ACQUIRED HEMOLYTIC ANAEMIAS

(1)Which are suggestive clinical features of Hemolytic anemia?

- **Anemia**
- **Jaundice**
- **Splenomegaly**

(2)Which investigations should be performed in case of suspected Hemolytic Anemia?

- **Complete blood count**
- **Reticulocyte count**
- **Blood film**

(3)What changes are seen in blood count in hemolytic anemia?

- **Decreased Hb Concentration**
- **Increased MCV**

(4)Why MCV is increased in Hemolytic anemia?

- **MCV is increased in Hemolytic anemia because Reticulocytes are larger than mature RBCs.**

(5) What is significance of Schistocytes on blood film in hemolytic anemia?

- **Schistocytes are seen in**
- **Microangiopathic hemolytic anemia**
- **Mechanical hemolytic anemia**

(6)What is significance of Spherocytes on blood film in hemolytic anemia?

- **Spherocytes seen in**
  - Autoimmune hemolytic anemia
  - Alloimmune hemolytic anemia
  -Drug induced hemolytic anemia
- **Burns**
- **Paroxysmal cold hemoglobinuria**

(7) What is significance of microspherocytes on blood film in hemolytic anemia?

- **Microspherocytes are seen in Burns.**

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(8)What is significance of marked RBC agglutination in hemolytic anemia?

- **Marked RBC agglutination are seen in Cold Antibody induced Hemolytic anemia.**

(9) What is significance of minor RBC agglutination in hemolytic anemia?

- **Warm autoimmune hemolytic anemia**
- **Paroxysmal Nocturnal Hemoglobinuria**

(10)What is significance of Hypochromic microcytosis and basophilic stippling in hemolytic anemia?

- **Lead poisoning**

(11)What is significance of erythrophagocytosis in hemolytic anemia?

- **Paroxysmal cold hemoglobinuria**

(12)What is significance of atypical lymphocytes in hemolytic anemia?

- **Cold antibody induced hemolytic anemia associated with infectious mononucleosis.**

(13)What is significance of lymphocytosis with mature small lymphocytes in hemolytic anemia?

- **Autoimmune hemolytic anemia with chronic lymphocytic leukemia.**

(14)What is significance of thrombocytopenia in hemolytic anemia?

- **Autoimmune hemolytic anemia**
- **Thrombotic thrombocytopenic anemia**
- **Microangiopathic hemolytic anemia associated with DIC**
- **Paroxysmal nocturnal hemoglobinuria**

(15)What is importance in neutropenia in hemolytic anemia?

- **Paroxysmal nocturnal hemoglobinuria**

(16)What is significance of irregularly contracted cells?

- **Oxidant Exposure**
- **Unstable Hb**
- **G_6PD deficiency**

(17)What is acquired immune mediated hemolytic anemia?

- **It is as a result of auto antibodies to patients own RBC antigen or alloantibodies in patient's circulation either present in plasma or bound to RBCs.**

(18)What are secondary causes of autoimmune hemolytic anemia?

- **Lymphoproliferative disorders**
- **Autoimmune disorder like SLE**
- **Following Mycoplasma pneumonia or infectious mononucleosis**
- **Paroxysmal nocturnal hemoglobinuria**
- **Drugs like Alfa methyldopa**

(19)Which types of antibodies are seen in autoimmune hemolytic anemia?

- **Warm antibodies which combines with RBC at 37^0C**
- **Cold antibodies which combines with antigen at 2-4^0C**

(20)Which is most common types of warm autoantibody?

- **IgG**

(21)In which cases there is problem in detecting warm antibody?

- **Warm autoimmune hemolytic anemia with complement or red cells –**
- **needs specific antisera specific for complement.**

(22)Which test is used for detecting warm antibodies in patient's serum?

- **Indirect coomb's test**

(23)Which type of Cold Autoantibodies are generally seen?

- **IgM**

(24)What is Cold Haemagglutinin syndrome?

- **Cold autoantibodies can cause intravascular hemolysis, the intensity of which is characteristically influenced by ambient temperature.**

(25)Which are common Cold Autoantibodies?

- **Anti I specific: react strongly with majority of adult RBCs and weakly with cord blood RBCs.**
- **Anti i specific: react strongly with cold blood cells and weakly with adult cells.**
- **Anti pr**
- **Anti – M**
- **Donath Landsteiner: IgG with anti P specificity**

(26)Which type of autoantibodies crosses placenta?

- **Ig G**

(27)Which type of autoantibodies causes activation of complements?

- **Ig G**
- **Ig M**

(28)Which type of autoantibody attaches to monocytes / macrophage?

- **Ig G**

(29)How many antigen binding sites are there on autoantibodies?

- **Ig G: 2**
- **Ig A: 2**
- **Ig M: 5 or 10**

(30)Which type of Autoimmine hemolytic disease is caused by IgG?

- **Warm autoimmune hemolytic anemia**
- **Paroxysmal cold haemoglobinuria**

(31)Which type of AIHA is caused by IgA?

- **Warm autoimmune hemolytic anemia**

(32)Which type of AIHA is caused by IgM?

- **Cold autoimmune hemolytic anemia**

(33)What is Paroxysmal cold hemoglobinuria?

- **Paroxysmal cold hemoglobinuria is caused by biphasic autoantibody with anti P specificity.**
- **It is seen in children as acute viral infection.**
- **Binds to RBCs in cold→activation complements →causes hemolysis on rewarming at 37^0C.**
- **DAT positive for complement and negative at 37^0C.**

(34)What care should be taken while collecting blood sample for cold agglutinins?

- **Blood sample should be collected and maintained at 37^0C until cell and serum are separated.**

(35)How blood is stored before investigation for agglutinins?

- **Washed RBCs are frozen at -20^0C for weeks or months.**

(36)What test is done to know whether RBCs are coated with immunoglobulins or complement?

- **DAT using polyspecific broad spectrum reagent containing both IgG and anti C. Result should be positive.**

(37)What test is done to confirm whether RBCs having immunoglobulin or complement coating?

- **If DAT is positive by polyspecific, then repeat DAT with monospecific antisera (anti IgG or Anti C_3d)**

(38)In which conditions we see false positive antibody screening test?

- **Protein electrophoresis**
- **ANA +ve**
- **Infectious mononucleosis**
- **Mycoplasma**

(39)Why EDTA blood is preferred in DAT?

- **EDTA blood is preferred in DAT because if serum is used from clotted blood there can be presence of complements which give false +ve results.**

(40)What are causes of positive DAT?

- **Autoantibody on RBC with or without hemolytic anemia**
- **Alloantibody on RBCs. Example: Hemolytic disease of newborn, incompatible blood transfusions.**
- **Drugs adsorbed to RBCs. Example :Cephalosporin, Alfa methyldopa**
- **Complements.**
- **Paroxysmal cold hemoglobinuria.**
- **ABO incompatibility**
- **Hypergammaglobulinemia, Multiple myeloma**
- **Antiphospholipid antibodies like SLE**
- **Sickle cell disease**
- **Elevated blood urea nitrogen**
- **Sample from other than EDTA.**

ANTICOAGULANTS, THROMBOLYTIC AND ANTI-PLATELET THERAPY

(1) Which investigations should be done before giving anticoagulant therapy?

- **One stage (quick) PT**
- **APTT**
- **TT**
- **Platelet count**

(2) What should be done for uniformity of anticoagulation tests?

- **Use of ISI (International Sensitivity Index) to assess sensitivity of given thromboplastin**
- **Use of INR (International Normalized Ratio)**

(3) What is International Sensitivity Index?

- **Slope of calibration line obtained when PTs obtained with reference preparation are plotted on vertical axis and PTs obtained by test thromboplastin are plotted on horizontal axis**

(4) What is INR?

- **PT ratio which by calculation would have been obtained, had the original primary human reference, thromboplastin been used to perform the PT**
- **INR= PT ratio obtained using the test thromboplastin to the power of ISI of test reagent**

(5) How PT ratio is obtained?

- **It is obtained by calculated patient's test result and log mean normal PT from normal 20 donors**

(6) What is the action of Heparin?

- **It binds to antithrombin thereby accelerating and enhancing antithrombin's inhibition of major coagulation enzymes**

(7) What is Heparin induced thrombocytopenia seen?

- **Most of the patients receiving heparin experience small immediate drop in their platelet count which is harmless**
- **This is due to binding of heparin to platelets**

(8) What is Hirudin?

- **It is direct thrombin inhibitor**

(9) Which are thrombolytic agents?

- **Streptokinase**
- **Recombinant tissue type plasminogen activator**

(10) What is streptokinase?

- **Purified fraction of filtrate from cultures of Streptokinase haemolyticus used as thrombolytic agent**
- **Causes fibrinogenolysis**

BASIC HEMATOLOGICAL TECHNIQUES

(1)What are pros and cons of manual and automated methods?

Manual	Automated
Adv: Low cost	Disadv: High cost depends on Calibration and use of reagents. Standardization needed.
Disadv: Laboratory extensive.	Adv: Rapid, Accurate,Precise.

(2)What is Oxygen Combining Capicity of blood?

- **1.34 ml of Oxygen / gram of Hemoglobin.**

(3) What is correlation between iron content and Hemoglobin?

- **0.347 gm iron = 100 gm Hemoglobin.**

(4) Which method of Hemoglobin estimation is standard and internationally accepted?

- **Cyanmethhemoglobin.**
- **Hb, Hi, Carboxyhemoglobin are converted into HiCN(Cyanmethhemoglobin).**
- **Absorbance measured at 540 nm or photoelectric colorimeters with yellow-green filter.**

(5)What is pH of Drabkin's solution?

- **pH - 8.6**

(6) What is Modified Drabkin's solution?

- **pH – 7.0 – 7.4 (Internationally Recommended)**
- **Advantage: Cause less turbidity, requires short conversion time.**

(7)What are disadvantages of Modified Drabkin's solution?

- **Detergent causes some frothing.**

Dr.CHERRY SHAH

(8) What are constitutes of Drabkin's Reagent?

- **Pottasium ferricyanide – 200 mg.**
- **Pottasium cyanide – 50 mg.**
- **Potassium dihydrogen phosphate – 140 mg.**
- **Nonionic detergent – 1 ml.**
- **Distilled / deionized water upto 1 litre.**

(9)Which nonionic detergents are used in Drabkin's solution?

- **Nonided P-40.**
- **Triton X-100.**

(10)What are criteria to good Drabkin's Reagent?

- **pH – 7.0 – 7.4 checked once a month by pHmeter.**
- **Diluent clean and yellow pale.**
- **Measured against water as blank at 540 nm – absorbance should be zero.**

(11) Where should Drabkins solution be stored ?

- **In amber brown coloured borosilicate glass bottle.**
- **At ambient temperature less than 30˚C.**

(12) How Hb standard is prepared ?

- **Specimen of Anticoagulated whole blood**
- **Hb is first determined by HiCN method**
- **Blood is diluted in 1:201 by pipetting 20ml of blood in 4 ml of ammonia**
- **Sequential dilutions**
- **Absorbance read at 540nm or photometer with yellowgreen filter**
- **Results plotted on graph**

(13) On what Hb standards specifications are based?

- **Specifications are based on relative molecular mass of human Hb**

(14) From whose blood standard of Hb are made ? why?

- **From Ox blood as its molecular mass is near to Human Hb.**

(15) How will you calibrate spectrophotometer for Hb estimation?

- **Checking wavelength with holmium oxide filter**
- **Absorbance with a set of calibrated neutral density filters**

(16) On which principle portable Hemoglobinometer is based on?

- **It is based on HbO_2 method**

(17)What is noninvasive screening test for Hb estimation?

- **Infrared spectroscopy is noninvasive screening test for Hb estimation.**

(18) What is microhaematocrit method?

- **It is carried out in blood contained in capillary tube of 75mm with internal diameter of about 1mm.**
- **It is accurate and precise method.**

(19) What attention should be paid in microhematocrit method?

- **K_2 – EDTA recommended as K_3 – EDTA causes shrinkage and thus reducing PCV .**
- **Excess anticoagulant causes (more than 2:2) false low PCV**
- **Test should be performed within 6 hours of sample collection**
- **Capillary calibration by tachometer is necessary**
- **Should be verified by regular checking of 5 to 10 minutes of normal and polycythemic blood.**
- **Reading should be taken immediately after centrifugation as RBCs swell**
- **Magnifying glass should be used to take reading.**

(20) What is plasma trapping?

- **Amount of plasma trapped between red cells in lower end of red cell column and red cell dehydration during centrifugation in PCV is plasma trapping.**

(21) In which conditions plasma trapping is seen?

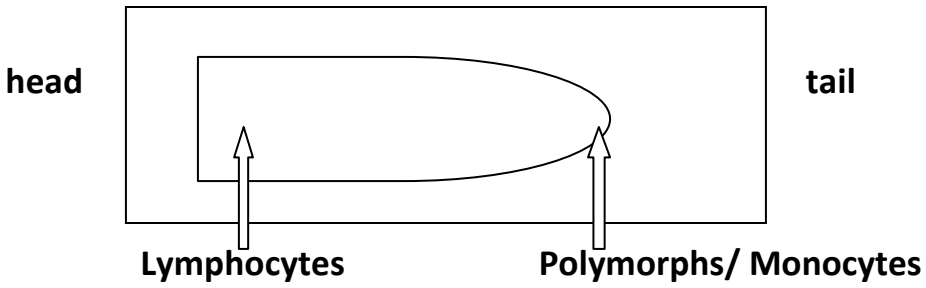
- **Macrocytic, Spherocytosis, Thalassemia, Hypochromic anemia, Sickle cell anemia. (more than 20%)**

(22) What happens if the smear film is thin or rough edged spreader is used?

- **More than WBCs accumulate at edge.**
- **Qualitative abnormality occurs: Neutrophils and monocytes at margin (tail) ; Lymphocytes at head.**

(23) Schematic drawing of blood film on slide.

- **Spread from left to right.**



(24) Chances of error in counting cells in Peripheral smear.

- **i) Thick smear : - cells cannot be counted**
  - **Donot allow excess neutrophils/ monocytes at edge**

ii) High count : eg., Leukemia (cells to be counted on well spread area)

iii) Artefactual differences

iv) Random distribution- out of 100, if neutrophils 50%, variation of ± 2SD; count falls between 36-64%.

v) 200 cells count more reliable.

(25) How will you repeat differential WBC count?

- ✓ **Repeated in absolute number (x 10^9)**
- ✓ **Myelocytes/ Metamyelocytes to be repeated separately**
- ✓ **Band cells to be counted as neutrophils (less than 6%);**
  Increases in inflammation even if there is no leucocytosis.

(26) What is total nucleated cell count (TNCC)?

- **When nucleated RBCs are included on total WBC and in differential count as percentage and reported as absolute number as leucocytes.**

(27) What is corrected WBC count?

- **If nucleated RBCs are present in significant number,**

  Eg.,WBC count – 8.0 x 10^9/ lit

  nRBCs _ 25%

  Than corrected WBC count = 8 – (8 x 25/100)

  = 6 x 10^9

  = WBC – (WBC x nRBCs/100)

(28) Which reference method id used to validate WBC count on automated system?

- **i) Manual procedure by counting atleast 200 cells by TWO independent observers from same sample.**

  ii) Flowcytometry.

(29) What are reticulocytes?

- **Juvenile RBCs with remnants of ribosomal RNA present in large amounts in cytoplasm of nucleated precursors.**

(30) Why reticulocytes are stained with basic dyes?

- **Because they contain ribosomal RNA which has property of reacting with basic dyes.**

(31)Which dyes are used to stain Reticulocytes?

- **Azure B.**
- **Brilliant Cresyl Blue.**
- **New Methylene Blue.**
- **Forming blue / purple precipitates of granules or filaments.**

(32)How stages of maturation are identified in Reticulocytes?

- **Most immature reticulocytes : largest amount of precipitable material.**
- **Least immature: dots or short strands.**

(33)Four groups are classified in form of immaturity of reticulocytes with large clump of reticulin in group I to most mature- few granules of reticulin (group IV)

(34)When does complete loss of basophilic material occurs in Reticulovytes?

- **Most in blood stream or in spleen after cells have left bonemarrow after 24 hours in circulation.**

(35)What are Stimulated Reticulocytes?

- **In case of stress the average maturation time taken by reticulocytes is long as 3 days so they are found in higher proportion in circulation.**

(36)What is Reticulocyte Index?

- **When there is severe anemia reticulocyte count should be corrected and expressed as Reticulocyte Index.**
- **Reticulocyte Index = $\frac{\text{Observed reticulocytes (\%) X Measures Hemoglobin or PCV}}{\text{Normal Hemoglobin or PCV}}$**

(37)Which is better Reticulocyte Stain?

- **New Methylene Blue is better than Brilliant Cresyl Blue as it stains reticulofilamentous material in reticulocytes more deeply and uniformly.**

(38)Which dye can be used in place of New Methylene Blue?

- **Azure B as it does not precipitate and is available in pure form.**

(39)For reticulocyte count large proportion of anemic blood is needed and small Proportion of polycythemic blood is needed.

(40)How will you calculate Absolute Reticulocyte Count?

- **Number of reticulocytes in n fields = x.**
- **Average number of red cells per field = y.**
- **Total number of red cells in n fields = n x y.**
- **Reticulocyte percentage = [x / (n X y)] X 100 %.**
- **Absolute Retic Count = % X RBC.**

(41) Which is alternative method for Absolute Retic Count?

- **Based on principle of balanced sampling using Miller ocular.**
  (Eyepiece giving a square field in corner of which is smaller ruled square, One-ninth the area of total square).
- **Reticulocytes are counted in large square.**
- **Red cells are counted in small square.**

(42) How will you differentiate between Reticulocyte and Red cell inclusion?

- **Pappenheimer type of granular material (Iron containing):**
  - **Present as single small dot -- dark shade of blue.**
  - **Perl's reaction -- Positive**
- **Heinz body : Denaturation by brilliant cresyl blue or New Methylene blue**
  - **Present as round inclusion bodies—greenish blue.**
  - **Light shade of blue.**
  - **Stain with Methyl violet.**

(43) Other methods of Reticulocyte count.

- **Fluorescence microscopy by Acridine orange:**
  - **RNA-- Orange red**
  - **DNA—Yellow**
- **Phase contrast- Stained as routine method.**
  - **Reticulocytes- Non-nucleated Red cells with atleast one or two blue staining particle.**

(44) Normal Reticulocyte count:

- **Adult & Children : 0.5-2.5%**
- **Infants (Full term, Cord blood) : 2-5%**

AUTOMATION

(45) What is the difference between Semiautomated and Automated instruments?

- **Semiautomated Instruments:**
  - **Certain steps like dilution of blood samople has to be carried out.**
  - **Measure only small number of component (eg., WBC, Hb)**

- **Automated Instruments:**
  - **No steps to be done manually**
  - **Measures 8-20 components**
  - **High level of precision**
  - **Accurate results**
  - **Flag from wrong results**

(46) What is Barcoding?

- **Automated procedures for sample recognition.**

(47) How is blood sampling done?

- **By piercing the cap of closed tube , so samples with any infection hazard can be handled with maximum safety.**

(48) How does automated counter measure Hb?

- **By modification of Manual HiCN (Hydrogen Cyanide) method with cyanide reagent and with nonhazards.**
- **Chemical as Sodium lauryl Sulphate; avoids possible environmental hazards from disposal.**

(49) What is basis of counting system?

- **RBCs are poor conductors of electricity.**
- **Diluents are good conductors.**
- **Difference form basis of counting system in Impedance Counting.**

(50) How is flow rate of required volume maintained in counter?

- **By Mercury siphon**
- **By displacement of tightly fitting pistons.**

(51) What is diluents in counter made up of?

- **It is made up of buffered electrolyte solution.**

(52) What is mechanism of action of cell counter?

- **Constant current between two electrodes – one in sample and another inside aperture.**
- **Blood cell goes in apertures- displaces conducting fluid- increases electrical resistance.**
- **Changes electrical potential between electrodes – lasts as long as red cell takes to pass through aperture.**
- **Height of pulses indicates volume of cells passing through.**
- **Pulses displayed on oscillograph, pulse height counted to determine volume**

(53) What is principle in light scattered cell counter?

- **RBCs and other cells are counted by means of electro-optical detectors.**

(54) What is the mechanism in light scattering?

- **Diluted cell suspension flows through aperture – in single file in front of light source**
- **Light scattered by cells**
- **Scattered light detected by photomultiplier/photodiode**
- **Converted into electrical impulses and counted.**

(55) How is volume of cell calculated?

- **Amount of light scattered x Surface area – volume of cell**

(56) What are chances of inaccuracy in cell counter?

- **Two cells pass through aperture and is counted as one**
- **Pulse generated during electronic dead time of circuit**
- **By recirculation of cell already counted**
- **By agglutination of RBCs where clump is counted as one , mostly due to cold agglutination (can be avoided by prewarming)**
- **Counting of bubbles, lipid droplets, microorganism or exogenous particles like cells.**
- **Faulty maintenance leading to variation in volume aspirated or flow rate.**

(57) What should be pH of diluents used in cell counter>

- **It should be between 7.0 – 7.5**

(58) What should be done if diluents is not available?

- **Physiological saline (NaCl – 9g/lit) can be used provided counts are performed immediately after dilution as it can lead to error of sphering**

(59) What is threshold?

- **Upper and lower limits of counting cells is threshold.**

(60) What is need of setting upper and lower thresholds in RBC count?

- **Platelets are counted as RBCs in absence of lower threshold**
- **WBCs are counted as RBCs in absence of upper threshold**
- **Microcytic RBCs are included with large platelets.**

(61)What is difference between Hematocrit and PCV (Packed Cell Volume)?

- **Both are same except that International Council for standardization in Hematology has suggested that Hematocrit is to be used in Automation while PCV is to be used manually.**

(62)How RBC, PCV and MCV are determined on cell counter?

- **Passage of cell through aperture leads to generation of electric pulse -> the height of pulse is proportional to cell volume.**
- **Number of pulse -> RBC count.**
- **Pulse height analysis -> PCV, MCV.**
- **Average pulse height – MCV.**
- **PCV = MCV X RBC.**

OR

Summation of Pulse Height – PCV

$$\text{MCV} - \frac{\text{PCV}}{\text{RBC}}$$

(63)How calibration for PCV and MCV done?

- **PCV - By manual determination.**
- **MCV – By means of pulse heights generated by latex beads stabilized cells or other particles of known size.**

(64)What is shape factor?

- **RBCs are biconcave and flexible.**
- **In aperture impendence system due to shape of RBCs apparent volume is greater than the true volume.**

(65)What is mechanism of Shape factor?

- **In Impendence Counters normal disc shaped RBCs become elongated and cigar shaped causing deformation of shear force.**
- **Cells with reduced Hemoglobin undergo more elongation -> leading to reduced shape factor -> reduced pulse height ->underestimation of MCV.**

(66)In which conditions MCV will be overestimated in cell counter?

- **In conditions like Spherocytosis where RBCs have rigid membrane and high Hemoglobin -> less deformation -> High MCV.**

(67)What is mechanism to avoid error in cell counter for MCV and PCV?

- **By light scattering instruments each cell is measured at two angles forward and at an angle of $90^{0.}$ This permits both volume and Hemoglobin.**

(68)What are causes of error in interpretation of MCV / PCV by Automated Cell? Counter?

- **Resulting from microclots.**
- **Extreme microcytosis.**
- **Presence of Cold agglutinins -> High MCV.**
- **Sickle cell anemia.**
- **Alternations in Plasma osmolarity e.g. In Hyperglycemia.**

(69)What is importance of doing Red Cell Indices?

- **Clarify anemia.**
- **Differentiate between Iron deficiency anemia and Thalassemia.**

(70)What does Red Cell distribution width in Automated Cell Counter indicate?

- **Quantitative measurements of variation in cell volume equivalent to microscopic assessment of degree of Anisocytosis.**

(71) How is RDW (Red Cell distribution width) expressed as standard deviation in fl or a CV?

- **In percentages (%).**

(72) What is significance of RDW?

- **Distinguish Iron deficiency anemia from Thalassemia trait.**
  - **In iron deficiency anemia—RDW is Increased.**
  - **In thalassemia trait—RDW is Normal.**

- **Also Distinguish Megaloblastic anemia from other caused of Macrocytosis.**
  - **In Megaloblastic anemia —RDW is Increased.**
  - **In other causes of Macrocytosis —RDW is Normal.**

(73) Which chemicals are used as red cell lytic agent for Total WBC count in automated cell counter?

- **Cetrimide + Formaldehyde.**

(74) What are chances of errors in automated cell counter for WBC count?

- **Giant platelets**
- **Nucleated RBC**
- **Failure to lyse RBCs.**
- **Increased WBC count in:**
  - **Uremia**
  - **Neonate cells**
  - **Abnormal Hb**
  - **Platelet clumping**
  - **Cryoglobulinemia**
- **Decreased WBC count in:**
  - **Leucocytic agglutination**
  - **Prolonged sample storage**
  - **Fragile cell (Leukemia).**

(75) What happens to RBCs in light scattering instruments?

- **They arenot lysed but are rendered transparent.**

(76) What does three part differential count mean?

- **It assigns cells to categories designated as;**
  a) Granulocytes (eosinophils, basophils) or Large cells
  b) Lymphocytes or Small cells
  c) Monocytes, Mononuclear cells or Middle cells

(77) What does five to seven part differential count mean?

- **It classify cells as Neutrophils, Eosinophils, Basophils, Lymphocytes, Monocytes and In extented DC (differential count). Also includes large immature cells (blasts), Atypical lymphocytes (small blasts).**
- **Flag or reject counts with nucleated RBCs.**

(78) Difference between Three part and Five/Seven part counters.

- **Three part counter has Single channel.**
- **Five/Seven part counter has Two or more channels.**

(79) On what does analysis of cell counter depend on?

- **Volume of cell**
- **Physical characteristics of cell**
- **Binding of certain dyes to granules**
- **Activity of cellular enzymes like Peroxidase.**

(80) With which cells degree of precision is more in manual count than in automated counter?

- **Monocytes**
- **Basophills**

(81)In which cases accuracy of cell counter is affected?

- **Unusual cell characteristics.**
- **Aging of blood specimen.**

(82)Which cells though abnormal are not flagged in cell counter?

- **Nucleated RBCs.**
- **Immature granulocytes.**
- **Atypical lymphocytes.**
- **Occasionally blasts.**

(83)What error can occur in WBC count in case of Nucleated RBCs?

- **As there is no function of flagging NRBCs in total WBC in cell counter, the total WBC count is neither true nor NRBC count is true – this leads to wrong Absolute count.**

(84)Which instruments available count NRBCs and correct WBC count?

- **Abott CellDyn 4000.**
- **Sysmex XE 2100.**
- **Beckman Coulter LH750.**

(85)What is disadvantage of manual count?

- **Slow method.**

(86)What does graphic display of automated instruments include?

- **Histograms of Red cells, White cell, Platelet, sometimes Hemoglobin concentration.**
- **Differential count as Scatter plots.**

(87)In which situations factitiously low automated platelet count is found?

- **Giant platelets when identified as RBCs.**
- **EDTA induced Platelet clumping.**

(88)In which situations factitiously high automated platelet count is found?

- **Microcytic or fragmented RBCs.**
- **Cell fragments in leukemia.**
- **Bacteria, fungi.**

(89)What is significance of MPV?

- **For differentiating essential thrombocytopenia from reactive thrombocytosis.**

(90)What is Platelet Distribution Width (PDW) in automated cell counter?

- **Measure of platelet anisocytosis.**

(91)How can we measure reticulocyte on cell counter?

- **By using various dyes and flurochromes that combine with RNA.**
- **E.g. Auramine O, Thiazole orange(fluorescent).**
  Oxazine 750, New methyelene blue(Non fluorescent).

(92)What are chances of error in measuring Retic count automated cell counter?

- **The dyes also stain DNA of nucleated cells.**
- **Inaccuracy occurs when leucocytes, platelets, Howell jolly bodies or malarial parasites are counted in retic count.**

(93)What are different methods to Calibrate Automated cell counter?

- **By using fresh normal blood specimen to which values have been assigned for Hb, PCV, RBC, WBC, Platelets by standard reference method.**
- **By using stable calibrant (preserved blood).**
- **By using commercial calibrant.**

(94)How will you assign normal reference range?

- **4 ml of blood from three normal volunteers in EDTA.**
- **Hb, PCV, RBC, WBC done.**
- **Mean found.**

(95)What does flagging mean?

- **Signalling that specimen being analysed have significant abnormality.**

BLOOD CELL ANTIGENS AND ANTIBODIES

(1) How many blood group systems exist?

- **Around 25**

(2) How blood group systems are determined?

- **By series of red cell antigens determined either by single genetic locus**

(3) What are alleles?

- **Alternative forms of gene coding for red cell antigens at a particular locus are alleles and individual may inherit identical or non identical alleles**

(4) Which chromosome is assigned to ABO and Rh system?

- **ABO- Chromosome 9**
- **Rh- Chromosome 1**

(5) How red cell antigens are determined?

- **By carbohydrate structure or protein structure**
- **Carbohydrate structure – indirect gene product coding for an intermediate product usually on enzyme which creates antigen specificity by transferring sugar molecules into protein or lipid**
- **Protein antigen – Direct gene products whose specificity is determined by the inherited aminoacid sequence**

(6) Which red cell antigens are erythroid specific?

- **Rh, LW, Kell, MNS**

(7) Where ABO antigens are expressed?

- **RBCs, endothelial and epithelial membrane**

(8) Which genes control expression of ABO antigens?

- **ABO located on chromosome 9**
- **FUT1 (H), FUT2 (Se) – located on chromosome 19**

Dr.CHERRY SHAH

(9) Which type of disaccharide chains occur on RBCs?

- **Four types**
- **Type 1- found in plasma and secretion is substrate of FUT2 (Se) gene**
- **Type 2,3,4- found on RBCs and are substrate for FUT1 (H) gene**

(10) How does A gene differ from B gene?

- **By consistent nucleotide substitutes**

(11) Which genes determine expression of A and B antigen?

- **H and Se genes giving rise to glycosyl transferrases that add L-Fucose producing H antigen.**
- **Further glycosyltransferase converts H substance into A and B antigens by the terminal adding of N acetyl-D-Galactosome – A and D- Galactose –B**

(12) Why O gene remains same as H?

- **Because O gene produces inactive transferase, therefore H substance persists unchanged.**

(13) What is Bombay Phenotype?

- **Oh Phenotype where individual is homozygous for h allele of FUT_1 and hence cannot form H precursor of A and B antigen.**
- **It is Rbc type O but plasma contains anti H in addition to Anti A, Anti B and Anti AB which are active at 37˚ C.**
- **Can be given only oh Red cells in transfusion.**

(14) Which are subgroups of Group A and AB?

- **A_1, A_1B , A_2 , A_2B**

(15) What is the cause of A_2 subgroup?

- **A_2 transferase is less efficient in transferring N-Acetyl-D-Galactosamine to available H-antigen sites and cannot utilize types 3 and 4 disaccharide chains.**

- **They have fewer A antigen sites than A_1**

(16) How subgroups A1 and A2 can be distinguished?

- **By lectin Dolichos Biflorus which reacts with only A_1 cells.**

(17) How agglutination reaction is assessed according to group?

- **H antigen content depends on ABO group.**
- **When assessed by anti H the strength of reaction tends to be graded as $O > A_2 > A_2B > B > A_1 > A_1B$**

(18) When A, B and H antigens are detected?

- **They are detectable early in fetal life but are not fully developed on Rbc at birth.**
- **Reaches adult level at 1 year of age and remains constant until old age.**

(19) What are secretors?

- **Secretors have 11 substances in saliva and other body fluids together with A substances, B substances or both depending on blood groups.**
- **This ability to secrete A, B and H substance in water soluble form is controlled by FUT_2**

(20) What are non secretors?

- **Only traces of A, B and H substance are found in saliva and other body fluids even though antigens are expressed normally on Rbcs and other tissues.**

(21) How individual's secretor status is determined?

- **By testing for ABH substance in saliva.**

(22) What is acquired B group?

- **Group A individuals acquire B antigen from bacterial infection due to release of deacetylase enzyme.**

- **This enzyme converts N-Acetyl-D-Galactosamine into α-galactosamine which is similar to galactose, the immunodominant sugar of Group B.**
- **Also found in cases of Carcinoma of GIT.**

(23) Which disease is predominant in individuals with A Antigen?

- **CA Stomach**

(24) Which disease is predisposed in individuals with O group?

- **Peptic Ulcer**
- **VWF and Factor VIII are 25% less in O group persons.**

(25) When does ABO Antibodies appear?

- **In first few months of birth due to exposure of ABH Antigen like substances in diet or environment.**

(26) Which type of Immunoglobulins are Anti A and Anti B?

- **IgM – react best at low temperature but lytic at 37˚C.**

(27) What are Hyper immune Anti A and Anti B?

- **Occurs in response to transfusion of pregnancy or following same vaccines.**
- **Of IgG class – usually produced by O group or A_2 group individuals.**
- **May cross placenta and cause hemolytic disease of newborn if produced in mother.**
- **Reacts in wide thermal range.**

(28) What is significance of Anti A_1?

- **Reacts only with A_1 and A_1B cells**
- **Found in serum of A_2 individuals**
- **Acts as Cold agglutinin, not reacting at 37˚C**
- **Limited Cell destruction.**

(29) What is significance of Anti H?

- **Reacts most strongly with group O and A_2 Rbcs.**
- **Acts as Cold Agglutinin.**

(30) What is significance of Anti H in Bombay Blood Group?

- **It is IgM type of Antibody and causes lysis at 37˚C**

(31) Why name Rhesus is given to Rh system?

- **The original antibody was raised by injecting red cells of Rhesus monkeys into rabbits and guinea pigs.**

(32) On which chromosome Rh is located?

- **Chromosome 1**
- **With closely linked genes RHDr, RHCE.**

(33) Which gene is absent in D negative individual?

- **Rh D**

(34) What is Du negative?

- **It is weak D antigen with quantitative reduction in D antigen sites.**
- **Arise from an uncharacterized transcriptional defect.**

(35) Which nomenclatures are used to describe Rh system?

- **Wiener's Rh H5 terminology**
- **Rosenfield's numeric notation**
- **Fisher's original theory – recommended by WHO**

(36) In which case Rh DNA typing is needed?

- **In case of Hemolytic disease of newborn for fetal D- status to describe the clinical management of pregnancy**

(37) Which are the sources of Rh DNA typing?

- **Amniotic fluid (Amniocentesis)**
- **Trophoblastic cell (chorionic villi)**
- **Maternal blood**

(38) What are other applications of Rh DNA typing?

- **Paternity testing**
- **Forensic medicine**

(39) Which method is used for Rh DNA typing?

- **PCR**

(40) What is Rh null phenotype?

- **Complete absence of Rh antigen**
- **Associated with hemolytic anemia with Spherocytes and stomatocytes in blood film, increased osmotic fragility.**

(41) Which are Rh Antigens?

- **C, D, E, c, d, e**

(42) Which Rh Antibodies are timed against Rh Antigens?

- **All except d**
- **anti -D, anti -C, anti –c , anti –E, anti –e**

(43) Which are naturally occurring Rh Antibodies?

- **Anti –E and anti –C**

(44) Which immunoglobulins are of Rh Antibodies?

- **IgG - react optimally at 37˚ C.**
  - **Do not bind to complement.**
  - **Detection is enhanced by use of enzyme treated Rbcs.**
  - **Extravascular hemolysis occurs in Spleen.**

(45) Which is most common Rh Antibody found? What is its significance?

- **Anti D**
- **May cause hemolytic Transfusion Reaction.**
- **Common cause of fetal death due to Hemolytic Disease of New Born.**

- **Anti D prophylaxis is needed.**

(46) Which is the second most common Rh Antibody found?

- **Anti – C**
- **Can give rise to severe Hemolytic Disease of Fetus and Newborn.**

(47) What is the severest form of Incompatibility seen?

- **O recipient and A red Cells.**

(48) On what mechanisms immune mediated hemolysis of Rbcs is dependent on?

- **Immunoglobulin class of Antibody.**
- **Ability of antibody to bind to complements.**
- **Interaction with reticuloendothelial system with maximum participation of macrophage in spleen.**

(49) How does intravascular hemolysis occur?

- **By binding of complement components cascade and formation of membrane attacking complex.**
- **Characteristic of IgM antibodies.**
- **Mostly in ABO transfusion reactions.**
- **Also occurs in Drug Induced Hemolysis.**

(50) Which Red Cell Autoantibodies cause Intravascular Hemolysis?

- **IgG Autoantibody of PCH.**
- **Autoantibodies of Haemagluttinin Disease.**

(51) How does extra vascular hemolysis occur?

- **By mononuclear phagocytic system**
- **Characteristic IgG antibodies**
- **Predominantly in spleen**
- **Caused by non complement binding IgG antibodies**

- **Form microspherocytes by phagocytosing sensitive RBCs**

(52) What should be done to activate complements if antibodies are not complete?

- **Antiglobulin test using appropriate anti complement antibody**

(53) Which are important sites for extravascular hemolysis?

- **Liver & spleen**

(54) Which factors affect interaction between sensitized cells and macrophages in hemolysis?

- **IgG subclass- IgG1 & IgG3 have more affinity**
- **Antigen density**
- **Regulation of macrophage activity**

(55) Which factors upregulate activity of macrophages?

- **Cytokines**
- **Interferon**
- **Interleukin 6**

(56) What is an end point of antigen antibody reaction?

- **Agglutination**
- **Lysis owing to complement**

(57) Which are the stages of antigen antibody reaction?

- **First stage when antibody binds to red cell antigen (sensitization)**
- **Second stage involving lysis and agglutination of sensitized cells**

(58) What is sensitization?

- **When antibody binds to antigen**
- **It is reversible**

(59) Which factors influence sensitization?

- **Temperature- cold antibodies (IgM) bind best to RBCs at 4˚C, while warm antibodies (IgG) bind efficiently at body temperature**
- **pH – usually pH 7 is perfect with range of 5.5-8.5**
- **ionic strength of medium- low ionic strength increases rate of antibody binding**

(60) On what factors agglutination or lysis of cells depend on?

- **Centrifugation**
- **Reducing intercellular distance by pretreatment of red cells with protease enzymes (papain or bromelin) y reducing surface charge**
- **Adding polymers e.g. albumin**
- **Bridging between sensitized cells with an antiglobulin reagent e.g. Coomb's test**

(61) What is negative charge on surface of Rbc owe to?

- **Sialic Acid Residues which keep cells apart.**

(62) What is normal distance between Rbcs in normal Saline?

- **18 nm**

(63) Why IgM antibodies agglutinate better than IgG antibodies?

- **Span between antigen binding sites of IgM molecules is around 30nm, which is sufficient to allow IgM antibodies to bridge between saline suspended cells while IgG molecules have a shorter span i.e 15nm and are unable to agglutinate.**

(64) What are the general aspects of Quality Assurance according to British Committee for standards in Hematology guidelines?

- **Laboratory should document its Quality System, appropriate to its requirements.**
- **Sensible inclusion of Internal controls in all tests should be undertaken.**
- **Participate in EQAS programmes.**
- **Use of Validated systems against documented requirements.**
- **Should cover the failure of Automated instruments.**
- **Should build checks at all critical points. E.g. Preserving Patient's Samples.**

(65) What are points of reagents reassuring quality of it?

- **Specifications according to International Society of Blood Transfusion or American Association of Blood Banks.**
- **Reagents should be in accordance with manufacturer's Instructions.**
- **Should be validated in accordance with BCSH guidelines.**
- **Record of all batch numbers and expiry dates of all reagents should be noted.**

(66) What care should be taken in techniques in view of Quality Assurance?

- **All procedures should be in accordance with recommended practices.**
- **All changes in techniques should be validated with BCSH guidelines.**
- **Implementation of new techniques should be done.**
- **Authorized SOP's covering all aspects of laboratory equipment must be documented.**

(67) What care should be taken in staff training and proficiency in view of Quality Assurance?

- **Documented programme for training laboratory staff covering all SOP's in use.**
- **Laboratory works to be undertaken by appropriately trained staff.**

- **Documented programme for assessing staff proficiency including details of actions.**

(68) What is importance of Auditing and reviewing in quality Assurance?

- **System for documenting and reviewing should be there.**
- **Reviewing all incidents of non compliance with procedures.**
- **Full audit trial of Laboratory step results, interpretation, authorization.**
- **Independent audits to assess compliance with documented in house procedures.**

(69) Why plasma is preferred over serum in microplate technology and automated systems?

- **In plasma Complement is inactivated by EDTA. This helps in detecting same complement binding antibodies.**

(70) What should be preferred for Red cell Alloantibodies, Serum or Plasma?

- **Serum**

(71) Which problems are encountered in case of Rbc Storage?

- **Red Cell Lysis**
- **Decrease in potency of Red cell antibodies, especially IgM**
- **Bacterial Contamination**

(72) For how many days blood group samples can be stored?

- **7 days at 4˚C**

(73) How patient's serum or plasma is stored?

- **Stored Frozen at -20˚C or below in 1-2 ml volumes in plastic vials.**

(74) For how much time complement remains active in frozen serum?

- **1-2 weeks**

(75) Which red cell suspension are routinely used for antibody titrations?

- **2-3% suspension of washed red cells in phosphate buffered saline at**

pH 7.0

(76) Why LISS (Low Ionic Strength Solution) is important for RBC suspensions?

- **Rate of association of antibodies with red cell antigens is enhanced. Therefore incubation period of IAT can be shortened.**

(77) What are the chances of false positivity in using LISS solution?

- **Nonspecific agglutination may occur when Nacl concentration <2g/lit are used.**
- **Complement compounds bind to Rbcs.**

(78) What should be done to avoid False positivity of LISS Solution?

- **Red cells suspended in LISS and serum or plasma should be incubated together in equal volumes to ensure optimal molarity.**
- **Red cells should be washed in saline twice and once in LISS before suspending in LISS.**
- **Working Solution of LISS should be freshly prepared.**
- **Centrifugation force and time should be optimal.**

(79) What is the significance of enzyme treated Rbcs? Which enzymes are generally used?

- **Papain and Bromelin are currently used.**
- **These enzymes increase activity of both IgM and IgG antibodies.**
- **Rbcs treated with enzyme are useful in detection and investigation of autoantibodies and alloantibodies.**

(80) Which are the methods of agglutination of Red cells by antibody?

- **Tubes**
- **Microtitre plated**

- **Slides – in emergency**

(81) What types of test tubes should be used for agglutination test?

- **Disposable plastic or glass test tubes of 75x10 or 12mm in size**

(82) What is the method of macroscopic reading of agglutination?

- **Tip & roll method**
- **Hand reading glass or concave mirror**

(83) What is scoring of results of red cell agglutination test?

	Score	
4+ or more	12	Cell button in clump
3+	10	Cell button dislodges in several large clumps
2+	8	Cell button dislodges in many small clumps
1+	5	Cell button dislodges in finely granular clumps
+ or weak	3	Cell button dislodges into finely granules only visible microscopically
-	0	Negative results

(84) How will you demonstrate RBC lysis?

- **With the help of fresh complements and using stronger (5%) red cell suspension**

(85) What is the use of antiglobulin test?

- **Used as a method of detecting incomplete Rh antibodies capable of sensitizing red cells but incapable of causing agglutination in saline suspended cells**

(86) What is the application of direct antiglobulin test in blood bank?

- **To demonstrate in vivo attachment of antibodies to red cells as in autoimmune hemolytic anemia, alloimmune HDN and alloimmune hemolysis following incompatible transfusion**

(87) What is application of indirect antiglobulin test in blood bank?

- **In blood transfusion serology including antibody screening, identification and cross matching**

(88) Which antiglobulin reagents are used in blood bank?

- **Polyspecific, monospecific**

(89) What are polyspecific reagents?

- **Containing anti IgG as majority of red cell antibodies are non complement binding IgG**

(90) Why anti IgA is not necessary in polyspecific reagents?

- **Because IgG antibodies are of same specificity**

(91) Why anti IgM is not required in polyspecific reagents?

- **Because IgM alloantibodies are easily detected by complements which are present in reagents**

(92) Why only anti IgG is required when plasma is used?

- **Because EDTA present in plasma prevents complement activation**

(93) What are nonspecific reagents?

- **They are prepared against heavy chains of IgG, IgM & IgA and are referred to as anti-ϒ, anti-μ & anti-α**

- **Specific antibodies against complement components C4 & C3 can be prepared**

(94) What is clinical application of monospecific reagents?

- **To define immunochemical characteristics of antibodies**

(95) Which criteria should be followed while doing quality control of antiglobulin regents?

- **Specificity and potency of anti IgG and complement antibodies**

(96) How will you measure specificity of reagent?

- **Reagent should only agglutinate red cells sensitized with antibodies or coated with significant levels of complement components**

(97) How will you measure potency of reagents?

- **By serological titration**

(98) Which technique is recommended for antiglobulin test?

- **A spin tube technique**

(99) Why antiglobulin test is carried out in glass test tubes?

- **Because plastic tubes adsorb IgG which could neutralize anti IgG of antiglobulin reagent**

(100) How red cells are sensitized for antiglobulin test?

- **Using normal saline- 2 volume serum+ 1 volume 3% cell suspension**
- **Using LISS- 2 volume serum+ 2 volume 1.5% suspended RBCs**

(101) What are used as positive control in Antiglobulin test?

- **An IgG anti D diluted to give 1+ or 2+ reactions with Rh D positive cells.**

(102) What is used as negative control in Antiglobulin Test?

- **An inert group AB serum with same Rh D positive cells.**

(103) What can be error of positive control showing negative reaction in Antiglobulin tests?

- **Washing Deficiency**

(104) Which are the other techniques of doing antiglobulin tests?

- **Solid phase red cell adherence method.**
- **Column Agglutination Techniques (Gel)**

(105) What are the advantages of Gel technique?

- **Easy to Use**
- **As no cell wash is required less chance of contamination.**
- **Results of cards can be preserved for 24 hours.**
- **Positive sample identification is easy.**

(106) How Secretor status of a person is determined from saliva?

- **Dilute Anti A and Anti B serum so as to give visible agglutination with A and B cells.**
- **Collect saliva in milliliters, place in boiling water for 10 minutes and centrifuge.**
- **Dilute supernatant serially ranging from 1:2 to 1:4.**
- **Allow them to stand at room temperature for 10 to 15 minutes.**
- **Then add A and B cell suspension**
- **Mix and allow them to stand for 1-2 hours.**
- **Inspect – Use pure saliva as control.**

- **If saliva contains A or B substances, agglutination is inhibited in all tubes except control.**

(107) What should be used to detect secretor status of H substance?

- **Extract of ulex eel serum or naturally occurring incomplete cold antibody.**

(108) What is the clinical significance of platelet specific alloantibodies?

- **Neonatal Alloimmune Thrombocytopenia (anti HPA- 1q)**
  (anti HPA - 5b)
- **Post Transfusion Purpura (mostly Anti HPA – 1a)**
- **Refractoriness of Platelet Transfusion.**

(109) What is the clinical significance of granulocyte specific alloantibodies?

- **Neonatal allo immune neutropenia.**
- **Febrile reactions due to transfusion.**
- **TRALI**
- **Autoimmune Neutropenia**

(110) What are isoantibodies?

- **Rarely after transfusion pregnancy patients make antibodies that react with platelet glycoprotein of their own platelets.**
- **E.g Glanzmann's disease or in Bernard Soulier Syndrome.**

BLOOD CELL MORPHOLOGY IN HEALTH AND DISEASE

(1)Why should a blood film be first examined macroscopically?

- **For Spreading and staining.**
- **Abnormal particles representing large platelet aggregates, cryoglobulin deposits or clumps of tumor cells.**

(2)What should be seen in peripheral smear on low power?

- **Idea of quality of preparation.**
- **Assess whether red cell agglutination excess Rouleaux formation.**
- **Platelet aggregation present.**
- **No distribution and staining of leucocytes.**
- **Detect scanty abnormal cells.**
- **Select suitable area for.**

(3)What should be seen in peripheral smear on high power?

- **Much better appreciation of variation in red cell size, shape and staining.**
- **Features like toxic granulation.**
- **Presence of Howell jolly bodies, Pappeniheimer bodies etc**

(4)What should be seen on oil immersion lens?

- **Final examination of unusual cells.**
- **Looking fine details like Auer rods.**

(5)What is normal diameter of RBCs?

- **6.0-8.5 micrometer.**
- **Same as nucleus of lymphocytes.**

(6) Pyknocytes: Irregularly contracted cells
Schistocytes(fragmented RBCs): Irregular shape
Anisocytosis: Variation in size
Poikilocytosis: Variation in shape (Target cells, Acanthocytes, Spherocytes)

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(7)What is importance of Poikilocytosis?

- **Seen mostly in abnormal Erythropoesis.**
- **Dyserythropoesis: Elliptocytes, Ovalocytes.**
- **Megaloblastic: Macro-ovalocytes.**
- **Iron deficiency: Pencil cells, Tear drop cells.**

(8)What are causes of Macrocytosis?

- **Main in Megaloblastic Anemia.**
- **Also on MDS, Aplastic anemia and Dyserythropoesis.**

(9)What are other causes of Macrocytosis?

- **Hydroxyurea / Hydroxycarbamine is drug causing macrocytosis.**
- **Alcohol: Macrocytosis.**
- **Chronic liver disease(Stomatocytes)**
- **CDA – Type III.**
- **Increased Reticulocyte.**

(10) What are causes of Microcytes?

- **Iron deficiency anemia.**
- **Various types of Thalassemia.**
- **Anemia due to chronic disease.**
- **Rare: Congenital / Acquired Sideroblastic Anemia.**

(11) What is basophilic stippling?

- **Numerous basophilic granules distributed throughout cell.**

(12) What does basophilic stippling indicates?

- **It usually indicates disturbed erythropoiesis.**

(13) In which conditions basophilic stippling is seen?

- **Thalassemia**
- **Megaloblastic anemia**
- **Infections**
- **Liver disease**

- **Lead and heavy metal poisoning**
- **Unstable Hb**

(14) What is Hypochromia?

- **Mainly due to lowered Hb concentration- due to impaired Hb synthesis**
- **Abnormally thin RBCs.**

  - ✓ **Causes of Impaired Hb synthesis:**
    - **Iron deficiency anemia**
    - **Thalassemia(Presence of target cells and basophilic stippling).**
    - **Sideroblastic anemia (less common)**
    - **Chronic Infection**

(15) What is Anisochromasia?

- **Abnormal variability in staining of red cells.**
- **Occur during development/ resolution of iron deficiency anemia or anemia of chronic disease.**

(16) What is Dimorphism?

- **Two distinct populations of cells are seen.**

(17) Conditions in which dimorphic picture is seen?

- **After transfusion of normal blood to hypochromic anemic patient.**
- **Iron therapy given to iron deficiency anemic patient.**
- **Sideroblastic anemia as feature of MDS (Myelodysplastic syndrome).**

(18) Hyperchromasia:

- **Unusually deep staining of red cells with lack of central pallor.**
- **Common in Macrocytosis. Eg., Neonatal blood- Megaloblastic (due to increased thickness of red cells).**
- **Rounded cells- Spherocytes ,irregularly contracted cells**

(19) Causes of Spherocytosis

- **Hereditary Spherocytosis (RBC membrane defect)**
- **ABO hemolytic disease of newborn**

- **Autoimmune hemolytic anemia**
- **Action of bacterial toxin. Eg., (Clostridium)**

(20) What are Spheroechinocytes?

- **Spherocytes which are crenated seen in:**
  - ✓ **As artifact in blood that is allowed to stand before films are spread.**
  - ✓ **Hereditary Spherocytosis with Splenectomy**
  - ✓ **Transfusion of stored blood**

(21) What are the causes of irregularly contracted red cells?
(Heinz bodies are found in these cells)

- **Drug/ Chemical induced hemolytic anemia**
- **Unstable hemoglobinopathies (Hb E)**
- **Hemolytic anemia due to G6PD Deficiency**
- **B-Thalassemia trait, Hb C**

(22) What are Heinz bodies?

- **They are pale pink staining bodies at cell margin and protruding from erythrocytes.**

(23) What is pyknocytosis?

- **Irregular contraction of RBCs due to unknown origin is known as pyknocytosis**
  - **In blood of infants(premature) infantile pyknocytosis due to transient hemolytic anemis(due to glutathione peroxidase and selenium deficiency)**

(24) What is infantile pyknocytosis?

- **Transient hemolytic anemia occurring especially in premature infants due to glutathione peroxidase and selenium deficiency is known as infantile pyknocytosis.**

(25) What are the causes of elliptocytosis?

- **Hereditary eliptocytosis**
- **Hereditary pyropoikilocytosis**

(26) What happens to reticulocytes when elliptocytes are present?

- **They will assume abnormal shape in late stage of maturation.**

(27) Which cells are included under terminology of spiculated cells?

- **Schistocytes**
- **Keratocytes**
- **Acanthocytes**
- **Echinocytes**

(28) What is difference between acanthocyte and echinocyte?

- **Echinocyte is crenated cell differentiated by number/shape and disposition of spicules.**

(29) What are causes of schistocytes of red cell fragments?

- **Genetically determined diseases like Thalassemia, CDA, hereditary pyropoikilocytois**
- **Acquired disorders of red blood cells formation when Megaloblastic erythropoeisis**
- **Due to mechanical stress e.g, Microangiopathic Hemolytic Anemia**
- **Direct thermal injury**
- **TTP**

(30)What happens to RBCs in case of burns?

- **Schistocytes becomes round with budding off small rounded blebs of cytoplasm.**

(31) What are keratocytes?

- **Keratocytes have pairs of spicules either one or two.**

- **Keratocytes are formed by removal of Heinz body or mechanical damage.**
- **Example: HELMET CELL OR BITE CELL**
- **Hemolytic anemia : G_6PD deficiency and Microangiopathy**

(32)What are Acanthocyte?

- **Abnormality of Red cell in which there are small number of spicules of inconstant length, thickness and shape, irregularity dispose on cell surface.**
- **Acanthocyte are seen :after Splenectomy**
  Severe liver diseases
  Abnormal phospholipid metabolism

(33)What are echinocytes?

- **Echinocytes are many short projections on RBCs mainly due to crenation.**
- **Causes: 1.Blood kept overnight at 20^{o}C before making smear**
  2. Uremia
  3. Cardiopulmonary Bypass
  4. Exchange transfusion in premature baby
  5. Artefact when RBCs washed with NaCl at high pH
  6. Unclear slides with traces of fatty substances or chemicals

If high degree of echinocytes – mostly ARTEFACT

(34)What is leptocytosis?

- **Leptocytes are usually thin red cells seen in severe iron deficiency anemia , and thalessemia.**

(35) What are target cells?

- **Target cells are central round stained area and peripheral rim of hemoglobinized cytoplasm separated by nonstaining or lightly staining cytoplasm.**

(36) On which conditions target cells are seen?

- **Thalessemia / Iron deficiency anemia**
- **Chronic liver diseases**
- **Hereditary hypobetalipoproteinemia**
- **Haemoglobinopathies**
- **After Splenectomy**

(37) What are stomatocytes?

- **Red cells in which central biconcave area appearance slit like in dried films (artefact)**
- **In wet film, they appear cup shaped.**

(38)In which conditions stomatocytes are seen?

- **Liver disease**
- **Alcoholism**
- **Myelodysplastic syndrome**

(39)When can a stomatocytes be artefact?

- **Stomatocytes can be artefact**
  - **When produced by decreased pH**
  - **As a result of exposure to cationic detergent like compounds**

(40) Which are common red cell inclusions?

- **Heinz bodies**
- **Hb crystal**
- **Howell Jolly bodies**
- **Peppenheimer bodies**

(41) What are Howell Jolly bodies?

- **It is nuclear remnant, usually single**
- **Mostly seen in RBCs of Pernicious anemia**

- **After Splenectomy**
- **Splenic atrophy**
- **Folate deficiency**

(42) What are Peppenheimer bodies?

- **Peripherally sited basophilic inclusion in RBCs(usually black)**
- **Composed of Hemosiderin**
- **Presence is related to iron overload and hyposplenism**
- **Confirmed by Perl's stain**

(43) How will you differentiate Peppenheimer bodies from Basophillia?

- **Not in large numbers**
- **With Pearl's stain Basophils stain- PINK**
  Peppenheimer bodies stain- BLUE

(44)What is meant by Polychromasia?

- **RBCs being stained by many colors**
- **Shades of bluish grey- Reticulocytes**
- **Seen in intense Erythropoesis and extramedullary Erythropoesis e.g, Myelofibrosis, Carcinomatosis**

(45) Which cells are not seen in Aplastic Anemia?

- **Erythroblasts**

(46) In which conditions except leukemia, erythroblasts are seen in large numbers?

- **Hemolytic anemia of newborn**

(47) In which blood erythroblasts are commonly found?

- **In infants cord blood**
- **Or in premature infants**
- **Extramedullary Erythropoesis in liver and spleen**
- **After Splenectomy**
- **Myelofibrosis**
- **Sickle cell anemia- in painful crisis**

- **Carcinomatosis**
- **Leukemia**
- **Leukoerythroblastic reactions**

(48) What is difference between Erythroblast and Normoblast?

- **Normoblast implies erythroid maturation**
- **Erythroblast is general term that includes megaloblast also**

(49) What are effects of Splenectomy or Hyposplenism seen in Peripheral smear?

- **Occurrence of target cells, Acanthocytes, Howell jolly bodies, Peppenheimer bodies**
- **Increase in all cell count (in early phase)**

(50) What is approximate diameter of a neutrophil?

- **About 13 µm**

(51)What is the normal % range of band cells in peripheral smear?

- **Approx 80%**

(52)What is drumstick?

- **In women 2-3% of neutrophils show appendage at a terminal nuclear segment.**
- **1-5 µm in diameter.**
- **Connected to nucleus by short stalk.**
- **Represents inactive X chromosome.**
- **Corresponds to Barr body of buccal cells.**

(53)In which conditions red cells adhere to neutrophils causing rosettes?

- **Immune hemolytic anemia.**
- **PNH**
- **Artefact.**

(54)What is meant by toxic granulation?

- **Increase in staining density and number of granules occurring regularly with bacterial infection and inflammation.**

(55)In which condition large coarsed granules are seen?

- **Aplastic anemia.**
- **Myelofibrosis.**

(56)In which conditions hypogranular or agranular neutrophils are seen?

- **MDS**
- **Other forms of myeloid leukemia.**

(57)Which are other conditions showing granular defects?

- **Alder-Reilly Syndrome: large discrete deep red granules with obscere nucleus neutrophil function normal**
- **Chediak Higashi Syndrome: Giant scanty , azurophilic granules, functional defect of neutrophils.**

(58)In which cells which bacteria are commonly seen?

- **In neutrophils cytoplasm: Meningococci, Pneumococci.**
- **In premature infants: Staphylococci.**

(59)What are Dohle bodies?

- **Small,round or oval pale blue grey structure found at periphery of neutrophil consisting of ribosomes and Endoplasmic reticulum.**
- **Seen in: bacterial infection, inherited condition of May Heglin anamoly**
- **But seen in all types of leucocytes except lymphocytes.**

(60)What happens to Dohle's inclusion in May Heglin's syndrome?

- **It is the only condition in which Dohle's inclusions are seen in all kinds of leucocytes except lymphocytes.**

(61) What does presence of vacuoles in neutrophils indicate?

- **Severe sepsis**

(62) Which neutrophil is marker to decide shift to left or right?

- **Neutrophil with three lobes.**

(63) In which physiological condition shift to left is seen in form of band cells?

- **Pregnancy**

(64) When a neutrophil is called hypersegmented?

- **When there are five or more nuclear segments.**

(65) In which conditions hypersegmented neutrophils are seen?

- **Most common megaloblastic anemia**
- **Uremia**
- **Uncommon in iron deficiency**
- **Cytotoxic drugs like methotraxate**

(66) What is Pelger Huet anomaly?

- **Neutrophil nuclei fail to segment properly.**
- **Inherited condition**

(67) What is Pseudo Pelger anomaly?

- **Similar type of cells are seen due to either**
  - **MDS (Myelodysplastic syndrome)**
  - **Acute myeloid leukemia with dysplastic mutation**
  - **CML (Chronic myeloid leukemia) – accelerated phase (hypogranular)**

(68) In which condition we see eosinopenia?

- **Prolonged steroid administration**

(69) In which conditions we see eosinophilia?

- **Allergic – moderate eosinophilia**
- **Parasitic – Severe eosinophilia**
- **Reactive – T cell lymphoma, B cell lymphoma, ALL**
- **Hypereosinophilic syndrome (Eosinophilic leukemia)**

(70) What does granules of Basophil contains?

- **Histamine, Serotonin, Heparin**

(71) What are the causes of Basophilia?

- **Myeloproliferative disorders**
- **Chronic Granulocytic Leukemia (Blast crisis)(more than10%)**

(72) Causes of Monocytosis.

- **Chronic infections**
- **Inflammatory condition like tuberculosis, Crohn's disease, CML, CMML,AL**

(73)Which type of lymphocyte constitutes a major portion of total lymphocytes?

- **T cell/ Natural killer cells- 85%**

(74)Which types of lymphocytes are seen in viral and bacterial infections?

- **Immunoblast/ Turk cells – Large round nucleus**
  - Abundant basophilic cytoplasm
- **May develop in Plasmacytoid cells/ Plasma cells**

(75)What are reactive lymphocytes?

- **Appear in viral infections**
- **Slightly larger nuclei with open chromatin and abundant irregular cytoplasm**
- **Also seen in Infectious Mononucleosis**

(76) How will you differentiate between CLL & Lymphocytosis?

LYMPHOCYTOSIS	CLL/LYMPHOMA
Less uniform cells	Uniform cells
	Indented, Irregularly lobed, cleaved nuclei, scanty cytoplasm- Lymphoma

(77)Lymphocytes are predominant in

- **Infants/ young children**
- **They are reactive with small number of lymphoblasts**

(78)In which condition large platelets are seen?

- **In any condition where platelet production is increased and hyposplenism e.g, Immune Thrombocytopenia**

(79) Very high platelet count is a feature of

- **Myeloproliferative disorders associated with extreme platelet anisocytosis- large agranular/Hypogranular platelets**
- **Acute inflammatory stress**

(80) Giant platelets with defective ristocetin & Platelet lacking granules are seen in which disorders?

- **Giant platelets with defective ristocetin→ Bernard Soulier syndrome**
- **Platelet lacking granules→ Grey platelet Syndrome having ghost like appearance**

(81) Thrombocytopenia and pseudothrombocytopenia are seen in

- **Thrombocytopenia – May heglin anomaly**
- **Pseudothrmbocytopenia- EDTA causing platelet clumping**
  - Give rise in Auto cell counter

(82) What are effects of EDTA on platelets?

- **Causes Pseudothrmobocytopenia**
- **Inhibit the staining of platelets**

BONE MARROW BIOPSY

(1) What is contraindication of bone marrow aspiration?

- **Haemophilia**

(2) Give advantage of bone marrow aspiration.

- **It is simple and safe.**
- **Can be repeated.**
- **Can be performed on out patients.**

(3)What is disadvantage of bone marrow aspirations in comparison to trephine biopsy?

- **Relationship between cells in marrow and between one cell and another is disrupted by process of aspiration.**
- **In fibrotic marrows blood is aspirated instead of cells.**
- **Trephine biopsy provides more information about structure.**

(4)What is advantage of taking imprint?

- **Morphological features of individual cells are identified.**

(5)Which are common sites of Bone marrow aspiration?

- **Sternum**
- **Illiac crest**
- **Anterior/posterior illiac spine**
- **Spines of vertebrae(lumber)**

(6)Which are common indications of using iliac spine?

- **When aspiration is not obtained**
- **In children**

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(7) What is a common indication for using sternum?

- **Obese immobile patients**

(8)What are dangers using sternum as site of Bone marrow aspiration?

- **Danger of perforating inner cortical layer, damaging underlying vessels and right atrium.**

(9)Which is the usual site of puncture in sternum?

- **Manubrium -1 cm above the sternomanubrial angle (more preferable as dense bone)- First or second parts of body of sternum**

(10)From which vertebrae bone marrow aspiration can be done?

- **Lumbar vertebrae in adults , but more pressure is required.**

(11) In children, which is common site of aspiration of Bone marrow?

- **Illiac puncture particularly in the region of posterior spine**
- **Obese children – Anterior ilium**
- **Small babies – Medial aspect of upper end of tibia just below tibial tubercle**
- **STERNAL PUNCUTRE AVOIDED**

(12) What are ideal specifications of Bone marrow needle?

- **Stout**
- **Hard stainless steel**
- **7-8 cm length**
- **Well fitting stillete**
- **Adjustable guard**
- **Reusable- Point of needle & bewel well sharpened**

(13) Most commonly used reusable needle

- **Salah/ Klima**
- **Islam – better grip**

(14) What should be ideal amount of Bone marrow aspiration?

- **0.3 ml. More than that brings diluted peripheral blood**

(15) How Bone marrow should be done in postmortem cases?

- **It is done as regular cells break down due to cellular autolysis occurring in first 15 hours.**
- **Small piece of marrow is suspended in 1-2 ml of 5% bovine albumin→ centrifuged→ deposit resuspended →Smears made**

(16) How will you grade cellularity of Bone marrow?

- **Less than 25% marrow particle → Hypocellular**
- **More than 75-80% → Hypercellular**

(17) How will you prevent dilutional effect of blood in marrow?

- **By aspirating small volume**
- **By counting the cells in trails left behind marrow particle as they spread on**

(18) When cells are under-represented in differentiated count?

- **When there is excess of reticulin**

(19) In which part of smear megakaryocytes are generally seen?

- **In tail portion of smear**

(20) In which condition there is abnormally high percentage of lymphocytes?

- **When lymphoid follicle is aspirated**

(21) What variations are seen in Bone marrow in early years of life?

- **Erythroblasts- Decreased from birth 10% at 2-3 weeks**
- **Myeloid – Increase during 1st two weeks**
  Decrease from 3rd week 60% by end of 1st month
- **Lymphocytes – 40% of nucleated cells in small infants**
  20% at 2 years
  15% rest of childhood
- **Plasma cells – low from infancy upto 5 years of age**

(22)Which maturation abnormalities are seen in bone marrow aspiration?

- **Giant Pronormoblast – Parvo virus**
- **Dysfunctional maturation –MDS**
- **Nuclear cytoplasmic asynchrony-Megaloblastic Anemia**
- **Changes in proportion of primitive to mature myeloid cells –response to treatment in leukemia**

(23)What is importance of known actual % of blast cells?

- **Significant in differentiation of Refractory anemia and 'Assessing leukemia prognosis'**

(24)How will you assess iron content in bone marrow?

- **Atleast 7 particles should be examined**
- **If fewer are available iron deficiency is tentative.**

(25)In which conditions trephine biopsy is more valuable?

- **Dry tap-Myelofibrosis , Infiltration**
- **Disrupted architecture - Hodgkin's lymphoma**

(26)Which is usual site for trephine biopsy?

- **Posterior iliac spine**

(27)Which needles are used for trephine biopsy?

- **Jamshidi , Islam**
- **Occasionally Vim-Silverman**

(28)What is advantage of Jamshidi niddle?

- **It has tapering end so it reduces crush effect.**

(29)What is advantage of Islam needle?

- **It has core securing device.**

(30)What is to be done in preterm neonates if bone marrow is needed to be?

- **For investigation of thrombocytopenia , neutropenia**
- **19 G half inch Osgood needle**
- **2 cm below tibial tuberosity**
- **Decalcification not required treated as adult biopsy specimen**

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COLLECTION AND HANDLING OF BLOOD SAMPLES

(1) What are causes of misleading results from discrepancies in specimen collection?

- **Precollection – Toilet within 30 minutes**
  - **Water and food intake within 2 hours**
  - **Smoking**
  - **Physical activity**
  - **Drugs**
- **Collection - Prolonged tourniquet**
  - **Posture**
  - **Time**
- **Handling of specimen – insufficient or excessive anticoagulant**
  - **-Inadequate mixing of blood with anticoagulant**
  - **-Patient indentification error**
  - **-inadequate storage**
  - **-dealy to transit**

(2) What are constituents of phlebotomy tray?

- **Syringe**
- **Needle**
- **Sterile swab**
- **Vaccuatte**
- **Request form**
- **Rack**

(3) What is ISO standard that relates diameter of needle?

ISO7864

- **19G – 1.1mm**
- **21G – 0.8mm**

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- **23G – 0.6mm**

(4) Which standard has to be followed for specimen collection containers?

- **International council for standardization in Haematology(non specific)**

(5) What are different types of vaccuattes?

- **Glass/ plastic with defined vacuum**
- **Silicon coated**

(6) What is role of vacuum in vacuattes?

- **Vacuum controls amount of blood giving adequate specimen and correct proportion of anticoagulants**

(7) Which tests should be performed in silicon coated vacuatte?

- **Routine coagulation screening tests.**

(8) While collecting blood when should tourniquet be released? Why?

- **It should be released as soon as the blood flows**
- **If delayed it leads to fluid shift and haemoconcentration due to venous stagnation – wrong results**

(9)What are requirements for successful venepuncture?

- **Site of puncture should be warm**
- **Sphygmomanometer cuff at diastolic pressure**
- **Tapping skin over site of collection few times**

(10) In obese patients, if veins are not seen what should be done?

- **Vein on dorsum of hand warming by immersion in warm water.**
- **Generally not recommended as it tends to bleed.**

(11) Why blood should be drawn slowly in sample?

- **Blood should be drawn slowly in sample to prevent hemolysis.**

(12) Why blood cannot be collected from indwelling line?

- **Blood cannot be collected from indwelling line as they are commonly flushed with heparin.**

(13) In which circumstances, we prefer to collect capillary blood?

- **Circumstances, we prefer to collect capillary blood are:**
  - **In infants**
  - **In obese patients**

(14) Why earlobe is not preferred as site of collection for capillary blood?

- **Earlobe is not preferred as site of collection for capillary blood as reduced blood flow renders it insufficient for representing reports.**

(15) Which part of heel should be punctured in infants for collection of capillary blood?

(16) Why central planter and posterior curvature should not be punctured?

- **Central planter and posterior curvature should not be punctured because of possible risk of injury and infection to tarsal bones.**

(17) If blood is collected from cold or cyanosed sites, how it will affect the results?

- **If blood is collected from cold or cyanosed sites, there will be:**
  - **High Hb**
  - **High RBCs**
  - **High WBCs.**

(18) What is the difference between capillary and venous blood?

- **In capillary blood, there are high platelet counts due to adhesions.**
- **In venous blood, there are high PCV, RBCs, Hb and WBCs.**

(19)What is difference between plasma and serum?

- **Serum lacks fibrinogen and some coagulation factors.**

(20)Can serum sample be used after 3 months?

- **Serum sample used after 3 months , if serum sample stored at -20 ^{0}C.**
- **Also used more, if stored at -40^0 C used after thawing.**

(21)How do you obtain plasma free Red cells?

- **Plasma cells are obtained by defibrinating whole blood.**

(22)How will you collect blood in case of cold agglutinins?

- **1. Blood must be kept at 37^{0}C until serum has separated.**
- **2. Bring patient to lab, collect blood and put immediately in prewarmed water bath at 37^{0}C.**
- **3. Collect blood in prewarmed syringe by keeping over preheated at 50^{0}C or incubator 37^{0}C for 30 minutes.**
- **4. Serum which is separated is transferred to tube that is warmed and rapidly centrifuged.**

(23)Until what temperature cold antibodies are capable of producing agglutination?

- **30^0C**

(24)Which anticoagulants act by removing Ca^+?

- **EDTA**
- **Sodium Citrate**

Ca^+ is either precipitated as insoluble oxalate or bound in nonionised form.

(25)How does heparin act as anticoagulant?

- **It binds to ANTITHROMBIN, thereby inhibiting interaction of several clotting factors.**

(26) Uses of different anticoagulants?

- **EDTA- CBC**
- **Citrate- Coagulation, ESR and in blood bank in combination with Dextrose.**

(27) Which salts of EDTA are powerful anticoagulants? Which one is better and Why?

- **Sodium , Potassium**
- **Dipotassium is better as it is very soluble. Dipotassium 1650gm dissolve in 1 liter, while disodium only 108gm dissolve in 1 liter.**

(28) What is concentration of EDTA needed to act as anticoagulant?

- **1:2 mg of anhydrous salt per ml of blood.**

(29) Why dilithium salt of EDTA is not used?

- **As it is less soluble.**

(30) Why tripotassium salt is not used?

- **It is in liquid form**

- **Blood is in more diluted form**
- **Produces some shrinkage of RBCs, PCV decrease by 2-3%.**

(31) Why trisodium EDTA cannot be used?

- **Because of it's High pH.**

(32) What happens if excess EDTA is used?

- **Normal range – 2mg/ml of blood.**
- **RBC , WBC Shrinkage, degenerative**
- **Decrease in PCV by centrifugation**
- **Increased in MCHC**
- **Platelets swell and disintegrate causing high platelet count.**

(33) Which anticoagulant fails to demonstrate basophilic stippling of red cells in lead poisoning?

- **EDTA**

(34) What is the proportion of sodium citrate used for coagulation studies?

- **9 volume blood + 1 volume sodium citrate of 109mmol/L**

(35) What proportion of sodium citrate is used for estimation of ESR?

- **4 volume blood + 1 volume sodium citrate (109mmol/L)**
  (Westergreen method)

(36)What salts and in what proportion of Heparin is used?

- **10-20 iu per ml for chemistry, blood gas analysis and emergency.**
- **It reduces lysis of RBCs so best for osmotic fragility.**

(37)Why Heparin cannot be used for Blood cell counting?

- **As it induces Platelet and Leucocyte clumping.**
- **It gives faint blue colouration to background when films are stained by Romanowsky dyes.**

EFFECTS OF STORAGE ON BLOOD

(38)For maximum how many hours blood test can be done in anticoagulant?

- **RBC, WBC, indices and Platelet count remains normal till 8 hours.**
- **At $4^{o}C$ – for 24 hours.**

(39)Which investigations are to be done preferably within 2 hours?

- **Leucocyte count and platelet count.**

(40)What happens to Reticulocyte count if blood is stored in EDTA?

- **Not affected for 24 hours at $4^{o}C$, but at room temperature count decrease within 6 hours.**

(41)What happens to Hemoglobin in stored blood?

- **Remains unchanged for days if not lysed.**

(42)Within what duration coagulation tests are to be performed?

- **2 hours - 22-24 ^{o}C.**
- **4 hours - 4 ^{o}C.**
- **2 weeks - -20 ^{o}C.**
- **6 months - -70 ^{o}C.**

EFFECTS OF STORAGE ON BLOOD CELL MORPHOLOGY

(43)Which circumstances affect the blood cell morphology?

- **Presence of anticoagulant for long.**
- **Defibrinated blood.**
- **Long standing blood.**

(44)What changes occur in Neutrophils?

- **Nuclei stain homogenously.**
- **Nuclear lobes become separated.**
- **Cytoplasmic margin become ragged.**
- **Small vacuoles in cytoplasm.**

(45)What changes occur in Lymphocytes?

- **Vacuoles in cytoplasm.**
- **Nucleus undergoes budding.**

(46)What changes occur in RBCs?

- **Progressive crenation and sphering.**

(47) How will yo distinguish between changes occurring due to storage from Th at of Apoptosis?

- **In Apoptosis -Cell shrinkage with cytoplasmic, condensation around nuclear membrane.**
  - Indentation in nucleus followed by fragmentation.
  -Cell remnants, basophilic apoptotic bodies.

Seen more in Leukemia.

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APPROACH TO THE DIAGNOSIS AND CLASSIFICATION OF BLOOD DISEASES

(1) In which conditions increase in blood cells occur in more than one cell line?

- **Myeloproliferative disorders**

(2) What are the causes of erythrocytosis?

- **Relative- owing to plasma volume**
- **Primary- as a part of Myeloproliferative disorder (Polycythemia)**
- **Secondary- due to chronic hypoxia**

(3) What is pseudopolycythemia?

- **It is relative increase in erythrocytes due to reduced plasma volume**

(4) Which causes of chronic hypoxia leads to erythrocytosis?

- **Chronic lung disease**
- **Congenital heart disease**
- **Aberrant erythropoietin production**
- **High affinity hemoglobin**

(5) In which infection monocytosis is seen?

- **Malaria**
- **Tuberculosis**

(6) What are the causes of reduction of cells in more than one cell line?

- **Aplastic anemia**
- **Lack of folate or vitamin-B12**
- **Leukemia**
- **Lymphoma**
- **Multiple myeloma**
- **Metastatic carcinoma**
- **HIV**

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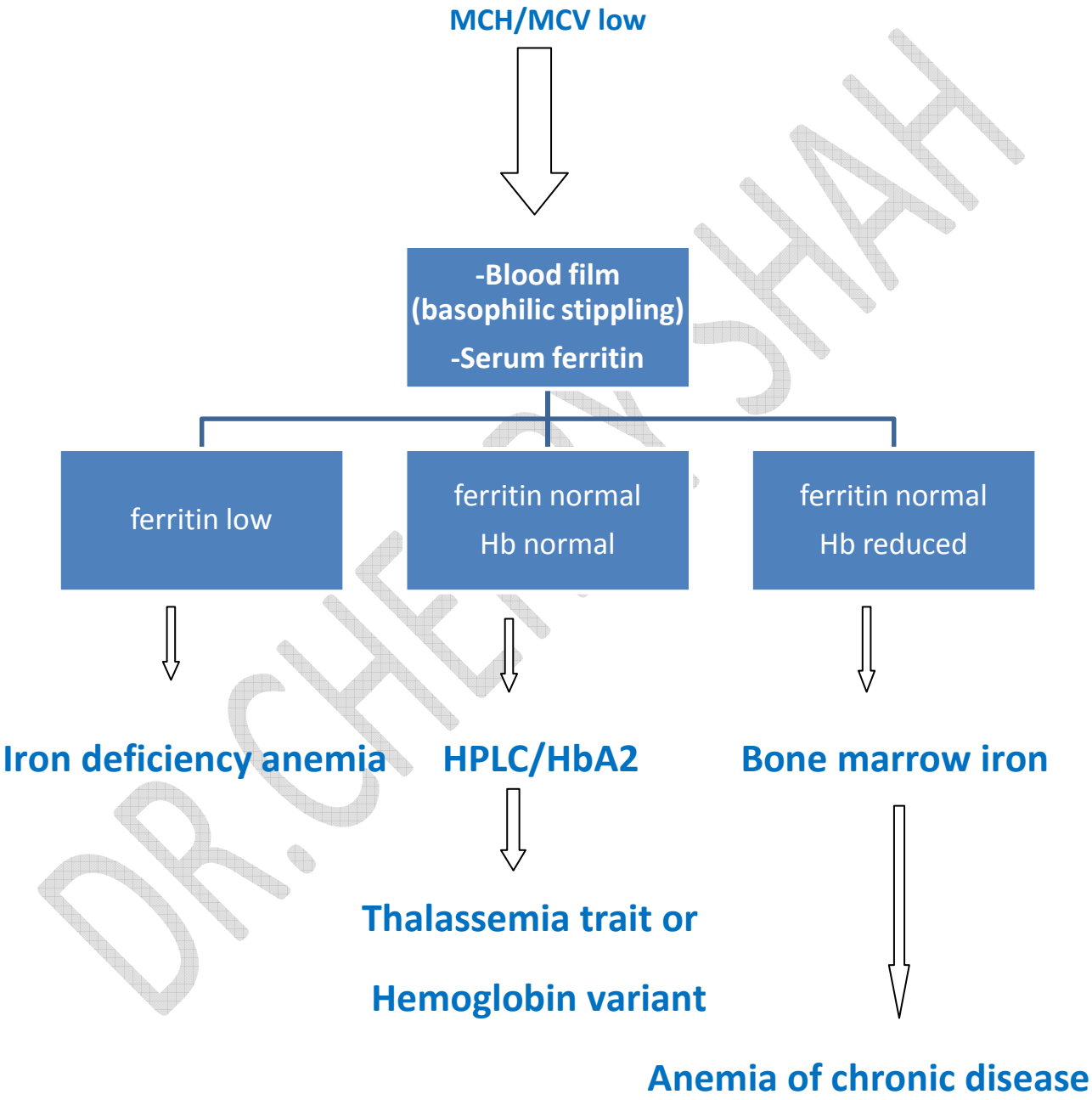
- **Myelodysplastic syndrome**
- **Myelofibrosis**

(7) How will you investigate microcytic hypochromic anemia?



(8) Which investigations are done for iron deficiency anemia?

- **Serum Ferritin**

- **Serum iron**
- **Total iron binding capacity**
- **Transferrin assay**
- **RBC protoporphyrin**
- **Bone marrow aspirates for iron staining**

(9) What is the differential diagnosis of iron deficiency anemia?

- **Anemia of chronic disease**

(10) What are investigating criteria of Microcytic anemia in inflammation?

- **Normal or high serum Ferritin**
- **Low serum iron**
- **Low transferritin or total iron binding capacity**

(11) How wii you differentiated between Thalassemia and Iron deficiency anemia?

In Thalassemia

- **High RBC count**
- **Normal hemoglobin**
- **Low MCV/MCH**

In iron deficiency anemia

- **Low hemoglobin**
- **MCV/MCH normal**

(12) Which investigations are to be done for Thalassemia?

β Thalassemia

- **Hb electrophoresis**
- **HPLC**
- **HbA2/HbF**

α Thalassemia

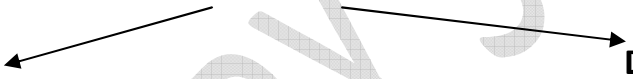
- **HBH inclusions**

- **DNA analysis**

(13) How will you investigate macrocytic anemia?

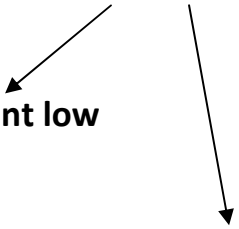
MCV/MCH high

Blood film
B12
Folate
Retic count
Drug history
Alcohol history
Liver function test
Thyroid function test



Diagnosis clear in MDS
B12 deficiency
Folate deficiency
Alcohol or drug effect
Liver disease
Hypothyroidism

Diagnosis is not clear



Retic count low

Retic count high

Acute blood loss
Hemolytic anemia

(14) In which condition hyper segmented neutrophils are seen along with macrocytes?

- **Folate or vitamin B12 deficiency**

(15) Which investigations are suggested in vitamin B12 or folate deficiency?

- **Malabsorption studies**
- **Study of celiac disease**
- **Test of intrinsic antibodies**
- **Schilling test for pernicious anemia**

(16) Which drugs causes microcytosis?

- **Hydroxycarbamide**
- **Zidovudin**

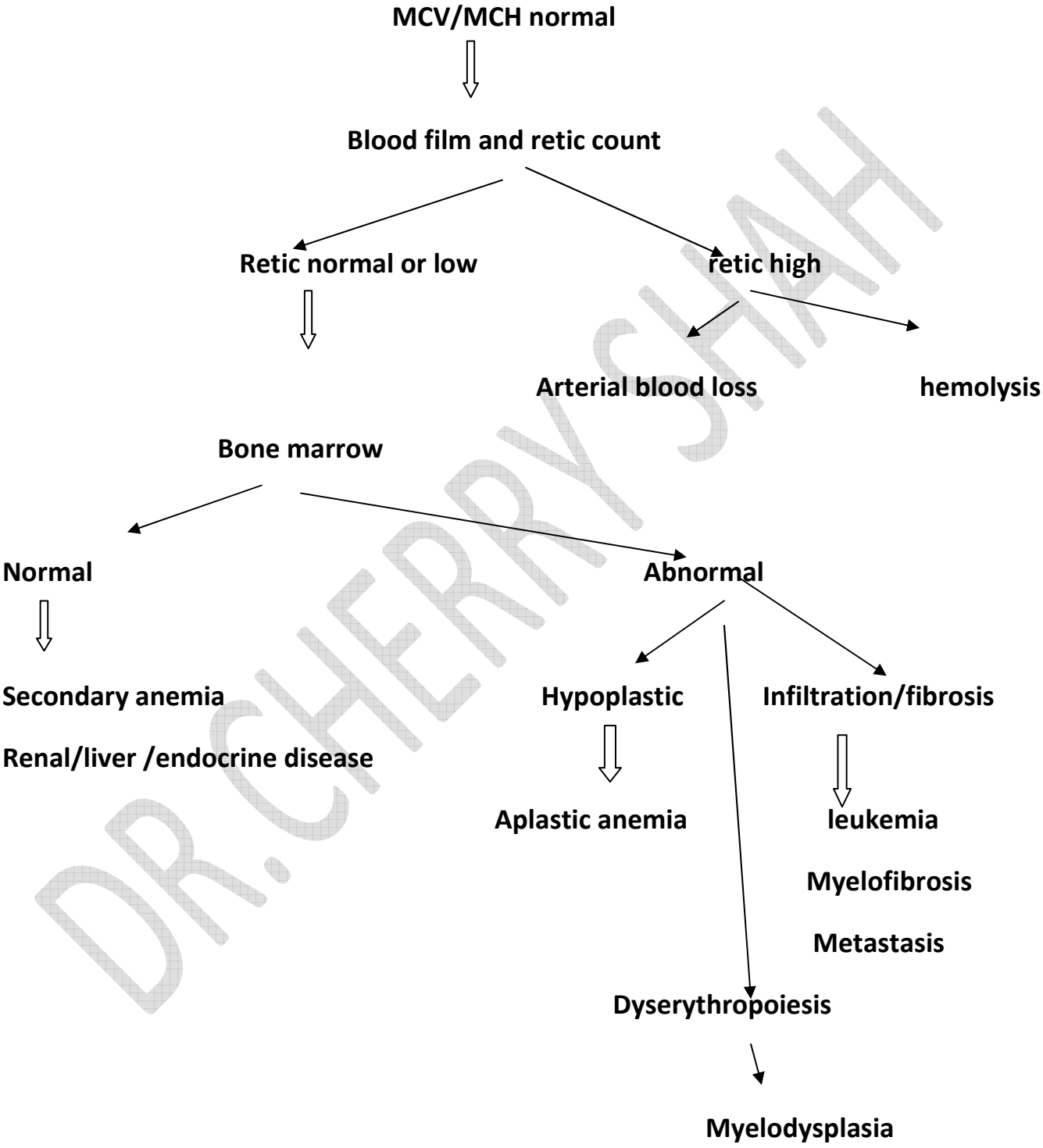
(17) How does microcytosis appear in chronic hemolysis?

- **Increased number of immature red cells**
- **reticulocytosis**

(18) What are the cause of normocytic normochromic anemia?

- **Chronic non-hemolytic disease**
- **Renal insufficiency**
- **Autoimmune disease**

(19)How will you investigate normocytic normochromic anemia?



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(20)Which are morphological causes of neutropenia?

- **Overwhelming infection**
- **Autoimmune disease**
- **Irradiation**
- **Drugs**

(21) What happens in severe acute respiratory syndrome?

- **Lymphoma with neutrophilia**

(22) In which conditions there is lymphopenia affecting CD4 cells?

- **HIV**
- **Renal failure**

(23)In which condition monocytopenia is seen?

- **Hairy cell leukemia**

(24) What are the causes of spurious thrombocytopenia?

- **Blood clots in sample**
- **Platelet aggregation**
- **Platelet satellitism**

(25) What are the causes of platelet aggregation?

- **Temperature dependent**
- **Anticoagulant dependent autoantibody**
- **Slides directly made from a fingerprick sample**

(26) What are the causes of true thrombocytopenia?

- **Anticancer therapy**
- **HIV**
- **Immune thrombocytopenic purpura**
- **Alcohol Excess**
- **Hypersplenism**
- **Myelodysplastic syndrome**

(27) What are the causes of pancytopenia?

- **Anticancer chemotherapy**
- **HIV**
- **Hypersplenism**
- **Bone marrow infiltration or failure**

(28) Which abnormalities are seen in Myelodysplastic syndrome?

- **Anisopoikilocytosis**
- **Mild microcytosis**
- **Hypergranular neutrophils with abnormal nuclear morphology**
- **Platelet anisocytosis**
- **Nuclear cytoplasmic asynchrony**

(29) Enumerate list of investigations to be done in Microcytic hypochromic anemia?

- **Serum Ferritin TIBC or transferrin**
- **Bone marrow with iron stain**
- **Stool for occult blood**
- **Tests for malabsoption, celiac disease**
- **Endoscopic biopsy**
- **Serum lead (for lead poisoning)**

(30) Enumerate investigations if thalassemia is suspected.

- **Hb electrophoresis**
- **HbA2/HBF by HPLC**
- **HbH preparations**
- **Family studies**
- **DNA analysis**

(31) Enumerate investigations in case of Aplasticanemia.

- **Bone marrow/Trephine**
- **Ham's test for PNH,if positive urine for hemosiderin and NAP.**
- **Vitamin B12 and folate**
- **Viral studies for Ebstain bar and hepatitis virus**

(32) Enumerate investigations if Fanconi's anemia is suspected.

- **Sensitivity of chromosomes to breakage of DNA crosslinkage agents**
- **Radiology of hands and forearm**

(33) Enumerate investigations done in acute leukemia.

- **Bone marrow/ Trephine**
- **Cytochemical stains**
- **Blood /bone marrow immunophenotyping**
- **Cytogenetic analysis**
- **Molecular studies(FISH) for specific oncogens**

(34) Enumerate investigations done in neutropenia.

- **Serum neutrophil count**
- **Antineutrophil antibodies**
- **Bone marrow/Trephine**
- **SLE investigations**
- **Vitamin B12 and folate levels**
- **Ham's test**

(35)Enumerate investigations to be done in chronic granulocytic leukemia.

- **Bone marrow aspirate**
- **Cytogenetic analysis**
- **Molecular studies(FISH for BCR-ABL rearragement)**
- **NAP score**

(36) Enumerate investigations for Lymphoproliferative disorders or lymphadenopathy.

- **Serological screening for infectious mononucleosis,CMV,HIV and toxoplasmosis**
- **Bone marrow/Trephine**
- **Immunophenotyping**
- **Serum protein electrophoresis and immunoglobulin concentration**
- **Serum urate ,calcium,LDH levels**
- **Lymphnode biopsy**
- **Cytogenetic molecular analysis for immunoglobulin heavy chain or T-cell receptor gene rearrangement**
- **Radiological studies(x-ray,CT scan,USG,MRI)**

(37) Enumerate investigations to be done in multiple myeloma.

- **Bone marrow/Trephine**
- **Protein electrophoresis/immunoglobulin concentration**
- **Albumin and calcium levels**
- **β 2 microglobulin**
- **urine for Bence John's protein**
- **RFT**
- **Radiological skeletal survey**
- **Serum free light chain and quantative ratio**

(38) Enumerate investigations for Myeloproliferative disorders.

- **Bone marrow/Trephine**
- **O2 saturation and carboxy hemoglobin**
- **USG abdomen**
- **NAP**
- **B12 level**
- **Urate**
- **JAK2 MUTATION**

(39) Enumerate investigations in Myelodysplastic syndrome.

- **Bone marrow/Trephine**
- **Cytogenetic analysis**

(40) What are FAB criteria for diagnosis of Acute myeloid leukemia?

- **Blast cells atleast 30% in bone marrow of total nonerythroid cells**
- **Cytogenetic features of acute hypergranular promyelocytic leukemia**
- **3% blast cells are positive for Sudan black, myeloperoxidase or non-specific esterase**

(41) What is FAB criteria for diagnosis of MDS?

- **Blast cells less than 30% in bone marrow with myeloid neoplasm and ineffective haematopoiesis.**

(42) What is WHO criteria for AML and MDS?

- **AML- Atleast 20% blast cells in bonemarrow**
- **MDS- Less than 20% blast cells in bonemarrow**

ERYTHROCYTE & LEUCOCYTE CYTOCHEMISTRY

(1) What are Siderocytes?

- **Red cells containing granules of non haem Iron.**

(2) What are granules in siderocytes contain?

- **Water insoluble complex of Ferric iron, Lipid, Protein and Carbohydrate. (Haemosiderin)**

(3) What is basis of Perl's reaction?

- **The siderotic material or haemosiderin reacts with potassium FerriFerrocynide to form blue compound.**

(4)What are the Pappenheimer bodies?

- **Haemosiderin when stained by Romanowasky dies appears as basophillic granules.**

(5)What is ferritin?

- **Water soluble nonhaem compounds of iron with protein apoferrritin namely present in all the cells of body.**

(6) Where is haemosiderin normally seen in the body?

- **Macrophages in bone marrow ,liver (Kupffer cells) and spleen**
- **When body is overloaded with iron as in Haemochromatosis or transfusion haemosiderosis.**

(7) What is Transferrin?

- **ß globulin helping in transportation of iron to bone marrow.**

(8) What happens to iron in bone marrow?

- **Divides in to Haem in cytosol and mitochondria and form nonheamferritin.**

(9) How is haemosiderin formed?

- **By degeneration of Ferritin seen as golden yellow in phagocytic cells and blue by Perl's stain.**

(10) Where are siderotic granules normally seen?

- **In cytoplasm of erythroblasts of human bone marrow and in marrow reticulocytes.**

(11) When are siderotic granules normally seen in RBCs?

1.After Splenectomy because reticulocytes after delivery from bone marrow are sequestreted in spleen where they complete haem synthesis and iron is stored in siderotic granules.

- **After spleenectomy above procedure takes place in blood stream.**

2. Spleen removes large sideroblastIc granules from RBCs by pitting but in its absence these granules remain in RBCs.

(12) Which are the methods to stain siderotic granules?

- **Potassium ferrocyanide**
- **Acid ferrocyanide for smears stained by Romanowsky dye.**
- **Combination of PAS and iron staining**
- **By 1% bromochlorphenol**

(13) In which condition sideroblasts are increased?

- **Haemolytic anemia**
- **Megaloblastic anemia**
- **Haemochromatosis**
- **Haemosiderosis**
- **Impaired synthesis of haemoglobin**

(14) What are ring sideroblasts?

- **When there is defect in haem synthesis iron granules are deposited in mitochondria and appears to be collar around nucleus.it is seen in Sideroblastic anemia.**

(15) Which are different types of Sideroblastic anemia?

- **Congenital (Hereditary)**
- **Pyridoxine(Vitamin B6 deficiency)**
- **B6 antagonists antituberculoid drugs**
- **Myelodysplastic Syndrome**

(16) In which conditions secondary Sideroblastic Anemia is seen?

- **Alcoholism**
- **Lead Poisoning**

(17) In which Hematologic abnormalities ring sideroblasts are seen?

- **Myelodysplastic Syndrome**
- **Idiopathic Myelofibrosis**
- **Acute Myeloid Leukemia**
- **Erythroleukemia**

(18) How will you differentiate Sideroblastic Anemia of Hematologic Origin from Non Hematological causes?

- **Erythroblasts at all stages of maturity are loaded with siderotic granules in Hematologic causes while in non Hematologic causes, maximum mature cells are affected.**

(19) How will you diagnose Anemia of Chronic Disease?

- **Increased Iron stores with much siderotic material in macrophages but absent in erythroblasts.**

(20) In which conditions you see excessive Iron in macrophages?

- **Anemia of Chronic Disease**
- **Thalessemia Intermedia**

- **Dyserythropoetic Anemia**

(21) In which Conditions Heinz Bodies are seen?

- **Chemical Poisoning**
- **Drug Intoxication**
- **G_6PD Deficiency**
- **Presence of Unstable HB**

(22) What are Heinz Bodies?

- **They are late sign of oxidative damage and represent an end product of degradation of Hemoglobin seen as inclusions in Red blood Cells.**

(23) How Heinz Bodies are demonstrated in Unstained Smears?

- **Refractile Objects on Low power**
- **Dark Ground Illumination**
- **Phase contrast Microscope**

(24) Which dyes are used to stain Heinz Bodies?

- **Methyl Violet**
- **Brilliant Green**
- **Rhodanile Blue**
- **Brilliant Cresyl Blue**

(25) How HbH inclusions are demonstrated and in which condition?

- **By staining with Brilliant Cresyl Blue in Alpha Thalessemia Major.**

(26) How fetal Hb is demonstrated?

- **Kleihauer test of acid –elution**

(27) What is the principle of Kleihauer test?

- **Identification of cells containing HbF depend on the fact that they resist acid elution in comparison to normal cells**

- **HbF containing cells appear isolated darkly stained among background of palely stained ghost cells(normal RBCs)**

(28) At what pH Kleihauer test is carried out?

- **1.5**

(29) What are used as positive and negative controls in Kleihauer test?

- **Smear prepared from mixed cord and adult blood- positive control**
- **Smear prepared from normal adult blood- negative control**

(30) What is myelo peroxidase?

- **Enzyme located in primary and secondary granules of neutrophils and their precursors, eosinophil granules and azurophilic granules of monocytes.**

(31)How will you differentiate between WBCS on basis of MPO?

- **Eosinophilic granules- MPO is cyanide resistant**
- **Neutrophils and monocytes-MPO is cyanide sensitive**

(32) Which anticoagulants are used for MPO?

- **Heparin**
- **Oxalate**
- **EDTA**

(33) Which chromogen is preferred for MPO Staining?

- **Diaminobenzidine(DAB)**

(34) Which reagents are used for counterstaining in MPO?

- **Aqueous Hematoxylin**
- **methyl green**

(35) How results are interpreted in MPO staining?

- **1)RBC and erythroid precursors- Diffuse Brown cytoplasmic stain**
- **2) Myeloblast-Negative**
- **3) Promyelocyte & myelocyte- Strongly positive(near Golgi region granules)**
- **4)Metamyelocytes & mature neutrophils-Fewer granules staining**
- **5) Eosinophils-Strongly positive**
- **6) Monoblasts and monocytes- negative or positive**
- **7) Basophils-negative**

(36) How will you differentiate between Neutrophil, Eosinophil and Basophil granules in MPO testing?

- **Eosinophil Granules – Larger than Neutrophil Granules**
- **Eosinophil peroxidase biochemically and immunologically distinct from Neutrophil peroxidase.**
- **Basophil granules may be positive or negative but if positive, granules are smaller than neutrophil and diffusely scattered.**

(37) What can be Pathological variation in MPO Staining?

- **Congenital deficiency of Neutrophil MPO.**
- **Dysplastic Neutrophils**

(38) What is the other use of DAB Staining?

- **It stains Auer Rods strongly.**

(39) What is the characteristic of Sudan Black B dye?

- **It is a lipophilic dye that binds irreversibly to an undefined granule component in granulocytes, eosinophils and some Monocytes and cannot be extracted by any organic solvents.**

(40) How will you interpret Sudan Black B stain?

- **Black and Granular stain is seen both in Leukemic and Normal cells.**
- **Eosinophil granules which cannot be stained by MPO can be stained by Sudan Black.**

(41) What is significance of Alkaline Phosphatase?

- **It is found predominantly is Mature Neutrophils and Metamyelocytes.**
- **Bone Marrow Macrophages are positive.**

(42) Which blood is used as control in Neutrophil Alkaline Staining?

- **Patient with Reactive Neutrophilia or Pregnant women.**

(43) What is reaction and interpretation in NAP staining?

- **Reaction is blue or granular.**
- **Negative - No Granules.**
- **Occasional granules - 1**
- **Moderate number of Granules - 2**
- **Numerous coarse granules – 3**
- **Heavy positivity with numerous coarse granules – 4**

(44) What are physiological variations seen in NAP Scoring?

- **Newborn Babies**
- **Children**
- **Pregnant Women**

(45) What is pathological significance of NAP Score?

- **CML**
- **High Score in – intecurrent infection**
  Blast transformation
- **Low Score – Chronic Phase**

(46) In which other conditions low score of NAP is seen?

- **PNH**
- **Hereditary Hypophosphataemia**

(47) In which other conditions high score of NAP is seen?

- **Neutrophilia of infection**
- **Polycythemia vera**
- **Leukemoid reaction**
- **Hodgkin's disease**
- **Aplastic anemia**

(48) What is significance of acid phosphatase?

- **In diagnosis of T-cell ALL & Hairy cell leukemia**

(49) What is reaction and interpretation in Acid phosphatase method?

- **Reaction is red with mixture of granular and diffuse positivity**
- **T cells- Early differentiation**
- **Granulocytes- strongly positive**
- **Bone marrow macrophage**
- **Plasma cells**
- **Megakaryocytes**

(50) What is significance of PAS?

- **Periodic acid oxidises 1-2 glycol to produce dialdehydes**
- **These dialdehydes give red reaction when exposed to Schiff's reagent (leucobasic fuchsin)**
- **Positive reaction occurs with glycogen mostly but also with other carbohydrates**
- **In hematopoetic cells, main source of positivity is glycogen**

(51) How will you distinguish PAS reaction from glycogen and other carbohydrates?

- **By sensitivity of glycogen to diastase digestion**

(52) What is result in PAS positivity?

- **Reaction product is red with intensity roughly from pink to bright red**

(53) How will you interpret PAS results?

- **Neutrophils- intense granular positivity**
- **Eosinophils- granules negative but cytoplasm positive**
- **Basophils- usually negative**
- **Monocytes- variable diffuse positivity with superimposed granules**
- **RBCs- negative**
- **Platelets- diffuse positivity**
- **Lymphocytes- 10-40% positive for granules**

(54) In which diseases PAS is helpful?

- **AML & MDS**

(55) What is significance of Toluidine Blue?

- **For Basophils & Mast cells**

(56) Which cytochemical stains are significant in MDS & AML?

- **Perl's reaction for demonstration of ring sideroblasts**
- **Double staining with Chloroacetate and ANAE for evidence of Auer rods**

(57) Which cytochemical stains are important in ALL?

- **Weak with SBB**
- **Strong positive with PAS**
- **Acid phosphatase- focal positivity**

HAEMOSTASIS

(1) What are the components of hemostasis?

- **Blood vessels, platelets, plasma coagulation factors, their inhibitors and the fibrinolytic system**

(2) What are the characteristics of endothelial cells?

- **Nonthrombogenic**
- **Supply nutrients to subendothelial structures**
- **Act as barrier to macromolecules**

(3) What are functions of endothelial cell?

- **Luminal surface has Heparin sulfate which activates anti thrombin, which is inhibitor of coagulation enzymes**
- **Tissue factor pathway inhibitor is also present in endothelial cell bound to heparin**
- **Thrombomodulin & Protein C – coagulation active proteins are present in endothelial cells**
- **Thrombus formation is due to binding with THROMBOMODULIN**
- **Also synthesize Protein S, a cofactor of Protein C**
- **Participates in vasoregulation**

(4) What are the functions of blood vessel in normal hemostasis?

- **All endothelial cell functions**
- **Vasoconstriction**

(5) What is the mechanism of vasoconstriction?

- **By formation of vasoconstrictors by endothelial cells like angiotensin VI**
- **Formation of Thromboxane A2 by platelets**
- **Muscular walls of vessels**

(6) Which type of granules are present in platelets?

- **α granules**
- **δ granules**
- **Lysosomal granules**

(7) What are Gp I- Gp X?

- **They are glycoprotein present in platelet membrane**

(8) What is mechanism of contraction of platelets?

- **By contracting system of dense microtubular system & circumferential microfilaments.**
- **Actin, Myosin and Calmodulin also helps in contraction**

(9) What are platelet functions in hemostasis?

- **Adhesion, activation and aggregation**

(10) Which are the mediators helping adhesion of platelets?

- **Surface bound VWF with GpIb**

(11) Which chemical mediators help in platelet aggregation?

- **Arachidonic acid metabolites – Thromboxane A2**
- **Platelet agonists like thrombin, collagen, ADP, adrenaline, serotonins activating actin & myosin & contractile system of platelets**

(12) Why platelets are not activated when they come in contact with healthy endothelial cells?

- **Due to prostacyclin which activates cAMP, which inhibit AA metabolites, not allowing platelets to aggregate**

(13) Which coagulation factor deficiencies prolong APTT?

- **Factor XII**
- **Factor VIII**

(14) On which factors coagulation factors are dependent on?

- **Surface receptors and phospholipids present on surface of platelets**
- **Calcium**

(15) What is contact activation system?

- **Factor XII, high molecular weight kininogen, Prekallikrein, Kallikrein**
- **Activate fibrinolytic system of coagulation and initiate coagulation cascade**

(16) What is tissue factor?

- **It is cofactor for extrinsic pathway and physiological inhibitor of coagulation**

(17) What are vitamin K dependant factors?

- **Coagulation factor II, VII, IX and X**
- **Natural anticoagulants like proteins S, C and Z**

(18) Which are most important cofactors?

- **Factor VIII and V**
- **Circulates in combination with VWF**

(19) What is fibrinogen?

- **It is protein found in platelets from which fibrin is formed by thrombin – helping in formation of clot**

(20) What is role of factor XIII in hemostasis?

- **Factor XIII activated by thrombin, crosslink fibrin monomers, helping the fibrin clot to be held together, not allowing it of solubilize naturally.**

(21) Enumerate natural inhibitors of coagulation pathway.

- **TFPI**
- **Antithrombin**
- **Heparin**
- **Thrombomodulin**

- **Protein S**

(22) Which are the plasminogen activators?

- **Tissue plasminogen activators**
- **Urokinase**
- **Factor XIIA**
- **Bradykinin**

(23) Which are the plasminogen inactivators?

- **PAI-1**
- **α-2 anti plasmin**

(24) Which equipments are used for study of hemostasis?

- **Water baths at 37˚C**
- **Refrigerators at 2˚- 4˚C**
- **Centrifuges**
- **Reagents and buffers(Buffers should be inspected for bacterial growth)**
- **Plastic and glass tubes**
- **Pipettes**
- **Stopwatches and clocks**
- **Automated coagulation analysers**

(25) What care should be taken in reagents and buffers while using them for hemostasis?

- **Freshly prepared, mainly $CaCl_2$**
- **Properly labeled and dated**
- **Buffers to be checked for bacterial contamination**
- **Chromogenic substances to be reconstituted by sterile distilled water**

(26) Which preservative is used to preserve buffers in hemostasis when it cannot be used?

- **Azide**

- **Azide preserved buffers cannot be used in reagents for platelet studies or Elisa substrate.**

(27) What care should be taken in pipettes while performing tests for hemostasis?

- **Graduated Class A certified glass pipettes**
- **Automatic pipettes**
- **Acids not to be pipetted**
- **Preservation of contamination while pipetting**
- **Record of accuracy and precision**

(28) Which are the different types of coagulation analysers?

- **Automated**
- **Semiautomated**

(29) Which blood id ideally preferred for coagulation studies?

- **Venous blood**

(30) What is the effect of stress and exercise on coagulation factors?

- **They increase Factor VIII, VWF and Fibrinolysis**

(31) Why pressure cuff should not be used while collecting blood for coagulation studies?

- **It causes haemoconcentration, increases fibrinolytic activity, platelet release and activation of certain clotting factors.**

(32) Why blood sample should not be collected from indwelling line or catheter for testing hemostasis?

- **Because they are prone to dilution or heparin contamination.**

(33) Which is the most common anti coagulant used for coagulation studies?

- **Trisodium citrate as calcium in it is neutralised rapidly.**

(34) Why EDTA, heparin or oxalate are not recommended as anticoagulant for coagulation studies?

- **Oxalate-labile factors Vand VIII are unstable**
- **Heparin -Directly inhibit coagulation process and interfere with end & point determination**

EDTA

(35) What is normal blood citrate ratio for coagulation studies?

- **9 volume of blood to 1 volume**
  (0.55ml citrate for 5 ml citrate blood)

(36) When blood citrate ratio should be adjusted in coagulation studies?

- **When Hematocrit is abnormal with either severe anemia or polycythemia**

(37)What is significance of time of blood collection in coagulation studies?

- **Fibrinolytic activity follow definite circadian pattern at around 6am**
- **Drug administration time should be taken in consideration e.g APTT for monitoring heparin therapy.**

(38) Which type of plasma is preferred for coagulation studies?

- **Platelet poor plasma**
- **Platelet count should be low$10^4/\mu l$**

(39) How samples can be stored for coagulation studies?

- **Small aliquots for plasma stored in liquid nitrogen at -40^{o} C or -80^{O}c for severe weeks**

(40) Which can be sources of error in coagulation studies?

- **Faulty collection**
- **Under filling or over filling of sample disturbing citrate plasma ratio**

- **Unsuitable anticoagulant**
- **Collection of sample from indwelling line**
- **Contamination of reagents**
- **Delay in sample analysis**
- **Incorrect concentration of Cacl2**
- **Machine mal function**

(41) What will happen if sample is collected from indwelling line or catheter?

- **Marked prolongation of APTT or TT if heparin is given by line.**

(42) What is minimum number of serum to be pooled for calibration?

- **20**

(43)Name some standard national institutes supply calibrated plasma as reference?

- **NATIONAL INSTITUE FOR BIOLOGICAL STANDARDS & CONTROL**

(44)What is the most important principal of calibration?

- **Repetition to minimize possible errors used for accuracy**

(45)What is procedure of calibration?

- **Atleast 4-6 independent assays to be done**
- **Each plasma tested in duplicate**
- **Two replicate assays carried out each day**
- **The whole procedure to be repeated every 4 days**
- **More than one operator should do the test**

(46) What is an independent assay?

- **As assay for which new ampoule of standard is opened or for which a new set of dilutions are prepared from frozen previous reference plasma.**

(47) What is the significance of control plasma?

- **Running controls will enable detection of non linearity in the standard curve used for precision.**

(48) What are the causes of variability of coagulation assays?

- **Result of dilution error**
- **Differences in the composition of reagents**
- **Lack of experience**
- **Difference in techniques between operators**
- **Differences in method.**

(49) What should be used to clean glass tubes if they are to be reused?

- **Chromic acid**
- **Detergent like 2% Decan90.**

(50) What is the end point of coagulation assay?

- **Formation of clot.**

(51) What care should be taken to detect clot formation as end point in coagulation assay?

- **Tube should be watched in lower panel under water and should be dipped in and out so as to avoid cooling and slowing down of clot formation.**

(52)What is the difference in clots when formed fast or slow?

- **Fast turning clot- opaque & easier to detect**
- **Slow turning clot-appear as fibrin wisps which are difficult to detect.**

(53)Which reagents are commonly used in coagulation studies?

- **CaCL2,Barbitone buffered saline**

(54) How work up solution of CaCL2 is prepared?

- **0.025mol/liter is prepared and stored for short periods to prevent contamination**
- **Pre warmed CaCL2 is discarded at the end of procedure.**

(55) What is the principle of prothrombin time?

- **It measures the clotting time of plasma in presence of optimal concentration of tissue extract (thromboplastin).**

(56)What does prothrombin time indication?

- **Overall efficiency of extrinsic clotting pathway.**

(57)On which factors prothrombin time is dependent on?

- **Prothrombin factor V,VIIand X and fibrinogen concentration of plasma.**

(58)Why plasma for prothrombin time should not be stored at $4^{o}C$?

- **As it may have shortened PT as a result of factor VII activation in cold**

(59) From where thromboplastin is derived?

- **From tissue extracts & rabbit brain or lung.**

(60)What is recombinant thromboplastin?

- **Manufacture using recombinant human tissue factor produced in E.coli & synthetic phospholipids which do not contain any other clotting factors like prothrombin, factor VII and factor X.**

(61) What are the advantages of using recombinant thromboplastin?

- **As it does not contain any clotting factors it is highly sensitive to factor deficiencies and oral anticoagulant treated patient plasma samples. It is mostly having International sensitivity index close to 1.**

(62) How do we express results of PT?

- **Samples should be run in duplication. Mean reading in seconds should be expressed.**
- **Ratio of mean patient's plasma time to mean normal control plasma time is expressed.**

(63) How control plasma is obtained for prothrombin time?

- **Twenty normal men and women (not pregnant or taking oral contraceptive) is taken and logarithmic normal mean normal PT is calculated.**

(64) What is normal range of PT?

- **11-16 secs but it also depends on thromboplastin used, exact technique and visual or instrumental end point reading is also to be taken in consideration.**

(65) What are the causes of prolonged PT?

- **-Oral anticoagulants drugs as they are vitamin K antagonist**
- **Obstructive liver disease**
- **Vitamin K deficiency**
- **DIC**
- **Factor VII, X, V or prothrombin deficiency.**

(66) What is principle of APTT?

- **Measure clotting time and plasma after activation of contact factor without adding thromboplastin.**
- **Indicates efficiency of intrinsic pathway.**

(67)Enumerate contact activator is APTT

- **Kaolin, silica,ellagic acid**

(68) What is significance of calcium in APTT?

- **Coagulation in APTT does not proceed beyond factor XI in absence of calcium.**

(69)On what does APTT depend on?

- **Depends on contact factor, factorVII, IX and also on reactions with factor X, V, prothrombin &fibrinogen.**
- **Also affected by presence of circulating anticoagulant & heparin.**

(70) Which buffered saline of what pH is used in APTT?

- **Barbitone buffered saline**
- **pH-7.4**

(71) What care should be taken while choosing reagent of APTT?

- **The activator phospholipid combination should be sensitive to deficiency of factors VIIC, IX and XI at concentration of 0.35to 0.40µ/ml.**
- **System should be reaspirasive to unfrationated heparin over the therapeutic range of approx 0.3-0.7µ/ml**
- **System should be sensitive to presence of lupus like anticoagulants.**

(72) How results of APTT are expressed?

- **As mean of test done in duplication**

(73) What is normal range of APTT?

- **26-40sec.**

(74) Which are causes of prolonged APTT?

- **DIC**
- **Congenital coagulation disorder**
- **Hemophilia**
- **Liver disease**
- **Massive transfusion of RBCs**
- **Administration or contamination with heparin or other anticoagulants**
- **Circulating antibody**
- **Deficiency of coagulation factor other than factor VII**
- **Oral anticoagulants ,Vitamin K deficiency**

(75) Deficiency of which coagulation factor does not affect APTT?

- **Factor VII**

(76) What should be done to differentiate between deficiency of coagulation factor and circulating anticoagulants in APTT?

- **50:50 mixture of normal & test plasma should be tested.**

(77)What is the principle of Thrombin time?

- **Thrombin is added to plasma and the clotting time is measured**

(78) Which factors affect Thrombin time?

- **Concentration & reaction of fibrinogen**
- **Presence of inhibitory substance like fibrinogen degradation products (FDP) & heparin**

(79) How results are expressed in Thrombin time?

- **Test is repeated two times along with duplication of controls also.Mean time of two is expressed as Thrombin time.**

(80) What is normal Thrombin time?

- **Within 2 sec of control(15-19sec)**

(81) Why plasma has to be diluted in fibrinogen assay?

- **To lowers levels of inhibitors (FDPs and heparin)**

(82) What is normal range of fibrinogen?

- **1.8-3.6 g/lit**

(83) Which are first lines of investigations in abnormalities of hemostasis?

- **PT, APTT, Thrombin time, Fibrinogen, Platelet count**

(84) If all first line of investigations are normal except prothrombin time which is prolonged, what will the interpretation of result?

- **Factor Vll deficiency**
- **Early oral coagulation**
- **Lupus anticoagulant**
- **Mild ll, V or X deficiency**

(85) If all first line of investigations are normal except APTT which is prolonged, what will be interpretation?

- **Factor Vlll, IX,Xl,Xll, prekallikrein, HMWK deficiency**
- **Von Willebrand's disease**

- **Lupus anticoagulant**
- **Mild ll, V or X deficiency**

(86) If both PT and APTT are prolonged with other first line investigations what will be the interpretation?

- **Vit K deficiency**
- **Oral anticoagulants**
- **Factor V, X and ll Deficiency**
- **Liver failure**

(87)What will be the results in first line of investigations, if there is massive transfusion ?

- **Chronic liver disease or DIC**
- **PT,APTT –Increased**
- **Platelets count-Normal**

(88)What will be the results in first line of investigations in case of DIC or acute liver disease?

- **PT,APTT,TT –Increased**
- **Platelets count, Fibrinogen-Decreased**

(89) If all first line of investigations are normal, still bleeding does not stop after injury or operation. What can be the causes?

- **Disorder of platelet function, congenital or acquired**
- **VWD (Factor Vlll not sufficient to prolong APTT)**
- **Mild coagulation disorder**
- **Factor Xlll deficiency**
- **Vascular disorder**
- **Severely damaged vessel**
- **Disorder of fibrinolysin like antiplasmin or PAI-I deficiency**
- **Administration of low molecular weight heparin**

(90) Which are second line investigations for hemostasis?

- **Specific factor assays, Bleeding time, clot solubility test**

(91) What is the principle of correction test using PT or APTT?

- **Correction test is doing test by mixing patient's plasma with normal plasma.**
- **It indicates possible factor deficiency.**

(92) What is the meaning of failure of Correction tests?

- **Presence of Inhibitor**

(93) Why normal plasma is used for Correction test of PT or APTT?

- **As normal plasma contains all coagulation factors, therefore it identifies presence of inhibitor or factor deficiency.**

(94) What is done in mixing correction test?

- **Perform PT, APTT on control.**
- **Perform PT, APTT with 50:50 mixture of patient's plasma and control.**
- **Duplication should be done.**

(95) How will you interpret mixing correction tests for PT or APTT?

- **Prolongation of result – Deficiency of Clotting Factor but will return within few seconds of normal – Factor Deficiency**
- **If APTT prolonged after correction – Inhibitor**

(96) In which cases mixing studies are misleading?

- **Inhibitors like antifactor VIII antibodies show positive results after 2 hours as they are time dependent.**
- **Lupus like anticoagulants being relative weak, become apparent in concentration of 25:75.**

(97) Which Factor assays are based on Prothrombin Time?

- **Factor VII**

(98) What is Protamine Sulphate?

- **A substance in corrected TT having net electropositive charge and interacts with Heparin, FDP neutralizing its inhibitory effect.**

(99) What is action of Toluidine Blue in corrected TT?

- **It neutralizes inhibitory action of Heparin.**

(100) How will you interpret results of corrected TT?

- **TT Plasma corrected with**

Saline	Normal Plasma	Protamine Sulphate	Toluidine blue	Interpretation
NO	YES	NO	NO	DEFICIENCY
NO	VARIABLE	NO	YES	Dysfibrinogenemia of Liver Disease
NO	VARIABLE	YES	NO	High Concentration of FDP

(101) Which factor assays are based on APTT?

- **Factor Vlll:C**

(102) In which conditions reduced factor Vlll:C is found?

- **Hemophilia**
- **Carrier Of hemophilia**
- **Acquired Hemophilia**

- **VWD**
- **Congenital combined deficiency**
- **DIC**

(103)How will you confirm factor Vlll deficiency due to hemophilia A?

- **VWF: Ag and VWF: RCo is measured with patient's family history to rule out hemophilia A from VWD.**

(104) What are the causes of acquired inhibitors of coagulation in body?

- **Autoimmune**

(105) When an inhibitors of coagulation is suspected in body?

- **When prolonged clotting time does not correct after mixing 50:50 normal plasma.**

(106)Which are the common anticoagulants seen in the body?

- **LAC-Predisposed to thrombosis**
- **Antibodies to factor Vlll C - causes bleeding**
- **Paraprotein-causes acquired VWF**

(107) Which tests are generally affected in Dysfibrinogenaemia?

- **APTT and TT**

(108)What are the causes of congenital fibrinogen abnormality?

- **DIC**
- **Hyperfibrinoysis**

(109)What is Reptilase or Ancord time?

- **Reptilase is a purified enzyme from snake and is used to replace thrombin in TT**
- **These are not inhibited by heparin and so give exact results.**

(110)What are vascular disorders? What are their causes?

- **Vascular disorders are those that arise as a result of a defect or deficiency of vessel wall.**
- **Common causes are-Inherited disorders of collagen -**

-Acquired disorder like amyloid or scurvy.

(111)Which test is most important in diagnosis of vascular disorders?

- **Bleeding time**

(112)What is the principle of bleeding time?

- **A standard incision is made on the volar surface of forearm and time of incision bleed is measured.**
- **Cessation of bleeding indicates formation of hemostatic plug which is dependent on adequate number of platelets and their ability to adhere to subendothelium and form platelet aggregate.**

(113)What is normal range of bleeding time?

- **2-7 minutes**

(114)Which are different methods of doing bleeding time?

- **Standardized Template method**
- **Ivy's method**

(115)What are the causes of prolonged bleeding time?

- **Thrombocytopenia- less than 50000**
- **Disorders of platelet function**

e.g. Congenital- thrombasthenia, storage pool defect

Acquired- drug use, uremia, presence of paraprotein, MDS,

Myeloproliferative disorders

VWD- defective platelet adherence

Vascular abnormalities- Ehler Danlo's syndrome

(116)On which factors bleeding time is dependent?

- **Standardization of sphygmomanometer pressure**
- **Longitudinal orientation of incision**
- **Blotting technique**

(117)What is the cause of shortening of BT?

- **If we repeat test within short period**

(118)Which are the first line investigations for platelets?

- **Peripheral blood count**
- **Bleeding time**
- **Fresh blood sample smear examination**

(119)In which groups platelet function tests are divided into?

- **Adhesion test**
- **Aggregation test**
- **Assessment for granular content**
- **Assessment for release reaction**
- **Investigation for prostaglandin pathways**
- **Tests for platelet coagulant activity**

(120)How granular content of platelets is assessed?

- **By electronmicroscopy**
- **By measuring substances released by platelets by different kits**

(121)Which drugs affect platelet functions?

- **Aspirin**

- **NSAIDs**
- **Steroids**
- **β blockers**
- **penicillin**
- **heparin**
- **cephalosporins**

(122)Which factors affect platelet aggregation?

- **Presence of chylomicra**
- **Chilled blood as cold activates platelets**

(123)Name platelet aggregative agents

- **Adenosine 5 Biphosphate**
- **Collagen**
- **Risticetin**
- **Arachidonic acid**
- **Adrenaline**

(124)How will you standardize Platelet Rich Plasma?

- **Platelet count performed on PRP**
- **It falls outside 200-400x10^9/L**
- **For high counts dilution of PRP in patients PPP is done**
- **Centrifugation should not be done as it activates platelets**

(125)Which factors affect platelet aggregation tests?

- **Centrifugation – should be at room temperature. At 4˚C large platelets are not removed**
- **Time – more reactiveness after 2 hours of centrifugation**
- **Platelet count – weak aggregation below 150 or over 400x10^9/L**
- **pH- < 7.7 inhibits platelet aggregation. >8 enhances platelet aggregation**

- **mixing speed - <800 RPM or >1200 RPM slows aggregation**
- **Hematocrit - >0.55-less aggregation**
- **Temperature - <35˚C-less aggregation**
- **Dirty cuvette**
- **Air bubbles in cuvette**

(126)In Bernard Soulier syndrome or thrombasthenia, which test is preferred?

- **Platelet membrane glycoprotein by flow cytometry**

(127)What is the principle of clot solubility test for factor XIII?

- **Fibrin clots formed in presence of factor XIII and thrombin are stable. Whereas clots formed in absence of factor XIII dissolve rapidly.**
- **Test is more sensitive with thrombin than calcium**

(128)What is DIC?

- **It is referred as consumptive coagulopathy because of its characteristic feature of consumption of clotting factors and inhibitors with loss of normal regulatory mechanism.**

(129)What happens to mechanism of clotting in malignancy?

- **The activation of clotting factors is less marked and partially compensated resulting in tendency to thrombosis in malignancy**

(130)How will you diagnose DIC?

- **PT, APTT, TT- prolonged**
- **Fibrinogen- reduced – late sign**
- **Thrombocytopenia- early sign**
- **FDP- raised**

(131) What is normal range of FDP?

- **Less than 10µg/ml**
- **10-40µg/lit-Acute venous thromboembolism**
  -Acute myocardial infarction
  - Severe pneumonia
  -Major surgery
- **>40µg/ml-DIC**

(132) What is normal range of D-Dimer?

- **<200µg/lit**

(133)What is significance of D-Dimer?

- **To exclude thrombosis**

(134) What is importance of family studies in hemophilia?

- **Hemophilia A and B are inherited by X-linked genes. So all sons are normal but all daughters are carrier.**

(135) In which factor deficiency heterozygots bleed severely?

- **Factor Xl**

➢

BLOOD FILM EXAMINATION

(1) What are the methods of making film?

- **Two slide method or Wedge method**
- **Coverglass method**
- **Spinner method**

(2) What are the criterias for good smear?

- **Thick and thin portion with gradual transition**
- **Smooth even appearance**
- **Free from ridges, waves and holes**
- **It must be without ragged tails**
- **RBCs must be evenly distributed and separated**
- **Fast or slow drying can lead to artifacts**
- **Labeling should be on thicker end**

BLOOD STAINS

(1) Which aniline dyes are used for blood staining?
Following are the aniline dyes:

- **Basic – methylene blue dye which stains nuclei and other structures**
- **Acidic – eosin dye**

(2) Name Romanowsky stains.

- **Giemsa stain and Wright stain with fixative**
- **Leishman, May Grunwald Giemsa stain**

(3) Which reagents are used in Wright's stain?

- **Potassium Phosphate and sodium phosphate are used as buffer**
- **Mixture of Thiazine (Methylene blue, Azure A, Azure B)**

(4) Which stain is excellent for malarial parasite ?

- **Giemsa stain is excellent for malarial parasite.**

(5) What are reasons for excessive blue stain and how can it be prevented?

Excessive blue stain may be because of:

- **Thick film**
- **Prolonged staining**
- **Inadequate washing**
- **Too high alkalinity**

It can be prevented by :

- **Shorter period staining**
- **Using less stain and more diluent**
- **Use new buffer with lower ph (many times buffer may be alkaline).**

(6) What are reasons for excessive pink stain?

- **Insufficient staining**
- **Prolonged washing time**
- **Mounting cover slips before drying**
- **Too high acidity**
- **Acidic buffer**

(7) What are the causes for precipitates on stain?

- **Unclear slide**
- **Drying**
- **Inadequate washing**
- **Failure to hold slide horizontally**
- **Inadequate filtration of stain**
- **Permitting dust to settle on slide or smear are causes of precipitates on stain.**

ABNORMAL SHAPES

(8) What is polychromatophilia?

- **Polychromatophil cells are young RBCs with retained RNA and it implies reticulocytosis seen in hemolysis and acute blood loss.**

(9) In which conditions elliptocytes are seen on smear?

- **Hereditary elliptocytosis**
- **Iron deficiency anemia**
- **Myelofibrosis with myeloid metaplasia**
- **Megaloblastic anemia**
- **Sickle cell anemia**

(10) In which condition spherocytes are seen on smear?

- **Hereditary Spherocytosis**
- **Autoimmune Hemolytic anemia**
- **Direct injury to cell like heat**

(11) In which condition target cells are seen?

- **Obstructive jaundice**
- **Post splenectomy**
- **Thalasemia**
- **Hb C disease**

(12) In which condition schistocytes are seen?

- **Hemolysis**
- **Burns**
- **Microangiopathic hemolytic anemia (helmet cells, Burr cells)**

(13) In which condition acanthocytes are seen ?

- **A betalipoproteinemia**
- **Liver disease**

(14) In which condition echinocytes (crenated cells) are seen?

- **Echinocytes seen on smear suggests artifact.**

(15)What is Basophilic Stippling? In which conditions it is seen?

- **Presence of Irregular basophilic granules.**
- **Stain deep blue with Wright's Stain.**
- **Fine Stippling: Increased Polychromatophilia.**
- **Coarse Stippling (instability of RNA in young cells): Lead Poisoning, Impaired Hemoglobin Synthesis, Megaloblastic Anemia.**

(16)What is Pappenheimer bodies? What is its significance?

- **Pappenheimer bodies are inorganic iron containing granules siderocytes.**
- **Seen in Splenectomy patients.**

(17)What are Howell Jolly Bodies? In which conditions they are seen?

- **Smooth round remnants of nuclear chromatin.**
- **Single: Megaloblastic Anemia, Splenectomy.**
- **Multiple: Megaloblastic Anemia, Abnormal Erythropoesis.**

(18) What are Cabot Rings? In which conditions they are seen?

- **Ring shaped, Figure of Eight, loop shaped.**
- **Rare -> Pernicious Anemia**
  -> Lead Poisoning
  -> Erythropoeisis
- **Probably microtubules remaining form a mitotic spindle.**
- **Evidence of abnormal Erythropoesis.**

(19)What is Malarial Stippling?

- **Seen in Plasmodium Vivax.**
- **Schuffner granules: Purplished.**

(20)When Roulex formation is seen on Peripheral smear?

- **Elevated plasma fibrinogen (Cryoglobulins).**
- **Elevated Erythrocyte Sedimentation Rate(ESR).**
- **Paraproteinemias. (Monoclonal Gammopathy)**
- **Cold agglutinin.**

(21)Which form of NRBCs are present is blood in disease?

- **Polychromatic.**

(22) Which conditions show Megaloblast?

- **Megaloblastic Anemia**
- **Pernicious Anemia.**

(23) In which conditions NRBCs present in blood?

- **Thalassemia Major.**
- **Hemolytic Anemia.**

(24) Which conditions showing Leucoerythoblastic reactions?

- **Indicates marrow involvement in tumor.**
- **Myelofibrosis with myeloid metaplasia**
- **Myeloproliferative Disorders.**
- **Metastasis.**
- **Multiple Myeloma.**
- **Gaucho's Disease.**
- **Leukemias.**

(25) What is difference between Segmented and Band Neutrophil?

- **Segmented Neutrophil: Atleast two lobes separated by filaments (56%).**
- **Band Neutrophil: Either a strand of nuclear material thicked than a filament connecting two lobes or U-Shaped nucleus of uniform thickness (3%).**

(26) What is shift to left?

- **Increase in percentage (%) of band cells which is normally 3%.**

(27) Universally stained granules of basophil resembles to which parasite?

- **Histoplasma capsulatum**

(28)What are basket cells?

- **Basket cells are nuclei of WBC without cytoplasm presenting fragile cells.**
- **Presents in Atypical lymphocytosis,**
  Chronic lymphocytic leukemia,
  Acute Leukemias.

(29)What are causes of Degenerative changes on smear?

- **Degenerative changes caused by EDTA effect or oxalated blood of longer duration.**

(30)Where do you see contracted cells on smear?

- **Contracted cells are seen in thicker part of wedge films where drying is delayed.**

(31)When do you see endothelial cells on smear?

- **Endothelial cells may appear in first day of blood in fingerprick specimen or venous blood mistaken for histiocytes.**

(32) What are sources of error in leukocyte count?

- **Mechanical errors-Variations in collection of blood samples**
  -Inadequate mixing
  -Irregular films
  -Poor staining

(33)What ideal requirement for automated cell counter?

- **1. Distribution of cells analyzed should be identical with blood.**
- **2. Leucocytes found in blood diseases should be accurately identified or detected.**
- **3. Speed of process enabling a large number of cells to be counted to minimize statistical error.**
- **4. Instrument should be cost effective.**

(34)What is digital image processing?

- **Uniformly made and stained blood film is placed on motordriven microscope stage and computer controls scanning of slide and stopping when leucocytes are in field recording optical detects like nuclear and cytoplasmic ratio, size, shape ,color by television camera analyzed by computer and converted into digital film comparing it with memory bank.**

(35)What is normal platelet count in peripheral smear?

- **One platelet/10 to 30 RBCs**
  Or
- **7 to 20 platelets/oil immersion field**

(36) What are changes of error in EDTA bulb for platelets?

- **Large and hypogranular paltelets are less than 3%, if smear is made within 10 to 60 minutes of blood collection.**
- **Large and hypogranular platelets are more than 3%, if smear is made immediately or after 3 hours. i.e. Activated platelets.**

(37) Which are conditions showing large platelets?

- **Conditions showing large platelets are:**
  - **ITP.**
  - **Bernard-Soulier's syndrome.**
  - **Myelophthisis.**
  - **Myeloproliferative disease.**

(38) When irregular shaped platelets are seen in blood films?

- **Irregular shaped platelets are seen when blood films are made from skin puncture wounds.**

(39) Why Hb level is more in males than in females?

- **Hb level is more in males than in females mainly due to effects of androgen in stimulating erythropoietic production and its effects on marrow.**

(40) Which are factors affecting Hb level?

- **Diurnal variation**
  - **Highest in morning.**
  - **Lowest in evening.**
- **Exercise and standing position**
  - **Hb increases due to loss of fluid.**
- **High altitude**
  - **Hb increases as anoxia increases erythropoietin production.**
- **Capillary blood**
  - **Hb is higher in capillary blood than in venous blood due to slowing of RBCs and loss of fluids.**

(41) Upto what age lymphocytes are predominant in blood?

- **Lymphocytes are predominant in blood upto seven years of age.**

(42) Which are factors affecting WBCs?

- **Factors affecting WBCs are:**
  - **Neutrophils: Highest in afternoon.**
    Lowest in morning.
  - **Exercise: Increases leukocytes.**
  - **Neutrophil concentration: Lower in blacks.**
    More in Whites.
  - **Smoking: Increases leukocytes.**
  - **Menstruation: Increases eosinophils and decreases neutrophils.**
  - **Ovulation: Decreases basophils.**

(43) Which are factors affecting platelets?

- **Factors affecting platelets are:**
  - **Menstruation: Decreases platelets.**
  - **At birth: platelets are low.**
  - **Women are having more platelets than men.**

(44) What is the basic cause of changes in ESR?

- **Increased levels of fibrinogen, α_2 and $ß_2$ Globulins causes increased ESR.**
- **Albumin decreases ESR.**
- **Cholesterol increases ESR.**

(45) Why ESR is increased in anemia?

- **Changes in RBC-Plasma ratio favor rouleux formation.**
- **Sedimentation rate∝ Weight of cell.**
- **Sedimentation rate 1/∝ Surface.**
- **Rouleux formation decreases surface area which in turn increases ESR.**
- **Microcytes are slowing rouleux formation.**
- **Macrocytes are fastening rouleux formation.**
- **Sickle cells and spherocytes decrease rouleux formation and decrease ESR.**

(46) What are the sources of error in ESR?

- **High concentration anticoagulants (heparin) – high ESR**
- **Bubbles in tube while filling**
- **Hemolysis**
- **Tilting of tube – high ESR**
- **Plastic ESR tubes – high ESR**
- **Temperature below 20˚C and above 25˚C – alter ESR**
- **Test is not done within two hours of collection - alter ESR**

(47) Interpretation of ESR

- **Sickle cell disease – low ESR**
- **Osteomyelitis – high ESR**
- **In stroke – high ESR - bad prognosis**
- **Prostate carcinoma- value >37mm/hr – bad prognosis**
- **Coronary artery disease – high ESR**
- **In cancer - value >100mm/hr – metastasis**
- **Arteritis – high ESR**
- **Hodgkins disease – ESR <10mm/hr – good prognosis,**
  >60mm/hr – bad prognosis
- **Renal cell carcinoma- high ESR before six years of diagnosis**

HEMOLYTIC ANEMIA

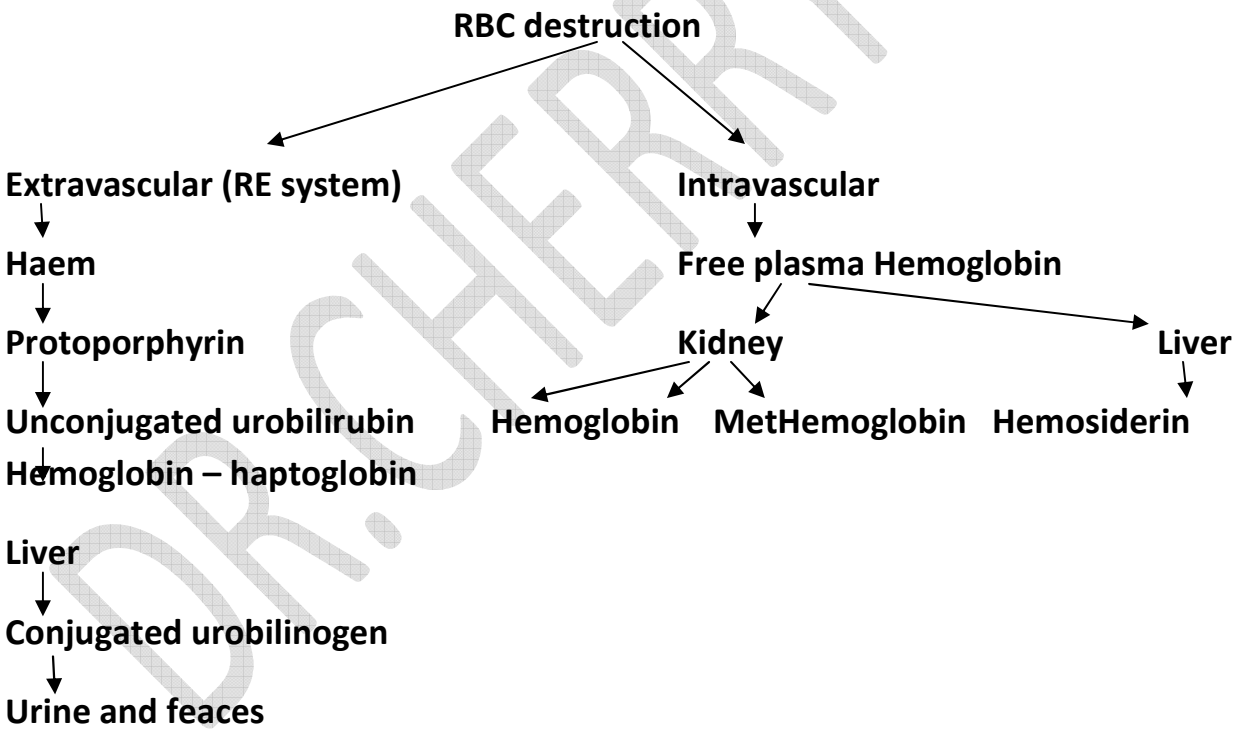
(1) After normal lifespan of 120 days where are RBCs destroyed?

- **RBCs are destroyed in cells of RE system (Liver, Spleen, Bone marrow)- Extravascular hemolysis.**
- **Within bloodstream - intravascular hemolysis**

(2) What is hemoglobinuria?

- **Excretion of Hemoglobin in urine due to excess hemolysis is known as hemoglobinuria.**

(3) Which investigation correlate with intravascular and extravascular hemolysis?



Dr.CHERRY SHAH

(4) Enumerate Series of investigation in Hemolytic anemia

- **Hemoglobin estimation, Retic count, PS for Spherocytes, Elliptocytes, Schistocytes, Autoagglutination**
- **Increased unconjugated Serum bilirubin (Extravascular)**
- **Urine Urobilinogen (Extravascular)**
- **Haptoglobin (Intravascular)**
- **Urinary Hemoglobin or Hemosiderin (Intravascular)**
- **Direct Antiglobulin Test**
- **Osmotic Fragility test**
- **Hemoglobin in urine, Plasma Hemoglobin**

(5) Precise diagnostic tests of hemolytic anemia

- **Hereditary hemolytic anemia**

  -Osmotic fragility after 24hr incubation
  -G6PD
  -PK assay
  -HemoglobinA2, HemoglobinF
  -Sickling test – demonstration of protein & red cell

- **Autoimmune acquired Hemolytic anemia**
  - **DAT**
  - **Cold Agglutination**
  - **Serum Proteins**
  - **Autoantibodies**
- **Drug Induced**
  - **Heinz bodies**
  - **G6PD**
  - **Meth Hemoglobin**
  - **Sulph Hemoglobin**
- **Mechanical Stress**
  - **RFT**

- **Coagulation study**
- **RBC morphology**
- **Fibrinogen assay**

- **Others – PNH – Acidified serum lysis test, sucrose analysis**
- **RBC life span , Spleenectomy**

(6) What are the methods to measure plasma Hemoglobin?

- **Peroxidase reaction**
- **Direct measurement of Hemoglobin by Spectrometry**

(7) What is normal range of Plasma Hemoglobin ?

- **10-40 mg/L**

(8) What is Significance of red plasma Hemoglobin ?

- **Intra and Extravascular hemolysis – breakdown of RBC**
- **In plasma Hemoglobin-Haptoglobin complex formed**
- **Removed by RE cells**
- **Excess unbound Hemoglobin filtrated by glomeruli causing hemoglobinuria**
- **Partly reabsorbed by tubules – iron is excreted in urine as Hemosiderin, Haem and globin is reabsorbed in plasma.**
- **Haem binds to albumin – methalbumin**
- **Globin competes with Hemoglobin to bind with haptoglobin – Hemoglobin level increase in hemolytic anemia.**

(9) In Which conditions plasma Hemoglobin increase ?

- **In PNH, Blackwater fever, March Hemoglobinemia, Paroxysmal cold hemoglobinuria – Plasma Hemoglobin increases.**

(10) In Which conditionS Plasma Hemoglobin is slightly increased?

- **In Warm type Autoimmune Hemolytic anemia, Sickle cell anemia and β-thalassemia, Plasma Hemoglobin is slightly increased.**

(11) In which case of hemolytic anemia Plasma Hemoglobin is not raised?

- **In case of Hereditary Spherocytosis, hemolysis occurs in spleen, hence plasma Hemoglobin is not raised.**

(12) What is significance of Hemosiderin in hemolysis?

- **Iron from haem released on hemolysis is retained and stored as Ferritin and Hemosiderin.**
- **In severe hemolysis there is accumulation of Hemosiderin intracellularly when cells slough and it is excreted in urine.**

(13) What is haptoglobin ?

- **It is glycoprotein synthesized in liver.**
- **It has two pairs of α and β chains.**
- **In case of hemolysis, α globin of Hemoglobin binds to β chain of haptoglobin.**

(14) How Haptoglobin is measured?

- **Colour chromatography**
- **Turbidometry**
- **Nephelometry**
- **Radial immunodiffusion**

(15)In electrophoresis at what position Hemoglobin-Haptoglobin complex band is seen?

- **At region of α_2 globulin, Hemoglobin –haptoglobin complex band is seen.**

(16) What is normal range of haptoglobin concentration?

- **Normal range varies in different methods of measuring Haptoglobin.**
- **Radioimmunodiffusion – 0.8 to 2.7g/l**
- **Turbidometry – 0.5 to 1.6g/l**
- **Nephelometry – 0.3 to 2.2g/l**

(17) What is significance of haptoglobin concentration?

- **Haptoglobin concentration decreases when daily turnover of Hemoglobin exceeds twice the normal rate.**
- **In case of small degree Intravascular hemolysis, turnover is not increased hence there is formation of methalbumin with decrease in haptoglobin concentration.**
- **Hepatocellular disease and congenital ahaptoglobinemia shows low haptoglobin count without hemolysis.**
- **Megaloblastic anemia shows low haptoglobin count with hemolysis.**

(18) Where is Hemoglobin-Haptoglobin complex cleared?

- **Liver is site of Hemoglobin-haptoglobin complex clearance.**

(19) When does the concentration of Haptoglobin increase?

- **In Pregnancy, malignancy, chronic infection, SLE, steroid, Oral contraception, Hodgkin's lymphoma there is increase in haptoglobin concentration.**

(20) What is haemopexin?

- **It is a β_1 glycoprotein synthesized by liver.**
- **Haem derived from hemoglobin when fails to bind with haptoglobin binds with haemopexin or albumin.**
- **Normal range : 0.5-1.15g/L**
- **Haemopexin is decreased in hemolysis when first haptoglobin is used and then hemopexin is used.**
- **Hemopexin is decreased in Thalessemia major, Renal and Liver disease causing hemolytic anemia.**
- **Increased hemopexin is seen in Diabetes mellitus, infection, carcinoma.**

(21) How is Plasma/ Serum methalbumin measured?

- **It is measured using hand spectroscope.**
- **It is not sensitive method.**
- **It shows weak band in red region (124nm).**

- **HbO_2 shows band in yellow-green region.**

(22) How will you differentiate between bands of methalbumin from Reduced hemoglobin Hi?

- **By reversing spectroscope, we can differentiate between methalbumin and Hi.**

(23) What is Schumm's Test?

- **Small quantities of methalbumin shows absorption band on hand spectroscope at 624nm which is too weak to recognize.**
- **Schumm's test is performed to recognize small quantity of methalbumin.**
- **It can be obtained by extracting pigment by ether.**
- **Converting it to an ammonium hemochromogen.**
- **This gives intense band in green region.**

(24) What is significance of Methalbumin?

- **Methalbumin is found in plasma when haptoglobin are absent in hemolytic anemia, predominantly intravascular hemolytic anemia.**

(25) How will you demonstrate Hemosiderin in urine?

- **Centrifuge 10 ml of urine at 1200rpm for 10-15minutes.**
- **Allow to dry**
- **Fix in methanol**
- **Stain by method of staining of siderocytes**
- **Appears as grouped blue staining granules 1-3µ in size intra and extracellularly**
- **Should be done on fresh sample.**

(26) What is significance of hemosiderinuria?

- **It is sequel of hemoglobin in glomerular filtrate.**
- **It is valuable sign of intravascular hemolysis.**

(27) Which are chemical tests of hemoglobin catabolism?

- **Serum bilirubin**
- **Urobilionogen, Urobillin**

(28) In which form bilirubin is present in serum?

Bilirubin is present in serum as:

- **Unconjugated prehepatic bilirubin**
- **Conjugate bilirubin (to glucuronic acid)**

(29) What form of bilirubin is found in hemolytic anemia?

- **Unconjugated bilirubin is found in hemolytic anemia.**

(30) What happens in Hemolytic disease of newborn?

- **There is high value of unconjugated bilirubin which is toxic to brain causing kernicterus.**
- **Bilirubin level is important to determine exchange transfusion.**
- **Normal newborn bilirubin level : 85µmol/L**
- **Infants with Hemolytic disease of newborn: 350 µmol/L and needs exchange transfusion.**

(31) In which condition bilirubin is moderately raised?

- **In pernicious anemia, bilirubin is moderately raised.**

(32) How urobilin and urobilinogen are formed?

- **By bacterial action on bile pigments in intestine, urobilin and urobilinogen are formed.**

(33) Apart from hemolytic anemia in which other condition urobilinogen is increased?

- **In dyshematopoietic anemia, pernicious anemia and in liver dysfunction, urobilinogen is increased.**

(34) Which is precursor of porphyrin?

- **Δ-aminolevulenic acid is precursor of porphyrin.**

(35) Which are porphyrins of clinical importance?

- **Protoporphyrin, uroporphyrin and coproporphyrin are porphyrins of clinical importance.**

(36) Which porphyrin is precursor of haem in hemoglobin and Myoglobin?

- **Protoporphyrin is precursor of haem in hemoglobin and Myoglobin.**

(37) In which conditions protoporphyrin is increased?

- **It is increased in deranged haem synthesis. Eg.: Sideroblastic anemia, Lead toxicity, Iron deficiency anemia, Thalassemia.**

(38) Which are abnormal hemoglobin pigment?

- **Methaemoglobin – Hi**
- **Sulphhaemoglobin – SHb**
- **Carboxyhaemoglobin – HbCO**
- **These abnormal haemoglobin can be demonstrated by Spectrometry between 400-700nm.**

(39) By which method abnormal hemoglobin pigments are detected?

- **Absorption Spectroscopy is the method of detecting abnormal hemoglobin pigment.**

(40) How will you differentiate between methaemoglobin and sulphaemoglobin on spectroscopy?

- **In the red band at 620-630nm, if drop of yellow ammonium sulphate is added to diluted blood solution after centrifugation, band of methaemoglobin will disappear but band of sulphaemoglobin will not disappear.**

(41) What is significance of Hi(Reduced hemoglobin) and SHb (Sulphemoglobin) in blood?

- **Hi – normally 1-2% of total Hemoglobin**
- **It increases due to oxidation of hemoglobin by drugs like sulphonamide, nitrate, nitrites.**
- **It leads to cyanosis.**

- **It can be converted to Oxyhemoglobin by reducing agents like methylene blue.**
- **Sulphaemoglobin is also formed at same time with Hi but it represents irreversible stage of hemoglobin degradation.**

(42) What is importance of Carboxy-Haemoglobin in blood?

- **Carboxyhemoglobin is usually < 1% in normal blood and upto 10% in smokers.**
- **It is increased in hemolytic anemia.**
- **It causes tissue hypoxia and can lead to death.**

-

IMMUNOPHENOTYPING

(1) What are the applications of immunophenotyping?

- **Diagnosis of acute and chronic leukemia**
- **Clues to pathogenesis of these disorders**
- **Possible monitoring of small numbers of residual leukemic cells**
- **Using Monoclonal antibodies as therapeutic tools**

(2) What is monoclonal antibody?

- **Antibodies that are made by identical immune cells that are all clones of unique parent cell**
- **They recognize binding sites on a single antigen**

(3) What are polyclonal antibodies?

- **They are made by using several different immune cells having affinity for same antigen but different binding sites**

(4) What are different methods to study immunological markers?

- **Flow cytometry- Test suspensions to variable cells or fixed cells**
- **Immunocytochemistry- Haematology**
- **Immunohistochemistry- Histopathology**

(5) On which samples immunophenotyping is done?

- **Isolated mononuclear cells**
- **Whole blood specimens**

(6) What does mononuclear cell fraction consists of?

- **Lymphocytes, Monocytes, Blasts etc.**

(7) What is immunophenotyping?

- **It is the method for detecting membrane antigens in viable cells and cytoplasmic and nuclear antigens in fixed and stabilized cells**

Dr.CHERRY SHAH

(8) Which are the methods of immunophenotyping?

- **Flow cytometry**
- **Fluorescence microscopy**

(9) Which are common immunocytochemical techniques?

- **Immunoperoxidase**
- **Antiphosphatase antialkaline phosphatase**

(10) Which are immunological markers in Acute Leukemia?

- **Acute B cell lymphocytic leukemia- CD19, CD10, cytoplasmic CD22, CD79a**
- **Acute T cell lymphocytic leukemia- CD2, CD7, cytoplasmic CD3**
- **Myeloid- CD34, CD33, CD117, cytoplasmic myeloperoxidase (anti MPO)**
- **Nonspecific in progenitor cells- CD34, HLA-Dr, TdT**

(11) Where immunological markers are expressed first?

- **Cytoplasm**

(12) What is significance of markers CD34 and TdT?

- **They are helpful in differential diagnosis between acute leukemia and large cell lymphomas.**
- **Positive in leukemia**
- **Negative in lymphoma**

(13) Which immunological markers are expressed in monoblastic leukemia?

- **CD14, Antilysozyme**

(14) Which immunological marker is expressed in erythroid leukemia?

- **CD36, antiglycophorin A**

(15) Which carcinomas mimic acute leukemia in bone marrow? How will you differentiate?

- **Neuroblastoma**
- **Oat cell carcinoma**
- **CD45 helps to differentiate**

(16) Which is immunological marker in Megakaryocytic leukemia?

- **CD41, CD42, CD61**

(17) What are mixed lineage leukemia?

- **Biphenotypic (AML+ALL)**
- **Dendritic cell leukemia**
- **Blastic natural killer cell leukemia**

(18) What is full form of TdT?

- **Terminal deoxynucleotidyl Tranferase**

(19) How will you differentiate acute lymphoid disorders from chronic lymphoid disorders?

- **TdT positive – generally acute**
- **TdT negative – generally chronic**

(20) Which are immunological markers for B cell in lymphoid disorders?

- **CD19, CD23 – first line**
- **CD25, CD103 – second line**

(21) Which are immunological markers for T cell in lymphoid disorders?

- **CD2, CD5 – first line**
- **CD3, CD4 – second line**

(22) Which immunological markers are important in CLL?

- **CD5, CD23**
- **Anticyclin D – mantle cell lymphoma**
- **P53 protein**

SPECTROPHOTOMETER

- **It is a device used to measure absorbed or transmitted light energy.**
- **Its measurement falls between 150-2500nm (Ultraviolet, visible and Infrared regions).**
- **Photometer is used to measure light intensity.**
- **Spectrophotometer measures absorption of monochromatic light produced by a grating monochromator.**
- **Flame photometer measures light emitted by single atoms burned in a flame.**
- **Fluorometer measure light of specific wavelength emitted by molecule after it has been excited by Electromagnetic rays of energy.**
- **Beer Lambert Law**
    - **Concentration of a substance is directly proportional to amount of light absorbed and it is inversely proportional to logarithm of transmitted light.**
- **Parts of spectrophotometer, their function and their composition**
- **Use of Nephelometer**
    - **It is used in measuring concentration of solution that contains particles too large for absorption spectroscopy.**

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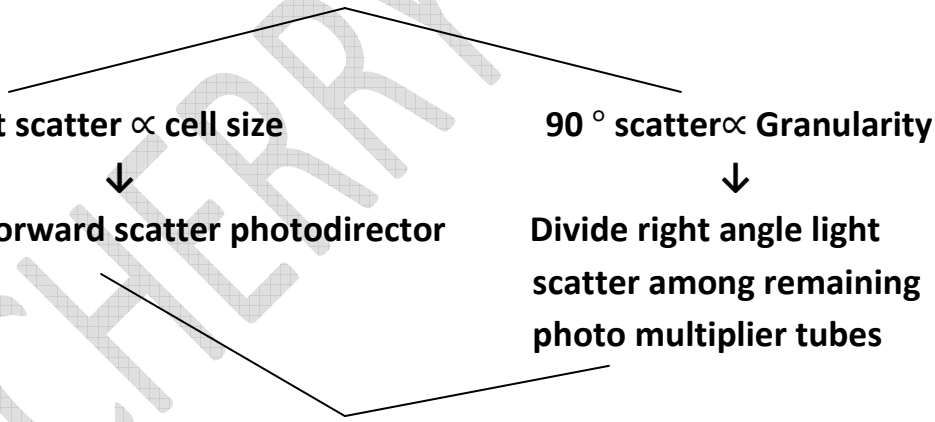
FLOW CYTOMETER

- **Instrument that measures some of properties of cells suspended in a moving fluid medium.**
- **Cells pass in single file through sensing point → Intercepted by argon laser beam→ Transmitted light consists of pulses of both scattered light and fluorescent light→ Light impulses are directed by lumen and focused on multiplier tubes → Converted into a digital signal → Computer can quantitise**
- **Samples to be analysed suspended in medium → Introduced in flow chamber using air pressure → Surrounded by low pressure sheath fluid → Forces specimen to centre → Results in single file alignment and individual cells known as HYDRODYNAMIC FOCUSING**
- **Cell → Intersected by laser beam**



Analysis of both scattered separate granulocytes, monocytes, lymphocytes based on size and granularity.

USES:

- **High speed: 70,000 events / second**
- **Multiparameter: Includes number of cells, cell size, presence of surface and cutoplasmic markers**
- **Used in cell cycle analysis by staining cells with fluorescent dye like propidium iodide.**

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HEMATOLOGY ANALYSERS BY ELECTRICAL IMPENDANCE

- **It is based on change in electrical resistance across an aperture when a particle in conductive liquid passes through this aperture.**
- **Used in hematology laboratory to enumerate leucocytes, erythrocytes and platelets.**
- **Aspirated blood divided into two separate volumes**
    - **One volume is mixed with diluents, where RBC and Platelet count are performed.**
    - **Other volume is mixed with diluents, lyses RBCs and Leukocyte counts are performed.**

      NRBCs can give false rise of WBCs
- **Electric current between electrode changes**
- **Each time cell passes -> Voltage pulse size is proportional to cell size**
- **Number of cell pulses -> cell count**

Examples: Abott- Cell Dyn

Coulter cell counter

- **Interferences are mediated by flag**

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ELECTROPHORESIS

- **Separation of charged compounds based on their electrical change.**
- **When voltage is applied to a salt solution an electrical current is produced by flow of ions**

  Cation → Cathode

  Anion → Anode
- **Greater the charge → faster they move towards oppositely charged electrode**
- **Net charge depend upon pH.**
- **It requires constant power supply.**
- **Buffer solution- Carefully controlled ionic solution**

Dilute, causes heat in cell **High ionic strength does not allow good separation of fraction**

- **Support media – Cellulose acetate**

  -Agarose

  -Poly acramide gel
- **Volume of specimen – 1 µl**
- **Support media dyed – Amino black**
  - **Ponceaus**
  - **Sudan black B**
- **Uses – Serum proteins**

  - Hemoglobins

  -Isoenzymes → CK, LD, Alkaline phosphatase

CALIBRATION OF HEMATOLOGY ANALYSERS

- **Using blood cell suspensions**
- **Success is checked by 3 level control (instrument results average)**
- **If commercial calibrant not available, then one can use fresh normal blood to measure**
    - **hemoglobin by HiCN method with standard photometer**
    - **Hematocrit by microhematocrit method**
    - **RBC and WBC by single channel analyser. For RBC 1:50000 dilution is made to reduce error.**

QUALITY CONTROL OF HEMATOLOGY ANALYSERS

- **Commercially available blood cell control may be used and charted every morning and at intervals during the day – this is expensive and not satisfactory.**
- **All parameters should be stable in EDTA at 4®C for atleast 24 hours**
- **5 to 10 normal samples are selected on day 1 and kept in refrigerator which are then reanalyzed on day 2.**
- **Change is detected using student 't' test**

$$T_n = \frac{\bar{d}}{sd}\sqrt{n}$$

n- number of observation

$\bar{d}$- mean of difference

sd - standard deviation

OR

$$\sqrt{\frac{\sum(d^2) - \frac{(\sum d)^2}{n}}{n-1}}$$

if t > 95% limits found

- **Tendency to drift by running 2-3 specimens from first morning batch at intervals throughout the day.**
- **Will check calibration also (by running 20 samples for MCV, MCH, MCHC)**

SOURCES OF ERROR

- **Carryover**
- **Increased WBC will lead to raised hemoglobin due to turbidity and raised Hematocrit and MCV as WBC are counted as RBC**
- **Voltage tube – MCV**
- **High glucose will give increased MCV value**
- **Hyperosmolality can lead to reduced MCHC**
- **Cold agglutinin - macrocytic RBC**
- **In Leukemia there will be fragile WBC which will give false low count**
- **In Uremia - false low count**
- **IgG, Ig M – shows increased leucoytes**
- **Increased lipids will lead to increased plasma turbidity and increased hemoglobin, MCH, MCHC**

RETICULOCYTE COUNT

(1) Which reagent is used?

- **1% new methylene blue**
- **Brilliant cresyl blue**

(2)What is reticulocyte?

- **Immature nonnucleated Red cell containing RNA which is precipitated by methylene blue or brilliant cresyl blue as dark blue network of reticulum.**

(3)What does reticulocyte count suggest?

- **It provides as estimate of a rate of red cell production.**

(4)How all controls of reticulocyte are made?

- **Hypotonic dialysis of RBC in presence of RNA followed by short period of hypertonic dialysis to reseal pores of RBC membrane.**

(5)How is absolute reticulocyte count determined?

- **By multiplying Reticulocyte percentage by RBC count**

(6)What does reticulocyte count indicate?

- **Estimate of rate of RBC production.**

(7)Which fluorescent dyes can be used in reticulocyte count?

- **Acridine orange and Thioflavin T which binds to RNA**

(8)What are causes of false positive reticulocyte count?

- **Presence of Howell Jolly Bodies**
- **NRBC**
- **Sickle cell**
- **Giant platelets**

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INVESTIGATION OF THE HEREDITARY HEMOLYTIC ANAEMIAS

(1) What does osmotic fragility test indicate?

- **It gives an indication of surface area/ volume ratio of erythrocytes.**
- **It is useful in diagnosis of Hereditary Spherocytosis.**
- **Also used in screening of Thalassemia.**

(2) Which tests demonstrate Red cell membrane defect ?

- **Osmotic fragility Test**
- **Glycerol lysis time**
- **Cryohemolysis**
- **Autohemolysis**
- **Membrane protein analysis**

(3) What is principle of osmotic fragility test?

- **Small volume of blood is mixed with large excess of buffered saline solution of varying concentration.**
- **Fraction of red cells lysed at each saline concentration is determined colorimetrically.**

(4) What is used as stock solution in osmotic fragility test?

- **Buffered NaCl equivalent to 100g/L (171mol/L) NaCl.**

(5) For how much duration stock solution of osmotic fragility can be preserved?

- **Stock solution of osmotic fragility can be preserved for 3-4months at 4˚C in well stoperred bottle**
- **Salt crystals may form which should be thoroughly redisolved before use.**

(6) How is hypotonic solution used in osmotic fragility test made?

- **10g/L solution is made from 100g/L NaCl stock solution by dilution with water.**

(7) Which dilution should be used for incubated samples in osmotic fragility test?

- **12g/L solution should be used for incubated samples.**

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(8) What are convenient dilutions in osmotic fragility test?

- **9.0, 7.5 ,6.5, 6.0, 5.5, 5.0, 4.0, 3.5, 3.0, 2.0 and 1.0 are convenient dilutions in osmotic fragility test.**

(9)Which concentrations are used in critical condition in osmotic fragility test?

- **4.75**
- **5.25**

(10)When should solution of osmotic fragility test discarded?

- **Solution of osmotic fragility test discarded when mould develop.**

(11) Which blood is used in osmotic fragility test?

- **Heparinized venous blood**
- **Defrinated blood**

(12)Why oxalated or citrated blood is not suitable for osmotic fragility test?

- **Oxalated or citrated blood is not suitable for osmotic fragility test because additional salts give wrong results.**

(13)Which wavelength is used in osmotic fragility test?

- **540nm**

(14)What is shape of curve plotted in normal osmotic fragility test?

- **Sigmoid**

(15)Why youngest RBCs are more resistant than older RBCs to hypotonic solution in osmotic fragility test?

- **Old RBCs have higher concentration of sodium and decreased capacity to pump out sodium.**

(16)Which factors affect osmotic fragility test?

- **1. Relative volumes of blood and saline.**
- **2. Final pH of blood in saline suspension.**
- **3. The temperature at which the test are carried out.**

(17)What is median corpuscular fragility (MCF)?

- **The concentration of saline causing 50% lysis.**

(18)What is the concentration of saline in normal osmotic fragility test?

- **Initial lysis at 5gm/litre Nacl**
- **Complete lysis at 3gm/litre Nacl**
- **Median corpuscular fragility at 4-4.5 gm /litre Nacl**

(19)How does normal RBCs withstand hypotonicity of water in fragility?

- **Because of its biconcave shape its volume increases 70% by stretching of membrane before lysis.**

(20)What happens in Spherocytes in osmotic fragility test?

- **As Spherocytes have increase volume to surface ratio their ability to take in water before stretching is limited than normal so they are more suspetible to lysis.**

(21)In which conditions osmotic fragility test decreases?

- **Decreased osmotic fragility test indicates presence of flattened red cells in which volume to surface area ratio is decreased.**
- **Leptocytes occurs in Iron deficiency anemia, Thalessemia with low MCV & MCH.**

(22)Which cells are resistant to osmotic lysis?

- **Reticulocyte.**
- **RBCs from patients being splenectomized**
- **Target cells –Example-In liver disease, there is lipid accumulation of cells.**

(23)What is the effect of incubation on osmotic fragility test?

- **It causes swelling of cells associated with accumulation of sodium exceeding potassium.**
- **RBCs become stressed due to relative lack of glucose.**

(24)In which conditions osmotic fragility test increases abnormally after incubation?

- **Hereditary Spherocytosis**
- **Hereditary Elliptocytes**
- **Hereditary Stomatocytes**
- **Autoimmune hemolytic anemia**
- **Seveer deficiency of pyruvate kinase enzyme**

(25)In which condition osmotic fragility test decreases abnormally after incubation?

- **Thalassemia major**
- **Thalessemia minor**
- **Iron deficiency anemia**

(26)In which conditions we get false negative results osmotic fragility test?

- **Atypical or mild Hereditary Spherocytosis**

(27)In which conditions we get false positive results in osmotic fragility test?

- **Pregnant patients**
- **Immune or hemolytic anemia**
- **Renal failure**

(28)Which dye is used in flow cytometric test of dye binding?

- **Eosin 5 maleinide**

(29)What is significance of glycerol lysis test?

- **Particularly useful in screening family members of patients with hereditary Spherocytosis where morphological changes are too small.**

(30)What is significance of cryohaemolysis test?

- **More specific for Hereditary Spherocytosis as depends on factors related to molecular defects in RBC membrane rather than surface area to volume ratio as in Osmotic fragility test.**
- **Normal 3-15%**
- **Increased lysis in Hereditary Stomatocytosis**

(31)What is significance of Autohaemolysis test?

- **Initial screening test in suspected causes of hemolytic anemia.**
- **Provides information about metabolic component of RBCs.**
- **Helps to distinguish membrane and enzyme defects.**

(32)By which method Autohaemolysis is measured?

- **Colorimeter**
- **Spectrometer**
- **Haemocue**

(33)What is principle of Autohaemolysis method?

- **Aliquots of blood are incubated both with and without sterile glucose solution at 37^0 for 48 hours and spontaneous haemolysis is measured calorimetrically.**

(34)What is role of glucose in Autohaemolysis?

- **It slows down Haemolysis.**

(35)Give causes of increased Autohaemolysis?

- **Membrane and metabolic defects of RBCs.**
- **Example-Hereditary Spherocytosis**
  -Pyruvate kinase enzyme deficiency
  -G_6PD deficiency
  -Oxidant drugs

(36)How will you interpret Autohaemolysis test?

- **Normal Results- Intrinsic RBC abnormality unlikely**
- **Abnormal hemolysis corrected by glucose-Metabolic Abnormality of RBC unlikely**
  -Membrane abnormality likely
- **Abnormal hemolysis not corrected by glucose-Metabolic Abnormality of RBC likely**

(37)Which are common hemolytic anemias associated with defects of Red cell membrane proteins?

- **Alfa Spectrin- Hereditary Elliptocytosis**
  - Hereditary Spherocytosis
  - Hereditary pyropoikilocytosis
- **Beta Spectrin- Hereditary Spherocytosis**
  - Hereditary Elliptocytosis
- **Ankyrin - Hereditary Spherocytosis**

(38)Which RBC membrane protein defect is associated with congenital dyserythropoetic Anemia type II?

- **Glycophorin A**

(39)Which RBC membrane protein defect is associated with Hereditary Stomatocytosis?

- **Stomatin**

(40)Which RBC membrane protein defects are associated with Hereditary Spherocytosis?

- **Alfa Spectrin, Beta Spectrin ,Ankyrin ,Pallidin**

(41 Which RBC membrane protein defects are associated with Hereditary Elliptocytosis?

- **Alfa Spectrin, Beta Spectrin ,Glycophorin C, Protein 4%**

(42)Which are screening nonspecific tests for diagnosis of RBCs enzyme defects?

- **Osmotic fragility test**
- **Autohaemolysis**

(43)What are the common variants of G_6PD enzymes?

- **Mediterranean type having low activity and lead to favism (acute intravascular hemolysis on ingestion of broad beans)**
- **A minus type leading to primaquinine sensitivity.**

(44)What is action of G_6PD enzyme?

- **It catalyses oxidation of glucose 6 phosphate to 6-phosphogluconate with reduction to NADP to NADPH.**

(45)What does screening tests for G_6PD deficiency are dependent on?

- **Inability of cells from deficient subjects to convert an oxidized substrate to reduced state.**

(46) Which are natural substrates used in G_6PD test?

- **NADP**
- **GSS_4**
- **Hi**

(47)Which Artificial dyes are used as substrates in G_6PD test?

- **Methylene blue**

(48)Which are the setbacks of fluorescent method of G_6PD deficiency?

- **False normal –In case of reticulocytosis**
- **False deficient-In case of anemia**

(49)What is the principle of Methhaemoglobin reduction test in G_6PD deficiency?

- **Sodium nitrite converts Hb to Hi.**
- **When no methylene blue is added, Methhaemoglobin persists.**
- **Incubation with methylene blue allows stimulation of pentose phosphate pathway in subjects with normal G_6PD level reducing Hi.**
- **This is blocked in G_6PD deficient individual.**

(50)How controls of Methhaemoglobin reductase test are produced for G_6PD deficiency test?

- **By adding 2 ml of blood without reagents to a tube containing 0.1 ml sodium nitrite –dextrose mixture without methylene blue.**

(51)What is interpretating colour of blood in normal patient in G_6PD test?

- **Clear red**

(52)What isinterpretating colour of blood in G_6PD deficient individuals in Methhaemoglobin reduction test?

- **Brown**

(53)What are advantages of Methhaemoglobin reduction test over fluorescent test for G_6PD deficiency?

- **Inexpensive**
- **Can be completed by cytochemical analysis in patients with reticulocytosis and heterozygotes.**
- **Only needs water bath.**

(54)Which blood is naturally deficient in G_6PD?

- **Sheep blood is naturally deficient in G_6PD. So it can be used as control.**

(55)What is the principle of cytochemical method for G_6PD deficiency?

- **Cells treated with sodium nitrite → Convert HbO_2 to oxyhaemoglobin (HbO_2) to Methhaemoglobin (Hi).**
- **In presence of G_6PD, Hi reconverts to HbO_2.**
- **In G_6PD deficiency, Hi persists.**
- **Blood incubated with soluble tetrazolium compound → will be reduced by HbO_2 but not Hi to insoluble Formazan form.**

(56)What is pyrimidine 5'nucleotidase? What is its significance?

- **Pyrimidine 5'nucleotidase is enzyme in RBCs.**
- **Its deficiency leads to Congenital Chronic Nonspherocytic Hemolytic Anemia.**
- **Pronounced Basophilic stippling are seen.**
- **Osmotic fragility test normal. Autohaemolysis is increased.**

(57)When does acquired deficiency of P_5N enzyme occurs in RBCs?

- **Acquired deficiency of P_5N enzyme occurs in Lead intoxication as it is inhibitor of P_5N.**

(58)How do you prepare Haemolysate?

- **1 Volume of washed RBCs with increased volumes of lysing solution consisting of 2-7 mmol /litre EDTA, pH7 and 7mmol/litre 2 mercaptoethanol.**

(59)Which buffer is recommended for RBC enzyme assay?

- **Tris HCL/EDTA buffer.**

(60)What is significance of G_6PD assay?

- **G_6PD is on X chromosome → so males having only one G6PD gene are normal or deficient heterozygote , but females are either normal homozygote or heterozygote with intermediate enzyme activity.**
- **RBCs hemolyse only if G_6PD enzyme activity is less than 20% of normal activity.**
- **G_6PD activity is higher in young RBC but decreases as RBCs age and is associated with reticulocytosis.**

(61)In which conditions G_6PD activity is decreased in males?

- **Pure red cell aplasia with reticulocytopenia**

(62)How will you interpret G_6PD value in females?

- **Normal-Female is normal homozygote**
- **Value less than 10% of normal activity → Deficient homozygote**
- **Value between 10 to 90% of normal activity →Heterozygote**

(63)How will you interpret pyruvate kinase assay?

- **Pyruvate kinase deficiency→ Chronic hemolysis, invariably have reticulocytosis**

(64)Which enzyme deficiency in RBCs is responsible for nonspherocytic haemolytic anemia?

- **Glutathione synthetase**

(65)In which conditions increased Glutathione synthetase is found?

- **Dyserythropoesis**
- **Myelofibrosis**
- **Congenital Anemia**

(66)What is significance of 2,3 Diphosphoglyconate in RBCs?

- **It is increased in Pyruvate kinase enzyme deficiency.**
- **It is decreased in Hexokinase deficiency.**

(67)In which conditions 2,3 Diphosphoglyconate concentration increases in RBCs?

- **2,3 Diphosphoglyconate concentration increases in RBCs in which arterial blood is undersaturated with oxygen.**
- **Example: -Congenital Heart Disease**
  - Chronic lung diseases
  -Acquired anemia
  -High altitude
  - Alkalosis

(68) In which conditions 2,3 Diphosphoglyconate concentration decreases in RBCs?

- **Acidosis**
- **Hereditary Spherocytosis**

(69)What is oxygen dissociation curve?

- **It is an expression of relation between partial pressure of oxygen and saturation of Hemoglobin.**

(70)In which organ Hb becomes 95-96% oxygenated?

- **Lungs**

(71)What is Bohr Effect?

- **An increase in CO_2 concentration , decrease oxygen affinity of Hb causing Bohr effect.**

(72)What is Hill's constant(H)?

- **It represents the number of molecules of oxygen that combine with one molecule of Hb. The value is 2-6.**

-

INVESTIGATION OF ABNORMAL HAEMOGLOBINS AND THALASSEMIA

(1)Which are transient embryonic hemoglobins?

- **Hb Gower-1**
- **Hb Gower-2**
- **Hb Portland-1**
- **Hb Portland-2**

(2)Which type of Hb is found in fetus ?

- **HbF**

(3)Which major Hb is found in adults?

- **HbA**

(4)What is normal proportion of HbA_2 and HbF in adults?

- **HbA_2- 2-3.3%**
- **HbF -0.2-1%**

(5)Which different chains are seen in different hemoglobin?

- **HbA- 2 alfa chains and 2 beta chains**
- **HbF- 2 alfa chains and 2 gamma chains**
- **HbA_2 - 2 alfa chains and 2 delta chains**

(6)On which chromosome genes of alfa Hb are seen?

- **Chromosome-16**

(7)On which chromosome genes of beta and gamma Hb are seen?

- **Chromosome-11**

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(8)What are causes of Haemoglobinopathies?

- **Structural variants of Hb. Example:HbS**
- **Failure to synthesize globin chain. Example: Thalassemia**
- **Failure to complete switch on fetal Hb to adult Hb. Example: HPFH**

(9)Give example of alteration in structure of Hb brought by point mutation.

- **HbS caused by substitution of Valine for Glutamic acid in position-6 of globin chain.**

-`

(10) Give example of Hb structural variation caused by shortening of globin chain.

- **Hb GunHill-5 amino acids deletion in beta chain.**

(11) Give example of Hb structural variant caused by lengthening of globin chain?

- **Hb constant spring – 31 aminoacids are added to α chain.**

(12) Give examples of Hybrid Hb.

- **Lepore and anti-Lepore Hb – β and δ chain combination.**

(13) Give examples of Hb with reduced solubility.

- **Hb S and Hb C**

(14) Why RBCs became sickle shaped with Hb S?

- **As Hb S has poor solubility in deoxygenated state, it polymerizes within RBCs and became rigid and distorted (Sickle shape).**

(15) What genetic change occurs in Hb C?

- **Glutamic acid is replaced by lysine in position 6 of β chain.**

(16) What are Heinz bodies?

- **Amino acid substitutions close to Haem group and at the point of contact between globin chains affect protein stability and result in intracellular precipitation of globin chains. These precipitated globin chain attach to RBC membrane giving rise to Heinz bodies.**

(17) Give example of unstable Hb.

- **Hb koln**

(18) What is Methamoglobinemia?

- **Hb M – hemoglobin from Methhaemoglobin by oxidation of ferrous iron to ferric iron incapable of binding to oxygen.**
- **Due to congenital NADH Methhaemoglobin reductase deficiency**
- **Exposure to oxidants and chemical like nitrate, nitrites etc.**

(19) What is Thalessemia Syndrome?

- **Heterogenous group of inherited conditions characterized by defects in the synthesis of one or more globin chains that form the hemoglobin tetramer.**

(20) What are the consequences of inadequate Hb production in thalassemia?

- **Hypochromia**
- **Microcytosis anemia**

(21) What are consequences of unbalanced accumulation of one type of globin chain in thalassemias?

- **Ineffective erythropoiesis**
- **Haemolysis**

(22) What is most common cause of β- Thalassemia?

- **Point mutations affecting globin gene.**

(23) What are characteristics of Hb production in man?

- **First 3 months of gestation- Embryonic Hb**
- **Last 6 months of gestation- HbF**
- **During 1st year of life- Transition from HbF to HbA with small amounts of HbA2 and HbF (0.2-1%)**

(24) Which inherited abnormalities show increased HbF levels?

- **HPFH (Hereditary Persistent of Foetal Hemoglobin)**
- **δB**
- **Congenital red cell aplasia (Blackfan Diamond Anemia)**
- **Congenital Aplastic Anemia (Fanconi anemia)**
- **CMML (Juvenile CML)**
- **MDS**

(25) Which are the series of investigations to be done in patient with suspected Hemoglobinopathy?

- **Hb concentration**
- **Red cell indies**
- **Detailed examination of peripheral smear**
- **Reticulocyte count**
- **Red cell inclusion**
- **Iron status by serum iron and TIBC**
- **Serum Ferritin**
- **Hb electrophoresis or Chromatography (HPLC)**
- **Sickling test**
- **Measurement of HbA2 and HbF percentage**
- **DNA hematology**

(26) Which red cell changes are seen in Hemoglobinopathies?

- **Target cells- HbE trait; Thalassemia**
- **Sickle cells- Sickle cell disease**
- **Irregularly contracted RBCs- Unstable Hb**

(27) In which pregnant women genetic counseling is needed antenatally in β-thalassemia trait?

- **With MCH less than 27pg.`**

(28) In which pregnant women genetic counseling is needed antenatally for α-thalassemia?

- **MCH less than 25pg.**

(29) How Haemolysate is prepare?

- **2 volumes of packed cells lysed with 1 volume of distrilled water and then adding 1 volume of carbon tetrachloride- shaking, centrifuging and transferring supernanent.**

(30) Why Haemolysate has to be made?

- **For quantification of Hb.**
- **Stability tests for qualitative electrophoresis.**
- **Quantitation of HbA2 and HbF or variant Hb.**

(31)Which are common controls used for assessing Haemoglobinopathies?

- **HbA + HbS**
- **HbA + HbC**
- **Normal cord blood (HbF + HbA)**
- **HbS + HbC + Normal cord blood(HbF+HbA)**

(32)What is ideal pH for Hb electrophoresis using cellulose acetate?

- **8.4 to 8.6.**

(33)What is principle of cellulose acetate electrophoresis at alkaline pH?

- **At alkaline pH ,Hb is negatively charged protein which migrate toward anode in electrophoresis.**
- **Structural variants that have a charge will separate from HbA.**

(34)Which buffer is used in electrophoresis?

- **Tris/EDTA-Borate(TEB) with pH 8.5**

(35)Which Hemoglobins migrate closely on cellulose Acetate electrophoresis?

- **1.HbS, HbD and HbG**
- **2.HbC,HbE and HbO**

(36)How will you differentiate between closely migrating hemoglobins?

- **By using acid citrate agarose gel, citrate agar electrophoresis**
- **HPLC**
- **Immunoelectrofluoroscence.**

(37)What are advantages of HPLC over electrophoresis in diagnosis of Hemoglobinopathies?

- **HPLC is Automated, so less man power and large batches.**
- **Small samples (5ul) needed , so also useful in pediatrics.**
- **Quantification of normal and variant Hb is available on every sample.**
- **Provisional identification of large proportion of variant Hb is made.**

(38)What is principle of HPLC?

- **It depends on interchange of charged groups of ion exchange material with charged groups of Hb molecule.**

(39)What is typical column made of in HPLC?

- **5um Spherical silica gel**

(40)How column is modified for separation of Hemoglobins in HPLC?

- **Silica gel column is modified by carboxyl groups having weak cationic charge which allows separation of hemoglobins.**

(41)How is rate of elution of different Hemoglobins determined in HPLC?

- **Rate of elution of different Hemoglobins are determined by pH and ionic strength of buffer applied to column.**

(42)Which factors affect results in HPLC?

- **Batch of buffer and column**
- **Age of column**
- **Laboratory temperature**

(43)Which Hb coelute in HPLC?

- **HbA_2 with HbE and Hb Leprae**

(44)What is other important use of HPLC?

- **Quantification of HbA_1C**

(45)On what tests for HbS depend on?

- **Decreased solubility of HbS at low oxygen tension.**

(46)Which reducing agents hasten sickling tests?

- **Sodium dithionate**

(47)What is principle of HbS solubility test?

- **HbS is insoluble in the deoxygenated state in a high molarity phosphate buffer. The crystals that form refract light and cause solution to be turbid.**

(48)How will you say that reagent for sickling has deterioted?

- **The blood reagent mixture should be ideally pink or red. If it is ORANGE indicates deteriotion of reagent.**

(49)Which hemoglobin run with HbS on cellulose acetate electrophoresis at alkaline pH?

- **HbD and HbG**

(50)What are the causes of false positive sickle test?

- **Severe leucocytosis**
- **In hyperprotenemia (Example. Multiple myeloma)**
- **Presence of unstable Hb especially after Splenectomy**

(51)What should be done to minimize false positive and false negative sickling test?

- **Packed cells should be used for testing.**

(52)What are the causes of false negative sickling test?

- **In infants younger than 6 months**
- **Low Hb**
- **Old outdated reagents**
- **When HbS is less than 20%**

(53) How neonatal screening should be done for sickle cell disease or thalassemia major?

- **Cord blood or heel prick sample is used**

(54) What care should be taken while collecting cord blood?

- **Sample should be collected by venipuncture and cleaned umbilical vein to avoid contamination with neonatal blood.**

(55) In which screening tests dried blood spot is used?

- **Congenital hypothyroidism**
- **Phenylketonuria**
- **Thalassemia major**
- **Sickle cell disease**

(56) Which tests are used to detect unstable HbS?

- **Heat stability test**
- **Isopropanol test**

(57) What is Methaemoglobin?

- **Iron present in ferric form in blood.**

(58)In which conditions we see Methaemoglobinemia?

- **HbMs (Inherited varients of Hb undergo oxidation to Methaemoglobin)**
- **Congenital Methaemoglobin reductase deficiency**

(59) Name very fast running Hbs .

- **Hb I**
- **Hb H**

(60) What common changes are seen in blood count and indices in thalassemias?

- **Elevation of RBC count**
- **Decrease in MCV and MCH**
- **MCHC and RDW normal in thalassemia trait**

(61) How will you differentiate between Thalassemia, Iron deficiency anemia from indices?

- **MCHC and RDW are normal in thalessemia trait but increased in Iron deficiency anemia.**
- **Anisochromasia is not seen in thalessemia but is present in Iron deficiency anemia.**

(62) What changes are seen on peripheral smear in thalessemia?

- **Target cells**
- **Basophilic stippling.**
- **Microcytosis in absence of hypochromasia**

(63)What are characteristic features of HbH on peripheral smear?

- **Anisochromasia**
- **Poikilocytosis**
- **Increased Reticulocytes**

(64) Which methods are used for Quantification of HbA2?

- **Elution after cellulose acetate electrophoresis (HPLC)**

(65) How will you interpret HbA2 values?

- **3.8-7.0→β thalessemia trait**
  - Unstable Hb
- **3.4-3.7→ Sever iron deficiency in β thalassemia trait**
  -Addition of chain with β thalassemias trait
  -Presence of HbS
  -Analytic error

- **2-3.3→ Normal**
- **< 2→α Thalassemia**
  - **-HbH**
  - **- Iron deficiency**

Iron deficiency Anemia

(1)What are different forms of iron present in body?

- **RBCs-Hb**
- **Muscle- Myoglobin**
- **Plasma ,Extra vascular fluid- Transferrin**
- **Liver , Spleen, Bone marrow – Haemosiderin , Ferritin**

(2)What is daily requirement of iron for making Hb?

- **30mg**

(3)What amount of iron is lost per day?

- **Iron lost is 1mg per day**
- **In menstruation and childbirth -2mg**

(4)What is meant by storage iron?

- **In men and postmenopausal women there iron in form of Ferritin or Haemosiderin which is available for Haem synthesis.**

(5)Which iron binding protein is responsible for extracellular transport?

- **Transferrin**

(6)How much iron is provided by a normal diet?

- **15mg**

(7) Is the most of dietary iron Haem or nonhaem?

- **Most of dietary iron is nonhaem. Haem iron is from meat and fish.**

(8)Where does maximum absorption of nonhaem iron occurs? Why?

- **Maximum absorption of nonhaem iron occurs in duodenum.**
- **Less absorbed in jejunum due to increased alkaline environment leading to formation of insoluble ferric hydroxide.**

(9)Which factors enhance iron absorption?

- **Vitamin C and Meat**

(10)Which factors inhibit iron absorption?

- **Phytates and tannins**

(11)What is significance of therapeutic iron?

- **Ferrous salts are well absorbed on empty stomach.**

(12)From which diet nonhaem iron is derived?

- **Cereals**

(13)From which diet haem iron is derived?

- **Meat and fish**

(14) What are Iron absorption steps?

- **1.Nonhaem iron from food released as Fe^{3+}(ferric)→reduced to Fe^{2+} (ferrous) by Ferric reductase**
- **2. Iron transported along brush border membrane of intestine by DMT.**
- **3.Then transported through serosal membrane by FERROPORTIN 1**
- **4. Fe^{2+} oxidized to Fe^{3+} by ceruloplasmin or nephaestin.**
- **5. Taken by Transferrin.**

(15)How iron bound transferrin is released?

- **Released by endocytosis.**
- **Transferrin / Apotransferrin returns to cell surface.**

(16) What is the role of macrophage in iron release?

- **They engulf senescent RBCs and release iron within using Haemoxygenase→ Haem released to plasma transferrin or stored as ferritin**

(17) What are different forms of storage iron in cells?

- **Soluble – Ferritin**
- **Insoluble – Haemosiderin**

(18) Which form of iron is visualized by Prussian blue stain?

- **Haemosiderin**

(19) What does Prussian blue stain contain?

- **Potassium Ferricyanide in acid**

(20) In which organs ferritin is found in highest concentration?

- **Liver, Spleen, Bonemarrow**

(21) Which substances are responsible for transport of Plasma Iron?

- **Haptoglobin- binds to Hb**
- **Hemoprexin**

(22) Which substance reflects the level of body iron stores?

- **Ferritin**

(23) What is meant by Maldistribution of Iron?

- **Eg., Anemia – associated with inflammation or infection where there is partial failure of erythropoiesis and iron release from phagocytic cells in liver, spleen and bone marrow --- results in accumulation of iron as Ferritin and Hemosiderin in cells.**

(24) Which test is required for determination of iron status?

- **Estimate of amount of Hb concentration in blood**
- **Serum Ferritin concentration for storage iron**

(25) What happens in anemia with infection or inflammation?

- **Partial failure of erythropoiesis – iron release from phagocytic cells in liver, spleen and bone marrow – accumulation of iron as Ferritin and Hemosiderin in cells.**

(26)What happens in iron deficiency anemia?

- **RBCs are Hypochromic and Microcytic**
- **In early stage, Normocytic and normochromic**
- **Increased concentration of proporphyrin (Zinc proporphyrin)- due to defective haemsynthesis**

(27)What happens in genetic Hemochromatosis?

- **Iron accumulation→Increased transferrin concentration→Increased serum Ferritin concentration**

(28)What happens in anemia with chronic disease?

- **Normal serum Ferritin concentration**

(29)What does serum Ferritin level reflect?

- **Body iron stores**

(30)What is normal serum Ferritin level?

- **15-300 microgram/litre**

(31)By which method serum Ferritin is measured?

- **Immunoassay-Elisa**

(32)Which are major Iron metabolism disorders?

- **I. Iron deficiency anemia-Deficient intake**
  Low availability in diet
- **II. Increased physiological requirement-Rapid growth in childhood**
- **III. Blood loss –Physiological (Menstruation)**
  -Pathological (GIT)
- **IV. Malabsorption of Iron –Partial gastrectomy due to**
  reduced acid secretion
  -Coeliac disease
- **V. Macrophage iron accumulation –anemia of chronic disease**
  - Iron overload
- **VI. Increased iron absorption –Hereditary Hemochromatosis**
- **VII. Multiple blood transfusion in refractory anemia, Example: Thalessemia, Aplastic Anemia-MDS**

(33)What investigations are to be done for assessing iron status?

- **1.Hemoglobin content**
- **2.RBC indices**
- **3.Serum iron -10-30ul**
- **4. Total iron binding capacity-47-70ul**
- **5.Trasferrin saturation-iron/TIBCx100-16-50%**
  (No.3,4 and 5 for iron supply)
- **6.Serum Ferritin -15-300ug/lit(For Iron stores)**
- **7.Perl's stain**

(34)What does Hemoglobin concentration measures?

- **Defines anemia and its severity**
- **Indicates response to therapeutic trial of iron**

(35)What is significance of RBC indices?

- **It's value indicates -iron deficient erythropoiesis**
  -Disorders of Hemoglobin synthesis other than iron deficiency

(36) What is significance of serum iron?

- **Normal range of serum iron is: 10-30 μmol/l.**
- **Decreased value is suggestive of iron deficiency.**
- **Increased value is suggestive of iron overload.**
- **Fasting sample should be used.**

(37) What is significance of TIBC?

- **Normal range of TIBC is: 47-70 μmol/l.**
- **Increased value is suggestive of iron deficiency.**
- **Decreased value is suggestive of iron overload.**

(38) What is significance of Serum Ferritin level?

- **Normal level of Serum Ferritin is: 15-300 μg/l.**
- **It correlates with body iron stores.**
- **Increased value is suggestive of liver disease.**
- **Decreased value is suggestive of Vitamin C deficiency.**

(39) How all standards of Ferritin are prepared?

Ferritin is obtained from liver/spleen cells (as these cells are iron overloaded) at postmortem.

↓

Stored at -20 ˚ C for 1 year.

↓

Purified at 75 ˚ C

↓

Further purified by precipitation from cadmium sulphate solution and gel filtration chromatography

↓

Purity assessed by polyacramide gel electrophoresis

↓

Stored at 4 ˚ C with concentration of 1 -4 mg/l in presence of Sodium azide for 3 years.

- **It is not frozen.**

(40) In which conditions, high concentration of serum Ferritin are found?

- **Conditions in which high concentration of serum Ferritin are found:**
  - **Iron overload**
  - **Liver disease**
  - **Inflammation**
  - **Malignant disease**
  - **Haemochromatosis**

(41) Which condition is masked showing normal serum Ferritin and why?

- **Condition, masked showing normal serum Ferritin is: Haemochromatosis**
- **It is because there is late onset of disease.**

(42) Serum Ferritin concentration upto

- **100 μg/l- Acute on chronic disease.**

(43) What is range of normal serum Ferritin concentration?

- **Range of normal serum Ferritin concentration is:**
  - **For adults: 15-300 μg/l.**
  - **For Children: 12 μg/l.**

(44) In which form, iron is carried in plasma?

- **Iron is carried in plasma bound to protein transferrin.**
- **It binds two atoms of iron as Fe^{3+}.**

(45) Why it is necessary to use disposable items in serum iron estimation?

- **It is necessary to use disposable items in serum iron estimation to prevent getting wrong results from contaminated tubes.**

(46) For serum iron concentration, what should be used?

- **) For serum iron concentration, deionised water should be used.**

(47) Which are interfering substances for serum iron concentration?

- **Interfering substances for serum iron concentration are:**
  - **EDTA Deferoxamine, as they are iron chelators.**

(48) In which conditions, low serum iron concentrations are found?

- **Conditions, in which low serum iron concentrations are found:**
  - **Iron deficiency anaemia**
  - **Chronic diseases (Inflammation, Infection, Cancer)**
  - **Acute phase response including response to surgery.**

(49) In which conditions, high serum iron concentrations are found?

- **Conditions, in which high serum iron concentrations are found:**
  - **Liver disease**
  - **Hypoplastic anaemia**
  - **Ineffective erythropoiesis**
  - **Iron overload**

(50) What is transferrin?

- **Transferrin is a iron-carrying substance. In plasma, iron is bound to transferrin.**

(51) What is total TIBC measure of?

- **Total TIBC is measure of transferrin.**

(52) What is unsaturated iron binding capacity (UIBC)?

- **Unsaturated iron binding capacity (UIBC) is an additional iron binding capacity of transferrin.**
- **TIBC= Serum iron concentration + unsaturated iron binding capacity (UIBC).**

(53) On what method, chemical analyzers are working?

- **Chemical analyzers are working on:**
  - **Non-precipitation method**
  - **Immunological Assay**

(54) What is benefit of measuring serum transferrin by immunological assay over non-precipitation method?

- **Benefits of measuring serum transferrin by immunological assay over non-precipitation method are:**
  - **Avoidance of high values of TIBC**
  - **Rapidity**
  - **Precise fullness**

(55)Give normal range of serum transferrin.

- **Serum transferrin- 2-3g/ litre**
- **1mg serum transferrin bind to 1-4microgram iron**

(56)In which condition TIBC increased?

- **Iron deficiency anemia**
- **Hemochromatosis**

(57)What is transferrin saturation?

- **Transferrin saturation= $\frac{\text{Serum iron concentration}}{\text{TIBC}}$**

  should be more than 16% if less ineffective erythropoiesis.
- **Most important use is in detecting genetic hemochromatosis.**

(58)Which is important test for screening of lead poisoning?

- **By measuring level of Erythrocyte protoporphyrin.**
- **Normal level is less than 70micromol/mol Haem**

(59)Which type of protoporphyrin increase in iron deficiency anemia?

- **Free-95% Zinc protoporphyrin**

(60)How many cells are analysed by Automated cell counter at a time?

- **Atleast 10,000 cells analysed by Automated cell counter at a time.**

(61)Which Vitamin deficiency causes lowering of serum Ferritin level?

- **Vitamin C deficiency causes lowering of serum Ferritin level.**

(62) Give predictive indicators of iron metabolism.

- **Chronic anemia(Due to mainly Rheumatoid arthritis ,Inflammatory bowel disease and renal disease)-normal (30-50mg)**
- **Or increased serum Ferritin, decreased serum iron, decreased or normal TIBC**

(63)Which test is considered Gold standard for iron concentration?

- **Storage iron in Bone marrow**

(64)Which is diagnostic test for diagnosis of iron deficiency?

- **Serum Ferritin Level measuring is diagnostic test for diagnosis of iron deficiency.**

(65)What do you mean by functional iron deficiency anemia?

- **Situation in which iron stores are adequate but iron supply for erythropoietin is inadequate. Example: Anemia –Erythropoietic deficiency.**

LABORATORY ORGANIZATION AND MANAGEMENT

(1)What is the test reliability?

- **Uncertainty of measurement of the analyte.**

(2) What is accuracy?

- **Closeness of agreement between the measurement that is obtained and the true value.**

(3) What is the bias or systemic error?

- **Extent of discrepancy from the accuracy is known as bias.**

(4) What are the causes of systemic errors?

- **Uncertain calibration of instrument**
- **Bias in equipment or glassware**
- **Faulty dilution**
- **Faults in steps of measurement**
- **Lack of critical resolution**
- **Preanalytical detoriation of specimen**
- **Environmental effects**

(5) How systemic errors can be rectified?

- **By using reference standard with test**
- **Internal quality control**
- **Regular checking by external quality control**

(6) What is precision?

- **Closeness of agreement when a test is repeated a number of times**

(7) What is imprecision?

- **It is a result of random errors in test.**
- **It is expressed as standard deviation and coefficient of variation.**

(8) What is Gaussian distribution of data?

- **When data are spread there is 95% probability that results fall within a range of +2SD and -2SD of target value.**

(9) What is sensitivity?

- **The fraction of true positive results when test is applied to patients known to have relevant disease or when results have been obtained by a reference method.**

  SENSITIVITY = TP / (TP + FN)

(10) What is specificity?

- **It is the fraction of true negative results when the test is applied to normal**

  SPECIFICITY = TP / (TP + FN)

(11) How the reliability of the test is calculated?

- **Reliability= (TP+TN/ total numbers of tests) X 100**

(12) What is normal value of specificity and sensitivity?

- **Near 1.0**

(13) What is the significance of CE mark on an instrument?

- **It indicates that it conforms to the defined specifications of the in vitro diagnostic medical devices directive.**

(14) How will you measure precision of the test?

- **Carry out appropriate measurement 10 times consecutively in three or more specimens including low, high and normal concentration.**
- **Calculate SD and CV.**

(15) What is linearity?

- **It demonstrates the effects of dilution.**

(16) What is carryover?

- **It indicates the extent to which the measurement of an analyte of the specimen is likely to be affected by preceding specimen.**

(17) What is accuracy and comparability?

- **It states whether the new method gives results that agree satisfactorily with an established procedure and reference method.**
- **It checks bias of the instrument.**

(18) What is the maintenance log?

- **Regular inspection and specific maintenance procedure of instrument to be documented including servicing, repairs and cleaning.**

(19) What is test turnaround time?

- **Time laps between arrival of a blood specimen in the laboratory and issue of a validated result.**

(20) What are the standard operating procedures (SOP)?

- **Written instructions that are intended to maintain optimal consistent quality of performance in the laboratory.**

(21) What should SOPS cover?

- **It should cover all aspects of work, some relating to test procedures and others relating to specimen collection, laboratory safety, handling of urgent requests, data storage and telephone reporting policy etc.**

(22) What are the SOPs based on?

- **On standard textbook description or an instrument manufacturer's instruction or manual.**

(23) How many times the SOPs should be reviewed?

- **Once a year with date**

(24) What is the suggested format of SOPs?

- **Cover page – Title, reference number, date of preparation, name of composer, name of authorizer**
- **Scope – Purpose of SOP, principle of test**
- **Specimen requirement – Type, amount, transportation, storage tempreture**
- **Safety precautions – protection*, High risk factors**
- **Equipments and reagnents**
- **Teat procedures**
- **Reporting – Routine/ Urgent**
- **Clinical significance with reference ranges**

- **Test limitations**
- **Maintenance of equipment**
- **Post test specimen storage**
- **List of relevant literature**
- **Date of review**

(25) What is meant by laboratory audit?

- **Systemic and critical analysis of the quality of laboratory service**

(26) What is Accreditation?

- **Allow external audit of a laboratory organization, management, quality assurance program and level of user satisfaction.**

(27) What is ISO?

- **Internatonal Standards Organization establishing guidelines for laboratory practice.**

(28) What is ISO 15189?

- **Medical laboratories particular requirements for quality and competence.**

(29) What is benchmarking?

- **Provides a reference point for laboratories to access their performance by comparison with their peers and leaders in field.**

LABORATORY SAFETY

(1) Which is the standard of safety management in medical laboratories?

- **ISO 15190**
- **WHO**
- **TES for electrical equipment**

(2) What care should be taken while deciding laboratory premise?

- **Sufficiently large**
- **Optimal lighting**
- **Adequate ventilation**
- **Protection from dust**
- **Adequate storage facilities**

(3) What care should be taken while deciding electrical connections in laboratory?

- **All electrical equipments should be certified by its manufacturer, meeting national and international safety standards.**
- **Should not interfere with medical devices**
- **Plugs and cabels should not be adjusted near to water taps.**
- **Preventive maintenance**

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(4) What should be done to prevent fire hazards in laboratories?

- **Alcohols and solvents should be kept away from flames.**
- **Not more than 400 ml reagents should be kept on open bench.**
- **Gas burners should be closed.**
- **Fire extinguisher suitable for electrical and chemical fumes, fire blankets should be placed.**

(5) What is meant by chemical safety in laboratory?

- **Strong acids and alkalis must be stored at floor level.**
- **Chemicals that are likely to react with each other must be stored apart.**
- **Poison should be stored in locked cabinets**
- **Carcinogenic products should be should be stored in restricted access areas.**
- **Weighing should be carried out in laminar air flow.**

(6) Which is the most commonly used disinfectant in laboratory? Why?

- **Sodium hypochlorite.**
- **As it is very active against all microorganisms.**

(7) What are the disadvantages of Sodium hypochlorite?

- **Less effective against fungi.**
- **Corrosive to metal**
- **Gradually lose their strength so fresh dilutions must be made daily.**

(8) What is the generally used concentration of sodium hypochlorite?

- **1g/lit (1000 ppm)**

(9) What concentration of sodium hypochlorite is used for blood spillage?

- **5g/lit (5000 ppm)**

(10) Which are the other disinfectants used in laboratory?

- **Formaldehyde – But less effective below - 20˚C**
- **Gluteraldehyde – Used for decontaminating equipments with metal components. Skin, eye, respiratory tract contacts should be avoided.**
- **Phenolic components – Active against fungus**
- **Alcohols – Effective in combination with formaldehyde and sodium hypochlorite?**

(11) What are the routine applications of disinfectants?

- **Working area should be wiped with freshly prepared 1% sodium hypochlorite.**
- **Reusable pipettes should be soaked in 2.5 % solution for 30 min.**
- **Blood spillage to be cleared with 10% hypochlorit solution.**
- **Sodium hypochlorite is to be freshly prepared daily.**

(12) How automated equipments should be disinfected?

- **By flushing several times with 10% sodium hypochlorite.**
- **2% Gluteraldehyde is used with instruments with metal surface to prevent rusting.**

(13) How centrifuges are disinfected?

- **Any spillage is to be dealt immediately.**
- **Bowl, head, buckets should be regularly disinfected by 2% Gluteraldehyde.**

(14) Why metal surface heavy instruments cannot be disinfected by sodium hypochlorite?

- **Because it is metal corrosive.**

(15) How syringes and needles are disinfected in laboratory?

- **Syringes – Soaked in 10% hypochlorite for atleast 30 min.**
- **Needles – Soaked in freshly diluted 2% Gluteraldehyde.**
- **Rinsed under running tap water and soaked in two changes.**
- **Sterilized by heating on the over at 120˚c for 30 min.**

(16) How gloves are disinfected?

- **Rubber gloves are washed.**
- **Decontaminated by soaking in 1 % hypochlorite solution for 30 min.**
- **Discarded if punctures or tears.**

(17) How blood and potentially infected body fluids are discarded?

- **Poured down a drain and drain should be immediately flushed with water followed by 250 ml of 10% hypochlorite and again flushed with water.**

LABORATORY ASPECTS OF BLOOD TRANSFUSIONS

(1) Which samples are required for blood transfusion?

- **Plain sample**
- **EDTA sample**

(2) Which problems arise if blood samples are stored for larger time in blood bank?

- **Lysis of Red cells**
- **Loss of complement in serum**
- **Decrease in potency of antibodies**

(3) For how many days the sample should be stored in blood bank?

- **7 Days**

(4) What is the use of anti AB reagent?

- **It acts as additional check on cells that were agglutinated by anti-A or anti-B but is also capable of detecting weak A antigens.**

(5) Which cells act as control in grouping?

- **O group cells.**

(6) Why O group should be done in duplication?

- **As there is no counterpart of reverse grouping of ABO testing.**
- **Should be done by monoclonal reagents using same anti-D reagent.**

(7) Which methods are used for Grouping?

- **Tube**
- **Slide**
- **Micro plates**
- **Columns**

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(8) How the sample should be mixed with reagent in test tube?

- **By Tapping**

(9) Why reverse grouping is not carried out for infants younger than 4 months of age?

- **Because corresponding antibodies are normally absent.**

(10) Why microplate method is not recommended for grouping?

- **Because splashing between wells happen in mixing reagents.**

(11) What are common causes of false positive ABO/D groups discrepancies?

- **Rouleaux formation**
- **Cold agglutinin**
- **Polyagglutination**
- **Acquired B**
- **Potentiators**
- **Contaminating antibodies**

(12) In which conditions Rouleaux formation occurs?

- **When ratio of normal albumin to globulin is altered in plasma e.g. multiple myeloma**
- **In presence of plasma substitutes e.g. dextran**

(13) How does Rouleaux formation look like?

- **Stacking of RBCs on top of one another in columns**

(14) How does Rouleaux formation disperse?

- **By adding drop of normal saline on slide**

(15) How does cold agglutinin interfere with blood grouping?

- **They are reactive at room temperature leading to autoagglutination in forward grouping and panagglutination in reverse grouping**

(16) How will you solve blood group problem with cold agglutinins?

- **Tests should be repeated using cells washed in warm saline or plasma/serum to be prewashed at 37˚C**
- **Autocontrol should be included**

(17) What is polyagglutination?

- **Agglutination of red cells by all or most of normal adult sera as the result of IgM antibodies reacting with a cryptantigen of red cells**

(18) Give example of T activation or polyagglutination interfering with blood grouping?

- **T-activation occurring due to neuraminidase produced by bacteria cleaves N-acetyl neuraminic acid from RBC membrane exposing T-antigen**

(19) In which conditions polyagglutination can occur?

- **In sick infants and young children (infants with necrotizing enterocolitis) while using polyclonal reagents**

(20) What is acquired B group antigen?

- **Acquired B antigen is caused by deacetylation of A red cells by bacterial red cell enzymes, resulting in discrepancy between forward and reverse grouping.**

(21) What are the causes of false negative discrepancies in blood groups?

- **Failure to add reagents**
- **Loss of potency**
- **Failure to identify lysis**
- **Mixed field appearance**
- **D Variant Phenotypes**

(22) In which conditions, the reagent loses its potency?

- **Inappropriate storage or freezing and thawing**

(23) What is mixed field appearance?

- **A dual population of red cells may be present in ABO and D grouping which should not be confused with agglutination.**
- **e.g In transfusion of non identical ABO or D red cells.**
- **It is the first indication of previous ABO incompatible transfusion.**

(24) How is mixed field detected?

- **In tube method microscopic reading reveals strong agglutinates in a sea of free cells.**
- **In column agglutination, line of agglutinated cells are on top with negative cells at bottom.**

(25) What is weak D?

- **D antigen is present on cells with fewer D antigen sites per cells.**

(26) In which condition chances of mistake are there with weak D?

- **When a D negative patient is transfused D positive blood group giving a mixed field reaction ; can give false weak D result.**

(27) What is partial D?

- **One or more epitopes of D antigen is missing.**
- **Differing reactions are obtained with two anti D reagents.**

(28) What is D^{v1}?

- **Lacks most epitopes of D.**

(29) What is significance of antibody screening?

- **Some antibodies react more strong with red cells of homozygous expression than those with heterozygous expression.**

(30) How antibody screening is done?

- **By indirect Antiglobulin Test**

(31) Why microcolumn method is preferred over test tube method of IAT?

- **Simpler to perform**
- **As sensitive as tube method**
- **Uses small volumes of serum/plasma and reagents**
- **More objective reading phase**

(32) What is used as control in IAT?

- **Weak anti-D**

(33) What should be done if an antibody is detected on antibody screening?

- **Should be tested against identification panel of reagent red cells**
- **Then each antibody is taken specifically and then excluded**
- **Phenotyping of red cells for relevant antigen- if positive presence of autoantibody is confirmed, direct AGT should be done**

(34) What is the purpose of cross matching blood before transfusion?

- **To prevent transfusion of incompatible red cells and subsequent hemolytic transfusion reaction**

(35) What is significance of indirect antiglobulin crossmatching?

- **It acts as a double check that blood bag is properly labeled negative for antigen**
- **It ensures serological compatibility**
- **Allows detection of antibodies to low frequency antigens**

(36) What is the significance of doing indirect Coomb's test?

- **To identify antibodies against low frequency antigens**

(37) What is the significance of immediate spin crossmatch?

- **To ensure that correct units of blood have been selected and the correct ABO group has been assigned**

(38) In which conditions false negative results are seen in immediate spin crossmatch?

- **Incompatibilities between A_2B donor cells and B patient sera**
- **C_1 interfering with agglutination if serum contains EDTA**

(39) What are the conditions in which you see false positive results in immediate spin crossmatch?

- **Cold reacting antibodies other than Anti A and Anti B**

(40) What should be done in emergency cases requiring transfusion?

- **Immediate forward and reverse grouping**
- **Immediate spin crossmatch**
- **If inadequately labeled specimen O+ve / O-ve should be given**
- **Group specific without crossmatching in extreme emergencies with retrospective crossmatch**

(41) What is massive transfusion?

- **More than one blood volume in 24 hours**

(42) What is hemolytic disease of newborn?

- **Hemolytic anemia of fetus and newborn infant that occurs when maternal alloantibody to fetal antigen crosses the placenta and causes hemolysis of fetal red cells.**

(43) Which type of immunoglobulin crosses placenta?

- **Ig G- This causes HDN**

(44) Which group of antibodies causes maximum hemolysis in HDN?

- **Anti D**

(45) Which other Rh antibody can cause hemolysis of fetus in uterus?

- **Anti C**

(46)When should be antenatal screening for grouping and antibody screening be done?

- **Early in pregnancy**
- **At 28 weeks**
- **In women with anti D - Every two weekly after 28 weeks**

(47) How can you predict fetal blood group and genetic basis of different types for HDN?

- **By DNA typing**

(48) How fetal anemia is assesed?

- **Amniocentesis by measuring optical density of amniotic fluid using spectrophotometry.**
- **Fetal blood samples by USG guided cordocentesis**

(49) Which tests should be performed on cordblood in pregnancies with red cell antibodies?

- **ABO → D grouping**
- **Direct antiglobulin test**
- **Hemoglobin**
- **Bilirubin**

(50) Which test should be performed on maternal blood in pregnancies with red cell antibodies?

- **Repeat ABO and D group**
- **Repeat antibody screen**
- **Test to determine degree of fetomaternal hemorrhage (Acid elution test OR Flow cytometry)**

(51) What tests should be done to measure fetomaternal hemorrhage?

- **Acid Elution test which depends on the HbF in fetal cells resisting acid elution to greater extent than HbA in maternal cells.**
- **Flow Cytometry**

(52) Which test is done in investigating intrauterine death or still birth with silent fetomaternal hemorrhage suspected?

- **Kleihauer Test**

(53) What happens in ABO hemolytic Disease of Newborn?

- **Neonatal Jaundice and Anemia with Spherocytosis**

(54) Which pregnancy is affected in ABO hemolytic Disease of Newborn?

- **First pregnancy as in contrast with Anti-D Hemolytic Disease of Newborn.**

(55) How should be the compatibility test be done in infants below 4 months?

- **As infants below 4 months do not make alloantibodies they may have passively acquired maternal antibodies.**
- **So investigations are to be done on**
- **Maternal sample – ABO and D group Antibody Screening**
- **Infant Sample – ABO and D group (Forward) Antibody Screening**
  Direct Antiglobulin Test
- **If DAT is positive – HDN should be considered**

(56) What is the choice of blood products to be administered in neonates and infants below 4 months of age?

Group	PCV	PRC	FFP
O	O	O	O
A	A or O	A	A or AB
B	B or O	B or A or O	B or AB
AB	AB or A or B or O	AB or A	AB

(57) What care should be taken during intrauterine (fetal) transfusion?

- **Antiglobulin crossmatch to be performed with maternal plasma/Serum, with every transfusion.**
- **Antibody identification to be performed on all maternal samples.**
- **Red cells of O, D negative group.**
- **Blood irradiated to prevent Graft vs Host Disease and transfused within 24 hours of irradiation.**

(58) What care has to be taken if patient requires transfusion at close intervals? Why?

- **As alloantibodies develop quickly following a transfusion, a new sample should be obtained every 3 days and an antibody screening test is to be done every 72 hours.**

(59) Which are acute transfusion reactions?

- **Acute Hemolytic Reactions**
- **Anaphylaxis**
- **Bacterial Contamination of Blood Products**
- **TRALI**
- **Acute Fluid Overload**
- **Allergic**
- **Febrile Non hemolytic Reaction**

(60) Which are chronic transfusion reactions?

- **Delayed Hemolyitc Reactions**
- **TTI**
- **Transfusion associated Graft vs Host Disease**
- **Post Transfusion Purpura**
- **Iron Overload**
- **Immunosuppression**

(61) What are causes of intravascular hemolysis following transfusion?

- **ABO incompatibility**
- **Other red cell antibodies activating complement through MAC, contamination**

(62) What are causes of extravascular hemolysis post transfusion?

- **A strong antibody which does not bind complement or only binds to C3 stage and is missed in pretransfusion testing**

(63) In which condition anaphylaxis reaction are common after blood transfusion?

- **When products containing large amount of plasma are given**

(64) What blood product is more prone to get infected? Why?

- **Platelets as they are stored at room temperature**

(65) Which investigations are to be done in acute blood transfusion reaction?

- **1. For hemolysis- visual examination of patient's plasma and urine**
  -Blood film: Spherocytes, red cell fragments
  -Bilirubin: increased
  -LDH: increased
- **2. For incompatibility- documentation of patient's identity**
  Pre and post transfusion -repeat ABO group
  -screen for patient RBC antibodies

-repeat cross match
-direct Coomb's test
-Eluate if DAT is positive

- **3. DIC- CBC**
  -peripheral smear
  -coagulation screen
  -FDP or D-dimer
- **4. Renal function –urea**
  -creatinine
  -electrolytes
- **5. Bacterial infection- blood culture**
  -gram stain
- **6. Immunological –IgA**
  -anti IgA antibodies

(66) Enumerate some non life threatening blood transfusion reactions?

- **Allergic- itchy, urticaria due to reaction to plasma proteins**
- **Febrile non hemolytic reaction- recipient's antibodies react with donor WBC increasing temperature by 1˚C or cytokines causing same reaction**

(67) In which condition of ABO incompatibility reaction is most severe?

- **Group A blood transfused to group O patient**

(68) When does a delayed hemolytic transfusion reaction occur?

- **When recipient is immunized to red cell antigen by previous transfusion or during pregnancy**
- **Antibody is present at low or undetected levels**
- **Secondary immune response is mounted to incompatible antigen**
- **IgG/ complement coated RBCs are destroyed in spleen or liver**

MEGALOBLASTIC ANEMIA

(1) Which are sensitive indicators of folate and cobalamin deficiency?

- **Sensitive indicators of folate and cobalamin deficiency are:**
  - **Plasma homocysteine**
  - **Serum methylmelonic acid**

(2) What is significance of elvated homocystine level?

- **Significance of elvated homocystine level is:**
  - **Associated with risk of idiopathic venous thrombosis**
  - **Vascular disease risk factor indicating MI and stroke.**

(3) What is cause of increased homocysteine level?

- **Cause of increased homocysteine level is:**
  Associated with B_{12} and folate deficiency
  ↓
  Reduced methionine synthesis
  ↓
  Increased homocysteine accumulation

(4) Which are the methods to measure plasma homocysteine levels?

- **The methods to measure plasma homocysteine levels are:**
  HPLC, Enzyme Immunoassays

(5) Which is specific marker of cobalamin deficiency?

- **Specific marker of cobalamin deficiency is:**
  Holotranscobalamine (B_{12} transcobalamine II Complex)

(6) What is cause of formation of megaloblast?

- **Cause of formation of megaloblast is an impaired DNA synthesis due to lack of Vit. B_{12} or folate.**

(7) When will you suspect pernicious anemia?

- **Pernicious anemia will be suspected in following conditions:**
  - **Increased MCV, more than 130 fl with oval mecrocytes, poikilocytes**
  - **Hypersegmentation of neutrophils (Greater than 5% of neutrophils with more than 5 nuclei)**

(8) Which are differential diagnosis of macrocytic anemia?

- **Differential diagnosis of macrocytic anemia are:**
  - **Myelodysplasia- Hypogranular neutrophils, Monocytosis**
  - **Excess Alcohol consumption- Round macrocytes but MCV rarely higher than 110 fl**
  - **Hypothyroidism**
  - **Liver disease, Aplastic anemia.**
  - **CDA-I and CDA-III – Erythroleukemia**
  - **Drugs- AZT, Anticonvulsants, OC pills.**

(9) What is significance of detecting clinical details?

- **Significance of detecting clinical details is:**
  - **If there is slight jaundice, it can be ineffective erythropoiesis**
  - **In cases of neurological deficiency, it can be Cobalamin deficiency**
  - **Vegetarians- B_{12}, Folate deficiency**
  - **Steatorrhea, Nocturnal bowel habits- Folate deifiency/ Crohn's disease**
  - **Mouth ulcers- B_{12} Deficiency**
  - **Glossitis, Koilonychia- Iron deficiency with Cobalamin deficiency**
  - **History of Alcohol- Vit B_{12} and /or Iron deficiency**

(10) Which history of Autoimmune diseases is relevant for anemia?

- **Following Autoimmune diseases are relevant for anemia:**

  Hypothyroidism and Coeliac disease- Relevant for Pernicious Anemia

(11) What is the role of gastrectomy in megaloblastic anemia?

- **In case of gastrectomy, there is an increased risk of Vit.B_{12} deficiency generally after 2 years of surgery.**

(12) What is significance of laboratory tests in Vit.B_{12} or folate deficiency?

- **Significance of laboratory tests in Vit.B_{12} or folate deficiency is:**
  - **Peripheral smear picture**
    - Macrocytes
    -Hypersegmented neutrophils
    ($\geq$ 5 lobes in more than 5% of neutrophils)
    -Howell-Jolly bodies- Hyposplenism
- **Differential diagnosis-**
  - **Myelodysplastic syndrome- Hypolobulated neutrophils**
  - **Alcoholic liver disease- Target cells, Stomatocytes.**

(13) What is importance of reticulocytes in cobalamin and folate deficiency?

- **Importance of reticulocytes in cobalamin and folate deficiency is:**
  - **Low in case of deficiency**
  - **Increased by 6th day of treatment**
  - **Will not increase if there is associated iron deficiency**

(14) What is significance of serum homocysteine level?

- **Significance of serum homocysteine level is:**
  - **Increased in Vit.B12 and folate deficiency**
  - **Differntial diagnosis- also increased in renal impairment**

(15) What is Schilling test?

- **Schilling test is a test which diagnoses B_{12} deficiency due to Malabsorption.**

(16) What is laboratory checklist for diagnosis of Pernicious Anemia?

- **Laboratory checklist for diagnosis of Pernicious Anemia is as follows:**
  - **Macrocytosis, Anemia, Increased Homocysteine, Gastric pH >6**
  - **B_{12} Deficiency, Megaloblastic Anemia not resulting due to folate deficiency**
  - **Schilling test**

(17) In which condition, Vit.B_{12} level is high?

- **Conditions in which Vit.B_{12} level is high are:**
  - **Liver disease**
  - **Myeloproliferative diseases**
  - **Acute inflammation**

(18) Mention causes of cobalamin deficiency.

- **Causes of cobalamin deficiency are:**
  - **Reduced intake (Vegetarian diet)**
  - **Malabsorption due to lack of intrinsic factor**
  - **Abnormal transport proteins**
  - **Acquired drug effects**

(19) Mention causes of folate deficiency.

- **Causes of folate deficiency are:**
  - **Reduced intake (Alcoholics)**
  - **Dieting (Tea and toast)**
  - **Malabsorption**
  - **Drug effects**
  - **Increased folate turnover**

(20) Which factors affect serum Homocyteine level?

- **Factors affecting serum Homocyteine level are:**
  - **Cigarrette smoking**
  - **Increasing Age**
  - **Renal disease**
  - **Drugs. E.g. Levodopa**

(21) What precautions should be taken for sampling blood for folate and B_{12} assays?

- **As folate is affected by recent dietery intake; fasting sample is to be taken. For B_{12} assay, this is not needed.**

(22) What preanalytical care is to be taken for doing folate assays?

- **Preanalytical care to be taken for doing folate assays is:**
  - **Light and temperature instability should not be there.**
  - **Hemolysed sample cannot be assayed.**

(23) What should be added to folate sample for more stability of sample?

- **Ascorbic Acid should be added to folate sample for more stability of sample.**
- **But it cannot be used for B_{12} assay as it destroys B_{12}.**

(24) What is MMA?

- **MMA- Methyl Malonic Acid**
- **It is a dicarboxylic acid present in urine and plasma due to deficiency of Cobalamin.**

(25) Why MMA is accelerated in B_{12} deficiency?

- **MMA is accelerated in B_{12} deficiency due to:**
  Absence of conversion of Methylmalonyl CoA to Succinyl CoA by enzyme MMA-CoA mutase.

(26) What is homocysteine?

- **Homocysteine is disulphide amino acid present in cells and plasma.**
- **It is increased in B_{12} deficiency as it is not converted to methionine by enzyme methionine synthatase.**

(27) What is significance of increased Homocysteine level?

- **Significance of increased Homocysteine level is:**
  - **It is a risk factor for vascular diseases, including MI and Stroke.**
  - **Also affected by age, smoking, renal disease, excessive coffee.**

(28) What care is to be taken while collecting blood for Homocysteine?

- **Care to be taken while collecting blood for Homocysteine is:**
  - **It should not be collected after venous stasis.**
  - **It should be collected following subject resting in supine position.**

(29) What is cause of Pernicious Anemia?

- **Cause of Pernicious Anemia is:**
  Antibodies against Gastric Parietal cells are present in 90% of cases, not allowing to secrete Intrinsic Factor (IF).

(30) How many types of antibodies are there against Intrinsic Factor?

- **There are two types of antibodies against Intrinsic Factor:**
  - **Type I- Blocks binding of B_{12} to Intrinsic Factor (IF).**
  - **Type II- Prevents attachments of IF or IF-B_{12} Complex to ileal receptors.**

(31) What is Schilling test?

It is absorption test for urinary excretion of radioisotope labeled B12 in follow up of B12 treatment.

Normal is >10% in first 24 hours

Pernicious anemia <5% in first 24 hours

MISCELLANEOUS TESTS

(1) Which are acute phase reactants?

- **Increase in fibrinogen**
- **Increase in haptoglobin**
- **Increase in ceruloplasmin**
- **Increase in immunoglobulin**
- **Increase in CRP**
- **Decrease in albumin**

(2) In which conditions acute phase responses occur?

- **Acute infection**
- **Active phases of chronic inflammation**
- **Malignancy**
- **Acute tissue damage(myocardial infarction)**
- **Physical injury**

(3) Which are common tests to measure acute phase responses?

- **CRP**
- **ESR**

(4) What are limitations of ESR as compared to CRP in acute phase response?

- **Slower to respond to active disease**
- **Insensitive to small changes**
- **Less specific because it is inflamed by immunoglobulins and anemia**
- **Rate of change is slower**

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(5) What are benefits of ESR over CRP?

- **Useful screening test**
- **Simple,cheap**
- **Not dependent on power supply**

(6) What is ESR? Which is standard recommended method?

- **It is measurement after 1 hour of sedimentation of red cells in diluted blood in open ended glass tube of 30 cm length mounted vertically on a stand**
- **Westergern method is preffered**

(7) What is standard length and diameter of Westergern ESR tube?

- **Length-30cm**
- **Diameter-2.55mm**

(8) Which sample is to be used for ESR estimation?

- **Venous blood in EDTA and then diluted in proportion of 1 volume of citrate to 4 volumes of blood**
- **Collect directly in citrate solution**

(9) Within which time ESR is to be performed?

- **4 to 6 hours if stored at 4˚ C**

(10) How reading of ESR is taken?

- **Nearest 1 mm the height of clear plasma above the upper limit of column of sedimenting cells**

(11) In which case there is poor delineation of upper layer of red cells?

- **When there is high reticulocyte count**

(12) Which phenomenon is incorporated in Automated ESR machine?

- **The tube is sloped, leading to quick sedimentation of Red cells.**
- **ESR can be measured after 20 minutes.**

(13) How quality control of ESR is done?

- **EDTA blood kept at 4˚ C (3-4 samples)**
- **By ICSH standardized method**
- **Stabilized whole blood preparation**

(14) What is ICSH standardized method?

- **It is a method intended to provide a reference method for verifying the reliability of any modification of test.**
- **It is carried out on EDTA blood sample not diluted in citrate using Westergren tubes.**

(15) How will you perform ICSH standardized method?

- **Select 10 samples with PCV 0.30-0.367 and ESR in range between 15 to 105 mm.**
- **Fill westergren tube to measure ESR.**
- **Sample should be collected in EDTA and not in citrate.**
- **Reading should be taken by formula-**
  Corrected ESR = (Undiluted ESR x 0.86) - 12

(16) Which is inhouse control for ESR?

- **Calculate daily cumulative mean when at least 100 samples are tested each day.**

(17) What is the mechanism of erythrocyte sedimentation?

- **Depends on difference in specific gravity between red cells and plasma.**
- **Influenced greatly by the extent to which red cells form rouleaux, which sediment more rapidly than single red cell.**

(18) Which factors affect erythrocyte sedimentation?

- **Ratio of Red cells to plasma**
- **Plasma viscosity**
- **Verticality of sedimentation tube**
- **Bore of tube**

- **Dilution of blood**

(19) Which factors affect rouleaux formation?

- **Concentration of Fibrinogen**
- **Other acute phase reactants- e. g - Haptoglobulin, Ceruloplasmin, α1 anti trypsin, immunoglobulin, CRP etc.**
- **Enhanced by immunoglobulins**

(20) Which factors retards rouleaux formation?

- **Albumin**
- **Defibrinated blood**

(21) What is effect of anemia on rouleaux formation and ESR?

- **Anemia alters ratio of red cells to plasma which encourage rouleaux formation and accelerate sedimentation**

(22) What are different phases of sedimentation of RBCs?

1. **Preliminary: few minutes during which rouleaux occur and aggregates form.**
2. **A period in which sinking of aggregates take place at constant speed**
3. **Rate of sedimentation slows as aggregated cells pack at the bottom of tube**

(23) What is the clinical significant of ESR?

- **Screening test**
- **Rheumatoid arthritis or Tuberculosis which provides index to progress of disease**
- **Diagnosis of temporal arteritis and polymyalgia rheumatica**
- **In suspected cases of multiple myeloma**
- **Early features of myocardial infarction**

(24) On which case of multiple myeloma ESR is normal?

- **When myeloma is nonsecretory of light chain**

(25)Why ESR is higher in woman than men?

- **Due to increased fibrinogen level which occurs in normal pregnancy**

(26) On which conditions ESR is low?

- **Polycythemia**
- **Hypofibrogenemia**
- **Congestive cardiac failure**
- **Abnormalities of red cells like Poikilocytosis, Spherocytes and sickle cell disease**

(27) What is the significance of plasma viscosity?

- **Reflects clinical severity of disease more closely than ESR**
- **Changes occur 24-48 hours before ESR**

(28) What are LE cells?

- **Seen in systemic lupus erythmatosus**
- **They are seen based on reaction between patient's autoantibody and nuclear antigens with subsequent phagocytosis by neutrophils**

(29) Which is immunological method for diagnosis of malaria?

- **Dipstick test- based on binding of monoclonal antibody to histidine rich protein which occurs specifically in plasmodium falciparum**
- **ELISA- less sensitive in low levels of parasitemia**
  -Negative when only gamatocytes are present
  -Unhelpful in immediate post- treatment follow up as it remain positive for 1-2 weeks after disappearance of parasites
- **Immunochromatography**

(30) Which kit is specific for plasmodium vivax?

- **With antibody against parasite glycolytic enzyme lactate dehydrogenase**

(31) Which is standard method of diagnosing malaria?

- **Examination of blood film -to obtain estimate of percentage of RBCs Affected**
  - to makeup the diagnosis
  - to monitoring of treatment

MOLECULAR AND CYTOGENETIC ANALYSIS

(1) What is the significance of DNA analysis?

- **In refining Hematological diagnosis and determining treatment.**

(2) In which cases DNA Analysis helps?

- **Identification of families at risk for hemoglobinopathies allowing early prenatal diagnosis.**
- **Assessment of genetic risk factors in Thrombophilia.**
- **Diagnosis and characterization of Leukemia.**
- **Monitoring host donor transplantation.**
- **Study of host donor chimerism following Bone Marrow Transplantation.**

(3) Which points of DNA are important in study of DNA Analysis?

- **Construction of proteins encoded by four Bases Adenine (A), Cytosine (C), Guanine (G) and Thymine (T) along sugar phosphate back bone of DNA.**
- **DNA is double stranded.**
- **Two strands are held together by hydrogen bonds between A and T and G and C.**
- **Sequences of bases are complementary.**
- **Strands have polarity, one end is $5'$ end and other end is $3'$ end. Both are in opposite direction.**

(4) Which enzyme manipulates DNA?

- **Recombinant molecules due to bacterial DNA modifying enzyme especially restriction enzymes.**

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(5) Which are the methods to determine sickle cell gene?

- **Hemoglobin Cellulose acetate electrophoresis**
- **Sickling Test**
- **DNA analysis (Prenatal Diagnosis at 10 weeks of Pregnancy)**
- **PCR**

(6) What are the restriction enzymes?

- **Endonucleases that cut DNA molecules wherever there is a short, specific sequence of bases.**

(7) What is PCR?

- **It has ability to amplify specific DNA fragments from small amounts of starting material. It is basis of DNA Analysis. It can be visualized by ethidium bromide staining on agarose gel.**

(8) How DNA can be extracted?

- **From any blood or tissue sample.**
- **3 ml of blood in EDTA is needed.**

(9) What is Genomic DNA?

- **DNA extracted from nucleated Cells.**

(10) Which mutation occurs in sickle cell disease?

- **In codon 6 of β Globin gene, GAG → GTG – resulting in loss of B_{54}36 I restriction enzyme site.**

(11) On which cells cytogenetic analysis and molecular techniques can be done (karyotyping)?

- **Skin fibroblasts**
- **Bone marrow cells**
- **Blood cells**
- **Lymphnode cells**

(12) What is FISH?

- **Bridge between cytogenetic analysis and molecular techniques.**
- **Chromosomes stained and visualized**
- **Specific DNA sequence recognized by means of florescent probe**
- **Can be carried out on metaphase or interphase of cells**

(13) What are the applications of FISH?

- **In detecting Trisomy, monosomy or chimerism**
- **Specific oncogenes**
- **Specific tumor suppressor genes**

(14) What are the advantages of FISH?

- **Many more cells are examined**
- **Abnormalities can be detected in nondividing cells**
- **Performed in shorter period of life**

(15) What is disadvantage of FISH?

- **Only those abnormalities that are specifically sought will be found**
- **All chromosomes cannot be evaluated**

(16) What is the significance of Philadelphia chromosome?

- **Chromosome 22q**
- **Present in 95% cases of CML**
- **Presence is confirmed by demonstration of BCR-ABL fusion gene by RT-PCR**
- **May be found in 25% and 5% of adult and childhood ALL where it is associated with poor prognosis**

(17) How small cleaved lymphoid conditions be differentiated?

- **Translocation involving BCL -1 → Mantle cell lymphoma**
- **Identification of BCL-2 → Follicular lymphoma**

(18) What is minimal residual disease? How it is detected?

- **One malignant cell in background of 1,00,000 normal cells is minimal residual disease.**
- **It is monitored by Quantitative real time PCR (QR-PCR)**

(19) What is the principal of QR- PCR?

- **It permits quantification of number of transcriptions of gene of interest at high levels of sensitivity**

(20) What are the advantages of QR-PCR?

- **Ability to detect accumulation of amplicones permiting quantification of DNA of interest**
- **Offers higher level of specificity.**
- **Eliminates need of carcinogenic epithelium-bromide stained gene.**

(21) Which enzyme deficiency can be measured by DNA analysis?

- **G6PD**

PREPARATION AND STAINING METHODS FOR BLOOD AND BONE MARROW FILMS

(1) What is normal measure of a glass slide?

- **75x25mm. 1mm in thickness.**
- **Ideally frosted on one side.**

(2) Which anticoagulant should be used for blood for slide preparation?

- **EDTA**

(3) What should be width of slide spreader?

- **18mm**

(4) What should be the angle of making slide ?

- **30˚**

(5) What should be ideal length of blood smear?

- **3cm**

(6) How is ideal thickness of smear indicated?

- **RBCs some overlap throughout film**
- **WBCs easily recognizable.**

(7) What are main components of Romanowsky dyes?

- **Preferrably:**
  - ✓ **Azure B (trimethylthionin)**
  - ✓ **Eosin Y (tetrabromofluorescein)**
- **May include Methylene blue (debatable)**

(8) What is usefulness of methylene blue in Romanowsky stain?

- **Enhance staining of nucleoli and polychromatic RBCs.**
- **In its absence normal neutrophil granules stain heavily and resemble toxic granules.**

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(9) What are different Romanowsky dyes?

- **Jenner**
- **Giemsa**
- **Leishman**
- **Wright**

(10) What should be minimum proportion of dye in stain?

- **80%**

(11)What should be the ideal pH for staining smear?

- **6.8**

(12) What should be the ideal pH for staining malarial parasite?

- **7.2 as it stains Schuffener's dots better.**

(13)How will you achieve ideal pH?

- **50 ml of 60mmol/L Sorenson's phosphate buffer is added to 1 litre of water. Used in diluting the stains and washing films.**

(14)What is the reason of too blue staining?

- **Eosin concentration low.**

(15) What is the reason of too pink staining?

- **Incorrect proportion of Azure-B.**
- **impure dye.**
- **Low pH of buffer.**

(16) What is the reason pale staining?

- **Old stain.**
- **High temperature.**

(17) What is the reason of precipitation on stain?

- **Stain uncovered.**
- **Stain unfiltered.**

(18)What are causes of blue background on stain?

- **Inadequate fixation.**
- **Blood collected in heparin anticoagulant.**

(19)Which are fixatives used to fix the peripheral smear?

- **Methanol.**
- **Ethyl alcohol (absolute alcohol).**

(20)What care should be taken for fixatives?

- **They must be store in bottle with tight fitting stopper to prevent contamination with absorbed water.**

(21)Which is Rapid staining method?

- **By staining with Field's stain.**
- **Useful especially for malarial parasites.**

(22)Which material is used for mounting coverglass?

- **DPX – miscible with Xylol.**
- **Temporary mount – Cedarwood oil.**

(23)How will you differentiate between Rouleax formation and Agglutination?

- **RBCs forming clumps are arranged side by side in Rouleax.**
- **Addition of normal saline causes disappearance of Rouleax.**

(24)What is Pitting?

- **Appearance of small crater like indentations on RBC cell surface.**
- **Seen in Splenic dysfunction (4 %).**
- **Normal – 2%.**
- **Demonstrated by electron microscopy or Nomarski illumination.**

(25)Crystals of which Hemoglobin are seen in wet preparation?

- **Hb C / Hb SC.**
- **If suspected incubate blood with 30 g/lit NaCL for 4 hours at 30^{O}C.**
- **Crystals seen as large amorphous material – shown by staining with Romanowsky stain.**

(26)How will you prepare buffy coat preparation?

- **By centrifuging EDTA blood sample for 5-10 minutes.**
- **Sedimenting blood with enhancing agents like fibrinogen, dextran.**

(27)What is use of buffy coat preparation?

- **To study leucocytes, platelets and abnormal cells.**
- **Atypical cells which are small in number can be studied better in buffy coat layer.**

(28)In which blood primitive cells are seen more in number?

- **Cord blood.**

(29)What are different types of cells are seen in buffy coat?

- **Megakaryocytes and immature cells of granulocyte series in disseminated carcinoma.**
- **Megaloblasts – Megaloblastic Anemia.**
- **Erythrophagocytosis – Autoimmune Hemolytic Anemia.**
- **LE cells – SLE.**
- **Bacteria, fungi, parasites in neutrophils.**
- **Ringed sideroblasts in Myelodysplastic syndrome.**
- **Hairy cells – Hairy cell leukemia.**

(30)Parasites found in blood?

- **Plasmodia.**
- **Leishmania.**
- **Babesiae.**
- **Trypanosoma.**
- **Micrifilaria.**
- **Ehrlichiosis.**

(31)What is advantage of making thick film?

- **Advantage of thick films, it detects parasite even when they are scanty.**
- **Microfilaria detection**
- **5minutes thick film = 1 hour thin film**

(32)What is disadvantage of making thick film?

- **Identification of species is difficult**
- **Mixed infection is missed**

(33)What is use of thin film?

- **For species identification**
- **For counting percentage of positive cells for severity of infection**

(34)For which parasite wet film is used?

- **Microfilaria as it is easy to detect it while it is moving.**
- **Others-trypanosome, spirochete**

(35)How correct thickness is achieved in thick films?

- **When slide put on newspaper , small print is just visible.**

(36)For staining of malaria how much pH of stain is very important?

- **7.2**

(37)Which fluorescent dye is used to stain malarial parasite?

- **Acridine orange(False positive-Howell jolly bodies, reticulocyte)**

(38)What are methods to diagnosis Leishmaniasis in Hematology?

- **Buffy coat preparation**
- **Amastigotes seen in monocytes**

(39)How wet preparation is prepared for microfilaria?

- **Capillary blood 1 ml + 9 ml 2% formalin**
- **Centrifugation**
- **Deposit placed on slide**
- **Drop of field's stain or 1% methylene blue**
- **Motile**

QUALITY ASSURANCE

(1) What is accuracy?

- **Closeness of the estimated value to that considered to be true.**

(2) What is precision?

- **Reproducibility of a result.**

(3) How precision is controlled?

- **By replicating tests and repeated tests on previously measured specimen.**

(4) How accuracy is controlled?

- **By using reference materials that have been analysed by reference methods.**

(5) What does quality assurance programme include?

- **Internal quality control**
- **External quality control**
- **Standardization**

(6) On what internal quality control is based?

- **Monitoring test procedure**
- **Measurement of specially prepared materials**
- **Repeated measurements on routine specimens**
- **Daily analysis of data obtained**

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(7) What is external quality control assessment?

- **Evaluation by outside agency of performance by numerous laboratories on specially supplied samples**

(8) What is proficiency testing?

- **Procedures by which EQA scheme functions**

(9) What is standardization?

- **Includes materials and methods**
- **Material standards are used to calibrate analytic instruments**

(10) What is reference method?

- **Technique that is used in association with a reference preparation when available to provide sufficiently precise and accurate data for scientific purposes and assessing validity of other methods.**

(11) What is selected method?

- **Method which is directly comparable with international reference methods**
- **Serves as an alternative to reference method**
- **Used for evaluation and validation of a proposed routine method.**

(12)What is a working or recommended method?

- **Method intended for use in routine practice**
- **Taking account of economy of labour and materials**
- **Easy to perform**
- **Validated and sufficiently reliable for intended purpose.**

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(13) What are controls?

- **Preparations that are used for either internal quality control or external quality assessment.**

(14) What is CE mark of instrument?

- **Certification that instrument will perform according to standard set by European parliament or Council directive.**

(15) What is ISO?

- **International organization of standardization has established standards for medical laboratory practice.**

(16) What is ISO 9000?

- **A set of standards that specify various aspect of quality management system for any organization.**

(17) What is ISO 15189?

- **Medical laboratories- particular requirement of quality & competence.**

(18) Which ISO series are important for laboratories?

- **ISO-9000**
- **ISO-15189**

(19) Which is reference preparation for Hb quality control?

- **Preparation of HiCN (Heaminglobincyanide)**

(20) Which control charts are used in laboratory?

- **Levy and Jennings**

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(21) How will you indicate fault in technique of instrument or reagent by Levy Jennig's Charts?

- **Outside 3SD- gross error**
- **One or two results outside +2SD or -2SD- Random error**
- **Comentive results on one side of mean- calibration fault**
- **Comentive value rising or falling by 2SD- Imprecision**

(22) Which is the another way of checking precision of routine work?

- **Duplicate tests on patient's specimen.**

(23) What is the method of doing duplicate testrips?

- **10 consecutive samples rerun**
- **Calculating differences between pair and derive SD**
- **Not more than 2SD**
- **Defects random errors**
- **Does not detect incorrect calibration**

(24) What is Delta check?

- **Blood count & patients should not differ much from the results obtained during previous 2-3 weeks**
- **Hb and RBC less than 10%**
- **WBCs less than 20%**
- **Platelet count less than 50%**
- **Detects deterioration of apparatus and reagents.**

(25) How patient data used for Quality control?

- **In hospital with more than 100 test requests each day there should not be significant daily variability in mean of RBCs indices**

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- **Indicates change in instrument calibration or fault in function**
- **Increased SD –loss of precision**
- **Drift in any three indices- instrument fault.**

(26)What is correlation check?

- **It implies that any unexpected result of a test must be checked to see whether it can be explained on clinical grounds or whether it correlates with other tests.**

(27) What is EQUAS?

- **Samples are sent from national or regional centers**
- **Results that are resumed are anlysed for target value& acceptable range**
- **Performance is judged.**

(28) What is Deviation Index?

- **It is analysis of EQUAS**
- **From results returned by participants median, mean and SD are calculated.**
- **Performance is compared with that of other laboratories & with self on previous sample by DEVIATION INDEX**
- **Calculated as difference between individual laboratories result and median or mean relative to SD**
- **DI score less than 0.5%- excellent result**

0.5 to1%- satisfactory
1to2%-acceptable
<2%- Analyser calibration
<3%- serial defect

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(29) What is target value or true value?

- **Result obtained by best performance of selected participants in survey using reference methods.**

(30)How percent bias is calculated?

- **Participants result (R), Tv-True value**
- **[(R-Tv)/Tv]x100**

(31)How often instruments should be calibrated routinely?

- **6 month interval**

(32) How often control should be run?

- **Daily or with each batch.**

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RADIO ISOTOPES IN HEMATOLOGY

(1) What are the applications of radioisotopes in Hematology?

- **Blood volume**
- **Red cell survival studies**
- **Vit B12 absorption (Schilling test)**
- **Ferrokinetic studies**

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REFERENCE RANGES AND NORMAL VALUES

(1) What is reference range?

- **It is established from measurements on a relatively small number of subjects assumed to be representative of population as a whole.**
- **Conditions same:- Standardized same time, same position etc.**

(2) If the pattern of sample analysed is symmetric, What is it called?

- **Gaussian**
- **If asymmetric - Non Gaussian**

(3) How arithmetic mean is detected?

- **By diving sum of all measurements by number of observations in Gaussian curve.**

(4) What is Mode?

- **Value that occurs more frequently.**

(5) What is Median?

- **Point at which there are equal number of observations above and below it.**

CONFIDENCE LIMITS

(6) In any analysis what are minimum and maximum number of samples needed for analysis?

- **Minimum- 40 samples**
- **Maximum- 120 samples**

(7) Why Hb and RBC count high immediately after birth?

- **Because of cessation of pulsation of umbilical artery in cord and uterine contracts resulting in much of blood contained in placenta reentering infant's circulation.**

(8) Why levels of Hb are less in females than males?

- **Hormonal influence in haematopoiesis.**
- **Menstruation- Iron deficiency.**

(9) Why does Hb decrease in pregnancy?

- **Inspite of increased erythropoietic activity due to increased plasma volume.**
- **Level reaches normal about a week after delivery.**

(10) What is cause of anemia in old age?

- **Decreased erythropoietic reserves with decrease in erythroid progenitors in Bone marrow.**
- **Chronic inflammatory disease.**
- **Chronic blood loss.**

(11) What is sports anaemia?

- **It occurs in long distance runners due to increased plasma volume.**
- **Endurance training leads to loss of iron in sweat- decrease serum iron and serum ferritin.**

(12) Why does hemoglobin increase in sprinter?

- **Due to decrease plasma volume as it requires high muscular exercise**

(13) What is March hemoglobinura or runners anaemia?

- **Hemolysis occurring as a result of pounding of feet on ground.**

(14) What is effect of posture on hemoglobin?

- **More Hemoglobin / PCV in sitting than lying as there is alteration of plasma volume.**

(15) Why resting for 5-10 minutes is necessary before blood collection?

- **As there can be variation in hemoglobin/ PCV due to change in position.**

(16) What are different seasonal and diurnal variation?

- **Diurnal variation – 20% variation in reticulocyte and leucocyte**
- **Erythropoietin – lowest at 8:00 am**
  Increase 40% at 4:00 pm
  Increase 60% at 8:00 pm
- **Also seen in serum iron and ferritin.**

(17) Which parameters are affected due to altitude?

- **Hemoglobin and PCV are altered due to hypoxemia that leads to Erythropoesis.**

(18) Which parameters are affected due to cigarette smoking?

- **Hemoglobin, RBC, PCV, MCV are affected due to cigarette smoking.**

LEUCOCYTE COUNT

(19) Till what age lymphocytes predominate in children?

- **Upto 5-7 years lymphocytes predominate.**

(20) Factors causing variation in leucocyte count

- **Adrenaline – increases leucocyte count**
- **Pregnancy – moderate increase(neutrophilia) with peak on 2nd semester**

- **EFFECTS OF SMOKING.**
  10 or more Cigarettes -> increase Hemoglobin, Packed cell volume, Mean cell volume due to accumulation of Carboxyhemoglobin.
  1 Cigarette -> 1% rise in Carboxyhemoglobin.
  Polycythemia.

- **White blood counts increase.**
  Increased Neutrophils and CD4 lymphocytes.

- **High platelet count.**
  Decreased platelet survival.

(21) Which are thing with decrease due to cigarette smoking?

- **Plasma volume – Protein.**

RETICULOCYTE COUNT

(1) Which reagent is used?

- **1% new methylene blue**
- **Brilliant cresyl blue**

(2)What is reticulocyte?

- **Immature nonnucleated Red cell containing RNA which is precipitated by methylene blue or brilliant cresyl blue as dark blue network of reticulum.**

(3)What does reticulocyte count suggest?

- **It provides as estimate of a rate of red cell production.**

(4)How all controls of reticulocyte are made?

- **Hypotonic dialysis of RBC in presence of RNA followed by short period of hypertonic dialysis to reseal pores of RBC membrane.**

(5)How is absolute reticulocyte count determined?

- **By multiplying Reticulocyte percentage by RBC count**

(6)What does reticulocyte count indicate?

- **Estimate of rate of RBC production.**

(7)Which fluorescent dyes can be used in reticulocyte count?

- **Acridine orange and Thioflavin T which binds to RNA**

(8)What are causes of false positive reticulocyte count?

- **Presence of Howell Jolly Bodies**
- **NRBC**
- **Sickle cell**
- **Giant platelets**

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SAMPLE COLLECTON

(1) What are the factors influencing laboratory results?

- **Diurnal variation**
- **Exercise**
- **Fasting**
- **Diet**
- **Ethanol**
- **Tobacco**
- **Posture**

(2) Which tests are affected by Diurnal Variation?

- **Serum Iron**
- **Neutrophil count**

(3) Which tests are affected by Exercise?

- **Increase CK**
- **AST**
- **Lactate Dehydrogenase**
- **Activate coagulation, Fibrinolysis**
- **Platelets**

Due to increased metabolic activities.

(4) How fasting affects the laboratory results?

- **48 hrs fasting -> increase serum bilirubin.**
- **72 hrs fasting -> decrease plasma Glucose.**

(5) What is the effect of eating in laboratory tests?

- **Eating leads to increase fat content -> increase Triglycerides and Glucose.**

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(6) Which test is affected in O+ve and B+ve blood groups ?

- **They are Lewis Secretor.**
- **Increase Alkaline Phosphtase.**

(7) What is the effect of Alcohol on laboratory results ?

- **Increase HDL**
- **Increase MCV**

(8) What is the effect of Tobacco?

- **It causes high Carboxyhemoglobin levels.**

(9) What is effect of Posture on laboratory results?

- **Supine to upright -> increase Albumin and Calcium**
- **Also increase in Hematocrit, Hemoglobin and WBC Counts.**

(10) What is effect of prolonged tourniquet binding on sample collection?

- **Increase serum enzymes**
- **Increase Proteins**
- **Increase cholesterol**
- **Increase calcium**
- **Increase Lactate Concentration.**

(11) What is difference between plasma and serum ?

- **Presence of Fibrinogen.**

(12)Which is ideal anticoagulant?

- **Heparin in form of Lithium Salt.**

(13) Why does Glucose estimation sample is taken in fluoride vacuatte?

- **Fluoride inhibits Glycolysis.**

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(14)What is importance of Silicone Gel material located at the base of tube?

- **Specific Gravity of the Silicone Gel is between RBCs and Serum.**
- **Gel rises and lodges between packed cells and serum forming INERT BARRIER**
- **Advantages: 1. Easy to use.**
  2. Higher serum yield.
  3. One centrifugation
  4. Transportation without disturbing.

(15) What is the effect of excess EDTA on blood sample?

- **Excess of EDTA – Alters RBC morphology**

(16) What are actions of various anticoagulant ?

- **K^+ oxalate- Variable dilution due to water transport from cells to plasma**
- **Ca^+ chelator -Inhibit different enzyme activities**
- **Oxalate, citrate – Inhibit amylase and acid phosphatase**
- **Oxalate, citrate and EDTA – Decrease Ca^+**

(17) Give differences between Arterial and Venous blood.

Arterial blood	Venous blood
➢ Uniform in composition	➢ Varies in composition
➢ Not O_2 deficient	➢ O_2 deficient
➢ Difficult to stop arterial bleeding	➢ Easy to stop venous bleeding
➢ Not easily obtained	➢ Easily obtained
➢ ABGA(Arterial blood gas analysis) - Does not differs in pH CO_2, PCV, Carbon of glucose, lactic acid, chloride.	➢ Differs in pH CO_2, PCV, Carbon of glucose, lactic acid, chloride.

(18) Which veins are preferred in blood collection?

- **First vein is preferred is medial cubital vein, then second is cephalic vein and then wrist, ankle and hand veins preferred for blood collection. (If IV set is present, then other hand veins are preferred)**

(19) Pattern to draw blood.

1. **Blood culture**
2. **Plasma(red)**
3. **Citrate(blue)**
4. **Heparin(green /black)**
5. **EDTA (lavender)**
6. **Fluoride(grey)**

(20) Which investigations are done from arterial blood? Which arteries are preferred?

- **Mainly used to measure O_2,CO_2 and Ph**
- **More difficult to stop bleeding leading to hematoma**
- **Radial, brachial and femoral arteries are preferred.**

(21) What is the substitute of arterial blood?

- **Capillary blood substitutes the arterial blood for Ph, pCO_2 but not for pO_2.**

(22) Which is the best method for blood gas collection in neonates?

- **Umbillical artery.**

(23)What are different specimen interferences?

- **Site of collection -- Important for glucose because as the capillary glucose is 10% to 30% higher than venous glucose**
- **Specimen collect from catheterized vein is not allowed**
- **Equipment avoid of detergents , plasticizers.**
  e.g., for lead free analysis – acid washed lead free container should be used
- **Lysis of RBC – overmixing**
  - Residual alcohol on skin
  - Exposure to heat or cold

(24) What cares should be taken while centrifuging the blood samples?

- **Blood specimen should be centrifuged as soon as the clot formation is complete (about 20 min at room temperature) - acceptable for 2 hours.**
- **Beyond 2 hours change in glucose, potassium and creatinine, etc.**

(25)What is ideal centrifugation?

- **Ideal centrifugation- 10 min at RCF- 850-1000**
- **If delayed store serum at 4^0 to 6^0 until analysis.**
- **Geometrically balance**

(26) What are benefits of Borrosil glass laboratory ware?

- **High degree thermal resistance**
- **Low alkali content**
- **Free for magnetic lime**
- **Zinc group**

(27) What are benefits of Corex glass laboratory ware?

- **Special alumina silicate glass strengthened chemically rather than thermally**
- **6 time stronger than borosilicate**
- **10 times conventional than borosilicate**

THROMBOTIC TENDENCY

(1) In which conditions investigation to exclude acquired or inherited thrombotic tendency is recommended?

- **In neonates, children and young adults who develop venous thrombosis**
- **In those who have strong family history**
- **Thrombosis at unusual sites**
- **Recurrent episodes of thromboembolism**

(2) What are lupus anticoagulants(LAC)?

- **An acquired autoantibody found in various autoimmune disorders**
- **Bind to complexes of various proteins with phospholipids active in coagulation- prolonging clotting times of phospholipid dependent tests e.g. PT or APTT**
- **Associated with recurrent venous thromboembolism, CVA, recurrent abortions, fetal loss etc.**

(3) Which abnormalities are seen in patients with LAC?

- **Thrombocytopenia**
- **Positive direct antiglobulin test**
- **Positive ANA**

(4) Which specific tests are done for LAC?

- **Dilute Rusell's viper venom time (DRVVT)**
- **Kaolin clotting time (KCT)**
- **Tissue thromboplastin inhibition time**

(5) Which sample is preferred to LAC testing?

- **Sample free of platelets**
- **Done by centrifugation of plasma at 2000g or passing plasma through 0.2µm microfilter**
- **Platelet count > $10x10^9$/L should be achieved**

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(6) Enumerate anticardiolipin antibodies.

- **β2 glycoprotein**
- **prothrombin**
- **annexin V**
- **Cardiolipin**
- **produce LAC effect**

(7) Which investigations are done for inherited thrombotic states?

- **Antithrombin III**
- **Protein C**
- **Protein S**

(8) What is antithrombin III?

- **It is the major physiological inhibitor of thrombin r factors IXa, Xa, XIa**

(9) What is protein C?

- **It is vitamin K dependent protein, activated by thrombin complexes with phospholipids and protein S to degrade factors Va and VIIIa**

(10) In which conditions acquired protein C deficiency is seen?

- **Vit K deficiency**
- **Oral anticoagulant**
- **DIC**
- **Liver disease**
- **Early postoperative period**

(11) What is Protein S?

- **It is Vitamin K dependent protein acting as a cofactor for activated protein C**

(12) When dysfibrinogenemia is suspected?

- **TT- increased**
- **Fibrinogen- decreased**

(13) What is significance of Homocysteine?

- **Homocysteinuria- venous or arterial thrombosis with vascular damage**
- **Performed by HPLC or mass spectroscopy or ELISA**

UNDER –RESOURCED LABORATORIES

(1)Which are different levels of laboratory?

- **Health centers**
- **District hospitals**
- **Central/regional hospitals**
- **National reference center**

(2) Which investigations are generally performed at Health Center?

- **Hb , Malaria & HIV**

(3) Which investigations are generally performed at District hospitals?

- **Hb, peripheral blood smear, platelet count, WBCs count, screening of sickle Hb, Malaria rapid test, CD4 count.**

(4) What is cost effectivement for any test?

- **CE=AX100/CX100/B A=Cost per test**
  B= its diagnostic reliability
  C= its clinical usefulment

(5)What is cost per test?

- **Price of reagents divided by number of tests performed.**

(6) What is Hemoglobin colour scale?

- **WHO has developed low grade colour scale representing Hb levels between 4&14g/dl.which is compared.**

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(7) What is difference between Neubar's chamber and improved Neubar's chamber?

- **In Neubar's chamber, in central area 16 groups of 16 small squares are separated by triple rulings while in improved Neubar chamber 25 groups of 16 small squares are separated by closely ruled triple lines.**

(8) Why platelet count done by skin prick are less than that of EDTA sample?

- **As platelets are lost at the site of skin puncture**

(9) Which diluent is used in manual platelet count?

- **1% ammonium oxalate**

(10) What happen to platelet count if sample is clotted?

- **Platelet count will be artificially low**

(11) Which are microscopic artifacts?

- **Dirt or clumped red blood cells may be mistaken for white cells & platelets**
- **Clumping of white cells if concentration of heparin is more than 25iu/ml**
- **Clumping is seen if blood is allowed to stand for several hours.**

(12) What are the reasons of error in observing peripheral blood smear?

- **Revised slide has trace of detergent showing misleading appearance**
- **Residual stain & scratched slides**

- **Methanol may absorb water causing gross artifactual changes**

(13) Which is simple test for screening of β-thalessemia trait?

- **Modified one tube osmotic fragility test**

(14) What % of buffered saline is used in modified one tube osmotic fragility test?

- **0.36%**

(15)What is the advantage of modified osmotic fragility test?

- **It also detects Hemoglobin E**

(16) Which is primary test for HIV in health centers?

- **CD4 count**

(17) How slides are cleaned in laboratory?

- **Leave slides in detergent solution overnight**
- **Wash well in running water**
- **Rinse in distilled water**
- **Store in 95% ethanol or methanol**
- **Dry with clean linen cloth**

(18) How glass ware is cleaned in laboratory?

- **Wash in running water**
- **Boil in detergent solution**
- **Rinse with acid**
- **Wash in hot running tap water**

(19) What is mean?

- **Sum of all the measurements divided by the numbers of measurements.**

(20)What is median?

- **A point that has an equal number of observation above and below.**

(21) What is Gaussian distribution?

- **It describes events or data that occur symmetrically about the mean**
- **This is expressed as standard deviation.**

(22) What is Confidence Intervals?

- **It indicates the upper and lower limits between which a specified proportion of results (95%) may be expected to occur.**

(23) What is Poisson distribution?

- **It describes the events that are random in their occurrence**

(24)What is Reverse Pipette? When it is done?

- **Used for plain, high viscosity fluids and /or very small volumes**
- **With plunger pressed and the way down, dip the tip below surface of fluid and release knob slowly**
- **Remove pipette ,wipe the tissue, put tip inside wall of receiving container, deliver the contents**

(25) How will you correlate focal length of microscope with its magnifying power?

- **FOCAL LENGTH** — **MAGNIFICATION**

2	X100
4	X40
16	X10
40	X4

(26) What is the working distance in microscope?

- **It is the distance between the objective and the object to be misualised**

OBJECTIVE	WORKING DISTANCE
X10	5-6mm
X40	0.5-1.5mm
X100	0.15-0.20mm

(27) What should be the size of cover glass to examine under oil immersion lens?

- **0.15mm thick**

(28) Should microscope be stored in wooden compartment?

- **No because it encourages fungal growth**

(29)How oil immersion lens should be cleaned?

- **By lens tissue absorbent, paper, soft cloth or medical cotton wool**
- **If other lens are smeared with oil, wipe with little toluene, 40% petroleum ether, 40% ethanol or 20% ether.**

(30)Why microscope lenses cannot be soaked in alcohol?

- **Because this may dissolve the cement**

(31) How condenser lens are cleaned?

- **In the same way as other lens**

(32) Where microscope should be stored?

- **Air tight duct cover with silica gel**
- **Warm tight fit tip doored cup board**

(33) How defibrinated blood is taken?

- **Taking blood in conical flask containing central glass rod on to which small pieces of glass capillary have been fused**
- **Blood is whisked by rapid rotation of flask**
- **Coagulation occur within 5 minutes with fibrin collected in central rod**

(34) What is Defibrinated blood?

- **Unclotted red cells without adding anticoagulant**
- **Used in certain types of hemolytic anemia**