KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed University Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2017 onwards)

DEPARTMENT OF BIOCHEMISTRY

STAFF NAME: Ms.P.LOGANAYAKI
SUBJECT : MOLECULAR BASIS OF NON INFECTIOUS HUMAN DISEASE- PRACTICAL

SEMESTER : IV
SUBJECT CODE: 16BCU514-A **CLASS: III B.Sc.(BC)**

1. Anthropometric measurements for normal and high risk individuals and identifications for Kwashiorkor, Marasmus and Obesity
2. Estimation of homocysteine levels in serum
3. Estimation of glycosylated hemoglobin
4. Permanent slides for different types of cancer
5. Diagnostic profile for assessment of CVS and Diabetes mellitus using case Studies.
6. Bone densitometry test demonstration (visit to a nearby clinic)

REFERENCES

Prescott, Harley, (2008). Klein's Microbiology 7th Ed., Willey, J.M., Sherwood, L.M., Woolverton, C.J. Mc Graw Hill International Edition (New York) ISBN: 978-007-126727.

Mandell, Douglas and Bennett.S, (2009). Principles and practices of Infectious diseases, 7th edition, Volume, 2. Churchill Livingstone Elsevier.

Kenneth, J., Ryan, C., and Ray, G., (2008). Sherris Medical Microbiology: An Introduction to Infectious Diseases, Publisher: McGraw-Hill

Patrick R., Ken, S., and Pfaller, M.A., (2008). Medical Microbiology by Elsevier Health Sciences

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LECTURE PLAN
DEPARTMENT OF BIOCHEMISTRY

STAFF NAME: Ms.P.LOGANAYAKI

SUBJECT NAME: PRACTICAL- MOLECULAR BASIS OF NON INFECTIOUS HUMAN DISEASE

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S. No	Duration of period	Topics covered	Books referred	Page No
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5	**1**	Diagnostic profile for assessment of CVS and Diabetes mellitus using case Studies.	-	-
6	**1**	Bone densitometry test demonstration (visit to a nearby clinic)	-	-
7	**1**	Model Exam		

REFERENCES

J1: http://www.biomedcentral.com/1471-2458/7/2. BMC Public Health 2007, 7:2.

J2: Trivelli, L.A., Ranney, H.M. and Lai,H.T., New Eng. J.Med. 284, 353, (1971).

W1: Pathology%20Reports%20-%20National%20Cancer%20Institute.html. National cancer institute, USA.

W2: https://www.cellbiolabs.com/sites/default/files/STA-670-homocysteine-elisa-kit.pdf

1. Anthropometric measurements for normal and high risk individuals and identifications for Kwashiorkor, Marasmus and Obesity

Anthropometric evaluation is an essential feature of geriatric nutritional evaluation for determining malnutrition, being overweight, obesity, muscular mass loss, fat mass gain and adipose tissue redistribution. Anthropometric indicators are used to evaluate the prognosis of chronic and acute diseases, and to guide medical intervention in the elderly.

Anthropometric measurements included weight, height, body mass index (BMI), body circumference (arm, waist, hip and calf), waist to hip ratio (WHR), elbow amplitude and knee-heel length.

Anthropometric measurements:

Weight:

A portable scale with a 125 kg maximum capacity and a +/- 100 g error margin was used. Individuals removed shoes and heavy cloths prior to weighing.

Height:

Subjects stood with their scapula, buttocks and heels resting against a wall, the neck was held in a natural non-stretched position, the heels were touching each other, the toe tips formed a 45° angle and the head was held straight with the inferior orbital border in the same horizontal plane as the external auditive conduct (Frankfort's plane).

Body circumferences:

Mid-brachial, calf, waist and hip circumferences were measured using a flexible non-elastic measuring tape. Individuals stood with feet together and arms resting by their sides. The hip circumference was measured from the maximum perimeter of the buttocks. The waist circumference was taken as the plane between the umbilical scar and the inferior rib border. The waist circumference was used to identify individuals with possible health risks based upon threshold values of ≥ 88 cm for women and ≥ 102 cm for men .

Knee-heel length:

To obtain the measurement, sits on the examination table with both legs dangling. Knee height in men average 21.3 inches. In a manner similar to the pattern for total height, it reaches a maximum of 21.6 inches by 25-34 years, then declines slowly to 20.6 inches for those men 75-79 years of age. About 90 percent of the in this population fall between 19.3 and 23.4 inches in this measurement, and probably 97 to 30 99 percent fall between 18.3 and 24.1 inches. In women, knee height averages 19.6 inches, average value as the total civilian population of or 1.7 inches less than in men, and changes little comparable age with age. The maximum average value of 19.7 inches occurs through the age groups 18-44 years, declines to 19.4 inches for those aged 55-64.

Body-mass index (BMI):

BMI was estimated by dividing weight (kg) by height^2 (m^2). Individuals were considered malnourished if their BMI was less than 18.5, normal from 18.5 to 24.9 and overweight if $\geq$ 25.

$$\textbf{Body-mass index} = \frac{\textbf{Weight in kg}}{\textbf{Height}^2\ \textbf{(m)}}$$

Waist to hip ratio (WHR):

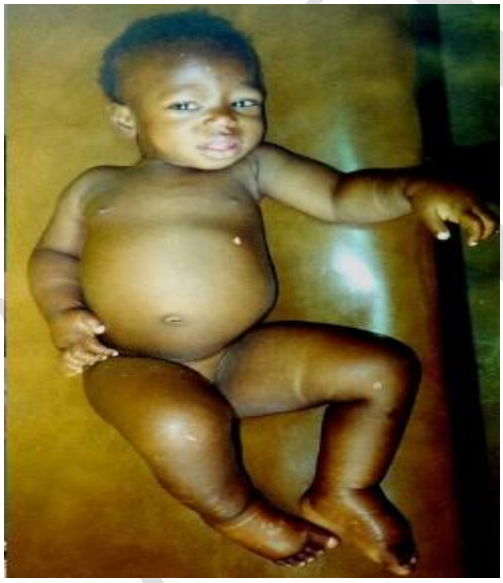
This was estimated by dividing waist circumference by hip circumference. The threshold WHR was $\geq$ 0.85 for women and $\geq$ 1.00 for men, above which superior distribution of adipose tissue was considered.

$$\textbf{WHR} = \frac{\textbf{Waist circumference}}{\textbf{Hip circumference}}$$

Anthropometric measurements for Kwashiorkor, Marasmus and Obesity:

Kwashiorkor:

A childhood disease that is caused by protein deprivation. Early signs include apathy, drowsiness, and irritability. More advanced signs are poor growth, lack of stamina, loss of muscle mass, swelling, abnormal hair (sparse, thin, often streaky red or gray hair in dark-skinned children), and abnormal skin that darkens in irritated but not sun-exposed areas. An enlarged and protuberant belly is common. Kwashiorkor disables the immune system, rendering the affected individual susceptible to a host of infectious diseases. It is responsible for much illness and death among children worldwide. Also known as protein malnutrition and protein-calorie malnutrition (PCM).

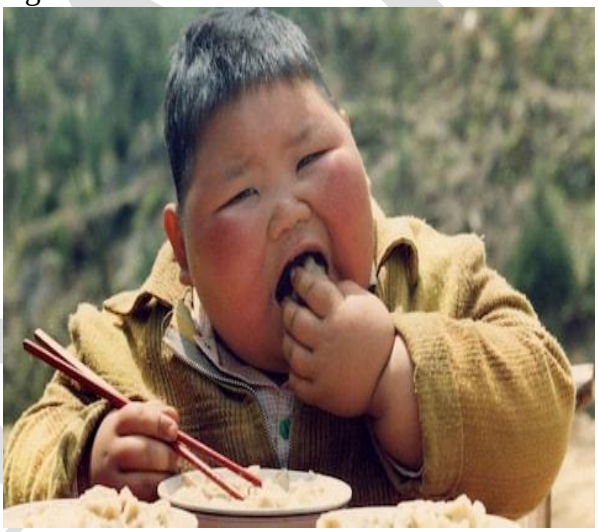


Marasmus:

Marasmus is a severe form of protein-energy malnutrition caused by a shortage of protein and calories in the body. Without these vital nutrients, the body becomes dangerously low in energy and important functions begin to stop. The main symptom of marasmus is being underweight. Children with this condition have lost a lot of muscle mass and subcutaneous fat.. Dry skin and brittle hair are also symptoms of marasmus.

Obesity:

Obesity is that physical state in which the amount of fat stored in the body is excessive or Obesity is due to excess of adipose tissue and is defined as that body weight over 20 per cent above mean ideal body weight.



Results:

2. Estimation of Homocysteine (ELISA Kit)

Principle:

Homocysteine is an amino acid intermediate formed during the production of the essential dietary amino acid methionine . Homocysteine is a homologue of cysteine, differing from cysteine only in that it contains an extra side chain methylene bridge. About 80% of homocysteine found in plasma is bound to protein. High levels of homocysteine in the blood have been associated with premature incidences of vascular disease, and homocysteine is likely to be a risk factor for heart disease. Homocysteine initially stimulates the production of nitric oxide in endothelial cells but ultimately reduces nitric oxide bioavailability and increases oxidative stress by blocking glutathione peroxidase activity as well as causing cellular oxidative degradation (increasing free radical generation). In addition, elevated homocysteine levels leads to increased platelet and leukocyte adhesion and activation, increased vasoconstriction, and increased proliferation of smooth muscle (a hallmark of atherosclerosis).

Preparation of reagents:

- Homocysteine Conjugate Coated Plate: Determine the number of wells to be used, and dilute the Homocysteine Conjugate 1:1000 into PBS. Add 100 µL of 1X homocysteine conjugate to each well of the 96-well Protein Binding Plate. Incubate for 2 hrs at 37ºC or overnight at 4ºC. Remove the diluted homocysteine conjugate, blotting plate on paper towels to remove excess fluid. Wash wells 3 times with 200 µL of PBS and blot on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hour at room temperature. Transfer the plate to 4ºC until ready to begin the assay. Preparation of Reagents Note: The Homocysteine Conjugate Coated Plate is not
- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity. stable long-term. We recommend using it within 24 hours after coating.
- Anti-Homocysteine Antibody and Secondary Antibody, HRP Conjugate: Immediately before use dilute the Anti-Homocysteine Antibody 1:500 and the Secondary Antibody, HRP Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Samples:

Plasma: Collect blood with heparin or EDTA and centrifuge for 10 minutes at 1000 g at 4ºC. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Normal plasma samples should be diluted 2- to 10-fold with PBS containing 0.1% BSA immediately before running the ELISA.

Serum: Harvest serum and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Normal serum samples should be diluted 2- to 10-fold with PBS containing 0.1% BSA immediately before running the ELISA.

• **Tissue homogenate**: Weigh and homogenize the tissue on ice in 5-10 mL cold PBS per gram of tissue. Centrifuge at 10,000 x g for 15 minutes at 4°C. Remove the supernatant and store on ice. Store any unused supernatant at -80°C for up to three months.

• **Cell lysate:** Collect cells by centrifuging at 2000 x g for 10 minutes at 4°C. Sonicate or homogenize the cell pellet on ice in 1-2 mL cold PBS. Centrifuge at 10,000 x g for 15 minutes at 4°C. Remove the supernatant and store on ice. Aliquot and store the supernatant for use in the assay. Store any unused supernatant at -80°C for up to three months.

• **Other biological fluids:** Centrifuge samples for 10 minutes at 1000 g at 4ºC and recover supernatant. Assay immediately or store samples at -80ºC for up to three months.

Assay protocol:

Prepare and mix all reagents thoroughly before use. Assay Protocol

2. Each unknown sample (see Preparation of Samples section), Homocysteine-BSA standard, and blank should be assayed in duplicate.

3. Remove the Assay Diluent from the plate and add 50 µL of unknown sample or standard to the Homocysteine Conjugate Coated Plate. Incubate at room temperature for 10 minutes on an orbital shaker.

4. Add 50 µL of diluted Anti-Homocysteine Antibody (see Preparation of Reagents section) to each well. Incubate at room temperature for 1 hour on an orbital shaker.

5. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After each wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.

6. Add 100 µL of the diluted Secondary Antibody, HRP Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker. During this incubation, warm Substrate Solution to room temperature.

7. Wash the strip wells 3 times according to step 5 above. Proceed immediately to the next step.

8. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).

10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Results:

3. Estimation of Glycosylated Hemoglobin

(Ion Exchange Resin Method)

Introduction:

Glycosylated Hemoglobin (GHb)is formed continuously by the addition of glucose by covalent bonding to the amino terminal valine of the hemoglobin beta chain progressively and irreversibly over the period of time and is stable till the life of the RBC. This process is slow, non enzymatic and is dependent on the average blood glucose concentration over a period of time.

A single glucose determination reflects the glucose level at the time. GHb on the other hand reflects the mean glucose level over an extended period of time. Thus GHb reflects the metabolic control of glucose level over a period of time unaffected by diet, insulin, other drugs, or exercise on the day of testing. GHb is now widely recognized as an important test for the diagnosis of Diabetes Mellitus and is a reliable indicator of the efficacy of therapy.

Principle:

A hemolysed preparation of whole blood is mixed continuously for 5 minutes with a weekly binding cation- exchange resin. The labile fraction is eliminated during the hemolysate preparation and during the binding. During this mixing, HBAo binds to the ion exchange resin leaving GHb free in the supernatant. After the mixing period, a filter separator is used to remove the resin from the supernatant. The percent Glycosylated hemoglobin is determined by measuring absorbance's of the glycosylated hemoglobin (GHb) fraction and the total hemoglobin (THb) fraction. The ratio of the absorbance of the glycosylated hemoglobin and the total hemoglobin fraction of the control and the test is used to calculate the percent glycosylated hemoglobin of the sample.

Reagent Preparation:

The ion exchange resin tubes and the lysing reagent are ready to use. Reconstitute the Control with 1 ml of distilled water. Allow to stand for 10 minutes, with occasional mixing. The reconstituted control is stable for at least 7 days when stored at 2-8^0 C tightly sealed, and at least 7 days when stored at – 20^{0}C.

Sample Preparation:

Whole blood, preferably fresh and collected in EDTA. GHb in whole blood is reported to be stable for one week at 2-8^{0}C.

A. Hemolysate Preparation:

1. Dispense 0.5 ml Lysing Reagent into tubes labeled as control (C) & Test (T).

2. Add 0.1 ml of the reconstituted control & well mixed blood sample into the approximately labeled tubes. Mix until complete lysis is evident.

3. Allow to stand for 5 minutes.

B. Glycosylated hemoglobin (GHb) separation:

1. Remove cap from the Ion- Exchange Resin tubes and label as Control and Test.

2. Add 0.1 ml of the hemolysate from step A into the appropriately labeled Ion exchange Resin tubes.

3. Insert a resin Separator into each tube so that the rubber sleeve is approximately 1 cm above the liquid level of the resin suspension.

4. Mix the tubes on a rocker, rotator or a vortex mixer continuously for 5 minutes.

5. Allow the resin to settle, then push the resin separator into the tubes until is firmly packed.

6. Pour or aspirate each supernatant directly into a cuvette and measure each absorbance against distilled water. (415 nm)

C. Total Hemoglobin (THb) fraction:

1. Dispense 5.0 ml of distilled water into tubes labeled as Control & Test.

2. Add to it 0.02ml of hemolysate from Step A into the appropriately labeled tube. Mix well.

3. Read each absorbance against distilled water. (415 nm)

Calculations:

$$\text{Ratio of Control } (R_c) = \frac{\text{Absorbance of Control GHb}}{\text{Absorbance of Control THb}}$$

$$\text{Ratio of Test } (R_T) = \frac{\text{Absorbance of Test GHb}}{\text{Absorbance of Test GHb}}$$

$$\text{GHb\%} = \frac{\text{Ratio of Test } (R_T)}{\text{Ratio of Test } (R_T)} \times 10 \text{ (Value of control)}$$

Result:

Ex: 4 Permanent Slides for different types of cancer

Cancer is a cellular tumour that, unlike benign tumour cells, can metastasize and invade the surrounding and distant tissues.Cancer has been a major cause of death in the USA for the past few decades, being second only to cardiac diseases. Approximately 20 per cent of all deaths in America are due to cancer. There are at least fifty different types of malignant tumours being identified. More than 50 per cent of the newly diagnosed cancers occur in five major organs:

(i)lungs, (ii) colon/rectum, (iii) breast, (iv) prostate and(v) uterus.

Cancers of the lungs, colon/rectum and prostate are the principal leading causes of deaths in males and in females, breast, colorectal and uterine cancers are the most common. Environmental factors play a very important part. Increased risk of certain cancers with occupational exposures to asbestos, naphthylamine, etc. Association of cancers of oropharynx, larynx, oesophagus and lungs with tobacco chewing and cigarette smoking.
Pathology reports play an important role in cancer diagnosis and staging (describing the extent of cancer within the body, especially whether it has spread), which helps determine treatment options.

Obtaining of tissue for examination:

In most cases, a doctor needs to do a biopsy or surgery to remove cells or tissues for examination under a microscope.

Some common ways a biopsy can be done are as follows:

- A needle is used to withdraw tissue or fluid.
- An endoscope (a thin, lighted tube) is used to look at areas inside the body and remove cells or tissues.
- Surgery is used to remove part of the tumor or the entire tumor. If the entire tumor is removed, typically some normal tissue around the tumor is also removed.

Tissue removed during a biopsy is sent to a pathology laboratory, where it is sliced into thin sections for viewing under a microscope. This is known as histologic (tissue) examination and is

usually the best way to tell if cancer is present. The pathologist may also examine cytologic (cell) material. Cytologic material is present in urine, cerebrospinal fluid (the fluid around the brain and spinal cord), sputum (mucus from the lungs), peritoneal (abdominal cavity) fluid, pleural (chest cavity) fluid, cervical/vaginal smears, and in fluid removed during a biopsy.

Tissue processed after a biopsy or surgery:

The tissue removed during a biopsy or surgery must be cut into thin sections, placed on slides, and stained with dyes before it can be examined under a microscope. Two methods are used to make the tissue firm enough to cut into thin sections: frozen sections and paraffin-embedded (permanent) sections. All tissue samples are prepared as permanent sections, but sometimes frozen sections are also prepared.

Permanent sections are prepared by placing the tissue in fixative (usually formalin) to preserve the tissue, processing it through additional solutions, and then placing it in paraffin wax. After the wax has hardened, the tissue is cut into very thin slices, which are placed on slides and stained. The process normally takes several days. A permanent section provides the best quality for examination by the pathologist and produces more accurate results than a frozen section.

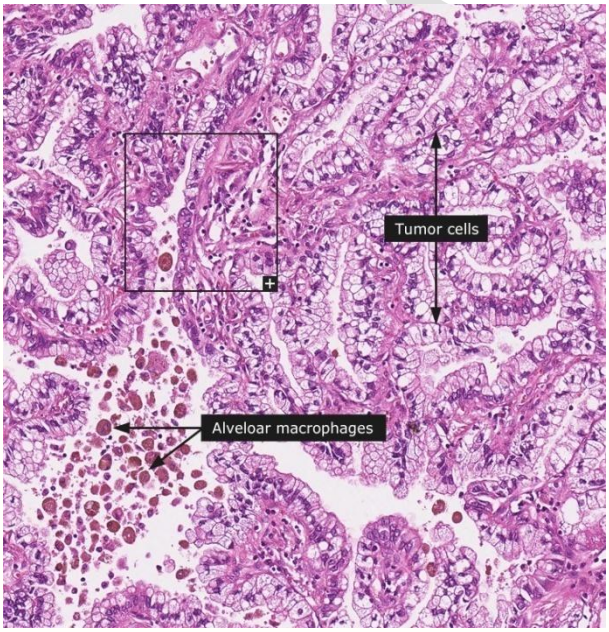
Frozen sections are prepared by freezing and slicing the tissue sample. They can be done in about 15 to 20 minutes while the patient is in the operating room . Frozen sections are done when an immediate answer is needed; for example, to determine whether the tissue is cancerous so as to guide the surgeon during the course of an operation.

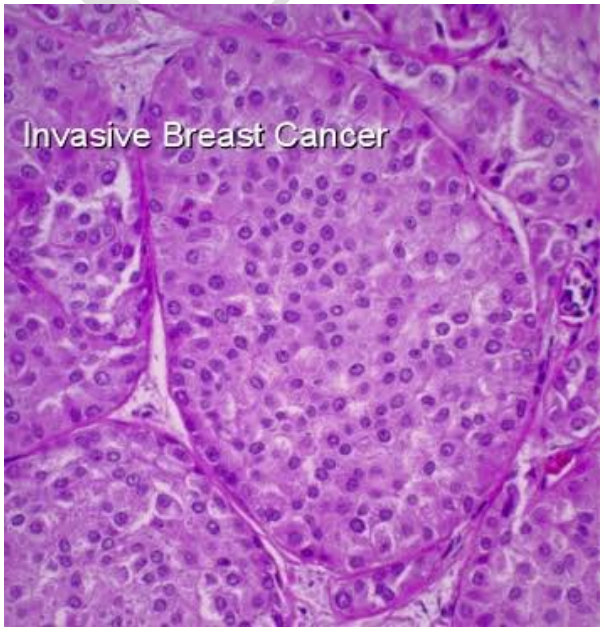
The pathology report may include the following information :

- Patient information: Name, birth date, biopsy date
- Gross description: Color, weight, and size of tissue as seen by the naked eye
- Microscopic description: How the sample looks under the microscope and how it compares with normal cells
- Diagnosis: Type of tumor/cancer and grade (how abnormal the cells look under the microscope and how quickly the tumor is likely to grow and spread)

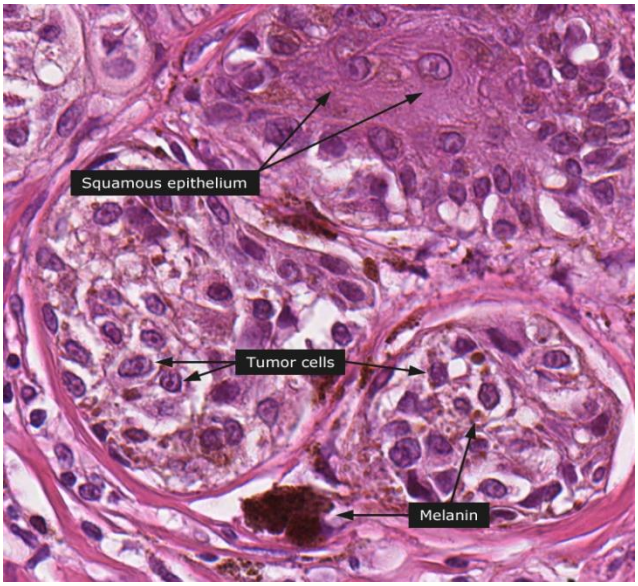
- Tumor size: Measured in centimeters
- Tumor margins: There are three possible findings when the biopsy sample is the entire tumor:
  - Positive margins mean that cancer cells are found at the edge of the material removed
  - Negative, not involved, clear, or free margins mean that no cancer cells are found at the outer edge
  - Close margins are neither negative nor positive
- Other information: Usually notes about samples that have been sent for other tests or a second opinion
- Pathologist's signature and name and address of the laboratory.

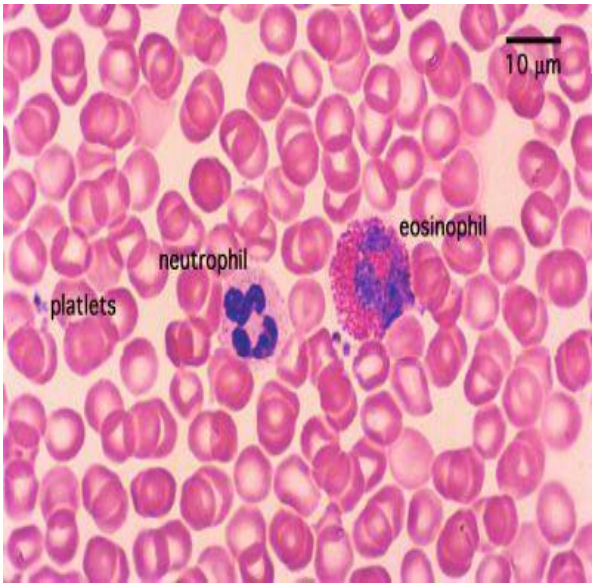
Lung Cancer **Breast Cancer**





Skin Cancer **Blood Cancer**


Squamous epithelium
Tumor cells
Melanin


10 µm
eosinophil
neutrophil
platlets

Ex: 5 Diagnostic profiles for assessment of CVS and Diabetes mellitus using case studies.

Case Studies

Ex: 6 Bone densitometry test demonstration (Hospital Visit)

- Bone densitometry, also called dual-energy x-ray absorptiometry or DEXA, uses a very small dose of ionizing radiation to produce pictures of the inside of the body (usually the lower spine and hips) to measure bone loss.
- It is commonly used to diagnose osteoporosis and to assess an individual's risk for developing fractures. DEXA is simple, quick and noninvasive. It's also the most accurate method for diagnosing osteoporosis.
- Doctor can help determine if you should have a bone density scan. They are recommended if you are age 65 or older regardless of risk.
- If under 65 years of age, should have a bone density scan if you have one or more of the following risk factors:
  - Calcium-deficient diet
  - History of amenorrhea, the abnormal absence of menstruation
  - History of malabsorption
  - Moderate to high alcohol intake
  - Poor nutrition
  - Postmenopausal
  - Prolonged treatment with steroids, certain anti-cancer drugs, thyroid hormone and some anti-seizure medications
  - Sedentary lifestyle
  - Significant caffeine consumption
  - Small-boned frame
  - Smoker

Procedure

A bone density scan is a simple, non-invasive and painless exam to measure bone mass in areas such as your spine, hip, wrist, finger, kneecap, shin bone and heel. The standard test uses a low dose X-ray to detect signs of bone thinning and mineral loss.

The scan measures the density of the spine and hip. The forearm is measured in people with hyperthyroidism or if either hip cannot be scanned. Some doctors will order just a hip scan as a screening study for patients under the age of 60.

There are several machines that measure bone density. Central machines measure density in the hip, spine and total body. Peripheral machines measure density in the finger, wrist, kneecap, shin bone and heel. At the UCSF, we offer screening with both central and peripheral machines, as well as ultrasound examinations of the heel to determine your risk for fractures.

A bone density scan, using a central machine, takes about 15 minutes, including registration. During the procedure, you will lie on a table scanner for five to eight minutes. A technologist will sit next to you throughout the procedure.

In addition to the standard scan, a CT bone density scan uses computed tomography to measure bone density. These scans provide detailed, 3-D images and can measure the effects of aging and diseases other than osteoporosis on your bones. For a CT test, you lie on a table that moves into a large tube-like area where images are taken. It typically takes about 10 minutes.

Preparation

A bone density scan requires little preparation. You may eat normally and take medications as prescribed by your doctor the morning of your test.

The only restrictions are:

- Do not take any vitamin pills or mineral supplements the morning of your exam.
- Must not have any exams involving barium or radioisotopes within the last month. These scans interfere with the bone density results.

Results

The results of bone density scan will be available within three to five days. This information will enable doctor to determine if you're at risk for fractures and require further evaluation. The lower bone density, the higher your risk for fracture.