## NORTH EASTERN INDIRA GANDHI REGIONAL INSTITUTE OF HEALTH AND MEDICAL SCIENCES,

#### **MAWDIANGDIANG, SHILLONG - 793018**

(An Autonomous Institute, Ministry of Health and Family Welfare, Government of India)



### PATHOLOGY INTEGRATED ATLAS WITH CASE LINKERS BASED ON SKILL COMPETENCIES

(as per Competency Based Medical Education curriculum)

#### **DISCLAIMER: FOR EDUCATIONAL PURPOSES ONLY**

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#### PATHOLOGY - SKILL DOMAIN COMPETENCY - OBJECTIVES

## COMPETENCY PA 2.8 Identify and describe various forms of cell injuries, their manifestations and consequences in gross and microscopic specimens

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#### **OBJECTIVES:**

PA 2.8.1.1 Describe the gross finding of the specimen (Fatty liver)

PA 2.8.1.2 Identify the organ and describe the type of cell injury seen in the slide provided

PA 2.8.1.3 Name the special stain used for identification of fat





Cirrhotic liver. Tissue shows hepatic nodules separated by fibrous septa



Masson Trichrome stain highlights the fibrous tissue



Reticulin stain highlights the reticular fibres surrounding the nodules

## CIRRHOTIC LIVER-EXAMPLE OF IRREVERSIBLE INJURY

Cell injury is reversible up to a point, but if the injurious stimulus is persistent or severe, the cell suffers irreversible injury and ultimately undergoes cell death

LINKER: 54 year old chronic alcoholic came with complaints of abdominal distension, pedal edema, shortness of breath and hematemesis. Read: Cirrhosis PA 2.8.2.1 Describe the gross finding of the specimen (Cardiac hypertrophy)

PA 2.8.2.2 Identify the organ and describe the type of cell injury seen in the slide provided



#### EXAMPLES OF IRREVERSIBLE CELL INJURY (NECROSIS)

PA 2.8.3.1 Describe the gross appearance of the specimen (tuberculous lymph node) PA 2.8.3.2 Identify the organ and describe the type of cell injury seen in the slide provided



Fig-3 Lymph node (arrow) showing multiple areas of cheese-like necrotic areas (Caseous Necrosis)



LANGHANS GIANT CELLS-Nuclei are arranged in a horseshoe-shaped pattern

**GRANULOMA-** Formed by the fusion of epithelioid macrophages

Most common cause of caseous necrosis is tuberculosis. Special stain for tubercle bacilli is Ziehl-Neelsen stain.

## COMPETENCY PA 6.7 Identify and describe the gross and microscopic features of infarction in a pathological specimen (SAME OBJECTIVE AS PA 2.8.4)

PA 2.8.4.1 Describe the gross appearance of the specimen (Myocardial Infarction) PA 2.8.4.2 Identify the organ and describe the type of cell injury seen in the slide provided



Linker: A 62 y/o man with complaints of chest pain and a "feeling of dread" was rushed to the casualty and expired soon after. A medical autopsy was performed.

**Read: Myocardial infarction** 

**Gross section** of ventricular wall of heart showing dark brown acute anteroseptal infarct (**Black arrow**) with extensive hemorrhage into the infarct.



Specimen showing hypertrophy of heart with left ventricular wall showing infarct is demonstrated by a lack of staining by triphenyltetrazolium chloride (**black Arrow**) and whitish fibrotic areas indicating areas of previous old infarct (**arrow head**)

Wavy fibers showing coagulative necrosis (**Blue Arrow**) with widened spaces between the fibers. Occasional neutrophils are seen (**Green Arrow**).

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#### PA3.2 Identify and describe Amyloidosis in a pathology specimen (Non core)

- PA 3.2.1 Identify the organ and describe the gross findings of the given picture (Fig1)
- PA 3.2.2 Write the microscopic findings of the given picture (Fig 2)
- PA 3.2.3 Name the stain used in the given picture (Fig 3)
- PA 3.2.4 Identify the technique used for confirmation in given picture (Fig 4)
- PA 3.2.5 Describe the appearance of amyloid in the given picture (Fig 4)



#### PA 4.4 Identify and describe acute and chronic inflammation in gross and microscopic specimens

#### **EXAMPLE OF ACUTE INFLAMMATION**

PA 4.4.1.1 Identify the organ and describe the gross findings (Acute Appendicitis)

PA 4.4.1.2. Describe your microscopic findings.



Linker: A 25 y/o man presented with peri-umbilical colicky pain which migrates to the right iliac fossa. This was associated with loss of appetite and nausea.

He was diagnosed with an inflamed appendix on radiology and underwent laparoscopic appendectomy. Specimen was sent for HPE

**Fig.1** -Appendix with attached mesoappendix, o/s- serosal congestion, c/s- patent lumen, thin walled. Outer surface of the appendix shows fibropurulent exudate **Read: Acute appendicitis** 



Fig.2-Dense mucosal acute inflammatory infiltrate (neutrophils) within lumen



Fig.3-Infiltration of neutrophils within the muscularis propria is characteristic

#### **EXAMPLE OF CHRONIC INFLAMMATION**

PA 4.4.2.1 Identify the organ and describe the gross findings (Chronic Cholecystitis)

PA 4.4.2.1 Describe your microscopic findings



Cut open specimen of gall bladder showing yellow dots on the mucosa, resembling strawberry



Foamy lipid laden macrophages expanding the lamina propria

CHOLESTEROLOSIS OF GALL BLADDER π

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#### PA 8.3 Observe diagnostic cytology and its staining and interpret the specimen (S, KH, Y)

- PA 8.3.1 Enumerate the types of specimens studied in the cytology section
- PA 8.3.2 Name 2 common stains used in cytology
- PA 8.3.3 Observe how to make a cytology smear from oral mucosa
- PA 8.3.4 Perform the staining of a given smear
- PA 8.3.5 Interpret the cytology smear given



**Oral Brush Cytology**: Lesion over the cheek is scraped and spread over a slide, which is subsequentlystained and examined.



Smear from *oral mucosa* showing superficial squamous cells

<u>Papanicolaou stain-Used in</u> cytology



May Grünwald Giemsa stain-Used in cytology. Smear of bronchial lavage showing respiratory Epithelial cells, Macrophages, Neutrophil and Lymphocyte

<u>Papanicolaou stain</u>- Cervical smear showing superficial and intermediate squamous cells, and parabasal cells

> Hematoxylin and Eosin stain-Used in cytology, FNAC from liver showing hepatocytes



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Fluid cytology – Giemsa stain-Pleural fluid showing monocytes, mesothelial cells, and lymphocytes



Fluid shows malignant cells arranged in clusters. These cells have highN:C ratio and hyperchromatic nuclei (Blue Arrow). One cellshows a prominent nucleolus (Black Arrow).

Intracellular mucin is also seen in one of the clusters (**orange Arrow**).

Background shows scattered benign lymphocytes (yellow arrowhead)

(MGG Stain; 400x)

#### PA 13.5 Perform, identify and describe the peripheral blood picture in anemia

- PA 13.5.1 Prepare a peripheral blood smear from the given blood sample
- PA 13.5.2 Stain the given smear with Leishman stain.
- PA 13.5.3 Identify the cells in the smear and describe the morphology of the red blood cells
- PA 13.5.4 Interpret the peripheral blood smear



Fig-2 -The diameter of a normocytic normochromic **RBC** (Dotted *black arrow*) ranges between 6-8 µm. It is therefore classically compared to the nucleus of a small lymphocyte (Solid black arrow) (7-9µm)

Linker: A 25-year-old man presentsto the OPD for a general annual health check-up. Peripheral smearwas performed.

**RBCs**- predominantly normocyticnormochromic

WBCs - within normal limits (*Red arrow-* **neutrophil**; *Red arrowhead-***small lymphocyte**; *Yellow arrow-* **monocyte**)

**Platelets** - adequate (*Yellow arrow head*- platelet clump)

**Impression- Normal smear** 



#### PA 14.3 Identify and describe the peripheral smear in microcytic anemia

- PA 14.3.1 Describe the features of microcytic anemia in a peripheral blood smear
- PA 14.3.2 Enumerate the differential diagnosis of microcytic anemia
- PA 14.3.3 Tabulate the laboratory findings and list the differences between the microcytic anemias



#### **RED CELL INDICES**

MCV (Mean corpuscular volume) is a measure of the average size of RBCs:  $92 \pm 9$  fL

MCH (Mean corpuscular hemoglobin) is the average amount of hemoglobin in the RBCs: 29.5  $\pm$  2.5 pg

MCHC (Mean corpuscular hemoglobin concentration) correlates the hemoglobin content with the volume of the cell: 33  $\pm$  1.5 g/dL

RDW (Red cell distribution width) measures the amount of red blood cell variation in size:  $12.8 \pm 1.2\%$ 

Condition	RBC Count	MCV	MCH	MCHC	RDW
	( $\vec{\bigcirc}: 5.0 \pm 0.5 \ x10^{6/} \ \mu L$	(83- 101 fL)	(27- 32 pg)	(31.5-34.5	(11.6-14%)
	$\label{eq:alpha} \begin{array}{l} \bigcirc : 4.3 \pm 0.5 \; x10^6 \! / \; \mu L) \end{array}$			g/dL)	
Iron deficiency anemia	↓	Ļ	Ļ	↓	↑
Thalassemia trait	Normal/↑	$\downarrow\downarrow$	Ļ	Normal	Normal

- Other causes of microcytic hypochromic anemia include sideroblastic anemia, anemia of chronic disease and lead poisoning.
- Definitive diagnosis of thalassemia trait is by hemoglobin electrophoresis or HPLC (High Performance Liquid Chromatography). HPLC findings of thalassemia trait: HbA 90-93%, HbA2 3.9-8%, HbF is normal/slightly increased to 1-4% in about half the cases.

#### PA 15.3 Identify and describe the peripheral blood picture of macrocytic Anemia

PA 15.3.1 Describe the features of macrocytic anemia in a peripheral blood smear



Red blood cell is larger than nucleus of small lymphocyte – so it is a macrocyte, oval in shape The central pallor is larger than one-third

of the cell size so it is hypochromic ( Hypochromic macro-ovalocyte seen in dual deficiency anemia – deficiency of B12/Folate/Fe) Small lymphocyte

Black arrow shows a hyper segmented neutrophil

White arrow shows a microspherocyte seen in autoimmune haemolytic anemia

Dx is Pernicious anemia (D/D macrocytic anemia)

Linker: A 57-year-old lady presented with marked fatigue, nausea and a sore swollen tongue. She recently has been feeling a tingling sensation on the toes. PBS showed above findings.

#### Read: Megaloblastic anemia

Causes of macrocytic anemia are Vitamin B12 deficiency, folate deficiency, liver diseases, alcoholism and hypothyroidism.

#### PA 16.6 Prepare a peripheral blood smear and identify hemolytic anemia from it (Certifiable skill)

#### (SAME OBJECTIVES AS PA 13.5)

PA 16.6.1-Describe the features of hemolytic anemia in peripheral blood smear.





Linker: A 2 y/o girl is brought to the casualty with complaints of pain abdomen and dyspnea. Her parents provide a history of recurrent blood transfusion requirements. O/E- marked pallor and icterus are noted.

## Hemogram: Hb- 7.8g/dL, RBC- 2.1 x10<sup>6</sup>/µl, MCV- 68 fl, MCH- 22 pg, MCHC- 25 g/dl, RDW-30%

Peripheral smear shows marked anisopoikilocytosis. Numerous target cells are seen (**Blue Arrows**) along with polychromatophils (**Red Arrows**) and nucleated RBC (**Green Arrows**)

Read: Thalassemia major



Linker: A 6 y/o boy is brought to the casualty with complaints of acute abdomen and joint pain.

Hemogram: Hb- 10.5g/dL, RBC- 3.1x10<sup>6</sup>/µl, MCV- 73 fL, MCH- 25 pg, MCHC- 29 g/dl, RDW-25%

Peripheral smear shows prominent sickle shaped RBCs (Blue Arrows), target cells (Red Arrows) and polychromatophils (Green Arrow) Read: Sickle cell anemia

PA 16.7 Describe the correct technique to perform a cross match (THIS TEST IS ALSO PART

OF THE COMPATIBILITY TESTING PROCEDURES)

#### PA 16.7.1 Define cross-matching

Principle: Cross match permits detection of clinically significant incompatibilities caused by complete orincomplete

(through the anti-globulin phase) antibodies.

#### PA 16.7.2 Describe different types of cross-match

**Types:** 

**1. Immediate spin cross match:** This test is performed at room temperature for detection of IgMantibodies and results are read within minutes.

**2.** Anti Human Globulin(AHG) cross match: This test is performed at 37°C with the use of AHGreagent for detection of IgG type of antibodies.

#### PA 16.7.3 Illustrate the steps of AHG Cross-match

#### STEPS OF CROSS MATCHING

Add 100  $\mu$ L of test serum + 50 $\mu$ l of 5% donor cell

**AHG Cross-match** 

Add 100µl of Polyethylene glycol(PEG)

Incubate for 15 min at 37°C in a water bath.

Wash 4 times with Normal saline

Add 100 µl or 2 drops of AHG.

Keep for 5 min at RT.

Spin at 1000 rpm for 10 sec.

Check under microscope.



Add 100  $\mu$ l of check cell Positive reaction: Report the result as **Negative** Negative reaction: **Repeat the test again** 

Negative

#### **Results:**

- 1. Positive test result is interpreted as INCOMPATIBLE.
- 2. Negative test result is interpreted as COMPATIBLE.

PA 19.3 Identify and describe the features of tuberculous lymphadenitis in a gross and microscopic specimen (SAME AS OBJECTIVE PA 2.8.3.1 and 2.8.3.2)

#### PA 19.5 Identify and describe the features of Hodgkin lymphoma in a gross and microscopicspecimen

- PA 19.5.1 Identify the specimen provided
- PA 19.5.2: Describe the gross features of the specimen provided PA

19.5.3: Describe the microscopic features on the slide provided



#### **Classical Reed Sternberg cells**

The classical Reed-Sternberg cell is a giant cell (20-30 microns) having two nuclei which may appear as mirror images, each nucleus contains a prominent inclusion-like eosinophilic nucleolus surrounded by a clear zone (owl-eyed nucleoli).

#### PA 19.7 Identify and describe the gross specimen of an enlarged spleen

PA 19.7.1 Identify the specimen provided

PA 19.7.2: Describe the gross features of the specimen provided PA

19.7.3: Describe the microscopic features on the slide provided



Microscopy shows thickened capsule, congested red pulp with atrophied white pulp in CVC (chronic venous congestion) spleen

Causes of CVC spleen: Cirrhosis of liver, right-sided heart failure

PA 20.1.5 List out the laboratory investigations in a suspected case of multiple myeloma

Linker: A 68-year-old male presented with anemia and lower backache. Hb 6.5g%, ESR 120mm/hr. Correlate the images and give the diagnosis.

**Read: Multiple myeloma** 



X-ray skull showing multiple lytic lesions





Serum Electrophoresis showing M spike

Abnormal plasma cells in bone marrow

#### PA 21.3 Differentiate platelet from clotting disorders based on the clinical and hematological features

- PA 21.3.1 Enumerate the causes of platelet disorders
- PA 21.3.2 Enumerate the causes of clotting disorders
- PA 21.3.3 Identify the platelet disorders based on clinical features
- PA 21.3.4 Identify the clotting disorders based on clinical features
- PA 21.3.5 Interpret the laboratory findings of patients to differentiate platelet disorders from clotting disorders



**Platelet disorders**: Immune thrombocytopenic purpura, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, Bernard-Soulier syndrome, Glanzmann's thrombasthenia, infections and drugs **Clotting disorders**: Hemophilia, Von Willibrand disease

## PA 22.2 Enumerate the indications, describe the principles, enumerate and demonstrate the steps of compatibility testing.

#### PA 22.2.1 Enumerate the indications of compatibility testing

A series of testing procedures and processes performed to ensure the best possible results of a blood transfusion are collectively known as **pre-transfusion testing or compatibility testing**. This is to ensure acceptable survival rate of transfused red blood cells and that no significant destruction of recipient's own red blood cells occurs. Although adverse events to blood transfusion cannot be totally avoided, pre transfusion compatibility testing, if carefully performed, ensures favorable outcomes.

#### Pre-transfusion testing consists of:

- 1. ABO blood grouping: forward and reverse grouping tests
- 2. Rh typing
- 3. Screening and identification for unexpected alloantibodies.
- 4. Cross-matching

#### PA 22.2.2 Describe the principles along with the steps of compatibility testing

1. ABO blood grouping (forward and reverse) and Rh typing:

#### Principle & steps:

ABO system is the only system in which there is a reciprocal relationship between the antigens on the red cells and the naturally occurring antibodies in the serum. Routine grouping of donors and patients must therefore include both cell and serum tests, each serving as a check on the other.



#### STEPS OF ABO FORWARD (CELL) GROUPING:



#### STEPS OF ABO REVERSE (SERUM) TYPING:

- 1. Put 50µl of reagent cells of groups A1, B and O in the appropriately labeled tubes.
- 2. Add to each tube, 100µl of test serum.
- 3. Mix the contents of the tubes gently and incubate at room temperature (RT) for 30 minutes or incubate at RT for 5 min and centrifuge at 1000 rpm for 1 minute.
- 4. Check all the negative reactions under microscope.

#### 2. Rh typing

#### **Principle:**

Testing with anti-D is necessary to determine if a patient possesses or lacks D blood group antigen on his/her red cells. When a person lacks D antigen, synthesis of anti-D may occur following sensitization to D Positive cells through blood transfusion or pregnancy.

#### **STEPS OF RH TYPING**



#### Forward grouping

#### **Reverse grouping**



#### Gradation:

Agglutination	Grading	
One single clump with clear back ground / Hemolysis	4+	
Three or four individual clumps with few free cells/ partial hemolysis	3+	
Many fairly large clumps with few free cells	2+	
Fine granular appearance visually, with definite small clumps (10-15 cells/LPF)	1+	
2 to 3 cells sticking together with uneven distribution	W	
All cells are free in LPF	0	

#### The interpretation of ABO group is as follows:

Cell typing			Serum typing		Blood			
Anti-A	Anti-B	Anti-AB	Anti A1	Anti H	A1 cell	B cell	O cell	group
+	0	+	+	X	0	+	0	"A1"
+	0	+	0	Х	0	+	0	"A2"
0	+	+	Х	Х	+	0	0	"В"
0	0	0	Х	+	+	+	0	"O"
+	+	+	+	Х	0	0	0	"A1B"
+	+	+	0	Х	0	0	0	"A2B"

+ = Agglutination (Weak to 4+), 0 = No agglutination, X – Not tested.

#### 3. Antibody screening and identification for unexpected antibodies:

#### **Principle:**

This procedure describes the method of detecting unexpected immunohematological antibodies in a patient directed against the minor antigens of the red cells which are not routinely typed in the Blood Bank. The serum of patients requiring blood transfusion are subjected to antibody screening with the three cell panel and if found positive, then identification of the specific antibody is performed with the 11cell panel.

#### STEPS OF ANTIBODY SCREENING Antibody screening/identification (tube technique)

Add 100 µL of test serum + 50µl of cell (screening cell 1, cell2, cell3)/ identification cells (1-11)

#### Add 100µl of PEG.

Incubate for 15 min at 37°C in a water bath.

Wash 4 times with Normal saline

Add 100 µl or 2 drops of AHG.

Keep for 5 min at RT.

Spin at 1100 rpm for 10 sec.

Check under microscope.



Positive Report the result Negative ↓ Add 100 µl of check cell

Positive reaction: Report the result as **Negative** Negative reaction: **Repeat the test again** 

#### 4. Cross-matching

**Principle:** Cross match permits detection of clinically significant incompatibilities caused by complete orincomplete (through the anti-globulin phase) antibodies.

#### **Types:**

**1. Immediate spin cross match:** This test is performed at room temperature for detection of IgMantibodies and results are read within minutes.

**2.** Anti Human Globulin cross match: This test is performed at 37°C with the use of AHG reagent fordetection of IgG type of antibodies.

#### STEPS OF CROSS MATCHING

#### AHG Cross-match

Add 100  $\mu L$  of test serum + 50  $\mu l$  of 5% donor cell

Add 100µl of PEG.

Incubate for 15 min at 37°C in a water bath.

Wash 4 times, with Normal saline

Add 100 µl or 2 drops of AHG

Keep for 5 min at RT.

Spin at 1100 rpm for 10 sec.

Check under microscope.

Positive Report the result

Add 100 µl of check cell

Negative

Positive reaction: Report the result as **Negative** Negative reaction: **Repeat the test again** 

#### **Results:**

- 1. Positive test result is interpreted as INCOMPATIBLE. (Agglutination of RBCs means positive result)
- 2. Negative test result is interpreted as COMPATIBLE (No agglutination of RBCs means negative result)



PA 23.1 Describe abnormal urinary findings in disease states and identify and describe common urinary abnormalities in a clinical specimen

PA 23.1.1 Enumerate the various collection methods for urine examination

Routine urine sample: first voided midstream morning sample is best as it is most concentrated, 100 ml

**24 hour urine specimen**: Start collecting urine voided from today's second sample till next day's first sample (8 amto 8 am). Total volume is measured and mixed well and sample taken for analysis of hormones, electrolytes, creatinine, 24 hour protein, parasites.



Ureteral Catheter

**Ureteral catheterization**: urine is removed from bladder, bladder is washed and urine is collected separately from right and left ureter with the help of a catheter.

**Suprapubic aspiration** – to obtain uncontaminated urine sample for diagnosis UTI in child who is not toilet trained. NOT A METHOD OF CHOICE



#### PA 23. 1.2 List name of preservatives used for urine collection

Ideally urine should be examined within 2 hours of collection. Preservatives used if >2 hours.

REFRIGERATION - for hormones, calcium, pigments- urobilinogen, bilirubin

#### CHEMICAL PRESERVATIVES

Formalin - 2 drops 40 % formalin / 30 ml urine , can give false positive for sugars.

HCL - 10 ml conc HCL in 24 hrs urine sample for calcium , amino acids and catecholamines

Chloroform – 50 drops/ 24 hrs urine – can interfere with Fehlings test (sugars)

Combination - Monopotassium phosphate, sodium benzoate, sodium bicarbonate and red mercuric oxide.

#### SPECIFIC PRESERVATION -

Sodium fluoride (NaF) – 24hrs glucose

estimationAcetic acid – vitamin C estimation

Sulphuric acid - serotonin and catecholamine estimation

# NEIGRIHMS

PA 23.1.3 Discuss the physical composition of normal urine PA 23.1.4 Describe abnormal urinary findings in term of volume, colour, odour, PH and specific gravity

	Normal	Pathological conditions			
Colour	yellow colour (urochrome & urobilin	See table below			
Odour	Aromatic odour	Pungent – bacterial overgrowth Lack of odour – acute tubular necrosis Maple syrup urine disease	Methionine malabsorption – cabbage smell Phenylketonuria – mousy smell Tyrosinemia – rancid smell		
Volume	600 to 2000 ml / day	Polyuria > 2000ml/24 hrs Oliguria - < 500ml / 24 hrs	Nocturia > 500ml at night with sp gravity 1.018 Anuria – complete suppression of urine formation for 12 hrs		
Urine pH	4.6 to 8	Alkaline urine – on standing for long duration (urea broken to ammonia and bicarbonate) - diet rich in citrus fruit, post meal - sodium bicarbonate, potassium citrate, acetazolamide (calculi dissolves in alkaline urine) - antibiotics – neomycin, streptomycin - metabolic and respiratory alkalosis	Acidic urine – diet rich in proteins -Ammonium chloride, methionine and methenamine – acidic urine which dissolves phosphate and calcium carbonate stones -Respiratory / metabolic acidosis -Diabetic ketoacidosis		
Specific gravity	1.016 to 1.022	Low – diabetes insipidus, Loss of concentrating ability of kidney High – Dehydration, Adrenal insufficiency	Isothenuric specific gravity – <b>fixed</b> at 1.010, due to loss of concentrating and diluting ability of kidney e.g., late stages of chronic renal failure		
Osmolality	500 to 800 mOsm/kg water	Low osmolality- diabetes insipidus	High osmolality- Excess intake of salt, GI loss of hypotonic fluid		

Colour of urine	Conditions	Colour of urine	Conditions
	Pale urine (diluted urine)- low specific gravity, high fluid intake) Dark urine (fluids withheld causing conc urine)- fever, thyrotoxicosis, starvation - increased metabolic rate	Nrc 1608222	Dark brown (cola coloured urine)- Acidic urine + Hb = myoglobin, Alkaptonuria, L-DOPA therapy
titios bla	Red urine – haematuria Haemoglobinuria, Myoglobinuria, Porphyria, Aniline dye, Beetroot intake		Green – bacteria, biliverdin, B complex, nitrofurantoin
MFG 1106282	Cloudy urine - WBC, bacteria, phosphates, mucous, sperms, prostatic fluid		Yellow – bilirubin, urobilin
	Milky urine – pyuria , chyluria	00	Orange – bile salts , rifampicin

#### PA 23.1.5 Enumerate the various chemical examination of urine with its interpretation

**PROTEIN** – Normally < 150mg/24 hrs protein is excreted in urine, one third – albumin, two third -globulin (alpha,beta and gamma). Excretion of >150mg/24 hrs is known as PROTEINURIA

**Methods of estimating – Heat and acetic acid test**, **FIG-1** – on heating there is coagulation and precipitation of proteins and phosphates, with the addition of acetic acid phosphates dissolve confirming protein in urine if turbidity persists.



**MICROALBUMINURIA** – is presence of albumin in urine above normal range but below the level at which proteincan be detected by conventional methods. Urinary protein of 30 to 300 mg / 24 hrs is microalbuminuria

GLUCOSE – normal glucose in urine < 100mg/24 hrs



#### Grading

0-100 mg /dl - clear blue or green opacity

100- 500 mg /dl – green background with yellow ppt +

500-1400 mg/dl - yellow to greenish yellow background with yellow ppt ++

1400- 2000mg/dl - muddy orange background with yellow ppt +++

>2000mg /dl – clear supernatant and orange red ppt ++++

#### **KETONES**

#### METHOD OF ESTIMATING: ROTHERA 'S NITROPRUSSIDE TEST



#### **REAGENT STRIP TEST**



Various chemical parameters can be detected in urine samples at the same time by using a reagent strip. The strip is dippedin urine and taken out and results are read within 60 secs.

The resultant change in colour is compared with the comparator on the bottle and a semi quantitative estimation can also be made.

#### **TEST FOR BILIRUBIN : FOUCHETS TEST**



**Fouchets test**: for detection of bilirubin in urine.

#### **BILE SALTS: HAYS TEST**





Control for comparison

Positive test

Hays test: Bile salts reduce surface tension

Dry sulphur powder added to urine -If bile salts are present, sulphur powder sinks due to lowsurface tension

# NEIGRIHMS

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#### PA 23.1.6 Enumerate the causes of increase protein, glucose and ketone bodies in urine

#### CAUSES OF PROTEINURIA

A. Physiological - Fever, cold, exercise, dehydration

B. **Transient** proteinuria- In pts with no abnormal history, normal physical examination and normal Renal Function Test.

C. **Postural** proteinuria - Occurs in particular recumbent position – in exaggerated lordotic position, due to high pressure on renal vein and artery, renal congestion and ischaemia

D. Renal proteinuria - Nephrotic syndrome Heavy/Massive proteinuria - Nephrotic syndrome, Rapidly Progressing Glomerular Nephritis (RPGN) Moderate proteinuria - Nephrosclerosis, Multiple myeloma.
Minimalproteinuria ( < 1gm / day ) - Chronic pyelonephritis , Nephrosclerosis</li>

#### CAUSES OF GLYCOSURIA

Renal Glycosuria –when renal threshold is reduced (Normal renal threshold is 180 mg/dl)

Causes - Pregnancy, galactosemia, lead poisoning, myeloma

Glycosuria with hyperglycemia - Metabolic - burns, Endocrine - Diabetes Mellitus, Drug - thiazide

CAUSES OF KETONURIA - Diabetic ketoacidosis, starvation

#### PA 23.1.7 Interpret the test results and renal charts to diagnose a disease

#### CASE 1

A 30-year-old woman presented with fatigue, malar rash, pain and swelling in the joints. On examination, she also had mouth ulcers. Her serum creatinine value was 6 mg/ dl. The immunofluorescence findings on the kidney biopsy is given here:



- 1) What is your diagnosis?
- 2) What is this immunofluorescence finding called?

**COMMENT**: Young female in reproductive age group with the above mentioned symptoms suggest systemic lupus erythematosus. Serum creatinine reflects deranged kidney function. IF shows full house positivity which is seen in lupus nephritis.

#### CASE 2

A 15-year-old child was admitted with symptoms of headache, pain in the flanks, anorexia (loss of appetite for food). He passed red coloured (cola coloured) urine and had edema around his eyes. His blood pressure was 170/110 mm. Results of laboratory test are as follows:

Laboratory test	Patient	Normal
Total Protein	7.0 g/dl	6-8 g/dl
Albumin	4.5 g/dl	3.5-5.0 g/dl
BUN	45 mg/dl	8-25 mg/dl
Serum Creatinine	3.0 mg/dl	0.6 -1.2mg/dl
Hb	9g/dl	14-18 g/dl
24-hour urine protein	2.0 g/24 hours	<150mg/24hours

1) Interpret the results and give your provisional diagnosis. (1)

Kidney biopsy revealed:



- 2) Write 1 light microscopic feature to favour your diagnosis.
- 3) What is the immunofluorescence findings seen in the picture below in this case?



- 4) What is the serum antibody test done in this case?
- 5) What is the type of hypersensitivity reaction seen in this case?

**COMMENT**: A young child passing cola coloured urine with periorbital edema and hypertension suggest post infectious glomerulonephritis.

#### CASE 3

A 2-year-old child came to paediatric OPD with symptoms of fever, fatigue, loss of appetite for food and generalized anasarca. Mother gave a history of passage of foamy urine by the child for last 7 days. Immunofluorescence study revealed no immune deposits. Results of laboratory test are as follows

Laboratory test	Patient	Normal
Total Protein	4.5 g/dl	6-8 g/dl
Albumin	1.2 g/dl	3.5-5.0 g/dl
Serum cholesterol	380 mg%	150-280 mg%
BUN	25 mg/dl	8-25 mg/dl
Serum Creatinine	1.0 mg/dl	0.6 -1.2mg/dl
Hb	9g/dl	14-18 g/dl
		PEV
24-hour urine protein	4.5 gm/ 24 hours	<150mg/24hours
-	13 4	

- 1) Interpret the results and give your provisional diagnosis.
- 2) Write 4 causes of this condition.
- 3) What is the electron microscopic findings for this case?

**COMMENT**: Frothy urine indicates proteinuria, combined with lab result of hypoalbuminemia and hypercholesterolemia suggest nephrotic syndrome.

PA 23.1.8 Describe the microscopic examinations of urine

Procedure of preparing urine for microscopic examination

12ml urine is centrifuged

Sediment is resuspended in 0.5 ml of urine

A drop of urine is transferred to a clean slide and covered with acover sli

Microscopic examination, with condenser in lowest point.



#### PA23.1.10 Discuss and identify various urinary abnormalities seen in microscopic examinations



Urinary crystals Alteration in pH, temperature, concentration of urine cause urinary salts to precipitate & form crystals



## PA 23.3 Describe and interpret the abnormalities in a panel containing semen analysis, thyroid function test, renal function test, liver function test

Name the vacutainer for collecting blood for the above tests

Vacutainer	Anticoagulant	Tests
	Ethylenediaminetetraacetic acid (EDTA)	Most hematological investigations: CBC, electrophoresis, PCR
	Trisodium citrate	Coagulation studies, ESR (Westergren)
	Heparin	Osmotic fragility test, flow cytometry
	Sodium fluoride (preservative) + potassium oxalate	Blood glucose levels
The second	No anticoagulant- for serum isolation	Blood bank tests, most biochemical tests Liver function tests Renal function tests Thyroid function tests

#### CHARTS WITH LINKERS, NORMAL VALUES AND INTERPRETATION

#### 1. Describe the normal findings in semen analysis (Given in the chart)

#### Interpret the given chart of semen analysis results

**Linker:** A couple came to OPD with inability to conceive after 5 years of marriage. Semen analysis of husband was advised and result showed

Parameter	Result	Normal (Reference value WHO-2010)
Count	<mark>5 million</mark>	15 -200 million / ml (<15million is oligospermia Absent sperms is azoospermia)
Total motility	40%	40-81%
Progressive motility	25%	38-75%
Non progressive motility	15%	Alma and a second s
Non motile	60%	IIIITE A
Volume	1 ml	1.5 – 6 ml
Vitality	<mark>45%</mark>	>58%
Morphology	6 1.28	
Normal	70%	▶ 4%
Defect Head Neck Tail	15% 5% 10%	AND IN

**COMMENT**: Semen analysis indicates oligospermia with low volume and vitality. Semen sample should be collected after 3-5 days of sexual abstinence.



## **2.** Describe the normal findings in thyroid function test (Given in the chart) Interpret the given chart of thyroid function test results

**LINKER**: A 50-year-old woman was admitted because of very rapid heart rate, severe weakness, weightloss and exophthalmos. She was extremely irritable, could not tolerate heat and was short of breath. Physical examination revealed bilateral eyelid lag. Thyroid profile shows:

Laboratory Test	Patient	Normal
Thyroid stimulating hormone (TSH)	<mark>0.2</mark>	0.5-7.2 mU/L
Total triiodothyronine(T3)	<mark>480</mark>	75-200 ng/dl
Total thyroxine (T4)	<mark>28</mark>	4.5-12 micro g/dl

**COMMENT:** The clinical presentation with low TSH and high T3,T4 indicates hyperthyroidism.

**LINKER**: A 12-year-old child presents in OPD with fatigue, lethargy, weight gain and cold intolerance. Mother also says his school performance is poor. On examination thyroid gland is enlarged. Thyroid profile shows:

Laboratory Test	Patient	Normal	Sec.
Thyroid stimulating hormone (TSH)	<mark>8.2</mark>	0.5-7.2 mU/L	E I
Total triiodothyronine(T3)	<mark>65</mark>	75-200 ng/dl	Et al d
Total thyroxine (T4)	<mark>3.5</mark>	4.5-12 micro g/dl	0

**COMMENT**: The clinical presentation with high TSH and low T3, T4 indicates hypothyroidism

#### **3.** Describe the normal findings in renal function test (Given in the chart)

#### Interpret the given chart of renal function test results

**LINKER**: A 11-year-old child was admitted with symptoms of headache, pain in the flanks, anorexia (loss of appetite for food). He passed red coloured (cola coloured) urine and had edema around his eyes. His blood pressure was 170/110 mm. Results of laboratory test are as follows:

Laboratory test	Patient	Normal
Total Protein	7.0 g/dl	6-8 g/dl
Albumin	4.5 g/dl	3.5-5.0 g/dl
BUN	45 mg/dl	8-25 mgl/dl
Serum Creatinine	3.0 mg/dl	0.6 -1.2 mg/dl
Hb	9g/dl	14-18 g/dl
24 hours urine protein	2.0 g/L	< 10mg/dl

**COMMENTS**: Abnormal values are highlighted. There is deranged renal function indicated by high ureaand creatinine. Patient has hypertension and hematuria (coca coloured urine) and 24 hour urine is < 3.5g/L which indicates a Nephritic syndrome, the most common in this age group being Post streptococcal glomerulonephritis.

**LINKER**: A 2-year-old child came to paediatric OPD with symptoms of fever, fatigue, loss of appetite for food and generalized anasarca. Mother gave a history of passage of foamy urine by the child for last 7days. Results of laboratory test are as follows

Laboratory test	Patient	Normal
Total Protein	4.5 g/dl	6-8 g/dl
Albumin	1.2 g/dl	3.5-5.0 g/dl
Serum cholesterol	<mark>380 mg%</mark>	150-280 mg%
BUN	25 mg/dl	8-25 mgl/dl
Serum Creatinine	1.0 mg/dl	0.6 -1.2 mg/dl
Hb	9g/dl	14-18 g/dl
24 hour urine protein	6.0 g/L	< 10mg/dl

**COMMENT**: Abnormal values are highlighted. Patient is a young child with generalized anasarca, massive proteinuria, hypercholesterolemia and hypoalbuminemia indicating Nephrotic syndrome. The most common cause in children is minimal change disease.

**LINKER**: A 70-year-old man came to emergency with symptoms of nausea, vomiting altered sensorium, gross edema and polyuria. He has history of uncontrolled diabetes for last 10 years. Results of laboratory test are as follows

Laboratory test	Patient	Normal
Serum cholesterol	180 mg%	150-280 mg%
Total Protein	6.5 g/dl	6-8 g/dl
Albumin	1.2 g/dl	3.5-5.0 g/dl
BUN	100 mg/dl	8-25 mgl/dl
Serum Creatinine	6.0 mg/dl	.6 -1.2 mg/dl
Urine for ketone	Positive	
Urine protein	6.0 g/L	and the second
Random Blood Sugar	400	<140 mg/dl
HbA1c	<mark>16 %</mark>	<6%

**COMMENTS**: Abnormal values are highlighted. A diabetic patient with altered sensorium with ketone bodies in the urine indicates Diabetic ketoacidosis. High Hb A1c suggests long term control of blood sugar levels is poor. Microalbuminuria is common in diabetics which accounts for low albumin.

#### 4. Describe the normal findings in liver function test (Given in the chart)

#### Interpret the given chart of liver function test results

**LINKER**: A 48-year-old man was hospitalized with the complaint of hematemesis. On physical examination the liver was enlarged with ascites and pedal oedema. He had history of alcohol intake forlast 15 years. Liver function test shows

Laboratory Test	Patient	Normal
Serum Bilirubin (Total)	<mark>5.0</mark>	0.2-1.0 mg/dl
Serum Bilirubin (Direct)	<mark>3.5</mark>	0.0-0.25 mg/dl
Serum Bilirubin (Indirect)	<mark>1.5</mark>	0.2-0.8 mg/dl
SGOT (AST)	<mark>550</mark>	0-40 IU/dl
SGPT (ALT)	<mark>320</mark>	0-40 IU/dl
Alkaline Phosphate	<mark>380</mark>	80-290U/L
Total Protein	<mark>4.0</mark>	6.3- 8.2 g/dl
Albumin	3.0	3.5-5.0 g/dl
Globulin	1.0	1.5-3.0 g/dl
A/G Ratio	1.2	1.2-1.5
GGT (gamma-Glutamyl transferase)	<mark>140</mark>	10-50 U/L (M),7-35 U/L (F)

**COMMENT**: Abnormal values are highlighted. History of ascites with hematemesis and prolonged alcohol intake suggest ALD with portal hypertension. Note the total protein value is low which contributes to ascites.



### PA 25.6 Interpret liver function test and viral hepatitis serology panel. Distinguish between obstructive from non-obstructive jaundice based on clinical features and liver function test (Certifiable skill)

#### (SAME OBJECTIVE AS PA 23.3.8 AND 23.3.9)

PA 25.6.1 Interpret the given viral hepatitis serology panel

PA 25.6.2 Distinguish obstructive from non-obstructive jaundice based on clinical features

PA 25.6.3 Distinguish obstructive from non-obstructive jaundice based on the given parameters of liverfunction test

**LINKER**: An 18-year-old boy presented with low grade fever, loss of appetite, nausea, vomiting and abdominal pain. He had history of travel to Delhi one week back. Liver function and serology shows

Serum Bilirubin (Total)	<mark>5.5</mark>	0.2-1.0 mg/dl
Serum Bilirubin (Direct)	<mark>3.5</mark>	0.0-0.25 mg/dl
Serum Bilirubin (Indirect)	2.5 ()	0.2-0.8 mg/dl
SGOT (AST)	<mark>1150</mark>	0-40 IU/dl
SGPT (ALT)	<mark>980</mark>	0-40 IU/dl
Alkaline Phosphate	<mark>340</mark>	80-290U/L
Total Protein	6.5	6.3- 8.2 g/dl
Albumin	3.5	3.5-5.0 g/dl
Globulin	2.5	1.5-3.0 g/dl
A/G Ratio	1.2	1.2-1.5
GGT (gamma-Glutamyl transferase)	50	10-50 U/L (M),7-35 U/L (F)
HBsAg	negative	P
IgM anti HBc	negative	
IgM anti HAV	positive	
IgM anti HCV	negative	

**COMMENT**: Abnormal values are highlighted. AST and ALT are markedly raised indicating hepatocyte injury. History of travel indicates change of eating places which may be unhygienic and lead to water/food borne disease. H/O fever with loss of appetite and nausea are seen in Hepatitis A infection.All other hepatitis serology apart from IgM anti HAV are negative ruling out other hepatitis virus.

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LINKER: A 20-year-old boy with history of drug abuse is admitted with jaundice. Liver function and serology shows

Serum Bilirubin (Total)	5.5	0.2-1.0 mg/dl
Serum Bilirubin (Direct)	3.5	0.0-0.25 mg/dl
Serum Bilirubin (Indirect)	2.5	0.2-0.8 mg/dl
SGOT (AST)	1150	0-40 IU/dl
SGPT (ALT)	<mark>980</mark>	0-40 IU/dl
Alkaline Phosphate	<mark>340</mark>	80-290U/L
Total Protein	6.5	6.3- 8.2 g/dl
Albumin	3.5	3.5-5.0 g/dl
Globulin	2.5	1.5-3.0 g/dl
A/G Ratio	1.2	1.2-1.5
GGT (gamma-Glutamyl transferase)	50	10-50 U/L (M),7-35 U/L (F)
HBsAg	positive	Uza A
IgM anti HBc	positive	IL Dr
IgM anti HAV	negative	
IgM anti HCV	negative	a star a

**COMMENT**: Abnormal values are highlighted. History of drug abuse with abnormal AST, ALT and positive serology for HBV indicates blood borne infection with HBV.

**LINKER**: 40-year-old lady presents with pain abdomen, jaundice and intense itching all over the body.Urine is dark colored. Liver function test shows

Laboratory Test	Patient	Normal	
Serum Bilirubin (Total)	5.5	0.2-1.0 mg/dl	
Serum Bilirubin (Direct)	5.0	0.0-0.25 mg/dl	
Serum Bilirubin (Indirect)	0.5	0.2-0.8 mg/dl	
SGOT (AST)	<mark>90</mark>	0-40 IU/dl	
SGPT (ALT)	110	0-40 IU/dl	
Alkaline Phosphate (ALP)	<mark>690</mark>	80-290U/L	
Total Protein	6.3	6.3- 8.2 g/dl	
Albumin	3.5	3.5-5.0 g/dl	
Globulin	1.5	1.5-3.0 g/dl	
A/G Ratio	1.2	1.2-1.5	
GGT (gamma-Glutamyl tranferase)	<mark>90</mark>	10-50 U/L (M),7-35 U/L (F)	

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**COMMENTS**: Abnormal values are highlighted. History of jaundice with intense itching and dark coloured urine is suggestive of obstructive jaundice. ALP and GGT will be markedly raised.

#### PA 27.8 Interpret abnormality in cardiac function testing in acute coronary syndrome PA 27.8.1 List the investigations you would perform in this patient

LINKER: A 65-year-old male came with acute onset of chest pain, sweating, dyspnea to the emergency

Complete blood count	Chest x ray
Serum electrolytes	and the second s
Random blood sugar	Cpublication of
Glycosylated haemoglobin	Bronshull softing
Serum cholesterol	Ken tag B Taper
Drugs – cocaine, methamphetamines	Hiter variousture
ECG - Electrocardiogram	- congestion Karles B Tree
CT coronary angiography	Kerdy B Tries
Cardiac proteins and enzymes	Caretorneyaly
Echocardiography	

**ECG** – arrhythmia – sinus bradycardia, ventricular tachycardia / fibrillation





**CT Coronary Angiography** 

PA 27.8.2 Enumerate the enzymes likely to be elevated in such a patient

Cardiac specific proteins – In MI necrosis of the cardiac muscle cause liberation of certain muscle proteins into blood

**TROPONIN** is the *most sensitive and specific* biomarker – aids in calcium mediated contraction of cardiac and skeletal muscles. Exists in two forms – cTnT (**Troponin T**), cTnI (**Troponin I**). It is normally not detectable.

CREATININE PHOSPHO KINASE (enzyme) - 2 isoforms - M and B forms

LACTATE DEHYDROGENASE - 5 isotypes, of which LDH1 is specific for heart

Cardiac Enzyme	Sensitivity and specificity	Rises at	Persists till	Comments
Troponin T and I	Most sensitive & specific	2 hours	10 days	Useful in all cases
CPK-MB	Sensitive not very specific	2-4 hours	2-3 days	Useful in acute cases
LDH 1 – Not very sensitive nor very specific		1-2 days 7 to 14 days Useful in patients presen after 48 hours of attack		Useful in patients presenting after 48 hours of attack

PA 27.8.3 Interpret the given chart of cardiac function enzymes and give your diagnosis



#### PA 31.3 Describe and identify the morphologic and microscopic features of carcinoma of the breast

- PA 31.3.1 Identify the organ and describe the gross specimen
- PA 31.3.2 Describe the microscopic features in the given slide
- PA 31.3.3 Distinguish between benign and malignant lesion of the breast



#### Linker: 48 y/o woman presented with a 5x6 cms fixed lump in the left breast. Overlying skin shows a "peau d'orange" appearance with retracted nipple.

Left Modified RadicalMastectomy was performed and specimen was sent for Histopathological examination.

Breast showing dimpled texture of skin-peau d'orange appearance ("French for skin of an orange"), along with nipple retraction

**Read: Carcinoma breast** 



**O/S**-shows specimen of breast (mastectomy) with a part of the skin with nipple and areola. The nipple looks puckered and retracted

C/S-shows a large grey white, partially circumscribed mass, with areas of necrosis and hemorrhage



Microscopy: Tumor cells arranged in glands with surrounding desmoplastic stroma in invasive breast carcinoma

**LINKER: 21-year-old girl with mobile lump in breast.** Lumpectomy specimen. Gross – 2 well circumscribed masses larger mass 3x4cms, smaller 1.5x1 cm.

Diagnosis - Fibroadenoma





Fibroadenoma (H&E 20x): uniform low stromal cellularity with no stromal cellular atypia



Whole microscopic section of the gross specimen above

#### PA 34.4 Identify, distinguish and describe common tumors of the skin

PA 34.4.1 Enumerate common tumors of skin

PA 34.4.2 List out fewdifferential diagnosis from the clinical images given

PA 34.4.3 Describe the microscopic features seen in squamous cell carcinoma, basal cell carcinoma and melanoma



#### PA 35.3 Identify the etiology of meningitis based on given CSF parameters (Certifiable skill)

PA 35.3.1 List out the normal parameters in normal CSF (Given in the chart)

PA 35.3.2 Identify the etiology of meningitis based on the given CSF parameters

Parameters	NORMAL	PYOGENIC	TUBERCULOUS	VIRAL
Proteins (mg/dl)	15-45 mg/dl	50-1500 mg/dl	45-300 mg/dl	Mild increase
Glucose (mg/dl)	45-80 mg/dl	<40 mg/dl	10-45 mg/dl	Normal
Microscopy	~100% lymphocytes with occasional monocytes (TLC- <5/µl)	Mostly neutrophils (TLC- >1000/µl)	Predominantly lymphocytes; or lymphocytes and neutrophils (TLC- 100- 600/µl)	Mostly lymphocytes (TLC- 5-300/µl)
Physical Appearance				

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