I. Routine Hematology Tests

All procedures have institution-established reference intervals—a range of values expected for an analyte or test parameter in healthy individuals and based on the patient population served. ‘Normal’ varies with age, sex, geographical location, and/or testing methodology. Reference ranges must be determined and validated by each laboratory. SEE Normals sheet.

Test results are used to assess health status (rule out disease), help establish a diagnosis or cause of disease, and to monitor treatment. Testing is automated unless doubtful automated results must be verified by microscopic exam of the blood smear or by manual cell counting methods, which delays turnaround time.

A. Complete Blood Count (CBC) // CBC without Diff
   • White blood cell count (WBC)
   • Red blood cell count (RBC)
   • Hemoglobin measurement (HGB)
   • Hematocrit measurement (HCT)
   • Red blood cell indices
     ◦ Mean Cell Volume (MCV)
     ◦ Mean Cell Hemoglobin (MCH)
     ◦ Mean Cell Hemoglobin Concentration (MCHC)
     ◦ Red Cell Distribution Width (RDW)
   • Platelet count (PLT)

B. Differential (DIFF)

C. Reticulocyte count (RETIC)

D. Coagulation studies (PT, PTT)

All tests must be reported using proper reporting format including decimals and units.

<table>
<thead>
<tr>
<th>CBC Parameters</th>
<th>Conventional Units (preferred)</th>
<th>SI Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC thousands/ccm</td>
<td>7,200/ccm</td>
<td>5,300,000/ccm</td>
</tr>
<tr>
<td>RBC millions/ccm</td>
<td>5,300,000/ccm</td>
<td>7.2 x 10^6/ccm</td>
</tr>
<tr>
<td>HGB grams/deciliter</td>
<td>15.0 g/dL</td>
<td>5.30 x 10^12/ccm</td>
</tr>
<tr>
<td>HCT percent</td>
<td>45.4 %</td>
<td>5.30 x 10^12/ccm</td>
</tr>
<tr>
<td>MCV femtoliters</td>
<td>86.8 fL</td>
<td>5.30 x 10^12/ccm</td>
</tr>
<tr>
<td>MCH picograms</td>
<td>28.8 pg</td>
<td>5.30 x 10^12/ccm</td>
</tr>
<tr>
<td>MCHC percent</td>
<td>33.1 %</td>
<td>5.30 x 10^12/ccm</td>
</tr>
<tr>
<td>RDW percent</td>
<td>12.8 %</td>
<td>5.30 x 10^12/ccm</td>
</tr>
<tr>
<td>PLT thousands/ccm</td>
<td>202,000/ccm</td>
<td>202,000/uL</td>
</tr>
</tbody>
</table>

Equivalent units: Cubic millimeters (cm³, mm³) = microliters (μL, mcL)
Thousands = K, Millions = M
To convert conventional units (cm³ or uL) to SI units (L), use a factor of x10^6

II. Acceptable/preferred anticoagulants for routine Hematology tests

Each test has specific/preferred specimen requirements. SEE page 17 for additional information.

A. Complete Blood Count
   Performed on whole blood drawn in EDTA which preserves cells best.

B. Differential

C. Reticulocyte count

D. Coagulation tests (PT/PTT)
   Performed on plasma drawn in Sodium Citrate which preserves labile coagulation proteins best.

What is the most common reason a Hematology sample is rejected?
III. Review of anticoagulants & blood collection tubes

A. In vivo, blood does not clot unless an injury occurs. Coagulation reactions involving platelets and clotting proteins are activated to transform fluid blood into a clot at the site of injury.

In vitro, freshly drawn blood is initially fluid but will clot. Coagulation reactions are triggered by the blood collection tube (surface contact and/or clot activator) or poor blood drawing technique.

Coagulation reaction:
Platelets & Clotting proteins + calcium \(\rightarrow\) thrombin which converts fibrinogen \(\rightarrow\) fibrin clot

1. Fibrinogen and other clotting proteins are ‘used up’ in the formation of fibrin.
2. Fibrin strands trap RBCs, WBCs and PLTs in the fibrin clot.

Cells and clotting proteins cannot be accurately measured if coagulation occurs.

3. ●To prevent activation of blood coagulation, tubes with an anticoagulant are filled with blood and immediately inverted to mix the blood with the additive.

B. Anticoagulants - chemical additives that irreversibly prevent blood coagulation by:

1. binding/chelating calcium - EDTA (purple top) and sodium citrate (light blue top).
2. inactivating/neutralizing thrombin - heparin (green top tube).

C. The use of different blood collection tubes with or without an anticoagulant determines the portion of the blood which can be analyzed: whole blood, plasma, or serum.

D. To obtain whole blood or plasma, blood is drawn in a tube that contains an anticoagulant:
1. Clotting is prevented by the anticoagulant if the blood has been drawn atraumatically (trauma-free) and properly mixed with the additive (blood flows smoothly upon tilting).
2. After mixing, the cells of whole blood can be analyzed.
3. Centrifugation of whole blood separates the cells from the fluid plasma.
4. After centrifugation, the fluid plasma can be analyzed. Plasma contains fibrinogen and all other clotting proteins.

E. Clotting occurs if blood is drawn in a red or gold top “clot” tube with no anticoagulant:
1. Fibrinogen is used to form fibrin strands which trap cells in the blood clot. Plastic clot tubes contain a clot activator such as silica to accelerate clotting.
2. Centrifugation separates the clot from the fluid serum.
3. After centrifugation, the fluid serum can be analyzed. Serum lacks fibrinogen.
IV. Potential sources of error

There are many potential sources of error that can cause erroneous laboratory results. The 1st step in obtaining accurate automated or manual test results is the proper collection and handling of the sample. The specimen test order, label, quality and quantity is evaluated prior to testing.

A poor quality sample will yield a poor quality result. Samples unacceptable for testing must be identified and redrawn to avoid pre-analytical errors which are the most common cause of invalid results. No matter who collects the specimen, the MLS is responsible for the integrity of all specimens analyzed.

Control samples do NOT detect blood collection errors or confirm the correct identity of patient specimens.

A. Sources of blood collection error that affect specimen integrity:

1. Wrong order or wrong patient identification/labeling error. NEVER pre-label!!

2. Wrong tube/anticoagulant drawn – blood drawn in heparin causes platelet clumping and a blue background of blood smears so cannot be used for platelet counts or differentials.

3. Insufficient fill - all tubes have minimum blood draw amounts to maintain the proper anticoagulant concentration to blood volume.
   a. EDTA tubes must be filled at least ¼ full for reliable results.
      Exception: EDTA tubes must be filled at least ½ full for the manual spun HCT test because excessive EDTA shrinks red cells.
   b. Sodium citrate tubes for coagulation tests must be full to maintain 9:1 ratio.

4. Hemoconcentration - prolonged tourniquet time (>1 minute) restricts blood flow causing falsely high cell counts.

5. Hemodilution with IV fluid causes false low cell counts due to specimen dilution and/or sample contamination can cause erroneous results (e.g., line draw).

6. Partially clotted blood - blood that is drawn too slowly (traumatic draw) or not mixed properly with anticoagulant-containing tubes may clot...large or micro-clots may form. When tilted, a tube with large clots will not flow smoothly.
   a. Cells are trapped in the fibrin clot causing falsely low cell counts. Platelets are the most affected when clotting is initiated → causes platelets to clump together.
   b. Clotting removes certain coagulation proteins such as fibrinogen.
7. Hemolysis - rupture of blood cells causes release of cell constituents.
   a. Caused by poor drawing technique, forcing blood through syringe needle or vigorous shaking of blood (rather than tilting to mix).
   b. One result of hemolysis is falsely low RBC counts.

8. Improper handling - certain tests have stability time limits for testing, e.g., blood smears should be made within 4 hours of drawing the blood.

B. Laboratory quality control employs control materials to check the reliability of patient test results. Controls monitor the actual testing process including equipment function, reagents, and individual testing technique, i.e. detects analytical errors.

1. Control samples are unknowns (like the patient samples) that have been assayed to determine a known target value and establish acceptable confidence limits. In certain tests, known positive and negative control samples are run with patient samples.
2. If a control sample result falls within ± 2 standard deviations of the control assay mean or exhibits the correct positive & negative results, results of patient samples are assumed to be correct and reportable. A control that ‘reads’ is “in control”.
3. Multiple levels of control are often used that contain analyte concentrations in medically significant ranges:
   a. Normal control (values are normal for ‘someone’; does not consider age, sex or a specific patient population)
   b. Abnormal low control (decreased values)
   c. Abnormal high control (increased values)

Note: If only 1 control level is run, the control value must fall within 2 standard deviations of the mean to be acceptable. If the control result falls between 2-3SD or >3SD of the mean, the control is “out” and unacceptable.

4. Controls confirm accuracy, monitor precision, and detect errors. Controls are NOT the same as standards/calibrators which are substances of known composition used to calibrate an instrument or test method. SEE QC handout.

C. Qualified personnel are essential to recognize specimens inadequate for testing or laboratory results that are “unbelievable” and require further testing or a sample redraw.

Which of the following is true of a quality control program?
1. Monitors the correct functioning of equipment, stability of reagents, and individual testing techniques.
2. Confirms the correct identity of patient specimens.
3. Detects clotted blood samples or other collection errors.

Is the range of acceptable values for a normal control level the same as the normal reference range of values established for a particular analytic test or method?
Anticoagulants Used in Hematology

A satisfactory anticoagulant for hematologic procedures should prevent blood from clotting without producing cellular change or alterations in the cell volume. It should also help preserve cells or labile coagulation proteins.

1. EDTA – ethylenediaminetetraacetate or ethylenediamine tetraacetic acid (spray-dried in plastic tubes)

Sodium and potassium salts of EDTA act as anticoagulants by forming insoluble salts with the calcium in the blood. EDTA is a much better preservative of cellular elements than any of the other anticoagulants.

CBC parameters are best done within 4 hours of drawing blood but are stable 24 hours if refrigerated. Acceptable smears for manual differentials may be made up to four hours after drawing the blood but not greater than 12 hours refrigerated. [According to some sources, blood smears should be made within 3 hours of the blood draw.] Changes associated with old blood include increased numbers of dying white cells, increased vacuoles in the white cells, and red cells can become crenated/spiculated (or may swell). Manual sedimentation rates must be done within 4 hours at room temperature or may be done on samples that have been refrigerated for up to 12 hours if brought to room temperature.

EDTA tubes must be filled at least ¼ full to meet minimum draw requirements and avoid alterations due to excessive anticoagulant. For the manual spun hematocrit and ESR tests, the EDTA tube must be at least ½ full with blood to obtain reliable results.

● EDTA anticoagulated blood is used for the following tests:
CBC (including WBC, RBC, HGB, HCT, RBC Indices and PLT), Differential, Retic count, Sedimentation rates (ESR), Sickle cell prep (SCP).

NOTE: When high numbers of smudge cells or broken cells are present, another smear is made using the patient’s blood to which 22% albumin has been added so these cells can be included in the differential.

2. Heparin

Heparin (with AT) inhibits coagulation by inactivating/neutralizing thrombin. It has the least effect on erythrocyte size of any of the anticoagulants and produces the least hemolysis. However, heparin is expensive and since it may not mix readily with blood, tiny clots form unless great care is taken in mixing. Heparin is NOT satisfactory for blood smears, as a blue background is produced upon staining and heparin CANNOT be used for platelet counts because platelets will clump.

● Heparinized blood is not the specimen of choice, but can be used for the tests done using EDTA blood with the exception of PLT counts and differentials. Heparin is NEVER suitable for coagulation studies.

3. Citrates (liquid)

Soluble citrates are salts of citric acid. They prevent coagulation by forming a double salt with calcium. Sodium citrate is an excellent anticoagulant for coagulation studies. It preserves some coagulation factors (V and VIII) better than oxalate, therefore, it is valuable for factor assays.

The 9:1 ratio (9 parts blood + 1 part anticoagulant) must be adhered to → tubes must be full and testing should be performed within four hours of drawing blood (time can vary depending on specific test ordered).

● Citrated blood is used for protimes (PT), APTTs, fibrinogen level, factor assays, thrombin times, and most other coagulation tests.
CBC PARAMETERS (WBC, RBC, HGB, HCT, RBC INDICES, PLT)

I. Leukocyte (WBC/White blood cell count) measurement - WBCs provide defense by phagocytic or immunologic mechanisms.

A. The total number of white cells are counted by an automated cell counter or may be counted manually. WBC counts are reported in thousands/cmm to the nearest hundred if no decimal, e.g. 4,900/cmm, and to the nearest tenth if a decimal, e.g. 4.9 K/uL or 4.9 x 10^9/uL (conventional units) or 4.9 x 10^9/L (SI units). **The total WBC count does not distinguish WBC types.**

B. Normal reference ranges and significance

<table>
<thead>
<tr>
<th>WBC Count Reference Ranges</th>
<th>Vary with age and testing procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>4.0-11.0 x 10^9/uL</td>
</tr>
<tr>
<td>Newborn</td>
<td>9.0-30.0 x 10^9/uL</td>
</tr>
<tr>
<td>Children (1-10 yo)</td>
<td>6.0-17.0 x 10^9/uL</td>
</tr>
</tbody>
</table>

1. Normals vary with age and testing procedure.

2. Significance
   a. **Leukopenia** - WBC value below normal
      - ↓ WBC production or ↑ WBC use; ↑ infection risk
   b. **Leukocytosis** - WBC value above normal
      - ↑ WBC production; response to infection, leukemia

3. Critical/life-threatening values: WBC <1,000/uL or > 40,000/uL *(varies with institution)*

4. Diurnal variation - WBC values are lower in the morning and higher in the afternoon.

C. Sources of error in counting WBCs

1. Pre-analytic/collection errors, e.g., clotted, hemodilution, hemoconcentration, wrong ID
2. Analytic/technical errors, e.g., instrument malfunction or manual cell counting errors

D. To assess the accuracy of automated or manual WBC counts, the number of WBCs can be estimated from the blood smear. The WBC estimate should correlate ± 20% with the total WBC count if results are accurate.

II. Erythrocyte (RBC/Red blood cell count) measurement - RBCs are vehicles for carrying hemoglobin and function to transport oxygen and remove CO₂.

A. The total number of red cells are counted by automated cell counters and reported in millions/uL to the nearest **hundredth**, e.g. 5.62 M/cmm or 5.62 x 10^6/uL or 5.62 x 10^9/L (SI units).

B. Sources of error in counting RBCs

1. Pre-analytic/collection errors, e.g., clotted, hemodilution, hemoconcentration, hemolysis
2. Analytic/technical errors, e.g., instrument malfunction

III. Hemoglobin (HGB) measurement - Each red cell contains 200-300 million hemoglobin molecules which transport O₂. Hemoglobinometry is the measurement of the concentration of hemoglobin in whole blood, reported in g/dL and to the nearest tenth. This is NOT the same as a free plasma hemoglobin test.

A. Hemoglobin concentration is determined using the cyanmethemoglobin method:

1. **Modifications of this reference method are used almost universally**, utilizing photometric semi-automated or fully-automated instruments. **Drabkin’s reagent** causes red cell lysis, release of hemoglobin, and conversion of hemoglobin to a stable pigment called cyanmethemoglobin.

2. Photometry - the absorbance of cyanmethemoglobin at 540 nm is directly proportional to the concentration of hemoglobin present in the blood. **All clinically significant forms of hemoglobin are measured** by this method (oxyhemoglobin, deoxyhemoglobin, methemoglobin, carboxyhemoglobin).
3. EDTA whole blood is diluted in Drabkin’s reagent which contains ferricyanide (FeCN) and potassium cyanide (KCN). Standards of known hemoglobin concentration are used for calibration.

\[
\text{Released Hgb + ferricyanide} \rightarrow \text{methemoglobin + potassium cyanide (Fe+2) oxidized (Fe+3)} \rightarrow \text{cyanmethemoglobin (stable pigment)}
\]

Read at 540 nm

4. The HemoCue photometer uses a dry reagent system to determine the concentration of azide methemoglobin photometrically and gives a digital display of hemoglobin in g/dL. Instrument calibration checks and control samples are run daily.

B. Sources of hemoglobin error
1. Pre-analytic/collection errors, e.g., clotted, hemoconcentration, hemodilution, wrong ID
2. Hemolysis has NO affect on Hgb measurement due to red cell lysis by the reagent
3. Analytic/technical errors, e.g., not mixing blood tube before obtaining specimen aliquot, sample mixup, instrument malfunction

C. Interference with photometric measurements causes falsely high hemoglobin results:
1. Lipemic blood (↑ fat/lipids) or icteric blood (↑ bilirubin) causes cloudiness.
2. Extremely high WBC count causes cloudiness. WBCs are present when hemoglobin is measured but normally at a concentration that does not interfere.
3. Invalid HGB results are detected by checking data correlation between the hemoglobin and hematocrit values.

IV. Hematocrit (HCT) or Packed Cell Volume (PCV) measurement

The hematocrit (HCT) is defined as the percentage of erythrocytes in a given volume of whole blood. The HCT obtained on automated instruments is reported to the nearest tenth. The manual spun hematocrit is the volume of packed red cells in a given volume of whole blood = HCT/PCV % and reported to nearest 0.5%.

Manual microhematocrit method - uses little blood and is very reproducible.
A. Whole blood anticoagulated with EDTA or heparin is acceptable.
   1. Fingerstick - draw treated tubes coated with heparin.
   2. Use untreated tubes that contain no additive for blood already anticoagulated (want to avoid excess anticoagulant).

B. Microhematocrit tubes are filled about 2/3 full with whole blood, sealed with clay, and centrifuged 5 mins @ 10-15,000 rpm. Centrifugation separates the blood into layers:
   1. The RBCs will be packed in the bottom of tube = HCT %.
   2. Above the red cells is a layer of white cells and platelets called the buffy coat, and above this is the plasma.
      a. The buffy coat will be large when WBC and/or PLT counts are increased.
      b. Plasma appearance can reveal patient conditions such as lipemia (creamy/white), bilirubinemia (dark yellow) or hemolysis (red/pink).
   3. Conditions influencing the degree of red cell packing include the radius of the centrifuge head, speed (rpm's) and duration of centrifugation. These must be adequate for maximal packing of the RBC’s with minimal amounts of plasma trapped among the red cells. Additional centrifugation does not reduce RBC packing. Centrifuge speed, the timer, and packing must be periodically checked.
C. Sources of error for manual spun hematocrits
   1. Pre-analytic/collection errors, e.g., clotted or hemolyzed blood, hemoconcentration, hemodilution from IV’s or tissue fluid contamination if drawn by fingerstick, ID error
   2. EDTA tube filled less than 1/2 full with blood has excess EDTA which causes red cell shrinkage and a falsely low manual spun HCT result.
   3. Analytic errors/improper testing technique
      a. Blood will leak out of tubes if not properly sealed with clay
      b. Not mixing blood sufficiently before filling HCT tubes or a sample mixup
      c. Improper use of HCT card reader → control will not be within acceptable range
      d. Including the buffy coat in the HCT reading can cause falsely high HCT results
      e. Mechanical error – insufficient centrifuge speed or time causes falsely high HCT results if maximal red cell packing is not achieved

<table>
<thead>
<tr>
<th>Complete Blood Count (CBC without Diff/Hemogram)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>7.2</td>
</tr>
</tbody>
</table>

V. The RBC, HGB, and HCT measurements parallel each other:

- Normal is RBC count, HGB, and/or HCT values within the reference range for age and sex.
- Anemia is a decrease in RBC, HGB and/or HCT values below normal for age and sex.
- Polycythemia/Erythrocytosis is an increase in RBC, HGB, and/or HCT above normal for age and sex.

A. Normal reference values vary with age and sex

<table>
<thead>
<tr>
<th>RBC Count, Hemoglobin, Hematocrit Reference Ranges</th>
<th>Vary with sex, age, altitude and testing procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>RBC Count: 4.40-5.80 million/cmm, HGB: 13.0-17.0 g/dL, HCT: 37.0-51.0 %</td>
</tr>
<tr>
<td>Females</td>
<td>RBC Count: 3.80-5.20 million/cmm, HGB: 11.5-15.5 g/dL, HCT: 35.0-46.0 %</td>
</tr>
<tr>
<td>Newborns</td>
<td>RBC Count: 5.00-6.50 million/cmm, HGB: 14.0-25.0 g/dL, HCT: 44.0-64.0 %</td>
</tr>
<tr>
<td>Children (1-10 yo)</td>
<td>RBC Count: 3.70-5.50 million/cmm, HGB: 11.0-14.0 g/dL, HCT: 34.0-42.0 %</td>
</tr>
</tbody>
</table>

B. Significance of RBC, HGB, and HCT measurements

1. Anemia - RBC, HGB, and/or HCT value below normal; ↓ O₂ transport ability
   - ↓ RBC production, ↑ RBC loss/destruction

2. Polycythemia/Erythrocytosis - RBC, HGB, and/or HCT value above normal
   - ↑ RBC production

3. Critical values: HGB <7.0 g/dL or HGB >18.5 g/dL (varies with institution)

4. Diurnal variation - RBC, HGB, and HCT values are higher in the morning and lower in the evening.
C. Adults

**CBC parameters:**

<table>
<thead>
<tr>
<th></th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC thousands/cmm</td>
<td>6.9</td>
<td>20.4</td>
<td>4.2</td>
</tr>
<tr>
<td>RBC millions/uL</td>
<td>4.61</td>
<td>1.95</td>
<td>7.05</td>
</tr>
<tr>
<td>HGB g/dL</td>
<td>13.7</td>
<td>5.8</td>
<td>18.8</td>
</tr>
<tr>
<td>HCT %</td>
<td>41.2</td>
<td>17.2</td>
<td>58.4</td>
</tr>
<tr>
<td>MCV fl</td>
<td>89.4</td>
<td>87.8</td>
<td>82.8</td>
</tr>
<tr>
<td>MCH pg</td>
<td>29.6</td>
<td>29.6</td>
<td>26.6</td>
</tr>
<tr>
<td>MCHC %</td>
<td>33.1</td>
<td>33.7</td>
<td>32.2</td>
</tr>
<tr>
<td>RDW %</td>
<td>11.4</td>
<td>13.5</td>
<td>12.1</td>
</tr>
<tr>
<td>PLT thousands/cmm</td>
<td>250</td>
<td>180</td>
<td>340</td>
</tr>
</tbody>
</table>

**Which patient has:**

a. Polycythemia  
b. Leukocytosis & anemia  
c. Normal WBC, RBC, HGB, HCT

VI. Correlation/relationship between RBC, HGB, and HCT measurements

A. Application of the correlation/relationship between the RBC, HGB, and HCT values can be used to estimate values OR to check the accuracy of these values. The basic equations are:

1. HGB x 3 = HCT ± 3 % - most commonly used equation  
   (3 % = 3 hematocrit percentage points)
2. RBC x 3 = HGB
3. RBC x 9 = HCT

B. These equations can be used to estimate values:

If the RBC = 3.00 million/cmm:
...the approximate HGB is 9.0 g/dL (3 x 3.00 = 9.0)
...the approximate HCT is 27.0% (9 x 3.00 = 27.0)
(Estimated RBC, HGB, or HCT values are not reported.)

C. RBC, HGB, and HCT values should correlate approximately using these equations if the red cells are normal in size and hemoglobin content which can be verified by inspection of the blood smear.

Example of values which correlate:

RBC = 5.00 million/cmm (3 x 5.00 = 15.0; 9 x 5.00 = 45.0)
HGB = 15.0 g/dL (15.0 x 3 = 45.0 ± 3 %)
HCT = 46.0% (falls within 42.0 to 48.0 %)

D. If the red cells are normal in size/hgb content but the values do not correlate, a problem exists and corrective action should be taken before reporting any results.

●Data correlation checks are done to prevent errors in the HGB measurement, HCT value and/or RBC count. Errors may involve the patient specimen (interferences), indicate an instrument malfunction, or be due to improper testing techniques such as labeling errors.

Which parameter does not correlate?

RBC = 4.00 million/cmm
HGB = 12.2 g/dl
HCT = 42.0%

E. NOTE: These ‘rules’ only apply to samples with normal erythrocytes. If the red cells are abnormal in size/hgb content (e.g., microcytic or macrocytic), the RBC, HGB, and HCT values will NOT always conform to these equations (most often it is the RBC that does not correlate).
Clinical Hematology

I. Erythrocyte Indices

A. RBC indices define the size and hemoglobin content of erythrocytes and include the MCV (mean cell volume), MCH (mean cell hemoglobin), and MCHC (mean cell hemoglobin concentration).
   1. RBC indices are calculated using accurately determined RBC, HGB, and HCT values.
   2. Invalid if there is an error in the RBC, HGB and/or HCT values used for calculation.

B. RBC indices, specifically the MCV and MCHC, are used to “describe” the red cells and classify types of anemia. The indices correlate with the appearance of red cells seen on the blood smear.
   1. According to the MCV, red cell size may be normal (nromocytic), small (microcytic), or large (macrocytic). Normal red cells are about the same size as the nucleus of a small lymphocyte.
   2. According to the MCHC, red cells may have normal hemoglobin concentration (normochromic), low hemoglobin concentration (hypochromic) or are spherocytes (no pallor area). The term hyperchromic is NOT used.

C. Generally, a normal RBC population is homogeneous with red cells that are uniform in size. Wide variations in red cell size due to a heterogeneous RBC population is called anisocytosis. RBC indices are mean/average values so have less meaning when RBC size variations are present.

II. MCV - Mean Cell Volume = Hematocrit % x 10 \[\text{fL}\] 
   RBC in millions/cmm

A. The result expresses the average/mean volume of erythrocytes in femtoliters (fL).
   1. Normal adult erythrocytes have an MCV of 82.0-98.0 fL...results between 82 and 98 indicate the red cells are normocytic.
   2. Results below 82 indicate the erythrocytes are microcytic.
   3. Results above 98 indicate the erythrocytes are macrocytic.
   4. Normal newborns often have MCV results around 110 fL and normal children often have MCV results around 77 fL.

B. The MCV is a mean volume measurement so it is important to interpret the reliability of the MCV along with an inspection of the blood smear. A wide variation in red cell sizes, with microcytic, macrocytic and/or normocytic red cells, can result in an MCV in the normal range (falsely normal).
   1. In this case, the RDW (Red cell Distribution Width) can be a valuable parameter since it is an index of variation in red cell sizes; the RDW is reported by automated cell counters.
   2. A normal/low RDW <14% indicates uniform RBC size, a homogeneous RBC population.
   3. An abnormal/high RDW >14% indicates variations in red cell size and the presence of anisocytosis due to a heterogeneous RBC population. The degree of anisocytosis is considered significant when the RDW is >22%.

III. MCH - Mean Cell Hemoglobin = Hgb in g/dl x 10 \[\text{pg}\] 
   RBC in millions/uL

A. The MCH result gives the average weight of hemoglobin per erythrocyte in picograms (pg). This indice varies with both size and hemoglobin content of the red cells. The MCH is not used to describe the red cells and is not too useful.
   1. A normal MCH in adults is 27.0-33.0 pg...results between 27 and 33 indicate the erythrocytes are normocytic and normochromic [or rarely macrocytic and hypochromic].
   2. Results below 27 indicate the red cells are microcytic and/or hypochromic, i.e. erythrocytes are microcytic and hypochromic or microcytic and normochromic or normocytic and hypochromic. Stated another way, if red cells are microcytic, the MCH is decreased due to decreased cell volume. If microcytic red cells are also hypochromic, the MCH is further decreased.
   3. Results above 33 indicate macrocytic and normochromic red cells (due to increased size).
B. High MCH values are obtained in uncomplicated macrocytic anemias. For the same reason, the MCH is higher in the newborn since their MCV is generally higher than adults. MCH values are consistently below normal in anemias with microcytic and hypochromic erythrocytes.

C. In most cases, changes in the average size of RBCs (MCV) parallel similar changes in the weight of hemoglobin in the RBCs (MCH). Consequently, the MCV and MCH show similar variations.

IV. **MCHC - Mean Cell Hemoglobin Concentration**

\[ \text{MCHC} = \frac{\text{Hgb in g/dl} \times 100}{\text{Hematocrit} \%} \]

A. The result expresses the average/mean concentration of hemoglobin per erythrocyte in percent. The MCHC may also be reported in g/dL (SI units).

1. Normal adult erythrocytes contain 32.0-36.0% hemoglobin.....results between 32 and 36 indicate the erythrocytes are normochromic.
2. Results below 32 indicate the erythrocytes are hypochromic.
3. An MCHC result above 36% should ONLY occur when spherocytes are present.

B. The normal red cell contains all the hemoglobin molecules it can, making a higher than normal hemoglobin concentration impossible unless the red cell is a spherocyte. On the other hand, regardless of erythrocyte size, the cell may have a low hemoglobin concentration, indicating that such a red cell is hypochromic.

C. An MCHC above 36.0% due to the presence of spherocytes is a valid cause for a high MCHC.

1. The MCHC can also be a valuable troubleshooting tool since MCHC results over 36% are often due to ERROR caused by the patient sample. The presence of lipemia, bilirubinemia or RBC agglutination can cause erroneous HGB, HCT and/or RBC results.
2. In these situations, the HGB and HCT values won’t match and the calculated MCHC result is over 36.0% due to an error in the HGB or HCT values.

V. **Calculation**

When calculating RBC indices, drop the units of the RBC, HGB & HCT values and report indices to the nearest tenth, with proper units. **Use MCV and MCHC values to describe the red cells** as to cell size and Hgb content. Example: RBC = 5.00 million/cmm, HGB = 15.0 g/dl, HCT = 45.0%

\[ \text{MCV} = \frac{\text{HCT} \times 10}{\text{RBC}} = 45.0 \times 10/5.00 = 90.0 \text{ fL} \] (82.0-98.0 fL)

\[ \text{MCH} = \frac{\text{HGB} \times 10}{\text{RBC}} = 15.0 \times 10/5.00 = 30.0 \text{ pg} \] (27.0-33.0 pg)

\[ \text{MCHC} = \frac{\text{HGB} \times 100}{\text{HCT}} = 15.0 \times 100/45.0 = 33.0\% \] (32.0-36.0%)

**Evaluation:** Normocytic and Normochromic red cells

VI. **Summary**

A. MCV - Volume
B. MCH - Mass or Weight (amount of hemoglobin in the red cell)
   Affected by both RBC size and HGB concentration so not too useful; tends to parallel the MCV.
C. MCHC - Concentration (% of red cell that is hemoglobin)
D. RDW – Size variation index (anisocytosis); generated by automated cell counters

F. **Adults CBC parameters:**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>1.0</td>
<td>16.8</td>
<td>8.0</td>
</tr>
<tr>
<td>RBC</td>
<td>2.90</td>
<td>3.57</td>
<td>2.35</td>
</tr>
<tr>
<td>HGB</td>
<td>8.8</td>
<td>8.2</td>
<td>10.0</td>
</tr>
<tr>
<td>HCT</td>
<td>26.9</td>
<td>25.7</td>
<td>27.2</td>
</tr>
<tr>
<td>MCV</td>
<td>92.8</td>
<td>72.0</td>
<td>124.0</td>
</tr>
<tr>
<td>MCH</td>
<td>30.2</td>
<td>23.0</td>
<td>42.6</td>
</tr>
<tr>
<td>MCHC %</td>
<td>32.7</td>
<td>31.8</td>
<td>34.4</td>
</tr>
<tr>
<td>RDW %</td>
<td>13.0</td>
<td>17.2</td>
<td>11.6</td>
</tr>
<tr>
<td>PLT</td>
<td>25.0</td>
<td>602</td>
<td>200</td>
</tr>
</tbody>
</table>

**Which patient has:**

a. Macrocytic anemia  b. Normocytic anemia  c. Microcytic anemia
Clinical Hematology

CBC PARAMETERS (WBC, RBC, HGB, HCT, RBC INDICES, PLT)

I. **Platelet (PLT) measurement** - Platelets (thrombocytes) play an important role in blood coagulation.

   A. The total number of platelets are counted by automated cell counters or may be counted by manual methods. Platelet counts are reported in thousands/cmm to the nearest hundred, e.g., 400,000/uL or 400 K/cmm or 400 x 10^3/uL or 400 x 10^9/L (SI units).

   B. Normal reference range and significance
      1. Normal is 150,000-450,000/cmm or 150-450 K/uL
      2. Significance
         a. **Thrombocytopenia** - PLT count below normal
            - ↓ PLT production, ↑ PLT use
         b. **Thrombocytosis** - PLT value above normal
            - ↑ PLT production
         c. **Critical**: PLT < 40,000/cmm or > 1 million/cmm *(vary with institution)*
            Patients with platelet counts <20 K/uL can have severe & spontaneous bleeding episodes.

<table>
<thead>
<tr>
<th>WBC thousands/uL</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC millions/cmm</td>
<td>2.76</td>
</tr>
<tr>
<td>HGB g/dl</td>
<td>8.2</td>
</tr>
<tr>
<td>HCT %</td>
<td>24.4</td>
</tr>
<tr>
<td>MCV fL</td>
<td>88.1</td>
</tr>
<tr>
<td>MCH pg</td>
<td>29.6</td>
</tr>
<tr>
<td>MCHC %</td>
<td>33.6</td>
</tr>
<tr>
<td>PLT thousands/uL</td>
<td>15</td>
</tr>
</tbody>
</table>

   C. Sources of error in counting platelets
      1. Pre-analytic/collection errors, e.g., hemodilution, hemoconcentration, patient ID error, clotted blood or heparinized blood
      2. A major source of error is false low platelet counts caused by platelet clumping:
         a. *Partially clotted EDTA blood due to a bad draw or improper mixing with the anticoagulant → platelet clumps and/or fibrin strands may be seen on the blood smear (edges).* The tube should be checked for clots and the blood sample must be REDRAWN if clotted.
         b. Heparinized blood - heparin is an unacceptable anticoagulant for platelet counts (causes platelet clumping) and should be rejected.
         c. Certain patients may exhibit EDTA-induced platelet clumping or EDTA-induced *platelet satellitism* in which the platelets stick to neutrophils.
            [For EDTA-induced clumping or satellitism, blood is redrawn in sodium citrate]
      3. Analytic/technical errors, e.g., instrument malfunction or manual cell counting errors

   D. To assess the accuracy of automated or manual PLT counts, the number of platelets can be estimated from the blood smear. The PLT estimate will correlate with the platelet count if results are accurate.
      1. Since platelets are the hardest cell type to count, all critically low or ‘flagged’ automated platelet counts are verified microscopically with a platelet estimate from the blood smear.
      2. To accept the platelet count (either automated or manual), it must agree with the platelet estimate from the blood smear as follows:
         a. ± 20% if PLT count is ≥ 50,000/cmm
         b. ± 10,000 if PLT count is < 50,000/cmm
      3. If the smear platelet estimate does not correlate with the platelet count, a problem exists that must be resolved...repeat platelet count, redo platelet estimate, redraw sample, etc.
MANUAL CELL COUNTING

Manual cell counts may be performed to verify a result “flagged” by the automated cell counter or done when a discrepancy exists between the instrument count and findings obtained on the blood smear. Cell counts are done manually with the use of a hemocytometer and dilutions of EDTA whole blood or dilutions of body fluids such as cerebrospinal fluid, pericardial fluid, peritoneal fluid, pleural fluid and synovial fluid.

● The principle is the same for counting leukocytes, erythrocytes, and platelets, however, the dilution, diluting fluid, or area counted can VARY.

A. Neubauer hemocytometer - dimensions
1. Each hemocytometer has two counting chambers, one on each side. When focusing, it may be helpful to focus on the groove, then move in to the counting chamber.
2. Each counting chamber is 3mm x 3mm (area of 9mm²).
3. Each chamber is divided into 9 squares - each square is 1mm x 1mm (area of 1mm²).
4. Each of four corner squares are divided into 16 smaller squares.
5. The center square (1mm²) is divided into 25 smaller squares bordered by double-ruled lines.
   a. Each of the 25 small squares in the center square is 1/5mm per side (area of 1/25mm²).
   b. Each of these 25 small squares is further subdivided into 16 smaller squares.
   c. Divisions (smaller squares within squares) help counting navigation.
6. The chamber is made so it is recessed and when a coverslip is placed over the counting chamber area there is a depth of 0.1mm.
7. Each chamber has a total area of 9mm² (3mm x 3mm).
8. Each chamber has a total volume of 0.9mm³.
   ▶ Chamber DIMENSIONS = 3mm x 3mm x 0.1mm.
9. The area (# of squares) to count and/or the blood dilution to make is determined by the number of cells present. Results may not be accurate if an insufficient area with too few cells is counted.
   a. When cells are numerous (millions), the cell dilution is larger and/or the area counted is smaller; fewer cells (thousands) requires a smaller dilution and/or a larger area counted.
   b. Cell size determines the magnification (objective lens) to use for counting.
B. **STANDARD FORMULA** to obtain # of cells/mm$^3$ (cmm) or # cells/uL.

1. Must correct for dilution used and area (squares) counted - this can **VARY**.

2. Standard formula:

   $$\text{cells/cmm} = \frac{\text{cells counted (both sides)} \times \text{dilution factor} \times \text{depth mm} \times \text{area counted sqmm (both sides)}}{\text{OR}}$$

   $$\text{cells/mm}^3 = \frac{\text{cells counted (both sides)} \times \text{dilution factor} \times \text{depth mm} \times \text{area counted sqmm (both sides)}}{\text{area counted sqmm (both sides)}}$$

   a. Dilution factor - invert dilution used. (If 1:20 use x20; if 1:100 use x100).

   b. Depth of 0.1 mm - use this to obtain # cells/cmm (a volume) rather than # cells/mm$^2$ (an area). Can use a depth factor of "x10" in numerator obtained by inversion of the denominator.

C. Diluents

1. Must be void of extraneous material and must not lyse or damage the cells to be counted.

2. Will often lyse cells not being counted so we can see the cells we want to count.

3. The preferred diluent for performing manual WBC and PLT counts is **1% ammonium oxalate**; the red cells will lyse but WBCs and platelets are left intact.

D. **Manual WBC counts**

Performed using a hemocytometer and a blood dilution prepared using a Thrombo-tic reagent vial.

1. Diluting (follow procedure protocol):
   The vial contains 990 uL of 1% ammonium oxalate (diluent) and 10 uL of blood is added with a capillary pipet $\rightarrow$ **1:100 dilution**. Allow a minimum of 5 minutes for RBC lysis.

2. Plating (follow procedure protocol):
   Place a coverslip on a clean hemocytometer. Plate/load the dilution by filling the entire counting area under the coverslip on each side of the hemocytometer without overfilling. Allow the cells to settle 3 minutes before counting.

3. Counting:
   a. **●Using 10x (low power) objective and LOW light, focus on hemocytometer groove, then move to the counting area.** Check for even cell distribution in counting squares.

   b. For consistency in counting – count dark cells which touch the top and left boundary lines; exclude light cells touching bottom and right lines as shown on diagram below. The diagram also has arrows that show a systemic approach to counting cells.

   c. **●Count cells in all 9 squares on each side of chamber...total area counted = 18 mm$^2$. Use 2 counter/tabulator keys...one key for counting the cells on each side.**

   d. The # of cells counted on each side must agree within $\pm 20\%$ to accept results.

   ![Counting Chamber (one side)](image)

   All 9 squares are counted on each side $= 9 \text{mm}^2 + 9 \text{mm}^2 = 18 \text{mm}^2$. 

Clinical Hematology
Standard formula: \( \# \text{cells (both sides)} \times \text{dilution factor} \times \text{depth} \times \text{area counted (both sides)} \)

4. Calculations and reporting:
   a. \( \text{WBC/cmm} = \frac{\# \text{cells (both)} \times 100}{0.1 \text{ mm} \times 18 \text{ sqmm}} \) or \( \frac{\text{total \# cells} \times 100 \times 10 \text{ mm}}{18 \text{ sqmm}} \)

   Report WBC counts to nearest hundred if no decimal or nearest tenth if a decimal, including proper units.

   b. Example: 1:100 dilution, counted cells in all squares on both sides

   \[
   \frac{105 \times 100 \times 10}{18} = 5,833 \rightarrow 5,800/\text{cmm} \text{ (rounded)}
   \]

   Side 1: 50
   Side 2: 55

   (Sides agree ± 20%)

   or \( 5.8 \times 10^3/\mu\text{L} \)

   or \( 5.8 \times 10^9/\text{L} \) (SI units)

   c. Cannot easily interpret WBC values if the WBC count is reported \( x \times 10^4 \) or \( x \times 10^5/\text{cmm} \), therefore, do NOT report WBC counts in this manner.

5. Manual WBC counts may be performed when the WBC count/\( \mu\text{L} \) is flagged as doubtful by the automated instrument and the WBC estimate from the blood smear doesn’t agree.

6. Sources of error
   a. Pre-analytic/specimen collection errors, e.g., clotted blood, hemolysis, hemoconcentration, etc.
   b. Analytic/technical errors
      1) Failure to mix blood specimen before taking blood aliquot or dilution is wrong due to poor diluting technique (bubbles in pipet or failure to wipe excess blood from pipet).
      2) Improperly filled chamber…cells counted on two sides won’t match \( \rightarrow \) must repeat.
      3) Wrong dilution or calculation; look at calculated result and check for proper units and reporting. Is the result believable??
      4) Dirty counting chamber, counting artifact as cells, counting cells in wrong area or wrong counting caused by incorrect light adjustment (won’t see any cells if light is too high).

E. Manual PLT Counts

Platelets are more difficult to count than WBCs and RBCs because of their small size. They stick to foreign surfaces and to each other, and are hard to distinguish from debris. Manual PLT counts are performed using a hemocytometer and a blood dilution prepared using a Thrombo-lic reagent vial.

1. Diluting (follow procedure protocol): The vial contains 990 \( \mu\text{L} \) of 1% ammonium oxalate (diluent) and 10 \( \mu\text{L} \) of blood is added with a capillary pipet \( \rightarrow \) 1:100 dilution. Allow a minimum of 5 minutes for RBC lysis.

2. Plating (follow procedure protocol): Place a coverslip on a clean hemocytometer. Plate/load the dilution by filling the entire counting area under the coverslip on each side of the hemocytometer without overfilling. Allow the cells to settle 10 minutes before counting. (The reference PLT method uses a phase microscope)

3. Counting:
   a. First use the 10 objective to focus on hemocytometer groove, then move to the counting area. ●Using 40x (high dry power) objective and LOW light, (adjust light for best contrast), check for even cell distribution in counting squares. You will see WBCs and if present, nucleated RBCs; you might see occasional unlysed red cells.

   ►When counting platelets, focus up and down; platelets appear greenish, NOT shiny.

   b. For consistency in counting – count dark cells which touch the top and left boundary lines; exclude light cells touching bottom and right lines.
c. **Count cells in the entire center 1 sq mm on each side** of chamber (count all 25 squares)...total area counted = 2 mm$^2$. Use 2 counter/tabulator keys...one key for counting the cells on each side.

d. The # of cells counted on each side must agree within $\pm 20\%$ to accept results.

(Center sqmm is divided into 25 squares with each subdivided into 16 squares)

All 25 squares of center square are counted on each side $= 1\text{mm}^2 + 1\text{mm}^2 = 2\text{mm}^2$.

Standard formula: $\frac{\#\text{cells (both sides)} \times \text{dilution factor}}{\text{depth} \times \text{area counted (both sides)}}$

4. **Calculations and reporting:**

a. $\text{PLT/cmm} = \frac{\# \text{cells (both) x 100 x 10 mm}}{2 \text{ sqmm}}$ or $\frac{\text{total } \# \text{ cells x 100}}{0.1\text{mm x 2sqmm}}$

Report to nearest thousand including units.

b. **Example:** 1:100 dilution, counted cells in center square on both sides

<table>
<thead>
<tr>
<th>Side 1</th>
<th>200</th>
<th>$410 \times 100 \times \frac{10}{2} = 205,000/\text{cmm}$ or $205 \times 10^3/\text{uL}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Side 2</td>
<td>210</td>
<td>$\frac{\text{Agrees $\pm 20%$}}{2}$ or $205 \text{ K/uL}$ or $205 \times 10^9/\text{L (SI units)}$</td>
</tr>
</tbody>
</table>

5. Manual platelet counts may be performed when the instrument has "flagged" the PLT count as doubtful and the PLT estimate from the blood smear does not agree with the automated platelet count/uL. For example, red cell fragments (called schistocytes) are being counted as platelets by the automated cell counter causing a falsely high platelet count.

6. **Sources of error**

a. Pre-analytic/specimen collection errors, e.g., clotted blood (clumped platelets), hemolysis, hemoconcentration, hemodilution, etc.

b. Analytic/technical errors

1) Failure to mix blood specimen before taking blood aliquot or dilution is wrong due to poor diluting technique (bubbles in pipet or failure to wipe excess blood from pipet).

2) Improperly filled chamber or has dried up...cells counted on the two sides won't match $\rightarrow$ must repeat.

3) Wrong dilution or calculation; look at calculated result and check for proper units and reporting. *Is the result believable??*

4) Dirty counting chamber, counting artifact as cells, counting cells in wrong area or wrong counting caused by incorrect light adjustment. A **clean chamber is especially important when performing manual PLT counts to prevent counting junk as platelets.**
F. Manual RBC counts - Example only

1. Manual RBC counts are not done on EDTA whole blood, HOWEVER, cell counts on bloody body fluids (spinal fluid) may require making a RBC dilution of 1:200 with isotonic saline (which prevents lysis of RBCs). Red cells in the RBC counting area of 0.4mm² are counted (5 small squares in the center square of the hemocytometer on each side).

2. Procedure
   a. MIX EDTA whole blood specimen. Make a 1:200 dilution with saline and mix.
   b. Plate dilution on each side of chamber and allow cells to settle 3 mins.
   c. Count 4 outer squares of center sqmm and the center square of center sqmm (shaded squares on chamber) on each side of the hemocytometer using 40x objective and low light.
   d. •Total area counted = 0.4 mm² → RBC counting area.

   Center sqmm is divided into 25 squares; each square in center square is 1/25mm²;
   5/25mm² counted on each side = 10/25mm²
   OR 1/5mm² + 1/5mm² = 2/5mm² = 0.4mm².

   e. Calculations:

   \[
   \text{RBC/cmm} = \frac{\#c\text{ells (both sides)} \times 200}{0.4 \text{ sqmm}} \quad \text{or} \quad \frac{\text{total \#cells} \times 200 \times 10}{0.1 \text{ mm} \times 0.4 \text{ sqmm}}
   \]

   Report to nearest hundredth.

   f. Example:

   Side 1  305 620 \times 200 \times 10 = 3.10 \text{ million/cmm} or 3.10 \times 10^6/\mu\text{L}
   Side 2  315 0.4 \quad \text{or} \quad 3.10 \text{ M/\mu\text{L}} \quad \text{or} \quad 3.10 \times 10^{12}/\text{L (SI units)}

SEE Calculations sheet for formulas, calculation examples and reporting units.