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Blood Report Analysis-A Review

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Abstract

To determine your general health status; to screen for, diagnose, or monitor any one of a variety of diseases and conditions that affect blood cells, such as anemia, infection, inflammation, bleeding disorder or cancer. The complete blood count (CBC) is a group of tests that evaluate the cells that circulate in blood, including red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs). The CBC can evaluate your overall health and detect a variety of diseases and conditions, such as infections, anemia and leukemia. Blood cells are produced and mature primarily in the bone marrow and, under normal circumstances, are released into the bloodstream as needed.

Keywords: WBC (White blood cells), CBC (Complete blood count), MCV (Mean corpuscular volume).

Introduction

Blood cells disorders can be classified as quantitative or qualitative. In quantitative alterations all cells appear normal but are present in abnormal quantities, either in excess or in defect of normal values, however, in qualitative defects, abnormal appearance, abnormal function of the cells, or extrinsic cells are found in circulation[4]. The Literature reveals that as much as 70% of clinical decisions and diagnoses are supported by laboratory medicine clinical information obtained from laboratory tests, which play a key role in the diagnosis and management of patients[5].

1. History

The first scientists such as Athanasius Kircher in 1657 described “worms” in the blood, and Anton van Leeuwenhoek in 1674 gave an account of RBCs [6], but it was not until the late 1800s that Giulio Bizzozero described platelets as “petites plaques” [7]. The development of Wright stain by James Homer Wright in 1902 opened a new world of visual blood film examination through the microscope. Although automated instruments now differentiate and enumerate blood cells, Wright’s Romanowsky-type stain (polychromatic, a mixture of acidic and basic dyes), and refinements thereof, remains the foundation of blood cell identification [8]. In the present-day hematology laboratory, RBC, WBC, and platelet appearance is analyzed through automation or visually using 5003 to 10003 light microscopy examination of cells fixed to a glass microscope slide and

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stained with Wright or Wright-Giemsa stain. The scientific term for cell appearance is morphology, which encompasses cell color, size, shape, cytoplasm inclusions, and nuclear condensation [9].

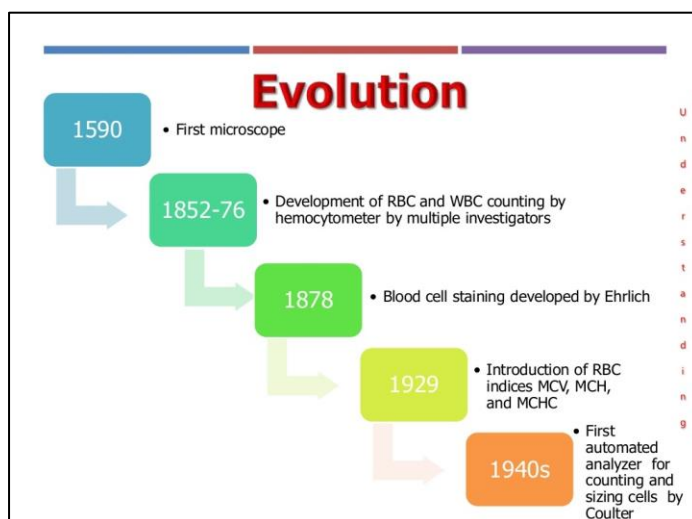


Figure1.1: evolution of microscope as well as study of blood

2. What is Complete Blood Count (CBC)/ Hemogram?

A complete blood count, or CBC, is an easy and very common test that screens for certain disorders that can affect your health. A CBC determines if there are any increases or decreases in your blood cell counts. Normal values vary depending on your age and your gender. Your lab report will tell you the normal value range for your age and gender [1].

3. How Sample is Collected?

During a CBC, a lab technician will draw blood from a vein, typically from the inside of your elbow or from the back of your hand. The test will take only a few minutes. The technician:

1. cleans your skin with an antiseptic wipe
2. places an elastic band, or tourniquet, around your upper arm to help the vein swell with blood
3. inserts a needle in the your and collects a blood sample in one or more vials
4. removes the elastic band
5. covers the area with a bandage to stop any bleeding
6. Label your sample and send it to a lab for analysis

A blood test can be slightly uncomfortable. When the needle punctures your skin, you might feel a prick or pinching sensation. Some people also feel faint or light-headed when they see blood. Afterwards, you may have minor bruising, but it will clear up within a few days[2].

In young infants, a nurse will typically sterilize the heel of the foot and use a small needle called a lancet to prick the area. The nurse will then gently squeeze the heel and collect a small amount of blood in a vial for testing [1].

4. It Assesses/Uses

A CBC is not a definitive diagnostic test. Blood cell counts that are too high or too low could signal a wide variety of conditions. Specialized tests are needed to diagnose a specific condition. Conditions that could cause an abnormal CBC and may require additional testing include:

- Anemia of various etiologies
- Autoimmune disorders
- Bone marrow disorders
- Dehydration
- Infections

- Inflammation
- Hemoglobin abnormalities
- Leukemia
- Low platelets
- Lymphoma
- Myeloproliferative neoplasms
- Myelodysplastic syndrome
- Sickle cell disease
- Thalassemia
- Nutritional deficiencies (e.g., Iron, B12 or folate)
- Cancer that has spread to the bone marrow

If your CBC shows abnormal levels, your doctor may order another blood test to confirm results. They may also order other tests to help further evaluate your condition and confirm a diagnosis [3].

5. Why CBC?



Figure1.2: several aspect of CBC

A healthcare practitioner typically evaluates and interprets results from the components of the CBC together. Depending on the purpose of the test, a number of additional or follow-up tests may be ordered for further investigation [1,9].

6. What are the various parameters of CBC?

A CBC is typically performed using an automated instrument that measures various parameters, including cell counts and the physical features of some of the cells. A standard CBC includes:

1. Red blood cell (RBC) tests: Red blood cell (RBC) count is a count of the actual number of red blood cells in your blood sample.
2. Hemoglobin measures the total amount of the oxygen-carrying protein in the blood, which generally reflects the number of red blood cells in the blood.
3. Hematocrit measures the percentage of your total blood volume that consists of red blood cells.
4. Red blood cell indices provide information on the physical features of the RBCs:
5. Mean corpuscular volume (MCV) is a measurement of the average size of your red blood cells.

6. Mean corpuscular hemoglobin (MCH) is a calculated measurement of the average amount of hemoglobin inside your red blood cells.
7. Mean corpuscular hemoglobin concentration (MCHC) is a calculated measurement of the average concentration of hemoglobin inside your red blood cells.
8. Red cell distribution width (RDW) is a measurement of the variation in the size of your red blood cells.
9. The CBC may also include reticulocyte count, which is a measurement of the absolute count or percentage of newly released young red blood cells in your blood sample.
10. White blood cell (WBC) tests: White blood cell (WBC) count is a count of the total number of white blood cells in your blood sample.
11. White blood cell differential may be included as part of the CBC or may be done in follow up if the WBC count is high or low. The WBC differential identifies and counts the number of the five types of white blood cells present (neutrophils, lymphocytes, monocytes, eosinophils, and basophils). The individual count can be reported as an absolute count and/or as a percentage of total.
12. Platelet tests: The platelet count is the number of platelets in your blood sample.
13. Mean platelet volume (MPV) may be reported with a CBC. It is a measurement of the average size of platelets.
14. Platelet distribution width (PDW) may also be reported with a CBC. It reflects how uniform platelets are in size.

CBC results that are outside the established reference intervals may indicate the presence of one or more diseases or conditions. Typically, other tests are performed to help determine the cause of abnormal results. Often, a blood smear will be examined using a microscope. A trained laboratory professional will evaluate the appearance and physical features of the blood cells, such as size, shape and color, noting any abnormalities that may be present. This information gives the healthcare practitioner additional clues as to the cause of abnormal CBC results [10,1,2,3].

MEHRA HOSPITAL
Dr. Ajay K. Mehra & Dr. Surbhi Mehra

Patient Name : Mrs. RANJITA ROY (MRN-16080424)
Age / Gender : 35 years / Female
Address : MAJRI, CHANDAPUR Maharashtra
Requesting Doctor : Dr. AJAY K. MEHRA

Request Date : 16-08-2016 05:10 PM
Collection Date : 16-08-2016 05:34 PM [HA1462]
Acceptance Date : 16-08-2016 05:34 PM | TAT: 00:54 [04 MM]

HAEMATOLOGY
Reporting Date : 16-08-2016 06:28 PM

Investigations	Result	Biological Reference Range
CBC		
HB	11.1 gms%	11.80 - 14.30
RBC	3.99 mill/ul	4.50 - 6.00
TLC	5100 /cu.mm	4,000 - 10,000
HCT	33.8 (%)	36.00 - 45.00
MCV	84.7 (fl)	75.00 - 98.00
MCH	27.8 (pg)	25.00 - 34.00
MCHC	32.8 (g/dl)	30.00 - 36.00
RDW	13.8% (cv)	11.00 - 15.00
Platelets	1.74 (Lacs/cu.mm)	1.50 - 4.00
PDW	18.1 (fl)	7.00 - 17.00
MPV	12.3 (fl)	6.00 - 13.00
PLCR	32.8	5.00 - 22.00
DLC		
Polymorphs	52 %	40.00 - 75.00
Lymphocytes	36 %	20.00 - 45.00
Eosinophils	12 % *	1.00 - 6.00

Peripheral Blood Film :
RBCs are Predominantly Normocytic Mild Hypochromic Mild Anisocytosis & Poikilocytosis.
TLC is normal. DLC shows Polymorphonuclear Predominance & Mild Eosinophilia (12 %)
Platelets Adequate. No Immature cells.No Parasites seen.
PS Suggestive of Normocytic Hypochromic Anaemia.

END OF REPORT.

Prepared and Checked by

Pathologist
Dr. Pramod Bangde
M.D. (Path)

Figure 1.3: Patient report

i.RBC

Red blood cells, also called erythrocytes, are produced in the bone marrow and released into the bloodstream when they mature. They contain hemoglobin, a protein that transports oxygen throughout the body [11]. The typical lifespan of an RBC is 120 days. Thus, the bone marrow must continually produce new RBCs to replace those that age and degrade or are lost through bleeding. A number of conditions can affect the production of new RBCs and/or their lifespan, in addition to those conditions that may result in significant bleeding. RBCs normally are uniform in size and shape, but their appearance can be affected by a variety of conditions, such as vitamin B12 and folate deficiencies and iron deficiency[12,13]. An example of a common condition affecting RBCs is anemia, which results from low red blood cell counts and low hemoglobin. Various diseases can lead to anemia, so additional tests are often needed to determine the cause[11,12,5].

Test	Reference Range	Example of Causes of Low Result	Example of Cause of High Result
Red Blood Cell Count (RBC)	<i>Conventional Units</i> Men: 4.5-5.9 x10 /microliter Women: 4.1-5.1x 10 microliter <i>SI Units</i> Men: 4.5-5.9 x 10 /L Women: 4.1-5.1x 10 /L	Known as anemia <ul style="list-style-type: none"> ● Acute or chronic bleeding ● RBC destruction (e.g., hemolytic anemia, etc.) ● Nutritional deficiency (e.g., iron deficiency, vitamin B12 or folate deficiency) ● Bone marrow disorders or damage ● Chronic inflammatory disease ● Chronic kidney disease 	Known as polycythemia <ul style="list-style-type: none"> ● Dehydration Lung (pulmonary) disease ● Kidney or other tumor that produces excess erythropoietin ● Smoking ● Living at high altitude ● Genetic causes (altered oxygen sensing, abnormality in hemoglobin oxygen release) ● Polycythemia vera—a rare disease
Hemoglobin (Hb)	<i>Conventional Units</i> Men: 14-17.5 g/dL Women: 12.3-15.3 g/dL <i>SI Units</i> Men: 140-175g/L Women: 123-153g/L	Usually mirrors RBC results, provides added information	Usually mirrors RBC results
Hematocrit (Hct)	<i>Conventional Units</i> Men: 41.5-50.4% Women: 35.9-44.6% <i>SI Units</i> Men: 0.415- 0.504 volume fraction Women: 0.359- 0.446 volume fraction	Usually mirrors RBC results	Usually mirrors RBC results; most common cause is dehydration
(MCV)Mean corpuscular volume	<i>Conventional Units</i> 80-96 micrometer <i>SI Units</i> 80-96 fL	Indicates RBCs are smaller than normal (microcytic); caused by iron deficiency anemia or thalassemias, for example.	Indicates RBCs are larger than normal (macrocytic), for example in anemia caused by vitamin B12 or folate deficiency, myelodysplasia, liver disease, hypothyroidism, etc.

(MCH) Mean corpuscular hemoglobine	<i>Conventional Units</i> 27.5-33.2 pg <i>SI Units</i> 27.5-33.2 pg	Mirrors MCV results; small red cells would have a lower value.	Mirrors MCV results; macrocytic RBCs are large so tend to have a higher MCH.
(MCHC) Mean corpuscular hemoglobine concentration	<i>Conventional Units</i> 33.4-35.5 g/dL <i>SI Units</i> 334-355 g/L	May be low when MCV is low; decreased MCHC values (hypochromia) are seen in conditions such as iron deficiency anemia and thalassemia.	Increased MCHC values (hyperchromia) are seen in conditions where the hemoglobin is more concentrated inside the red cells, such as autoimmune hemolytic anemia, in burn patients, and hereditary spherocytosis, a rare congenital disorder.
RBC Distribution Width (RDW, RDW-SD, RDW-CV)		Indicates that RBC are uniform in size.	Indicates mixed population of small and large RBCs; young RBCs tend to be larger. For example, in iron deficiency anemia or pernicious anemia, there is high variation (anisocytosis) in RBC size (along with variation in shape – poikilocytosis), causing an increase in the RDW
Reticulocyte Count	<i>Conventional Units</i> 0.5-1.5% or 25-125 x 10 /microliter <i>SI Units</i> 0.005-0.015 number fraction or 25-125 x 10 /L	In the setting of anemia, a low reticulocyte count indicates a condition is affecting the production of red blood cells, such as bone marrow disorder or damage, or a nutritional deficiency (iron, B12 or folate).	In the setting of anemia, a high reticulocyte count generally indicates peripheral cause, such as bleeding or hemolysis, or response to treatment (e.g., iron supplementation for iron deficiency anemia).

ii. WBC

White blood cells, also called leukocytes, are cells that exist in the blood, the lymphatic system, and tissues and are an important part of the body's natural defense (immune) system. They help protect against infections and also have a role in inflammation, and allergic reactions. There are five different types of WBCs and each has a different function. They include neutrophils, lymphocytes, basophils, eosinophils, and monocytes [16].

WBCs are present in the blood at relatively stable numbers. However, these numbers may temporarily shift higher or lower depending on what is going on in the body. For instance, an infection can stimulate your bone marrow to produce a higher number of neutrophils to fight off a bacterial infection. With allergies, there may

be an increased number of eosinophils. An increased number of lymphocytes may be produced with a viral infection. In certain diseases, such as leukemia, abnormal (immature or mature) white cells may rapidly multiply. For additional details, see the articles White Blood Cell Count and WBC Differential [13,14,15,1].

Test	Reference Range	Examples of Causes of a Low Count	Examples of Causes of a High Count
White Blood Cell Count (WBC)	<i>Conventional Units</i> 4,500-11,000 white blood cells per microliter (mcL) <i>SI Units</i> 4.5-11.0 x 10 per liter (L)	Known as leukopenia <ul style="list-style-type: none"> ● Bone marrow disorders or damage ● Autoimmune conditions ● Severe infections (sepsis) ● Lymphoma or other cancer that spread to the bone marrow ● Dietary deficiencies ● Diseases of immune system (e.g., HIV/AIDS) 	Known as leukocytosis <ul style="list-style-type: none"> ● Infection, most commonly bacterial or viral Inflammation ● Leukemia, myeloproliferative neoplasms ● Allergies, asthma ● Tissue death (trauma, burns, heart attack) Intense exercise or severe stress
Absolute neutrophil count, % neutrophils (Neu, PMN, polys)	<i>Conventional Units</i> Percent (mean): 56% Absolute count (per microliter): 1800-7800 <i>SI Units</i> Mean number fraction: 0.56 Absolute count X 10 per liter: 1.8-7.8	Known as neutropenia <ul style="list-style-type: none"> ● Severe, overwhelming infection (sepsis) ● Autoimmune disorders ● Dietary deficiencies ● Reaction to drugs ● Immunodeficiency ● Myelodysplasia ● Bone marrow damage (e.g., chemotherapy, radiation therapy) ● Cancer that spreads to the bone marrow ● Congenital neutropenia 	Known as neutrophilia <ul style="list-style-type: none"> ● Acute bacterial infections Inflammation ● Trauma, heart attack, or burns ● Stress, rigorous exercise ● Certain leukemias (e.g., chronic myeloid leukemia) ● Cushing syndrome
Absolute lymphocyte count, % lymphocytes (Lymph)	<i>Conventional Units</i> Percent (mean) 34% Absolute count (per microliter): 1000-4800 <i>SI Units</i> Mean number fraction: 0.34 Absolute count X 10 per liter: 1.0-4.8	Known as lymphocytopenia <ul style="list-style-type: none"> ● Autoimmune disorders (e.g., lupus, rheumatoid arthritis) ● Infections (e.g., HIV, viral hepatitis, typhoid fever, influenza, Covid-19) ● Bone marrow damage (e.g., chemotherapy, radiation therapy) ● Corticosteroids 	Known as lymphocytosis <ul style="list-style-type: none"> ● Acute viral infections (e.g., chicken pox, cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes, rubella) ● Certain bacterial infections (e.g., pertussis (whooping cough), tuberculosis (TB)) ● Toxoplasmosis ● Chronic inflammatory disorder (e.g., ulcerative colitis) ● Lymphocytic leukemia, lymphoma ● Stress (acute)

<p>Absolute monocyte count, % monocytes (Mono)</p>	<p><i>Conventional Units</i> Percent (mean) 4% Absolute count (per microliter) 0-800 <i>SI Units</i> Mean number fraction 0.04 Absolute count X 10 per liter 0-0.80</p>	<p>Usually, one low count is not medically significant. Repeated low counts can indicate:</p> <ul style="list-style-type: none"> ● Bone marrow damage or failure ● Hairy cell leukemia ● Aplastic anemia 	<ul style="list-style-type: none"> ● Chronic infections (e.g., tuberculosis, fungal infection) ● Infection within the heart (bacterial endocarditis) ● Collagen vascular diseases (e.g., lupus, scleroderma, rheumatoid arthritis, vasculitis) ● Monocytic or myelomonocytic leukemia (acute or chronic)
<p>Absolute eosinophil count, % eosinophils (Eos)</p>	<p><i>Conventional Units</i> Percent (mean) 2.7% Absolute count (per microliter) 0-450 <i>SI Units</i> Mean number fraction 0.027 Absolute count X 10 per liter 0-0.45</p>	<p>Numbers are normally low in the blood. One or an occasional low number is usually not medically significant.</p>	<ul style="list-style-type: none"> ● Asthma, allergies such as hay fever ● Drug reactions ● Parasitic infections ● Inflammatory disorders (celiac disease, inflammatory bowel disease) ● Some cancers, certain acute or chronic leukemias or lymphomas ● Addison disease ● Connective tissue disorders
<p>Absolute basophil count, % basophils (Baso)</p>	<p><i>Conventional Units</i> Percent (mean) 0.3% Absolute count (per microliter) 0-200 <i>SI Units</i> Mean number fraction 0.030 Absolute count X 10 per liter 0-0.20</p>	<p>As with eosinophils, numbers are normally low in the blood; usually not medically significant</p>	<ul style="list-style-type: none"> ● Rare allergic reactions (hives, food allergy) ● Inflammation (rheumatoid arthritis, ulcerative colitis) ● Some leukemias Uremia

iii. Platelets

Platelets, also called thrombocytes, are actually tiny cell fragments that circulate in blood and are essential for normal blood clotting. When there is an injury and bleeding begins, platelets help stop bleeding by adhering to the injury site and clumping together to form a temporary plug. They also release chemical signals that attract and promote clumping of additional platelets and eventually become part of a stable blood clot at the site of the injury that remains in place until the injury heals [17,14].

If you have a disease or condition that causes low platelets (thrombocytopenia) or dysfunction of platelets, you may be at an increased risk of excessive bleeding and bruising. An excess of platelets (thrombocytosis) can cause excessive clotting. For more information, see the article Platelet Count [18,16,15].

Test	Reference Range	Examples of Causes of Low Result	Examples of Causes of High Result
Platelet Count (Plt)	<i>Conventional Units</i> 150-450 x 10 /microliter <i>SI Units</i> 150-450 x 10 /L	Known as thrombocytopenia: <ul style="list-style-type: none"> ● Viral infection (mononucleosis, measles, hepatitis) ● Rocky mountain spotted fever ● Platelet autoantibody ● Drugs (acetaminophen, quinidine, sulfa drugs) Cirrhosis <ul style="list-style-type: none"> ● Autoimmune disorders (e.g., ITP) Sepsis <ul style="list-style-type: none"> ● Leukemia, lymphoma Myelodysplasia <ul style="list-style-type: none"> ● Chemo or radiation therapy 	Known as thrombocytosis: <ul style="list-style-type: none"> ● Cancer (lung, gastrointestinal, breast, ovarian, lymphoma) ● Rheumatoid arthritis, inflammatory bowel disease, lupus Iron deficiency anemia <ul style="list-style-type: none"> ● Hemolytic anemia ● Myeloproliferative disorder (e.g., essential thrombocythemia)
(MPV) Mean Platelet Volume		Indicates average size of platelets is small; older platelets are generally smaller than young ones and a low MPV may mean that a condition is affecting the production of platelets by the bone marrow.	Indicates a high number of larger, younger platelets in the blood; this may be due to the bone marrow producing and releasing platelets rapidly into circulation.
Platelet Distribution Width (PDW)		Indicates uniformity in size of platelets.	Indicates increased variation in the size of the platelets, which may mean that a condition is present that is affecting platelets.

7. Methodology

Clinical laboratory hematology has evolved from simple observation and description of blood and its components to a highly automated, extremely technical science, including examination at the molecular level. However, some of the more basic tests have not changed dramatically over the years [10].

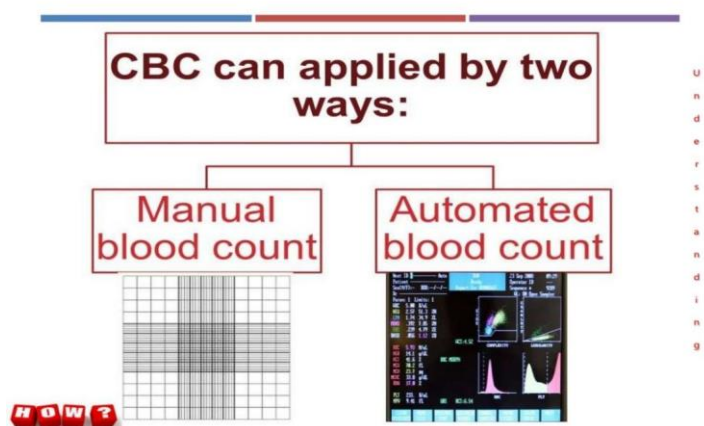


Figure 1.4: Difference between automated and manual blood count

A. Manual Method

Manual cell counts are performed using a hemacytometer, or counting chamber, and manual dilutions made with calibrated, automated pipettes and diluents (commercially available or laboratory prepared). The principle for the performance of cell counts is essentially the same for white blood cells (WBCs), red blood cells (RBCs), and platelets; only the dilution, diluting fluid, and area counted vary [10, 19].

i. RBC count

The most common one is the Levy chamber with improved Neubauer ruling. It is composed of two raised surfaces, each with a 3 mm × 3 mm square counting area or grid (total area 9 mm²), separated by an H-shaped moat. As shown in Figure 1.5, this grid is made up of nine 1 mm × 1 mm squares. Each of the four corner (WBC) squares is subdivided further into 16 squares, and the center square subdivided into 25 smaller squares. Each of these smallest squares is 0.2 mm × 0.2 mm which is 1/25 of the center square or 0.04 mm²[10].

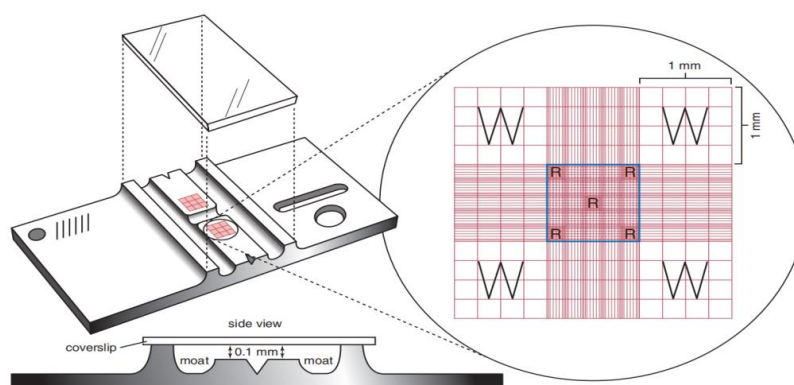


Figure 1.5: Hemacytometer

Calculation: The general formula for manual cell counts is as follows and can be used to calculate any type of cell count:

$$\text{Total count} = \frac{\text{cells counted} \times \text{dilution factor}}{\text{area (mm}^2\text{)} \times \text{depth (0.1)}}$$

Or

$$\text{Total count} = \frac{\text{cells counted} \times \text{dilution factor} \times 10^*}{\text{area (mm}^2\text{)}}$$

The calculation yields the number of cells per mm³. One mm³ is equivalent to one microliter (mL). The count per mL is converted to the count per liter (L) by multiplying by a factor of 10[20].

ii. MCH Count

The MCH is the average weight of hemoglobin in a red blood cell, expressed in picograms (pg), or 10⁻¹² g. For example, if the hemoglobin = 16 g/dL and the RBC count = 5 × 10⁻¹², the MCH = 32 pg. The reference interval for adults is 26 to 32 pg. The MCH generally is not considered in the classification of anemias [10,24].

Calculation

$$\text{MCH} = \frac{\text{HGB (g/dL)} \times 10}{\text{RBC count} (\times 10^{12}/\text{L})}$$

iii. MCV Count

The MCV is the average volume of the red blood cell, expressed in femtoliters (fL), or 10^{-12} L. For example, if the HCT is 45% and the RBC count is $5 \times 10^{12}/L$, the MCV = 90 fL. The reference interval for MCV is 80 to 100 fL. RBCs with an MCV of less than 80 fL are microcytic; those with an MCV of more than 100 fL are macrocytic [24].

Calculation:

$$\text{MCV} = \frac{\text{HCT (\%)} \times 10}{\text{RBC count} (\times 10^{12}/L)}$$

iv. MCHC

The MCHC is the average concentration of hemoglobin in each individual red blood cell. The units used are grams per deciliter (formerly given as a percentage). For example, if the HGB = 16 g/dL and the HCT is 48%, the MCHC = 33.3 g/dL.

Values of normochromic red blood cells range from 32 to 36 g/dL; values of hypochromic cells are less than 32 g/dL, and values of “hyperchromic” cells are greater than 36 g/dL [10,26].

Calculation:

$$\text{MCHC} = \frac{\text{HGB (g/dL)} \times 100}{\text{HCT (\%)}}$$

v. ESR

The erythrocyte sedimentation rate (ESR) is ordered with other tests to detect and monitor the course of inflammatory conditions such as, rheumatoid arthritis, infections, or certain malignancies. It is also useful in the diagnosis of temporal arteritis and polymyalgia rheumatica [26].

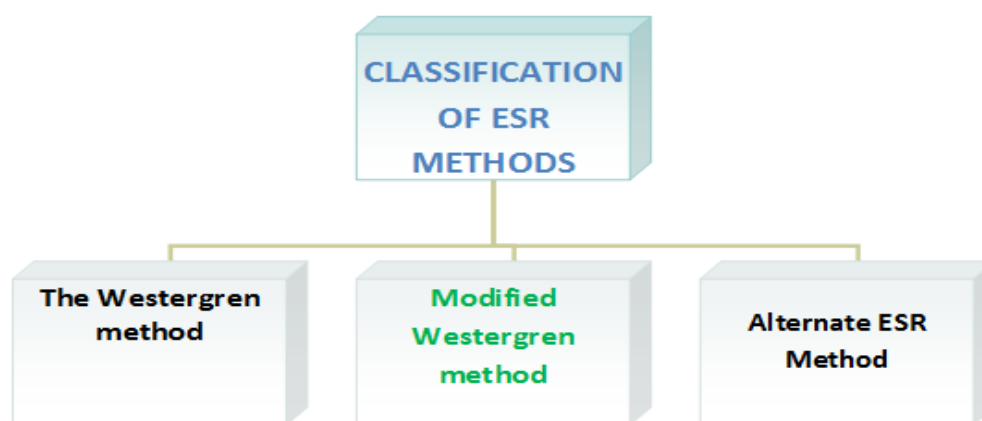


Figure 1.7: Classification of ESR methods

vi. WBC Count

The WBC or leukocyte count is the number of WBCs in 1 liter (L) or 1 microliter (mL) of blood. Whole blood anticoagulated with ethylenediaminetetraacetic acid (EDTA) or blood from a skin puncture is diluted with 1% buffered ammonium oxalate or a weak acid solution (3% acetic acid or 1% hydrochloric acid). The diluting fluid lyses the nonnucleated red blood cells in the sample to prevent their interference in the count. The typical dilution of blood for the WBC count is 1:20 [10,21].

Calculation: The average number of cells of the two sides of the chamber is 96. Using the average in the formula:

$$\begin{aligned} \text{WBC count} &= \frac{\text{cells counted} \times \text{dilution factor}}{\text{area counted (mm}^2\text{)} \times \text{depth}} \\ &= \frac{96 \times 20}{4 \times 0.1} \\ &= 4800/\text{mm}^3 \text{ or } 4800/\mu\text{L or} \\ &4.8 \times 10^3/\mu\text{L or } 4.8 \times 10^9/\text{L} \end{aligned}$$

Sources of Error:

1. The hemacytometer and coverslip should be cleaned properly before they are used. Dust and fingerprints may cause difficulty in distinguishing the cells.
2. The diluting fluid should be free of contaminants [21,22].

vii. Platelets Count

A platelet count is the number of platelets in 1 liter (L) or 1 microliter (mL) of whole blood. Platelets adhere to foreign objects and to each other, which makes them difficult to count. They also are small and can be confused easily with dirt or debris. In this procedure, whole blood, with EDTA as the anticoagulant, is diluted 1:100 with 1% ammonium oxalate to lyse the nonnucleated red blood cells [10,21,22].

Calculation: The platelet count is calculated by using one of the equations given earlier. Using the first equation as an example, if 200 platelets were counted in the entire center square [23].

$$\begin{aligned} &\frac{200 \times 100}{1 \times 0.1} \\ &= 200,000/\text{mm}^3 \text{ or } 200,000/\mu\text{L} \\ &\text{or } 200 \times 10^3/\mu\text{L or } 200 \times 10^9/\text{L} \end{aligned}$$

Sources of Error:

If fewer than 50 platelets are counted on each side, the procedure should be repeated by diluting the blood to 1:20. If more than 500 platelets are counted on each side, a 1:200 dilution should be made. The appropriate dilution factor should be used in calculating the results [22].

Cells Counted	Diluting Fluid	Dilution	Objective	Area Counted
White blood cells	1% ammonium oxalate	1:20	10×	4 mm ²
	or	1:100	10×	9 mm ²
	3% acetic acid			
Red blood cells	or			
	1% hydrochloric acid			
Red blood cells	Isotonic saline	1:100	40×	0.2 mm ² (5 small squares of center square)
Platelets	1% ammonium oxalate	1:100	40× phase	1 mm ²

Figure 1.6: Manual Cell Counts with Most Common Dilutions, Counting Areas

B. Automated Methods

Since the 1980s, automated blood cell analysis has virtually replaced manual hemoglobin, hematocrit, and cell counting, due to its greater accuracy and precision, with the possible exception of phase platelet counting in certain circumstances [10]. Hematology analyzers are marketed by multiple instrument manufacturers. These analyzers typically provide the eight standard hematology parameters (complete blood count [CBC]), plus a three-part, five-part, or six-part differential leukocyte count in less than 1 minute on 200 mL or less of whole blood. Automation allows more efficient workload management and more timely diagnosis and treatment of disease [27].

i. Microhematocrite Reader

The hematocrit is the volume of packed red blood cells that occupies a given volume of whole blood. This is often referred to as the *packed cell volume* (PCV). It is reported either as a percentage (e.g., 36%) or in liters per liter (0.36 L/L) [24,10].

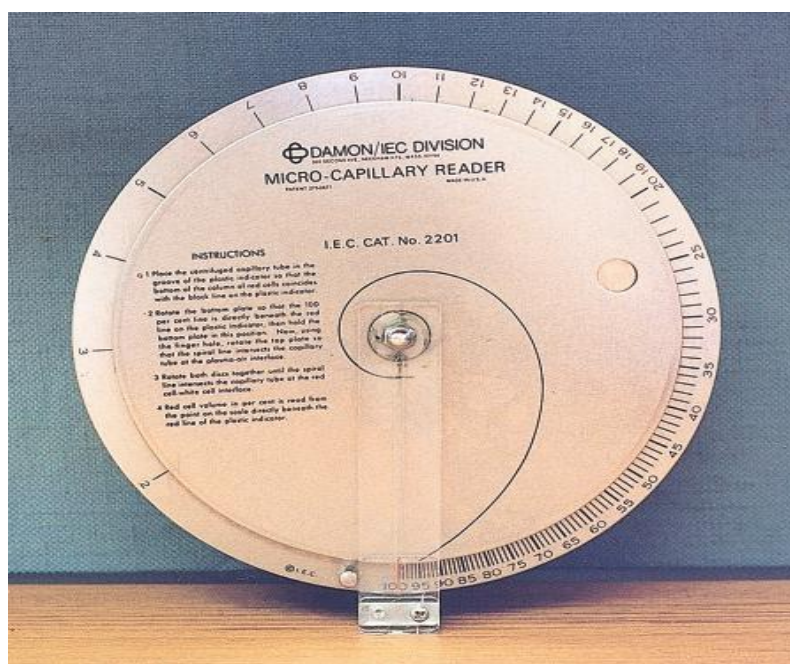


Figure 1.6: Microhematocrite reader

Sources of error:

1. An increased concentration of anticoagulant (short draw in an evacuated tube) decreases the hematocrit reading as a result of red blood cell shrinkage.
2. A decreased or increased result may occur if the specimen was not mixed properly[24].

ii. Centrifugal capillary tube

The time to obtain maximum packing of red blood cells should be determined for each centrifuge. Duplicate microhematocrit determinations should be made using fresh, well-mixed blood anticoagulated with ethylenediaminetetraacetic acid (EDTA). Two specimens should be used, with one of the specimens having a known hematocrit of 50% or higher. Starting at 2 minutes, centrifuge duplicates at 30-second intervals and record results. When the hematocrit has remained at the same value for two consecutive readings, optimum packing has been achieved, and the second time interval should be used for microhematocrit determinations [24].



Figure 1.7: Readcrit centrifuge with built-in capillary tube compartments and hematocrit scales. (Courtesy and © Becton, Dickinson and Company, Franklin Lakes, NJ.)

Sources of Error:

Many disorders, such as sickle cell anemia, macrocytic anemias, hypochromic anemias, spherocytosis, and thalassemia, may cause plasma to be trapped in the red blood cell layer even if the procedure is performed properly. The trapping of the plasma causes the microhematocrit to be 1% to 3% (0.01 to 0.03 L/L) higher than the value obtained using automated instruments that calculate or directly measure the hematocrit and are unaffected by the trapped plasma [23].

iii. Automated ESR

There are several automated ESR systems available using the traditional Westergren and Wintrobe methods, as well as alternate methods such as centrifugation. Automated ESR analyzer is the Sedimat 15 (Polymedco, Cortlandt Manor, NY), which uses the principle of infrared measurement. It is capable of testing one to eight samples randomly or simultaneously and provides results in 15 minutes [27].



Figure 1.8: Sedimat 15 (Polymedco) automated sedimentation rate system. (Courtesy Polymedco, Cortlandt Manor, NY.)

8. Result and Discussion

- In cold agglutinin disease, red cell agglutination is observed. In such situations, a clump of red cells may be counted as one red cell. Thus, the RBC count will be falsely low, and the MCV will be falsely elevated. However, when the red cells are lysed, a true hemoglobin result should be observed. Thus, a clue to cold agglutinin disease is disproportionate low RBC count compared to hemoglobin level. In cold agglutinin disease, MCHC should also be high. The lab uses abnormally high.
- MCHC as an indicator of possible cold agglutinin disease and warms the blood prior to repeating the CBC run on the analyzer[29].
- In patients with severe hyperglycemia (glucose > 600 mg/dL), osmotic swelling of RBCs may spuriously elevate the MCV.
- MCH is decreased in patients with anemia caused by impaired hemoglobin synthesis. The MCH may be falsely elevated in blood specimens with turbid plasma (usually caused by hyperlipidemia) or severe leukocytosis.
- RDW is elevated in iron deficiency anemia, myelodysplastic syndromes, and macrocytic anemia secondary to vitamin B12 or folate deficiency. In contrast, RDW is usually normal or only mildly elevated in thalassemia.
- MCHC is decreased in microcytic anemias in which the decrease in hemoglobin mass exceeds the decrease in the size of the RBC. It is increased in hereditary spherocytosis and in patients with hemoglobin variants, such as sickle cell disease and hemoglobin C disease.
- Pseudothrombocytopenia may be due to traumatic venipuncture and activation of clotting, a significant number of large platelets (platelets being counted as RBCs), EDTA-induced platelet clump, or EDTA-dependent platelet satellitism (in which platelets form a satellite ring (rosetting) around neutrophils).

9. Conclusion

The CBC is a basic, routine test that is not diagnostically specific in its individual values. It is the most commonly performed tests in health care due to the vast amount of data obtained through the various components. The CBC is a group of mostly interrelated tests that is meant to be examined as a whole and then correlated with the clinical picture. An infection can occur with a high WBC or a low one. Blood loss can present with or without a change in the Hgb and Hct. Blinding oneself to all that the CBC reveals, by narrowly focusing on only a few specific values, doesn't begin to use the wealth of information it actually contains [28]. The fluctuation in patient's report shown in figure 1.1 indicates that RBCs are predominantly normocytic mild anisocytosis and poikilocytosis suggesting normocytic hypochromic anemia. More simply patient is suffering from iron deficiency and thalassemia. The report highlights other components of blood such as adequate platelets count, WBC count is normal as well except eosinophils which is quite high pointing towards the clinical profile of patient already suffering from rheumatoid arthritis.

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