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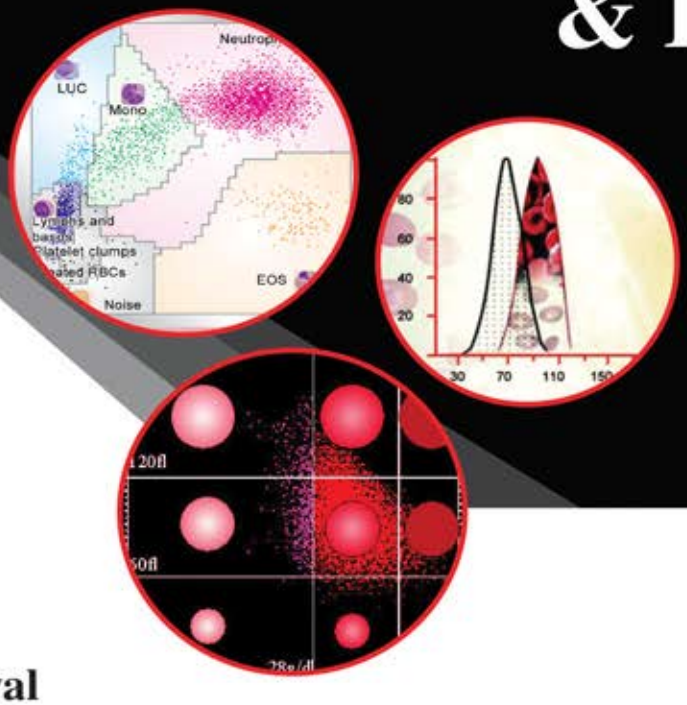
2nd Edition

DP Lokwani

The ABC of CBC

Interpretation of Complete Blood Count & Histograms



Foreword
MB Agarwal

JAYPEE

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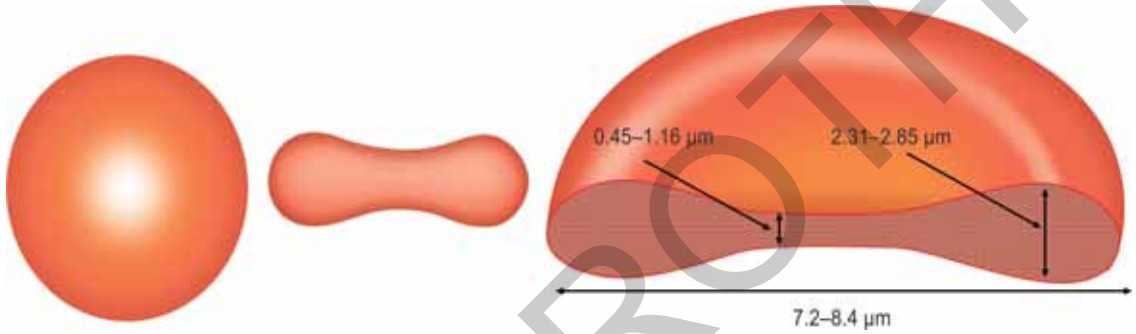
CHAPTER 2

Red Blood Cells

RED BLOOD CELLS



The diagram of red blood cell including cross section.

ERYTHROCYTE (RBC)

- RBCs are produced in the bone marrow with the help of metals (iron, cobalt, manganese), vitamins (B_{12}, B_6, C, E, folate, riboflavin, pantothenic acid, thiamine), and amino acids.
- Erythropoiesis is regulated by erythropoietin, thyroid hormone, and androgens.
- The biconcave erythrocyte is thinner in the middle, creating a central pallor on blood smears that is ordinarily less than 1/3rd of the cell's diameter. Such a cell, possessing the normal amount of hemoglobin, is called normochromic.
- The mature red blood cell (also known as an erythrocyte) carries oxygen attached to the iron present in hemoglobin.
- The mature red blood cell is an anucleate structure with no capacity to synthesize protein, yet it is capable of a limited metabolism, which enables it to survive for 120 days.
- Assessment of the RBC is to check for anemia and to evaluate normal erythropoiesis (the production of red blood cells).
- The number of red blood cells is determined by age, sex, altitude, exercise, diet, pollution, drug use, tobacco/nicotine use, kidney function, health and disease status, etc.
- The clinical importance of the test is that it is a measure of the oxygen carrying capacity of the blood.

Normal Red Blood Cells Values at Various Ages	
Newborn	: 4.1–6.1 million/mm^3
Children	: 3.6–5.5 million/mm^3
Adult	: (Males): 4.6–6.0 million/mm^3
	: (Females): 4.2–5.0 million/mm^3
Pregnancy	: Slightly lower than normal adult values

Broadly, the causes of decreased RBC count may be classified as:

- Impaired red blood cell (RBC) production
- Increased RBC destruction (hemolytic anemias)
- Blood loss
- Fluid overload (hemodilution).

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(I) Decreased RBC Count Due to Impaired Production

- **Disturbance of proliferation and differentiation of stem cells:**
  - Pure red cell aplasia
  - Aplastic anemia (affecting all kinds of blood cells)
  - Fanconi anemia (a hereditary disorder featuring aplastic anemia and other abnormalities)
  - Anemia of renal failure (insufficient erythropoietin production)
  - Anemia of endocrine disorders
- **Disturbance of proliferation and maturation of erythroblasts:**
  - Pernicious anemia (macrocytic anemia due to impaired absorption of vitamin B_{12})
  - Anemia of folic acid and vitamin B_{12} deficiency (megaloblastic anemia)
  - Anemia of prematurity (diminished erythropoietin response in premature infants)
  - Iron deficiency anemia (resulting in deficient heme synthesis)
  - Thalassemias (causing deficient globin synthesis)
  - Anemia of renal failure (erythropoietin deficiency)
- **Other mechanisms of impaired RBC production:**
  - Myelodysplastic syndrome
  - Anemia of chronic inflammation
  - Myelophthisic anemia or Myelophthisis (a severe type of anemia resulting from the replacement of bone marrow by malignant tumors or granulomas)

(II) Decreased RBC Count Due to Increased Destruction: Hemolytic Disorders

- **Intrinsic (intracorpuscular) abnormalities**
  (Where the red blood cells have defects that cause premature destruction)
  - Hereditary spherocytosis (a hereditary membrane defect, causing RBC to be sequestered by the spleen)
  - Hereditary elliptocytosis(defect in membrane skeleton proteins)
  - Abetalipoproteinemia (causing defects in membrane lipids)
  - Enzyme deficiencies
    - Pyruvate kinase and hexokinase deficiencies (causing defective glycolysis)
    - Glucose-6-phosphate dehydrogenase deficiency (causing increased oxidative stress)
  - Hemoglobinopathies
    - Sickle cell anemia
    - Hemoglobinopathies causing unstable hemoglobins
  - Paroxysmal nocturnal hemoglobinuria
- **Extrinsic (extracorpuscular) abnormalities**
  - Antibody-mediated
    - Warm autoimmune hemolytic anemia is an anemia caused by autoimmune attack against red blood cells, primarily by IgG
    - It is the most common of the autoimmune hemolytic diseases. It can be idiopathic, drug-associated or secondary to systemic lupus erythematosus, or a malignancy, such as chronic lymphocytic leukemia (CLL)
    - Cold agglutinin hemolytic anemia (IgM mediated,can be idiopathic or secondary)
    - Hemolytic disease of the newborn
    - Mismatch transfusion reactions
  - Mechanical trauma to red cells
    - Microangiopathic hemolytic anemias including TTP, HUS
    - Disseminated intravascular coagulation (DIC)
    - Infections, including malaria
    - Prosthetic valve/heart surgery

(III) Decreased RBC Count Due to Blood Loss

- Trauma or surgery, causing acute blood loss
- Gastrointestinal tract lesions (causing chronic blood loss)
- Gynecologic disturbances (also generally causing chronic blood loss)
- Hypervolemia causes spurious low RBC count (due to hemodilution)

(IV) Causes of High Red Blood Cell Count

- Polycythemia vera (often a hereditary problem)
- Excessive smoking
- High altitudes
- Renal neoplasia (the kidneys are producing too much erythropoietin)
- Heart failure or some heart diseases
- Lung diseases like emphysema, pulmonary fibrosis, etc.
- Dehydration
- Erythropoietin doping by athletes to boost their performance
- Hemoglobinopathies
- Adrenal cortical hyperfunction
- Anabolic metabolism

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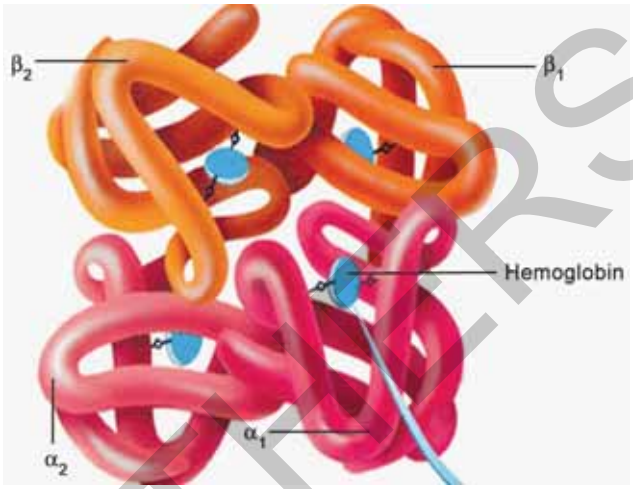
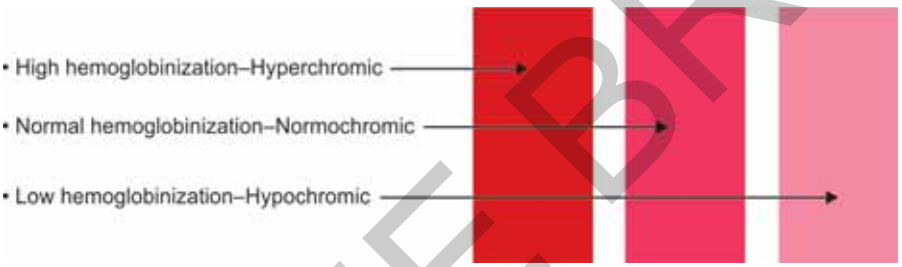
HEMOGLOBIN

- Hemoglobin is the oxygen-carrying component of the red cell. Each organ in the human body depends on oxygenation for growth and function.
- By measuring the hemoglobin concentration of the blood, the oxygen-carrying capacity of the blood is evaluated.
- Both high and low hemoglobin counts indicate defects in the balance of red blood cells, and may indicate disease.
- Hemoglobin is synthesized at the polychromatic normoblast stage of red cell development.
- This synthesis is visualized by the change in cytoplasmic color from a deep blue to a lavender-tinged cytoplasmic color.
  - *The hemoglobin molecule consists of two primary structures:*
  - *Heme portion and globin portion.*
  - *Each heme molecule consists of four heme structures with* iron at the center and two pairs of globin chains.
- The normal range for hemoglobin is highly age and sex-dependent with men having higher values than women and adults having higher values than children except neonates which have the highest value of all.
  - Hemoglobin, bound to O_2 gives RBCs their "chrom" color; therefore variations in hemoglobin are referred to as "chromic" changes.



Hemoglobin normal range	Male	Female
	13.3–16.2 g/L	12.0–15.8 g/L



Hemoglobin Concentration Measurement

To measure hemoglobin concentration (Hb), a known volume of well-mixed whole blood is added to a diluent which lyses red cells to produce a hemoglobin solution. Lysis occurs because of the hypotonicity of the diluent, but may be accelerated by the inclusion in the diluent of a nonionic detergent to act as a lytic agent. The Hb is determined from the light absorbance (optical density) of the solution of hemoglobin or its derivative at a selected wavelength.

Cyanmethemoglobin Method

The International Council for Standardization in Hematology (ICSH) has recommended a reference method in which hemoglobin is converted to cyanmethemoglobin.

Method

The basis of the method is the dilution of blood in the solution of potassium cyanide and potassium ferricyanide.

A volume of 20 µL of blood is added to 5.0 mL of diluents (1:250), mixed well, and allowed to stand at room temperature for at least 5 minutes the absorbance is measured, against the reagent blank, in the photocalorimeter at 540 nm and compared against the absorbance of HiCN standard. Calculate the Hb concentration by

Hb (g/dL) = (Test sample abs./Standard abs) × Concentration of standard × Dilution factor.

Advantages

- Hemoglobin, methemoglobin, and carboxyhemoglobin are all converted to cyanmethemoglobin and are therefore included in the measurement.

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- Cyanmethemoglobin has an absorbance band at 540 nm, which is broad and relatively flat and thus measurements can be made either on a narrow-band spectrophotometer or on a filter photometer or colorimeter that reads over a wide band of wavelengths.
- (Stable secondary) International Standard are readily available for calibration.

Disadvantages

- The presence of sulfhemoglobin will lead to a slight underestimation of total hemoglobin.
- The slow conversion of carboxyhemoglobin to methemoglobin.
- The maximum possible error that could be caused if 20% of the hemoglobin were in the form of carboxyhemoglobin, a degree of abnormality that may be found in heavy smokers, would be 6%.
- Increased turbidity causes a factitiously elevated estimate of Hb.....
  - **When the WBC is high**, turbidity effects are circumvented by centrifugation or filtration of the solution prior to reading the absorbance.
  - **When turbidity is due to a high level of plasma protein** (either when a paraprotein is present or when there is polyclonal hypergammaglobulinemia resulting from severe chronic infection or inflammation), it can be cleared by the addition of either potassium carbonate or a drop of 25% ammonia solution.
  - **When turbidity is due to hyperlipidemia**, the lipid can be removed by diethyl ether extraction and centrifugation.
  - **When turbidity is due to red cells containing hemoglobin S or C**, which may fail to lyse in the diluent and, produces a factitiously high reading of Hb making a 1:1 dilution in distilled water ensures complete lysis of osmotically resistant cells.

Other Methods

Alternative methods of measuring Hb are not widely used except when they have been incorporated into hemoglobinometers. Such methods usually require standardization by reference back to a cyanmethemoglobin standard.

- Hb can be measured as hematin produced under alkaline conditions. The alkaline–hematin method measures carboxyhemoglobin, sulfhemoglobin and methemoglobin, although it does not adequately measure hemoglobin F or hemoglobin Bart's, which are resistant to alkaline denaturation.
- Hemoglobin can be converted into a sulfated derivative with maximum absorbance at 534 nm by the addition of sodium lauryl sulfate. Conversion is almost instantaneous and methemoglobin, but not sulfhemoglobin, is converted. This method correlates well with the reference method that is employed for calibration. This method is suitable for use with a spectrophotometer and has also been incorporated into several automated instruments.
- Hb can also be measured following conversion to acid methemoglobin by the addition of sodium nitrate and sodium azide. This is the method permit compensation for turbidity.
- Hb can be measured as oxyhemoglobin; in this case concentration of carboxyhemoglobin, sulfhemoglobin and methemoglobin will not be measured accurately.
- Acid hematin method (Sahli's hemoglobinometer) is commonly used but less reliable.

Hemoglobin Measurement by Sahli Acid Hematin Method

Materials required for Hb measurement:

- Distilled water.
- Sahli-Hellige hemoglobinometer kit containing:
  - Small bottle of dilute (approx. 0.1N) hydrochloric acid.
  - Graduated tube, with a scale on two sides. On one side is the percentage scale, and on the opposite side is the Gram scale. The percentage scale reads from 0 to 170. The Gram scale reads from 0 to 24.
  - Pipette, marked at the 20 mm level.
  - Stirring rod.
  - Color comparator, with a window on the side. On the right and left sides of this opening is the color standard for comparison. The center has an open slot to hold the graduated tube.



Procedure

- With a dropper, place 5 drops of the 0.l NHCl in the bottom of the graduated tube. Place the tube in the color comparator.
- Using well-mixed venous blood or fingertip blood, fill the pipette to the 20 mm mark.
- Wipe the blood from the outside of the pipette. Transfer blood to the Sahli tube.

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- Aspirate distilled water into the pipette two or three times and transfer these washings to the tube.
- Shake until the blood is well-mixed and the tube is a uniform color.
- Add distilled water, drop by drop, each time mixing the solution with the stirring rod. Keep adding water and mixing until the color of the solution matches the standards on either side. Remove the stirring rod from the tube each time before comparing. Natural light makes more accurate readings possible.
- Five minutes after the time noted, read the result from the scale on the tube by noting the graduation mark at the lower edge of the meniscus.

Hemoglobin is decreased in:
Area of central pallor takes up more than 1/3rd of the area of the RBC (hypochromia)
- Blood loss (lung, gastrointestinal, hemorrhoids, ulcers, colitis, uterine, menses, in urine via kidneys, hemorrhage)
- Deficiency (protein malnutrition, iron, copper, vitamin C, vitamin B_1, folic acid, B_{12}), chronic disease (liver, kidney, rheumatoid arthritis, carcinoid, etc.)
- Bone marrow insufficiency (infiltration with tumor or tuberculosis, toxic, immune or drug-induced hypoplasia)
- Digestive inflammation (with occult or obvious blood loss) as might occur with parasites, colitis, hemorrhoids, etc.
- Free radical pathology
- Adrenal cortical hypofunction
- Hereditary anemia(s)
- Hemodilution (pregnancy, edema)

Hemoglobin is increased in:
Area of central pallor takes up less than 1/3rd of the area of RBC (hyperchromia)
- Polycythemia vera
- High altitude adaptation
- Pulmonary pathology
- Splenic hypofunction
- Testosterone supplementation
- Dehydration as might occur with prolonged or severe diarrhea
- Emphysema, severe asthma, and other long-standing respiratory distress
- Macrocytosis (deficiency of B6, B12, folic acid, or hypothyroid)
- Adrenal cortex overactivity

RETICULOCYTE

In the journey of erythropoiesis, a reticulocyte is a cell just one stage prior to the mature erythrocyte.

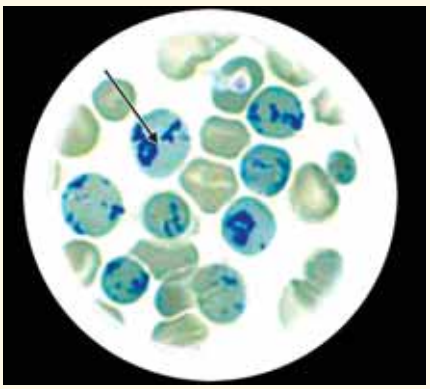
Normal range of reticulocyte count is - 0.5% to 1.5%

An increased number of reticulocytes is seen when the marrow is churning out RBCs at excessive speed (presumably to make up for those lost to hemolysis or hemorrhage).

The reticulocyte count is usually performed when patients are evaluated for anemia and response to its treatment.

The lifespan of erythrocytes is approximately 120 days, and about 0.8% of the red cells need to be replaced daily by young cells released from the bone marrow.

Manual reticulocyte count
- These immature cells (reticulocytes) contain ribosomal RNA that can be precipitated and stained by supravital dyes, such as new methylene blue or brilliant cresyl blue.
- The blood and stain are mixed and allowed to stand for about 15 minutes before the films are prepared and air-dried.
- The cells containing blue granules or reticulum are counted as a percentage of total red cells or as an absolute number per volume.



Automated Reticulocyte Count by Fluorescent Method

Automated methods employ a substance, such as thiazole orange, that binds to RNA and fluoresces.

With the use of fluorescent technology, the retic channel provides:
- Accurate reticulocyte counting in both % and absolute count
- Improved immature reticulocyte information (IRF-Immature Reticulocyte Fraction) for earlier diagnosis and treatment by clinicians.
- Elimination of common interferences such as Howell-Jolly bodies, Pappenheimer bodies, and immature reticulocytes to avoid manual count errors.

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Reticulocyte Production Index or Corrected Reticulocyte Count

- Reticulocyte count must be adjusted for the level of anemia to obtain the reticulocyte index, a more accurate reflection of erythropoiesis.
- Reticulocyte index = Reticulocyte count × Patient's hematocrit/Normal hematocrit.
  For example: Reticulocyte count 6%, hematocrit 15, then Reticulocyte index = (6 × 15)/45 = 2%.

Reticulocyte Proliferation Index and Shift Correction Factor

Normally reticulocytes survive 3.5 days in the marrow and one day in peripheral circulation at normal PCV. In case of anemia and reduced hematocrit, the survival time in circulation is increased and this may cause a poor correlation of retic count with marrow response. This situation can be dealt with the calculation of corrected retic count or reticulocyte proliferation index.

Reticulocyte proliferation index =
Corrected retic count/shift correction factor
For example, Corrected retic count 7%, PCV—25, RPI = 7/2 = 3.5

Reticulocyte proliferation index	
PCV %	***Maturation time in days***
45	1
35	1.5
25	2
15	2.5

Increased Retic Count

- Hemolytic anemias
- Response to treatment of iron and Vit B_{12} and folic acid in anemia
- Recent hemorrhage
- Thalassemia
- Pregnancy
- Erythroblastosis fetalis
- HbC disease
- Leukemias
- Hypoxia

An increased count indicates active erythropoiesis

Retic Count is Decreased in:

- Decreased adrenocortical and anterior pituitary activity
- Aplastic anemia
- Cirrhosis
- Megaloblastic anemia
- Exposure to radiation
- Anemia of chronic diseases
- MDS

Reticulocyte Hemoglobin Measurement (RET-He)

RET-H*e* is a parameter measured in the reticulocyte channel and is used to measure the incorporation of iron into erythrocyte hemoglobin beyond traditional biochemical tests (e.g. iron, ferritin, TIBC, % Sat).

The RET-H*e* parameter supports:

- Assessment of anemia and is an established parameter used in KDOQI (Kidney Disease Outcomes Quality Initiative) guidelines for assessing the initial iron status of patients.
- Rapid, direct analysis of an earlier stage of RBC development for prompt clinical follow-up.
- Accuracy and sensitivity in measurement of red cell production that supports effective monitoring of costly drug protocols for cell stimulation.
- Screening of infants for iron deficiency,
- Evaluation of iron deficiency in end stage renal disease (ESRD)/chronic dialysis patients
- In other chronic diseases associated with inflammation in which traditional biochemical tests may be misleading.
- Ret-He is a Sysmex instrument parameter and CHr is a Siemens instrument parameter
- While not identical, these parameters essentially measure the same thing in reticulocytes
- Reticulocyte Hemoglobin content provides an idea regarding the amount of iron available for RBC production in the bone marrow. It is a newer parameter that is being used in the research area to check the utility in diagnosing iron deficiency.

Low, Medium and High Fluorescence Reticulocytes

The newer fluorescence flow cytometry technique now allows us to categorize the reticulocytes based on their degree of maturation.

This is done by measuring the RNA content of the reticulocyte as fluorescence intensity. The higher the RNA content, the less mature the cells are:

- Low fluorescence reticulocyte: Mature
- Medium fluorescence reticulocyte: Medium maturity
- High fluorescence reticulocyte: Most immature

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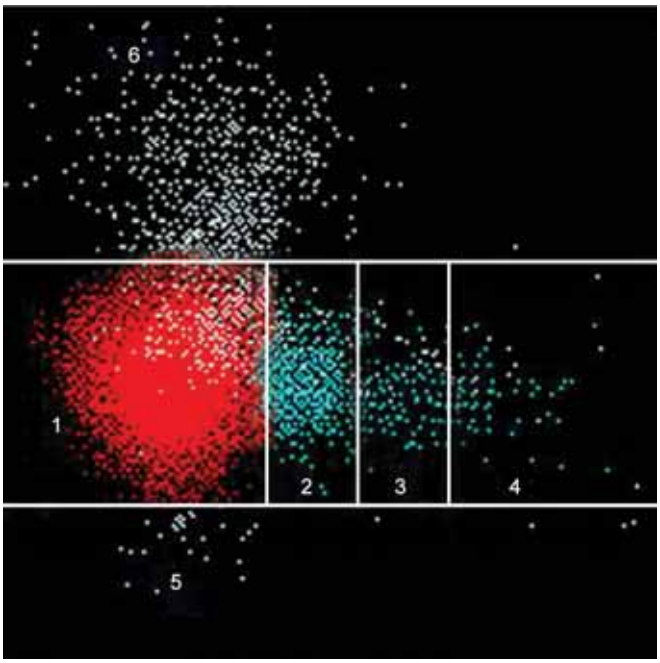

Retic scatter absorption

Courtesy: © Siemens Healthcare GmbH 2020.

1. Mature RBCs
2. Low absorption retics
3. Medium absorption retics
4. High absorption retics
5. Platelets
6. Coincidence events

Immature Reticulocyte Fraction (IRF)

- IRF is a marker to show the effectiveness of erythropoiesis in a patient.
- IRF is a calculated ratio of newly released reticulocytes to total reticulocytes.
- It is a very sensitive measure of marrow erythropoietic activity.
- Evaluate patients after bone marrow transplantation to indicate early engraftment
- In patients treated with EPO to measure the response to EPO therapy
- Red cell inclusions (e.g. Howell-Jolly bodies, Pappenheimer bodies, basophilic stippling) may cause interference with this parameter
- For most practical purposes, evaluation of the combination of IRF along with reticulocyte count (RET) may be used to define the etiology of anemia in a particular patient.

For example:

- **Low RET + Low IRF** would suggest *aplastic anemia or renal failure.*
- **High RET + High IRF** would suggest *hemolytic anemia or blood loss.*
- **Low/normal RET + High IRF** would suggest *iron, folate, or B-12 deficiency.*
- **Low/normal RET + Normal IRF** would suggest *anemia of chronic disease.*

To summarize in terms of practical diagnosis,

The reticulocyte count *is a very valuable measure of assessment of the rate of production of the red cells, and efficacy of the marrow in terms of responding to anemia.*

To oversimplify,

- ***If the disease-causing the anemia is inside the marrow, the reticulocyte count is decreased.***
- ***If the disease-causing the anemia is outside the marrow, the reticulocyte count is increased.***

HEMATOCRIT—PACKED CELL VOLUME (PCV)

- The hematocrit (Hct) or packed cell volume (PCV) or erythrocyte volume fraction (EVF) is the ratio of the volume of erythrocytes to that of the whole blood and is reported as a percentage.
- This is the earliest RBC parameter identified and is used for red cell disorders differentiation.
- The hematocrit is one of the most precise methods of determining the degree of anemia or polycythemia, i.e. increase or decrease in RBC concentration.

Hematocrit normal range	Male	Female
	38.8–46.4%	35.4–44.4%

- The manual method of measuring Hct has proved to be a simple and accurate method of assessing red cell status.
- It is easily performed with little specialized equipment – The spun.
- However, several sources of error are inherent in the technique.
- The spun Hct measures the red cell concentration, not red cell mass.
- Therefore, patients in shock or with volume depletion may have normal or high Hct measurements due to hemoconcentration despite a decreased red cell mass.
- Technical sources of error in manual Hct determinations usually arise from inappropriate concentrations of anticoagulants, poor mixing of samples, or insufficient centrifugation.

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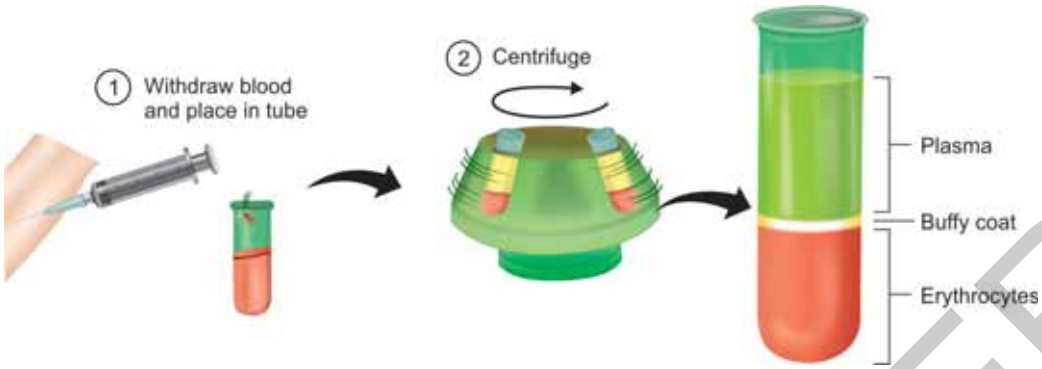
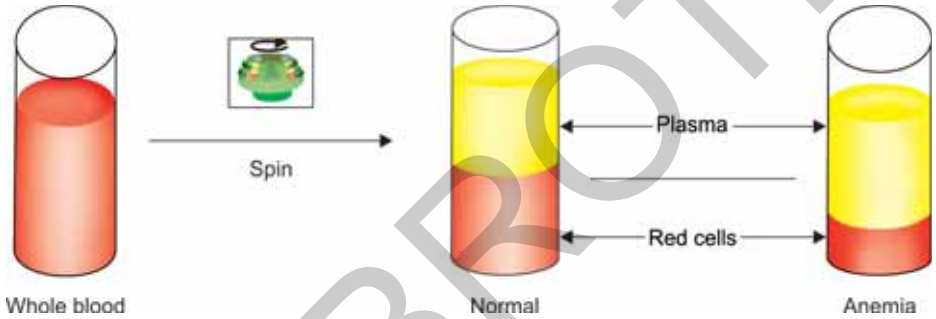


Another inherent error in manual Hct determinations arises from the trapping of plasma in the red cell column. This may account for 1–3% of the volume in microcapillary tube methods, and more trapping of plasma is noted in the macrotube methods.

- Abnormal red cells (e.g. sickle cells, microcytic cells, macrocytic cells, or spherocytes) often trap higher volumes of plasma due to increased cellular rigidity, possibly accounting for up to 6% of the red cell volume.
- Very high Hct, as in polycythemia, may also have excess plasma trapping. Manual Hct methods typically have a precision [coefficient of variation (CV)] of approximately 2%.



- Automated analyzers do not depend on centrifugation techniques to determine Hct, but instead calculate Hct by direct measurements of red cell number and red cell volume (Hct = Red cell number × red cell volume).
- Errors of automated Hct calculation are more common in patients with polycythemia or abnormal plasma osmotic pressures. Manual methods of Hct determination may be preferable in these cases.
- The precision of most automated Hct is less than 1% (CV).

High Hematocrit is due to:
◆ Dehydration (such as from severe diarrhea) ◆ Kidney disease with high erythropoietin production ◆ Low oxygen level in the blood ◆ Congenital heart disease ◆ Cor pulmonale ◆ Pulmonary fibrosis ◆ High altitude ◆ Dengue hemorrhagic fever ◆ Polycythemia vera ◆ Smoking

Low Hematocrit is due to:
◆ Blood loss (hemorrhage) ◆ Bone marrow failure (for example, aplasia or hypoplasia due to radiation, infections, drugs, neoplasia, autoimmune, etc.). ◆ Erythropoietin deficiency (usually secondary to kidney disease) ◆ Hemolysis (RBC destruction due to varied etiologies) ◆ Leukemia ◆ Malnutrition (nutritional deficiencies of iron, folate, vit. B_{12}, or vit. B_6) ◆ Multiple myeloma ◆ Autoimmune/collagen-vascular diseases, such as lupus erythematosus or rheumatoid arthritis

An elevated hematocrit may be due to spleen hyperfunction, and a reduced hematocrit may indicate low thymus function.

Rule of three

Correlation checks between the Hgb and Hct are a significant part of quality assurance for the CBC and are known as the "rule of three." The formulas for correlation checks/rule of three are as follows:

Hgb × 3 = Hct ± 3, and RBC × 3 = Hgb.

Example: 14.8 (***Hgb***) × 3 = 44 (patients actual hematocrit result is 45%
11.0 (***Hgb***) × 3 = 33 (patients actual hematocrit result is 32%

The exception to this rule is in patients with hypochromic red cells. These patients will have hematocrits that are more than three times the hemoglobin

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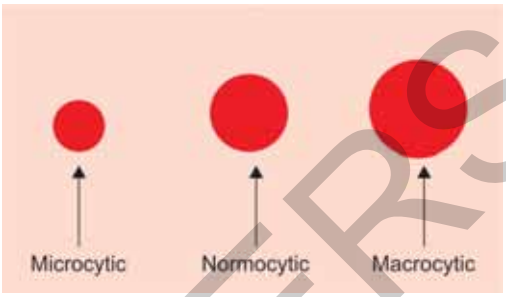
MEAN CORPUSCULAR VOLUME

Mean corpuscular volume (MCV) is the average volume of the red blood cell

- MCV = (hematocrit/red cell count) × 100.
- Can be directly measured by automated cell counters.
- With impedance analyzers, the MCV is measured by averaging the amplitude of the pulses created as the cells pass through the aperture of the counter.
- Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were first introduced by Wintrobe to define the size (MCV) and hemoglobin content (MCH, MCHC) of red blood cells.



MCV normal range	Men and Women
	79–98 fL (femtoliters)

Cells are classified as microcytic if MCV < 79 fL and macrocytic when > 98 fL and normocytic when indices are within normal limits.

- MCV is higher at birth, decreases rapidly during first 6 months of life.
- The blood analyzer gives us a superior determination of the red cell size whereas the microscopic observation gives us a crude impression of the same.
- Anemias in which red cells are normal in both size and hemoglobin content are called "normocytic normochromic".

- *Presence of microcytic and macrocytic cells in the same sample may result in a normal MCV.*
- *MCV <72 fL without heterogeneity, i.e., with normal RDW, has sensitivity and specificity (S/S) of 88% and 84% respectively as a predictor of thalassemia trait.*

Normal MCV (Normocytic Anemia)

- Following acute hemorrhage
- Dimorphic anemia
- Hemoglobinopathies
- Anemias due to inadequate blood cell formation
  - Myelophthisic
  - Hypoplastic
  - Aplastic.
- Endocrinopathies—(hypopituitarism, hypothyroidism, hypoadrenalism, hypogonadism)
- Anemia of chronic disease (chronic infections, neoplasms, uremia).

Increased MCV (Macrocytic Anemia - MCV >98 fL)

- Megaloblastic anemia
- Pernicious anemia (vitamin B_{12} or folate deficiency)
- Sprue (e.g., steatorrhea, celiac disease, intestinal resection or fistula)
- Macrocytic anemia of pregnancy
- Megaloblastic anemia of infancy
- Di Guglielmo disease
- Myelodysplastic syndromes (aplastic anemia, sideroblastic anemia)
- Myelophthisic anemia
- Post splenectomy
- Infants and newborns
- Nonmegaloblastic macrocytic anemias
- Alcoholism
- Liver disease
- Anemia of hypothyroidism
- Drugs:
  - Oral contraceptives
  - Anticonvulsants (e.g., phenytoin, primidone, phenobarbital)
  - Antitumor agents (e.g., methotrexate, hydroxyurea, cyclophosphamide)
  - Antimicrobials (e.g., sulfamethoxazole, sulfasalazine, trimethoprim, zidovudine, pyrimethamine).

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Decreased MCV (Microcytic Anemia – MCV <79 fL)
◆ Usually hypochromic – Iron deficiency anemia – Thalassemia (major or combined with hemoglobinopathy) – Lead poisoning – Disorders of porphyrin synthesis ◆ Usually normochromic – Anemia of chronic diseases (<1/3rd of patients) – Heterozygous thalassemia and hemoglobinopathies

Interferences in MCV
◆ Cold agglutinins (increased values) ◆ Warm autoantibodies ◆ Marked hyperglycemia (>600 mg/dL) (increases MCV) ◆ Marked leukocytosis (>50,000/µL) (increased values) ◆ In vitro hemolysis or fragmentation of RBCs (decreased values) ◆ Methanol poisoning (increased values) ◆ Marked reticulocytosis (>50%) from any cause (increases MCV)

MEAN CORPUSCULAR HEMOGLOBIN

Mean corpuscular hemoglobin (MCH) is the amount of hemoglobin in a single red blood cell.

MCH = (hemoglobin/red cell count) × 100

MCH normal range	Men and women
	26.7–31.9 pg/cell

- MCH if low—Hypochromasia
- MCH if high—Hyperchromasia.

MCH decreases in:
◆ Microcytic and normocytic anemias

MCH increases in:
◆ Macrocytic anemias ◆ Infants and newborns

Interferences in MCH
◆ Lipemia ◆ Marked leukocytosis (>50,000/µL) ◆ Cold agglutinins ◆ In vivo hemolysis ◆ Monoclonal proteins in blood ◆ High heparin concentration

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC)

- The average hemoglobin concentration per unit volume (100 mL) of packed red cells is indicated by MCHC.
- In contrast to MCH, MCHC correlates the hemoglobin content with the volume of the cell.
- It is expressed as g/dL of red blood cells.

MCHC = (Hemoglobin/Hematocrit) × 10

MCHC normal value	Male and female
	32–36 g/dL

MCHC decreased in (decrease is defined as ≤ 30.1 g/dL):
◆ Hypochromic microcytic anemia. Normal value does not rule out any of these anemias. ◆ Low MCHC may not occur in IDA when performed with automated instruments.

MCHC increased in:
◆ Hereditary spherocytosis ◆ In spherocytosis, the MCHC is increased due to loss of membrane and the consequent spherical shape assumed by the cell ◆ Infants and newborns ◆ Autoagglutination ◆ Artifactual (abnormal MCHC may be most valuable clue to artefact)

Interferences: MCHC decreased in:
◆ Marked leukocytosis (>50,000/µL)

MCHC increased in:
◆ Hemolysis (e.g., sickle cell anemia, hereditary spherocytosis, autoimmune hemolytic anemia) with shrinkage of RBCs making them hyperdense ◆ Conditions with cold agglutinins or severe lipemia of serum ◆ Rouleaux or RBC agglutinates ◆ High heparin concentration

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The microcytic-to-hypochromic ratio
(Siemens %HYPO, Sysmex %HYPO-He)

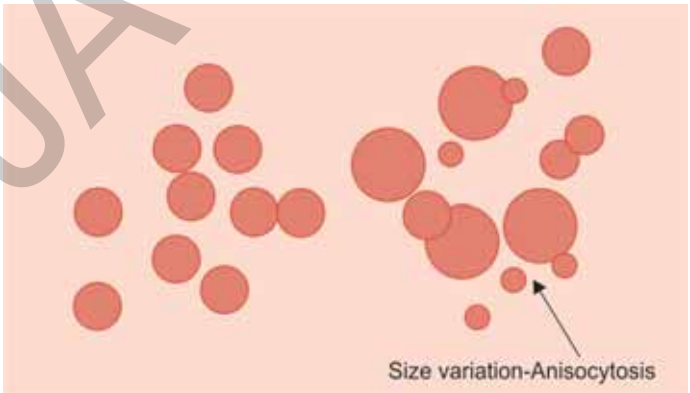
- To distinguish thalassemia from iron deficiency anemia (IDA), as the former tends to show more prominent microcytosis, while severe hypochromasia is more characteristic of the latter.
- Iron deficiency superimposed on certain thalassemia variants can result in marked hypochromia, yielding a ratio similar to that of a non-thalassemic patient with IDA.

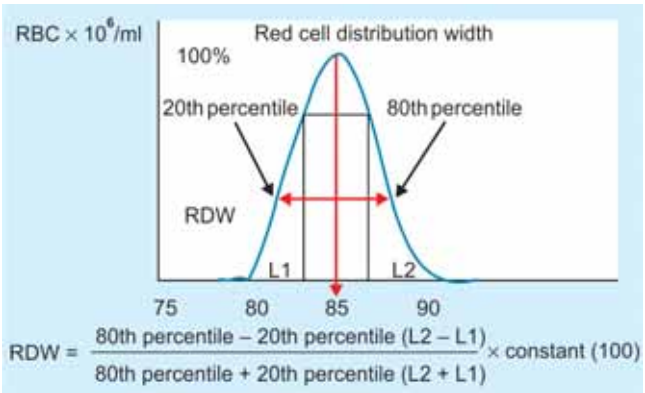
- $\% \text{ Macro} = 100 \times \frac{\text{Cell count in RBC volume histogram} > 120 \text{ fL}}{\text{Total cell count in RBC volume histogram}}$
- $\% \text{ Micro} = 100 \times \frac{\text{Cell count in RBC volume histogram} < 60 \text{ fL}}{\text{Total cell count in RBC volume histogram}}$
- $\% \text{ Hyper} = 100 \times \frac{\text{Cell count in RBC HC histogram} > 41 \text{ g/dL (*HC = Hemoglobin conc.)}}{\text{Total cell count in RBC HC histogram}}$
- $\% \text{ Hypo} = 100 \times \frac{\text{Cell count in RBC HC histogram} < 28 \text{ g/dL}}{\text{Total cell count in RBC HC histogram}}$

RED CELL DISTRIBUTION WIDTH

Red cell distribution width (RDW) is a quantitative measure or numerical expression of anisocytosis. It is a coefficient of variation of the distribution of individual RBC volume, as determined by an automated blood cell counting instrument.

- The RDW value reflects size variability in red cells.
- High RDW values mirror a large range in red cell size.
- The RDW can provide insight into the basis of anemia because some processes increase their value while others do not.
- Microcytosis, for instance, exists with either iron deficiency or thalassemia minor. The former condition increases the RDW while the latter does not. Therefore, marked microcytosis with a normal RDW suggests thalassemia minor early in the work-up.
- Anisocytosis of RBCs is a variation of cell size in a given population of cells or excessive heterogeneity on smear or the visual equivalent of increased CV (coefficient of variation).
- The coefficient of variation (CV) means how reproducible repetitive measurement is.
- The reproducibility of values among a cell population reflects not only variability in the measurement but also the biological heterogeneity of the cells.
- Cells excessively heterogeneous in size would not present a reliable amount of oxygen-diffusion surface to the capillary endothelium. On the other hand, the more rigidly the red cell size is controlled, the large number of cells would be quickly destroyed by the spleen.
- The heterogeneity of a red cell property, therefore, is a result of a balance of acceptable function versus the cost of quality control.
- The coefficient of variation (CV) of red cell size is reported as "red cell distribution width" or RDW.
- The index of red cell heterogeneity should not be simply the width of the histogram, because this measures only the SD.
- The CV is the ratio of the standard deviation–SD (width of the histogram) to the "mean corpuscular volume" (MCV).





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RDW-CV:

The RDW-CV is calculated from the formula:
RDW-CV = (Standard deviation of RBC volume/mean MCV) × 100
1SD reflects the size variation of the erythrocytes round the mean. As the 1SD is divided by the MCV, the RDW-CV also depends on the mean size (MCV) of the erythrocytes.
Reference values: 11.5–14.5%

The RDW is markedly elevated in newborns, ranging from 14.5% to 19.5%, gradually decreases, and reaches adult levels by the age of 6 months.

RDW-SD:

The determination of the RDW-SD is an actual measurement of the width of the erythrocyte distribution curve. This measurement is performed at a relative height of 20% above the baseline. The wider the curve is spread by erythrocytes of different sizes, the higher the RDW-SD value will be.

Reference values: 35–45 fL.

Thus, a given SD may be normal or abnormal depending on MCV, therefore, more important is the calculation of RDW-CV.

Unlike most variables, in which, these are abnormally high and abnormally low values – No disorder is known to have abnormally low RDW.

Role of RDW in Modern Classification of RBC disorder

Anemia has been classified by two main methods.

1. **Morphologic**: In which classification is based on RBC size and is classified into:
   - Normocytic, (normal MCV)
   - Microcytic (low MCV)
   - Macrocytic (higher MCV).

   Usually, MCV is the classifier in this classification but this parameter can be deceptive in conditions like mixed nutritional deficiency, where microcytic and macrocytic RBCs, average the MCV to the normocytic range.
2. **Physiologic**: Usually bone marrow studies, biochemical markers and reticulocyte count are used as a classifier in this classification and is classified as:
   - Hypoproliferative or bone marrow production defect
     - Marrow aplasia
     - Chronic disease
     - Myeloproliferative disorder
     - Organ failure, (e.g. renal failure)
     - Blood dyscrasias.
   - RBC maturation defect or ineffective erythropoiesis
     - Cytoplasmic: Hypochromic
     - Nuclear: Megaloblastic
     - Combined: Myelodysplastic syndrome
   - Decreased RBC survival (hypoproliferative)
     - Hemorrhagic: Acute blood loss
     - Hemolytic: Primary, immune, hemoglobinopathies, enzymopathies, and parasites, etc.
     - Dilutional : Pregnancy, splenomegaly

Bone marrow studies help to differentiate hypoproliferative anemia (no erythroid hyperplasia) from maturation disorder (erythroid hyperplasia).

Biochemical markers such as serum iron, ferritin, TIBC, folate and vit. B_{12} assay help to distinguish various maturation disorders.

Reticulocyte production distinguishes between hypoproliferative and hemolytic anemia.

But bone marrow studies are invasive, biochemical markers are expensive and reticulocyte count has a high duplication error without automation.

Older parameters like MCHC, MCH, and HCT have always shown variable disproportionate findings towards the diagnosis. Therefore, the discussion of red cell disorders focuses primarily on directly measured parameters of RBC.

The modern time classification based on two indices – *MCV and RDW was proposed by Bessman.*

Although this classification method needs to be prospectively validated.

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RDW is mathematically derived from the RBC histogram with a portion of each of the extreme ends of the histogram curve excluded. This allows for a computation to exclude platelets, platelet clumps, and electrical interference from left side of the curve.
The exclusion of the right side of the curve eliminates clumped RBCs (from Rouleaux or agglutination) or overly large RBCs.
The MCV is calculated using the entire RBC histogram.
The RDW independent of the MCV may be normal when the MCV is normal, low, or high.

The six possible combinations classify almost all major possible causes of anemia.

- Low MCV with Normal RDW or High RDW
- Normal MCV with Normal RDW or High RDW
- High MCV with Normal RDW or High RDW

	MCV Normal (Normocytic)	MCV High (Macrocytic)	MCV Low (Microcytic)
Normal RDW (Nonheterogeneous)	◆ Anemia of chronic disease ◆ Acute blood loss ◆ Hemolysis ◆ Chronic lymphocytic Leukemia (CLL) ◆ Chronic myelogenous leukemia (CML) ◆ Hemoglobinopathy ◆ Normal variant	◆ Aplastic anemia ◆ Preleukemia ◆ Myelodysplastic syndrome	◆ Anemia of chronic disease ◆ Thalassemia (heterozygous)
High RDW (Heterogeneous)	1. RDW increases before MCV becomes abnormal – Early iron deficiency anemia – Early vitamin B_{12} deficiency – Early folate deficiency 2. Anemic globinopathy like Sickle cell anemia	◆ Vitamin B_{12} deficiency ◆ Folate deficiency ◆ Immune hemolytic anemia ◆ Liver disease ◆ Cold agglutinins ◆ Alcoholism	◆ Iron deficiency anemia ◆ RBC fragmentation ◆ HbH ◆ Thalassemia intermedia ◆ G6PD deficiency
Low RDW	Low RDW is not observed normally but theoretically, it is possible only at the marrow level when RBC produced by marrow has less heterogeneity than the accepted normal. Some observers quote low RDW low MCV in thalassemia minor and low RDW with high MCV in aplastic anemia but the author includes both these under normal RDW		

The classification may be grossly summarized as:

- Hypoproliferative disorders—Independent of MCV have normal heterogeneity.
- Nutritional disorders—Independent of MCV have increased heterogeneity.
- Hemolytic disorders—Independent of MCV have heterogeneity that is increased in direct proportion to the degree of anemia caused by the disorder.

Hemoglobin distribution width (HDW)

- HDW is a measurement of anisochromia, or variation in hemoglobin concentration, similar to the red cell distribution width (RDW) which is an automated measurement of anisocytosis (variation in red cell size),
- The HDW is useful to differentiate between causes of microcytosis and macrocytosis and is only available on haematology analysers that use optical red cell technology.
- The normal range for HDW is 22 to 32 g/L.
- Cases of microcytosis caused by iron deficiency anaemia often have a raised HDW (and RDW), whereas microcytosis caused by thalassaemia trait usually has a normal HDW (and RDW).
- In cases of macrocytosis, the HDW is typically below the reference range in alcoholic liver disease (ALD), normal (or borderline raised) in megaloblastic anemia and raised in hemolytic anemia.
- Different HDW values for different causes of microcytosis and macrocytosis, as well as different cytogram appearances are helping parameter in diagnosis.

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Salient Features

- ABC of CBC is a keen attempt on interpretation and clinical correlation of various traditional and novel parameters in a complete blood count report.
- Enriched with all relevant text and graphic information and its interpretation.
- Encompassing various less understood and ignored parameters, such as Large undefined cells (LUC), Mean platelet volume (MPV), Immature platelet fraction (IPF), Red cell distribution width (RDW), histograms and cytograms.
- There is a lot of hidden information generated by newer and sophisticated cell counters, which these parameters reveal, because "Eyes can only see what the mind knows!"
- Extremely useful to the undergraduates, postgraduates, practicing clinicians and practicing consultants of various medical fraternities.
- It still remains the first ever published elaborated document on the subject of CBC, with profound information on many useful parameters for diagnostic skills.
- Proper interpretation of CBC is of great utility in narrowing the differential diagnosis, also aid in avoiding expensive and invasive battery of tests like bone marrow aspiration and biopsy.



DP Lokwani MD is the Founder Vice-Chancellor of MP Medical University, Professor and Head, Department of Pathology, Netaji Subhash Chandra Bose (NSCB) Medical College and Hospital, Jabalpur, Madhya Pradesh, India. He is also Former Dean of Academics, Diplomate of National Board courses, Jabalpur Hospital and Research Centre. He is the only Pathologist to date who has been bestowed with the highest honor by IMA, MP, the "CHARAK AWARD", in October 2019. He has reported over half a million CBCs in his career, with excellent clinic-pathological communication.

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Dr DP Lokwani has done a laudable job in writing this volume on "CBC" and histograms in the era of sophisticated laboratory techniques. I have no hesitation in recommending the book to be on the shelf of everyone even remotely connected with CBC.

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Printed in India

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JAYPEE BROTHERS
Medical Publishers (P) Ltd.
EMCA House, 23/23-B, Ansari Road,
Daryaganj, New Delhi - 110 002, INDIA
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ISBN 978-93-90595-98-3

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