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 10^3/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
      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 10⁶/uL or 5.62 x 10¹²/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 plasma hemoglobin test.

   Hemoglobin methods:
   A. Copper Sulfate - screening method used for potential blood donors.
      1. Based on specific gravity - drops of blood will rise or fall in copper sulfate solutions of known specific gravity.
      2. Fast, simple; accuracy depends on number of solutions used.
   B. **Cyanmethemoglobin** - modifications of this reference method are used almost universally.
      1. Hemoglobin is measured by photometric semi-automated or fully-automated instruments. *Drabkin’s* cyanmethemoglobin 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 (oxyhgb, deoxyhgb, methgb, carboxyhg). 

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.

```
Released Hgb + ferricyanide → methemoglobin → KCN
(Fe+2) oxidized (Fe+3)
```

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.

C. 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

D. Interference with photometric measurements causes falsely high hemoglobin results:
1. Lipemic blood with ↑ fat/lipids or icteric blood with ↑ bilirubin **causes clouddiness.**
2. Extremely high WBC count **causes clouddiness.** 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

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>RBC Count</td>
<td>HGB</td>
</tr>
<tr>
<td>Males</td>
<td>4.40-5.80 million/cmm</td>
</tr>
<tr>
<td>Females</td>
<td>3.80-5.20 million/cmm</td>
</tr>
<tr>
<td>Newborns</td>
<td>5.00-6.50 million/cmm</td>
</tr>
<tr>
<td>Children (1-10 yo)</td>
<td>3.70-5.50 million/cmm</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**

<table>
<thead>
<tr>
<th>CBC parameters:</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>
</tbody>
</table>

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)

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).
**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 and are reported to the nearest **tenth**.
2. • RBC indices are invalid if there is an error in the RBC, HGB and/or HCT values used for calculation.

B. RBC indices are used to classify types of anemia and correlate with the characteristics of red cells observed on the blood smear.

1. According to the MCV, red cell size may be normal (normocytic), 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) or low hemoglobin concentration (hypochromic). The term **hyperchromic** is **NOT** used.

C. RBC indices are average/mean values so have less meaning when wide variations in RBC size are present called **anisocytosis**.

II. **MCV - Mean Cell Volume**

\[ \text{MCV} = \frac{\text{Hematocrit} \times 10}{\text{RBC in millions/cmm}} \text{ fL} \]

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. It is imperative to interpret the reliability of the MCV along with an inspection of the blood smear, since the MCV is a mean volume measurement.

1. It is possible to have a wide variation in red cell sizes, with microcytic, macrocytic and/or normocytic red cells, and have an MCV in the normal range (MCV is falsely normal).
2. 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.
3. **A normal/low RDW \(<14\%\) indicates uniform RBC size**, a homogeneous RBC population.
4. An abnormal/high RDW >14% indicates variations in red cell size and the presence of **anisocytosis** due to a heterogeneous RBC population (or dimorphism with two RBC sizes).

III. **MCH - Mean Cell Hemoglobin**

\[ \text{MCH} = \frac{\text{Hgb in g/dl} \times 10}{\text{RBC in millions/cmm}} \text{ pg} \]

A. The 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, so not very 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.
3. Results above 33 indicate macrocytic and normochromic red cells.
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 be a valuable troubleshooting tool since MCHC results over 36\% are more often due to **ERROR**. The presence of lipemia, bilirubinemia or RBC agglutination may 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. **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. The "normal" erythrocyte is described as normocytic and normochromic…this is a description of cell size and hemoglobin content.

1. ► **MCV = 82.0-98.0 fl**
2. **MCH = 27.0-33.0 pg**
3. ► **MCHC = 32.0-36.0 \%**

When calculating RBC indices, drop the units of the RBC, HGB & HCT values and report indices to the nearest tenth.

E. **Adults**

<table>
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<tr>
<th>CBC parameters:</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC thousands/cmm</td>
<td>1.0</td>
<td>16.8</td>
<td>8.0</td>
</tr>
<tr>
<td>RBC millions/uL</td>
<td>2.90</td>
<td>3.57</td>
<td>2.35</td>
</tr>
<tr>
<td>HGB g/dL</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 fl</td>
<td>92.8</td>
<td>72.0</td>
<td>124.0</td>
</tr>
<tr>
<td>MCH pg</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 thousands/cmm</td>
<td>25.</td>
<td>602.</td>
<td>200.</td>
</tr>
</tbody>
</table>
**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 10^9/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.

   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 when a parameter is below the automated instrument's linearity, to verify a doubtful result "flagged" by the instrument, or when smear findings don't agree with the automated result. Manual cell counts are performed with the use of a hemocytometer and blood dilutions (or dilutions of body fluids such as cerebrospinal fluid).

- The principle is the same for leukocytes, erythrocytes, and platelets, however, the dilution, diluting fluid, and area counted can VARY. Dilutions are often made using Unopettes.

A. Neubauer hemocytometer - dimensions
   1. Each hemocytometer side has a counting chamber (two). 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 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 number of cells are 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. Size of the cells determines the magnification (objective lens) to use for counting.
B. **Standard Formula** to obtain # of cells/mm³ (cmm) or # cells/uL.

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

2. Standard formula:
   
   \[
   \text{cells/cmm} = \frac{\text{cells counted (both sides) x dilution factor}}{\text{depth mm x area counted sqmm (both sides)}}
   \]
   OR
   
   \[
   \text{cells/mm}^3 = \frac{\text{cells counted (both sides) x dilution factor x depth mm}}{\text{area counted sqmm (both sides)}}
   \]

   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/mm² (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, damage, or agglutinate the cells to be counted.

2. Will often lyse cells not being counted so we can see the cells we want to count, e.g., red cells are lysed to count white cells and platelets.

3. **PLT/WBC unopettes** can be used for manual platelet or manual WBC counts and contain 1% ammonium oxalate (diluent); red cells will lyse but platelets and WBCs are left intact.

D. **Manual WBC counts**

1. Performed with the use of a hemocytometer and a blood dilution usually made with a PLT/WBC unopette. The reservoir contains 1.98 ml of 1% ammonium oxalate (diluent) and 20 ul of blood is added → **1:100 dilution.** RBCs lyse leaving WBCs and PLTs.

2. Procedure:
   
   a. **Diluting and plating**
      
      MIX EDTA whole blood specimen. Using proper technique, dilute a PLT/WBC unopette making a 1:100 dilution. Mix & label unopette; let stand 10 minutes to allow RBC lysis.

   b. **CLEAN** hemocytometer chamber and place coverslip on top. After 10 minutes, remix unopette, convert to dropper and discard a few drops. Plate/load the unopette dilution by placing pipet in groove and slowly squeezing. Smoothly fill the entire area under the coverslip on each side of the chamber without overfilling. Allow cells to settle 3 minutes in petri dish with damp cotton ball before counting.

   c. **Counting**
      
      - 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.

   d. 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. This diagram also has arrows that show a systemic approach to counting cells.

   e. **Count cells in all 9 squares on each side of chamber....total area counted = 18 mm².**
      
      Use 2 counter/tabulator keys…one key for counting the cells on each side.

   f. The # of cells counted on each side must agree within ±20% to accept results.

   ![Counting Chamber Diagram](image)

   For a normal number of WBCs and a 1:100 dilution, 9mm² is counted on each side = 9mm² + 9mm² = **18mm².**
Standard formula: \[ \# \text{cells (both sides)} \times \text{dilution factor} \times \text{depth} \times \text{area counted (both sides)} \]

g. Calculations:

\[
\text{WBC/cmm} = \frac{\# \text{cells (both)} \times 100}{0.1 \text{ mm} \times 18 \text{ sqmm}} \text{ or total } \# \text{ cells} \times 100 \times 10 \text{ mm} = \frac{18 \text{ sqmm}}{
\]

Report WBC counts to nearest **hundred** if no decimal or nearest **tenth** if a decimal.

h. Example:

\[
\begin{align*}
\text{Side 1} & \quad 50 \\
\text{Side 2} & \quad 55 \\
(\text{Sides agree } \pm 20\%) & \\
\end{align*}
\]

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

or \[\frac{5.8 \times 10^{3}}{\mu \text{L}}\]

or \[\frac{5.8 \times 10^{9}}{\text{L}} \text{(SI units)}\]

i. **Cannot easily interpret WBC values if the WBC count is reported x 10^4 or x 10^5/cmm, therefore, WBC counts should not be reported in this manner.**

3. Manual WBC counts may be performed when:

a. WBC is extremely low – make 1:20 dilution (rather than 1:100) and count all 18 squares.

b. WBC is flagged as doubtful by the automated instrument and the WBC estimate from the blood smear doesn’t agree with the automated total WBC result.

4. 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**

1. 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 platelet counts are performed with the use of a hemocytometer and a blood dilution usually made with a PLT/WBC unopette. The reservoir contains 1.98 ml of 1% ammonium oxalate (diluent) and 20 ul of blood is added \( \rightarrow \) 1:100 dilution. RBCs lyse leaving WBCs and PLTs.

2. Procedure (The reference method for manual PLT counts uses a phase microscope)

   a. MIX EDTA whole blood specimen. Using proper technique, dilute a PLT/WBC unopette making a 1:100 dilution. Mix & label unopette; let stand 10 minutes to allow RBC lysis.

   b. CLEAN hemocytometer chamber and place coverslip on top. After 10 minutes, remix unopette, convert to dropper and discard a few drops. Plate/load the unopette dilution by placing pipet in groove and slowly squeezing. Smoothly fill the entire area under the coverslip on each side of the chamber without overfilling. Allow cells to settle 10 mins in petri dish with damp cotton ball before counting.

   Counting

   c. **● Using 40x (high dry power) and LOW light (adjust light for best contrast), focus on hemocytometer groove, then move to the counting area.** Check for even cell distribution in counting squares. You will see WBCs and if present, nucleated RBCs; might see occasional unlysed red cell. **● When counting platelets, focus up and down; platelets appear greenish, NOT shiny.**

   d. For consistency in counting – count dark cells which touch the top and left boundary lines; exclude light cells touching bottom and right lines.
e. Count cells in center 1 square mm on each side of chamber...total area counted = 2 mm². The center square is divided into 25 smaller squares. Use 2 counter/tabulator keys...one key for counting the cells seen on each side.

f. The # of cells counted on each side must agree within ±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 = 1mm² + 1mm² = 2mm².

Standard formula:

\[ \text{#cells (both sides)} \times \text{dilution factor} \times \text{depth} \times \text{area counted (both sides)} \]

\[ \text{PLT/cmm} = \frac{\text{# cells (both)} \times 100 \times 10 \text{ mm}}{2 \text{ sqmm}} \text{ or } \frac{\text{total # cells} \times 100}{0.1 \text{mm} \times 2 \text{sqmm}} \]

Report to nearest thousand.

h. Example:

Side 1 200 410 x 100 x 10 = 205,000/c/mm or 205 x 10³/uL
Side 2 210 2 = 205 K/uL or 205 x 10⁹/L (SI units)

(Agrees ± 20%)

Manual platelet counts may be performed when:

a. PLT count is below the automated instrument's linearity, i.e., a very low number.
b. 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/c/mm. For example, red cell fragments (called schistocytes) are being counted as platelets by the automated cell counter causing a falsely high platelet count.

4. 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 ⇒ 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 0.4 mm² 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.

   ![Image of hemocytometer](image_url)

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

   e. Calculations:

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

   Report to nearest hundredth.

   f. Example:

   Side 1 305  \[ 620 \times 200 \times 10 = 3.10 \text{ million/cmm} \text{ or } 3.10 \times 10^6/\mu\text{L} \]

   Side 2 315  \[ 0.4 \text{ or } 3.10 \text{ M/\muL} \text{ or } 3.10 \times 10^{12}/\text{L (SI units)} \]

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

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**Complete Blood Count with Differential**

<table>
<thead>
<tr>
<th>CBC parameters</th>
<th>WBC</th>
<th>RBC</th>
<th>HGB</th>
<th>HCT</th>
<th>MCV</th>
<th>MCH</th>
<th>MCHC</th>
<th>RDW</th>
<th>PLT</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>8.8</td>
<td>5.01</td>
<td>15.0</td>
<td>44.9</td>
<td>86.6</td>
<td>29.0</td>
<td>33.5</td>
<td>11.5</td>
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</table>

<table>
<thead>
<tr>
<th>Differential (WBC types)</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Eosinophils</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>56%</td>
<td>34%</td>
<td>8%</td>
<td>1%</td>
<td>1%</td>
</tr>
</tbody>
</table>

**Equivalent, Conventional (preferred) & SI Units**

**Equivalent units**
- cmm (cubic millimeters) = mm³ = uL (microliters) = mcL (conventional units)
- thousands/uL = x10⁵/cmm = x10E5/uL = K/cmm

**Examples:**
- WBC 6,400/uL = 6.4 x10⁵/cmm = 6.4 x10E5/uL = 6.4 K/cmm
- PLT 250,000/uL = 250 x10³/cmm = 250 x10³/uL = 250 K/cmm

- millions/uL = x10⁶/cmm = x10E6/uL = M/cmm

**Example:**
- RBC 3.45 million/uL = 3.45 x10⁶/cmm = 3.45 x10E6/uL = 3.45 M/cmm

To convert conventional units (cmm or uL) to SI units (L), use a factor of x10⁶

**Examples:**
- WBC 6.4 x10⁵/uL (x10⁶) = 6.4 x10⁶/L (SI units)
- RBC 3.45 x10⁶/uL (x10⁶) = RBC 3.45 x10⁶/L (SI)
- PLT 250 x10³/uL (x10⁶) = 250 x10³/L (SI = System of International)