 µm for noninvasive ("minuta") forms, appearing actively motile with some containing erythrocytes while nuclei are small with a central karyosome located centrally or at the periphery. Cytoplasm is finely granular and compared to *E. coli*, they move much faster. While invasive trophozoites contain red cells, noninvasive forms contain bacteria. *E. histolytica* cysts are 10-20 µm in

diameter with cytoplasm containing glycogen vacuoles which stain with PAS. Immature cysts with 2 nuclei usually show a large glycogen vacuole displacing nuclei to the periphery. Mature cysts contain 4 nuclei. In contrast, mature cysts of *Entamoeba coli* contain 8 nuclei.

Serological tests may be useful in extraintestinal infection. Hemagglutination and Enzyme immunoassay methods are available. Culture is not used in practice.

Giardia lamblia

G. lamblia commonly causes diarrhea in travellers and children and is transmitted via contaminated water. *G. lamblia* trophozoites multiply in small bowel attaching to mucosa by a concave sucker. Infection may be asymptomatic or cause mild diarrhea and malabsorption syndrome.

Diagnosis: Stool examination shows trophozoites or cysts of *G. lamblia*. Trophozoites are detected in fresh diarrheal specimen and the latter in formed stools. Occasionally trophozoites of *G. lamblia* are found in duodenal or jejunal biopsies. Trophozoites of *G. lamblia* are pear shaped with 2 nuclei appearing as owl eyed with 2 ventral, 4 lateral and 2 caudal flagellae. Cysts are oval and usually quadricnucleate with its cytoplasm often retracted from the cyst wall.

Cryptosporidium spp.

Cryptosporidium parvum develops in the brush border of intestinal epithelium and occasionally spreads to gall bladder and respiratory tract. It commonly causes transient diarrhea especially in children. Outbreaks occur by contaminated water. *Cryptosporidium* causes chronic diar-

rhea in patients with AIDS and contributes to death in those patients.

Diagnosis: Stool examination shows trophozoites of cysts of *Cryptosporidiun* oocytes using concentration method and acid fast or immune (IF or EIA) stain. Spherical oocyte is 4-6 µm in diameter.

Microsporidia

An obligate intracellular, spore forming protozoan parasite, *Microsporidia* are pathogenic in immunocompromized patients especially in AIDS.

Diagnosis: Intracellular spores are very small, 1.5-3 µm, elliptical and stain red against a background of pale green in a special trichrome stain in stool specimens. Detection of *Microsporidia* requires meticulous search in clinically suspected cases.

Ascaris lumbricoides

Ascaris lumbricoides is a round worm which infects the intestinal tract, seen mainly in children but also in adults. It is prevalent in areas with poor sanitation. *Ascaris lumbricoides* infection is usually asymptomatic but can cause severe diarrhea. Heavy lung infection by larvae of *Ascaris lumbricoides* causes pneumonitis or Loffler's syndrome, characterized by diffuse pulmonary infiltration and eosinophilia. Heavy intestinal infection may cause abdominal pain, diarrhea or even intestinal obstruction, especially in children. Female worm measures up to 12 mm in length and the male is slightly shorter.

Adult worms inhabit duodenum and jejunum, are distinguished by presence of 3 lips in their anterior end. Females produce 200,000 eggs per day which mostly pass to the environment, since eggs do not hatch before the passage of 4 weeks. Eggs hatch their larvae which pass through intestinal epithelium into blood stream reaching pulmonary capillaries, mature and enter alveoli. Larvae move up epiglottis and are swallowed to enter small bowel. The egg-mature worm cycle takes about 2 months time.

Diagnosis: Ascaris lumbricoides eggs are detected in stool specimens. Counts of <20 per slide indicate mild infection and >100 cysts a heavy infection. Fertile eggs are round to oval, up to 75X50 µm exhibiting an irregular external mamillated layer with females showing thinner shell with irregular mamillations.

Hookworms

Hookworms occur in tropical and subtropical regions and some temperate zones with the same geographic distribution as that of *Ankylostoma duodenale*. Skin disease with itching develops at site of larval penetration. Heavy larval infection of lung can cause Loffler's syndrome. The most important effect is chronic blood loss through the intestinal tract leading to iron deficiency anemia, especially in children within endemic zones.

Adult worms inhabit small intestine for years and shed eggs which pass out in feces; after several days larvae develop and under the right conditions re-enters through skin where it causes itching, migrating through blood to reach lung capillaries and then migrate up trachea to be swallowed travelling to small intestine. Adult worms measure up to

12cm in length; males are slightly smaller and possess a fan shaped bursa at the posterior end. Anterior end of hookworms show a buccal capsule containing teeth or cutting plates.

Diagnosis: In feces, shelled eggs are detected and measure 60-75 µm long and 35-40 µm wide. Male and female eggs are indistinguishable, but adult worms can be distinguished by the bursa in the male. Egg counts <5 per slide denote mild infection and >25 per slide indicate heavy infection with risk of developing iron deficiency anemia.

Strongyloides stercolaris

Strongyloides stercolaris occurs in tropical and subtropical zones and some temperate areas. Affected individuals may have intestinal symptoms such as peptic ulcer, abdominal pain and diarrhea. Malabsorption may occur. Skin penetration by larvae may cause itching at site of entry and in heavy lung infection may cause Loffler's syndrome.

The worm inhabits the duodenum. Eggs hatch primarily in the small intestines, producing larvae which are passed in feces. Under the right conditions, larvae penetrate skin of exposed individuals and migrate through the circulation to the lung capillaries, enter alveoli and move up the respiratory tract to be swallowed and then develop in the small intestine. Adult female worm is 2-3 mm long and the male is slightly shorter.

Diagnosis: Rhabditiform larvae of *Strongyloides stercolaris* can be detected in stool specimens. *Strongyloides* larvae are distinguished from larvae of hookworms by their short buccal cavity and a prominent genital promordium, with a notched tail and an esophagus about ½ the length of

the body. Duodenal aspirates may yield larvae as well as in sputum or bronchio-alveolar lavage specimens, especially in cases with heavy lung infection.

Serological tests include Enzyme immunoassay and agglutination methods, but such tests do not distinguish between current and past infection.

Enterobius vermicularis

The nematode *Enterobius vermicularis* or pinworm commonly infects children. Infection may be asymptomatic or may cause pruritis ani, irritability and loss of sleep. It can underlie development of enuresis in children. Adult worms may migrate to peritoneal cavity or other sites where they die but at the same time induce tissue reactions with inflammation and granulation tissue formation.

Residing in cecum and adjacent regions, laid eggs rapidly mature so that transmission readily occurs to other family members or inmates in institutions. Eggs become infective within hours after shedding and, after ingestion, maturation to gravid status is reached within one month.

Diagnosis: Recovery of eggs or adult worms is usually made by placing a cellulose tape on perianal skin soon after the child goes to sleep or first thing in the morning. Several trials may have to be made to obtain a positive tape test. Only in a minority eggs or worms can be detected in stool specimens. Eggs are 50-60 µm long and 20-40 µm wide, while adult female worm reaches 13mm in length. The worm shows prominent lateral alae and may sometimes be identified in appendectomy specimens.

Trichuris trichiura

Of worldwide distribution, infection by *Trichuris trichiuria* or whipworm may be asymptomatic, but heavy infection (>300 worms) leads to diarrhea with dysentery-like symptoms, consequent dehydration and anemia. Rectal prolapse may occur.

Adult worms live in the colon, especially cecum, but in heavy infection may populate colon and rectum. Anterior end of worm hooks onto intestinal mucosa and posterior end remains free in the intestinal lumen. The worms may live for up to 10 years. Eggs are passed unembryonated into soil and require several weeks to hatch larvae, which in turn may be ingested to mature into adult worms in the colon.

Diagnosis: Fecal specimens, in direct mounts or after concentration, show typical eggs of *Trichuris trichiuria*. Eggs are barrel shaped with refractile ends measuring 50-55 µm long and about 23 µm wide. Adult worm measures up to 50mm long and its posterior end is thicker than the anterior end. Tails of male worms are coiled in contrast to straight shape in the female worm. Quantitation may be required in cases of heavy infection.

Taenia saginata and Taenia solium

Cestodes or tapeworms are of worldwide distribution. *Taenia saginata* infection is acquired by eating uncooked or inadequately cooked beef and *Taenia solium* infection by ingestion of raw or incompletely cooked pork. *Taenia saginata* rarely produces abdominal pain and diarrhea in man. Ingestion of raw beef containing larvae (cysticercus) leads to development of adult worm in the small intestine within 2-3 months. However, ingestion of eggs of *Taenia saginata*

is noninfective to man but infects cattle in whom larvae (cysticerci) develop which in turn infect man to reside in small intestine and develop into mature worms. On the other hand, *Taenia solium* goes through a similar life cycle as that of *Taenia saginata* with similar abdominal symptoms when present, but eggs of *Taenia solium* are infective to man whereby their ingestion in contaminated pork may lead to cysticercosis.

Diagnosis: Eggs are indistinguishable, spherical, 30-40 µm in diameter, detected in direct or concentrated fecal mounts. Adult worms are distinguished by their scolices, which in *Taenia saginata* show 4 suckers and no hooks on the rostellum, while *Taenia solium* shows four suckers and two rows of hooks. Gravid uterus shows 15-20 lateral branches in the former and 7-13 lateral branches in the latter. The adult worm reaches several meters in length.

Cysticercosis

Cysticercosis results from human infection by larvae of *Taenia solium* subsequent upon the ingestion of their eggs contaminating food or water. Larvae penetrate intestinal mucosa and reach via blood distant sites such as skeletal muscle, brain, heart and so on. The larvae induce local inflammation and seizures occur with brain involvement.

Diagnosis: Cysticerci are oval sacs 5mm in diameter with a single inverted scolex. Computed tomography aids in diagnosis and recovery of cysticerci at surgery confirms the diagnosis. Serological tests include immunoblot as well as enzyme immunoassay methods. However such tests do not distinguish active from old infection and are not useful in monitoring therapy.

Hydatidosis

Hydatid disease in man, sheep, goats, cattle and camels occurs due to ingestion of eggs of *Echinococcus granulosus*, which is the prevalent species in the Middle East and other regions of the world. Other species include *Echinococcus multilocularis* prevalent in Europe and Northern America that produces multilocular hydatid disease but with no brood capsules developing in the hydatids, and *Echinococcus vogeli* which is limited to South America and produces invasive disease with brood capsule formation in the cysts. The mature *Echinococcus granulosus* worm is small and inhabits the intestine of dogs, often stray or non-vaccinated dogs, in whom hundreds or thousands of eggs are passed in the dog's stools contaminating environment. Ingested eggs by humans or herbivores are passed to intestine where larvae develop, which in turn penetrate intestinal wall through blood to reach liver, lung and other organs. Unilocular hydatid cysts are more commonly encountered in liver and lung, but hydatids may be encountered in any organ, including brain, eye, bone and cardiac muscle. Hydatid cysts may be single or multiple, may recur and reach a huge size. Prevention is by strict control measures on stray and infected canine population.

Diagnosis: Clinical diagnosis is made by clinical history, examination and radiologic methods including computed tomography and ultrasonography. Hydatid cysts contain fluid harbouring brood capsules with proscolices or scolices bearing hooklets but such are not found in nonfertile hydatids. Cyst aspiration is discouraged for fear of dissemination or cyst rupture, which rarely can lead to anaphylactic shock. The cyst lining consists of a thin germinal cell layer that produces the proscolices and scolices, supported by a thick

laminated layer which in turn may be bordered by a thick collagenous layer formed by reaction of surrounding tissue. Apart from cyto-histological diagnosis of hydatid cysts and identification of laminated and germinal layers or scolices/hooklets in direct crush smears or routine paraffin sections, serological tests include hemagglutination inhibition and enzyme immunoassay methods, which may be confirmed by immunoblot method.

Leishmaniasis

Leishmania is a parasite which which accounts for cutaneous or visceral disease. About 30 species of *Leishmania* exist, 21 of which can cause human infection. The different species can be distinguished by specific antibody testing or molecular methods. The disease is prevalent in the Middle East and parts of Africa, Asia, Central and South America. In cutaneous leishmaniasis, one or more sores develop, commonly on the face, which eventually leave a scar. Sores may be painful or painless. Some persons develop regional lymphadenopathy. In visceral leishmaniasis, children are most frequently affected; it is a disease of the reticuloendothelial system, usual presentation fever, weight loss and hepatosplenomegaly. The parasite is transmitted by the small sized sandfly which after biting the infected animal, usually a rodent or dog, makes its way to bite human skin, subsequent to which skin sore(s) develop within a few weeks after being bitten while visceral leishmaniasis can develop months or perhaps years after infection. Visceral leishmaniasis is usually seen in India, Nepal, Sudan and Brazil. Cutaneous leishmaniasis, if untreated, may leave ugly scars. Rarely, it may spread to nose or mouth leading

to mucocutaneous leishmaniasis; this form of the disease is usually seen in South America.

Diagnosis: In smears from cutaneous lesions, amastigotes are detected. The samples are best taken from the viable margin of the lesion, through a small incision or, preferably, by fine needle aspiration whenever possible. Leishman or any Romanowsky stain will be suitable. In visceral leishmaniasis, buffy coat layer, aspirates from lymph node, bone marrow, spleen or liver may yield a positive result.

Culture may be made in Novy-McNeal-Nicolle medium or in Schneider *Drosophila* medium with fetal calf serum supplement. Cultures yield promastigotes in a few days and should be held for four weeks. However, sensitivity of culture does not exceed 60% while all methods combined do not exceed 70% sensitivity.

Malaria

Malaria is a disease caused by *Plasmodium spp.* Four species cause human malaria: *P. vivax*, *P. falciparum*, *P. malariae* and *P. ovale*. *P. vivax* is the commonest species in man. Malaria is prevalent worldwide and although the World Health Organization had attempted to eradicate malaria, the disease remains a major health problem in regions between 45° North and 40° South. Emergence of mosquitos resistant to insecticides has posed a formidable problem. Resistance to anaphylactic drugs poses another problem. Persons with sickle cell trait are less susceptible to *P. falciparum*. Malaria can be transmitted via transfused blood or placenta. Travelers to endemic zones are advised prophylaxis, since *P. falciparum* is potentially fatal. The classical presentation of malaria is fever and chills often accompa-

nied by splenomegaly. Initially febrile attacks are irregular but later develop characteristic patterns: tertian in *P. vivax*, *P. falciparum* and *P. ovale* infections or quartan in *P. malariae* infection. Asexual stage lasts 48 hours in tertian and 72 hours in quartan malaria. Hemolytic anemia develops, sometimes with other manifestations such as headache, muscle pain, abdominal pain or diarrhea. *P. falciparum* can lead to severe hemolysis with hemoglobinuria and marked anemia (blackwater fever). It can cause widespread capillary thrombosis and severe anoxia; cerebral malaria is a well recognized complication of *P. falciparum* resulting in coma and often death. Exchange transfusion is urgently required and can be life saving. Therefore, untreated fatal cases are mostly caused by *P. falciparum*. Otherwise, malarial infection may lead an indolent course with febrile attacks which come and go. Intervals of attacks may be long, in terms of months or years, in *P. vivax* and *P. ovale* infections. In *P. falciparum* and *P.* malariae infections, symptoms tend to vacillate to a small extent due to persistent low grade parasitemia. Relapses in *P. vivax* and *P. malariae* result from reinvasion of blood from infected liver cells.

Malaria parasites pass through a sexual stage in the mosquito *Anopheles* and an asexual stage in humans. In the mosquito it produces infectious sporozoites and in humans it produces schizonts and merozoites. The latter develop into gametocytes in the blood of infected persons, whereby the female mosquito pierces skin and sucks the gametocytes from the blood of an infected person. In the mosquito gametocytes mature into male (micro-) and female (macro-) gametes, which in turn fuse, followed by migration outside the mosquito's stomach wall to form an oocyst, in which

many sporozoites are formed. On rupture into body cavity, sporozoites migrate through tissues into the salivary gland from where they are injected into the host via the mosquito's bite.

Diagnosis: Malaria parasites are detected in erythrocytes of thin or thick blood films. The best time to collect blood is just before or at the beginning of a febrile paroxysm. In *P. falciparum*, only rings and /or gametes are found in the peripheral blood. In each of the other three species, all stages of the parasite are found in a single blood film. In *P. falciparum* and *P. malariae* the size of infected red cells remains unchanged, while in *P. vivax* and *P. ovale* they are enlarged. In *P. falciparum* the rings are small and show two chromatin dots with crescent shaped or elongated gametes. In other types trophozoites are either rounded (*P. malariae* and *P. ovale*) or ameboid, that is large and irregularly shaped (*P. vivax*). Pigment tends to be black in *P. falciparum* infection. Presence of Schuffner's dots excludes *P. falciparum* and *P. malariae*. Schuffner's dots are small pink granules that occur in red cells infected by *P. vivax* and *P. ovale*. Multiple infection of same red cell is frequently seen in *P. falciparum* and only rarely in *P. vivax* infection. Mixed infection occasionally occurs and such diagnosis should be made in a specialized center. Caution should be made not to confuse artefacts for malaria parasite; they include platelets, bacterial clumps or precipitated stain. Serologic tests, including complement fixation, indirect hemagglutination inhibition or immunofluorescence antibody testing, are available that identify *Plasmodium* species, which may be used in tracking infected blood donors or confirming diagnosis in a recently treated febrile illness. However, such tests do not distinguish active from

past infection. In chronic malaria, tests for ANA and VDRL may be falsely positive.

Schistosomiasis

A snail-mediated disease, schistosomiasis or bilharziasis is caused by the trematode *Schistosoma* which is prevalent in tropical and subtropical regions in the world. In the Middle East, it is specially prevalent in Egypt, Saudi Arabia, Oman, Yemen and Iraq. *Schistosoma hematobium* affects urinary tract while *Schistosoma mansoni* affects intestinal tract.

Schistosoma hematobium inhabits vesical and pelvic veins causing hematuria. Adult female worm is 26×0.5 mm and the male is slightly shorter. Adult schistosome deposits its eggs in vesical, ureteric or urethral wall some of which pass out into urine or may penetrate rectum to pass with feces to enter water in the environment where the eggs hatch into larvae (miracidia) which in turn require an intermediate host, the snail belonging to the genus *Balinus*, to continue its development or otherwise larvae will die. In the snail, *S. hematobium* or *S. mansoni* produces sporocysts which develop into cercaria, 0.2mm long, that are free swimming forms which can survive in contaminated water for 1-3 days. If they come in contact with a human they may penetrate skin and produce mild dermatitis (swimmer's itch).

Cercaria then travel via blood to liver where adult schistosome worm develops and in turn migrate to vesical or pelvic venous plexus.

Toxemia develops about one month after infection by cercariae with fever, pain and urticaria; eosinophilia is seen. Several months later localized symptoms develop due to presence of *Schistosoma* eggs. In *Schistosoma hematobium*

infection, hematuria and painful micturition occur and in chronic cases irreversible obstructive lesions of ureters and kidneys may develop. Clinical symptoms vary between individuals. In *Schistosoma mansoni* infection, acute toxemic phase is more severe than in *Schistosoma hematobium* infection. Fever, nausea, vomiting, diarrhea, abdominal pain and tenderness occur with a dry cough, dyspnea and urticaria; eosinophilia is also more marked than in *Schistosoma hematobium* infection. Toxemic symptoms are not usually seen in persons living since birth in endemic zones so that the disease in those persons develops insidiously into a chronic phase. The latter is represented by hepatic fibrosis leading to portal hypertension and development of esophageal varices. Bleeding esophageal varices constitutes the most common cause of death in those patients. Some cases develop numerous colonic polyps with severe diarrhea, or large inflammatory masses in the intestines may be seen, mimicking malignant tumors. But in general most infected persons do not suffer severe effects.

Diagnosis: Schistosoma hematobium eggs with terminal spines are found best in a midday urine sample at the time of peak urinary egg excretion. Eggs are 110-170 µm long and 40-70 µm wide. Successful treatment is marked by disappearance of not only eggs but also of hematuria. Serological tests include complement fixation test, indirect hemagglutination, indirect immunofluorescence and radiometric methods.

Schistosoma mansoni eggs in direct fecal smear exhibit lateral spine is relatively insensitive and is superceded by concentration method of examining stool specimens with repetition three times before recording a negative result in suspected cases. Rectal biopsy is a useful method for diag-

nosis when fecal examination is negative. Fresh rectal mucosal specimen is crushed on a glass slide and examined under the microscope. Serological tests for *S. mansoni* are the same as described for *S. hematobium.*

Trichomonas vaginalis

A common cause of vaginitis, *Trichomonas vaginalis* causes prurutis with vaginal discharge and occasionally dysuria. Males develop urethritis-prostatitis with balanitis but infection may be asymptomatic. The protozoan is transmitted by sexual intercourse.

Diagnosis: Direct wet smears from vaginal discharge, first specimen urine or prostatic fluid, show small actively motile and flagellate propelled parasite. In view of the low sensitivity of direct wet mounts, culture may be attempted or immunoenzyme or immunofluorescence testing can be used. Pap smears are not reliable in finding the parasite.

Trypanosomiasis

Trypanosomiasis is caused by one of two *Trypanosome* species, namely, *T. brucei* and *T. cruzi*. The former is known to occur in Africa and the latter in America. *T. brucei rhodesiense* is transmitted by the tse tse fly *Glossina* whereby a transient chancre develops at the site of the bite. A rapidly developing fever with lymphadenopathy is followed by central nervous system involvement and death. *T. brucei gambiense* classically causes sleeping sickness. It begins with intermittent fever, night sweats and generalized weakness. Lymphadenopathy develops and in time central nervous involvement leads to drowsiness, confusion, stupor, coma and death. *T. cruzi* causes Chagas' disease,

which may be acute or chronic. Acute disease is commonly seen in young children, characterized by malaise, chills, fever, hepatosplenomegaly and myocarditis. Tissue swelling at site of inoculation is seen. Romana's sign is a unilateral swelling of the eyelid following penetration of *T. cruzi* through mucous membranes and is an early diagnostic sign of Chagas' disease. In older persons, the acute phase is less severe and may be asymptomatic. Chronic disease manifests with megaesophagus, megacolon and cardiac dysfunction due to parasympathetic conduction defects. Chronic disease may be exacerbated by immunosuppressive status.

Diagnosis: Geographic and clinical history with relevant clinical findings should suspect the diagnosis. Detection of parasites is made in thin and thick blood films, or in buffy coat, lymph node or bone marrow aspiration or in CSF. In the acute phase, smears from sites of insect bites (chagomas) may also be examined. Films are stained with Giemsa or other Romanowsky stain. Culture or animal inoculation may be tried to establish the diagnosis. Culture may be made in Novy-McNeal-Nicolle medium. In chronic cases, serologic tests for diagnosis include enzyme immunoassay, immunofluorescent and complement fixation methods. However, such tests cannot distinguish acute from chronic phase of the disease.

SECTION 6.

SEROLOGY

SEROLOGICAL TESTS IN USE FOR BACTERIA, VIRUSES & FUNGI

For individual tests, also refer to Tests list, Collection of samples for microbiology and Panels in important clinical situations.

Introduction

Specimens for viral antibodies are collected in both the acute and convalescent phases of infection. Acute phase covers the first week after onset of infection and convalescent phase starts at least one week after the acute phase falling 14-28 days after onset of the infection. Serological diagnosis of a viral infection relies on a 4-fold rise or more of the antibody titer during the convalescent phase.

Diagnosis of a viral infection can be made at 5 different levels: First, *viral culture*, which is the diagnostic test of choice when it is available. Second, *antigen detection*, using immuno-enzyme, immuno-chemolumenescent, fluorescent antibody and other techniques. Third, *serological tests*, which include demonstration of IgM antibody or a significant rise in IgG antibody in convalescent compared to acute phase. Fourth, *molecular tests*, employing DNA or RNA

probes and techniques. Fifth, *electron microscopy*, which has been used in conjunction with culture in the diagnosis of smallpox.

The following is a practical list of how to approach the diagnosis of various types of viral infection:

(a) Cell culture

Adenovirus (Respiratory system, Eye), *CMV, Enteroviruses* (Coxsackie, Echo, Polio) *Herpes simplex, Influenza & Parainfluenza, Measles, Mumps, Varicella zoster.*

(b) Antigen detection

Adenovirus (Enteric), *Hepatitis B & D, HIV, Influenza A & B, Measles, Respiratory syncytial virus, Rotavirus.*

(c) Serology

Arboviruses, EBV, Hepatitis A, B, C & D, Human Herpes Virus Type 6, HIV, Measles, Mumps, Parvovirus, Rubella.

Avian Influenza Virus

At the time of writing this book, an 'avian influenza virus' pandemic is feared to be inevitable and possibly imminent.

Definition: Avian influenza is a contagious viral infection which affects all species of birds. It is caused by type A strain of the influenza virus which was initially identified more than 100 years ago in Italy. The virus is 80-120nm in diameter and contains 8 strands of RNA. 15 subtypes of influenza virus are known to infect birds. To-date, all outbreaks in birds have belonged to subtypes H5 and H7.

Viruses of low pathogenicity can mutate to be more virulent. Influenza virus subtypes are genetically labile and may swap or interchange (reassort) genetic material to produce a genetic shift, resulting in novel subtypes of the virus; this can occur on an intra-species or inter-species basis. Besides birds, pig and man can become infected. The offender in humans, so far, has been reported to be influenza virus strain A subtype H5N1, which poses a serious danger since it mutates rapidly, acquires genes from viruses infecting other species and is able to cause severe disease in man with a high mortality rate.

Epidemiology: Outbreaks in poultry were reported from Pennsylvania, USA, Australia, Pakistan, Mexico, Italy, China, Netherlands and Belgium between the years 1983 and 2003. Currently, it is reported to be affecting poultry in Japan, China, Korea, Laos, Vietnam, Thailand, Cambodia and Indonesia. The virus is transmitted to man by direct contact with infected poultry, dead or alive, but no man-to-man transmission has not been documented. Previously known only to infect birds, avian influenza virus strain A type H5N1 was first isolated from a Hong Kong child in 1997, who subsequently died of Reyes syndrome during an acute respiratory illness. The World Health Organization issued on February 6th 2004 a confirmed list of 20 and 16 known human cases of avian influenza virus A type H5N1 infection in Thailand and Vietnam, 15 and 11 of whom died, respectively. It is believed that such lists will grow with time.

However, mass culling of flocks of poultry affected plays an important part in containing the spread of the virus. Adequate surveillance systems by affected countries and scientific bodies is required. In affected zones, avoidance of

live poultry and their droppings (on the egg shell) is advised. Hygienically prepared cooked poultry and eggs is mandatory. Traveling to hit areas does not pose a risk to the traveler if adequate precautions, as aforementioned, are taken.

Clinical features: Initial clinical presentation is not different from ordinary influenza. Patients develop fever, sore throat, cough and, in several fatal cases, severe respiratory distress syndrome and viral pneumonia. Renal failure may ensue. Previously healthy persons, as well those with pre-existing chronic disease, may be affected. Published data on the clinical course of this disease are, so far, limited. However, infection with H5N1 avian influenza appear to be associated with high morbidity and mortality rates. No vaccine is currently available to protect humans against H5N1 type infection. However, research in this direction is underway on a prototype strain, remembering the great tendency for this virus to mutate. Treatment is as for any viral illness. Antiviral drugs may be useful, such as amantadine (Symmetrel).

Specimen collection: Detection of viral antigen and viral isolation is best made on samples taken soon after, or within 3 days, and not later than 7 days after onset of symptoms. Samples should be refrigerated and processed within 1-2 hours. For viral isolation, inoculation of susceptible cells is made immediately after sample collection or, alternatively, stored in deep freeze below -70 C. An acute phase serum (3-5ml of whole blood) should be collected within the first week after onset of symptoms and a convalescent phase serum 14 days after onset of symptoms. When patients are near death, an additional sample is taken. Stan-

dard precautions should be always followed in collection and handlings of such specimens.

Upper respiratory samplings are taken from nose, nasopharynx and throat. Swabbing should be vigorous; wash specimens of nose and nasopharynx may also be collected.

Lower respiratory tract samplings include tracheal aspirate, bronchoalveolar lavage, lung biopsy and postmortem respiratory tissue. Virus transport medium contains veal broth, bovine albumen, gentamicin and amphotericin B in sterile, distilled water.

Diagnosis: Tests available are rapid and reliable. Serological diagnosis, on acute and convalescent sera, can be made by a hemagglutination test. ElLISA test kits are also commercially available; they yield occasional false positive results. Agar gel precipitation test may be performed although this test method is non-group-specific. Direct detection of viral antigen can be made by immunofluorescent method. Viral isolation in chick embryos can be used to confirm the diagnosis.

Brucella

A zoonotic disease, brucellosis in man is caused by *B. melitensis, abortus, suis and canis* with the first two the most common and *B. melitensis* as the prevalent species in Jordan. Endemicity of the disease in Jordan was first proved by culture in 1986 (Dajani YF & Halabi MK. 19 culture positive cases of brucellosis in Jordan. *Bulletin of the Consulting Medical Laboratories* 1986; 4 (3): 1-2). The disease remained highly endemic during the 1980s in the region

around Jordan, with seasonal variation well documented (Dajani YF, Masoud AA & Barakat H. Epidemiology and diagnosis of human brucellosis in Jordan. *Journal of Tropical Medicine and Hygiene* 1989; 92: 209-214); then a decreasing incidence with downward trend in a 9-year longitudinal study was reported (Oumeish OY, Saliba E & Dajani YF. The 5th International and Pan Arab Seminar on Leishmaniasis and other Zoonoses. *Bulletin of the Consulting Medical Laboratories* 1996; 14 (1): 4). Fall of brucella cases was related to a nationwide REV1 vaccination program for animal livestock initiated in June 1990.

Culture remains the mainstay for a definitive diagnosis of brucellosis and guides antibiotic therapy and emergence of resistant strains. Cultures are usually taken from blood during high fever, bone marrow or any suspected body fluid or tissue. Special culture technique is required.

However, serological tests are invaluable in clinical practice:

Screening test Rose Bengal test is a quick 2-minute test which is highly sensitive. However, prozone phenomenon poses a problem while cross reaction with *Salmonella* is much less frequent than in tests for *Salmonella*.

Agglutination titer A titer of 1/160 is taken as potential positive while in endemic zones the limit is pushed up to 1/320. Cross reaction is seen with *Salmonella*, *Yersinia*, *Cholera and Francisella tularensis*.

ELISA test Commercial preparations are now readily available that test for IgM and IgG antibodies to *Brucella*. Values are reported in index units. Results falling in the marginal zone should be repeated on a fresh sample for

confirmation. The ELISA test is sensitive and specific. Molecular PCR testing can also be used.

Chlamydia (Psittacosis)

Chlamydial (psittacosis) serology testing can be made for antibodies or antigens.

Antibody tests include complement fixation (CFT), immunofluorescence (IF) or ELISA. Presence of antibody indicates past infection, while a fourfold or greater rise between acute and convalescent sera indicates recent infection. However, antibodies tend to remain persistently elevated, while recurrent mild genital affection may not cause a rise in the antibody level. Moreover, CFTs often do not differentiate between *Chlamydia* species but are useful in the diagnosis of lymphogranuloma venereum. IF and ELISA tests are sensitive; they identify IgM and IgG antibodies and are useful in the diagnosis of chlamydial pneumonia.

Antigen tests include enzyme immunofluorescence (IF), immunoassay (EIA) or molecular testing by PCR. They detect organisms whether dead or alive and are performed on samplings of tissue infected by *Chlamydia*. EIA test shows 60-98% sensitivity but is highly specific, reaching 90-100%. This test is acceptable for eye and nasopharyngeal but not rectal samplings. IF test is used for genital and rectal as well as oculo-pharyngeal swabs. IF test shows 70-100% sensitivity depending on represented samplings and 80-100% specificity. This test is useful for nasopharyngeal and conjunctival specimens. Both EIA and IF are not rec-

ommended for rectal or vaginal samplings from children suspected of sexual abuse.

Clostridium difficile

Responsible for pseudomembranous colitis.

Latex fixation and ELISA tests are available though not as sensitive as culture, but may serve as initial screening tests in suspected cases of pseudomembranous colitis. Besides culture, tissue culture cytotoxicity assay is the diagnostic test on stool specimen. Besides laboratory tests, endoscopy and radiology are used in the diagnosis of the aforementioned condition.

CMV

CMV may be tested directly, identifying intranuclear inclusion in infected cells, culture of which is not practicable, or by serological tests. The latter in current use include indirect hemagglutination, ELISA, radio-immunoassay, immuno-fluorescence and chemolumenescence. The latter method is recommended for routine use because of its accuracy, speed and practicality.

Presence of IgM indicates current infection. However, CMV IgM antibodies can persist for many years. IgG antibody levels fluctuate in normal individuals but a four-fold increase or more is indicative of active infection.

Immunocompromised patients may have generalized and fatal CMV affection. Congenital CMV infection is probably the most common acquired fetal infection. The fetus can be

infected in an exacerbated episode of long standing CMV infection in the mother. IgM in the fetus indicates current infection. CMV IgG crosses the placenta and remains detectable up to 3 months after birth, but a four-fold rise in a newborn indicates a congenital infection. Most affected infants are asymptomatic while some develop hepatomegaly with elvated liver enzymes and jaundice.

False positive results occur due to ANA and EBV infection.

Epstein-Barr Virus (EBV)

The old hemagglutination test for heterophil antibodies using horse red cells has become obsolete and its details are outside the scope of this guide. Culture of throat swab or leukocytes are theoretically possible but not available in clinical practice.

Monospot test is an agglutination test that uses sheep or horse red cells. The test is positive in EBV infection, serum sickness and as a manifestation of a nonpathological phenomenon termed Forssman antibodies. Persistence after absorption with guinea pig kidney suggests infectious mononucleosis, with beef red cells suggests Forssman antibodies, while by both suggests serum sickness.

Sensitivity of the test is high. Tests for specific antibodies to EBV have superceded the monospot test.

EBV antibodies are:
(1) *anti-VCA* (anti-viral capsid antigen) IgM & IgG.
(2) *anti-EA* (anti-early antigen).
(3) *anti-EBNA* (anti-EB nuclear antigen).

Methods used to detect EBV antibodies include fluorescent, ELISA and more developed techniques such as chemo-lumenescent method.

EBV causes infectious mononucleosis. It is implicated by collaborative data in the epidemiology or etiology of Burkitt lymphoma and nasopharyngeal carcinoma. EBV antibodies testing detects 10% of cases missed by the monospot test.

EBV antibodies reach high levels in the acute phase of the disease, anti-VCA IgM peaking during the second week of the illness and disappears after 2-3 months. As with other infection, a four-fold increase of anti-VCA IgG antibodies indicates active disease. Anti-EA antibodies indicate active infection. Anti-EBNA antibodies start rising in the second month of illness and persist for life.

Clinical presentation of infectious mononucleosis includes mailaise, fever, sore throat and lymphadenopathy. Mild to moderate hepatitis and splenomegaly may develop. In a minority, pharyngitis and lymphadenopathy are seen.

Blood film shows reactive lymphomonocytoid cells or Downey cells which, to the novice, appear worrying. Such cells are also seen in other viral infections such as CMV, hepatitis, rubella, mumps and so on as well as in toxoplasmosis and drug induced hepatitis.

Complications include serious *neurological* sequelae such as meningoencephalitis or *cardiac* such as myocarditis, *respiratory* such as interstitial pneumonia, *renal* such as nephritis, *hematologic* such as hemolytic anemia, disseminated intravascular coagulation etc., *gastro-intestinal* such

as hepatitis, pacreatitis and so on, *immunologic* such as fatal X-linked lymphoproliferative disorder.

Fungi

Invasive fungi may occur in normal or immunocompromized individuals, including *Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis, Cryptococcus neoformans, Pneumocystis carinii, Aspergillus* and *Candida* species. Serological tests are useful in the diagnosis of some of the aforementioned infections.

For *Candida* antigen and antibody testing is unreliable and diagnosis is made by direct examination and culture.

Tests for antibody show 50% sensitivity and include RAST (Radioallergosorbent test), Counter immunoelectrophoresis and double diffusion. Tests for antigen include latex agglutination, radioimmunoassay and enzyme immunoassay. As with antibody testing, antigen testing is not sufficient to be of clinical value.

For *Aspergillus* immunodiffusion method is the most popular and may aid in the diagnosis of allergic bronchopulmonary aspergillosis, with a >90% sensitivity. For non-invasive disease, complement fixation test, IgE and IgG antibodies to *Aspergillus fumigatus* are detectable. In addition, blood eosinophilia and elevated serum IgE are found. Clinically, the condition presents with episodic bronchospasm, pulmonary infiltrate and central bronchiectasis.

For *Blastomyces dermatitidis* direct examination and culture are the methods of choice. Various antibody tests are available including complement fixation, immunodiffusion,

enzyme immunoassay, western blot and radioimmunoassay, reportedly with a sensitivity for the first of 40% and the second method 65%, while each of the others are reported to be >80% sensitive.

For *Coccidioides immitis* are available but not specific. They may be done on serum or CSF. Methods include precipitin IgM and complement fixation IgG tests, latex agglutination (6% false positive rate), immunodiffusion and counter immunoelectrophoresis. IgG antibody titer of 1:16 or over suggests disease. Increasing titer is diagnostic. Precipitin IgM antibody is detected in 90% of cases during second or third week of illness. Direct testing includes microscopy which shows the diagnostic spherules. It should be noted that infection is asymptomatic in 60% of cases.Infection usually affects the lungs but may spread to involve any organ.

For *Cryptococcus neoformans* latex agglutination test detects antigen in more than 90% of cases. False positives occur in other infections while a negative result does not exclude infection. It is a common infection in AIDS patients affecting 7% of cases. It enters via lungs and commonly infects CSF producing meningitis. Culture of the organism can be made and histopathology reveals the encapsulated organism. Antibody testing is not useful for diagnosis.

For *Pneumocystic carinii,* direct stain by Grocott-Gomori, toluidine blue or Giemsa are useful. ELISA test for IgG can be used; latex agglutination test for antigen is available but unreliable. Molecular PCR testing can also be used.

Pneumocystic carinii pneumonia is the most frequent cause of life-threatening opportunistic infection in AIDS patients, with fatality rate reaching 50%.

For *Histoplasma capsulatum* complement fixation antibody and antigen testing may be useful. The former is often used for diagnosis with a sensitivity of 80% or more. The latter detects antigen in urine, serum or CSF and allows rapid diagnosis but false positive results occur with other fungal infections. Alternatively, immunodiffusion test detects H and M antigens present in active histoplasmosis. Radioimmunoassay and enzyme immunoassay can be used to detect IgM, IgA and IgG antibodies. But antibody tests may be falsely negative or false positive results may be seen in other fungal and tuberculous infection.

Gonococcal Antigen Assay

This test uses an EIA method and is used on cervical and urethral swabs only. The test takes less than one hour and is highly sensitive, though less sensitive than cervical culture. It is unfit for use as a screening test in the general population.

Herpes Simplex (HSV)

Herpes simplex is probably the most frequent viral infection in man, most of no clinical import. Two viral strains exist, namely, Types 1 and 2. HSV 1 affects upper half of body, most frequent transmission through kissing and Type 2 causes venereal disease through sexual contact. Another

mode of transmission is longitudinal during normal birth by an infected mother.

Serological tests include immuno-fluorescent and chemolumenescent methods, while culture on human embryo or Hela cells can be done but is not readily available in clinical laboratories.

In genital infection, incubation period ranges from 1-4 weeks. Ulcers heal within 3 weeks. Recurrence is spontaneous and not due to reinfection. Acyclovir orally is a first line treatment.

In neonates, infection may be localized to skin, eyes or mouth, cause encephalitis or disseminate leading to potentially fatal consequences such as disseminated intravascular coagulation or pneumonia. Rarely adults can develop encephalitis due to HSV-1 leading to coma and death in a substantial proportion of cases.

Human Immunodeficiency Virus (HIV)

HIV is an RNA retrovirus which has gained great fame at the end of the 20th century. It has become a world problem despite its low infectivity, largely due to its vast prevalence and drastic consequences in affected individuals. This has been potentiated by changing life style and deviational behavior by certain groups who are not commonly accepted by the mainstream of many societies on this planet. The virus is transmitted by exposure to certain body fluids, especially blood and genital secretions. It is most commonly seen in homosexuals and drug users but is transmitted transplacentally in 50% of infected mothers, while trans-

mission through transfusion of infected blood is not a major problem due to strict screening measures undertaken by blood banks. HIV-1 has been reported worldwide while HIV-2 mainly in West Africa and some European countries.

Screening for HIV-1 and HIV-2 is simultaneously made by ELISA (Enzyme linked immunoassay) test and is confirmed by Western blot test. A negative result does not rule out HIV infection, since the incubation period lasts for up to 6 months. A recent influenza shot can cause a false positive reaction in the ELISA but not in the Western blot reaction. In the ELISA test, core and envelope proteins of HIV-1 and HIV-2 are both tested. A reactive result should be retested in duplicate to confirm a reactive result. PCR is an essential tool and HIV viral load is used to detect very low levels of the virus and is routinely used for monitoring response to treatment.

Immune response to HIV

The Antigens: In the first 1-3 weeks, viremia develops and blood becomes reactive to p24 antigen and PCR (polymerase chain reaction) test for HIV becomes positive. The reaction peaks at 6 weeks and then falls down to very low levels at 12-24 weeks, which may pose difficulty in detecting viral activity in peripheral blood during this period of time, during which HIV viral activity can be detected in lymph nodes. This so-called period of latency may last up to ten years.

The Antibodies: Antibody response starts at about 6 weeks and peaks at 12-24 weeks. The antibodies react against multiple HIV antigens and their function is still unclear. The best studied antibodies are those against envelope antigens gp160, gp120, p88 and gp41, which can be either pro-

tective or pathogenic. Antibody to gag protein (p24, p17 and p55) is the first to appear. Appearance of p24 antibodies associates with very low level of p24 antigen in blood (Figure 1).

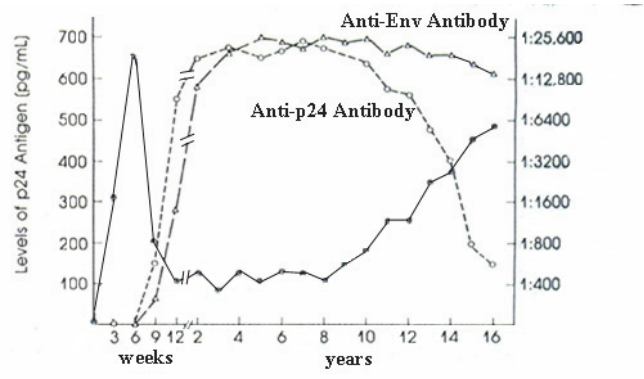

Figure 1.

Lymphocyte Cell Response: CD4 counts in peripheral blood falls gradually with progress of time. The slope of this decline is highly predictive of the clinical course and development of the advanced stages of the disease (Figure 2). CD4:CD8 ratio gets depressed with progress of the disease. Total lymphocyte count in peripheral blood also falls, and a critical level of 800 calls for alarm in susceptible individuals. Severe cases show lymphocyte counts below 400.

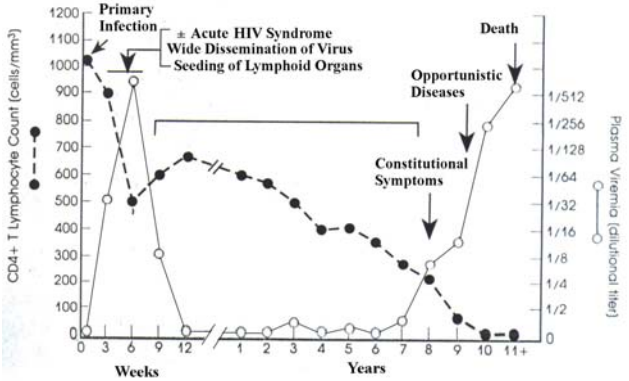

Figure 2.

Legionnaire's Disease

The disease is caused by *Legionella pneumophilia* and related species.

Culture in sputum or bronchio-alveolar lavage or lung needle or tissue samplings are highly sensitive (80-90%) and 100% specific, while in blood sensitivity is low but with absolute specificity.

Serological methods include *indirect* IF method for *antibody* in blood and *direct* IF method for *Legionella organism* in tissue, for example lung, sputum, pleural fluid and pus (25-80% sensitivity and 95-99% specificity). An enzyme immunoassay (EIA) test is also available for detecting *Legionella* antigen in urine. The EIA test may be performed after initiation of antibiotic therapy; the method

is 80-99% sensitive and 99% specific and *Legionella* excretion is concentrated in the urine, persisting for several weeks after infection.

During the first week of infection, antibody level tends to be low and rises during second or third week. Antibody levels remain elevated for years following recovery. Also, cross reaction occurs with other gram negative organisms and tuberculosis.

Lyme Disease

Lyme disease is caused by the spirochete *Borrelia burgdorferi*. The organism is transmitted via a tick vector. Lyme disease is a clinical diagnosis supported by serological testing. A characteristic skin manifestation is "erythema chronica migrans", also acrodermatitis chronica atrophicans and cutaneous lymphoid hyperphasia. Spirochetes can be demonstrated by silver stain. It can cause infectious arthritis and organisms may be detected in synovial fluid by PCR. The heart and nervous system may be involved with response to antibiotics.

The most common serological test used is the ELISA method, by which IgM rises initially to peak at 3-6 weeks while IgG rises later. However, the test shows variable sensitivity due to lack of standardization of preparations used. IF test was replaced by ELISA method. Like for HIV, Western blot method can be used to confirm a positive ELISA test result.

Culture and direct identification of the organism by using monoclonal probes can be done and they show poor sensi-

tivity due to poor recovery rate. PCR is used as a research tool only.

Mycoplasma pneumoniae

A common cause of upper and lower respiratory tract infection. See 3.66 and 67.

Mycoplasama pneumoniae is a frequent cause of tracheobronchitis in children and adults with epidemics seen especially in the autumn. Pneumonia occurs in one-third of infected persons. Infection manifests by gradual onset of fever, malaise, headache, upper respiratory symptoms with persistent dry cough. Lower lobe pneumonia appears on chest X-ray. The illness may require hospitalization because of severity of symptoms. Complications include pleural effusion, lung abscess and secondary bacterial pneumonia. Hemolytic anemia may develop and typically very high serum cold agglutinin titers are detected. Other complications include erythema multiforme, erythema nodosum, urticaria, peripheral neuritis and arthritis.

Diagnosis can be rapidly made by detection of cold agglutinins in serum, detected 7-10 days after infection, confirmed by elevated level of IgM antibody. A 4-fold rise of IgG antibody between acute and convalescent serum levels is also diagnostic. Isolation by culture is laborious and takes several weeks so that it is not commonly used in clinical practice.

Pertussis

Presence of *Bordetella pertussis* can be detected by direct IF method, in which 3 or more viable organisms are identified in nasopharyngeal secretions. Alternatively, IF antibody testing is more rapid but more elaborate requiring specially trained personnel. However, the IF antibody method has high false positive and false negative rates.

Rocky Mountain Spotted Fever (RMSF)

RMSF is caused by *Rickettsia rickettsii* transmitted to man via a tick bite. The organism enters blood stream reaching vascular endothelium where it causes systemic vascular damage. It is prevalent in certain geographic zones in America and was named after Rocky Mountains although it is only rarely seen there. Rickettsial antibodies are first detected after the first week of infection. Fatality rate of 10% is usual. Treatment is with tetracycline or chloramphenicol which should be promptly started on suspicion of a case without waiting for test results.

Rapid diagnosis can be made by a full-thickness skin biopsy with frozen section subjected to fluorescent labeled antiserum to *Rickettsia rickettsii*. LDH is invariably elevated in this disease.

Serological tests include latex agglutination, indirect hemagglutination, and indirect IF methods. Sensitivity is reported for the aforementioned tests as 71-94%, 91-100% and 94-100%, respectively, with specificity of 96-99%, 99% and 100%, respectively. A fourfold increase in anti-

body level indicates recent infection. A single IF titer of 1:64 and higher is considered to indicate active infection.

Rotavirus

Rotavirus is the most frequent cause of viral gastroenteritis, accounting for about 70% of cases in infants and young children 3 months to 2 years old with peak incidence in winter. Incubation period is 3 days and diarrhea lasts for about one week with vomiting and fever. In infants the infection is commonly mild but severe case rarely occur with severe diarrhea and serious complications such as necrotizing enterocolitis, intestinal perforation and even death.

Viral tests include culture which is difficult, direct electron microscopy, immune electron microscopy and serologic tests, namely, latex agglutination and ELISA methods. The latter has 95-100% sensitivity and specificity, while the latex test is less sensitive and specific.

RSV (Respiratory Syncytial Virus)

Direct tests are available either by *culture of the organism* on HEp-2 cells, monkey kidney, human fibroblast or other cell line, or by *testing for antigen* can be made using ELISA or direct IF method. Samples are obtained from respiratory epithelium, upper or lower, and bronchoalveolar lavage.

Sensitivity of the ELISA method varies between 75-95% and specificity between 5-100%, while for the direct IF method sensitivity and specificity are generally between 85-

95%. Antigen detection is best attempted at the time when abundant, namely, in early infection. RSV presents clinically as a common cold. However, it may cause bronchopneumonia. The illness is more severe in premature infants or immuno-compromised patients. Antiviral therapy with ribavirin is indicated in some patients.

Rubella

Rubella or German measles is a mild acute viral infection which most children acquire. However, first exposure during the first trimester of pregnancy is associated with fetal infection leading to abortion, stillbirth and congenital anomalies - the Rubella syndrome. The earlier the infection, the higher risk of fetal affection, with a 50% risk during the first month falling to 10% risk during the second or third months of pregnancy. Rubella syndrome is characterized by cataract or glaucoma, congenital heart disease, hearing loss and pigmentary retinopathy. Associated features include mental retardation, microcephaly, splenomegaly, jaundice, purpura and translucent bones.

Detection can be made by ELISA and other methods described for Toxoplasma. Presence of IgM antibodies indicates active infection. A four-fold rise in IgG antibody titer also indicates acute Rubella infection. Immune status of pregnant women exposed to actively infected cases may be sought. An IgG titer indicates immunity while susceptible pregnant women and those who are at risk of exposure, such as hospital workers, should be vaccinated.

Salmonella

Widal test

Agglutination testing for Salmonella O (somatic) antigen and H (flagellar) antigen may show a rise following typhoid vaccination or infection. The cut off point for a positive titer may be regarded as 1:32 or 1:64, but there are two essential points to consider.

Firstly, a rise in H antigen is of no significance and may occur with any inflammatory condition. A significant rise in O antigen is more meaningful but cross reaction is known to occur, especially with Brucella infection. It should be stressed that a positive Widal test is not diagnostic of typhoid; culture is the golden standard. A lot of overdiagnosed cases in practice are seen in practice due to lack of understanding and overplay on the role of the Widal test in making a diagnosis of typhoid.

SARS (Severe Acute Respiratory Syndrome)

Definition: SARS is a severe acute respiratory illness caused by a previously unrecognized human coronavirus, called SARS-associated coronavirus (SARS-CoV). It is characterized by fever, severe respiratoty symptoms and pneumonia. However, it is possible that other pathogens, such as human metapneumovirus, might have a role in some cases of SARS. A dual infection was noted in some Canadian patients. It is transmitted by airborne and contact infection. However, the mode of transmission has still to be more precisely defined.

Specimen collection: The earlier collection the better, since viral load diminishes with time. 3 types of specimens are collected from the upper respiratory tract, namely, nasopharyngeal wash or aspirates, nasopharyngeal swabs and oropharyngeal swabs. Nasopharyngeal aspirate is the specimen of choice especially in small children. From the lower respiratory tract, 3 types of specimens are also collected, namely, broncho-alveolar lavage, tracheal aspirate and pleural tap. The specimen is divided in two parts: one spun and pellet fixed in formalin and the other retained capped in a sterile container. Blood samples are collected in two containers, plain and EDTA bottles. Convalescent serum should be collected at least 4 weeks after onset of fever. 5ml for each of EDTA blood and serum samples are sufficient in adults and 1ml for children.

Diagnosis: Evaluation includes chest roentgenogram, pulse oximetry, blood cultures, sputum Gram stain and culture, as well as testing for viral respiratory pathogens, especially influenza A and B and respiratory syncytial virus. Legionella and pneumococcal urinary antigen testing may be considered. Additional tests may be designed afterward on any samplings saved when required. Acute and convalescent sera should also be ensured.

Currently, 3 different modes of testing are available for SARS-CoV, but the tests are still being refined, while sensitivity and specificity remain uncertain and have still to be determined. It also is not known which tests perform best at which time after the onset of a patient's illness. The following tests are being used:

Serum antibody tests, including both enzyme immunoassay (EIA) and indirect fluorescent-antibody (IFA) methods.

Some do not consider a test positive until more than 28 days after onset of illness. Therefore, a negative test result can be considered a true negative only if the specimen was collected more than 28 days after the patient's onset of illness. For patients with a negative antibody test result whose specimens were obtained 28 or fewer days after illness onset, an additional antibody test should be done on a specimen drawn more than 28 days after onset to determine if they are negative or positive for SARS-CoV. Recommended timing of the second sample may be adjusted as more information becomes available.

Reverse transcription-polymerase chain reaction (RT-PCR). This test can detect SARS-CoV RNA in clinical specimens, including serum, stool, and nasal secretions.

Viral isolation for SARS-CoV. Clinical specimens from SARS patients are co-cultured with well-characterized cell lines, and then evidence of SARS-CoV replication is looked for in these cultured cells.

The number of tests that can be done is limited by the amount and type of specimens and the test type. If there is sufficient specimen, both antibody testing and the RT-PCR are done. Viral isolation is highly sophisticated, time-consuming and cannot be done on all patients.

Interpretation: A positive test result suggests that the patient has or recently had an infection with SARS-CoV. False positive test results occur. As testing becomes more refined, initially false positive results on same samplings from patients may later show negative results. Also, false negative results occur. This can be due to infection by other virus causing similar clinical presentation; for example, half the cases of pneumonia have an undetermined cause. False

negative results may be due to shortcoming of current methodology. Moreover, samples may not have been obtained at a point of time when the test has turned positive. In addition, viral RNA is not always present in collected specimens while antibody tests do not become positive before 28 days of disease onset.

Several laboratories have reported positive test results for human metapneumovirus in some patients with SARS. Human metapneumovirus is a recently recognized virus that belongs to the paramyxovirus family of viruses, which cause a broad range of respiratory and childhood illnesses, including mumps, measles, and croup. Human metapneumovirus is genetically related to respiratory syncytial virus, a common cause of lower respiratory tract infection in children. However, the role of human metapneumovirus, if any, remains undetermined in some cases of SARS.

Streptococcal Antibodies

ASO (Anti Streptolysin O) titer A latex fixation test with <100iu/ml for adults and <200iu/ml for children as the reference range.

A rise over an interval (acute to convalescent) exceeding 3-fold is consistent with an immunologic response to Group A Streptococcus. A rise in antibodies to streptolysin O is a very sensitive test valuable for documentation of streptococcal pharyngitis, which when untreated develops a raised ASO titer in 80% of cases. It is also frequently raised in acute rheumatic fever and post-streptococcal glomerulitis.

A persistent rise in ASO titer indicates a focus of streptococcal infection or complication. In absence of complications, ASO titer falls within 6-12 months.

False positive ASO titers occur in lipemic, contaminated or long standing serum.

Streptozyme test The test uses prefixed sheep red cells coated with Group A streptococcal antigens, DNA-ase, streptokinase, streptolysin O, hyaluronidase and NAD-ase. A positive result indicates serum antibodies to various streptococcal markers. It is positive in post-streptococcal glomerulitis, acute rheumatic fever, streptococcal pharyngitis, dermatitis and bacterial endocarditis.

Sensitivity in streptococcal infection is 95% and rises earlier than ASO titer.

Anti-DNAase-B test Reference range 1-5 yrs <60units;6-15yrs <170;adults <85

DNA-ase as antigen is incubated with the patient's serum at various dilutions and a positive reaction is indicated by disappearance of colorant tagged to the antigen. The result is represented by the highest titer in terms of units.

Elevation of DNA-ase is especially seen in streptococcal pyodermal affection and acute glomerulonphritis. DNA-ase titers rise slower than those of ASO titers, peak levels seen at 4-8 weeks after onset of infection.

Syphilis

About one-third of sexual contacts with early syphilis are expected to be infected, with a lower risk in latent stage.

The incubation period is 3-10 weeks during which serological tests would be negative. The typical primary lesion is the chancre at site of inoculation. In the secondary stage, generalized affection of skin and mucous membranes and multiple organ involvement is seen. During this stage, reactivity of serological tests is maximal. A latent stage follows, lasting for several years. In the early part of this latent stage transient muco-cutaneous lesions may appear. 5 to 20 years after infection tertiary syphilis develops. The most frequent complications at this stage are neural and cardiovascular with gummatous lesions appearing in multiple organs such as skin, bone and etc.

(a)RPR (Rapid Plasma Reagin)

A serological agglutination test for syphilis, which can be used for screening or to follow up therapy. Sensitivity of this test is 30% during the first week rising to 90% after 3 weeks, 100% during the secondary and 90% in the tertiary stage of syphilis. A positive result should be confirmed by hemagglutination inhibition (TPHA) or fluorescent antibody (FTA) tests. RPR should not be used in CSF.

RPR testing is sometimes routinely used on all cord bloods. A positive test should be followed by maternal RPR testing. A positive test in the infant may represent passive antibody transfer or early infection. RPR has been recommended in newborns with hepatomegaly, aseptic meningitis and fever of undetermined cause.

Reversion (four-fold decrease within 3 months) is frequent in treated primary and secondary stages but is infrequent in those treated with tertiary stage of syphilis.

False positive RPR reaction is seen in *lupus erythematosus*, *pregnancy*, drug *addiction* and *several other infections* including malaria, infectious mononucleosis, hepatitis A, brucellosis, HIV, Lyme disease and others.

(b) VDRL (Veneral Disease Research Laboratory)

A reaginic agglutination test for syphilis. It is employed in screening, monitoring response to therapy, detection of central nervous system involvement and congential syphilis. A positive result should be confirmed by hemagglutination inhibition (TPHA) or fluorescent antibody (FTA) tests. Use of all treponemal tests in CSF is controversial and not encouraged. On the other hand, a negative VDRL in CSF does not exclude neurosyphilis.

VDRL becomes reactive in early syphilis, peaks during the secondary or early tertiary stage then converts in late syphilis. VDRL is not the test of choice for early or late syphilis.

In follow up of patients on treatment, a 3-fold decline in titer indicates success of therapy.

False positive VDRL is seen in many conditions, mostly at <1/8 titer, including other infections (bacterial or viral), collagen diseases, some drugs and inflammatory conditions.

(c) TPHA (Treponema pallidum Hemagglutination Inhibition)

A hemagglutination inhibition test which is used to confirm the diagnosis of syphilis, with a sensitivity of up to 70% compared with 80% sensitivity for FTA. However, it is simple, reliable, relatively cheap and demands little equipment.

The test is of value in diagnosis but is not useful in monitoring treatment since test reactivity remains for life, even in those adequately treated.

(d) FTA (Fluorescent Treponemal Antibody-Absorption)

A fluorescent antibody method for the diagnosis of syphilis and the most sensitive test available in practice. On the other hand, TPI (*Treponema pallidum* Immobilization) test, although the most specific of all antibody tests for syphilis, remains a research tool and is not readily available in clinical laboratories, since it requires a source of live organisms.

Toxoplasma gondii

Culture can be done, but more readily available tests in clinical laboratories are based on antibody testing by indirect immunofluorescence, ELISA or chemo-lumenescent techniques. Complement fixation test is obsolete and direct agglutination method is unreliable. Antigen testing is not used in clinical practice.

Toxoplasma gondii is an intracellular protozoan transmsitted via uncooked meat and domestic animals such as cats. Infection is symptomless in the majority, while clinical presentation is typically by sore throat, fever, rash, malaise and cervical, especially occipital, lymphadenopathy. The disease is self remitting but immuno-deficient patients may be severely affected and die. For example, AIDS patients may have severe systemic affection, chorio-retinitis, meningo-encephalitis and abscesses.

In pregnancy transplacental transmission after acute infection in the mother may lead to infection of the fetus in 50% of such cases. In only 10% of cases the mother shows symptoms of the acute infection. Fetal infection during the second trimester is more serious than during the third trimester. Those events are not repeated in subsequent pregnancies, namely, it can occur only once. However, stress on toxoplasmosis as a cause of recurrent abortion has been overplayed in recent years, so that the test has fallen into disrepute with regard to its usefulness in screening for causes of recurrent abortion.

Active infection is indicated by a positive IgM test; this appears within one week of infection, peaks at 3-4 weeks and disappears after 3-4 months. Evidence of active infection can also be deduced by elevation of IgG antibody, which appears 3 weeks after infection, peaks at 2 months and subsides later but may persist for a long time with the possibility of anamnestic response to other infections. Immunodeficient patients and newborns may not show an IgG response.

False positive test results from anti-DNA antibodies and rheumatoid factor.

SECTION 7.

BASIC STAINS IN MICROBIOLOGY

Gram Stain

This is routinely performed on all samples submitted for bacterial culture or when specifically requested. Specimens are either fluid or semifluid such as exudates or, alternatively, solid tissue.

Technique

(1) Make a thin smear of the material onto a pre-cleaned slide and leave to air dry.
(2) Fix using a flame 3-4 times or by placing on a slide warmer set at about 70°C.
(3) Overlay with crystal violet solution for 1 minute.
(4) Wash thoroughly with water.
(5) Overlay with Gram iodine for 1 minute.
(6) Wash thoroughly with water.
(7) Decolorize with acetone-alcohol until violet color stops appearing- takes up to 10 seconds.
(8) Wash thoroughly with water.
(9) Overlay with safranin counterstain for 1 minute.
(10) Wash the slide thoroughly under running tap water, then allow to air dry or blot dry for speed.
(11) Microscopic examination is made first by screening under X10 then X100 oil immersion.

(12) Interpretation: Gram positive bacteria stain dark blue to purple Gram negative bacteria stain pink red, like nuclei of neutrophils.

EXAMPLES OF IMPORTANT GRAM POSITIVE & GRAM NEGATIVE BACTERIA

Gram Positive Bacteria	Gram Negative Bacteria
Cocci	*Cocci*
Staphylococcus aureus/epidermidis	*Neisseria gonorrhoeae/meningitidis*
Streptococcus pyogenes/Group B/viridans/ bovis/pneumoniae/anerobic	*Moraxella catarrhalis*
	Anerobes
Bacilli	*Bacilli*
Bacillus anthracis (anthrax)	*Hemophilus influenzae/ducreyi*
Clostridium perfringens/tetani/difficile	*Pseudomonas aeruginosa/actinobacillus pseudomallei*
Corynebacterium diphtheriae	*Bordetella pertussis*
Listeria monocytogenes	*Brucella melitensis /abortus*
	Gardenella vaginalis
	Legionella
	Enteric bacilli: Proteus/E.coli/Klebsiella
	Campylobacter Enterobacter/Salmonella
	Bacteroides fragilis/Providencia/Serratia
	Yersinia pestis
	Vibrio cholerae
	Acinetobacter
	Pasteurella

Ziehl-Neelsen Stain

Procedure

(1) Take fixed smear / deparaffinized section and lay with freshly filtered *Carbol-fuchsin* solution and heat up to evaporating point short of boiling, leaving for 5 minutes without drying.
(2) Wash in running water.
(3) Decolorize with 5% *acid alcohol (or 1% H_2SO_4)* until color turns pale pink.

(4) Wash thoroughly in running water for 8 minutes.
(5) Counterstain by dipping in *methylene blue* working solution until color turns pale blue.
(6) Wash with tap water, then with distilled water.
(7) Dip twice in *95% ethanol* and then twice in *absolute ethanol*.
(8) Take through 2 changes in *xylene*, 2 minutes each.
(9) Mount.

Results

Acid fast bacilli bright pink or red and non-birefringent; red cells yellow orange; other cells blue.

Acid fast bacilli include Mycobacterium species and Nocardia asteroids.

Material

Carbol-fuchsin: 2.5ml melted phenol, 5ml absolute ethanol, 0.5g basic fuchsin, 50ml distilled H_2O.

1% acid alcohol: 1ml HCl in 99ml 70% ethanol.

Methylene blue: Stock solution: 1.4g methylene blue, 100ml 95% ethanol. Working solution: 10ml stock solution, 90ml tap water.

Notes

Overcounterstaining will obscure the bacilli while with undercounterstaining background fades out. The latter effect is produced also by excessive rinsing in tap water.

Fungal Stains

Selected Staining Techniques versus Some Fungi

Name of Fungus	Suitable Staining technique
Candida albicans	H&E/ PAS/ GMS/ Gridley
Aspergillus fumigates	GMS/ PAS/ Gridley
Actinomyces spp	PAS/ Giemsa/ GMS
Histoplasma capsulatum	GMS/ Gridley
Cryptococcus neoformans	Mucicarmine/ AB-PAS/ GMS/ Gridley
Blastomycetes	GMS/ PAS/ Gridley
Nocardia asteroides	GMS/ Giemsa

H&E = Hematoxylin & Eosin **PAS** = Periodic Acid Schiff Stain. **GMS** = Grocott's Methenamine Silver Nitrate Method for Fungi. **Gridley** = Gridley's Method for Fungi. **Giemsa** = Giemsa Romanowsky Stain. **Mucicarmine** = Mayer's Mucicarmine Method. **AB-PAS** = Alcian Blue-PAS

Above staining techniques and others are found in any standard manual or laboratory techniques textbook.

We choose to give an account of the PAS stain and KOH direct smear below.

PAS

Procedure

1. Immerse in periodic acid solution for 5 minutes.
2. Rinse in distilled water.
3. Place in Schiff reagent for 15 minutes.

4. Wash in lukewarm tap water for 10 minutes.
5. Counterstain in Mayer's hematoxylin solution for 15 minutes (or Harris' hematoxylin 6 mins).
6. Wash in tap water for 15 minutes.
7. Dehydrate and clear through 95% ethanol, absolute ethanol, xylene 2 changes 2 minutes each.
8. Mount.

Results

Fungi red to purple; Glycogen, mucin, basement membrane red to purple; Nuclei blue.

Material

Periodic acid solution 0.5% solution: 0.5g periodic acid, 100ml distilled water.

Schiff reagent 1g Basic fuchsin, 200ml distilled water at 60°C: bring to boil, cool and then add 2g Potassium metabisulfite, 1N HCl. Allow to bleach for 24 hours then add 0.5 Activated carbon, Shake for 1 minute, Filter through coarse filter paper. Repeat filtration until solution is colorless. Store in the fridge. For *Mayer's or Harris' hematoxylin* refer to any standard Histotechnology manual.

KOH Direct Smear for Fungi

KOH Preparation:

1- Place a drop of 15-20% KOH (Potassium hydroxide with or without Calcofluor white) on a clean glass slide.

2- Immerse the specimen in the drop of KOH already on slide.
3- Coverslip & heat the specimen/KOH mixture by passing the slide through a Bunsen burner flame several times. Do not allow the mixture to boil.
4- Examine the preparation under 10X & 40X. Excessively tenacious specimens may require additional heating.
5- KOH may also be used as a simple mounting medium without heating.

SECTION 8.

SAFETY IN THE MICROBIOLOGY LABORATORY

Laboratory safety is established to protect laboratory personnel against infectious biohazards and toxic material. Risk is compounded by two factors, namely, amplification of microorganisms by culture and direct contact with body fluids & blood containing microorganisms such as hepatitis B and HIV. Thus, bio-safety practice in the microbiology laboratory is essential.

The following basic precautions should be noted:

1. Aerosol and organisms in open culture should be handled in a containment cabinet.
2. Staff must have specific training on how to handle pathogenic agents.
3. Access to the laboratory should be limited to staff personnel.
4. Lab attire, such as coats and masks, should provide adequate protection, not to be rough, woollen or to have a furry surface.
5. Sign posts on biohazard should be adequately placed.
6. Gloves should be worn when working with clinical material.
7. Face should be protected whenever there is a chance of splatter.

8. Use of sharp articles should be greatly restricted and finger nails well trimmed.
9. Basal serum samples for all employees should be tested for HBV, HCV and HIV.
10. Safety manual should be within easy reach for all laboratory personnel.
11. Curtailing undue body movement to cut down contamination.
12. Not to carelessly touch or lean on bench tops.
13. Bench cleansing to be done by technologists working in that area.
14. Long hair should be kept covered and never let loose.
15. Strictly no food to be taken into the microbiology laboratory.
16. Visitors and chatting to be kept to a minimum.
17. Noise distracts workers and their concentration. Quietness is golden.
18. Disposal of infectious material should be immaculate and to rule.

CHAPTER 5

CLINICAL GENETICS

A SHORT GUIDE TO GENETIC TESTS

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INTRODUCTION

- Who should have genetic testing, that is, who are the targets for testing their genes?

  a) With a history in the family e.g. thalassemia, muscular dystrophy, high cholesterol etc.
  b) Some conditions arise unexpectedly in the young e.g. breast cancer, thromboembolism.

  In such cases, genetic testing is offered to any member of the family, especially those at high risk.

  There are many facets to genetic counselling, including investigations that can be simple and cheap or elaborate and costly, implications of a diagnosis when ascertained and supportive care with follow up of the case or family members involved. It also involves aswering questions like future health of individuals, marital and conceptual issues, heritability, preventive measures as well as psychological effects. The handling of such cases requires a team work approach with the realization that, whatever the diagnosis, impact on the index case and family can be serious and careful work up needs to be established with first contact individuals.

- How is genetic testing done?

  By taking a blood sample most often. Occasionally, other than blood is sampled.

  The DNA is separated from the person's cells and then analyzed for a suspected disorder.

- What use can be made of genetic testing results?

  Positive result: genetic mutation identified; confirms suspected diagnosis and helps screen other members for risk and in planning any treatment.

  Negative result: does not rule out possibility of an inherited condition due to mutation undetected by techniques employed or as yet unidentified.

  Screening is made on the basis of personal and family history.

  Inconclusive Result: genetic alteration detected is uncertain to be responsible for the disorder of interest. Further tests may have to be done or continued screening be recommended to the family.

The person seeking genetic testing is entitled to know the delay time for the results and should be interviewed with the aim of drawing a family tree and taking advice on risks once genetic testing results become available.

In order to achieve the best results for genetic testing, an accurate diagnosis should be attempted. A family tree should always be made in the first instance, and that may take more than one interview to establish two generations preceding as well as same and lower generation(s) of the index case whenever present. Detailed medical history and physical examination are basic requisite in order to plan a diagnostic work up.

Laboratory investigations: Depending on clinical presentation, investigations are geared accordingly:

1- Radiological diagnosis and ultra-sound for skeletal deformity.
2- Biochemical tests include a full systematic check up (look up full health laboratory screen performed at the Consulting Medical Laboratories) as well as for specific disorders such as lactate, pyruvate, ammonia, amino acid, organic acids, fatty acids, assay for glycolytic enzymes, lysozomal enzymes and so on, as the case may indicate.
3- Biopsies may be taken from tissue such as muscle to perform relevant histo- and immunohisto-chemical analyses, or skin to grow fibroblasts and run assays, for example, mucopolysaccharidases, etc.
4- Karyotyping for disorders associated with chromosomal numerical or structural abnormalities.
5- Molecular DNA analysis for specific genetic disorders are performed to detect mutations, gene aberration or rearrangement.

CML (Consulting Medical Laboratories) provide over 40 genetic tests on routine basis for medical specialists to use in their practice. The *Clinical Genetics Service* is a product of years of preparation by a specialized team using most up-to-date techniques with quality control regularly employed. New cases requiring full investigations for mutations concerned may be time consuming and costly, and there is seldom readiness for those concerned to spend large sums of money, knowing that subsequent tests after identifying the mutation sequence in a family will enable testing in other family members at a relatively cheap rate and with a short turnaround time. With this understanding, laboratory

and clinical specialists will have to work together in realizing accurate diagnosis, optimal management, reliable prognosis and sound genetic counselling for genetically determined disorders.

This guide provides a section on genetics aimed at exploitation by clinical practitioners, since in recent years, medical genetics has undoubtedly moved from basic sciences to the clinical scene.

Clinical genetic testing is readily available at the Consulting Medical Laboratories. For example, we can use genetic tests to prevent thrombo-embolic disasters at early or late age. We can provide accurate diagnosis and genetic counselling on hemoglobinopathies and inborn errors of metabolism. Genetic tests will eventually become part of assessing lipid profile. Metabolic disorders can be circumvented in several situations by early genetic diagnosis. And so on …

1. Cytogenetics Service

This is a flourishing section of CML laboratory services and the first that had ever been established in Jordan. Analysis of blood lymphocytes commenced in 1987 and prenatal cytogenetic diagnosis in 1989 (Sawwaf H. Initiation of prenatal diagnosis. *Bulletin of the Consulting Medical Laboratories*, 1990;8(1):1).

Within CML cytogenetics service, all routine cytogenetics tests are performed, namely, culture, banding and staining with use of the most up-to-date technology. A variety of specimens, such as blood, bone marrow, tissue like CVS (chorionic villus sampling) and abortus, as well as amniotic

fluid are handled. Comprehensive reporting includes high-resolution photographs of all the 46 chromosomes with highlight on any abnormality present.

Culture

Specimen: Fresh heparinized whole blood in sterile vacutainer, fresh bone marrow aspirate in heparinized RPMI, amniotic fluid, products of conception in sterile or RPMI.

Cells of interest are cultured and metaphase spreads prepared. Number and structure of the karyotype are reported with diagnosis and comment. Turnaround time for karyotyping varies between 10 and 20 days, depending on the case.

2. FISH (fluorescent in situ hybridization)

Specimens: as for cytogenetic testing.

Useful in the diagnosis of trisomy where the turnaround time is 24-48 hours as well in the diagnosis of specific chromosomal abnormalities and sexing.

This method has also been used in our laboratories in PGD (pre-implantation diagnosis).

FISH is applied to cultured and uncultured cells to detect microdeletions not visible by routine cytogenetics methodology, as well as to pick out complex translocations especially in malignancy, for example, Philadelphia chromosome, or for speeding up results to obtain a workable answer within one to two days, for example in the diagnosis of trisomy 21.

FISH can be applied in the diagnosis of genetic diseases and to detect certain oncogenes related to tumor genesis, diagnosis, natural progression and treatment. Samplings can be from peripheral blood, bone marrow or specific tissue of interest.

3. PGD (Pre-Implantation Diagnosis)

Specimens: 1-2 *blastomeres* from 4-16 cell stage embryos are fixed on slide(s). PCR +/- sequencing and FISH can be employed on the blastomere. Report will either inform on the sex of embryo by FISH or determine presence or absence of trisomy 21. Testing enables information on chromosome 13,18,21, X, Y and etc. Turnaround time is same day or within 24 hours.

Pre-implantation diagnosis is a relatively recent achievement in the precise diagnosis of genetic disorders even before implantation of the fertilized ovum onto the uterine mucosa. Genetic disorders which are candidate for screening include chromosomal anomalies (<1%), DNA or genetic mutations leading to abnormal product (1%) and the remaining majority (>98%) which comprise multi-factorial disorders. The Consulting Medical Labortaories were the first to introduce PGD technique in Jordan (Hasan BI. Pre-implementation diagnosis: A preliminary report. *Bulletin of the Consulting Medical Laboratories* 1998;16(1):1.

GENETIC TESTS LIST
FOR CLINICAL PRACTITIONERS

A. Hematological disorders

Genetic tests for thromboembolism:

A1. MTHFR gene, homocysteine mutation
A2. Factor II, prothrombin mutation
A3. Factor V, Leiden factor

Genetic tests for coagulation:

A4. Hemophilia A
A5. Hemophilia B

Genetic testing for HbS/thalassemia

A6. Beta-globin gene mutations/hemoglobinopathies

B. Genetic tests for inborn errors of metabolism:

B1. Cystic fibrosis
B2 Hereditary haemochromatosis
B3. Alpha-1 antitrypsin deficiency
B4. Osteoporosis
B5. Adrenogenital syndrome
B6. ACE (Angiotensin converting enzyme)
B7. Familial thyroid hormone resistance
B8. Maturity onset diabetes mellitus in Young I,II,III
B9. Cystinuria

C. Gene tests for abnormal lipid metabolism:

C1. Apolipoprotein AI/ CIII, APO AI/CIII gene
C2. Apolipoprotein B, APO B gene
C3. Apolipoprotein E, APO E gene
C4. Familial hypercholesterolemia, LDL receptors gene
C5. Sandhoff's disease (hexosaminidase decifiency)

D. Gene testing for azoospermia:

D1. Azoospermia factor

E. Gene tests for neoplasia:

E1. MEN (multiple endocrine neoplasia) type I
E2. MEN type II
E3. ALL (acute lymphoblastic leukemia)
E4. Pre B-cell ALL
E5. CML (chronic myeloid leukemia)
E6. Follicular lymphoma
E7. Familial nonpolyposis colon cancer

F. Gene tests for neuromuscular disorders:

F1. Muscle dystrophy
F2. Mitochondrial encephalopathy, MELAS
F3. Myoclonal epilepsy, MERRF gene
F4. Myotonic dystrophy, DMK gene
F5. Fragile-X syndrome
F6. Spinocerebellar ataxia
F7. Amyloidotic polyneuropathy
F8. Huntington's disease
F9. Spinal muscular atrophy
F10. Friedrich's ataxia
F11. Hereditary Motor & Sensory Neuropathies or Charcot-Marie-Tooth Disease

K. Gene tests for kidney disorders:

K1. Autosomal dominant polycystic kidney disease
K2. Autosomal recessive polycystic kidney disease
K3. Autosomal dominant medullary cystic kidney disease

L. Familial mediterranean fever

A. Gene Tests for Hematological Disorders

A1. MTHFR (Methylene-tetra-hydro-folate reductase) GENE

Specimen: 2-5 ml EDTA blood. *Turnaround time*: 3 days.

Indications:

(1) Thrombo-embolic episode(s) at young age.
(2) Screening women before administration of oral contraceptives.
(3) Recurrent thrombo-embolic episode(s) of unknown cause.
(4) Screening relatives of index patients.

Methodology: Detection of mutations in Chromosome 1 using PCR technique.

Comment:

MTHRF-gene mutations are associated with hypercysteinemia and increased risk for thrombo-embolic phenomena. The homozygous state is more serious than the heterozygous form and is inherited as an autosomal recessive trait. The former presents with homocystinuria and hypomethioninemia at early age with mental retardation and vascular accidents. The trait predisposes to venous thrombosis. Homozygous forms carry 2-3 fold increase of thrombo-embolic episodes. When other risk factor(s) coexist, such as Factor V or Factor II, the risk is accentuated.

A2. Prothrombin or Factor II Gene:

Specimen: 2-5 ml EDTA blood. *Turnaround time:* 3 days.

Indications:

(1) High incidence of thrombosis in a family.
(2) Thrombo-embolic episode(s) at young age.
(3) Recurrent thrombo-embolic episode(s) of unknown cause.
(4) Screening women before administration of oral contraceptive pills.

Methodology: Detection of specific mutations in Chromosome 11 by PCR.

Comment:

Factor II is the inactive form of thrombin and elevated levels in plasma pose a risk for development of venous thrombosis. Heterozygous status causes 2-8 fold increased risk of thrombosis. Factor II gene mutations are found in 18% of patients with thrombosis compared to 2-3% of healthy individuals. Association with Factor V-Gene or MTHFR-Gene mutations further increases the risk of developing thromboembolic episodes.

A3. Leiden Factor or Factor V Gene

Specimen: 2-5 ml EDTA blood. *Turnaround time:* 3 days.

Indications:

(1) High familial incidence of thrombo-embolic episodes(s).
(2) Thrombo-embolic episode(s) at young age.
(3) Screening women prior to administration of oral contraceptive pills.
(4) Thrombo-embolic episode(s) of unknown origin.

(5) Screening relatives of index patients.

Methodology: Detection of mutations in Chromosome 1 by PCR.

Comment:

Patients who have no obvious risk factor(s) for thrombo-embolic episode(s), such as postoperative bed rest, underlying neoplasia, high cardiolipin antibodies and so on, may show a high familial incidence of thrombosis episodes. Genetic defects seen in this group of patients include mutation in Antithrombin-III gene, Protein-S and Protein-C genes, all of which are rare but the genetic defect which is commonly seen involves a mutation in Factor-V gene, which in turn causes resistance to active Protein C and, consequently, an increased risk of developing thrombo-embolic accidents. The incidence of Factor V mutation has been estimated to be 20% in patients with thrombo-embolism and in < 4% of controls. This genetic anomaly appears in young individuals with recurrent thrombo-embolic episodes and/ or pulmonary embolism. Women have a 30-fold increased risk after taking oral contraceptive pills. This test is not affected by oral contraceptives or anticoagulant therapy.

A4. Hemophilia A, Factor VIII Gene

Specimen: 5-10 ml EDTA blood. *Turnaround time:* 2-3 weeks.

Indications:

(1) Diagnosis of new hemophilia cases.
(2) Screening of female carriers.

(3) Counselling of hemophilia patients and female gene-carriers.
(4) Prenatal diagnosis.

Methodology: Detection of mutations on X-chromosome, Xq28, with capped markers, Southern-Blot or sequencing.

Comment:

Hemophilia A is a relatively common inherited blood coagulation anomaly, with an incidence of 1:5000-1:10000 male newborns. It is the result of a defect in Factor VIII-Gene at X chromosome. 30-40% of the hemophilia cases are caused by new mutations where no family history exists. The defect results in diminished or complete absence of coagulation Factor VIII activity in blood is described below:

Severe hemophilia A	Factor VIII activity	<1%
Less severe hemophilia A	Factor VIII activity	1-5%
Mild hemophilia A	Factor VIII activity	5-15%
Sub hemophilia A	Factor VIII activity	15-50%

The disease is inherited as X-linked chromosomal recessive, affecting only male individuals. In case of a hemophiliac male marrying a healthy woman all sons will be healthy, while daughters will all be gene carriers. Marriage of a female carrier to a healthy man results in 50% of their sons hemophiliac, and 50% of their daughters carriers. Severe form of Haemophilia A is usually diagnosed early because of bleeding episode(s). Indirect Gene analysis on capped genetic markers for the identification of gene-carriers is commonly sufficient. However, more information is provided by direct DNA sequencing.

A5. Hemophilia B, Factor IX Gene:

Specimen: 2-5 ml EDTA blood. *Turnaround time:*3 weeks.

Indications:

(1) Investigation of new hemophilia cases.
(2) Genetic counselling to hemophilia patients and female gene-carriers.
(3) Detection of gene-carrier status in suspected female patients.
(4) Prenatal diagnosis.

Methodology: DNA sequencing by PCR.

Comment:

Haemophilia B is the second most common inherited blood coagulation anomaly, accounting for 1:50,000 male newborns. It results from a genetic defect in Factor IX-Gene, leading to a diminished or completely absent activity of this factor in plasma. The clinical course of the disease is similar to that of Haemophilia A, as well as correlation of severity of the disease with percentage of factor activity in blood. The disease is X-linked, autosomal recessive, affecting only males. The same inheritance rules for Hemophilia A apply to Hemophilia B. The causative mutation of the disease can be identified by DNA sequencing and, in this way, detect female gene-carrier status. Modern transfusion medicine has contributed to prolongation of symptom-free life span.

A6. Sickle cell anemia and β-thalassemia:

Specimen: 2-5 ml EDTA blood or 20 mg chorionic villi sampling in ice.

Turnaround time: 2-3 weeks.

Indications:

(1) Screening family members for the trait.
(2) Prenatal diagnosis of carriers.
(3) Differential diagnosis of hemoglobinopathies.

Methodology: Detection of mutations on Chromosome 11 by PCR, FISH or sequencing.

The nine most frequently found β-globin gene mutations, in the Mediterranean, Nord and Middle East regions are the following; -87, HbC, HbS, IVS1-1, IVS1-6, IVS1-110, cd 39,ICSII-1, IVSII-745 and which are screened for routinely.

Comment:

Hemoglobinopathies result from β-Globin gene deletions and various mutations in the β-globin chain. Sickle cell anemia (HbS), HbC and β-Thalassemia are frequently observed in Mediterranean, African and South-East Asian populations; carried through an autosomal recessive mode of inheritance. In homozygous Sickle cell anemia, substitution of nucleotide base pair A→T, accounts for defect in erythrocyte formation, which relates to poor oxygenation and recurrent infections as well as chronic anemia leading in the late stages to heart failure or liver damage with a shortened life span. In the heterozygous state, HbS mutation in one locus is often associated with other β-globin gene-variants (HbC or β-Thalassemia). In β-Thalassemia many mutations, low synthesis or complete absence of β-chain occur. Asymptomatic carrier status can be detected by simple hematologic tests. Homozygous or 'mixed heteroge-

neous gene-carriers' of β-Thalassemia mutations can have severe clinical manifestations and become transfusion-dependent with chronic anemia and extramedullary erythropoiesis leading to hepato-splenomegaly, accompanied by malnutrition and predisposition to infection. The disease becomes manifest between 6^{th} –12^{th} months after birth with a poor life expectancy. However, blood transfusions may prolong viability to the third decade. Identification of carrier state and prenatal diagnosis form the basis for prevention of the disease. In a routine genetic screen depending on the population at hand, depending on mutant variants, associated to different ethnic populations, 65-90% of β-Thalassemia mutations and 90% of β-globin gene-mutations, are detected.

B. Gene Tests for Inborn Errors of Metabolism

B1. Cystic fibrosis (Mucoviscidosis), CFTR-Gene

Specimen: 2-5 ml EDTA blood, buccal scrapings on 2 swabs or 10mg chorionic villi in ice. *Turnaround time:* 2 weeks.

Indications:

(1) Sweat-test results at upper-limits in patients presenting with features of mucoviscidosis.
(2) Differential diagnosis of a positive sweat test.
(3) Screening newborn babies with big abdomen and fatty, voluminous stools.
(4) Investigation of meconium ileus in newborn babies.
(5) Recurrent respiratory infections of undetermined cause.
(6) Family screening and heterozygous status identification.
(7) Prenatal diagnosis
(8) Obstructive azoospermia or severe oligospermia.

Method: Detection of 31 mutations on Chromosome 7 by PCR or capillary electrophoresis. In subfertile males, additional testing is made of the poly-T region of intron 8.

Comment:

Mucoviscidosis is the most common inherited mucopolysaccharidosis with a multi-organ disease in Caucasians. It is

inherited as autosomal recessive, with a frequency of 1: 2500 newborns. Heterozygous gene-carriers are 1: 25, 85% of whom can be identified. Mucoviscidosis is the result of changes in the cell membrane proteins (CFTR-protein) which regulates transport of chloride through cell walls. Malfunction of these proteins leads to pathological changes in exocrine glands with multiple organ affection. The respiratory tract is most frequently affected. Life span of those patients has dramatically improved by modern therapy. Nevertheless, the majority of patients die as a consequence of advanced bronchopulmonary disease. In addition, 85% of patients develop pancreatic insufficiency. 10-20% of mucoviscidosis-newborns have meconium ileus. Liver involvement can occur with cirrhosis developing in adulthood. Heterozygous males may be infertile due to obstructive azoospermia, while female patients have a reduced fertilization rate due to secretory malfunction.

B2. Hereditary Hemochromatosis, MHC-Class I-Like Gene

Specimen: 2-5 ml EDTA blood. *Turnaround time:* 2 weeks.

Indications:

(1) Identification of homozygous or gene-carriers in family members.
(2) Confirming diagnosis in suspected patients with liver cirrhosis, hepatomegaly, cardiomyopathy, diabetes mellitus, hypogonadism, skin hyperpigmentation, arthropathy and high transferrin levels.

Methodology: Sequence analysis of the most common mutations in the MHC Class I like-gene on Chromosome 6 by PCR.

Comment:

Hemochromatosis results from excessive deposition of iron in parenchymal cells, leading to damage in liver, heart, pancreas, endocrine organs, skin and joints. The most common clinical manifestations are hepatomegaly, cirrhosis, cardiomyopathy, diabetes mellitus, hypogonadism, skin hyperpigmentation and arthropathy. Hereditary hemochromatosis in Nord Europeans is the most common gene-defect of clinical importance. Diagnosis is usually delayed for several years after appearance of symptoms. Toxic levels of iron can be reached with irreversible damage to tissue before diagnosis is made. Hemochromatosis is a potentially fatal disease and requires early diagnosis for its prevention. Genetic diagnosis of gene carriers provides an excellent tool for prevention.

B3. Alpha 1-Antitrypsin deficiency, AAT-Gene

Specimen: 2-5 ml EDTA blood, 20mg chorionic villi in ice or fetal cultured cells. *Turnaround time:* 2-3 weeks.

Indications:

(1) Molecular genetic diagnosis of AAT deficiency.
(2) Differential diagnosis of hepatitis and liver cirrhosis of unknown cause.
(3) Differential diagnosis of obstructive lung disease and emphysema.

(4) Differential diagnosis of prolonged neonatal jaundice in newborns and small children as well as severe, premature, infantile liver cirrhosis.
(5) Prenatal diagnosis in suspected families.

Methodology: Detection of Z and S mutations on Chromosome 14 by PCR and DNA sequencing. Detection of other mutations is also possible.

Comment:

AAT is an acute-phase protein and is the most important protease-inhibitor in plasma. It is synthesized mainly by hepatocytes, alveolar macrophages, and blood monocytes. Normal plasma concentration is between 0.9-2.0 g/l. AAT levels down to 30-40% of normal values are accompanied by clinical symptoms. More than 70 known alleles and subtypes are involved in the formation of AAT. Gene-carriers of AAT deficiency present with lung and liver problems. AAT deficiency of childhood involves predominantly liver and, to a lesser extent, lungs and kidneys. AAT of adulthood is dominated by lung emphysema. Initial investigation of AAT deficiency determines plasma concentration and Pi-typing in the isoelectric focus. Hereditary AAT deficiency results from mutations in the AAT-Gene on Chromosome 14 with an autosomal dominant mode. In Europe, the incidence of the disease is 1: 2000. Two mutations of clinical relevance code phenotypes PiS and PiZ, both of which may cause a homozygous or heterozygous status, and lead to chronic respiratory disease. Rarely, so-called 'null' mutations cause severe depletion or absence of AAT activity with early onset of physical signs. The homozygous Z-mutation leads to severe liver disease and, less frequent, lung disease. Screening families with a positive history al-

lows presymptomatic diagnosis of gene-carriers, while prenatal, or better, pre-implantation diagnosis enables prevention.

B4. Osteoporosis, Vitamin D Receptor Gene

Specimen: 2-5 ml EDTA blood. *Turnaround time:* please enquire.

Indication:

Familial predisposition to osteoporosis.

Methodology: Estimation of polymorphism by PCR.

Comment:

Bone density in humans is genetically determined, as supported by twin genetic studies. Mineral content, bone density and stability appear as inherited characteristics in 60-80% of people at large. However, a single gene responsible for bone instability has not been identified, although vitamin D Receptor-Gene mutations have been described and appear responsible for defective bone formation and osteoporosis in adults. Epidemiological studies suggest that genetic capping occurs between different alleles polymorphisms determining bone density of individuals. For example, genotype B/B has a higher bone density than genotype b/b.

B5. Adrenogenital Syndrome, 21-hydroxylase deficiency Gene

Specimen: 2-5 ml blood or 20 mg chorionic villi in ice. *Turnaround time:* 3-4 weeks.

Indications:

(1) Clinical signs of adrenal hyperplasia.
(2) Existence of AGS in a family.
(3) Genetic counselling to couples with a family history.
(4) Prenatal Diagnosis.
(5) Hirsutism, premature puberty, acne, seborrhea, tall stature, accelerated bone aging and clitoris hypertrophy.

Methodology: Direct detection of gene mutations on Chromosome 6 by PCR. Detection of all known mutations on 21- hydroxylase gene by DNA sequencing.

Comment:

AGS is caused by a genetic defect in steroid hormone synthesis affecting 21-steroid-hydroxylase enzyme. The incidence of this disease is 1: 5,000 -1:15,000 of newborns in western countries. It is autosomal recessive and manifests in different clinical forms. In severe forms, AGS causes premature virilization of the female fetus in-utero, which can be prevented by early administration of glucocorticoids (dexamethasone). Late-onset, non classical form of the disease presents after puberty, Adolescents and young men are usually asymptomatic, while girls show signs of premature puberty, acne, seborrhea, hirsutism, tall stature, accelerated bone aging and clitoris hypertrophy. The 21-steroid-hydroxylase-gene is located on the short arm of chromosome 6. Prenatal diagnosis is indicated when suspicion of a female fetus or a homozygous carrier exists. Glucocorticoid suppression therapy is mandatory in such cases. In the non-classical form of the disease, two different genetic phenotypes can occur, namely, a heterozygous state or a com-

pound heterozygous type, whereby more than one gene defect can lead to multiple enzymatic defects in steroid biosynthesis and different clinical syndromes.

B6. Angiotensin Converting Enzyme, ACE Gene

Specimen: 2-5 ml EDTA blood. *Turnaround time:* 2 weeks.

Indications:

Risk factors for hypertension and coronary thrombosis.

Methodology: Detection of deletions/insertions polymorphism, in intron 16 on Chromosome 17, by PCR.

Comment:

The Renin-Angiotensin-Aldosterone System plays a big role in the pathogenesis of hypertension and coronary thrombosis. Renin function is Angiotensin II mediated, which under the influence of ACE is converted to Angiotensin I. Vasoconstrictive activity of Angiotensin II regulates blood pressure in the body, so that treatment of hypertension includes ACE inhibitors. ACE-Gene contains 26 exons and 25 introns. In intron 16, insertion/ deletion-polymorphism exists and regulates ACE level in the blood. Three different genotypes of ACE-Gene are described: I/I, I/D, D/D. Patients with D/D have higher circulating ACE level, as well as a higher risk of developing myocardial infarction compared to the two other genotypes. The D/D genotype is seen in 25% of western people. Patients with end-stage heart failure and ischemic dilatation of cardiac muscle usually belong to the D/D genotype. Early detection of these genotypes can help in timely diagnosis, prevention

and prognosis of both hypertension and coronary thrombosis.

B7. Familial thyroid hormone resistance, Thyroid Hormone Receptor B-Gene

Specimen: 2-5 ml EDTA blood. *Turnaround time:* 2-3 weeks.

Indications:

(1) Elevated free T4 and T3 serum levels without TSH suppression.
(2) Screening families with index cases.

Methodology: DNA sequencing of the THR-B-Gene on Chromosome 2 by PCR.

Comment:

Two genes encode for Thyroid Hormone Receptors: the α-receptor gene TR-α on chromosome 17 and the β-receptor gene TR-β on chromosome 2. Each gene, through a specific pathway, generates a different isoform which is tissue-specific. Molecular changes in those receptors cause total resistance of target organs to hormonal induction. Patients are euthyroid or have only a partial hyperthyroid state. Mutations of the B-Gene have been described in about 200 families. Mutations of the TR-α-gene are lethal in-utero. Correlation between the clinical picture of resistance and the molecular genetic defect has not been classified.

B8. MODY, Maturity onset diabetes mellitus in the young I,II,III

Specimen: 2-5 ml EDTA blood. *Turnaround time:* 8 weeks for new case.

Indications

(1) Early onset of NIDDM (Non Insulin Dependent Diabetes Mellitus) with high familial incidence.
(2) Family screening of index cases.

Methodology: Detection of mutations by PCR and sequencing on Chromosome 20 (Type I), Chromosome 7 (Type II) and Chromosome 12 (Type III).

Comments:

NIDDM accounts for 5-7% of cases of diabetes mellitus in western countries. An underlying genetic component is suspected to be transmitted through an autosomal dominant trait, but this has not yet been proven. MODY (Maturity Onset Diabetes in the Young) is most frequently seen during the third decade of life. Genetic studies have shown capped DNA markers on Chromosomes 20, 7 and 12 in MODY Types I,II and III, respectively. MODY I is caused by mutated transcription factor HNF1α (Hepatic nuclear factor, 1α) which is synthesized in liver and β-pancreatic cells and functions as a regulator of glucokinase and insulin genes. MODY II is caused by mutated glucokinase gene. Glucokinase is also synthesized in liver and β-pancreatic cells; it plays a key role in gluconeogenesis. MODY III is caused by mutated transcription factor HNF 4α (Hepatic nuclear factor, 4α). It is synthesized in liver and pancreas

and regulates the HNF 1α, namely, MODY I gene. MODY types are not usually associated with obesity, while renal and vascular complications are less frequent than in other diabetics. Early diagnosis aids timely detection and improves outcome.

C. Gene Tests for Errors in Lipid Metabolism

C1. Apolipoprotein AI/CIII, Apo AI/CIII Gene

Specimen: 2-5 ml EDTA blood. *Turnaround time:* 2 weeks.

Indications:

(1) Familial hypertriglyceridemia.
(2) Combined familial hyperlipidemia.
(3) Evaluation of risk for coronary thrombosis.
(4) Increased familial incidence of myocardial infarction.

Methodology: Detection of mutations in the Apo AI/CIII gene by PCR.

Comment:

Combined familial hyperlipidemia or hypertriglyceridemia result from a defect in lipid metabolism causing diminished lipoprotein catabolism. Affected patients have an increased risk of coronary thrombosis. The Apolipoprotein genome is located on the long arm of the chromosome 11, in a 15kb DNA segment, containing the Apo AI, Apo CIII,and Apo AIV complex. In 1985, a gene variant of ApoCIII was described, the S2allele, present in 10 of 48 patients with myocardial infarction while, only 2 of 47 control persons were carrying the gene. The heterozygous carrier of this variant, S1/S2- genotype is associated with a higher triglyceridemia level compared to carriers of the S1/S1 genotype. The S2 allele is associated with 3-8 fold increased risk of hypertriglyceridemia.

C2. Alipoprotein B, Apo B Gene

Specimen: 2-5 ml EDTA blood. *Turnaround time:* 2 weeks.

Indications:

(1) Differential diagnosis of increased LDL-Cholesterol level in blood.
(2) Hyperlipidemia.
(3) Risk factors for atherosclerosis.

Methodology: Detection of specific mutations in Chromosome 2 by DNA sequencing.

Comment:

Apolipoprotein B forms part of LDL (Low-density-lipoproteins) and constitutes its binding site to the LDL-Receptor, playing an important role in lipid transport. Disturbances in lipid metabolism and transport are due to mutations in the Apo-B-Gene, inducing defective binding between LDL-particles and LDL-receptors. A mutation within the ApoB-Gene involves a base-pair substitution, namely, glutamine for arginine (A→G) in Exon 26, position 3500, which results in defective lipid metabolism and transport throughout the body. Another mutation in position 3531 associated with high LDL-Cholesterol levels in blood, where arginine is substituted by cysteine (A→C). As a result hypercholesterolemia and hyperlipidemia is seen in 2-5% of cases positive for the ApoB-Gene mutation. Incidence of ApoB mutation in position 3500 is 1:500 in western countries, rising to 1:210 in Switzerland, which ranks this as the commonest single-base-pair mutation that causes

increased risk of hyperlipidemia and cardiovascular disease within families.

C3. Apolipoprotein E, ApoE Gene

Specimen: 2-5 ml EDTA blood. *Turnaround time:* 2 weeks.

Indications:

(1) Differential diagnosis of high LDL-Cholesterol levels in plasma.
(2) Hyperlipidemia Type III.
(3) Obesity.
(4) Diabetes mellitus.
(5) Risk factors for atherosclerosis.
(6) Alzheimer disease.

Methodology: Detection of E2,E3,E4 genotypes on Chromosome 19 by PCR.

Comment:

ApoE (Apolipoprotein E) plays an important role in cholesterol transport through binding to different lipoproteins and interaction with LDL-receptor and ApoE-receptor in the liver. ApoE influences metabolism of IDL(intermediate density lipoprotein), chylomicron remnants and HDL. It is responsible for transport of cholesterol from peripheral tissues to the liver. Persons with ApoE4-isoform have a higher LDL-Cholesterol plasma level in comparison with persons having ApoE3-isoform. Patients with hyperlipidemia Type III often have the ApoE2 isoform. Studies suggest a close association between ApoE4 isoform and Alzheimer disease, as well as an inherited risk factor for

atherosclerosis, coronary thrombosis and cerebrovascular accidents.

C4. Familial hypercholesterolemia, LDL Receptor Gene

Specimen: 2-5 ml EDTA blood. *Turnaround time:* please enquire.

Indications:

(1) Therapy-resistant hypercholesterolemia total >250 mg/dl or LDL >180 mg/dl.
(2) Familial incidence of coronary thrombosis.
(3) Investigations in relatives of gene carriers.

Methodology: DNA sequencing of LDL Receptor gene on Chromosome 19 by PCR.

Comment:

Increased plasma levels may involve cholesterol, triglycerides or both. Below is a Table which illustrates the different clinical phenotypes met in clinical practice.

Phenotype	Chylomicron	VLDL	LDL	Cholesterol	Triglycerides
I	⇑	--	--	↑ 320*	⇑ 4000*
II a	--	--	⇑	⇑ 370*	--90*
II b	--	↑	↑	↑ 350*	↑ 400*
III		↑IDL		↑ 500*	↑ 700*
IV	--	↑	--	↑ 220*	↑ 400*
V	⇑	↑	--	↑ 700*	⇑ 5000*

* mg/dl

Clinical signs of genetically inherited hyperlipidemia include whitish-sickle form lipid deposition in the cornea (arcus lipoides corneae), yellowish lipid deposition in the orbits (xanthelasma palpebratum) and nodular lipid deposits in skin (xanthomas). These xanthomatous changes should always be investigated in association with hyperlipoproteinemia. Secondary hyperlipoproteinema can be associated with other diseases, such as diabetes mellitus Type II, hyperthyroidism, myocardial infarction, nephrotic syndrome, cholestasis, hepatitis, burns sepsis, AIDS, alcohol and medicament abuse. Under primary hyperlipidemias are II a, II b, IV Types. Familial hypercholesterolemia (HLP II a) results from a genetic defect in LDL-Receptor-Gene, whereby LDL-Cholesterol catabolism is blocked and cholesterol biosynthesis in the liver continues despite high plasma cholesterol levels. Its incidence is about 1: 500 among Europeans. Homozygous patients show cholesterol concentration in plasma ≅ 600 mg/dl. Heterozygous individuals show cholesterol levels in plasma 280-550 mg/dl. Homozygous patients develop at 10-15 years of age heart infarction, while heterozygous individuals, 85% of men and 50% of women before the age of 60, suffer with myocardial infarction. At the LDL-Receptor-Gene about 150 point-mutations, deletions, inversions and insertions can be detected on full gene analysis. Early diagnosis enables prevention and early therapy.

C5. Sandhoff's Disease (GM2 Gangliosidosis)

Specimen: 5-10 ml EDTA whole blood. *Turnaround time:* 2 –3 weeks.

`Indications

Progressive dystonia, mental deterioration, behavioral disturbances and cherry red foveolar spots.

Methodology:

PCR for detection of the most common mutation which is 16 Kb deletion.

Comment:

A lipid storage disease autosomal recessive disease; has a classical infantile form but also juvenile and adult forms exist. Due to deficiency of both hexosaminidase A&B leading to accumulation of GM2 gangliosides. Histopathology shows pleomorphic storage cytosomes in retinal neurons and glial cells of optic nerve.

Both carrier diagnosis and prenatal diagnosis can be done.

D. Gene Tests for Azoospermia

D1. Azoospermia factor

Specimen: 2-5 ml EDTA blood. *Turnaround time:* 2 weeks.

Indications:

(1) Subfertile men with azoospermia or severe oligospermia (sperm count <1 mill/ ml)
(2) Family study of index cases.

Methodology: Detection of microdeletions in Yq 11 region, (evidence of 27 loci) using PCR technique.

Comment:

Y- chromosome carries different genes responsible for germ cell growth and maturation. In the Yq 11 region, some of the genes are concerned with germ cell function. Disturbance in the genetic make-up of this region has been reported in 5-20% of cases with azoospermia and oligospermia. Such genetic anomalies are considered de-novo mutations which are not detected in normospermic men. 3 gene loci are described related to spermatogenesis abnormalities, namely, loci **a**, **b** and **c** respectively. Any genetic deletion on those loci is expected to cause abnormality of spermatogenesis. In the management of subfertile males, the advent of ICSI (Intracytoplasmatic sperm injection) and TESE (Testicular sperm extraction) have revolutionized results of assisted reproduction methods. However, such procedures allegedly run the risk of carrying sperm

with defective Y-chromosome, which could lead to subfertility problems in the male progeny.

E. Gene Tests for Neoplastic Disorders

E1. Multiple Endocrine Neoplasia Type I, MEN I Gene

Specimen: 2-5 ml EDTA blood. *Turnaround time:* 2 weeks.

Indications:

(1) Familial incidence of primary hyperparathyroidism associated with hypersecretion of pancreatic- peptide hormones, e.g.s gastrin, insulin and pancreatic peptide.
(2) Pituitary hyperfunction, e.g.s elevated levels of prolactin, growth hormone, TSH or ACTH.
(3) Adrenocortical hypertrophy, carcinoid, lipoma or pinealoma.
(4) Multiple tumors in the parathyroid gland, endocrine pancreas or anterior lobe of the pituitary gland.

Methodology: DNA sequencing of MEN I gene on Chromosome 11 by PCR.

Comment:

MEN I (Multiple Endocrine Neoplasia Type I) is an autosomal dominant inherited disorder, characterized by combined neoplasia of the parathyroid gland, neuroendocrine component of pancreas and duodenum as well as pituitary gland. Clinical manifestations are largely related to the affected organ(s) involved with consequent hyperfunction or hypofunction of the relevant hormone(s). Hypercalcemia occurs as a result of exaggerated production of parathyroid

hormone and which is the most common biochemical abnormality seen in MEN I. Secretion of pancreatic peptide hormones, gastrin or insulin leads to defined clinical syndromes, for example, Zollinger-Ellison syndrome, hypoglycemia and so on. Pituitary hyperfunction manifests as hyperprolactinemia and, rarely, acromegaly (GH), hyperthyroidism (TSH) or Cushing syndrome (ACTH). Furthermore, MEN I patients show increased risk of developing tumors in other organs, for example, adrenocortical hyperplasia, carcinoid tumor, lipomas, and pinealomas. Prevalence of MEN I is estimated at 0.02-0.175/1000 inhabitants. The predisposing genetic defect responsible for the disease is located on the long arm of Chromosome 11 (11q13). Detection of these mutations makes possible early treatment and prevention of the above neoplasms in the presymptomatic stages.

E2. Familial medullary thyroid carcinoma, MEN II Gene

Specimen: 2-5 ml EDTA blood. *Turnaround time:* 2 weeks.

Indications:

(1) Familial or sporadic medullary carcinoma of the thyroid gland.
(2) Pre-symptomatic diagnosis of MEN II in relatives of FMTC (familial medullary thyroid carcinoma).
(3) Pheochromocytoma, bilateral or at early age, or association with Von-Hippel-Lindau-Syndrome or Neurofibromatosis 1.

Methodology: Detection of all known MEN II/MTC mutations in Ret-Proto-oncogene (Exons: 10,11,13,14,15,16) on Chromosome 10 by PCR.

Comment:

Approximately 25% of MTC cases are familial. This form of thyroid carcinoma is found in combination with other neuroendocrine tumors and neuroectodermal defects. Characteristic of MEN IIa is a combination of MTC, pheochromocytoma and hyperparathyroidism. Variant MEN IIb shows in addition neuroectodermal defects. MEN IIa is autosomal dominant. Gene carriers manifest clinically with MTC in 70% by the age of 70 years. The disease is usually first diagnosed during the fourth decade of life. Early diagnosis is based on biochemical screening by the pentagastrin stimulation test for calcitonin secretion, which unveils C-cell hyperplasia or occult MTC. The test has been recommended in MEN IIa family members once every 6 to 12 months for early diagnosis. A positive Pentagastrin stimulation test calls for further assessment of the endocrine system to enable timely surgical intervention.

E3. Acute lymphoblastic leukaemia, ALL t(9;22)abl Oncogene

Specimen: 2-5 ml EDTA blood or bone marrow aspirate in dry ice. *Turnaround time:* please enquire.

Indications:

(1) ALL.
(2) Difficult diagnostic cases of leukopenia and thrombocytopenia.

(3) Differential diagnosis of cytochemically similar forms of leukemia.
(4) Follow up for minimal residual disease in treated ALL cases.

Methodology: RNA reverse transcription and PCR.

Comment:

ALL involves proliferation of immature lymphocytes and lymphoid precursor cells. The disease is commonly seen in children between 2-10 years old and in people over 60 years old. About 50% of cases show chromosomal translocations or deletions. 4% of childhood and 20% of adulthood ALL are Philadelphia chromosome positive with respect to translocation between the long arm of Chromosome 9 and 22, transferring with it the c-abl-Proto-oncogene from Chromosome 9 to Chromosome 22. Molecular analysis of this translocation enables specific typing of the disease and detects the So-called 'Fusions gene', a 6.5kb long mRNA coding a 190kD fusions-protein. Detection of the 9;22 translocation by PCR makes possible the detection of bcr/c-abl mRNA. Total RNA –fraction is extracted from peripheral blood or bone marrow aspirate, after which by reverse transcription, complementary DNA copy (c DNA) is prepared. Gene product sequences are amplified with the help of highly specific oligonucleotide primers, after which cells with the translocation are amplified by PCR. This procedure is very useful for detecting minimal residual disease and has a high sensitivity, detecting one positive cell in 1 million negative cells. This is of utmost importance in the follow up of transplanted patients.

E4. Pre B-Cell ALL, Pre-B ALL t(1;19) EZA/PRL Oncogene

Specimen: 2-5 ml EDTA blood or bone marrow aspirate in dry ice. *Turnaround time:* please enquire.

Indications:

(1) ALL with neutropenia,and thrombocytopenia.
(2) Pre-B-cell markers (CD19,CD10) positive by immunocytochemical staining.
(3) Differential diagnosis of cytochemically similar forms of leukemia (Common-ALL, B-ALL).
(4) Follow up of treated cases.

Methodology: RNA reverse transcriptase, PCR.

Comment:

Pre-B-Cell ALL is associated with a translocation t(1;19) (q23;p13.3) which is found in 6% of all pediatric leukemias and 30% of all pre-B-Cell phenotype leukemias. Genetic detection of this translocation, based on detection of the ancient E2A/PRL mRNA, using PCR technique, makes possible specific typing of the disease. The high sensitivity of this test provides an optimal diagnostic aid for monitoring minimal residual disease after bone marrow transplantation.

E5. Philadelphia Chromosome t(9;22), CML bcr/abl Oncogene

Specimen: 2-5 ml EDTA blood or bone marrow aspirate in dry ice. *Turnaround time:* one week.

Indications:

(1) Leucocytosis with suspected CML (chronic myeloid leukemia).
(2) Follow up of treated patients.

Methodology: RNA reverse transcriptase and PCR.

Comment:

CML involves proliferation of pluripotent stem cells, characterized by a cytogenetic anomaly represented by Philadelphia chromosome (Ph'). In 90% of cases, diagnosed clinically as CML, translocation is seen between long arms of Chromosomes 9 and 22. C-abl-Proto-oncogene (Chromosome 9) is translocated to Chromosome 22. The break-point on Chromosome 22 is situated in a 5.8 kb gene-region represented by Ph'. This is called the break-point-cluster-region (bcr-210). After translocation a hybrid-mRNA of 8.5 kb, coding for 210-kDa-protein is formed, which in turn represents the oncogene responsible for the malignant transformation of cells. Molecular genetic analysis of this translocation makes possible specific typing of the disease. Detection of t(9;22) translocation using PCR, based on detection of bcr/c-abl-mRNA, has a peak sensitivity of one positive cell in one million negative cells. This renders it ideal for detection of minimal residual disease after bone marrow transplantation.

E6. Follicular Lymphoma t(14;18) bcl-JH Oncogene

Specimen: 2-5 ml blood, lymph node biopsy or bone marrow aspirate in dry ice. *Turnaround time:* one week.

Indications:

(1) Differential diagnosis of cytochemically similar leukaemias or lymphomas.
(2) Differential diagnosis of different forms of lymphomas.
(3) Follow up of treated patients.

Methodology: DNA amplification by PCR to identify translocation.

Comment:

Follicular lymphoma is a B-cell tumor which in 85% of cases shows a translocation t(14;18) (q32;q21) where the Oncogene bcl-2, from chromosome 18 on the J-region of the Immunglobulin-Heavy Chain-Gene, is translocated to Chromosome 14. A so-called major break-point-region (mbr) of 15 bp in 3'nontranslated region of Exon 2 is found in 60% of cases. The break-point on Chromosome 14 is located in the 5'end of one of six JH-Gene segments of the Immunoglobulin-Heavy Chain. The result of this translocation is a Fusion-gene, the bcl-2/JH-oncogene. In 25% of cases, a break-point-region of 2 kb of this gene, identified as minor-cluster-region (mcr), is present. On the other hand, bcl-2 is a mitocondrial protein responsible for cell programming in apoptosis. Following t(14;18) translocation, irregular expression of bcl-2 takes place with consequent prolongation of the malignant B-lymphocytes life-span. Molecular genetic amplification has proved to be highly sensitive and useful in the detection of minimal residual disease.

F. Gene Tests for Neuromuscular Disorders

F1. Muscle dystrophy, Duchenne/Becker

Specimen: 2-5 ml EDTA blood. *Turnaround time:* 2 weeks.

Indications:

(1) Differential diagnosis of muscle diseases of unknown origin, especially at young age and in children with delayed walking.
(2) Family screening and confirmation of diagnosis in female gene carriers.
(3) Prenatal diagnosis.

Methodology: Direct detection of deletions in male patients by PCR. Indirect analysis for carrier status in suspected females by capped DNA markers.

Comment:

Muscle dystrophies, of Duchenne and Becker types, are the most common forms of inherited muscle disorder. It is linked to X-Chromosome, affecting only males with females affected as gene carriers of the disease. One-third of patients have evidence of a new mutation. Incidence of the disease is estimated at 1:3500 newborns. Dystrophin gene is in 73 exons, 2300 kb on X-chromosome (Xp21). In 60% of cases, a deletion of one or more exons is detected, in 5% duplication of more exons in the genome is seen, while in 35% of cases point mutations, are present. Such mutations or deletions are responsible for absence or severe structural

changes in the dystrophin protein with consequent disease effects. In case of BMD (Becker muscle dystrophy), a shortened but functioning dystrophin molecule is formed after deletion, leading to a mild form of the disease. Children with DMD (Duchenne muscle dystrophy) are symptoms free for the first two years of life. Nevertheless, changes in serum creatinine kinase concentration, as well as in muscle biopsy changes are present by then. Manifestations of the disease begin between 2-4 years of age, and consist of a waddling gait, inability to climb stairs or get up from the floor unaided; simultaneously, remarkable general muscle weakness with hypertrophy of some weight bearing musculature develops. Progressive muscular weakness takes place so rapidly that, at 8-12 years of age, patients are unable to walk and, before the 25^{th} year of life, die of cardiac or respiratory failure. About 20% of these patients also have mental retardation. In BMD cases, symptoms appear in early puberty with severe muscular weakness. Molecular genetic diagnosis enables early therapeutic measurements and improves quality but does not extend life span.

F2. Mitochondrial Encephalopathy, MELAS tRNA leu UUR Gene

Specimen: 2-5 ml EDTA blood, biopsy material. *Turnaround time:* 2-3 weeks.

Indications:

(1) Stroke-like episodes, lactic acidosis, dementia, often headache accompanied by vomiting, impaired hearing, cortical blindness, hemiparesis.
(2) Ragged red fibers in muscle biopsies.

(3) Family members of MELAS patients in the maternal inheritance line.
(4) Diabetes mellitus with maternal inheritance and neurological symptoms.

Methodology: Detection of mutations using PCR and DNA sequencing.

Comment:

MELAS (Mitochondrial Encephalopathy, Lactic Acidosis, Strokes) is an inherited metabolic disorder which belongs to the mitochondrial cytopathy group. The disease is inherited through the maternal line, caused by mutations in the mitochondrial genome. 80% of patients show a mutation in the t-RNA Leu-UUR Genome (position 3243 A>G). The clinical picture is dominated by stroke-like episodes, brain ischemia, hemiparesis, vessel-related visual disturbances as well as cortical blindness and hemianopia. In all cases ragged-red fibers are found in the muscle biopsy.

F3. Myoclonal Epilepsy, MERRF t-RNA lys Gene

Specimen: 2-5 ml EDTA blood, biopsy material. *Turnaround time:* 2 weeks.

Indications:

(1) Myoclony with ataxia and muscle weakness associated with fits, Hyperlactasemia and ragged-red fibers in muscle biopsies.
(2) Relatives of MERRF patients in the maternal line.

Methodology: Detection of mutations by PCR and DNA sequencing.

Comment:

MERRF (Myoclonal Epilepsy with Ragged Red Fibers) is an inherited defect in mitochondrial biosynthesis, producing mitochondrial encephalomyopathies inherited through the maternal line. The underlying cause in 85% of cases is a mitochondrial mutation in t-RNA-Genes. Clinical features are rhythmic epileptic myoclonic attacks, ataxia and general weakness. Severely affected family members suffer with dementia, respiratory difficulty due to hypoventilation, cardiomyopathy and renal failure. The disease affects predominantly (95%) young individuals at a median age of 20 years. Molecular diagnosis may require muscle biopsy in addition to blood sampling.

F4. Myotonic Dystrophy, DMK Gene

Specimen: 5-10 ml EDTA blood. *Turnaround time:* 2-3 weeks.

Indications:

(1) Myotony, cataract, mental retardation.
(2) Screening of families with index cases.

Methodology: Determination of CTG-Repeats number on Chromosome 19, by PCR and Southern Blot.

Comment:

Myotonic dystrophy is the most commonly inherited neuromuscular disease. It is autosomal dominant with an incidence of 1:25000-1:8000 individuals. Myotonic reactions are frequently found in Myotonic Dystrophy (Curschmann-Steinert), Myotonia congenita, Chondrodystrophic myoto-

nia, Dyskalemic paralysis and PROMM (proximal myotonic myopathy, the autosomal dominant type). The clinical picture of those disorders are protean but characterized by simultaneous appearance of myotonia, muscular weakness, muscular atrophy with pathological changes in bones, hair follicles, eyes and endocrine glands. Muscular weakness and atrophy affects all muscles, including facial, oropharyngeal, neck, as well as those at extremities and in severe grades the respiratory musculature. They almost always show cataract on slit-lamp examination. 83% of males and 16% of females show frontal boldness and the majority have cardiac lesions such as heart block, atrial fibrillation, cardiomyopathy and mitral valve prolapse. Congenital dystrophy is the most severe form of disease, manifesting at early age with hypotonia, delayed mental and motor development. The majority of myotonic dystrophies begin at young age, with progressive pathological changes and deterioration of the general condition with a shortened life span extending to 45-50 years. Molecular genetic diagnosis aids in specific determination of CTG-repeats number and identification of affected individuals with provision of a meaningful tool for genetic counselling.

F5. Fragile-X or Martin Bell Syndrome, FMR1 Gene

Specimen: 5-10 ml EDTA blood. *Turnaround time:* 2-3 weeks.

Indications:

(1) Differential diagnosis of mental retardation, especially in males.
(2) Identification of mutations and pre-mutations in female carriers.

(3) Confirmation of cytogenetic test results.
(4) Family screening of index cases.

Methodology: Determination of CGG-Repeats by PCR. Examination of CGG-Repeats and methylation of regulatory sequences of FMR1-Gene on X-Chromosome by Southern Blot Hybridization.

Comment:

Fragile X-syndrome is the most common genetic cause of mental retardation, whose spectrum ranges from mild learning disability to severe mental retardation and autism. The incidence has been estimated at 1:1250 in males and 1:2000 in females, while 1:300 females are carriers. The disease is caused by excessive repeats of the sequence CGG in the genome. In the normal population the number of the CGG trinucleotide repeats is between 5-59. The region between 60-200 CGG trinucleotide repeats is called premutation region. Premutations female carriers are healthy but transmit this defect to their children who in turn could develop a full mutation of the FMR1 gene and thereby manifest the disease. A region with more than 200 CGG-trinucleotide repeats as well as hypermethylation of regulatory sequences is called full mutation region, and leads to the fragile X syndrome. In 80% of cases there is learning disability and mental retardation. Direct molecular genetic analysis provides confirmation of cytogenetic diagnosis of fragile X syndrome. Moreover, by detecting mutations and premutations, prevention can be improved through better informed genetic counselling.

F6. Spinocerebellar ataxia (Types 1,2,3,6,7,8,10,12,17)

Specimen: 5-10 ml EDTA whole blood *Turnaround time:* 2-3 weeks

Indications

Unstable gait, dysarthria, hyporeflexia, cerebellar atrophy on head MRI with normal brainstem.

Methodology: PCR followed by sequencing and hybridization to identify CAG repeats.

Comment:

Spinocerebellar ataxia (SCA) is an autosomal dominant cerebellar ataxic disorder which is clinically, pathologically and genetically heterogeneous. The disease is of late onset (>20 years). Cerebellar ataxia is usually associated with opthalmoplegia, optic atrophy, pigmentary retinopathy, mild dementia, peripheral neuropathy and extra-pyramidal dysfunction. Symptoms are usually progressive. It is accompanied by dementia. SCA is caused by CAG trinucleotide repeat expansions (>37 repeats, usually 43-81) in the ataxin gene on chromosome 6p and is inherited as an autosomal dominant character. 10 responsible genes have been identified for spinocerebellar ataxia types SCA 1, 2, 3, 6, 7, 8, 10, 12, 17 and dentatorurbral palliduluysian atrophy (DRPLA), respectively. Still, about 20% of cases have unidentified mutation. There is unstable expansion of trinucleotide (CAG), the normal being 19-37 repeats, whereas in type I, for example, it is 43-81 repeats. In contrast, SCA III, known as Machado-Joseph disease, is charaterized by late-onset cerebellar ataxia with dementia and is caused by repeat expansions on chromosome 14q.

F7. Amyloidotic Polyneuropathy

Specimen: 5-10 ml EDTA whole blood *Turnaround time:* 2-3 weeks.

Indications

Sensorimotor polyneuropathy, digestive autonomic disturbances, cardiomyopathy.

Methodology: PCR is mainly used to detect FAP mutations with sequencing in the prepositus cases.

Comments:

Familial Amyloidotic polyneuropathy (FAP): a heterogeneous group of autosomal dominant multisystem disorders which usually present with small fiber peripheral neuropathy affecting the limbs and autonomic function. Mutant protein is called TTR (Transthyretia). Many point mutations in TTR protein are more common in certain ethnic groups than in others, e.g., FAP mut 30-exaon of TTR mainly in Portuguese, also ALA 60, Glu 89 and many other types of substitution proteins.

F8. Huntington's Disease (HD)

Specimen: 5-10 ml EDTA whole blood. *Turnaround time:* 2-3 weeks.

Indications

(1) Positive family history, consistent with autosomal dominant inheritance.

(2) Progressive motor disability involving both voluntary and involuntary movement.
(3) Mental disturbance and/ or changes in personality.

Methodology: PCR for the detection of HD gene - expansion of CAG repeats in the 4p16.3 region.

Comment

Diagnosis is made primarily on clinical examination. CT and MRI provide support for the diagnosis. HD (Huntington's disease) is an autosomal dominant condition caused by CAG repeat expansions on chromosome 4p16.3. It is the most important type of late-onset neurodegenerative diseases that result in extrapyramidal movements and progressive dementia.

Detection can be readily offered to those at risk, since the nature of HD mutation is known. However, if testing for the mutation is waived in a woman at risk who is pregnant, linkage analysis using a polymorphism close to the HD gene could be accepted for prenatal diagnosis in the fetus, since it is may be possible to exclude the disease in the fetus without appreciably changing the parent's risk.

F9. Spinal muscular atrophy (SMA)

Specimen: 5-10 ml EDTA whole blood. *Turnaround time:* 2-3 weeks.

Indications

Family history of neuromuscular disease or suspected neuromuscular disease in children.

Methodology: PCR amplification of three regions 253300, 253400, 253550 (Types I,II,III) on chromosome 5, followed by hybridization with region specific probes.

Comments

Spinal muscular atrophy is a relatively common disorder affecting 1/10,000 births with a carrier frequency rate of up to 1/80 births.

It is an autosomal recessive disorder which results in anterior horn cell degradation of the spinal cord. Type I (Werdnig-Hoffman disease), the most severe form of SMA, presents with progressive weakness and hypotonia, leading to death in infancy.

The mutation consists of a complex of large genomic deletions involving SMN (Survival Motor Neuron) gene in 5q13 region. The gene encompasses the atelomeric (SMNT) region where >97 % of deletions occur, and a highly homologous centromeric (SMNC) region where < 5 % of deletions occur and with no obvious phenotypic effect.

F10. Friedreich's ataxia

Specimen: 5-10 ml EDTA whole blood. *Turnaround time:* 2-3 weeks.

Indications

Unsteady gait before puberty, difficuly in walking, abnormal reflexes, incoordination, slurred speech and nystagmus.

Methodology: PCR for GAA repeat expansions and linkage analysis.

Comment

In most large studies, Friedrich's ataxia accounts for at least 50 % of cases of hereditary ataxia. It is an autosomal recessive disorder. Cases in which no family history can be induced may represent new mutation. Symptoms usually develop before 20 years of age.

The defective mutant gene, X25 or Frataxin, is located on long arm of chromosome 9 (9q13), which causes the ataxia, most commonly resulting from GAA trinucleotide repeat expansions in the first intron. Symptoms are caused by degeneration of nerve fibers in spinal cord and peripheral nerves.

F11. Hereditary Motor & Sensory Neuropathies or Charcot-Marie-Tooth Disease

Specimen: 5-10ml EDTA blood. *Turnaround time*: 3-5 weeks

Indications

(1) Toe-walking in a young child or deformity of hands or feet, e.g. pes cavus.
(2) Abnormal gait due to dorsiflexor weakness, slapping gait.
(3) Impaired tendon reflexes.
(4) Nerve hypertrophy in onion fashion on sural nerve biopsy.
(5) Symmetrically reduced nerve conduction velocity: < 40 meters/second.

Methodology: Pulsed field gel electrophoresis for DNA segment separation and Southern blotting using specific probes for CMT1A duplication.

Comment

Patients complain of pain, skin injury due to sensory loss and unsteady gait, frequently with autonomic disorders. Hereditary motor and sensory neuropathies (Types I, II & III) or Charcot-Marie-Tooth disease (CMT) constitutes the most common inherited peripheral neuropathy, with an estimated prevalence of 1 in 2500.

The most common form is CMT Type 1A, the majority of which are accounted for by DNA duplication in a region on the short arm of chromosome 17 (17p11.2). A small proportion is accounted for by point mutation at the PMP22 gene in nonduplication patients with clinical CMT1A. Also, CMT1B cases have been rarely identified, caused by aberration on the long arm of chromosome 1 (1q21.22). CMT Type II cases are much rarer than Type I and are characterized by mild to moderate reduction of nerve conduction velocity. And although the vast majority are autosomal dominant, occasional cases are X-linked and more rarely autosomal recessive. No genetic tests are available for X-linked or autosomal recessive CMT cases. Testing for CMT1A duplication in 17p11.2 is possible and detects 70-90% of CMT Type 1 patients. A positive result is indicated by 500kb CMT1A duplication specific junction fragment and is diagnostic of CMT Type I disease. However, some patients with clinical CMT Type I show point mutation at peripheral nerve myelin protein gene, PMP22, site in the same region instead of CMT1A duplication; therefore a negative result for duplication does not exclude the diagnosis of CMT Type I disease since it may be due to point mutation.

K. Gene Tests for Kidney Disorders

K1. Autosomal dominant polycystic kidney disease (ADPKD)

Specimen: 5-10 ml EDTA whole blood. *Turnaround time:* 2-3 weeks.

Indications

Family history of hypertension, especially with renal impairment or cerebrovascular accident in one parent. 3 or more kidney cysts on ultrasound strengthened by family history.

Methodology: PCR based methodology and linkage analysis.

Comments:

Autosomal dominant, usually symptoms starts in the fourth or fifth decade of life with hematuria, hypertension, abdominal pain, abdominal mass and uremia. Although abnormalities in the kidney are present since infancy and childhood they may be discovered by chance. Hypertension often develops before onset of kidney cysts. Cysts grow out of nephrons eventually separating them causing enlarged but preserved kidney shape. Patients may develop urinary tract infection, nephrolithiasis, liver and pancreatic cysts, abnormal heart valves, aneurysms and diverticular disease of colon.

Many patients may lack positive family history, indicating a new mutation.

Diagnosis can be easily made by abdominal ultrasound and CT, both prenatally and postnatally.

K2. Autosomal recessive polycystic kidney disease (ARPKD)

Specimen: 5-10 ml EDTA whole blood. *Turnaround time:* two - three weeks.

Indications

Kidney failure in early life and even in utero, though rare, leads to death in the first hours of life in severe cases. Children with less severe form may develop renal impairment in their teens and show liver cysts.

Methodology: PCR based methodology with linkage studies.

Comment:

In the infantile form, carrier frequency is 1:49 associated with liver abnormalities. The gene responsible for some case of ARPKD was found to reside on chromosome 6. Diagnosis should be confined to those individuals who present with bilateral renal cystic disease or renal tubular ectasia and congenital hepatic fibrosis of varying degrees either at birth or during the first year of life.

Parents of affected child may be normal where a 25% chance exists for any subsequent child to have the disease.

Prenatal ultrasonography is a reliable screening procedure. Ultrasound of relatives may be helpful, for example, a parent or grandparent. The liver may show scarring on a scan.

K3. Autosomal dominant medullary cystic kidney disease (MCKD)

Specimen: 5-10 ml EDTA whole blood. Turn around time: two - three weeks.

Indications:

Polyuria- especially salt losing, also chronic renal failure in children (second most common cause).

Methodology: PCR based method.

Comment:

An autosomal dominant disease that usually starts in adulthood and is not associated with any other renal or nonrenal manifestations. MCKD Type 1 is autosomal dominant caused by gene MCDK1 located on chromosome 1q21, with unknown mutation and genetic defect. MCKD Type 2 is also autosomal dominant and is caused by gene MCKD2 located on chromosome 16p1, at which same site gene for familial juvenile hyperuricemic nephropathy is located. Mutation and genetic defect have not yet been identified. MCKD accounts for 15 % of cases with childhood end-stage renal failure. It is a chronic progressive disorder that starts with inability to concentrate urine, anemia, growth retardation with small kidneys exhibiting either medullary cysts or tubulointerstitial fibrosis. Numerous small cysts develop deep within the kidneys. First, symptoms may not appear before adulthood, but this form may show an auto-

somal dominant mode of inheritance. About 15% of persons with medullary cystic disease have retinal degeneration (renal-retinal dysplasia).

L. Gene Testing for FMF (Familial Mediterranean Fever)

Specimen: 5-10 ml EDTA whole blood. *Turnaround time:* 2-3 weeks.

Indications:

Fever or serositis of unknown cause, especially in Arabs, Armenians, Turkish and Eastern Jewish stock. Recurrent abdominal pain with fever is a common presentation.

Methodology:

ARMS (PCR based technique) is used to identify the FMF gene, designated MEFV, which was recently cloned on chromosome 16, with three main mutations M6801, M 694V and V726 accounting for the vast majority of patients with FMF. In addition, PCR followed by enzymatic digestion is used to screen for two more mutations, namely E148Q and F479L. Both heterozygous (carrier) and homozygous (affected) individuals can now be identified.

Comments:

FMF is a rare autosomal recessive disorder characterized by episodes of fever and acute inflammation of serous membranes manifesting as abdominal pain, pleurisy, pericarditis, arthritis or skin rash, usually diagnosed clinically by exclusion. The main complication is amyloid which accumulates in kidneys causing impairment of renal function.

DNA Extraction Methods

DNA Extraction from Whole Blood

1. Collect ~20ml blood in a 50ml tube containing 0.5ml of 0.11M Di-potassium EDTA (SIGMA cat. No. ED2P - 40g/L, 0.11M).
2. Add 0.9% saline (9g NaCl/L) to 50ml and centrifuge for 5' at 2500 RPM. Remove the supernatant. Avoid removing the buffer coat layer.
3. Add RBC (Red Blood Cell) lysis solution (0.18M NH_4Cl; Ammonium Bicarbonate - 9.54g Ammonium Chloride; 0.237g Ammonium Bicarbonate/L H_2O to 50ml). Mix for 10-15 min until haemolysis is complete.
4. Centrifuge for 5' at 2500 RPM to pellet the white cells. Remove supernatant.
5. Add 10ml WBC (White Blood Cell) lysis buffer (NaCl 100mM; Disodium EDTA 25mM - 10ml of 4.0M NaCl + 20ml 0.5 EDTA pH8.0 up to 400 ml H_2O), and mix well to resuspend the pellet. Add 500µl 10% SDS, and 10µl of Protease (200 mg/ml). Digest for at least 2h at 37^{O}C (Protease type XXV, Cat. No. 6911, Sigma, 200 mg/ml in H_2O, autodigested for 1 h at 37^{O}C).
6. Add 0.37ml of 9.5M Ammonium Acetate per ml of protease-digested DNA. Mix well.
7. Centrifuge for 20 min at 4000 RPM.

8. Collect the supernatant. Add 2 volumes of 100% EtOH. Mix well and spool DNA. Transfer DNA to a 2 ml tube and wash with 70% EtOH. Drain well.
9. Dry by standing or on speed vac. for 1-2 min.
10. Redissolve in ~1ml of sterile H_2O (peripheral blood) depending on sample size.

Modified from: Miller, S.A., Dykes, D.D., Polesky, H.F. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* 1988;16(3): 1215.

DNA Extraction from CVS

Collect microvilli in heparinized saline solution.

If the sample is bloody, allow microvilli to settle by gravity, remove the supernatant and add fresh sterile saline. Repeat until the sample is fairly clear. Place in a petri dish and clean microvilli from debris, small blood clots and any suspect maternal tissue by microdissection under a stereo microscope. Rinse dissected microvilli with saline under normal gravity and then once by centrifugation.

Resuspend clean microvilli in WBC lysis buffer (1.0 - 2.5 ml, according to sample size). Proceed as for DNA extraction from whole blood (paragraph 5) adjusting the volumes appropriately.

Notes:

Transport of samples:

Fresh blood is best sent on ice and should be delivered within 48 hours. Good DNA may be recovered even after a few days at room temperature.

DNA can be sent under 70% ethanol as a precipitate by ordinary post if samples are not urgent. It can also be sent dissolved in sterile water and sent within a few days.

CHAPTER 6

HISTOPATHOLOGY - CYTOLOGY

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1- Routine Cytohistopathology Service

Same-day or 24-hour turnaround time whenever possible.

Over-the-phone results and discussion of cases is commonly practiced.

Collection of specimens from hospital and clinics are made on the basis of call order.

Routine fixative (buffered formalin) is readily available, free of charge, for clients.

Results and reports are issued direct to patients or by courier to clinics, hospitals, or by fax.

Alternatively, e-mail delivery is made on request with captions of interest.

Pricing is in accordance with the Ministry of Health price list for clinical tests and the Jordan Medical Association for cytohistopathology.

Standard quality control methods constitute a strong back-up in the different fields of laboratory medicine. This is supported by CME programs in discussion groups, conferences and research work and which includes modern communication as support tools.

For critical tests as well as pathology, relevant clinical history, even in a few words, would be invaluable in reaching a precise diagnosis in the shortest time. Minimum data would include patient's name, age, sex, site of origin, clinical diagnosis and doctor's name. This applies to common as well as rare lesions (Captions 32 & 33).

Brief notes on gross pathology

Every sample should be handled gently, taking care to incur least damage to tissue, in order that microscopy is least affected by crush artifacts.

Some opt to adopt special strategies in handling different samples, which would be a good idea. For instance, attempt should be made to make adequate dissection leaving at the same time room to reassemble the specimen back to its original shape and form, whenever possible.

Approaching dissection of a specimen should follow a fairly systematic pattern. For example, in the alimentary tract longitudinal sections should be taken rather than cross sections to give more useful information along the alimentary tube. In addition, it is best to section the lymph node across its hilum, at which level relation of the different lymph node zones can be better identified.

Every sample should be weighed and its weight recorded, as well as its maximum dimensions. Comment on the shape of the specimen sometimes may be useful. Texture and color should be described as a routine.

Gross pathology should be performed by the pathologist and not the technologist.

Despite textbooks and many chapters in textbooks written on gross pathology, the author of this guide believes that its value is greatly underestimated by workers in pathology. A reference book or key articles on gross pathology should always be at hand in the pathology department. In fact, proper grossing is sometimes the key to successful handling of a difficult case in surgical pathology. The importance of clinicopathologic correlation cannot be overemphasized,

and importance of a biopsy being representative of the disease process underlies the degree of diagnostic precision.

2- Frozen Sections

Specimen sampling and result over the phone are given within less than one hour on out-patient basis and half hour on an in-patient basis for any hospital in a city like Amman. Samples for frozen section are sent direct from the operating theater to the laboratory by courier, hospital transportation or patient's relatives.

The frozen section service at the CML has been running very efficiently for the past 23 years. It is stipulated that results of frozen section are only given to attending medical staff and the telephone line should only be disconnected when the surgeon in charge releases the pathologist from the case at hand and after acknowledging receiving the frozen section result by himself or by a delegated medically qualified person.

3- Immunohistochemistry Service

A wide panel of antibodies for immunohistochemistry used in the investigative diagnosis of hematologic and solid tumors as well as identification of specific antigens in body tissue. Moreover, organ specific markers are available, such as for prostate and thyroid. Markers for hormonal receptors in breast cancer (estrogen and estrogen receptors and HER-2 neu) on sections from paraffin blocks are routinely done for prognostic and treatment purposes, while marker studies of round cell tumors are indispensable. (See Captions 22&34).

Immunohistochemistry testing on individual tumors or cases are designed individually, with use of a special panel in each case as guided by H & E sections. For consult cases, paraffin blocks are required as a rule.

A new method called “tissue microarray technique” is gaining wide use in busy and large immunohistochemistry departments. It involves taking 0.6mm tissue core samples, one or more per case, then their insertion into holes in the recipient block, the TMA (tissue microarray) block. More than 200 cores are borne in one TMA block representing scores of cases. TMA blocks save on reagent consumption and extremely expedites results, whether by manual or automated method. A large number of samples is examined promptly, while it poses itself as a strong research tool.

Pathology Report (Employing immunohistochemistry)

Round cell tumor: Ewing’s sarcoma

An 8-year old boy presented with loss of weight. X-ray and U/S suspected lung hydatid. Histopathology reported a malignant round cell tumor with the possibility of Ewing sarcoma. The case was referred for confirming diagnosis.

Microscopic examination **See Caption 34**

Sections show a necrotic and infiltrating small cell tumor with evidence of rosette formation. The cells showed monotonous morphology with strong PAS positivity.

Immune Reactions of tumor cells:

CD99= Strong Positive *with a membranous staining pattern.*

S100, Synaptophysin, Vimentin, Desmin and EMA: positive reactions of various intensity. LCA & Myosin= Negative

Conclusion

PRIMITIVE NEUROECTODERMAL TUMOR (EWING’S SARCOMA).

Yahia F. Dajani, M.B. Ch.B. (Bristol), F.R.C.Path. (London)

September 14, 2002.

In special circumstances, immunohistochemistry markers are performed on frozen sections or cell smears obtained by FNA, cytospin or touch imprints, for example bone marrow. Immunohistochemistry is essential in the diagnosis of round cell tumors. (See Pathology Report overleaf).

4- Consultations Service

* Consultations on problem cases in pathology should be open to doctors and the public at large. Any physician may refer a case, usually by submission of:

1- A relevant clinical history, glass slides and preferably:
2- Paraffin block(s) or tissue material from the case in question, since further testing may be required, such as examination of further material in search of additional features of the case or to perform other tests, for example hormonal receptors for breast cancer, or tumor markers by immunohistocheminal stains to reach a more precise diagnosis or classification in the case of interest, such as often seen in spindle or round cell tumors.
3- Further material on the case, for example, x-rays or tests relevant to the case. In the case of bone or intracranial tumors, to mention two examples, provision of radiological material is mandatory for reaching an adequate diagnosis.

* *The CML consultation service is supported by high expertise and modern methods, such as immunohistochemistry, flow cytometry, cytogenetics, molecular methods and telepathology.*

5- Telepathology Unit

Telepathology started operating in June 1997, the first of its kind in Jordan.

Telepathology may be defined as practicing diagnostic pathology at a distance by telecommunication.

Telepathology may use static or dynamic methods. The former is employed at the CML Telepathology Unit, since it is time-convenient, less costly and of adequate diagnostic accuracy. In fact, telepathology is integrated into routine pathology service, backing up an evidence-based mode of practice.

Innovation of worldwide internet has enabled transmission and capture of microscopic images which allows ready availability of expertise at remote or distant sites. This acts as backup to the CML consulting service in cases where special expertise is required. The telepathology unit provides a unique opportunity to attend courses in specific subjects, enable review of reference and illustrated material at one's own convenience.

For educational purposes, courses are available on the internet on different topics with illustrated cases showing images that can be enlarged for more detail and can provide means of continuing education in pathology, economizing with travel expenses and learning at leisure, with use of either problem solving case approach or looking up material in atlas form and which is readily available from several centers. This enriches the referral library of the practicing pathologist and allows a ready access to illustrated reference histology material that can be used during his or her everyday practice in pathology. Many sites are

available on the web to meet such purpose, such as Bristol Biomed Image Archive http://www.brisbio.ac.uk, or Web Path www.medlib.med.utah.edu/WebPath/webpath.html#-MENU, and so on.

Quality assessment programs at the AFIP and the Royal College of Pathologists and most useful for their users. Such facilities will inevitably be required in the future practice of pathologists. Also, distant learning as part of daily activities, whether service or education oriented, has recently taken new strides. For example in the Telemedicine Department at the AFIP, Washington D.C., a schedule can be found at the AFIP web site http://www.afip.org/-grandrounds/schedule.htm which enables attending lectures by world renowned persons through a special subscription. Grand rounds through videoteleconference can be attended. In-planning projects include provision of educational modules for the most common diagnostic problems, emerging diseases and quality assurance, all of which are invaluable in daily pathology practice. Database in the making will provide an extensive supply of still pictures, lectures, short movies and background information which are easily retrievable on demand and will help diagnosis and patient care, irrespective of location of your practice.

The following are two examples of cases referred to the AFIP Telemedicine Department, Washington, D.C.

Case 1

A 60-year old man had a 20 year history of working in the textile industry. For the past 6 months he developed anorexia, lethargia, weight loss, fever, left sided chest pain, progressive dyspnea and muscle weakness in his lower limbs. Physical examination showed cachexia with proximal muscle wasting and weakness. Chest examination showed dullness on percussion of the left chest with absent breath sounds. CT scan of the chest (Figures 1-3) showed encasement of left lung

by extensive nodular pleural thickening with enhancing areas and regions of encysted fluid involving the whole hemi-thorax. The mass involved the mediastinal pleura with a large area of mixed solid and hypodense portions abutting the aortic arch, great vessels and encasing the descending aorta and some of the left pulmonary veins. Some pericardial fluid was noted. The changes were in keeping with the diagnosis of mesothelioma. It was apparently unresectable. Superior vena cava was patent. No adenopathy was seen. The right side was clear. A trucut biopsy from left pleura was taken. *Histological examination* showed an infiltrative lesion with a varying pattern consistent with the diagnosis of mesothelioma, epithelial type. Immune reactions did not exclude adenocarcinoma. However, a diagnosis of mesothelioma, epithelial type, of left lung was favored. Caption 35.

The case was sent to the Telemedicine Department of the AFIP for a definitive diagnosis.

AFIP Preliminary report

Pleura, left lung, needle biopsy – Malignant epithelial neoplasm.

We agree with your assessment of the case. The static images of the biopsy of pleura shows diffuse replacement by large epithelioid cells that focally form glands and papillary structures. Tumor cells are immunoreactive with keratin and EMA. S100 and CEA show weak staining, the importance of which cannot be determined in the submitted images. The differential diagnosis is between malignant mesothelioma and carcinoma. Unfortunately, we are unable to render a definitive diagnosis on these images. *Our usual panel of stains to separate the two lesions include: mucicarmine, pankeratin, CEA, CD15, Ber EP4, B72.3, calretinin, CK5/6 and TTF-1.* In this case, mesothelial markers are not provided (calretinin and CK5/6 in our panel). Thus, we classify this tumor as malignant epithelial neoplasm until a complete panel is available.

3 axial CT images were reviewed by our Department of Radiologic Pathology and they report a large soft tissue mass centered in the anterior mediastinum with extension into the pleura associated with pleural effusion. The radiologic impression is that this does not appear to be a lung based lesion and mesothelioma or metastatic malignancy is favored.

Afterward, one paraffin block containing the pleural biopsy and one H& E stained slide were sent by courier to the AFIP.

AFIP Final report

Pleura, left, biopsy: Poorly differentiated nonsmall cell carcinoma.

Paraffin block was received.

The biopsy of pleura shows diffuse replacement by large epithelioid cells that focally form glands, papillary structures and solid sheets. No intracytoplasmic mucin is seen on the mucicarmine stained section. On immunohistochemical examination, the tumor cells are positive for pancytokeratin, Cam5.2, CK 7, CD15, Ber-EP4, B72.3, MOC31 and calretinin, while the CK20, CEA, TTF-1, PAP and PSA were negative. Based on the light microscopic morphology and immunophenotyping we classify this lesion as poorly differentiated nonsmall cell carcinoma and favor adenocarcinoma. In our most recent study, up to 60% of some types of lung carcinomas were positive for calretinin. Given that 4 carcinoma epitopes are positive in this tumor we interpret the lesion as a carcinoma with calretinin positivity rather than mesothelioma. While the histology is compatible with a lung primary, other primary site should be excluded.

Comment

Diagnosis of mesothelioma remains, until this day, a difficulty area. So much so that a panel of experts in one report had showed conflict of opinion in 30% of cases[(1)].

Mesothelioma is divided into 3 histologic types: epithelial, biphasic and sarcomatous. The epithelial type may be subdivided into tubulopapillary, acinar, polygonal, small cell, clear cell, microcystic, decidua like, desmoplastic and lymphohistiocytic. In the case reported here, several patterns were seen (Figure 5). And although many immuno-histochemical panels have been used over recent years, definitive markers for distinguishing malignant mesothelioma from adenocarcinoma have not been demonstrated. Even with clinical data at hand, difficulties are still seen, as exemplified in this case. The argument in the final report of the AFIP on this case is hard to beat. The panels used were valid in attempting to make a distinction between features of malignant mesothelioma and carcinoma. However, conclusive diagnosis was made at the end by inference and one may argue that an absolutely final diagnosis may be kept open with regard to the exact primary location although lung was favored. The case is a typical example of a still an ongoing confusion over adenocarcinoma-mesothelioma saga, although the final diagnosis in this case has been reached with good reason. It should be added than the majority of cases can be solved and difficulties may be met in a minority of cases after a thorough clinical, radiologic and histopathological study. The role of immunohistochemistry remains to have limitations, while search for the ideal antibody or antibodies to single out cases of mesothelioma from carcinoma has to continue.

Reference

1. **McCaughey W, Colby T, Battifora H *et al*. Diagnosis of diffuse malignant mesothelioma: Experience of a US/Canadian mesothelioma panel. *rn Pathology* 1991; 4: 342-353.**

Case 2

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Case 2

A 3-year old boy developed sudden onset of quadriparesis 4 months ago associated with difficulty in swallowing and recuurent attacks of respiratpry tract infection. Clinical examination showed bulbar palsy, paresis of the lower cranial nerves and spastic quadriparesis. At surgery, suboccipital craniectomy for total excision of the left cerebellar tumor was done. The tumor had cleavage line in the brain stem and 4th ventricle but was arising from the right cerebellar hemisphere. Postoperatively the bay started to walk and use his hands.

Brain MRI showed a huge heterogeneous posterior fossa tumor on the right side measuring 4x4.2cm compressing the 4th ventricles causing hydrocephalus. The lesion extended through the foramen magnum compressing the medulla oblongata. Surrounding edema was noticed. The radiological conclusion was a posterior fossa tumor consistent with an astrocytoma.

Gross pathology : 7g of soft whitish tissue was received and totally embedded in 3 blocks. Microscopic examination: The tumor showed papillary branchings covered by a single layer of cubical epithelium with a falt surface. It appeared densely packed in areas with occasional microcystic change. Little fibro-vascular stroma was noted and fibrous cores. No glial component could be seen. No anaplastic change was evident. Immune reactions showed positivity for cytokeratin and vimentin and negative for GFAP. PAS reactivity was focal near the cell surface. Caption 36.

In view of the discrepancy between the clinical impression and the histological diagnosis, the case was referred to the AFIP, Washington D.C. for settling the issue.

Referral diagnosis **Choroid plexus papilloma**
Returned diagnosis **Agreed with histological diagnosis.**

In this case, the histological diagnosis was confirmed and this was essential in view of the implication off treatment and prognosis based on a confirmed diagnosis.

6- Retrieval of Archival Records and Material

For every case referred to the CML, copies of signed reports, glass slides and paraffin blocks are filed, each in their own respective slot.

Reports are retrievable from our computer files, while copies of signed reports on paper are issued on request.

Glass slides corresponding to the signed report are kept in the lab indefinitely. From the paraffin block, a glass slide copy is issued when requested and the paraffin block is kept in the archives. If any block is to be issued, the pathologist has to be convinced by the treating physician and whoever it is issued to should sign a declaration to this effect.

Extra paraffin blocks can be made in some cases, and whenever possibile, from tissue available, to be issued out of the laboratory on request. Cytology slides, unless replicas are available, are kept in the archives of the laboratories indefinitely. This is especially important for the sake of any possibility of future litigation on the case.

Tissue material left over after issuing the signed report is kept in the lab for several months after which it is disposed. Fluid for cytology is kept for one week after a signed report is issued. The reader is referred to “CML Protocol on Handling Biopsy Samples”.

7- Postmortem of Perinates

Facilities for postmortem gross and histopathology examination are available on request, either in the laboratory or hospital. Whenever possible, placenta should also be submitted for examination.

Adequate maternal history is of paramount importance; any further information on test results, x-rays or drug intake is useful.

Fresh blood or tissue samples are required for chromosomal analysis or genetic testing. Prior discussion with the Genetics Unit is advised to present the right sample in the right

container for testing. Any special transportation medium will be provided by the laboratory at the time of taking the sampling for cytogenetic diagnosis.

The prepositus or cadavar is usually returned within 24 hours.

FETAL MEASUREMENTS

Foot length is the best indicator of fetal gestational age (Table 1). Weight of the fetus is a poor parameter for gestational age.

Table 1. Foot length in Relation to Fetal Gestational Age

Foot length mm	Weeks of amenorrhea (32-40) Immature	Premature	Mature
32	20		
37	21		
40	22		
44	23		
47	24		
49	25		
52	26		
54	27		
56	28		
59		29	
60		30	
63		31	
65		32	
67		33	
69		34	
72		35	
74		36	
77			37
79			38
81			39
83			40

Ratios between organ weights can indicate normal development irrespective of gestational age (Table 2).

Table 2. Normal Ratios of Certain Fetal Organs.

Organs Compared	Approximate Reference Range
Lungs/heart	2 – 4
Brain/liver	2 - 4
Liver/heart	4 – 7
Thymus/spleen/adrenals	1 - 1

Fetal death takes place in three modes, namely: acute, subacute and chronic.

In *acute intra-uterine death*, organs weights and ratios are relatively normal.

In *subacute intra-uterine death*, there is variable change in the weights of liver, brain/liver and liver/heart ratios accompanied by decreased thymus weight.

In *chronic intra-uterine death*, there is reduction in the weights of thymus, liver and adrenals, an increase in brain/liver ratio and a decrease in liver/heart ratio.

Grossly, subcutaneous fat appears nodular and scattered in the immature, nodular and confluent in the premature and solid in the mature. Meconium is absent in the first, present in distal colon in the second and throughout colon in the third gestational phase.

Microscopically, immature phase shows tubular lung parenchyma with alveolar spaces developing during the premature phase and reaching fully developed form in the mature phase. The liver, on the other hand, shows diffuse hematopoiesis in the immature phase, abundant focal in the premature and only sporadic focal hemopoiesis in the mature phase.

8- Hematopathology

Blood films: Abnormal blood film is read by a hematologist. Infectious mononucleosis sometimes creates a problem in blood film interpretation to the novice (Caption 20).

Bone marrow aspiration and trephine biopsies have been routinely performed at CML since estalishment in 1981. Facilities are available for performing bone marrow biopsies and aspirates in infants and children as well. Such procedures are usually done under local anesthesia. Usually, samples from the iliac crests are taken. A full hematologic workup and full clinical examination are routinley done on referred patients, while samples are processed, read and reported with the full co-operation by the referring clinician or hematologist.

Follow up of patients on anticoagulant therapy is also done. Moreover, a full battery of tests for coagulation and thrombosis, including genetic tests, are also available.

Flow cytometry is employed hand in hand with other techniques in the hematologic workshop of leukemias, lymphomas and other related conditions, while DNA analysis is available for solid tumors.

Lymph node pathology is specially catered for, with full support by FCT (immunophenotyping), FNA (Fine Needle Aspiration) and, when required, back up by expert interpretation of equivocal cases.

A selection of cases in hematology are presented in Captions 12-20.

9- Cytology Service

Cervical smears. Caption 37

See: Pap smear in-house Form (Form 7)

Pap smears: reports are given the same day or within 24 hours and copies of reports reach the doctor clinic by courier. All pap smears are screened by a consultant cytopathologist.

Essential history and relevant information are helpful when given, for example, age, any recent delivery or IUD wear.

Adequate cellularity in a smear is essential requirement for a valid report. Discrepancies between cytology and clinical findings are discussed in the spirit of a working team. Systems used in the classification of pap smears: CIN and Bethesda.

Cyst aspirate or effusion:

Brief comment on clinical and imaging findings are useful. Cytospin smears of fluid samples are routinely prepared and reported promptly.

Routine stains used: Romanowsky, H&E and Pap. Other as necessary.

Samplings need to be representative of the lesion.

Advice from laboratory is freely available.

Air-dried smears generally adequate for a cytological diagnosis. (See Captions 27 & 38).

FNA (Fine Needle Aspiration)

Samples may be aspirated in the laboratory, clinic or in hospital under imaging guidance, for various purposes. Also, FNA smears on glass slides are accepted for interpretation and reporting. FNA smears commonly reported are taken from breast, testis, thyroid and deep tissue masses (Caption 21, 39 & 40). Lymph nodes are aspirated for cytology immunophenotyping in cases suspected of malignant lymphoma. However, for a primary diagnosis before initiating any treatment, a histopathological diagnosis is mandatory and cytology does not suffice. Scouting FNA testis technique with a new classification of azoospermia have both emanated from the Consulting Medical Laboratories. See classification of azoospermia in 2.24 and Caption 21.

10- Immunopathology Service

Renal and skin biopsies are readily tested during the work-up of glomerular disease and pemphigus vulgaris, respectively.

Methods used are immunofluorescence or immunohistochemical, in which latter case paraffin block material can be used with permanent record of the results.

Turnaround time for immunofluorescence or immunohistochemical antibody testing of renal and skin biopsies is 48 hours. (See Captions 41 & 42).

11- Cell Immunology FCM: Flow Cytometry (Cell Immunophenotyping)

Liquid Phase Cell Labelling

Specimens: Blood lymphocytes or from whole blood in EDTA or heparin.

Bone marrow from marrow aspirate in EDTA or heparin.

Lymph node aspirate or from any organ or tumor mass in RPMI.

Surface antigen detection by flow cytometry marks out the pattern of the immune cell type with expression of their proportions by percentages. In this way, comprehensive panels for leukemias and lymphomas are available as well as diagnosis of immune deficiency.

Turnaround time is same day or within 24 hours.

FCM is routinely used in the diagnosis of lymphoproliferative disorders and leukemias as well as dyshemopoietic conditions.

DNA Analysis (Cell Ploidy)

Liquid or Solid Phase Labelling for Ploidy.

Tumor ploidy has recently been suggested to be associated with clinical progression of a number of human malignancies. An increased proportion of aneulpoid cells within a tumor appears to exert a negative effect on prognosis. DNA analysis flow cytometry can be performed on fresh cells and tissue as well as archival material from paraffin blocks,

the latter enabling DNA analysis of tumors in routine pathology. Turnaround time for flow cytometric DNA analysis is 48 hours.

12- Research & Development

The following synopsis is aimed at doctors and health personnel who read articles and works related to the health fields and in which several important terms are often mentioned in the results. For a full account the reader is referred to the literature.

Evidence-based Cellular Pathology

Evidence-based cellular pathology is a state-of-the-art term related to participation of pathologists in clinical diagnosis. This implies that a histopathological test is evaluated in the same way as other diagnostic tests, with application of reproducibility, sensitivity and specificity. It involves laying a foundation for a systematic approach in the recording of histological parameters relevant to the case, taking into account pertinent clinical data with temporal and geographical changes and any possible biologic modification of histological parameters due to various influences, such as iatrogenic effects. A systematic structure for such approach is protracted and complicated, but eventually an evidence-based approach will lead to standardized pathology data that bears optimal effect on clinical assessment of the case. It will help to circumvent any uncertainties or ambiguities in pathology reporting and reduce inter-observer variation with better communication between pathologists themselves on the one hand and clinical attendants on the other.

13- Selected Issues on Cancer

The principal purpose of international agreement on staging anatomic extent of disease is to provide a method of conveying clinical experience to others without ambiguity.

(a) TNM System: General Rules[1]:

T= Extent of primary tumor T0, T1,T2,T3, T4

N= Lymph node status N0, N1, N2, N3

M= Presence of absence of distant metastasis M0, M1

1. All cases must have microscopical confirmation.
2. 4 classifications are used: clinical cTNM, pathologic pTNM, retreatment rTNM and autopsy aTNM.
3. For primary therapy, both clinical and pathologic stages are used; one does not replace the other.
4. In the case of the primary tumor being of unknown origin, staging will be on the clinical suspicion of the primary origin e.g. T0,N1,M0.

Although histopathologic grading has been devised as G0 (in situ), G1 to G4 well, moderately, poorly and un- differentiated, respectively, in several instances grading system has been justifiably modified with convincing results.

(b) Histological Grading of Rare Soft Tissue Tumors:

In the relatively rare soft tissue tumors, where precise histological diagnosis in some cases may not be easy to reach, attempts have been made to correlate common morphologic features with clinical outcome. In this direction, histological grading of soft tissue tumors was made to correlate with prognosis, where such stratification may correlate with

prognosis as groups; this does not necessarily apply to individual cases. In all instances, such grading systems do not replace a precise diagnostic classification. For soft tissue tumors, two recent grading schemes were devised, one from the 'National Cancer Institute' in Bethesda, Maryland, U.S.A.[1] and the other from the French "Federation Nationale des Centres de Lutte Contre le Cancer'[2,3,4]. The two proposed systems attempt to predict biologic behavior by histological appearance alone. Recent genetic studies appear to support the proposed histological groups. It remains to be seen if such approach will withstand the test of time. Histological grades in both of the above classification systems are 1, 2 and 3, whereby criteria used for grading include:

1- Tumor differentiation. 2- Tumor necrosis. 3- Mitotic rate.

In the French proposal, a score 1-3 are given to each aforementioned criterion: the first according to the degree of differentiation and resemblance to normal tissue; the second according to the amount of necrosis and the third according to mitotic activity. The total score is the sum of the 3 scores assigned to each of the 3 criteria. Accordingly, 3 grades may be deduced, namely, *Grades 1, 2 and 3*.

For illustrating the above, the French grading system will be used with examples:

Tumor differentiation

Score= 1: Well differentiated (resemblance to normal tissue):

WD (well differentiated) liposarcoma, WD leiomyosarcoma

Score= 2: Certain histological types:

Myxoid liposarcoma, malignant peripheral nerve sheath tumor (MPNST)

Score= 3: PD (poorly differentiated):

PD leiomyosarcoma, rhabdomyosarcoma, round cell liposarcoma, Ewing's sarcoma/ PNET, synovial sarcoma, extraskeletal osteosarcoma, PD MPNST, mesenchymal chondrosarcoma

Tumor necrosis

Score 0: Absent necrosis.

Score 1: Less than 50% of tumor necrotic

Score 2: Necrosis > 50%

Mitotic rate

Score 1: 0-9 mitoses per 10 high power fields

Score 2: 10-19 mitoses per 10 high power fields

Score 3: 20 mitoses or more

Therefore, resultant scores correlate with the following histological grades: Score 2 or 3 Grade 1; score 4 or 5 Grade 2; score 6-8 Grade 3.

References

1. AJCC Cancer Staging Handbook. 5th edition by Fleming ID, Cooper JS, Henson DE *et al*. American Joint Committee on Cancer, American Cancer Society, American College of Surgeons. Lippincott-Raven, Philadelphia, New York, 1998.
2. Pollock RE, Karnell LH, Menck HR & Winchester DP. The National Cancer Data Base report on soft tissue sarcoma. *Cancer* 1996; 78: 2247-2257.
3. Coindre J-M, Terrier P, Binh Bui N *et al*. Prognostic factors in adult patients with locally controlled soft tissue sarcoma: a study of 546 patients from the French Federation of Cancer Centers Sarcoma Group. *Journal of Clinical Oncology* 1996; 14: 869-877.
4. Guillou L, Coindre J-M, Bonichon F *et al*. Comparative study of the National Cancer Institute and French Federation of Cancer Centers Sarcoma Group grading system. *Journal of Clinical Oncology* 1997; 15: 350-362.

(c) Cancer Survival Analysis

Survival rate: refers to a particular point in time.

Survival curve: refers to a pattern of survival rates over time.

Both of the above refer to patients from time of diagnosis to time of death.

Difficulties with above methods of survival analysis:

1. Patients diagnosed at end of study stand better chance of being alive.

2. Patients entering beginning of study might not all stay- some may leave; those patients are called censored cases; those staying to the end of the study are known as uncensored cases.

In order to circumvent the above difficulties, taking account of censored and uncensored cases, we have two basic procedures, namely *Life table* and *Kaplan-Meier method*, respectively. More sophisticated methods of survival analysis generating graphs are also available in most computer software packages which are easily accessible.

Life table: a set period of time over which a group of patients is observed divide set period into fixed intervals : in terms of months or years etc. At end of each interval, record % surviving ,i.e., # dead / # entering interval for each succeeding interval srvival rate is similarly calculated.

Cumulative survival rate equates with probability surviving the last interval x probability of surviving all prior intervals

e.g. When 1^{st} interval= 90% 2^{nd} interval= 90% 3^{rd} interval= 90%

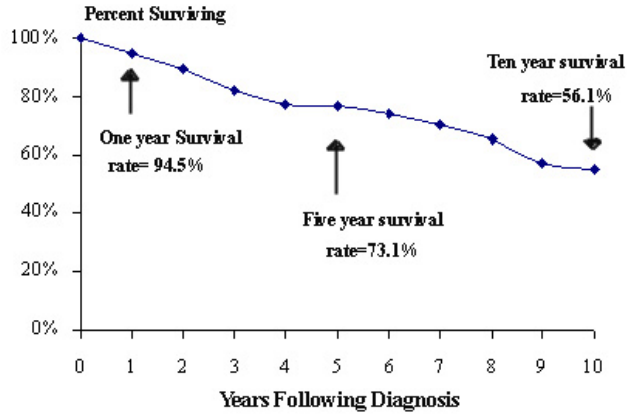
cumulative survival rate= 0.9x0.9x0.9= <u>0.729</u>

Figs 1 & 2 for Ca breast & lung show different survival patterns.

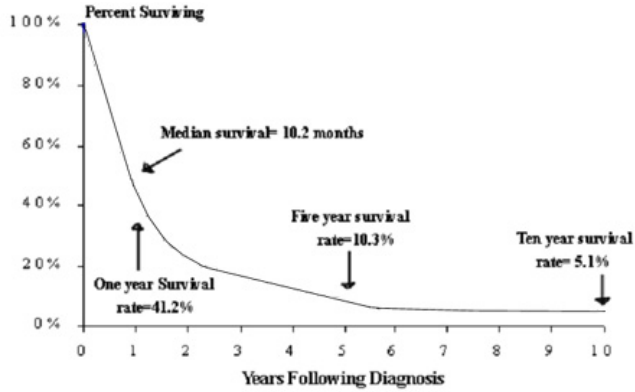
Kaplan-Meier method Similar to life table method except:

- it provides for calculating proportion surviving to each point in time that a death occurs.
- therefore, in Kaplan Meier method, the end point is death event not time.

- stepwise changes in cumulative survival rate independent of follow up time axis.



Fig(1) Ten year survival of 1,543 breast cancer patients

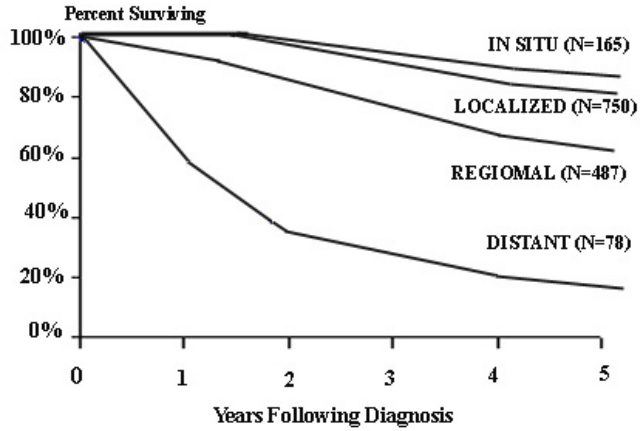


Fig(2) Ten year survival of 1,275 lung cancer patients

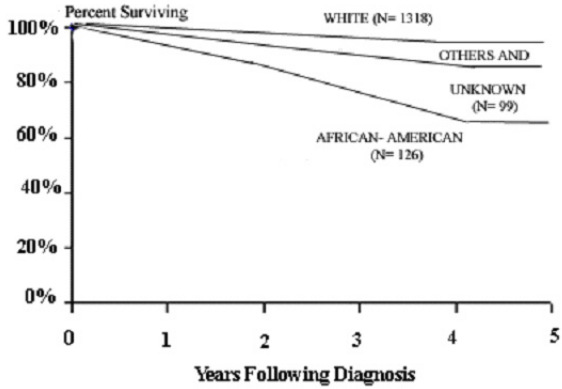
Notes:-Life table and Kaplan Meier give identical results in absence of censored patients.

Any variable or subclass can be used to calculate survival rates in order to produce more meaningful results e.g. stage-specific curves or other.

Figs 3 & 4 illustrate use of some variables.



Fig(3) Five year survival by stage of disease at diagnosis



Fig(4) Five year survival by race of 1,543 breast cancer pa

Observed survival rate accounts for all deaths regardless of cause.

Adjusted survival rate accounts for patients dying of causes other than the disease under study, who are treated as censored patients.

Illustrative example: For poor prognosis lung cancer, observed and adjusted survival rates are almost same, since lung cancer is efficient at killing. In contrast, the better prognosis breast cancer patients live long enough to be at risk of dying of other disease so that adjusted will be higher than observed survival rate, reflecting a positive effect.

Multiple regressive analysis studies joint effect of multiple variables on a single outcome. It is inapplicable to censored observations.

Cox regression analysis provides a method for estimating the influence of multiple Covariates on survival distribution, including censored observations. Covariates may be categorized, such as race, lab results or time interval measures e.g. age.

Note:- Many computer packages for statistical analysis are readily available.

Standard error of a survival rate

If we take two comparable groups of patients from same general population and estimate true values of their survival rates, they are unlikely to be the same. The difference between the two values is referred to as sampling variation or sampling error.

Standard error means the extent to which sampling variation influences the computed survival rate. The true survival rate will fall within the range of two standard errors on either side of the computed rate about 95 times in 100. This range is called the 95% confidence interval.

Standard error appraises the question: what is the probability that the observed difference may have occurred by chance? If the *95% confidence intervals* of two survival rates do not overlap, then the observed difference would be considered statistically significant.

Note:- Care should be taken in accepting statistical results per se. A difference in survival rate may be due to difference in patients or disease characteristics rather than treatment efficacy. *A more definitive approach to therapy evaluation requires a randomized, double blind clinical*

trial in order to ensure comparability of the two treatment groups and their disease.

(d) NonHodgkin Lymphoma as Illustration of Cancer Staging at Specific Anatomic Site

Anatomy

Major sites include lymph nodes, spleen, thymus, Waldeyer's ring, appendix and Peyer's patches. Other sites include lymphoreticular system and other body organs.

Classification

Diagnosis requires biopsy of lymph node or extranodal tumor. FNA and frozen sections are never considered sufficient.

Clinical staging involves clinical (history and physical examination), radiologic (abdominal CT-scan, bone scan +/- CT chest) and surgical procedures. Lab workup to include CBC and bone marrow biopsy. Other investigation as the need may arise e.g. endoscopy, ultrasound, etc.

Pathologic staging Initial diagnosis in nodal or extranodal site by surgical biopsy. Laparotomy is done in cases presenting with abdominal disease. Liver biopsy is done when liver is suspected.

Retreatment evaluation Suspected recurrence or relapse require biopsy diagnosis.

Ann Arbor Staging (abridged)

Stage I Single lymph node or single extranodal organ involved

Stage II >1 lymph node or single extranodal site and its associated lymph nodes on same side of diaphragm involved

Stage III Lymph nodes on both sides of diaphragm +/- extranodal site/spleen involved

Stage IV One or more extranodal organs +/- lymph node (s) with distant involvement

Prognostic factors

International Prognostic Index uses age, Ann Arbor stage, number of extranodal sites, performance status and serum LDH. Other markers are currently under study. Beta-2 microglobulin, cytogenetic and gene rearragement are among markers which may in the future be added to the *I.P.I.*

Systemic symptoms

Remarkably fewer symptoms occur than in Hodgkin's lymphoma. Each stage is divided into category A who are without and B who are with symptoms. Category B applies to the following patients:

1. Those who have lost >10% of body weight in <6 months before the diagnosis.
2. Unexplained fever with temperature >38°C.
3. Drenching night sweats.

(e) Histopathologic type: WHO Lymphoma classification

The concensus currently depends on the WHO (World Health Organization) classification, which no doubt will undergo modification in the future to emphasize the genetic characteristics of tumor subtypes. However, the one below is currently in fashion.

WHO Classification of Neoplastic Diseases of Hemopoietic & Lymphoid Tissues

B-CELL NEOPLASMS

Mature (peripheral) B-cell neoplasms

B-CLL / small lymphocytic lymphoma

B-cell prolymphocytic leukemia

Lymphoplasmacytic lymphoma

Mantle cell lymphoma

Follicular lymphoma (Caption 43)

Nodal marginal zone B-cell lymphoma

(+/- monocytoid B-cells)

Extranodal marginal zone B-cell lymphoma

(MALT type)

Splenic marginal zone B-cell lymphoma

(+/- villous lymphocytes)

Hairy cell leukemia

Plasma cell myeloma / plasmacytoma

Diffuse large B-cell lymphoma (Caption 44)

Morphologic variants

Centroblastic

Immunoblastic

T-cell / histiocyte-rich

Lymphomatoid granulomatosis type

Anaplastic large B-cell

Plasmablastic

Subtypes

Mediastinal (thymic) large B-cell lymphoma

Primary effusion lymphoma

Intravascular large B-cell lymphoma

Burkitt's lymphoma / Burkitt cell leukemia

Morphologic variants

Burkitt-like with plasmacytoid differentiation (AIDS associated)

Subtypes, clinical and genetic

Endemic

Sporadic

Immunodeficiency-associated

T-CELL AND NK-CELL NEOPLASMS

Precursor T-cell neoplasms

Precursor T-lymphoblastic leukemia / lymphoma

Mature (peripheral) T-cell neoplasms

Predominantly leukemic / disseminated:

T-cell prolymphocytic

T-cell granular

Aggressive NK-cell

Adult T-cell lymphoma / leukemia (HTLV1 +)

Predominantly nodal:

Angioimmunoblastic T-cell lymphoma

Peripheral T-cell lymphoma, not otherwise typed

Anaplastic large-cell lymphoma,T/null-cell,1y systemic disease

Predominantly extranodal:

Mycosis fungoides / Sezary syndrome

Anaplastic large cell lymphoma,T/null cell,1y cutaneous type

Subcutaneous panniculitis-like T-cell lymphoma

Extranodal NK/T-cell lymphoma, nasal type

Enteropathy-type T-cell lymphoma

Hepatosplenic gamma-delta T-cell lymphoma

HODGKIN LYMPHOMA

(Hodgkin's disease)

Nodular lymphocyte predominance Hodgkin lymphoma

Classical Hodgkin lymphoma

Nodular sclerosis Hodgkin lymphoma (Grades I and II)

Lymphocyte-rich classical Hodgkin lymphoma

Mixed cellularity Hodgkin lymphoma

Lymphocyte depletion Hodgkin lymphoma

Illustrative examples:

Case 1

A 51 year old man presented with cervical and inguinal lymphadenopathy. Biopsies from the two sites showed lymph nodes with preserved capsule, compressed subcapsular sinuses and marked follicular hyperplasia with active germinal centers associated with striking parafollicular, strongly pyroninophilic lymphocytic cell proliferation extending to the outside of the lymph nodes. Reticulin stain showed distorted lymph nodal pattern with increased vascularity. *Immunohistochemical study* showed strong reactivity to CD20 with nodular reactivity to bcl-2 and CD43 in a parafollicular distribution. Blood film showed lymphocytosis with notched nuclei in small lymphocytes (cleaved cells). A bone marrow biopsy was taken; BM aspirate was dry. Bone marrow imprints showed predominant small cleaved lymphocytes accounting for >95% of BM cells. BM biopsy showed fibrosis (Grade III/IV) with BM spaces

replacement by closely packed lymphoid follicles of small cleaved cells leaving a few narrow spaces containing normal bone marrow cells. The lymphoid follicles were strongly reactive to CD20 and CD43, also to bcl-2 and were negative to CD3. *Diagnosis*: Grade I (out of III) Follicular Lymphoma (small cleaved type) , sweeping bone marrow also infiltrating parafollicular zone of lymph nodes with evidence of lymphocytosis (follicular lymphoma cells) in peripheral blood.

Case 2

A 70 year old lady presented with a right lateral chest mass. The case was diagnosed malignant lymphoma and referred for classification.

Microscopic examination Caption 44

Sections show a diffuse tumor composed of large cells with pleomorphic vesicular, cleaved nuclei having membrane bound nucleoli (centroblasts) and a lesser number of large, noncleaved cells with central nucleoli. Mitoses average 37/10 hpf in 80 hpf examined (range= 28-44 mitoses/10 hpf). There are areas of necrosis and reticulin framework shows vague nodularity in places, suggesting a pre-existing nodular lymphoma. *Immune Reactions of tumor cells:* POSITIVE for LCA, CD20 & CD30 WEAK for IgM NEGATIVE for CD43& IgG. *Conclusion:* Diffuse Large B-Cell Lymphoma.

14- CML PROTOCOL FOR HANDLING BIOPSY SAMPLES

As of January 2000, the following protocol, for handling pathology and cytology specimens, has been followed at the CML (Consulting Medical Laboratories) in Jordan. The CML first proposed a system relating to the whole issue in April 1998 to the Minister of Health in Jordan, but no action was taken. Also recently, guidelines were published in relation to ownership, storage and release of pathology results on the one hand, and disposing biopsy and surgical resection material on the other, by the Royal College of pathologists (RCP) in London: guidelines relating to handling of pathology results were initially published in 2000 and revised guidelines approved by Council in November 2002 (Anonymous. Advice relating to the ownership, storage and release of pathology results - revised guidelines. *The Bulletin of the Royal College of pathologists* 2003; 121: 27-28.), while guidelines on tissue disposal, undertaken by a Specialty Advisory Committee on Histopathology, was endorsed by Council in September 2002 (Anonymous. Interim guidelines for the disposal ot tissue, blocks and slides from biopsies and surgical resections. *The Bulletin of the Royal College of pathologists* 2003; 121: 26); some comments on those guidelines will be referred to at the end of the CML protocol. To-date, no such guidelines have been published by any Jordanian body other than the CML.

When tissue is extirpated from the human body for medical diagnostic purpose, there are responsibilities and rights which befall upon three parties concerned, namely, the attending pathologist, the attending physician and the patient, respectively. Ultimately, smooth and maximal benefit to the

patient can only be attained by full trust among all parties concerned. The following guidelines are designed to promote such trust. The protocol may be modified with time.

The Attending Pathologist

For maintaining good faith and standard in medical practice, the pathologist must shoulder certain duties but also has certain rights. The following guidelines outline such inferences.

1. The pathologist should submit a written and clear report, duly signed, on any specimen submitted to him/her for medical diagnosis, whether tissue, cells or other body component.
2. The pathologist is entitled to advise the attending physician on any further investigations as he/she sees useful in attempt to reach a precise diagnosis.
3. The pathologist is responsible for keeping samples, related slides and reports within a proper filing system, copies for which should be retrievable whenever the need arises.
4. If the patient or his/her deputy or, alternatively, the attending physician requests copies of glass slides or paraffin blocks for the case of interest belonging to that particular patient, the attending pathologist has to honor such request, ensuring sufficient material, as far as possible, to help reach an accurate diagnosis; in such a case, the patient, deputy or requesting physician has to return the paraffin block, after finishing work on them, to the attending pathologist, since the latter's duty is to store them in an organized archive for the purpose of protecting both patient's and pathologist's rights of ownership.

5. The attending pathologist reserves the right to consult on any case referred to him/her with any other pathologist, physician or health personnel without taking permission from any other party concerned, provided that the attending pathologist respects the patient's secrecy code of ethics. Moreover, the name of any persons consulted should not be written in the pathology report unless the person(s) have agreed to be added to the pathology report.
6. The attending pathologist is responsible for maintaining archives of referred cyto-histopathology material (wet tissue 3 months, glass slides for 10 years and paraffin blocks for 20 years).

The Attending Physician

1. The treating physician has to send *all* material taken out of the human body, for medical diagnostic purposes, to the pathologist. In case such material has not been sent and a problem arises, the treating physician takes full responsibility for such action and is liable to any penalties that ensue subsequent to failure to send such material for pathological diagnosis.
2. Specimens have to be sent in toto to the individual pathologist, and the attending physician has no right to divide the sample and send the divided samples to different pathologists. If such procedure is undertaken and a genuine difference in the diagnosis is proven due to sampling division between pathologists, then the attending physician is solely liable for such mix up. In cases where more than one opinion is required by patient, patient's deputy or attending physician, the whole sample should be submitted to one pathologist

and then the latter may be asked to seek other opinion or the concerned parties have the option, after receiving a written report signed by the attending pathologist, to take copies of glass slides and are entitled to borrow the paraffin blocks of the case whenever possible, provided that all borrowed paraffin blocks are returned in due time to the original attending pathologist.

3. The attending physician has to ensure, without any element of doubt, that the specimen label includes the name and other basic data which correspond to the patient from whom that very specimen was taken. Also, the attending physician is responsible for the accuracy of data about the patient on the request form and should ensure an appropriate fixative for the sample as well as awareness of a sound transportation system for the specimen to reach the pathologist in reasonable time, if necessary with the aid of laboratory personnel concerned or staff attending the operating theater.
4. The treating physician has to be certain that the attending pathologist, to whom he refers the sample(s) to, is properly qualified and registered to practice his/her specialty according the regulations of the licensing bodies in the country of interest. If the attending physician accepts a written and signed report from a nonqualified pathologist, then the former is fully liable. The attending physician can obtain a list of qualified pathologists, readily available from the official licensing bodies.

The Patient

1. The patient has the right to receive a written and duly signed report from the attending pathologist. As to the time of receiving such report, this will be determined by the attending pathologist.
2. The patient or patient's deputy has the right to obtain copies of glass slides that should be sufficient, whenever possible, to reach a sound conclusion relative to the material available. The patient or deputy has the right to borrow any paraffin block(s) pertaining to the case belonging to the patient, and in adequate amount as the case permits, with freedom to refer such material to whomever he or she wishes, provided that all such borrowed material is returned to the attending pathologist. On the other hand, the attending pathologist has the right to retain as much material as to guarantee adequate reference material in his/her files or archives. The attending pathologist has the right to hold a sum of money against any borrowed material, which is returnable after the paraffin blocks or any other essential items borrowed have been returned. In some cases, extra glass slides or paraffin blocks are made subject to additional fees.
3. Collection of residual pathology specimens by patients from the original pathology laboratory for referral to external pathologist(s) should be discouraged, since examination of a temporized sample often does not allow full assessment of a case and may sometimes be misleading.

A Brief Look at the RCP Guidelines on Pathology Results: Ownership, Storage and Release (Abridged)

Ownership: A laboratory does not own pathology results but acts as a guardian of data with storage of material in proper archives. Results are returned to the referring physician. Situations arise which are exceptions to this dictum and the attending pathologist should use reasoning. Health personnel officially delegated by the attending physician should have access especially when the patient has given permission, also notifiable disease; in some circumstances to patients themselves.

Release of results directly to patients: Patients are entitled to receive an informed opinion, preferably by their own attending physician, and may wish to partake in healthcare decisions and treatment options. However, problems may arise from giving results directly to patients. First, the requester may be a false pretender, or the patient may misunderstand the significance of the pathology result, while results on HIV and cancer raise sensitive issues. Moreover, the possibility of undermining the attending physician should be considered. However, there is good reason to give routine results on established conditions to patients direct (for example, fasting blood sugar or HbA1C for a diabetic or prothrombin ratio for a patient on anticoagulation regime).

Release of results to doctor patients: Complications can arise when the patient is medically qualified. Anyway, they are not encouraged to self-investigate but should go through their own attending physician.

A Brief Look at the RCP Guidelines on Disposal of Tissue, Blocks and Slides (Abridged)

The guidelines of the RCP on disposal of tissue, blocks and slides apply to human material on live patients at the time of its removal. Recommended minimum periods of retention by the RCP that had had been made in a 1999 publication were as follows: Wet tissue 4 weeks, frozen sections 10 years, paraffin blocks 30 years, blood films 7 days, bone marrow smears 20 years, cytology slides 10 years, histology slides 10 years.

There is no obligation to determine the wishes of the patient regarding disposal except for fetuses and fetal tissues including products of conception. Where the patient wishes to have the material returned to them (for disposal), such material should be returned to them, provided that the hospital authority (in the United Kingdom authority is delegated to hospital administration rather than to the attending pathologist) is satisfied that the proposed method of disposal or keeping is safe and lawful. The patient should be informed in writing of any hazards associated with the material.

Material for disposal should be incinerated in an organized way and separately from those of clinical waste. A written record of what has been disposed should be kept.

The United Kingdom government is currently reviewing the law on human organs and tissue. These guidelines published by the RCP form part of a broader framework of interim measures being developed by key national bodies.

15- Tests List: Pathology-Cytology

1 Adrenal Gland
2 Anal Biopsy
3 Anal Fistula
4 Appendix
5 Bladder Biopsy
6 Bone Biopsy
7 Bone Marrow Aspiration
8 Bone Needle Biopsy
9 Brain Biopsy
10 Breast + Axillary LNS.
11 Breast Biopsy
12 Breast, Reductive
13 Bronchial Biopsy
14 Buccal Smear
15 C Ascitic Fluid
16 C Breast Discharge
17 C Bronchial Wash
18 C CSF
19 C Ascitic Fluid
20 C Pleural Fluid
21 C Sputum
22 C Synovial Fluid
23 C Urine
24 C. Breast
25 C. Lymph Node
26 C. Other
27 C. Thyroid
28 Cervical Biopsy
29 Cervical Polyp

30 Colectomy
31 Colon, Segment Removal
32 Colorectal Biopsy
33 Colorectal Polyp
34 Cone Biopsy
35 Conjunctiva
36 Consultation
37 Cyst
38 Cystectomy
39 Cytology N.O.S.
40 Duodenal Biopsy
41 Endometrial Polyp
42 Endometrium Biopsy
43 ER/PR Report
44 Esophagectomy.
45 Esophagus Biopsy.
46 Estrogen Receptors
47 Extremity, Amputation
48 Eyeball
49 Fallopian Tubes
50 Fetus
51 Fetus+Placenta
52 Fibroid.
53 Frozen Section
54 Gall Bladder
55 Gastrectomy Partial
56 Gastrectomy Total
57 Gastrointestinal Biopsy
58 Heart Valve
59 Heart, Endocardial Biopsy
60 Immuno Histochemistry
61 Kidney Biopsy
62 Kidney

63 Laryngeal Biopsy
64 Larynx
65 Liver Biopsy
66 Lung Lobe.
67 Lung Total Resection
68 Lung, Biopsy
69 Lymph Node
70 Mass up to 7 cm
71 Mastectomy Simple/ Radical/ Lumpectomy
72 Mucosa Biopsy
73 Muscle Biopsy
74 Nasal Biopsy
75 Nasal Polyps
76 Nasopharyngeal Biopsy
77 Neck Tumor
78 Nerve Biopsy
79 Omental Biopsy
80 Oral Biopsy
81 Orchidectomy
82 Ovarian Biopsy Ovarian Cyst
83 Ovarian Tumor
84 Pancreas
85 Pap Smear
86 Pelvic Mass
87 Pericardial Biopsy
88 Perineal Biopsy
89 Pharyngeal Biopsy
90 Pinna
91 Placenta
92 Pleural Biopsy
93 Polyp
94 Products of Conception
95 Prostate

96 Prostatectomy
97 Salivary Gland Tumor
98 Serosal Membranes
99 Skin Biopsy
100 Skin Tumor or Lesion
101 Small Bowel Resection
102 Small Intestinal Biopsy
103 Soft Tissue Mass
104 Splenectomy
105 Synovial Membrane Biopsy
106 Testicular Biopsy
107 Testis Biopsy-one Testis
108 Testis Tumor
109 Thymus
110 Thyroid
111 Thyroid Total Resection
112 Tongue Biopsy
113 Tonsil
114 Tracheal Biopsy
115 Tubal Pregnancy
116 Ureter
117 Urethral Biopsy
118 Uterine Fibroids 3-7 cm.
119 Uterine-Fibroids <3 cm
120 .Uterus
121 Uterus-Tubes-Ovaries
122 Vaginal Biopsy
123 Vulval Biopsy

CHAPTER 7

TRANSFUSION MEDICINE

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BLOOD GROUP SYSTEMS

Erythrocytes *Leukocytes* Human Leukocyte Antigens
Platelets Human Platelet Alloantigens

ERYTHROCYTES

Red cell antigens

The ABO system is the most important antigen system in transfusion medicine.

H determinant is a precursor to A and B antigens and is found on all human red cells except rarely in subjects with O-Bombay phenotype. Those who lack A carry B antibodies and B antigen carriers show A antibodies, while those lacking A and B are typed O and carry both A and B antibodies. Those who have A and B antigens on red cells are typed AB and lack the two aforementioned antibodies. ABO incompatibility, such as by erroneous transfusion, can lead to severe hemolysis but hemolytic disease of the newborn is rarely caused by ABO incompatibility, since most A and B antibodies are of IgM class added to the fact that A and B antigens do not sufficiently develop before birth.

The Rhesus system is an important blood goup system. Rh (Rhesus) positive subjects have DCE, DCe, Dce or DcE genotype while Rh negative subjects only possess dCE, dCe, dce or dcE genotype. 8 antigen combinations are possible: Rh0 (Dce), Rh1 (DCe), Rh2 (DcE), Rhz (DCE), rh (ce), rh' (Ce), rh" (cE) and rh^y. "d" represents absence of D antigen while anti-d has never been detected. For practical

purposes it is sufficient to determine if the subject is Rh positive (D +) or Rh negative (D-). D antigen is strongly immunogenic with all the implications of incompatible blood transfusion or intrauterine transfer of D+ to D- subject. On the other hand, C, c, E and e antigens are less immunogenic, becoming relevant with incompatible transfusion, while over 40 other Rh antigens are of no clinical effect.

Other red cell group systems: Some 200 others are known, for example MNSs, Kell, Lewis, Duffy, Kidd and so on. Such systems become important with incompatible blood transfusion; any atypical antibodies detected should be investigated in specialized blood banks to assess their clinical significance. While such may be induced by multiple transfusions or pregnancies, others are naturally occurring.

Disease associations Some blood groups show statistical association with certain illnesses, such as Group A persons and cancer of stomach, colon and salivary glands while Group O subjects and peptic ulcer, rheumatoid arthritis and von Willebrand disease. Certain phenotypes show resistance to malaria parasite, for example lack of Fy^b antigen deprives a critical receptor for *P. vivax* on red cell membrane, thereby cell penetration cannot take place. Antigen expression, whether inherited or acquired, can be altered in some diseases. For example, ABH expression can be weakened in acute myelocytic leukemia and returns after remission. In another example, acquired Group B activity has been associated with *E. coli* and *Proteus vulgaris* infection. Aquired A activity may also be seen after infection with *P.mirabilis*. In Rh*null* red cells, most are stomatocytes or spherocytes with increased osmotic fragility, since D antigen plays a significant role in membrane structure. A com-

prehensive list of such examples will be found in specialized textbooks.

Red cell antibodies

Red cell antibodies may be natural or immune, the latter following sensitization. Autoantibodies are related to self antigens, while alloantibodies are related to antigens of other individuals present on red cells. Anti-A and Anti-B are usually detected 2 to 6 months after birth, since fetal antibody production only begins shortly before birth with IgM, IgG and IgA appearing in this order. Therefore, mother's serum is used when cross matching for the newborn. Natural antibodies are mostly IgM with few being IgG. Saline suspended red cells are agglutinated by IgM antibodies and sometimes by IgG antibodies at high concentration. Except for anti-A and anti-B, naturally occurring antibodies do not agglutinate red cells at body temperature and thus are of no clinical significance. Immune antibodies are mostly IgG but may be IgM and sometimes IgA, so that they often react at body temperature.

Hemolytic transfusion reactions Caused by transfusing ABO incompatible blood and less commonly with Kidd incompatiblity, though with the latter transfusion reaction is delayed. Rarely P and Lewis blood groups may be involved.

Hemolytic disease of newborn Caused by incompatible blood groups between mother and fetus. It occurs with IgG1 and IgG3 antibodies that cross the placenta, most frequently in relation to Rh incompatibilty subsequent to the first pregnancy, while ABO incompatibilty, though most common, is of no clinical significance since the fetus does

not fully express ABO antigens. Moreover, most Anti-A and Anti-B are IgM antibodies that do not cross the placenta. The disease has become largely preventable by giving anti-D immunoglobulin during labor.

Autoimmune hemolytic anemia Caused by '*warm*' or '*cold*' antibodies directed at red cell antigens. Causative factors include viral infections, drugs, immune defect, lymphoma or alloantigen that cross reacts with self red cell antigens. Warm (mostly IgG) autoantibodies react at 37°C, such as seen in some cases of lymphoma, while cold (mostly IgM) autoantibodies react at below 25°C and have anti-I activity with hemolysis occurring on exposure to cold, such as in paroxysmal cold hemoglobinuria.

Blood grouping and Rhesus typing

ABO grouping and Rhesus typing is the most frequent test in blood banking. In ABO typing, forward testing is made by incubating patient's red cells with anti-A and anti-B sera, respectively. Positive test is indicated by hemagglutination or hemolysis. The result should be confirmed by reverse testing, whereby patient's serum is incubated with known group A and group B red cells, respectively.

In Rhesus typing, individuals who are Rhesus type D positive are called Rhesus positive. Blood donors and expectant mothers who are Rhesus negative should be further tested by the antiglobulin (indirect Coombs) test to confirm Rhesus negativity; a positive antiglobulin test indicates weak D antigen and such subject should be considered Rhesus positive. A positive direct Coombs test is seen in autoimmune hemolytic anemia, drug induced hemolytic anemia, hemolytic disease of newborn, transfusion reac-

tions and in transplantation. However, a small percentage of the general population shows a positive direct Coombs test with no demonstrable clinical significance.

Typing other blood groups, for example Cc, Ee, Kell, Duffy, MNS and so on may be indicated in those requiring long term blood transfusions such as for thalassemia or sickle cell anemia.

HUMAN LEUKOCYTE ANTIGENS

HLA (Human leukocyte antigens) comprise a large number of antigens controlled by a cluster of genes located on the short arm of chromosome 6. *Six HLA groups* are recognized, namely, *HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DP and HLA-DQ*. The first three aforementioned HLA antigens are Class I and the latter three are Class II.

Class I are found on most nucleated cells while Class II are found on B lymphocytes, macrophages, monocytes, dendritic and endothelial cells. HLA types can be tested for by selorological and molecular methods.

The HLA cluster of genes falls within a complex of genes-*MHC* (Major Histocompatibilty Complex) on short arm of chromosome 6 that encodes for immune regulation and response.

In organ transplantation, such as of kidney or bone marrow, allografts are selected to be as HLA compatible as possible with the recipient, the best match an identical twin while closer blood relations stand better chance for compatibility. The more matched the donors, the less the rejection rate in recipients. Recent advances in immunosuppressive therapy have obviated the need for a strong match in kidney trans-

plantation though long term survival in matched supercedes that of mismatched donors. In bone marrow transplantation, partially matched donors increase the chance of GVH (Graft versus Host) reaction. Therefore, matched DR and DQ donors are sought to decrease the incidence of GVH disease.

PLATELETS

Five HPA (Human Platelet Allontigen) systems are known, namley, HPA1- through to HPA-5. Each HPA system has two alleles and allelic frequencies show racial variation. Patients who are homozygous for one allele can develop alloantibodies to the contralateral allele after receiving platelets that express the latter. One half of patients receiving multiple platelet transfusions develop platelet antibodies against antigens of transfused platelets. Some patients do not develop platelet antibodies despite repetitive platelet transfusions. Others develop anti-HPA or/and HLA antibodies so that cell sorting and inactivation of leukocytes by ultraviolet light can minimize immunization. Moreover, platelets from HLA matched donors survive longer in highly immunized recipients than from non HLA matched donors.

BLOOD TRANSFUSION SERVICE

A central blood bank service supplies several types of blood and its products, but autologous blood transfusion is always encouraged such as by preoperative collection and preservation provided this does not adversely affect the patient, or by blood salvage procedures and methodologies. For example there are cardiac surgical centers which operate on transfusion free basis. However, blood banks provide various preparations as follows: *Whole blood* up to 21 days old or fresh (less than 5 days old), *Red cell concentrates* (less than 3 weeks old), *Washed red cells* which should be used within 24 hours after washing, *Filtered red cells* that is leukocyte depleted and should be used within 24 hours after filtration, *Frozen red cells* which can be stored in glycerol at below 120C for 10 years, *Random donor platelets* suspended in a small amount of plasma and given within 5 days of preparation, *Cell separator single donor platelets* to be given within 5 days of donation, *Fresh frozen plasma* which is valid for one year, *Single donor plasma* which is valid for 5 years, *Cryoprecipitate* which is valid for one year, *Irradiated blood products* and *Plasma substitutes, such as normal saline, Ringer lactate, Dextrose 40%, dextrose 70%, Glucose-saline, HES.* Ability of a blood bank to separate the blood unit into separate components will act in the best interest of the patient to fulfill his or her need and curtail side effects of undesired blood components, also providing for more people in this way.

For a comprehensive treatise on therapeutic activities in a blood transfusion service, the reader is referred to specialized textbooks and literature devoted to this subject.

Indications for blood transfusion

Whole blood fresh is given to newborns or to patients with massive acute hemorrhage such as in dissecting aneurysm. *Stored whole blood* is largely replaced by giving the required blood component.

Packed red cells is given to replace lost blood, chronic anemias or for exchange transfusion in the newborn.

Washed red cells given to raise oxygen uptake such as for patients with high fever or sensitivity due to previous blood transfusion as well as in paroxysmal nocturnal hemoglobinuria.

Filtered red cells given to raise oxygen uptake in those who had two or more post-transfusion reactions or to reduce the possibility of delayed post-transfusion reactions.

Frozen red cells used for those with rare blood groups, long term autologous transfusion and in IgA deficiency. The latter are at risk of developing anaphylactic shock on receiving blood containing IgA.

Random donor platelets Red and white cells poor and rich in platelets with all coagulation factors, used for the treatment of thrombocytopenia with relentless bleeding or in cancer patients receiving chemotherapy or when platelet count falls below 20.000 ^9/l.

Fresh frozen plasma which contains all plasma proteins and coagulation factors and is given to patients lacking co-

agulation factors including hemophilia A, thrombotic thrombocythemic purpura, also for acute liver failure, correction of coumarin overdose and in DIC (disseminated intravascular coagulation).

Cell separator single donor plasma rich in platelets and used solely in treatment of patients who do not respond to random donor platelet preparations due to circulating leukocyte antibodies.

Single donor plasma which contains Factor VIII, Factor I, von Willebrand Factor and Factor XIII. It is used for cases as for fresh frozen plasma and for hemophilia B.

Cryoprecipitate containing Factor VIII, Fibrinogen and von Willebrand Factor and is used for treatment of hemophilia A, acute hypofibrinogenemia, von Willebrand disease and DIC.

Irradiated blood products which prevents GVH reaction (graft versus host disease), given in the premature, intrauterine fetal transfusion, acquired immunodeficiency due to chemotherapy, irradiation and in bone marrow transplantation.

Plasma substitutes used to increase blood volume, namely, plasma expanders, and to avert use of plasma for this purpose due to concomitant risks involved with plasma transfusion.

PRETRANSFUSION MEASURES

Blood donation

Blood donation, on volunteer basis, is encouraged through public education and campaigns as well as incentives in some cases. Paid donors are discouraged due to higher risk of hepatitis transmission.

Volunteers aged 17- 50 years, or in some cases up to 65 years old, are usually registered by blood banks with donations accepted at regular intervals, as often as 3-monthly. A standard screening procedure is performed every time. The hemoglobin should not be below 14 g/dl in males or 12 g/dl in females, pulse 50-100, blood pressure not higher than 100mm Hg diastolic and temperature not to exceed 37.5°C. Body weight should exceed 50 kg, since a safe donation should not exceed 10 ml blood per kg body weight.

Whole blood Donor Screening

Hb, PCV, ABO grouping & Rhesus typing.

Specific tests are routinely performed on blood donors: HIV I&II, HBsAg, HCV Abs, RPR for syphilis, Blood film for malaria. Some countries have certain common pathogen that is to be screened before accepting the unit for donation.

Blood is collected in special bags in a closed system to minimize microbial contamination. 450ml +/- 10% of whole blood is collected and mixed with 63ml anticoagulant of choice, for example ACD (acid-citrate-dextrose), CPD (citrate- phosphate-dextrose) or CPD-adenine, the

latter to increase shelf life of stored blood. Additive solutions like adenine-dextrose, mannose and so on can extend shelf life to 42 days.

Crossmatching

Major crossmatch consists of testing red cells of donor against patient's serum.

Minor crossmatch consists of testing serum of donor against patient's red cells.

ABO grouping

Group A	Anti-B in serum	Group B	Anti-A in serum
Group AB	No Anti A/B in serum	Group O	Anti-A (or -A1) & Anti-B in serum

Note

Group A: 80% A1 and 20% A2: A2 gives weak reaction with main Anti-A serum. Therefore, A2 and A2B can be mistaken for Group O and Group B, respectively.

Testing for subgroup A, natural Anti-A1 serum (strong) is better used for Group B donor. Ordinary Anti-A can be removed by adsorption with a suitable quantity of A2 cells.

Besides Anti-A and Anti-B, a large number of hemagglutinins have been described to occur in human serum. Only few may be detected in routine blood grouping. Such occurrence may cause discrepancy between the group red blood cells as determined by reactions against its serum. Commonest extra agglutinins are anti-P, anti-O, anti-Li^a, anti-M.

Table 1. ABO & Rhesus Compatibility in Blood Transfusion.

Recipient's Blood Type	Donor's Blood Type			
	O	**A**	**B**	**AB**
O	Yes	No	No	No
A	W	Yes	No	No
B	W	No	Yes	No
AB	W	W	W	Yes
	Donor D+		**Donor D-**	
D-	No		Yes	
D+	Yes		Yes	

W= Can transfuse after washing red cells.

Table 2. Agglutinogens & Agglutinins of A1, A2, B, O, A1B & A2B Blood Groups

Blood Gp	Reactions with Agglutinins			Serum Agglutinins	Serum Agglutinins
Sub-group	Anti-A	Anti-A1	Anti-B	Always Present	Sometimes Present
A1	+	+	-	Anti-B	------
A2	+	-	-	Anti-B	Anti-A2 (2% of cases)
B	-	-	+	Anti-A / -A1	------
A1B	+	+	+	NONE	------
A2B	+	-	+	NONE	------
O	-	-	-	Anti-A/ -A1/ -B	------

Antihuman Globulin Test (AGT):

The antiglobulin test (Coombs' test) is based on the fact that specific antiglobulin antibodies act as a bridge between red cells and complement to induce agglutination.

Direct Coombs' test refers to detecting antibodies tagged on red cells in vivo.

Indirect Coombs' tests refers to detecting reaction between antibody and red cells in vitro.

AGT is a very sensitive method so that a negative result excludes antibodies on red cells. False positive results may result from contaminated reagents, over-centrifugation, presence of strong cold agglutinins or saline stored in metal or glass containers. False negative reactions occur due to reagent infidelity, improper washing, failure to add antiglobulin reagents, improper centrifugation, low serum/cell ratio or delayed washing in presence of already eluted weakly attached antibody.

Direct Coombs' test is used in the diagnosis of hemolytic transfusion reactions, hemolytic disease of the newborn, investigation of cold or warm auto-antibodies as well as in drug induced hemolytic anemia.

Indirect Coombs' test is used in the detection of erythrocyte antibodies in serum, typing red cell antigens and in cross-matching.

For technical details, the reader is referred to specialized manuals and textbooks.

TRANSFUSION REACTIONS

Serious administrative moves have been made in certain countries to curtail serious effects of transfusion, advocating safer transfusion practice. Several vigilance schemes have been created, for example in the United Kingdom SHOT: Serious Hazards of Transfusion and in Denmark a scheme called DART. Such bodies have to be introduced to less emancipated countries with provision of a formal control program to keep surveillance of any serious transfusion reactions in hospitals with interchange and feedback of mandates on the local, regional and international levels aiming at universally circumventing as many serious incidents of transfusion as possible.

Immediate Transfusion Reactions

1- Acute hemolysis (a) Immune (b) Nonimmune

(a) Immune: poses a serious threat to life and is due to ABO incompatibilty. It results in intravascular hemolysis, shock, DIC and later renal failure. In anesthesized patients there is hypotension and generalized bleeding. The most serious complication to treat is DIC. This complication is almost always due to clerical error.

(b) Nonimmune: donor red cell damage may have resulted for several reasons, such as overheating or frozen unduly after misplacement. Alternatively, packed red cells in hypotonic solution may also be damaged, also by certain toxins such as *Clostridium welchii*.

2- Febrile reactions

An increase of temperature by 1°C in immediate relation to transfusion may be accompanied by chills. Factors such as TNF (tumor necrosis factor), Interleukins-1B and -6 may act as endogenous pyrogens. However, a full workup is indicated with febrile reaction to exclude bacterial contamination. Antipyretic administration prior to transfusion can sometimes prevent febrile reactions.

3- Allergic reactions

Usually IgE mediated and manifests by urticaria and itching. Histamine release is involved with headache, flushes, hypotension and may progress to wheezing and stridor. In addition to histamine, serotonin and platelet activating factor may be involved. Treatment is by antihistamines.

Severe allergic reaction represented by anaphylactic shock can occur after receiving only a small amount of blood and may manifest by acute respiratory distress, stridor due to laryngeal edema and cough due to bronchospasm. Ig A deficient persons with anti-IgA immunoglulin are susceptible. Treatment is by adrenaline and steroids.

4- Pulmonary edema

Transfusion related acute lung injury is characterized by bilateral pulmonary edema in the absence of heart failure. It is caused by leukocyte agglutinins and activation of complement resulting in histamine release from basophils and platelets. Treatment is by high dose steroid and general supportive measures.

5- Bacterial contamination

Bacterial contamination induces blood clot which can be seen with the naked eye. Gram negative organisms can cause serious reaction and rapid diagnosis can be made with Gram stain from contaminated container or tubing. Toxic shock manifests by fever, hypotension, abdominal pain and vomiting with diarrhea and severe shock. The latter is a severe complication with a high fatality rate. Although uncommon with use of disposable bags, higher incidence is noted in platelet concentrates stored at room temperature.

6- Circulatory overload

Excess blood transfusion produces hypervolemia especially in patients with heart failure. It provokes heart failure with pulmonary edema. Also, patients with severe anemia and rapidly transfused are susceptible.

7- Air embolism

A rare complication after the use of closed systems, only large volumes can produce clinically significant effect. Dyspnea may result and atrial air aspiration may be required.

8- Reactions against massive transfusions

Massive transfusions lead to calcium depletion with myocardial suppression, while citrate intoxication can be prevented by intravenous calcium gluconate.

9- Bleeding syndromes

DIC due to incompatible blood (see acute hemolysis above) or coagulation factor defeciency due to transfusion of large amounts of stored blood may lead to bleeding. However,

the most common surgical cause of bleeding is a severed blood vessel.

Delayed Transfusion Reactions

1- Disease transmission

This is the most frequent risk in transfused patients, whereby transmission of viruses such as hepatitis, HIV as well as other organisms such as malaria is possible.

2- Delayed Hemolysis

Development of new alloantibodies lead to hemoytic reaction during the fiest or second week after transfusion. Such cases have been previously sensitized by a previous transfusion or pregnancy, but at very low levels. It manifests by jaundice and a positive direct Coombs' test. Such reactions are not as severe as acute hemolytic reaction and frequently occur at subclinical level.

3- Posttransfusion purpura

This is a rare complication due to development of platelet antigen in a platelet $P1^{A1}$ appearing about one week after the transfusion.

4- Graft versus host reaction

This is an uncommon complication of transfusion which can be prevented by giving irradiated blood.

5- Other delayed reactions

Hemochromatosis can result from chronic transfusion, while alloimmunization to red cells and histocompatibility antigens can occur. Investigating alloimunization reactions requires specialized units.

INVESTIGATION OF TRANSFUSION REACTION

In order to investigate a transfusion reaction, *pretransfusion serum* and *residues of donor's blood* should be available.

History first

Study of
a) Cross-matching and other tests carried out and includes revision of pre-transfusion clotted specimen, post-transfusion blood sample and the blood bag.
b) Handling of blood: all events from donor to recipient.
c) The transfusion, which should be immediately stopped.
d) The reaction.

Further specimens required

Post-transfusion venous blood as soon as possible after reaction:

1- About 20ml placed in dry sterile bottle: check for any evident hemolysis.
2. Few drops placed in heparin bottle.

Possible causes of reaction

1- Serological incompatibilty : must be ruled out.
2- Lysed donor cells from excessive temperature changes.
3- Massive transfusion with outdated blood.

4- Contamination of the donor's blood with bacteria.

The attending physician and blood bank should work hand in hand on the case.

TESTS

Blood tests

1- Group Donor cells for ABO and Rhesus.
2- Group Patient's cells in *pretransfusion* and *post-transfusion samples* for ABO and Rhesus.
3- Direct Coombs' (Anti-Human Globulin) on *Pre-transfusion(a)* and *post-transfusion sera(b)* of recipient versus *Donor red cells*.

   If *(a)* is negative and *postransfusion serum* positive: suggests incompatible serum with *Donor cells*

   If (a) is positive and post-transfusion serum negative: suggests the reverse
4- Using Pre-transfusion sample as control, test Post-transfusion serum for: Hemoglobin content present if immediately after the reaction Bilirubin content: later after the reaction; positive if > 2mg/dl Schumm's test for methemalbumen; positive in considerable intravascular hemolysis.
5- Full cross-matching of both Pre-transfusion and Post-transfusion sera against:
   (i) *Donor red cells* (from each bottle and pilot tube used).
   (ii) Recipient's red cells.
6- Posttransfusion serum tested for haptoglobin, methemoglobin, hemoglobin and hemosiderin.
7- Posttransfusion anticoagulated specimen may have to be screened for DIC if suspected. Also, frequent

Hb and PCV estimations may be necessary if hemolysis appears to be continued.

Bacteriological examination

Gram's stain to be done on blood in the bag with culture to rule out bacterial sepsis.

Urine tests

Look at color for hemoglobinuria.

May need to test for urobilinogen, bilirubin and urine hemoglobin.

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Table 1a. Complete blood count: Normal values*

Age	Hb (g/dl)	PCV (l/l)	RBC (10^{12}/l)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Platelet count x10^9/l
1-6 Day	14.5-22.5	0.45-0.67	3.9-5.4	98-118	31-37	30-36	
1-4 Weeks	13.5-21.5	0.42-0.66	4.0-6.6	95-121	31-37	29-37	
1-5 Months	10.0-18.0	0.31-0.55	3.9-6.3	88-126	28-40	28-38	
6-11 Months	9.5-13.5	0.29-0.41	3.0-5.4	85-123	28-40	29-37	
1-5 Years	10.5-13.5	0.33-0.39	3.1-4.5	74-108	25-35	30-36	
6-12 Years	11.5-13.5	0.34-0.40	3.7-5.4	70-86	23-31	30-36	
12-15 Years	11.5-13.5	0.35-0.45	3.9-5.3	75-87	24-30	31-37	
			4.5-5.2	77-95	25-34	31-37	
Adult Female	11.7-15.7	0.35-0.47	3.8-5.2	76-100	27-34	32-36	>150.000
Adult Male	13.5-17.5	0.40-0.52	4.4-5.9	76-100	27-34	32-36	>150.000

* Modified from Baker J, Cornbleet PJ, Erythrocyte Disorders. *In*: Howanitz JH, Howanitz PJ, editors. Laboratory medicine: Test selection and interpretation. New York: Churchill Livingstone, 1991: 447-498.

Table 1b. Absolute white cell and differential counts; Normal values* (10^9/l)

Age	WBC	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
At Birth	9.0-30.0	6.0-26.0	2.0-11	0.4-3.1	0.02-0.85	0.0-0.64
1-6 Day	9.4-34.0	5.0-21.0	2.0-11.5	0.2-3.1	0.05-1.00	0.0-0.30
1-4 Weeks	5.0-21.0	1.5-10.0	2.0-17.0	0.3-2.7	0.07-1.10	0.0-0.25
1-5 Months	5.0-19.5	1.0-9.0	2.5-16.5	0.15-2.0	0.07-0.90	0.0-0.20
6-11 Months	6.0-17.5	1.0-8.5	4.0-13.5	0.10-1.3	0.07-0.75	0.0-0.20
1-5 Years	6.0-17.5	1.5-8.5	4.0-10.5	0.05-1.1	0.07-0.70	0.0-0.20
6-12 Years	5.0-14.5	1.5-8.0	1.5-7.0	0.0-0.8	0.0-0.65	0.0-0.20
12-15 Years	4.5-13.5	1.8-8.0	1.2-6.0	0.0-0.8	0.0-0.55	0.0-0.20
Adults	4.5-11.0	1.8-7.7	1.0-4.8	0.0-0.8	0.0-0.45	0.0-0.20

* Modified from Combleet J, Astania R, Wolf PL, White blood cells and platelet disorders. *In*: Howanitz JH, Howanitz PJ, editors. Laboratory Medicine: test selection and interpretation. New York: Churchill Livingstone, 1991; 553-618.

Table 2a. Pathological leukocytosis*

Cause	White cell type
Allergy	Eosinophil
Brucellosis	Lymphocyte, monocyte
Convulsions	Neutrophil or lymphocyte
Drugs and poisons:	
ACTH	Neutrophil
Camphor	Neutrophil and eosinophil
Copper sulphate, phosphorus	Eosinophil
Tetrachloroethane, epinephrine	Monocyte, neutrophil and lymphocyte
Other: (acetanilid, arsenicals, benzene, carbon monoxide,digitalis,lead, phenacetin, turpentin, venoms)	Neutrophil
myeloid growth factors	Neutrophil, monocyte
Hemolysis	Neutrophil
Hemorrhage	Neutrophil
Hodgkin's disease	Neutrophil, eosinophil and monocyte
Infectious lymphocytosis	Lymphocyte
Infectious mononucleosis	Lymphocyte, atypical changes
Leukemia	Granulocyte, lymphocyte or monocyte

(Continued on page 4)

Table 2a. cont'd

Cause	White cell type
Loeffler syndrome, periarteritis nodosa, pernicious anemia	Eosinophil
Polycythemia vera	Neutrophil, eosinophil, basophil
Toxemias: diabetic acidosis, eclampsia, gout, uremia	Neutrophil
Tuberculosis	Neutrophil, eosinophil lymphocyte, monocyte
Tumors involving:	
Marrow and serous cavity	Neutrophil and eosinophil
Ovary	Eosinophil
GI tract and liver	Neutrophil
Typhoid fever	Lymphocyte

* Adapted from Finch SC. *In* Williams WJ, Beutler E, Erslev AJ and Lichtman MA (editors): Hematology, 3rd ed. New York, Mcgraw-Hill,1983.

Table 2b. Classification of neutropenia*

I. Myeloid hypoplasia
   a. Infantile genetic agranulocytosis (Kotsmann); familial neutropenia; cyclic neutropenia: chronic (hypoplastic) neutropenia; neutropenias associated with lymphocytic disorders, myelophthisic neutropenia
   b. Drug induced
      1. Cytolytic
         a. Alkylating agents (nitrogen mustard, cyclophosphamide, chlorambucil, busulfan)
         b. Ionizing radiation
         c. Mitosis inhibitors (colchicine, vinblastine, vincristine)
         d. DNA depolymerization (procarbazine)
      2. Metabolic interference with DNA synthesis
         a. Purine and pyrimidine antagonists (cytosine arabinoside,† methotrexate,† 6-mercaptopurine, azathioprine, hydroxyurea)
         b. Phenothiazine type (phenothiazines, benzodiazepines, antithyroid compounds,† sulfonamides,† antibiotics, anticonvulsants)
         c. Others (chloramphinicol,† benzene†)
      3. Idiosyncratic
         a. Acute, days to weeks (quinine, quinidine, indomethacin, procainamide, thiazides, sulfonamides,† phenylbutazone,† antithyroids†)
         b. Chronic, months to years (chloramphenicol,† phenylbutazone,† benzene,† gold salts†)

(Continued on page 6)

Table 2b. Cont'd

II.	Marrow hyperplasia with ineffective granulocytopoiesis
	a. Chediak-Higashi syndrome; megaloblastic anemia; myeloproliferative disorders (these may belong in IV)
	b. Drug induced
	1. Impaired nucleic acid synthesis (cytosine arabinoside,† methotrexate,† phenytoin)
	2. Others (alcohol, chloramphenicol†)
III.	Decreased survival in circulation due to increased utilization or increased destruction
	a. Bacterial infection; viral infection; protozoal infection; chronic benign neutropenia of childhood; chronic idiopathic neutropenia in adults; splenic neutropenia; neonatal isoimmunization neutropenia; acquired immunoneutropenia
	b. Drug induced (immunologic mechanism)
	Aminopyrine, amidopyrine, phenylbutazone,† sulfapyridines†
IV.	Combination of impaired production (I or II) and decreased survival (III)
	a. Megaloblastic anemia; severe bacterial infections; mycobacterial infections; chronic idiopathic myelokathexis
	b. Drug induced (very likely)
	Alcohol, purine and pyrimidine inhibitors, aminopyrine
V.	Pseudoneutropenia (shift from circulating granulocyte pool [CPG] to marginal granulocyte pool [MGP])
	a. Endotoxin
	b. Drug induced: anesthetic agents, ether, pentobarbital

* Adapted from Finch SC. *In:* Williams WJ, Beutler E, Erslev AJ and Lichtman MA (editors): Hematology, 3rd edition. New York, Mcgraw-Hill,1983.

† Drugs cited for more than one mechanism.

Table 3. Some combinations of Hb S and α-Thalassemia, *β*-Thalassemia, or Globin chain structural abnormalities

Hemoglobinopathy	Percentage in adults					Sickle cells	Clinical severity*
	Hb A	Hb S	Hb X†	Hb A_2	Hb F		
Sickle cell trait (Hb AS)	55-60	40-45	0	Normal	N	0	1
Sickle cell anemia (Hb SS)	0	90-95	0	Normal	5-10	+	3
Hb S α-Thalassemia 1	75	25	0	Normal	N	0	1
Hb S β^0 Thalassemia	0	90-95	0	Increased	5-10	+	3
Hb S β^+ Thalassemia	5-30	60-90	0	Increased	5-10	+	2
Hb S HPFH	0	70-80	0	Normal	20-30	0	1
Hb SC	0	50	50	Normal	Normal	+/-	2
Hb $SD_{LOS\ ANGELES}$	0	50	50	Normal	Normal	+/-	2
Hb SN	0	50	50	Normal	Normal	0	1
Hb SO	0	50	50	Normal	Normal	+	3
Hb AS -$G_{PHILADELPHIA}$	25	25	50‡	Normal	Normal	0	1

* Clinical severity 1: benign; 2: moderately severe; 3: similar to sickle cell anemia.

† Hb X, abnormal hemoglobin other than Hb S.

‡ Approximately 25% Hb G (migrating with Hb S) and 25% of the hybrid α2G β2S (migrating as Hb C).

Table 4. Selected clinical characteristics and morphologic features of four types of malaria

Characteristic	*P. falciparum*	*P. vivax*	*P. ovale*	*P. malariae*
Usual incubation period (days)	8-11	10-17 or longer	10-17 or longer	18-40 or longer
Severity of primary attack	Severe in nonimmune	Mild to severe	Mild	Mild
Periodicity (hours)	None	48	48	72
Duration of untreated primary attacks (weeks)	2-3	3-8	2-3	3-24
Duration of untreated infection	6-17 months	5-7 years	12 months	20+ years
Average parasitemia (/ul)	20,000 or greater	10,000	9,000	6,000
Anemia	Frequent & severe	Mild	Mild	Mild
CNS involvement	Yes, severe	Rare	Rare	Rare
Nephrotic syndrome	Rare	Rare	No	Frequent
Infected erythrocyte				
Enlarged	-	+	+/-	-
Oval, fimbriated	+/-	+/-	+	-
Schuffner's dots	-	+	+	-
Maurer's dots	+	-	-	-

(Continued on page 9)

Table 4. Cont'd

Characteristic	*P. falciparum*	*P. vivax*	*P. ovale*	*P. malariae*
Parasite				
Multiple in single erythrocyte	+	+/-	-	-
Multiple forms	-	+	+	+
Only ring forms	+	-	-	-
Large amoeboid rings	-	+	+	+
Double chromatin dots	+	+/-	-	-
Peripheral location in erythrocyte	+	+/-	-	-
Band forms	-	-	-	+
Banana-shaped gametocytes	+	-	-	-
Number of merozoites	8-24	12-24	8-12	6-12

Table 5. Normal ranges of immunoglobulins IgG, IgA and IgM at different ages (g/l)[*]

Age	IgG	IgA	IgM
Cord blood	7.45-16.02	0.00-0.08	0.04-0.26
1/2-3 months	2.93-8.06	0.03-0.57	0.16-1.64
3-6 months	1.39-9.34	0.04-0.78	0.20-1.20
6-12 months	4.10-10.81	0.13-0.82	0.48-2.29
1-2 years	3.49-11.39	0.13-1.02	0.40-2.29
2-3 years	4.82-12.00	0.22-1.18	0.54-2.09
3-6 years	5.53-13.07	0.33-1.8	0.56-2.18
6-9 years	6.46-14.51	0.28-2.22	0.55-2.32
9-12 years	6.13-15.12	0.57-2.56	0.70-2.84
12-16 years	6.67-14.64	0.77-2.19	0.49-2.61
Adults	6.58-18.37	0.71-3.60	0.40-2.63

[*] Lenter, C (editor). 1984 Geigy Scientific Tables, 8th edition, volume 3. Published by Ciba-Geigy Ltd., Basle, Switzerland. Table 36, page 154.

Table 6. IgG subclasses: Reference values in mg/dl

Age	IgG1	IgG 2	IgG 3	IgG4
Cord	435-1084	143-453	27-146	1-47
Children				
0-2 month	218-496	40-167	4-23	1-33
3-5 month	143-394	23-147	4-100	1-14
6-8 month	190-388	37-60	12-62	1-30
9-24 month	286-680	30-327	13-82	1-65
3-4 year	381-884	70-443	17-90	1-116
5-6 year	292-816	83-513	8-111	1-121
7-8 year	422-802	113-480	15-133	1-121
9-10 year	546-938	163-513	26-113	1-121
11-12 year	456-952	147-493	12-179	1-168
13-14 year	347-993	140-440	23-117	1-83
Adult	422-1292	117-747	41-129	1-129

Table 7. Oral GTT (Glucose Tolerance Test) diagnostic criteria for Diabetes Mellitus*

	Fasting mg/dl (mmol/l)	1 hr mg/dl (mmol/l)	2 hr mg/dl (mmol/l)	3 hr mg/dl (mmol/l)
Adult				
Diabetes mellitus				
Fasting	>140 (>7.8)	-	-	-
Glucose tolerance test (75 g glucose or 1.75 g/kg body weight)	>140 (>7.8)	>200 (>11.1)	>200 (>11.1)	-
Impaired glucose tolerance (75 g glucose)	>200 (>11.1)	140-200 (7.8-11.1)	-	-
Gestational diabetes mellitus				
Screening (50 g glucose)	-	>140 (>7.8)	-	-
Definitive (100 g glucose)	<105 (>5.8)	>190 (>10.5)	>165 (>9.2)	>145 (>8.1)
Pediatric				
Diabetes mellitus				
Fasting	>140 (>7.8)	-	-	-
Glucose tolerance test (75 g glucose or 1.75 g/kg body weight)	>140 (>7.8)	>200 (>11.1)	>200 (>11.1)	-

* Also refer to Lab Form 3.

Table 8. Gastric acid output in various conditions*

Status	Basal acid output (mmol h+/hr)	*Maximal acid output (mmol h+/hr)†
Control	0.0-5.0	5.0-20.0
Achlorhydria	0	0
Gastric carcinoma	0.0-5.0	0.0-10.0
Gastric ulcer	0.0-5.0	0.2-15.0
Duodenal ulcer	0.0-5.0	5.0-30.0
Zollinger-Ellison syndrome	5.0-70.0	10.0-100.0

* Modified from Craig RM. Alimentary tract and exocrine pancreas. *In*: Laboratory Medicine, Noe DA, Rock RC (editors), Baltimore, Williams & Wilkins; pp 383-400.
† 15 min after pentagastrin injection.

Table 9. AFP levels in pregnancy

Period of gestation	Normal range serum (ng/ml)	Median serum (ng/ml)	Normal range amniotic fluid (µg/ml)
15 weeks	Up to 54	27	Up to 35
16 weeks	Up to 58	29	Up to 31
17 weeks	Up to 67	34	Up to 25
18 weeks	Up to 76	38	Up to 21
19 weeks	Up to 92	46	Up to 17
20 weeks	Up to 109	55	Up to 15
21 weeks	Up to 140	70	Up to 12

Table 10. *B*-HCG serum values (detection limit: 5 iu/l)

	Time after conception	*B*-HCG (miu/ml)
Males	-	<10
Nonpregnant females	-	<10
Pregnant women	1st week	10 - 50
	2nd week	50 - 400
	3rd week	100 - 3,000
	4th week	1,000 - 20,000
	2nd month	4,000 - 100,000
	3rd month	3,000 - 150,000
	2^{nd} trimester	7,000 - 100,000
	3^{rd} trimester	1,000 - 60,000

Table 11: Serologic markers in the diagnosis of hepatitis

	HAV Ab IgM	HAV Ab total	HBs Ag	HBs Ab	HBc Ab IgM	HBc Ab total	HBe Ag	HBe Ab	HBV DNA	HCV Ab	HDV Ab	HEV Ab
Acute infection												
HAV	+	+								-		
HBV												
Early			+	-	+	+	+	-	-			
Window			-	-	+	+	+/-	-	-			
Resolving			-	+	-	+	+	+	-			
HEV										+/-		
HDV												
Coinfection			+/-	-	+	+	-	-	+		+	
Superinfection			+/-	-	-	+	+/-	+/-	+/-		+	

(Continued on page 17)

Table 11: Cont'd

	HAV Ab IgM	HAV Ab total	HBs Ag	HBs Ab	HBc Ab IgM	HBc Ab total	HBe Ag	HBe Ab	HBV DNA	HCV Ab	HDV Ab	HEV Ab
Chronic infection												
HBV												
Nonreplicating			+	-	-	+	-	+	-			
Replicating			+	-	-	+	+	-	+			
Reactivation			+	-	+	+	+	-	+			
HEV										+		
HDV			+	-	-	+	+/-	+/-	+/-		+	
Past infection												
HAV	-	+										
HBV			-	+	-	+	-	+	-			
HCV										+		
HDV			-	+/-	-	+	-	+	-		+	
HEV												+

Table 12. Cerebrospinal fluid findings in meningitis*

Meningitis	Leukocytes 10^9/l	Protein (mg/dl)	Glucose (mg/dl) †	Comments
Acute bacterial	1.000-10.000 or more; mainly PMNs.	100-500 in most cases	Usually <40	Partially treated cases may convert to lymphocytosis
Viral	0.005-0.300 mainly lymphocytes; PMNs may predominate initially.	<100 in most cases	Normal (50-70)	Reduced glucose seen in 25% of cases of mumps and some hsv.
Fungal	0.040-0.400; lymphocytes and/or polys. Eosinophilia may be found in coccidioides.	50-300	Usually <40	Neutrophilic pleocytosis most common with mycelial fungal forms.
Tuberculous	0.100-0.600 up to 1.200 mainly lymphocytes; polys may predominate initially.	50-300 marked increase with spinal block.	Decreased; <45 in many cases.	Findings vary depending on clinical stage.
Acute syphilitic	0.300-0.700 mainly lymphocytes.	Mildly increased, usually <100	Normal (50-70)	Up to 15% have normal CSF parameters

(Continued on page 19)

Table 12. Cont'd

Meningitis	Leukocytes 10^9/l	Protein (mg/dl)	Glucose (mg/dl)	Comments
Amebic (naegleria)	Mildly increased to grossly purulent (>20.000); PMNs.	Increased, may reach 1000	Normal to mildly decreased	RBCs suggest brain hemorrhages.
Lyme disease (stage II)	0.005-0.400; lymphocytes predominate.	Moderately increased; <300	Normal to mildly decreased	CSF normal in stage I
Malignant	0.00-0.300; lymphocytes predominant; variable number of tumor cells may be seen.	Moderately increased; <500	Usually <50	Marked neutrophilic pleocytosis may be seen in large tumors.

* Adapted from Fishman RA: Cerebrospinal fluid in disease of the nervous system, 2nd ed. Philadelphia, W.B. Saunders, 1992.

† In presence of normal serum level.

Table 13a. Ascitic, pleural and pericardial fluids*

Findings	Transudates	Exudates
Appearance	Clear or pale yellow	Cloudy, turbid, purulent or bloody
Specific gravity	<1.016	>1.016
Clot formation	Absent	Present
Cells: White cells	Few lymphocytes	>0.500 10^9/l
Red cells	Few red cells	Variable, may be high.
Glucose	As in serum	Less than in serum
Cholesterol	<60 mg/dl (<1.55 mmol/l)	>60 mg/dl (>1.55mmol/l)
Fluid/serum cholesterol ratio	<3	>3
Protein	<3.0 g/dl	>3.0 g/dl
Fluid/serum protein ratio	<0.5	>0.5
Fluid/serum LDH ratio	<0.6	>0.6

* Adapted from Kjeldsberg CR, Knight JA: Body Fluids: Laboratory examination of amniotic, cerebral, seminal, serous and synovial fluid, 3rd edition. American Society of Clinical Pathologists, Chicago, 1993.

Table 13b. Recommended tests in ascitic, pleural and pericardial fluids*

Routine Includes gross examination, fluid/serum protein ratio and examination of stained smear.
Useful in most patients Gram & Z.N. Stains, Baterial & T.B. Cultures, cytology, fluid cholesterol and fluid/serum cholesterol ratio.
Useful in certain circumstances Biopsy; other tests such as pH, LDH, amylase, lipase, lipid analysis, immunologic studies, tumor markers and fluid/serum bilirubin ratio.

* Adapted from Kjeldsberg CR, Knight JA: Body fluids: Laboratory examination of amniotic, cerebral, seminal, serous and synovial fluid, 3rd edition. American Society of Clinical Pathologists, Chicago, 1993.

Table 14. Typical biochemical findings associated with disorders of Porphyrin metabolism*

Disorders	Urinary contents			
	ALA	PBG	UP	CP
Acute intermittent porphyria (AIP)	++	++	+	+ or N
Hereditary coproporphyria	+	+	N	+
Variegate porphyria (acute attacks)	+	+	+ or N	+ or N
Congenital erythropoietic porphyria	N	N	++	+
Erythropoietic protoporphyria	N	N	N	N
Symptomatic porphyria	N	N	++	+
Lead poisoning	+	+ or N	N	+

* ALA= *D*-aminolevulinic acid; UP= uroporphyrin; CP= coproporphyrin; PBG= porphobilinogen; += increased; ++= large increase; N= normal.

Table 15 Free Estriol (E3) in Late Pregnancy: Study results for reference values (ng/ml).

Stage in 3rd Trimester Pregnancy (weeks)	Number of cases	Median levels	95% Percentile ranges
27	21	6.5	2.9-12.7
28	21	7.3	3.3-14.3
29	19	8.2	3.7-16
30	19	9.2	4.1-17.9
31	22	10.3	4.6-19.9
32	18	11.4	5.1-22.1
33	18	12.7	5.7-24.4
34	20	14.0	6.3-27.0
35	19	15.5	7.0-29.7
36	20	17.0	7.7->30
37	19	18.7	8.5->30
38	20	20.4	9.3->30
39	18	22.3	10.2->30
40	14	24.3	11.1->30

Table 16. Hormonal Testing in Menstrual Cycles of Assisted Reproduction Protocols

Time in Menstrual Cycle (Day)	Hormonal test(s)
3	E2, FSH, LH
4-12	E2
10-14	E2, Progesterone, LH
14-28	E2, Progesterone
28	E2, Progesterone, HCG
28-70	Progesterone, HCG

Table 17. Aminoacid Profile Chromatograms by Thin Layer Chromatography (Qualitative).

BAND NO.	AMINO ACIDS MIGRATION PATTERN	DISORDERS
1.	Leucine, Isoleucine	Maple syrup urine disease (msud), Hartnup syndrome
2.	Phenylalanine	Phenylekotonuria, Hartnup syndrome
3.	Valine, Methionine	Maple syrup urine disease (msud), Hartnup syndrome
4.	Tryptophan, Aminoisobutyric acid	Hartnup syndrome
5.	Tyrosine	Tyrosinemia Types I, II and III Hartnup syndrome
6.	Proline	Hyperprolinemia
7.	Alanine ethanolamine	Histidinemia, Citrullinnuria, Hartnup syndrome
8.	Threonine, Glutamic acid	Histidinemia, Citrullinuria
9.	Homocitruline, Glycine Serine, Hydroxyproline, Aspartic acid, Glutamine, Citrulline	Argininosuccinic aciduria, Hyperprolinemia, citrullinuria, Hartnup syndrome
10.	Homocystine, Asparagine	Homocystinuria
11.	Argininosuccinic acid, Histidine, Arginine, Lysine, Ornithine, Cystathionine, Cystine, cysteine, Hydroxylysine	Cystinuria, Argininosuccinic aciduria, Histidinemia, Citrullinuria Hartnup syndrome

Note: Generalized aminoaciduria is also found in Wilson's disease, Lowe's syndrome, Galactosemia, Cirrhosis and renal dystrophies. Adapted from: Laboratory Corporation of America, Holdings & Lexi-Comp Inc.

Table 18. Mucopolysaccharidoses.

TYPE	DISORDER	CAUSE (ENZYME DEFICIENCY)	INCIDENCE/ LIVE BIRTH
MPS I			
MPS I H	Hurler syndrome	α-l-iduronidase	1/100,000
MPS I H/S	Hurler-sheie syndrome	α-l-iduronidase	1/115,000
MPS I S	Sheie syndrome	α-l-iduronidase	1/50,000
MPS II	Hunter syndrome	iduronate-2-sulfatase	1/110,000 mails
MPS III			
MPS III A	Sanfilipo syndrome type A	heparan N-sulfatase	1/325,000
MPS III B	Sanfilipo syndrome type B	N-acytle- α -D glucosaminide	v. rare
MPS III C	Sanfilipo syndrome type C	acytl-coa-alpha glucosamine acytle transferase	v. rare
MPS III D	Sanfilipo syndrome type D	N-acytleglucosamine-6-sulfatase	v. rare
MPS IV			
MPS IV A	Morquio syndrome type A	galacosamine-6-sulfatase	1/300,000
MPS IV B	Morquio syndrome type B	beta-galactosidase	1/300,000
MPS VI	Maroteaux- Lamy syndrome	N-acytleglucosamine-4-sulfatase	v. rare
MPS VII	Sly syndrome	β-glucuronidase	v. rare
MPS IX	Hyaluronidase deficiency	hyaluronidase	v. rare

APPENDICES

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APPENDIX I
Firsts in Jordan by CML
(Consulting Medical Laboratories)

(1) 1981: the setting up of comprehensive medical laboratory service in private practice in Jordan, supported by a complementary team of experts.

(2) 1981: installing quality control in laboratory medicine as part of routine practice in all laboratory disciplines. For instance, objective reporting of routine urine examination was first issued from our laboratories with giving of cell counts backed by in-house studies that had established reference ranges, namely, up to 10 and 2 for urinary white and red cells per microliter, respectively (Barakat HF & Dajani YF. Urine cell counts. *Bulletin of the Consulting Medical Laboratories* 1986;4(1):3). Since then, we saw several medical laboratories in town adopting this method.

(3) 1981: installation of a cell immunology laboratory.

(4) 1981: provision of frozen section service in private practice.

(5) 1981: provision of door-to-door service to clinics for laboratory results.

(6) 1981: establishing research as part of daily routine practice in private medical laboratories in Jordan.

(7) 1982: introduction of semithin plastic-embedded technique to pathology.

(8) 1983: publication of a quarterly scientific medical bulletin which has earned an international reputation and is now in its 22nd year.

(9) 1983: isolation of brucella in bone marrow and blood and proof of its endemicity with a seasonal pattern in Jordan.
(10) 1985: performance of chormosomal analysis in blood, amniotic fluid, trophoblast and bone marrow.
(11) December 1985: performance of HIV screening test by ELISA.
(12) 1988: introduction of FNA technique of testis in a way to supercede the traditional testicular biopsy.
(13) 1989: testing for ER PR status of breast cancer.
(14) 1996: introduction of immunohistochemistry service in private practice in Jordan.
(15) 1997: launching of telepathology in the region for consultative and educational purposes through the internet.
(16) 1997: performance of pre-implantation testing for sexing and Down's syndrome on blastomeres.
(17) 1997: performance of FISH on amniotic fluid.
(18) 1998-1999: setting up of a comprehensive diagnostic molecular genetics laboratory.
(19) 1981-now: introducing and innovating new techniques and methods in medical laboratory practice in the private Jordanian medical sector, with continuing educational activities at various levels through publication of the scientific periodical, the 'Bulletin', at the personal level by lectures, seminars, participation of staff in medical conferences, medical committees, undergraduate and postgraduate teaching as well as education by use of modern methods in information technology.
(20) 1981-now: active office for several medical scientific organizations.

CML takes pride in their staff, efficient turnaround results, prompt collection arrangements, easy access to consultant advice, in-built and time honored quality control structure, promptly alerting referees about results of importance, clear communication policies based on exemplary ethical grounds, high ranking and in-house built computer program with links, internet and cybernetic facilities, while billing and pricing is in fact more money and time saving than many people can imagine.

APPENDIX II
CML WEBSITE

Refer to home-page above (Page 1 of our web site) to select your entries.

WEB SITE ADDRESS IS www.cml-jordan.com

The following guidelines help you enter into our web site to see facilities available in our establishment as well as enter your queries which will be answered via your given e-mail address.

A BIG WELCOME. Our motto is :

Hi Technology

Patient comes first

Teamwork a baseline

Back to Home Page المختبرات الطبية التخصصية

If you like to read the current message from the Chairman, click in

After reading the Chairman's comment, click Back to Home Page

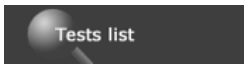
To read about some of the achievements by the CML, click

Here are some examples:

- 1981: Setting up a comprehensive medical laboratory service for the first time in private medical practice in Jordan, including a "Cell immunology" lab, "Frozen sections" for the pathology service and development of "serious research" as part of the daily routine practice.

- 1983: Publication of the quarterly scientific '*Bulletin*', now 20 years old.
- Isolation of *Brucella* by culture for the first time in Jordan and proof of its endemicity.
- 1997: The launching of "Telepathology" as a pioneering project in pathology in the region.
- Performance of PGD (pre-implantation genetic diagnosis) on blastomeres and FISH (Fluorescent in situ hybridization) on amniotic fluid as well as "Clinical genetics" lab.

After reading about us you can click Back to Home Page and can then enter the following icon on left of home page.



All test names are arranged in alphabetical order. More than 400 tests are in this list. To look up information on an individual test, double click on the first letter of the test. The chosen letter will open all test names which start with that very letter. Then, we can choose the individual test. Example: for inquiry about "VALPROIC ACID" go to letter "V". When a list of tests that start with the letter "V" appears such as:

VALPROIC ACID (DEPAKENE, CONVULEX)

VANILMANDELIC ACID (VMA)

VARICELLA ZOSTER (IgG & IgM)

and so on…

Choose the test sought in the list, where you will find the following information for VALPROIC ACID:

NAME OF TEST	SPECIMEN	UNITS	NORMAL RANGE	CLINICAL SIGNIFICANCE
VALPROIC ACID (DEPAKENE, CONVULEX)	SERUM	UG/ML UMOL/L	50-100 350-700	VALPROIC ACID IS AN ANTICONVULSANT THAT HELPS TO CONTROL CERTAIN TYPES OF SEIZURES ASSOCIATED WITH EPILEPSY.

Back to test list

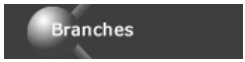
Back to Home Page المختبرات الطبية التخصصية

If you want to read about our departments, click

Departments include 9 categories 1. Clinical 2. Cytohistopathology 3. Referrals 4. Consultations 5. House Call Service 6. Administrative 7. Publications 8. Research & Development 9. Training

Back to Home Page المختبرات الطبية التخصصية

If you like to know where our branches are located, click



1. Jabal Amman (established 1981): Queen Misbah Street Telefax 4644414/ 4644480
2. Zerka (established 1982): King Hussein Street Telefax 05-3981117/ 05-53993446

3. Jabal Hussein (established 1984): Sukaina Square Telefax 5669614/ 5685614
4. Ibn Khaldoun (established 1996): Opposite Khalidi Hospital Telefax 4611124/ 4654122
5. Central (established 2000): Opposite Specialty Hospital Telefax 5671146/ 5671147

Back to Home Page المختبرات الطبية التخصصية

To know about our personnel, click

Employees : *Total number*= 50 *Technologists* = 31 *Administrative*= 19

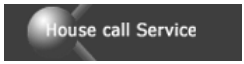
Board of Directors

* Yahia F. Dajani, M.B.Ch.B. (Bristol) F.R.C.Path (London) Chairman
* A. Aziz Masoud, M.Sc.
* Hani Barakat, M.S, M.Sc.
* Basma Hasan, M.B.B.S., Ph.D.

(CVs are available).

Back to Home Page المختبرات الطبية التخصصية

For more information about our house call service,



Appointments for house calls can be made on the phone between 8 am to 8 pm daily except Fridays.

Call 962-6-5671147 or mobile 079-5556424

Blood and other samples are collected for laboratory testing by our designated technologists who are experts in their field. Our fleet has become integrated over the years into many families with great and warm relationships. Members within those families have contributed to improvisions here and there for developments in our institution over the past 23 years.

Reports may be relayed through fax, e-mail or, alternatively, delivered to your door.

Back to Home Page المختبرات الطبية التخصصية

To look up our “Consultation service” click

Clinical pathology Fine needle aspiration Bone biopsy Educational whose details are given within.

Back to Home Page المختبرات الطبية التخصصية

For Telepathology, click

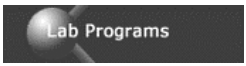
Back to Home Page المختبرات الطبية التخصصية

For more information related to our Publications and R&D click

This icon opens data on "Quality control", "Research programs", "Publications", "Monitoring"

Back to Home Page المختبرات الطبية التخصصية

To read more about our original home-made lab programs, click



For the last 20 years, CML was involved in designing and commissioning 5 generations of computed laboratory programs for management and work. In 1999, the last (5th) generation went into full operation.

Back to Home Page المختبرات الطبية التخصصية

For more information about our training programs click

CML is a recognized center for training medical technologists and pathology training, locally and on an international scale.

Back to Home Page المختبرات الطبية التخصصية

For specific questions relating to lab tests or other lab issues click

Questions

CONSULTING MEDICAL LABORATORIES E-mail

info@cml-jordan.com

For contact, can use one of the phone numbers or email addresses listed below:

(1) dajani@cml-Jordan (2) 00962-6-5671147 (3) 00962-6-5671146
(4) 00962-79-5556424

For question(s), fill in the form provided as illustrated below and send it to info@cml-Jordan.com or the e-mail address given above:

"QUESTIONS FORM" TO FILL

►Name ►Age ►Sex ►Address ►P.O.BOX ►E-mail ►Telephone ►Country ►Matter of Urgency (Please indicate by x) -- urgent or -- normal ►Query ►Question(s):

Back to home page; then can enter the item of your choice in our web site, as you please.

You are all welcome to visit our website.

APPENDIX III

CML BRANCHES AND ADDRESSES

Jabal Amman (established 1981):
Queen Misbah Street Telefax 4644414/ 4644480

Zerka (established 1982):
King Hussein Street Telefax 05-3981117/
05-53993446

Jabal Hussein (established 1984):
Sukaina Square Telefax 5669614/ 5685614

Ibn Khaldoun (established 1996):
Opposite Khalidi Hospital Telefax 4611124/ 4654122

Central (established 2000):
Opposite Specialty Hospital Telefax 5671146/
5671147

APPENDIX IV
Reference weights & measurements used in the Guide

g = *Gram*

kg = Kilogram= 1x1000g

mg = milligram= $1x10^{-3}$g

ug = microgram= $1x10^{-6}$g

ng = nanogram= $1x10^{-9}$g

pg = picogram= $1x10^{-12}$g

l = *Liter*

ml = milliliter= $1x10^{-3}$l

ul = microliter= $1x10^{-6}$l

fl = femtoliter= $1x10^{-9}$l

$$\text{Body mass index} = \frac{\text{Body Weight (kg)}}{\text{Body Surface Area (sq m)}}$$

Calculations and formulas

Creatinine clearance

Creatinine clearance reflects glomerular filtration rate and is usually performed on a 24 hour urine.

Calculation

$$\textit{Creatinine clearance}\ (\text{ml/min}) = \frac{\text{Urine volume(ml/min)}}{\text{Serum creatinine}} \times \frac{\text{Urine creatinine}}{\text{Body surface area}} \times 1.73$$

To obtain urine volume in ml/minute, we divide 24 hour urine volume by 1440, the number of minutes in 24 hours.

Excretion fraction of filtered sodium

This test is sensitive and specific for acute tubular necrosis versus pre-renal uremia, whose excretion fractions are indicated by > 2 and < 1, respectively.

Requirements: Random serum and urine samples.

Calculation

$$\text{Excretion fraction of filtered Na} = \frac{\text{Urine Na}}{\text{Serum Na}} \times \frac{\text{serum creatinine}}{\text{urine creatinine}} \times 100$$

Amylase clearance/ creatinine clearance

Reference range= up to 4%.

Useful in confirming or excluding the diagnosis of acute pancreatitis.

Requirements: Random serum and urine samples.

It is the amylase renal clearance expressed as percentage of creatinine clearance.

Calculation:

$$\frac{\text{Amylase clearance}}{\text{Creatinine clearance}}\ (\%) = \frac{\text{Urine amylase}}{\text{Serum amylase}} \times \frac{\text{Serum creatinine}}{\text{Urine creatinine}} \times 100$$

When acute pancreatitis subsides, urine amylase remains elevated after serum amylase returns to normal. In

macroamylasemia, serum amylase rises but the amylase-creatinine clearance ratio is <1%.

Correction of serum calcium for low albumen

Serum albumen is required for interpreting serum calcium level.

Calculation

Adjusted calcium= Serum calcium – serum albumen + 4.0

This equation corrects for serum calcium level, whereby serum Ca is increased by 1mg/dl for every 1g/dl drop of albumen level below 4g/dl.

The anion gap

Reference ranges: Normal 5-15, borderline 15-20, increased >20.

The anion gap is a calculation of diagnostic convenience representing the amount of unmeasured anions and cations in a clinical sample.

The anion gap depends on the principle of electroneutrality, whereby total number of cations in a solution should equal that of anions.

Commonly measured cations are Na and K, anions measured Cl and HCO_3.

Calculation

Anion gap = Measured cation – measured anions

namely (Na) – (Cl + CO_2 content) (K usually not included).

An increase of anion gap beyond 30 is usually due to known cause such as lactic acidosis or keto-acidosis. In multiple myeloma, strongly basic monoclonal Igs serve as positively charged cations resulting in decreased anion gap. Anion gap may be used as quality control of a low serum sodium.

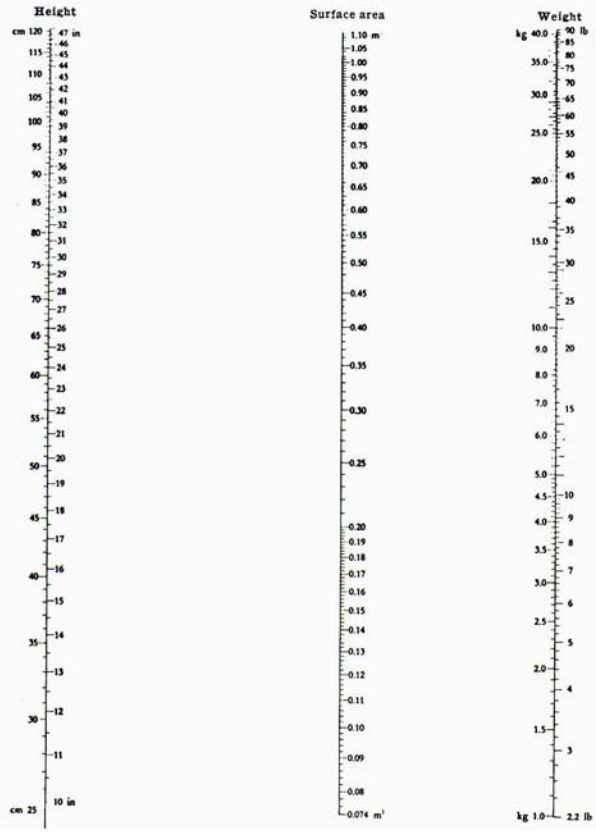
Traditional versus SI units conversion & vica versa

$$\text{CF (Conversion factor)} = \frac{1000}{\text{Mol. Wt.}}$$

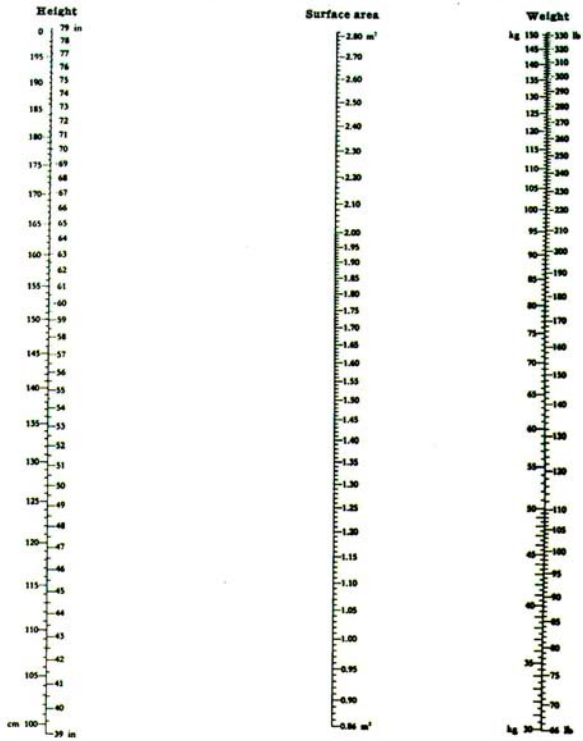
Traditional units to International units:
ug/ml (microgram/ml) × CF= I.U. (micromole/liter)

International units to Traditional units:
I.U. (microliter/liter) × CF= ug/ml (microgram/ml)

Nomogram for Calculating the Body Surface Area of Children



Nomogram for Calculating the Body Surface Area of Adults



APPENDIX V
SOUND PRACTICE OF VENESECTION (BLOOD TAKING)

1. *Before you begin a venesection:* make sure that all necessary gadgets and collection receptacles available, tests request form having been already well groomed. No blood taking should be made before prior writing a request form, even if that is done upon the patient's request, such as for estimation of blood sugar serum level.
2. *Selecting the vein:* To begin a venesection, the vein of interest has to been seen or felt under the skin. In frequently tested cases, e.g in ICU, it is advised to ensure by soft palpation that blood flow in the vein of interest is maintained, in order to avoid hitting a thrombosed vein. This can be done by applying and releasing the tourniquet, watching whether the vein swells or remains at same diameter, while any hard feel should induce looking for another vein. In case of difficulty, always ask for senior help.
3. *Cleansing the skin:* Alcohol and iodine are ideal combination for skin sterilization, but iodine is messy and alcohol suffices. In fact, sterile-needle prick of unprepared skin imparts no harm, but an alcohol sweep does appear to give psychological repose for both venesectionist and patient. Psychology of the patient should always be taken into consideration and an amiable approach is mandatory, especially in children, a task that needs an experienced person.
4. *Applying the tourniquet:* The purpose of the tourniquet is to help insert the needle in a roomier vein. Once aspiration begins and blood starts coming up,

the tourniquet should be released to avoid effect of obstructing blood flow in the aspirated vein. This is necessary for certain tests, such as serum calcium.

5. *Pressure on site after venesection:* Pressure should be applied to the frequently punctured antecubital site with the elbow extended, to enable free venous flow and facilitate closure of the vein punctured. We often see patients made to flex their arms strongly in attempt to stop bleeding, which in fact increases intravenous pressure and leads exactly to the opposite! With any bleeding point at the site of venepuncture, stopping bleeding should be ensured by even and firm pressure on the spot. Afterwards, application of a plaster on the site is optional.
6. *Blood tubes:* It is amazing how some believe in one kind of collection tubes or another. In fact, once you properly adopt any method of collecting blood, by using vacuum or not, it will be sufficient for the purpose. Also, a very frequent mistake is made, especially in hospitals, by distributing already labeled bottles to patients then taking the samples. Labeling must be made immediately after blood taking and never before, making a habit of asking the patient's name as you label the bottle(s), even when you know the patient's name. When serum and EDTA collection are required for the same sample fill the former first to avoid contaminating serum by EDTA.
7. *Mixing the blood in the anticoagulated tubes:* Mixing should be gentle without shaking. Gentle mixing by rotation clockwise and anticlockwise, also by tilting and swerving the tube, are admissible. 1-2 minute mix usually suffices. The right amount of blood should be ensured in relation to anticoagulant.

8. *Centrifugation:* Preparing serum or plasma should be done prompt in order to avoid hemolysis or change in serum composition.

APPENDIX VI
FORMS

GENERAL HEALTH LABORATORY SCREEN

A general health laboratory screen can never be complete without a full physical examination. The following list has been recommended by the Consulting Medical Laboratories for well over 20 years. This test panel is recommended in men and women over 50 years, but may be done in those over 40 years or perhaps in younger persons as may seem suitable.

Example :

المختبرات الطبيـــــة التخصصيـــــة

CONSULTING MEDICAL LABORATORIES

AMMAN –PHONE: 4644414, 4644480, 5669614, 5685614, 4611124, 4654122, 5671146, 5671147

E-mail < **medlab@go.com.jo> web: www.cml-jordan.com**

LABORATORY TESTS

Patient's Name: ***Age:*** **years** ***Sex:***

Dr. YAHIA F. DAJANI

Date:

CBC

***Hb*= /dl PCV= l/l MCHC= mg/dl MCH= pg MCV= fl RBC= 10^12/l**

***WBC*= 10^9/l Neutrophils= % Lymphocytes = % Monocytes= % Eosinophils= %**

***Platelet count*= 10^9/l *ESR*= mm 1st hour mm 2nd hour**

Chemical tests

***Fasting blood sugar*= mg/dl (70-120) *Cholesterol total*= mg/dl (up to 200)**

***Cholesterol HDL*= mg/dl (31-71) *LDL*= mg/dl (50-190)**

***Triglycerides*= mg/dl (up to 160) Cholesterol Ratio=**

***Urea*= *mg/dl* (<50) *Creatinine*= mg/dl (0.6-1.2) *Uric Acid*= mg/dl (2.0-6.0)**

***Sodium*= mmol/l (135-147) *Potassium*= mmol/l (3.5-5.5)**

***Chloride*= mmol/l (95-105) *Calcium*= mg/dl (8.8-10.2) *Phosphate*= mg/dl (2.5-5.0)**

***GOT*= u/l (up to 35) *GPT*= u/l (up to 35) *GammaGT*= u/l (up to 54)**

***Alkaline Phosphatase*= u/l (30-120) *Bilirubin total*= (<1.1) *direct*=0.12 (<0.2)**

PSA total=??ng/ml (up to 4.0)

Urine Analysis

***Color* *Appearance* *Specific gravity*= *Reaction* *pH*=**

Normal reactions to *nitrite, protein, glucose, ketone, urobilinogen, bilirubin, blood.*

***Microscopy*: First portion White CC = /ulRed CC= / ul**

Second portion WhiteCC = /ul RedCC= /ul

Stool Analysis

Appearance :

Ova & Cyst :

***Occult blood* :**

Yahia F. Dajani, M.B.Ch.B. (Bristol), F.R.C.Path. (London)

April 12 , 2003.

Consulting Medical Laboratories Established 1981
Central, Opposite Specialty Hospital, Amman Telefax 5671146 / 5671147 email: mediab@go.com.jo
Board of Directors: *Yahia F. Dajani, M.D. (Chairman)* *Hani F. Barakat. M.Sc.*
Basma I. Hasan, M.D. *Abdul Aziz H. Masoud, M.Sc.*

URINE ANALYSIS ROUTINE

COLOR *APPEARANCE*

SP. GRAVITY *REACTION* *PH*

CHEMISTRY:

NITRITE *PROTEIN*

GLUCOSE *KETONE*

UROBILINOGEN *BILIRUBIN*

BLOOD

MICROSCOPY:

	INITIAL	*MEDSTREAM*	*LAST PORTION*	*NORMAL RANGE*
WHITE CELLS COUNT /UL	----------	----------	----------	*(UP TO 10)*
RED CELLS COUNT /U L	----------	----------	----------	*(UP TO 3)*

OTHERS ----------
COMMENT ----------

Consulting Medical Laboratories Established 1981
Central, Opposite Specialty Hospital, Amman Telefax 5671146 / 5671147 email: medlab@go.com.jo
Board of Directors: *Yahia F. Dajani, M.D. (Chairman)* *Hani F. Barakat. M.Sc.*
Basma I. Hasan, M.D. *Abdul Aziz H. Masoud, M.Sc.*

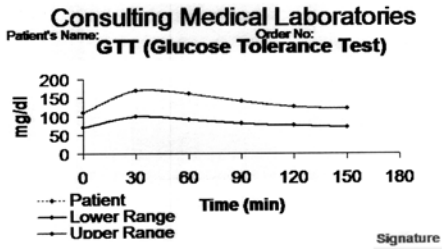
GTT (GLUCOSE TOLERANCE TEST) (See also Table 7)

GLUCOSE TOLERANCE TEST :

ORAL DOSE OF GRAMS GLUCOSE WERE GIVEN.

	SERUM	URINE
FASTING	*MMOL/L* *MG/DL*	
30 MINS. AFTER GLUCOSE :	*MMOL/L* *MG/DL*	
60 MINS AFTER GLUCOSE	*MMOL/L* *MG/DL*	
90 MINS AFTER GLUCOSE	*MMOL/L* *MG/DL*	
120 MINS AFTER GLUCOSE	*MMOL/L* *MG/DL*	
150 MINS AFTER GLUCOSE	*MMOL/L* *MG/DL*	

Patient	Lower Range	Upper Range
0	70	110
30	100	170
60	90	160
90	80	140
120	75	125
150	70	120



SEMEN ANLYSIS

HISTORY.

Marriage : Yrs. Smoking: Cigs/d for Yrs Children Abstinence: days.

Collection method : MASTURBATION AT LAB Medication:

SEMEN

Volume ml Liquefaction complete mins / hrs.

pH : Fructose : mg/dl (Normal > 120 mg/dl)

SPERM DENSITY mill / ml (Normal > 20 mill /ml)

MOTILITY :

Screen : Initial : Good (Normal : GOOD)

At 6 hrs Good (Normal : GOOD)

3-hrs. Motility : Darters : % Nonlinear fast : %

Slow : % Immotile : %

Total motile count : millions (Normal > 50)

Motility index : (Normal > 150)

(Viability : %)

MORPHOLOGY: (No of sperm counted :)

Normal : % (Reference > 50)

Pathological : %

Head % Round head %

Tail : % Cyto. droplet : %

CELL COUNTS :

RED CELLS : 10^9/ML (Normal < 0.100)

WHITE CELLS.: 10^9/ML (Normal < 0.250)

Lymphocytes :

Neutrophils (Normal for Neutrophils < 10)

COMMENT :

Normal values at the Consulting Medical Laboratories**

Volume= 1.5-8ml pH= >7 *Liquefaction*= < 1hour *Sperm density*= >20 millions/ml *Motility*= >40% (or 30%)*
Total motile count= 25-400 million sperm *Motility index*= >150 *Morphology*= >40% (30-75% mean 44%)*
Viability >30% (done when motility is below 30%) *Fructose* >120mg/dl red cell count= 0.100-0.350 X10^9/l
White cell count= 'total' 0.100-0.350 X10^9/l 'differential neutrophilic cell count' <30% usually <10%; lymphocytes >80%
Carnitine= >2.5mg/dl

*Limits depend on individual laboratory bias.

Consulting Medical Laboratories Established 1981

Central, Opposite Specialty Hospital, Amman Telefax 5671146 / 5671147 email: medlab@go.com.jo

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Basma I. Hasan, M.D. *Abdul Aziz H. Masoud, M.Sc.*

FNA TESTIS

MARRIAGE DURATION : yrs **NATIONALITY:**

AZOOSPERMIA : **NO. OF BROTHERS:**

FSH : miu/ml *married:* *: fertile:*

TESTICULAR VOLUME : **Lt** ml **Rt** ml

POSITION : **ORCHIDOPEXY:**

BIOPSY :

EPIDIDYMIS : **Lt** **Rt**

SKIN & TUNICA :

CASE # :

LEFT TESTIS : CELL YIELD = AMOUNT & TYPE OF CELLS

Lower zone :

Middle zone :

Upper zone :

RIGHT TESTIS: CELL YIELD* = AMOUNT & TYPE OF CELLS

Lower zone :

Middle zone :

Upper zone :

* MAXIMAL 9 : OPTIMAL > 4

CONCLUSION: Dajani Grade

Reported azoospermia; FSH= miu/ml. Biopsy:

Comment: Above form was designed during the 1990s to meet developments in the field of assisted reproduction and clarify terminology that had been often been confusing and of little practical use in cases of azoospermia. Cell yield portrays quantity irrespective of type of cells. A to D grading system of azoospermia depends on objective findings and quantification by making sperm counts in each testicular zone present, which proved very helpful at the time of sperm extraction for ICSI in Grade A2 cases. Captions of some sperm present are always illustrated on the report to remove any doubts. The A-D grading system of azoospermia is applicable to both cytological and histopathological testicular samplings.

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PAP SMEAR

SPECIMEN NO.:
SPECIMEN DATE:

Origin of Specimen:

Brief Clinical History & Diagnosis :

(Maturation Index = / /)

Case #

Cell Yield = Adequate / Moderate/ Unsatisfactory

Lactobacilli = / +++ Neutrophils = /+++ Red cells = /+++

Endocervical cells = / +++ Parabasal cells = /+++

Metaplasia Negative/Positive ASCUS Negative/Positive

There is / no evidence of neoplasia. In positive cases cytological description must be made of cellular and nuclear changes.

Conclusion:

NEGATIVE FOR NEOPLASIA.

(System of reporting used: Bethesda and CIN systems combined, with personal modifications).

***Comment*: Presently, no ideal classification of pre-invasive cervical neoplasia exists for pap smears. We have seen Papanicolaou classes I-V reduced to CIN I-III, then came the Bethesda 2-grade classification system LISL & HISL with ASCUS and AGUS as wastebasket entities in borderline cases. More recently, a proposal was made to divide ASCUS into ASC-US "atypical squamous cells of undetermined significance" and ASC-H "atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion"; similarly AGUS into AGC,NOS "AGUS, not otherwise specified" and "AGC, favor neoplasia". Shelving of "Reactive changes" as a category is suggested. Recent recommendations of guidelines for managing women with abnormal and atypical smears are being debated (1). Interpretation of mild or borderline changes in pap smears greatly depends on the quality of the reader as well other factors, for example, use of IUD. Liquid-based cytology and HPV testing may add an advantage but is not always practicable. Use of common language in practice and teamwork usually lead to prompt and sound conclusions. And with changing trends in terminology, we have to make the best out of current concensus in our daily practice. *Reference* 1. Wright TC Jr, Cox JT, Massd LS, Twiggs LB, Wikinson EJ. 2001 Concensus Guidelines for the management of women with cervical cytological abnormalities. *JAMA* 2002; 287: 2120-2129.**

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BONE MARROW REPORT

Clinical History :

PERIPHERAL BLOOD:

Hb : g/dl RBC : 10^12/1 PCV : 1/1 MCV : fl MCHC: g/dl
MCH : pg WBC : 10^9/1 Platelets. : 10^9/1 Reticulocytes : %

DIFFERENTIAL Neutrophils: % Lymphocytes: % Monocytes: %
Eosinophils % Basophils %

Blood Film:

BONE MARROW : IRON IN MARROW STORES : / ++++.
MARROW FRAGMENTS: MANY / FEW CELLULARITY= ADEQUATE/MODERATE/LOW/INADEQUATE

Differential Marrow Cell Count:

ERYTHROBLASTS : % BLASTS : % LYMPHOCYTES : %
PROMYELOCYTES : % MYELOCYTES : % METAMYELOCYTES: %
NEUTROPHILS : % EOSINOPHILS : % PLASMACYTES %

MYELOID:ERYTHROID RATIO = (NORMAL 2-10)

ERYTHROPOIESIS: NORMOBLASTIC/MEGALOBLASTIC : H-J BODIES, MEGALOBLASTS
HYPERPLASTIC /NORMAL
BASOPHILIC STIPPLING , INCREASED MITOSES
MYELOPOIESIS: HYPERPLASTIC / NORMAL/ MATURATION LAG
CYTOPLASTIC GRANULES
NO PROLIFERATION OF CELLS: BLASTS/ PLASMA CELLS/ TUMOR CELLS/ OTHER
OTHER FINDINGS
BONE BIOPSY: RETICULIN FRAMEWORK: GRADE (OUT OF IV) =
MARROW CELLULARITY: %
NO INFILTRATION BY GRANULOMA OR TUMOR IS EVIDENT.
OTHER FINDINGS :
CONCLUSION :

QUALITATIVE MARROW CELL COUNTS

Normal differential	%	Normal differential	%	Normal differential	%
Hematocytoblasts	0.1-1	Myeloblast	0.1-3.5	Promyelocyte	0.5-5
Myelocyte	5-20	Metamyelocytes (Young forms)	10-30	Stab (young) forms Neutrophils	10-30
Neutrophils segmented	7-25	Eosinophilic myelocyte	0.1-3	Adult eosinophil	0.2-3
Basophilic myelocyte	0-0.5	Adult basophil	0-0.5	Lymphocyte	5-20
Lymphoblast	Nil	Plasma cell	0.1-3.5	Myeloma cell	Nil
Monocyte	0-0.2	Megakaryocyte	0.1-0.5	Reticulum cell	0.1-2
Pro-erythroblast	0.5-5	Basophilic normoblast	2-20	Polychromatic normoblast	2-20
Orthochromic (pyknotic) normoblast	2-10	Megaloblast	Nil		

GASTRIC BIOPSY

Specimen No.:
Specimen Date:

Origin of Tissue:

Brief Clinical History & Diagnosis:

Gross Examination

No. of soft tissue fragments: ( )

Microscopic Examination

No. of fragments: Gastric body ( ) Antral ( ) Other ( )
Chronic inflammatory cell infiltration of lamina propria: Normal

Exocytosis:
Glandular atrophy:
H. pylori:
Metaplasia
Neoplasia

Antral versus Gastric body involvement:

Comment:

Conclusion

Fetus + Placenta

EXTERNAL EXAMINATION :

PLACENTA:

Wright : g. Dimension: cm
Membranes: Cord :
Form :
Cutsurface :

FATUS

Weight: Kg . Length : cm
Head circumference: cm
Foot Length : cm

FACIES :
SKIN :
EXTREMITIES :
EXTERNAL ORIFICES:
ANOMALIES :

INTERNAL EXAMINATION:

FETUS:

CARDIOVASCULAR SYSTEM:

Pericardium:

Heart : Weight : Great vessels:

Ductus : Chamber and valves :

RESIPRATORY SYSTEM:

Pleura : Lungs: Left: Right:
Description:

GASTROINTESTINAL SYSTEM:

Liver: Pancreas: Mouth and palate:
Esophagus and stomach:
Intestines :
Gall bladder and bile ducts:

HEMOPLETIC SYSTEM:

Spleen: Thymus:

ENDOCRINE SYSTEM:

Thyroid: Adrenals: Left: Right:

GENITOURINARY SYSTEM:

Kidneys : Left: Right:
Urinary tract :
Genitalia :

SKELETAL SYSTEM:

Axial bones :
Peripheral bones:
Digits :

CENTRAL NERVOUS SYSTEM:

Brain :
Brain surface:
Brain stem :
Base of skull :

HISTOLOGY:

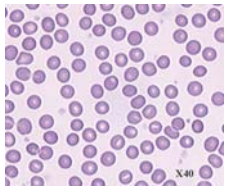
PLACENTA:
CORD AND MEMBRANES:
CYTOLEDONS:

FETUS:

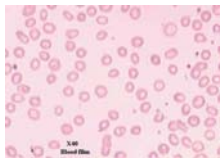
COCLUSION:

CAPTIONS

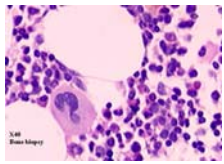
Caption 1. A case of iron deficiency anemia due to menometrorrhagia.



(a) X40 *Blood film*: Normal red cells in a normal control,



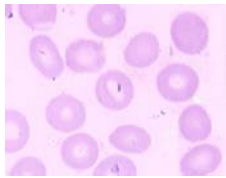
(b) X40 *Blood film*: Hypochromia and microcytic red cells.



(c) X40 *BM biopsy:* An actively proliferating bone marrow in iron deficiency with erythroid hyperplasia.

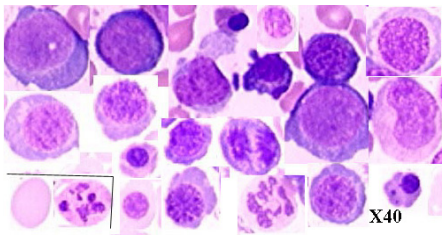
Note on the case: Female, 36 yrs, menometrorrhagia,; Hb 6.3g/dl MCHC 27g/dl MCH 19pg MCV60fl Retic Ct= 0.9% WCC 4.500×10^9/l Pl Ct 150.000×10^9/l *Blood film:* Microcytic, hypochromic red cells. *BM smear:* normoblastic erythroid hyperplasia; M/E ratio= 0.8 *BM bx:* Reticulin framework= I/IV Cellularity= 60% with preserved architectural pattern. Diagnosis: Iron deficiency anemia. Serum ferritine = 5mg/ml.

Caption 2. Dichromatic red cells after blood transfusion.



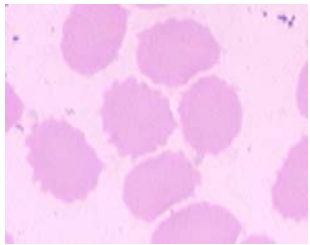
X100 *Blood film*: Hypochromic red cells coincidental with normochromic (transfused) red cells.

Caption 3. A case of megaloblastic anemia due to gastric atrophy.



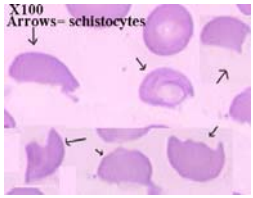
X40 *Plate of Blood film & BM smear*: Peripheral blood left bottom corner with an oval macrocyte and a hypersegmented neutrophil. BM smear cells show early, intermediate and late megaloblasts, mitotic figure, blue polychromasia, Howell-Jolly bodies, giant metamyelocyte and a hypersegmented neutrophil.

Caption 4. Chronic renal failure.



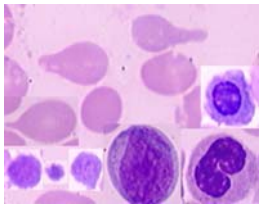
X100 *Blood film*: Crenated red cells in chronic renal failure; normochromic anemia.

Caption 5. Red cells in a case of DIC (Disseminated Intravascular Coagulation).



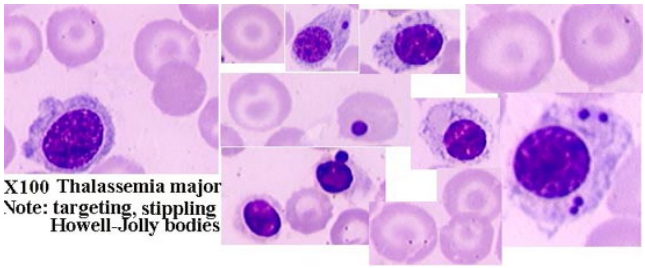
X100 *Blood film*: Schistocytes (fragmented red cells) are typically seen in DIC.

Caption 6. A case of myelofibrosis.



X100 *Blood film*: Tear drop cells or drumsticks are seen in myelofibrosis. In this case, there is typically a leuko-erythroblastic blood picture with immature red and white cells present. Moreover, two giant megakaryocytes are placed in the left lower corner on each side of a nongiant platelet.

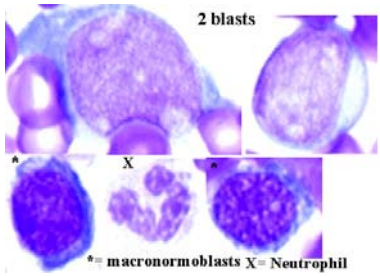
Caption 7. A case of thalassemia major.



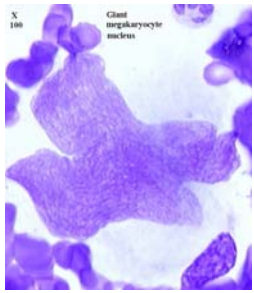
X100 *Blood film*: Target cells, erythroblasts, basophilic stippling and Howell-Jolly bodies in red cells.

Caption 8. MDS (Myelodysplastic Syndrome): RAEB with ring sideroblasts.

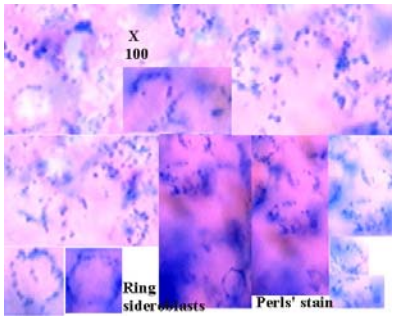
(a) X100 *BM smear*: Blast cells, macronormoblasts and hypogranular neutrophil.



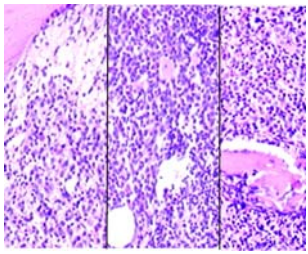
(b) X100 *BM smear*: Giant megakaryocyte with abnormally shaped and large nucleus.



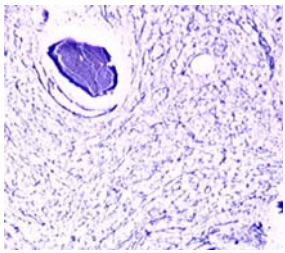
(c) X100 *BM smear: Perls' stain*: Ringed sideroblasts easily seen.



(d) X10 *BM biopsy*: Hyperplastic bone marrow with disorganized mixing of the bone marrow cells and an increase in the blast cell component: 3 different BM areas.

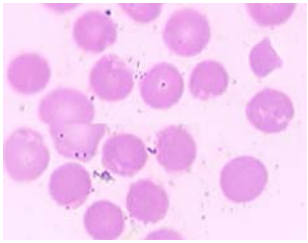


(e) X10 *BM biopsy: Reticulin stain*: Grade III/IV diffuse deposition of fine reticulin fiber laying.



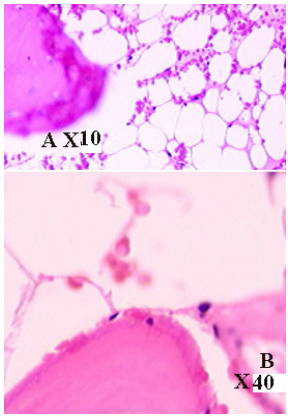
Note on the case: Male, 36 year old, Hb 9.3g/dl, WCC 2.300×10^9/l, Platelet Ct 44.000×10^9/l, Reticulocyte count 0.8%. *Blood film*: Pancytopenia. Red cells normochromic with schistocytes and teardrops. No blast cells seen. CD34 positive cells were only 1% of white cells in peripheral blood. *BM smear*: Iron stain: ring sideroblasts easily seen. Few BM fragments, moderate cellularity with few megakaryocytes. Macronormoblastic erythropoiesis with granule depletion in myeloid series with cytoplasmic vacuolation and blast cells accounting for 12% of bone marrow cells. *BM biopsy*: Reticulin framework Grade III/IV with diffuse fine fiber deposition. Cellularity 100% with panmyeloid hyperplasia in a disorganized fashion and evident rests of immature large cells present. *Conclusion*: MDS: RAEB (Refractory Anemia with Excess Blasts) and ringed sideroblasts; peripheral pancytopenia.

Caption 9. A case of hereditary spherocytosis.



X100 *Blood film*: Spherocytes in peripheral blood.

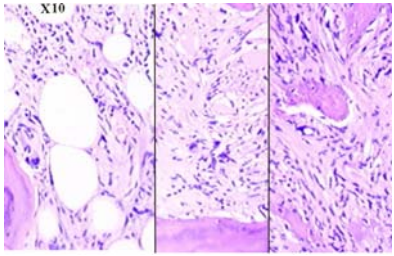
Caption 10. A case of aplastic anemia.



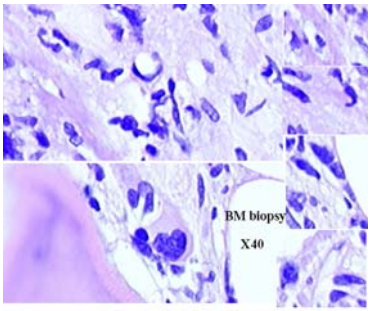
BM biopsy intermediate(X10) and high(X40) powers: Predominant fat cells with suppression of marrow cells.

Note on the case: Female, 13 year old, Hb 13.7g/dl, WCC 3.700×10^9/l, Platelet Ct 46.000×10^9/l, Reticulocyte count 0.7%. *Blood film*: Mild leukopenia and moderately marked thrombocytopenia. No blast cells seen. Red cells unremarkable. *BM smear*: Dry tap. *BM biopsy*: Reticulin framework Grade I/IV not increased. Cellularity grossly deficient and near 0%. *Conclusion:* Bone marrow aplasia.

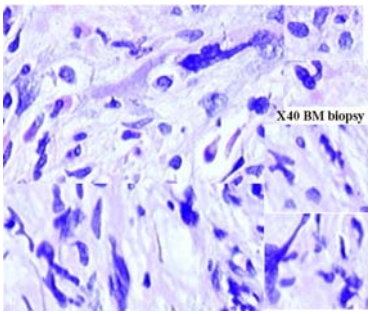
Caption 11. Myeloproliferative disorder – myelofibrosis.



(a) X10 *BM biopsy*: 3 areas of varying cellularity in same bone core.

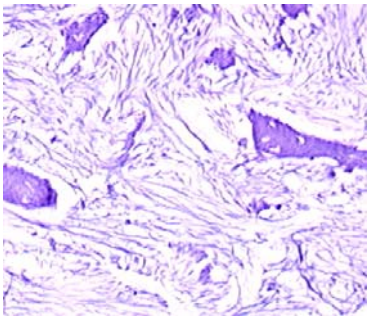


(b) X40 *BM biopsy:* BM infiltrated by edematous fibrous tissue. Note: abnormal megakaryocytes.



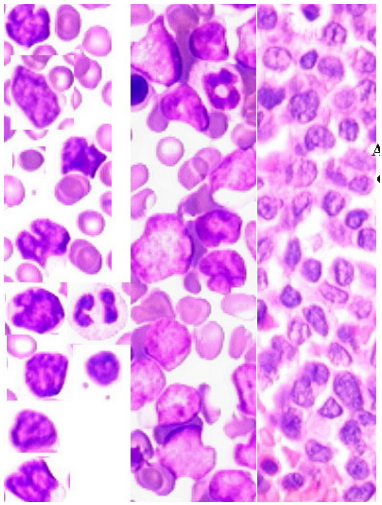
(c) X40 *BM biopsy:* BM infiltrated by irregular cells in edematous fibrous tissue

(d) X10 *BM biopsy (Reticulin stain):* Grade IV out of IV fibrosis in BM.



Note on the case: Male, 44yrs, diagnosed as a case of leukemia, Hb 10.9g/dl, PCV 0.33 l/l MCV 79fl, MCHC 33g/dl, MCH 26.1pg, WCC 2.400×10^{9}/l, Platelet Ct 126.000×10^{9}/l *Blood film:* Blast cells 7% with tear-drop red cells; platelets slightly suppressed; 1normoblast/ 100WBCs. *BM smear:* Highly diluted with blood. *BM bx:* Reticulin = IV/IV diffuse severe fibrosis; cellularity difficult to assess but appears severely suppressed; megakaryocytes show atypical forms. No infiltration by granuloma or tumor. Chromosomal analysis of bone marrow showed a 46, XY, t(12,14)(q15q24), del(13)(q13) karyotype, indicating a translocation between the long arm of chromosome 12 and long arm of chromosome 14 (15,24) with deletion in the long arm of chromosome 13 at 13q. *Diagnosis:* Myeloproliferative disorder – myelofibrosis.

Caption 12. Acute lymphocytic leukemia.

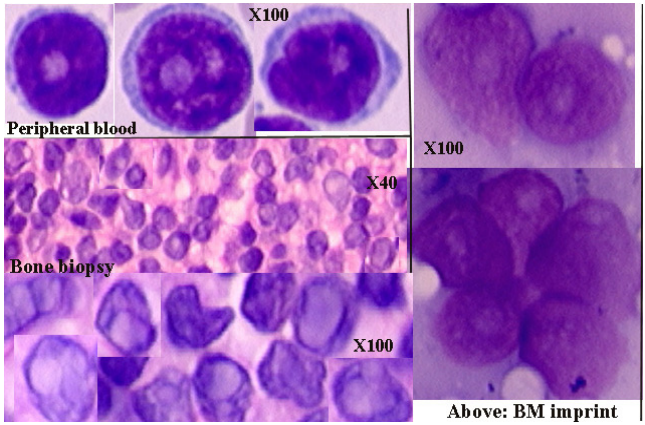


X40 *Bl film, bone marrow smear & bx:* lymphoblasts with indented nuclei.

Note on the case: Male, 74yrs, Pancytopenia and splenomegaly. Hb 9.1g/dl PCV 0.27l/l MCHC 33.7 g/dl MCH 28.7pg MCV 85fl WCC 3.400×10^9/l Platelet Ct 44.000×10^9/l Retic Ct 0.7%

Blood film: 20% of white cells are blast cells. *BM smear:* Many marrow fragments, cellularity adequate, megakaryocytes hard to find; 90% of marrow cells are blast cells with a positive reaction to CD19 97% CD10 90% HLA-DR 95% *Diagnosis:* Acute lymphocytic leukemia, B-cell type. Morphology fits with ALL 2.

Caption 13. Acute prolymphocytic leukemia.

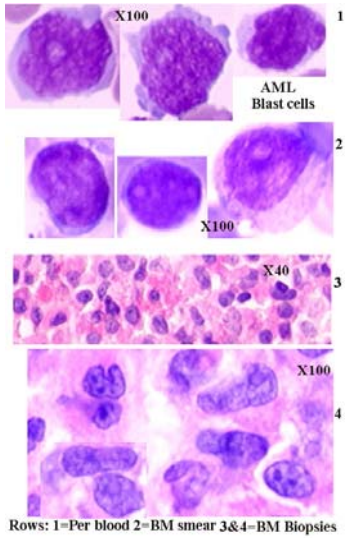


X100 *Bl, BM imprint & bx:* Prolymphocyte leukemic cells.

Note on the case: Male, 75yrs, High WCC. Hb 8.1g/dl, PCV 0.24l/l, MCHC 33.7g/dl, MCH 27.7pg, MCV 82fl, WCC 131.600×10^9/l Plat Ct 54.000×10^9/l Retic Ct 0.5%. *Blood film:* 86% of white cells prolymphocytes with a fair amount of cytoplasm and single, large nucleoli in presence of chromatin clumping. Red cells normochromic with moderate thrombocytopenia. *BM imprint* shows predominant prolymphocytes and a relatively small number of myeloid and erythroid precursors, while megakaryocytes are difficult to find. *BM bx:* Reticulin III/IV with diffuse fine fiber laying and coarse condensations. Cellularity full with lymphocytic cell infiltrate sweeping over 80% of the bone marrow areas examined; infiltrating lymphocytes are of small to intermediate size and show single prominent nucleoli,

while precursor bone marrow cells, including megakaryocytes, account for a minor proportion of the bone marrow cells. Immunophenotyping showed monoclonal kappa light chain expression on the surface of infiltrating cells. *Diagnosis:* Acute prolymphocytic leukemia.

Caption 14. Acute myeloid leukemia.



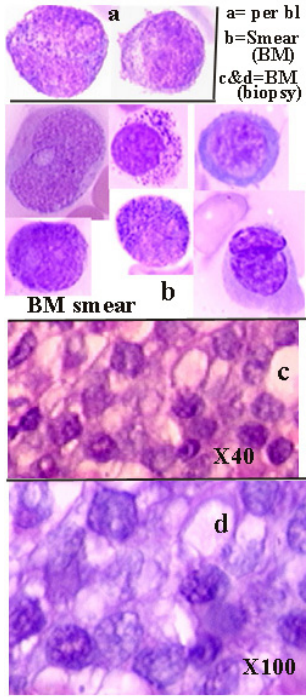
Rows: 1=Per blood 2=BM smear 3&4=BM Biopsies

X40 & X100 *Bl BM & Bx:* Appearance of myeloblasts.

Note on the case: Male, 80 yrs, Pancytopenia, Hb 9.8g/dl PCV= 0.29l/l MCHC 33.7g/dl MCH 33.5pg MCV 99fl WCC 4.800×10^9/l Platelet Ct 84.000×10^9/l Retic Ct 0.8% *Bl film:* Blast cells 18% of white cells with occasional erythroblast seen. *BM smear:* Many BM fragments with poor cellularity and hypocellular smears so that only occasional blast cells are picked up. *BM bx:* Reticulin framework III/IV with diffuse fine fiber laying. Cellularity near 100% with a pleomorphic cell infiltrate of large abnormal cells exceeding 30% of the bone marrow cells, while distribution of the normal BM cell elements is lost. Immunophenotyping showed the following reactions: CD34+ 98%, CD33+ 98%, CD13+97%, HLA-DR+ 96% with low expression of CD5 in 50% of

blast cells, indicating a myeloid lineage of blast cells. Chromosomal analysis of bone marrow showed a 46,XY karyotype with no structural or numerical abnormality. *Diagnosis:* Acute myeloid leukemia.

Caption 15. Acute promyelocytic leukemia.

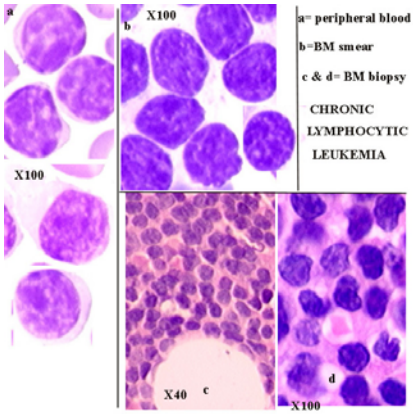


X40 & X100 *Bl BM smear & Bx:* Acute promyelocytic leukemia.

Note on the case: Male, 38yrs, Pancytopenia.Hb 6.2g/dl PCV 0.19l/l MCHC 32.6g/dl MCH 34.4g/dl MCV 105fl WCC 0.700×10^9/l Plat Ct 49.000×10^9/l Retic Ct 2.1% *Blood film:* Pancytopenia with occasional abnormal promyelocytes in peripheral blood. *BM smear:* Scanty fragments with low cellularity and megakaryocytes hard to find. Differential marrow cell count not possible due to dilution with blood but an evident increase of hypergranular promyelocytes with abnormal nuclear appearance, multiple large nucleoli but no Auer bodies, were detected. Erythropoiesis was suppressed and megaloblastic while myelopoiesis was suppressed apart from increased number of hypergranular promyelocytes. *BM bx:* Reticulin framework III/IV with diffuse increase of

fine fibers. Cellularity near 100% with obliteration of BM spaces and diffuse infiltration by immature cells which sometimes show nuclear indentation and in some places showing patches of infiltrating immature cells. Megakaryocytes are noted in reduced number and other BM elements appear suppressed. *Diagnosis:* Acute promyelocytic leukemia.

Caption 16. Chronic lymphocytic leukemia.

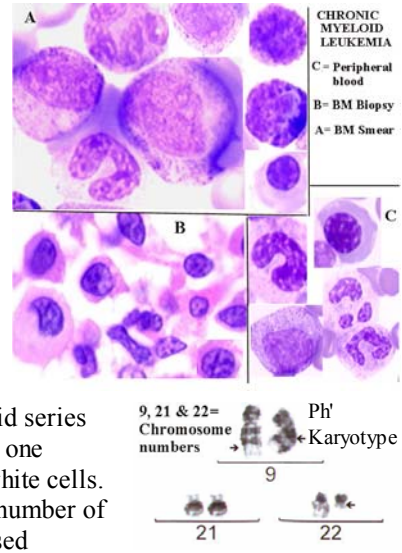


X40 & X100 *Bl BM smear & Bx:* See plate.

Note on the case: Male, 48yrs, Lymphadenopathy, splenomegaly. Hb 11.9g/dl PCV 0.36l/l MCHC 33 g/dl MCH 28.4pg MCV 86fl WCC 186.000×10^9/l Plat Ct 97.000×10^9/l Retic Ct 0.5%. *Blood film:* There is proliferation of small mature lymphocytic cells. *BM smear:* Deficient fragments, markedly diluted with blood and megakaryocytes hard to find. >95% of marrow cells are lymphocytic cells with only a small number of erythroid or myeloid cells. *BM bx:* Reticulin framework III/IV with diffuse fine fiber laying and coarse foci. Cellularity 80% with diffuse infiltration by small lymphocytic cells sparing only a small area in this BM biopsy. Immunophenotype reactions of infiltrating cells: Positive for CD19, CD20 & HLA-DR expressing

surface IgM and CD5, consistent with CLL. *Diagnosis:* Lymphoproliferative disorder, chronic lymphocytic leukemia and diffuse infiltration of bone marrow.

Caption 17. Chronic myeloid leukemia, Ph' positive.

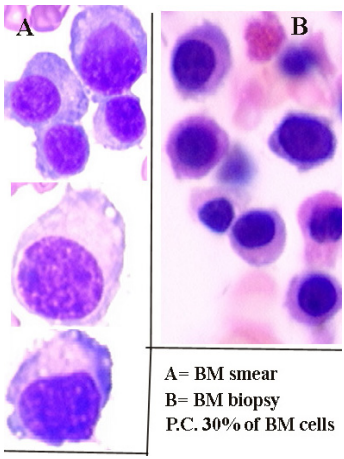


X100 *Bl BM smear & Bx:* See plate.

Note on the case: Female, 32 yrs, Tiredness, splenomegaly. Hb 11.5g/dl PCV 0.35l/l MCHC 32.8g/dl MCH 27.9pg MCV 85fl WCC 92.000×10^9/l Plat Ct 538.000×10^9/l Retic Ct 0.8%. *Blood film:* Proliferation of myeloid series with no blast cells and one erythroblast per 100 white cells. *BM smear:* Adequate number of fragments with increased cellularity and a moderate number of megakaryocytes, blast cells 1%. M/E ratio 28 (normal 2-10). Erythropoiesis normoblastic with myeloid hyperplasia and proliferation of all the granulocytic series which in turn display hypogranulation but no blast cell proliferation. *Bone bx:* Reticulin framework III/IV with diffuse fine fiber deposition. Cellularity practically 100% with only an occasional residual fat cell present. There is diffuse myeloid hyperplasia with a moderate number of megakaryocytes present. No blast cell

proliferation is evident. Chromosomal analysis on routine G-banding showed a 46,XX t(9,22)(q11,q22) karyotype with all metaphases exhibiting a translocation between the long arm of chromosome 9 and chromosome 22, indicating a positive result for Philadelphia chromosome. By PCR, the bone marrow and peripheral blood showed presence of bcr/abl fusion gene. *Diagnosis:* Myeloproliferative disorder, chronic myeloid leukemia, Ph' chromosome positive.

Caption 18. Multiple myeloma.

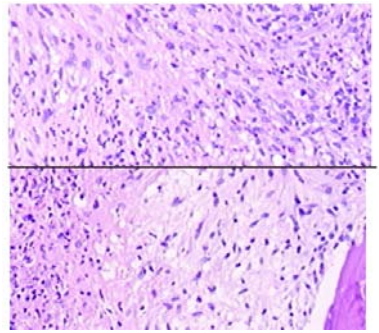


X100 *BM smear & Bx:* Proliferating plasma cells.

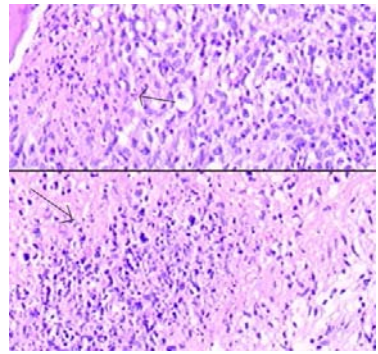
Note on the case: Female, 60yrs, backache, lytic lesion in lumbar spine. Hb 10.1g/dl PCV 0.30l/l MCHC 33.6g/dl MCH 31.3pg MCV 93fl WCC 4.600×10^9/l Plat Ct 170.000×10^9/l Retic Ct 1.2% ESR 62mm 1st hour *Blood film:* noninformative. *BM smear:* Iron in marrow stores= ++/+++ moderate increase. Adequate fragments and cellularity with a good number of megakaryocytes; plasma cells 30% of marrow cells with aggregated and double nucleated forms; normoblastic erythropoiesis and unremarkable myelopoiesis. *BM bx:* Reticulin II/IV slightly increased, marrow cellularity 35% overall with diffuse increase of plasma cells and patchy increase of cellularity in places. *Protein EP:*

Total protein 6.8g/dl. A monoclonal band in gamma region (kappa monoclonal band 2.6g/l), accompanied by decreased IgA, IgM and lambda and normal IgG levels on IEP. Urine immunoelectrophoresesis showed a kappa band but absent lambda, IgG, IgA and IgM. *Diagnosis:* Multiple myeloma.

Caption 19. Hodgkin lymphoma infiltrating BM.

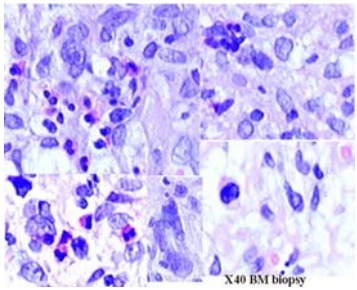


(a) X10 *BM bx:* Hodgkin infiltrate of varying density.



(b) X10 *BM bx:* Hodgkin infiltrates with focal necroses (arrows).

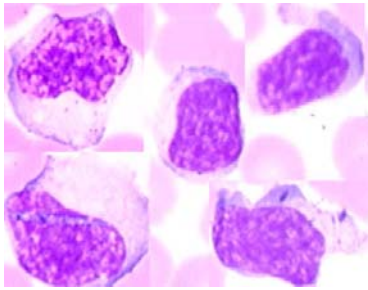
(c) X40 *BM biopsy:* infiltration by Hodgkin tissue.



Note on the case: Female, 6yrs, Cervical lymph node diagnosed Hodgkin lymphoma, nodular sclerosis type; BM for staging. Hb 15.2g/dl PCV 0.46l/l MCHC 33g/dl MCH 24.4pg MCV 74fl WCC 14.500×10^9/l Plat Ct 377.000×10^9/l Retic Ct 0.7% *Blood film:* unremarkable. *BM smear:* Iron in marrow stores moderately increased. Many fragments with good cellularity and number of megakaryocytes. M/E ratio 2.4 with normoblastic erythropoiesis and unremarkable myelopoiesis; no proliferation of blasts or tumor cells evident. *BM bx:* Reticulin framework IV/IV with diffuse fibrosis and the bone marrow in this bone core totally replaced by a pleomorphic cell infiltrate including histiocytes, a relatively small number of lymphocytes, neutrophils and eosinophils in varying proportions as well as multinucleated cells in places, accompanied by microfoci of tumor necrosis. Occasional Hodgkin like cells seen. No granuloma noted. The BM clot showed prominent eosinophilic cell infiltrate. *Diagnosis:* BM infiltration by Hodgkin lymphoma.

Caption 20. Infectious mononucleosis cells.

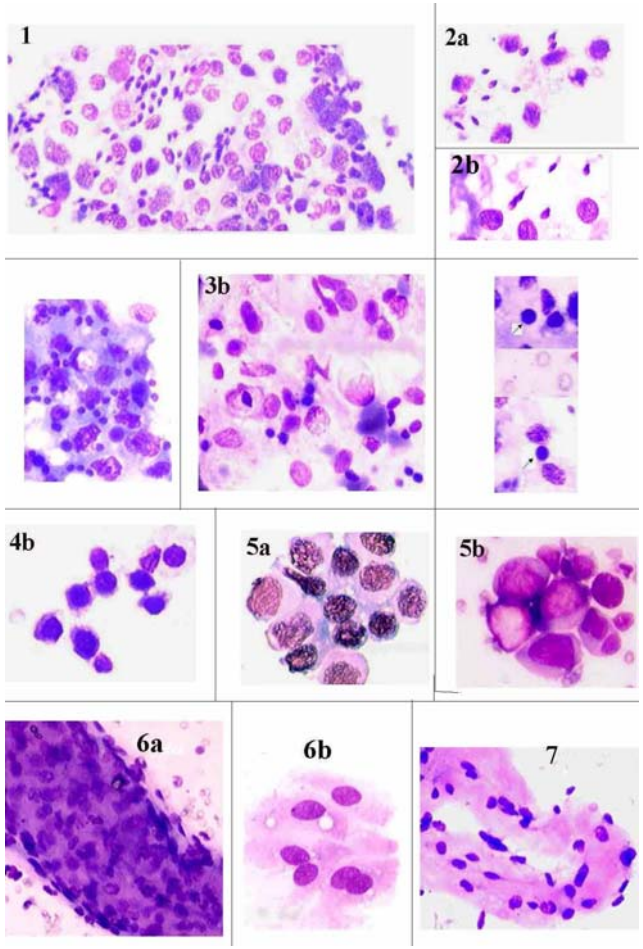
X100 *Per bl:* Infectious mononucleosis cells.



Caption 21. Seminiferous tubular cell elements.

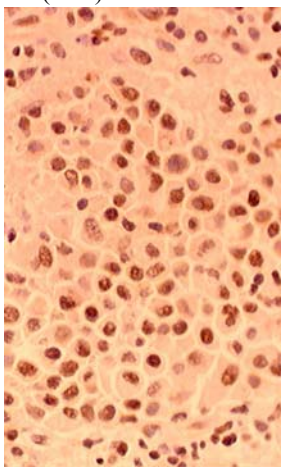
1 A good number of sperm: Grade A1: obstructive azoospermia.
2a Reduced number of sperm: Grade A2: nonobstructive azoospermia.
2b Scanty sperm: Grade A2: nonobstructive azoospermia.
3a Transitional forms: Grade B*.
3b Phase III spermatids: Grade B*.
4a Phase II spermatids.
4b Phase I spermatids.
5a Spermatocytes.
5b Spermatogonia.
6a Seminerous tubule in 2-dimension. Grade C.
6b Sheet of Sertoli cells. Grade C.
7. Sclerotic seminiferous tubule: Grade D.

* Maturation arrest (Grade B) occurs usually at phase III spermatid stage and occasionally at the transitional phase.

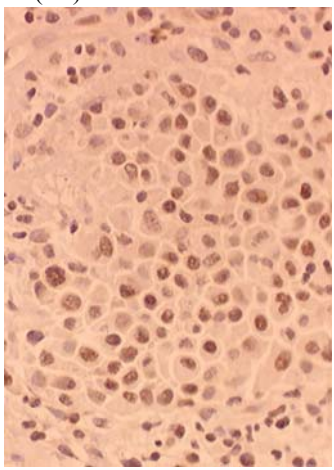

1
2a
2b
3b
4b
5a
5b
6a
6b
7

Caption 22. X40 ER PR & HER-2/neu in breast cancer.

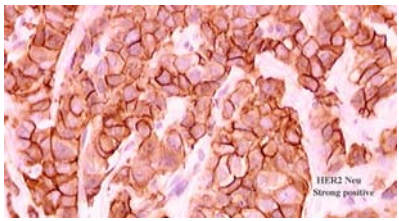
(a) ER Strong Positive. (+++).



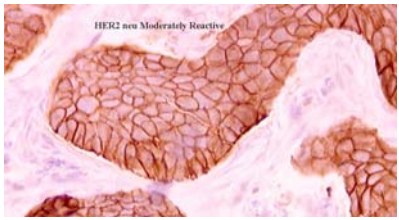
(b) ER Moderately reactive (++).



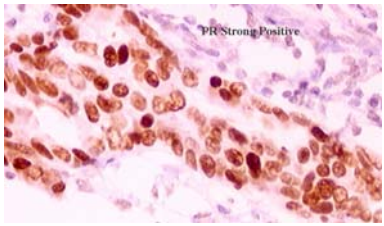
(c) HER2 neu Strong positive.



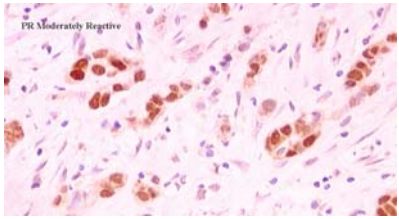
(d) HER2 neu Moderately reactive



(e) PR Strong positive (+++).

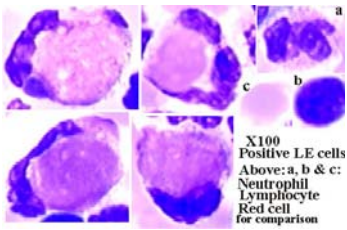


(f) PR Moderately reactive (++).

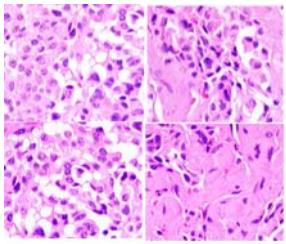


Caption 23. LE cells.

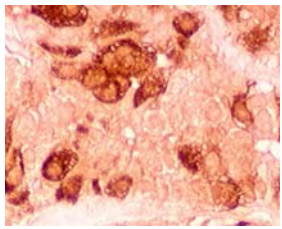
X100 *Per bl* LE cells: of historical interest; replaced by ANA and anti-DNA.



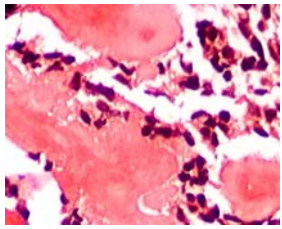
Caption 24. Medullary carcinoma of thyroid gland.



(a) X40 Medullary carcinoma with amyloid deposition in stroma.



(b) X40 Calcitonin positive cells.

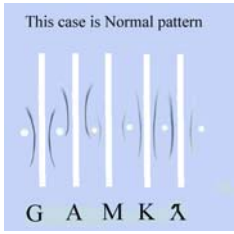


(c) X40 Congo red stain positive stroma.

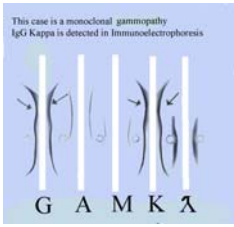
Note on the case: Male, 70yrs, presented with a neck mass, suspected thyroid tumor. Histology showed infiltrating acini, trabeculae and sheets of round to elongate, moderately sized cells with mildly pleomorphic, vesicular and finely stippled nuclei tending to locate eccentrically, accompanied by moderate amount of eosinophilic to amphophilic, somewhat vacuolated cytoplasm. Irregular deposition of pink hyaline material in stroma stained orange to Congo red with green birefringence under the polarized light, while many of the tumor cells stained positively for calcitonin. *Diagnosis:* Medullary carcinoma of thyroid gland.

Caption 25. Immunofixation tracks in two cases of gammopathy with normal control.

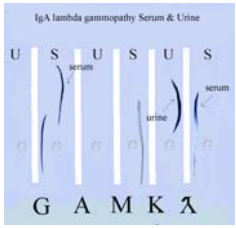
(a) Normal pattern.



(b) Monoclonal gammopathy. see plate.

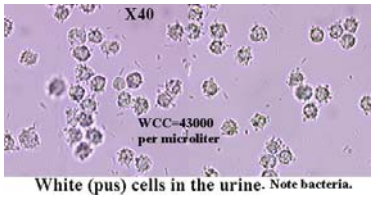


(c) IgA lambda gammopathy serum and only lambda in urine.



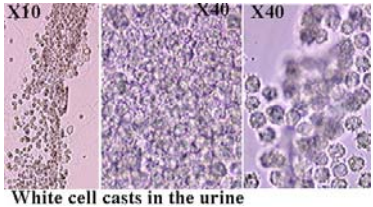
Caption 26. Urine microscopy: some microscopic findings.

(a) White cells in urine-pyuria. White cell count 43000 per ul X40.



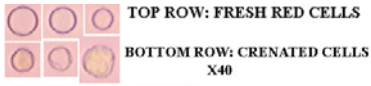
White (pus) cells in the urine. Note bacteria.

(b) White cell casts in urine X10 & 40.

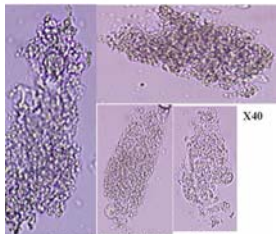


White cell casts in the urine

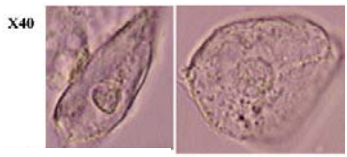
(c) Microscopic hematuria. Fresh and crenated red cells X40.



MICROSCOPIC HEMATURIA

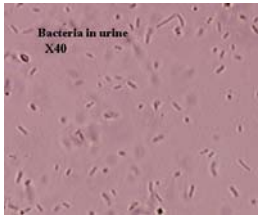


(d) Granular casts in urine X40.

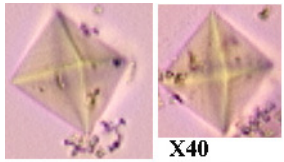


(e) Epithelial cells in urine X40

(f) Bacteria in urine X40.

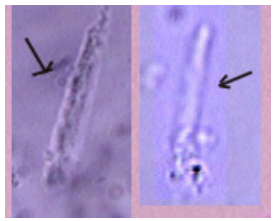


(g) Urine Calcium oxalate crystals X40.

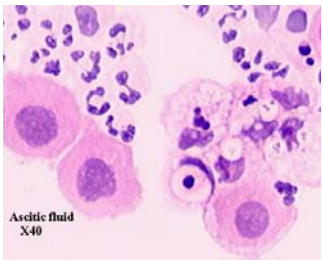


(h) Amorphous material in urine X40.

(i) Hyaline cast in urine X100.

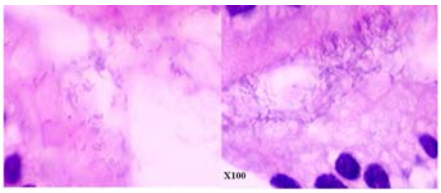


Caption 27. Peritonitis.



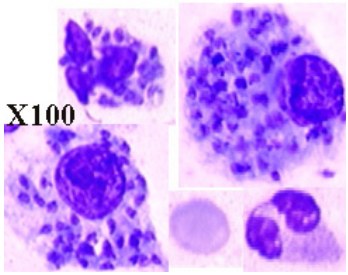
X40 Ascitic fluid in peritonitis. Note predominance of neutrophils.

Caption 28. *H.pylori* in gastric mucosa.



X100 *Helicobacter pylori* appearing as short delicate and slightly bent eosinophilic rods in mucus overlying gastric mucosa.

Caption 29. X100 Leishman bodies in macrophages from a skin lesion.



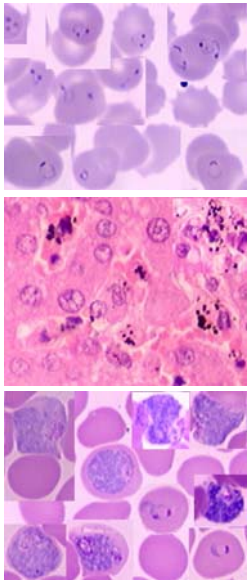
X100 Leishman bodies in smear.

Caption 30. Peripheral blood: *Plasmodium vivax* and *Plasmodium falciparum*.

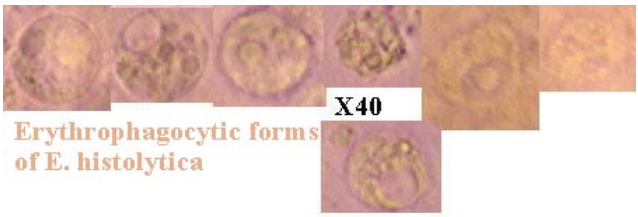
(a) X100 *Plasmodium falciparum* rings in red cells.

(b) X40 *Pl falciparum* black dark brown pigment in Kupffer liver cells.

(c) X100 *Pl vivax* multistage infection of red cells by rings and trophozoites of irregular shape and enlarged red cells.

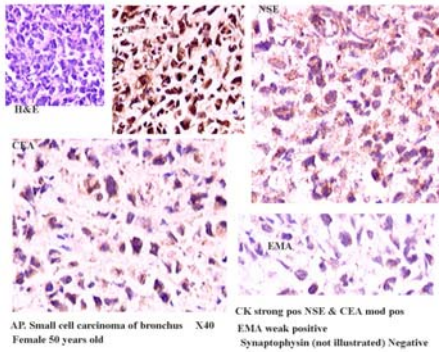


Caption 31. *E. histolytica* in stool sample.



X40 E*ntamoeba histolytica* in a fresh stool sample from a 26 year old man with diarrhea.

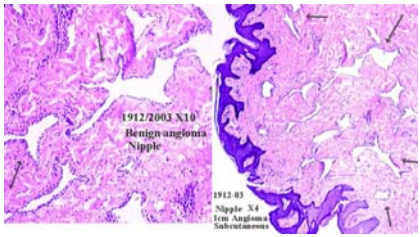
Caption 32. Small cell carcinoma of lung.



X40 Small cell carcinoma of left lung.

Note on the case: Male, 48yrs, Cough and left hilar mass on chest X-ray. Tru-cut biopsy showed a cellular small dark cell tumor forming ribbons, trabeculae and masses with frequent mitoses and a delicate stroma. Focal necrosis seen. Vascular permeation detected. Histology and immune stains confirmed the diagnosis of infiltrating small cell carcinoma of left lung.

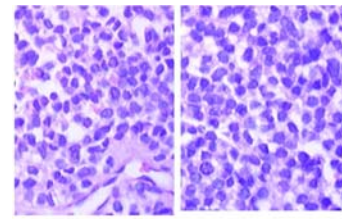
Caption 33. Nipple angioma.

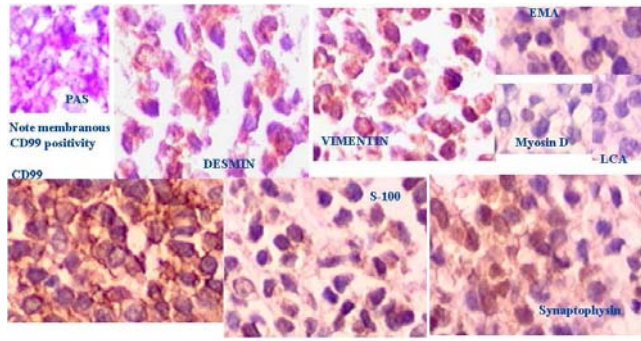


X4 & X10 Angioma of nipple; benign. The lesion is composed of irregular thin walled vessels in the nipple with a poorly defined border, supported by fibrous stroma.

Caption 34 Ewing's sarcoma, PNET.

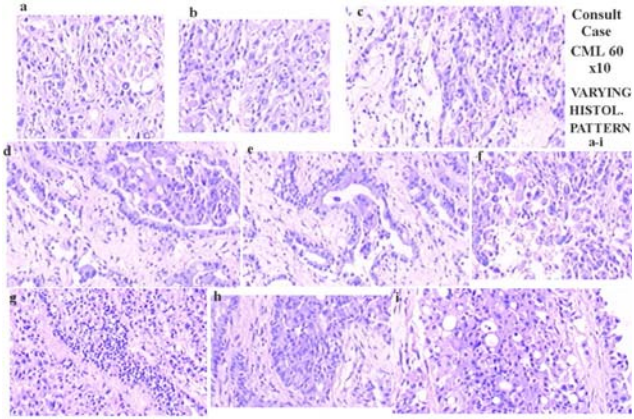
(a) X40 HE Small cell tumor. Note rosetting.



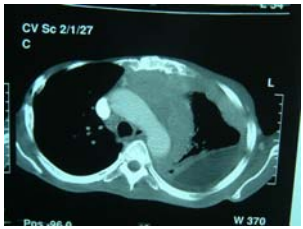


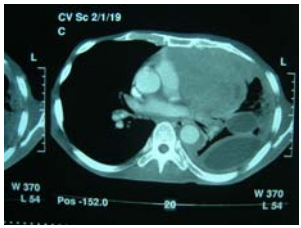
(b) X40 Strong positive reactions of tumor cells to PAS and CD99. Variable positive reactions of tumor cells to S100, Synaptophysin, Vimentin and Desmin. Weak reaction to EMA. Negative reactions of tumor cells to LCA and Myosin.

Caption 35. Telepathology Case 1: Pleural tumor. See text.



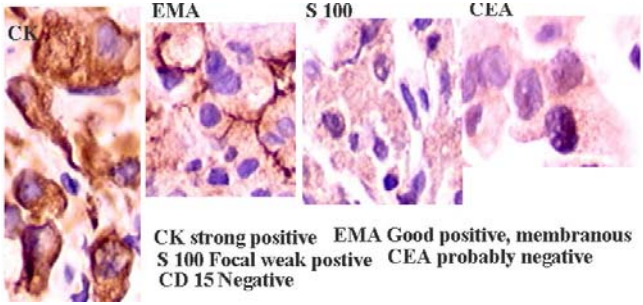
(a) X10 Varying histology patterns.





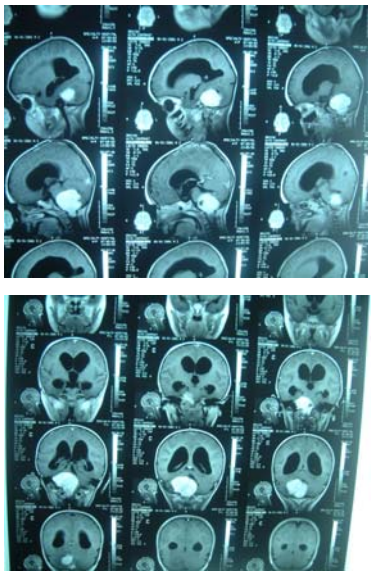
(b) CT-scans of chest.

(c)X40 Immunostains.

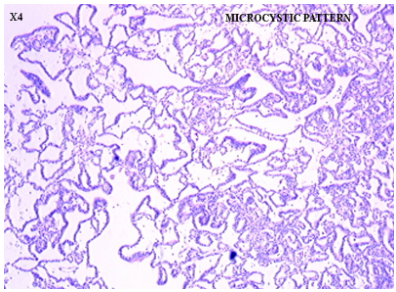


Caption 36. Telepathology Case 2. Brain tumor. See text.

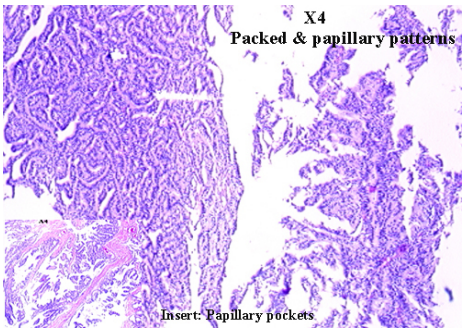
(a&b) Brain MRI images.



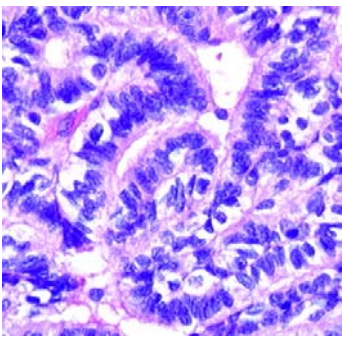
(c) X4 Microcystic pattern of tumor.



(d) X4 Diffuse and papillary patterns.

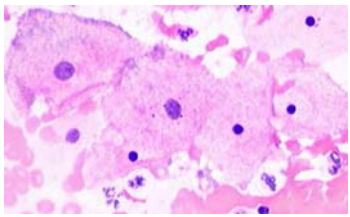


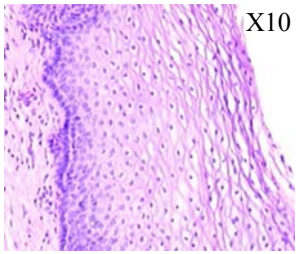
(e) X40 Packed pattern.



Caption 37. Cervical cytology vs. histology.

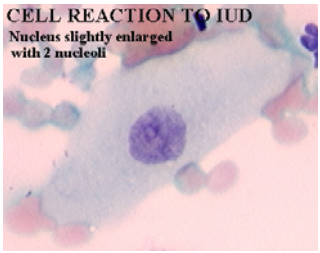
40 Normal intermediate & superficial squamous cells.



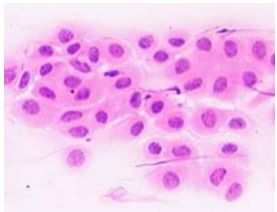


X10

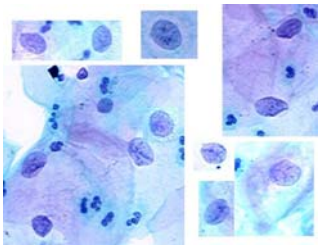
X40 Cell Reaction to IUD.

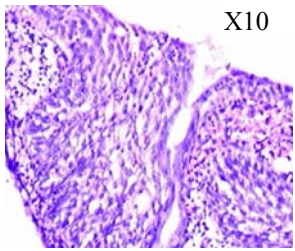


X40 Parabasal cells.



X40 ASCUS 34yrs No IUD Asymptomatic P3 Normal gyne exam

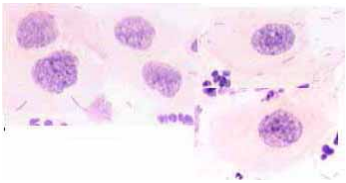




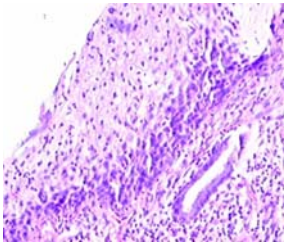
X10

CIN I.

X40

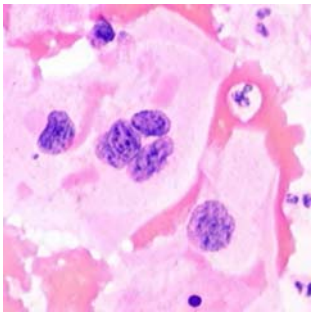


X10

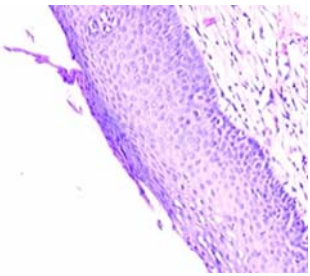


CIN II.

X40

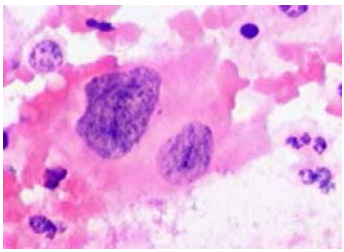


X10

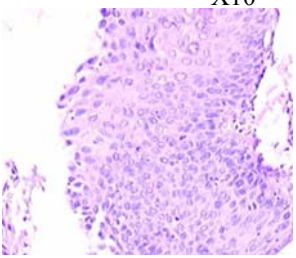


CIN III

X40

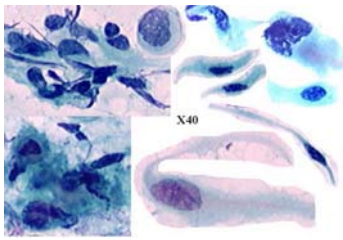


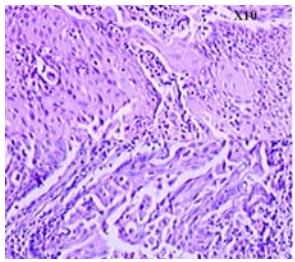
X10



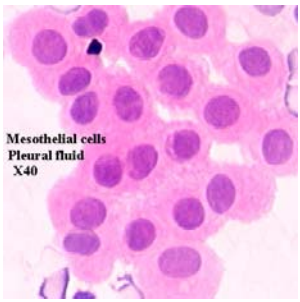
Squamous carcinoma of cervix.

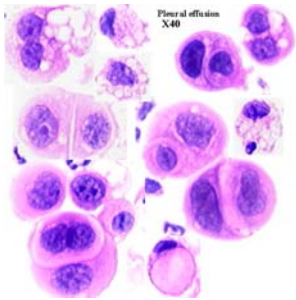
X40 X10





Caption 38. Pleural effusions, benign and malignant.

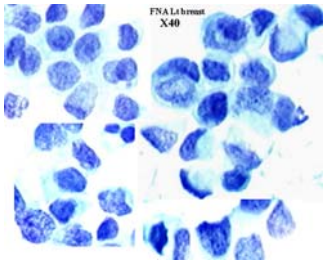




(a) HE X40 Pleural fluid: benign mesothelial cells.

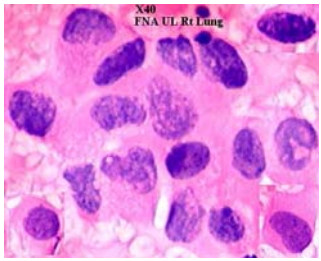
(b) HE X40 Pleural effusion AdenoCa pancreas male 70yr

Caption 39. FNA smear: Breast cancer.



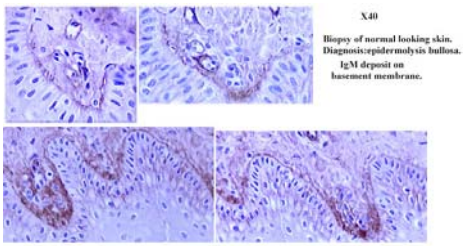
Pap X40 FNA left breast female 60 yrs. Moderate cell anaplasia.

Caption 40. FNA smear: Bronchial nonsmall cell lung carcinoma.



HE X40 FNA Upper lobe Rt lung: Nonsmall cell carcinoma, with pleomorphic and hyperchromatic nuclei.

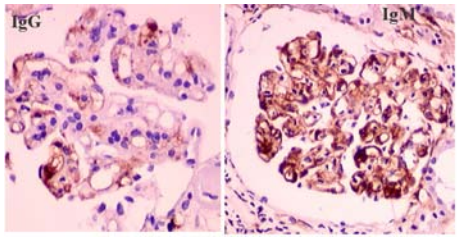
Caption 41. Epidermolysis bullosa.



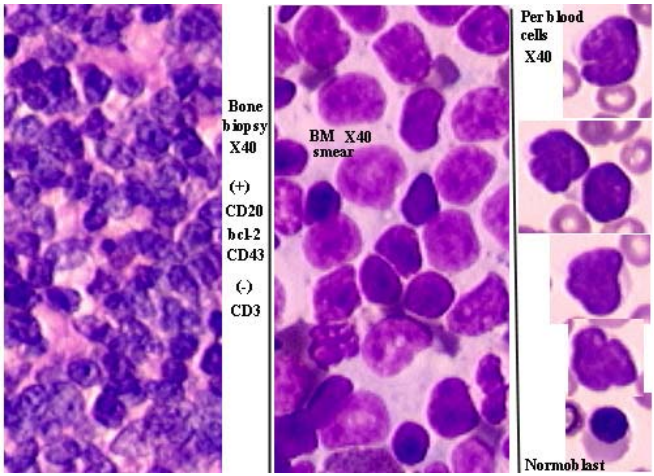
X40 Epidermolysis bullosa IgM deposit on basement membrane of skin.

Caption 42. Proliferative glomerulopathy.

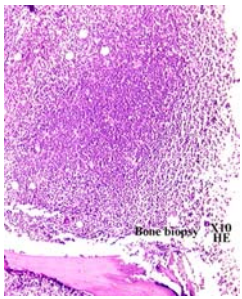
Renal bx X40
Immune deposits IgG + & IgM +++ granular deposits.



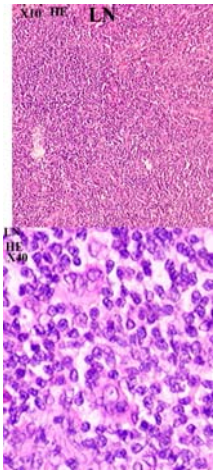
Caption 43. A case of follicular lymphoma. See text.



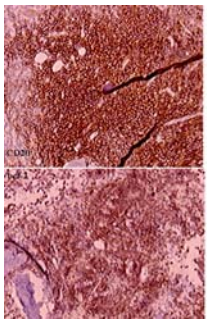
(a) X40 Follicular lymphoma cells in *Per bl and BM imprint*.



(b) X10 Nodular lymphocytic infiltrate in BM.



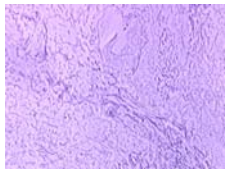
(c) LN infiltrate.



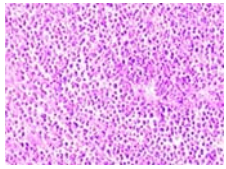
(d) X40 Immune reactions of follicular lymphoma in BM: CD20 & bcl-2.

Caption 44. A case of diffuse large B-cell lymphoma. See text.

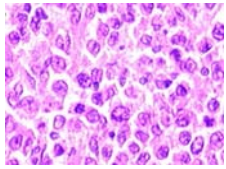
(a) X4 Reticulin frame: vague follicular remains.



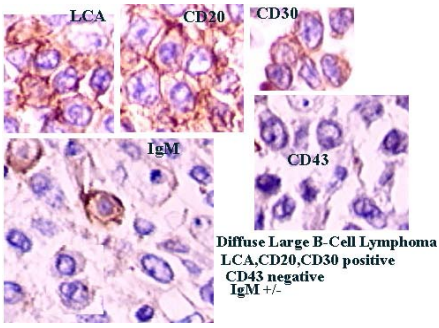
(b) X10 Diffuse large cells with many mitoses.



(c) X40 Large centroblasts, fewer immunoblasts.



(d) X40 Immune reactions of tumor cells.



PICTURES OF CML PERSONNEL

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