Magnification: The total magnifying power of the microscope is = power of eyepiece × power of objective.

Focusing Procedure

Under Low Power (10X)

a. Place the slide to be focused on the stage and fit it into the mechanical stage.
b. Focus the light on the object by adjusting the concave mirror. Lower the objective (10X) and partially close the aperture.
c. The aperture of the objective lens is to be kept above the point that has to be focused on the slide.
d. The condenser should be lowered from the top position till the object is properly illuminated, iris diaphragm is partially opened and focus is adjusted using the fine adjustment for a sharper focus.

For High Power Magnification (40X)

a. First focus under low power and select the required area.
b. Turn the high power objective lens into visualization position.
c. The iris diaphragm is to be half opened and plane mirror is used to condense the light on the object.
d. Raise the condenser to high position
e. Use the fine focusing knob to focus the object.

For Oil Immersion (100X)

a. Condenser should be at the highest position.
b. Iris diaphragm should be fully opened.
c. Adjust the plain mirror to focus the light or object.

Note: The students should note that in self-illuminated microscope having inbuilt light

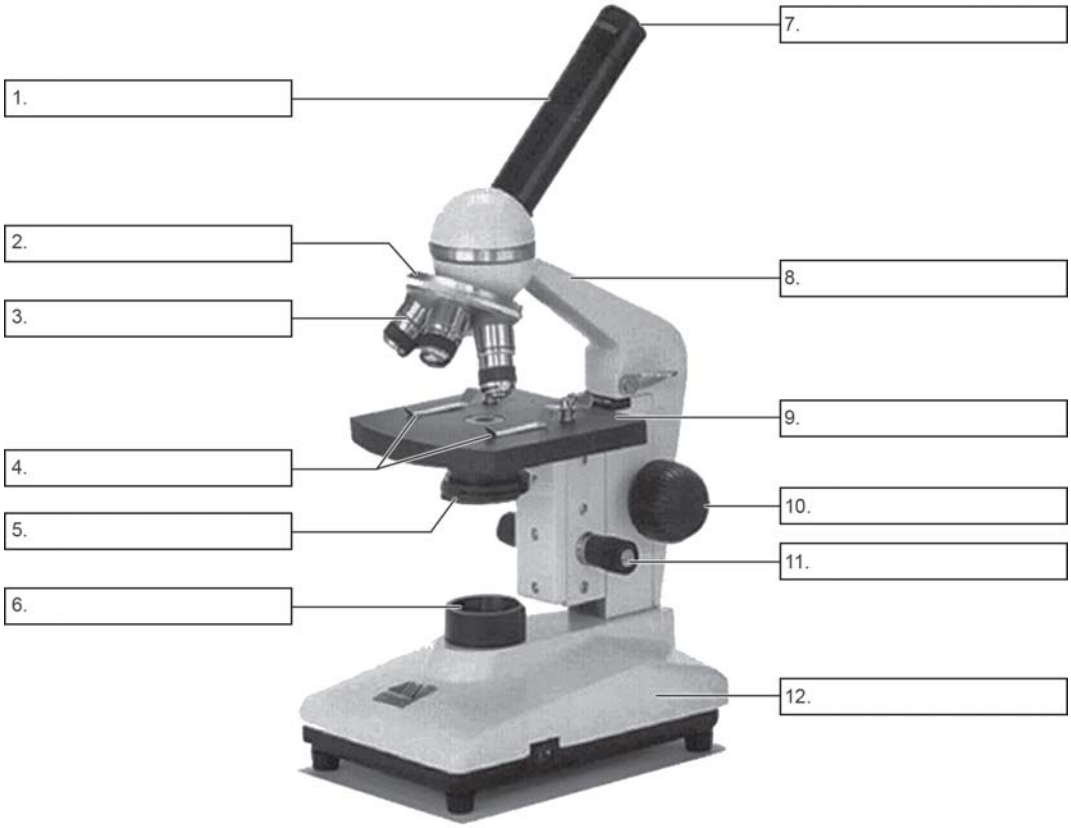


Fig. 1.2: Indentify the parts of the compound microscope

c. Tip of the pipette should be wiped before dipping it into the diluting fluid.
d. Dilution of blood with the respective diluting fluid must be done quickly to avoid clotting of blood.

Q 5. Which among the following is incorrect regarding charging of the Neubauer chamber?

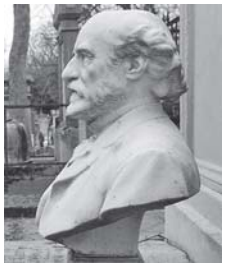
a. The chamber must be completely covered by the diluting fluid.
b. If the chamber is overcharged, it must be washed, dried and recharged again.
c. The first two drops of fluid in the pipette must be discarded before charging the Neubauer chamber.
d. The cells must be counted immediately after charging the Neubauer chamber.

ANSWERS

1. c **2. b** **3. a**
4. b **5. d**

HISTORICAL ASPECTS

LOUIS-CHARLES MALASSEZ



Louis-Charles Malassez was born on September 21, 1842. He was a French anatomist.

Malassez is known for his studies of microscopic anatomy, histology of blood cells, as well as for inventing the hemocytometer. In dentistry, he studied some ligament cells which are now named after him, called the epithelial cell rests of Malassez (ERM). A genus of fungi, Malassezia is also named after him, and includes species that can cause skin irritation.

RECENT UPDATES

1. Though utility of the manual hemocytometer cannot be sidelined as it interprets results by human eye observation; but with increased workload in labs the automated cell counters are now being used for sampling the blood and describing cell populations using electrical and optical techniques for counts of RBCs, WBCs, and platelets. The advanced cell counters today also report Cell Population Data within the leukocyte morphological information and clue for suspecting diseases on basis of observed cell abnormalities.
2. The disposable plastic counting chamber with a grid pattern exactly the same as the improved Neubauer chamber are preferred today because they have
   a. Precise fixed depth counting chamber having superior accuracy and reproducibility.
   b. Reduces the risk of exposure to potentially infectious material.
   c. Increases productivity as cleaning work interruptions are eliminated.
   d. These are light and nonbreakable.

The student should leave the tube in the comparator for acid hematin formation. The technician will take care of further step.

OSPE I AND OSPE II

Question: Dilute the blood (from green sample) for estimation of hemoglobin.

A technician after waiting for 10 minutes has kept the hemoglobinometer tube ready. Golden brown color of acid hematin is visible.

1. Dilute the acid hematin by adding distilled water drop by drop. Mix it with the stirrer. Match the color of the solution in the hemoglobinometer tube with the standards of the comparator. **(Yes/No)**

2. After addition of every drop of distilled water, the student mix the solution and compare the color of the solution with the standard of the comparator till it matches with that of the standard. **(Yes/No)**

3. Take care to hold the stirrer above the level of the solution. At no stage should the stirrer is taken out of the tube. **(Yes/No)**

4. Note the reading when the color of the solution exactly match with the standard and express the hemoglobin content in gm%. **(Yes/No)**

MULTIPLE CHOICE QUESTIONS

Q 1. The haem is an iron porphyrin complex called:

a. Iron ..... Protoporphyrin VII
b. Iron ..... Protoporphyrin IX
c. Iron ..... Protoporphyrin X
d. Iron ..... Protoporphyrin XI

Q 2. The iron in haem is in:

a. Ferrous (Fe^{2+}) form
b. Nitrous form
c. Sulfurous form
d. Potash form

Q 3. Lifespan of fetal hemoglobin is around:

a. 2–3 weeks b. 1–2 weeks
c. 3–4 weeks d. 4–6 weeks

Q 4. Haem is synthesized in:

a. Golgi complex b. Ribosomes
c. Lysosomes d. Mitochondria

Q 5. Sickle shaped cells

a. Decrease blood viscosity thereby decreasing blood flow to the tissue.
b. Increase blood viscosity thereby decreasing flow to the tissue.
c. Increase blood viscosity thereby increasing blood flow to the tissue.
d. In which the blood viscosity remains unaltered

Q 6. One hemoglobin molecules contain _____ iron atoms.

a. 8 b. 4
c. 2 d. 1

Q 7. The affinity of hemoglobin is _____ times for carbon monoxide than oxygen.

a. 20 b. 50
c. 250 d. 125

Q 8. Hemoglobin combines with oxygen to form:

a. Oxidation b. Oxygenation
c. Oxyhemoglobin d. Deoxyhemoglobin

Q 9. Adult hemoglobin has _____ chains.

a. 2 alpha, 2 beta
b. 2 alpha, 2 delta
c. 3 alpha
d. 6 alpha

Q 10. Beta thalassemia minor:

a. HbF level remains unchanged
b. HbF level is markedly increased
c. HbF level is markedly decreased
d. HbF chains are absent

Q 11. Hemoglobin iron content is:

a. 2% b. 3%
c. 0.33% d. 4%

Q 12. The commonest form of thalassemia is:

a. Beta minor b. Beta major
c. Alpha d. Beta

Ans. The types of leukocytes are lymphocytes, monocytes, neutrophils, eosinophils and basophils. The neutrophils, eosinophils and basophils are granulocytes in whose cytoplasm have granules (when viewed under electronic microscopy). Agranulocytes are lymphocytes and monocytes.

Q 5. What is the generic function of leukocytes? What leads to leukocytosis and leukopenia?

Ans. The generic function of leukocytes is to actively participate in the defense of the body against pathogens.

Leukocytosis: It is due to increase in the number of WBCs beyond 11000/cu mm of blood; irrespective of the type of cells (granulocytes, monocytes, lymphocytes, etc.)

a. Physiological leukocytosis is seen in normal infants, after intake of food and after digestion, physical exercise, pregnancy, mental stress, parturition, etc.
b. Pathological leukocytosis is seen in acute pyogenic bacterial infection (Streptococcus, Staphylococcus), myocardial infarction, acute hemorrhage, burns, malignancies or postsurgical postoperative rise.

Leukopenia: It is a decrease in the number of white cells below the normal lower limit of 4000/cu mm.

a. Physiological leukopenia is rare and marginal decrease may be seen in extreme cold conditions as in arctic environment exposure.
b. Pathological leukopenia are seen in:
   - Infection with non-pyogenic organisms (typhoid and paratyphoid fevers, protozoa infection like malaria, etc.)
   - Viral infections (influenza, mumps, smallpox, and acquired immunodeficiency syndrome).
   - Drugs (chloramphenicol, sulphonamides, aspirin, penicillins, phenytoin, etc.)
   - Repeated exposures to X-rays and radium during radiotherapy in cancers.
   - Malnutrition (deficiency of vitamin B and folate), etc.

MCQs

Q 1. The normal WBC per cu mm of blood is:

a. 4000–6000 b. 6000–7100
c. 4000–11000 d. 2000–3000

Q 2. The pathological cause of leukocytosis is:

a. Pyogenic infection b. Stress
c. Pregnancy d. Exercise

Q 3. The ratio of WBC and RBC is:

a. 1 : 6 b. 1 : 60
c. 1 : 600 d. 1 : 6000

Q 4. The most abundant granulocyte in human blood is:

a. Neutrophil b. Basophil
c. Eosinophil d. None of the above

Q 5. Which of the following organs is the most essential for proper immune maturation and functioning?

a. Spleen b. Liver
c. Thyroid d. Thymus

Q 6. WBCs squeeze through pores in capillary wall by:

a. Chemotaxis b. Diapedesis
c. Pinocytosis d. Opsonization

Q 7. Which of the following is not phagocytes?

a. Dust cells b. Eosinophils
c. Microglia d. Plasma cells

Q 8. Regarding granulopoiesis, which statement is not *true*?

a. Duration is 2 weeks
b. Occurs exclusively in red bone marrow
c. Wholly extravascular process
d. Dead granulocytes play an important role in its regulation

Q 9. Which of the following does not belong to white blood cells category?

a. Eosinophil b. Basophil
c. Histiocyte d. Neutrophil

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