Diffrential Leucocyte count

Laboratory Department
General Hospital, Meshana
Differential leucocyte count is done to know relative proportion of different WBC’s. It is expressed in terms of percentage of each type of leucocyte.

**Specimen :**
Capillary blood by pinprick or venous blood in EDTA bulb.

**Equipments :**

1. **Slide :** Slides must be chemically clean and free of any grease. They can be cleaned by 95% alcohol, dried with clean linen and warmed on flame.

2. **Spreader :** Spreader may be a glass slide. It should have absolutely smooth spreading edge which is narrower than the slide for preparation of blood smear.

3. **Stains :** The smears can be stained by various staining methods:-
**Romanowsky stains** are universally employed for staining blood film.

The remarkable property of Romanowsky dyes of making subtle distinction in shades of staining and staining granules differentially depend on two components namely azure B and Eosin Y.

Some of the Romanowsky stains used are:

1) Leishman stain
2) Alternatively, Field’s stain containing polychromed methylene blue and eosin is used.
3) Giemsa stain
4) Wright stain
(a) Leishman’s staining technique:

- Flood the smear with Leishman’s stain and leave it for 2 minutes. During this time, the stain must not dry on the smear otherwise it will precipitate.
- Using Pasteur pipette add double the quantity of either Sorenson’s buffer (pH 6.8) or distilled water taking care that it does not overflow from the slide. It should be thoroughly mixed with stain so that a shining scum forms on the smear surface.
- Allow the diluted stain to remain on the slide for 10 minutes.
- Wash the slide in running tap water.
- Remove any stain on the other side of the slide using cotton wool.
- Dry the smear in upright position.
Dye solution

Buffer

Buffer

wash

[Image of a tissue sample]
Criteria of good staining:

• A well stained smear is rose pink in colour to the naked eye.

• It is free from any precipitate stain.

• The red cells should stain pink, nuclei of leucocytes should be purple, granules of eosinophils, neutrophils and basophils should be red, pink and blue respectively.
Field’s stain provides better staining of thick smears. It can also be used for staining the slides routinely as it is a rapid procedure.

Method of staining:
Step 1: Fix the smear with methanol for 10 – 15 seconds.
Step 2: Allow it to air dry.
Step 3: Dip the smear 3 – 4 times in stain B (eosin) and immediately rinse in tap water.
Step 4: Dip the smear in stain A (polychromed methylene blue) for 6 – 8 dips.
Step 5: Wash the slide with tap water.
Step 6: Allow it to air dry.
Step 7: See under low power magnification of the microscope for distribution of cells and scrutinise under oil immersion lens.
Examination of peripheral blood smear:

1) Examine the stained smear under low power to make sure that distribution of cells and staining are satisfactory.

2) For differential count: The different types of leucocytes are examined from head to tail under oil immersion lens.

3) Record each type of leucocyte by means of a cell counter (or on paper) till at least 100 leucocytes are counted.
Neutrophils are 10-15 μm in diameter. The nucleus of mature cells will have 2-4 lobes.
Eosinophils are slightly larger than neutrophils (12-17 μm). They usually have a bilobed nucleus and many eosinophilic (orange) cytoplasmic granules. The granules are membrane-bound organelles.
Monocytes are large (12-20 μm in diameter), usually have a horseshoe-shaped nucleus. The grey-blue cytoplasm may contain faint azurophilic granules and be vacuolated.
The small lymphocyte is agranular and has a high N/C ratio.
Lymphocytes

- Large granular lymphocytes have a lower N:C ratio and contain azurophilic granules.
Basophils

- **Basophils** (10-14 μm) are characterised by purple-black granules.
Eosinophil & basophil
4. Arneth Count:

It is based on nuclear indices. Polymorphonuclear leucocytes show variations in degree of lobulation depending upon the age and maturation of the cells. Number of the segmentation is directly proportional to the age of the cell. In mature cells there are three to four nuclear lobes and at times five.
<table>
<thead>
<tr>
<th>Class</th>
<th>characteristics</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>One lobe ( band cell &amp; Metamyelocyte )</td>
<td>10-12%</td>
</tr>
<tr>
<td>II</td>
<td>Two lobes jointed by thin filament</td>
<td>2-5%</td>
</tr>
<tr>
<td>III</td>
<td>Three lobes</td>
<td>15-19%</td>
</tr>
<tr>
<td>IV</td>
<td>Four lobes</td>
<td>15-16%</td>
</tr>
<tr>
<td>V</td>
<td>Five lobes</td>
<td>2-3%</td>
</tr>
</tbody>
</table>
Total 100 leucocytes counted

a) Normal index I + II + III = 60%

b) Increase in class I and II: Shift to left, indicates infection. It is also found in leukemia.

c) Increase in multilobed forms occurs in pernicious/megaloblastic anemia. (Hypersegmented neutrophils more than 5%) which is called shift to right.
Absolute leucocyte count =

Percentage of leucocyte X
Total leucocyte count/ml
Abnormal values for differentiated leucocytes:

A. **Leucocytosis**: It is an increase in total number of leucocytes above 11,000/cmm of blood.

   The term leucocytosis is interchangibly used with reference to increase in Neutrophils since for the increase of other types of leucocytes there are respective names.

I. **Leucocytosis**:-

   (Neutrophilic leucocytosis = Absolute neutrophil count > 7500/µl)

   #Infection:
   - Pyogenic infection e.g. Bacterial staphylococcal, streptococcal (like appendicitis, otitis media, Infected burns, salpingitis, etc).
   #Fungal infection.
   #Viral infection (lymphocytic leukocytosis could be present)
(b) Inflammatory:
- Rheumatic fever
- Ischemic Necrosis of heart, Crohn’s disease
- Collagen disease

(c) Haematological:
- Chronic Myeloid leukemia,
- Leukemoid reactions, sometimes acute leukemias

(d) Physiological: Heat, exercise, pain.

(e) Malignant neoplasia – Myeloproliferative disease.

(f) Others: Diabetic ketoacidosis
- Metabolic disorder

(g) Severe hemorrhage
II. Leucopenia:-

Leucopenia refers to decreased leucocyte count below 4000/cmm.

**Causes:**

- Enteric fever
- Influenza, measles
- Kala azar
- Overwhelming infection
- X-ray, irradiation, arsenic poisoning, anti-cancer drugs.
- Drug induced – chloramphenicol
- Myeloid hypoplasia, aplastic anemia
- Nutritional deficiency – Malnutrition
  - Starvation
  - Cachexia
    - Debility

Acute leukemia in some cases.
B. Eosinophilia: (Eosinophilic leucocytosis = absolute eosinophilic count > 600/µl)

Parasitic infection:
Intestinal helminthiasis, filariasis, guineaworm infestation.

Allergic disease:
Asthma, hay fever, urticaria, pulmonary hypersensitivity syndrome,
Loffler’s syndrome

Skin diseases:
Scabies, eczema, exfoliative dermatitis, pemphigus etc.

Tropical Eosinophilia
Haematological:
Chronic myeloid leukemias.
Hodgkin’s lymphoma
Eoisinophilic leukemia.

**Eosinopenia**: Acute stress.
Acute inflammatory state.
C. Basophilia: (Basophilic leucocytosis):

Chronic Myeloid leukemias.
Allergic condition.
Polycythemia vera.
Hemolytic anemia.
Hypothyroidism.
Following splenectomy.

Basopenia:
Acute stress.
D. **Monocytosis**: (Monocytic leucocytosis = absolute monocyte count > 1000/µl)

Preleukemia, AML, HL, and NHL

Bacterial endocarditis.

Mycotic, rickettsial, protozoal infection.

In TB: suggestive of poor prognosis.

Recovery of acute infection.

Autoimmune diseases – Myositis, ulcerative colitis, SLE.

**Monocytopenia** :- Prednisolone therapy.

Hairy cell leukemia.
E. Lymphocytosis: (Lymphocytic leucocytosis = absolute lymphocyte count > 4000/µl in adults; > 7200/µl in adolescent; >9000/µl in children and infants)

Viral infection: Epstein barr virus
   Cytomegalovirus, mumps, herpes,
   Infectious mononucleosis, Infective hepatitis.

Bacterial: Typhoid, leucopenia associated with lymphocytosis (Relative lymphocytosis)
   Tuberculosis
   Rickettsial infection
   Toxoplasmosis

Normal in infancy

Lymphocytic leukemia-CLL
Lymphocytopenia:

Congnital immunodeficiency syndrome.

Acquired immunodeficiency syndromes.

e.g. AIDS
Hodgkin’s lymphoma
Chemotherapeutic drugs.
Myeloma
Prothrombin time

- Indicator of common and extrinsic coag. Pathways.
- Thromboplastin is added to plasma in the presence of calcium and the time taken for clotting of plasma is noted.
- Used for monitoring of oral anticoagulant therapy.
- Reagents: 1) citrated test plasma
  2) citrated control plasma of normal person
  3) thromboplastin reagent & calcium chloride
  4) water bath at 37°, stopwatch or coagulometer.
Method:

- Place all reagents and test tubes at 37 C for 15 min.
- Deliver 100 ul of plasma into one test tube and add 100 ul of thromboplastin reagent into it and keep in 37 C incubation.
- After 1 min add 100 ul of ca. chloride sol to the tube and start stop watch and gently tilt the tube while submerging lower end in waterbath.
- As soon as fibrin strands appear, record time.
- Also run control plasma in the same manner.
Reporting

- Normal range: 11-16 seconds
- PT - patient: 16 seconds
- control: 13 seconds
- INR (International Normalized Ratio) = ratio of the pt. PT compared to mean PT normal raised to the power of the ISI.
- INR is used to monitor the effectiveness of warfarin and INR should be 2-3.
- Prolonged in liver dis. Obstructive jaundice, HDN, DIC, Coumarin derivatives.
Thank You