NORMALS SHEET

All procedures have *institution-established* reference intervals....a range of values expected for an analyte or parameter in healthy individuals that are based on the population served. Normal reference ranges vary with age, sex, ethnicity, geographical location and/or test methodology. Reference ranges must be determined and verified by each laboratory.

EQUIVALENT UNITS

cmm (cubic millimeters) = mm^3 = uL (microliters) = mcL
thousands/uL = $x10^3$/cmm = x10E3/uL = K/cmm
millions/uL = $x10^6$/cmm = x10E6/uL = M/cmm

NORMAL REFERENCE RANGES — **VALUES VARY, *ESTABLISHED BY INSTITUTION***

WBC	Adult	4.0-11.0 x 10^3/cmm or K/uL			Report to nearest tenth if decimal, e.g. 7.7 x 10^3/uL
	Newborn	9.0-30.0 x 10^3/uL			
	Children (1-10y)	6.0-17.0 x 10^3/cmm			Report to nearest hundred if no decimal, e.g. 7,700/cmm
	(10-20y)	4.5-13.5 x 10^3/cmm			
RBC	Males	4.40-5.80 x 10^6/cmm or M/uL			Report to nearest hundredth
	Females	3.80-5.20 x 10^6/uL			
	Newborns	5.00-6.50 x 10^6/cmm			
	Children (6mo-11y)	3.70-5.50 x 10^6/cmm			
	(12-15y)	3.90-5.70 x 10^6/cmm			
HGB	Males	13.0-17.0 g/dL			Report to nearest tenth
	Females	11.5-15.5 g/dL			
	Newborns	14.0-25.0 g/dL			
	Children (1mo-1y)	10.0-15.0 g/dL			
	(1-10y)	11.0-14.0 g/dL			
HCT	Males	37.0-51.0%			Report to nearest tenth
	Females	35.0-46.0%			
	Newborns	44.0-64.0%			
	Children (1mo-10y)	34.0-42.0%			
MCV		82.0-98.0 fL	Newborns ~110 fL Children ~77 fL		Report all indices to nearest tenth fL = femtoliters; pg = picograms
MCH		27.0-33.0 pg			
MCHC		32.0-36.0% [or g/dL]			
RDW		≤ 14.0%			
PLTS		150-450 x 10^3/cmm or K/uL			Report to nearest thousand, e.g. 150,000/uL or 150 x 10^3/uL
RETICS	Adults	0.5-2.0% (Relative)	Newborns 2.0-6.0%		
		25,000-100,000/cmm or 25-100 K/uL (Absolute)			Report to nearest thousand
ESR	Males	0-10 mm/hr (Westergren)	0-20 mm/hr (>50y)		
	Females	0-20 mm/hr (Westergren)	0-30 mm/hr (>50y)		

DIFF

Adults				
Segs	43-74%	}Neutrophils 45-75%		
Bands	0-10%	Absolute neutrophils	1800-7500/cmm or 1.8-7.5 K/uL	
Lymphs	15-45%	Absolute lymphocytes	1000-3400/cmm	
Monos	1-12%	Absolute monocytes	100-800/cmm	
Eos	0-6%	Absolute eosinophils	50-400/cmm	
Basos	0-2%	Absolute basophils	0-200/cmm	
Children				
Segs	25-70%	(5-16y)		
Bands	5-11%	(7d-14y)		
Lymphs	45-75%	(1-4y)	25-55% (5-14y)	
Monos	1-12%			
Eos	0-8%	(0-14y)		
Basos	0-2%			

PROTHROMBIN TIME (PT)	11.0-13.4 seconds		
APTT	22.0-37.0 seconds		
BLEEDING TIME (BT), TEMPLATE	≤ 8 minutes		
PLATELET FUNCTION ASSAY (PFA)	< 185 seconds		
FIBRINOGEN	150-450 mg/dL		
HGB ELECTROPHORESIS	Hgb A Majority (~97%)	Hgb A_2 2-3.5%	Hgb F <2.5% (> 2yo)
SERUM IRON	50-190 ug/dL		
IRON BINDING CAPACITY	230-400 ug/dL		
FERRITIN	10-100 ng/ml (female < 45y)	25-250 ng/ml (female > 45y)	35-250 ng/ml (male)

CALCULATIONS SHEET

EQUIVALENT, CONVENTIONAL & SI (System of International) UNITS

cmm (cubic millimeters) = mm^3 = uL (microliters) = mcL; conventional units (preferred)
thousands/uL = $x10^3$/cmm = x10E3/uL = K/cmm
millions/uL = $x10^6$/cmm = x10E6/uL = M/cmm
e.g. 5,500/uL = 5.5 $x10^3$/uL = 5.5 K/cmm; 4.33 million/uL = 4.33 $x10^6$/uL = 4.33 M/cmm

To convert conventional units (cmm or uL) to SI units (L), **use a factor of $x10^6$**
e.g. WBC count of 5.5 $x10^3$/uL (**x 10^6**) = 5.5 x 10^9/L (SI units)
e.g. RBC count of 4.33 $x10^6$/uL(**x 10^6**) = 4.33 x 10^{12}/L (SI)
e.g. PLT count of 255 $x10^3$/uL (**x 10^6**) = 255 x 10^9/L (SI)

CORRELATIONS

HGB X 3 = HCT ± 3 3 X RBC = HGB 9 X RBC = HCT
(Data may not correlate when red cells are abnormal, i.e., not normocytic & normochromic)

FORMULAS

MANUAL CELL COUNTS

$$\#\ \text{cells/cmm} = \frac{\text{total \# cells (both sides) x dilution factor x 10\^{} mm (depth)}}{\text{total area counted mm}^2\ \text{(both sides)}}$$

^10 = inversion of 0.1 mm

RBC INDICES

$$\text{MCV (fL)} = \frac{\text{HCT x 10}}{\text{RBC}} \qquad \text{MCH (pg)} = \frac{\text{HGB x 10}}{\text{RBC}} \qquad \text{MCHC (\%)} = \frac{\text{HGB x 100}}{\text{HCT}}$$

RELATIVE RETICS

$\frac{\text{\# retics}}{\text{1000 RBCs}}$ x 100 **OR** $\frac{\text{\# retics in 1000 RBCs}}{10}$ = **Relative Percent reticulocytes (%)**

ABSOLUTE VALUES

Absolute Retic # in thousands/cmm = Retic % x RBC count (millions/cmm)

e.g. 4.0% retic (40 retics in 1000 RBCs) & RBC of 3.01 million/uL gives an absolute retic count of:

$\frac{\text{\# retics}}{\text{1000 RBCs}}$ x RBC/cmm = 0.04 x 3,010,000 = 120,400/uL = 120,000/uL

OR

$\frac{\text{retic \%}}{100}$ x RBC/cmm = $\frac{4.0}{100}$ x 3,010,000 = 120,400/cmm or 120 K/uL or 120 x 10^3/uL

Absolute # of WBC type in thousands/cmm = Differential % x WBC count (thousands/cmm)

e.g. 31% lymphs and WBC count of 8,000/cmm would give an absolute lymphocyte count of:

$\frac{31}{100}$ x 8000 = 2,480/cmm = 2,500/cmm **OR** 0.31 x 8.0 = 2.5 x 10^3/uL

WBC ESTIMATE (from smear)

Find average # WBCs per high power field (HPF); Avg # WBC/HPF x 2,000 = **WBC estimate/uL**

PLT ESTIMATE (from smear)

Find average # of platelets per oil immersion field (OIF):

•Take the 1st 10 PLTs (from Avg # PLT/OIF) x 20,000 each (= 200,000) **PLUS** each additional PLT above 10 x 10,000 each = **PLT estimate/cmm**

PLT COMMENT RANGES

150,000-450,000 - Adequate
100,000-150,000 - Slt decreased
50,000-100,000 - Decreased
<50,000 - Mk decreased
450,000-500,000 - Slt increased
500,000-650,000 - Increased
>650,000 - Mk increased

WBC CORRECTION FOR NUCLEATED RBCS (if needed)

$\frac{100}{100 + \#\text{NRBCs/diff}} = \frac{\text{Corrected WBC/ul}}{\text{Uncorrected WBC/ul}}$ **OR** $\text{Corrected WBC/ul} = \frac{\text{uncorrected WBC/ul x 100}}{100 + \#\ \text{NRBCs/diff}}$

CRITICAL VALUES

VERIFIED, CALLED & DOCUMENTED - *VALUES VARY PER INSTITUTION POLICY*

WBC	< 1,000/cmm OR > 40,000/cmm
HGB	< 7.0 g/dl OR > 18.5 g/dl
PLT	< 40,000/cmm OR > 1,000,000/cmm
Differential	Absolute neutrophil count (ANC) < 500/cmm; Blasts, lymphoma cells, highly unusual findings
PT	INR > 4.0
PTT	> 100 seconds

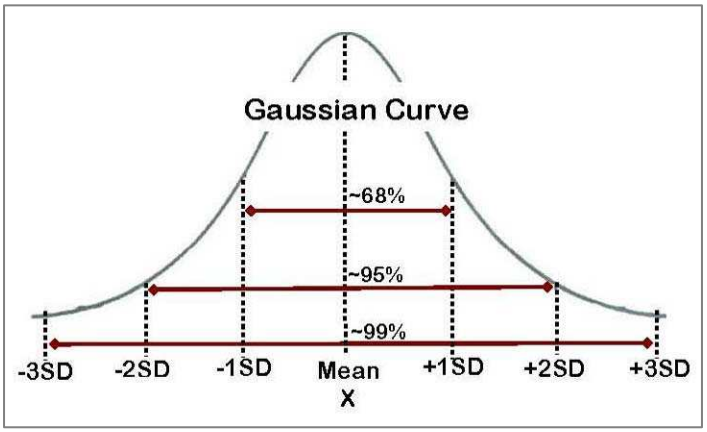
TEST	**INCLUDES:**
CBC without DIFF/Hemagram	WBC, RBC, HGB, HCT, RBC INDICES (MCV, MCH, MCHC, RDW), PLT
CBC with DIFF	WBC, RBC, HGB, HCT, RBC INDICES, PLT, WBC DIFFERENTIAL, CELL MORPHOLOGY

Quality Control (QC)

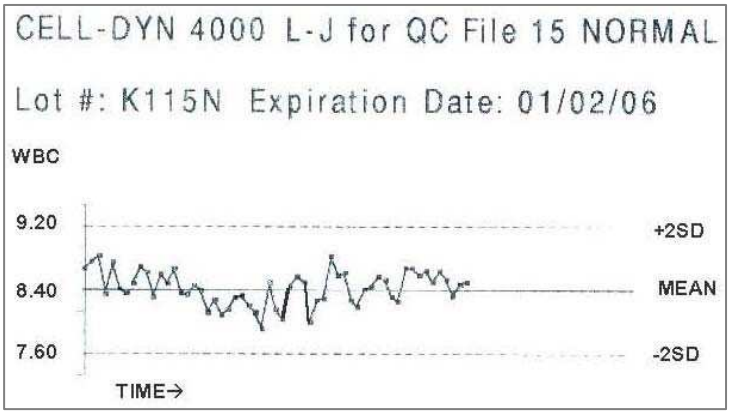
Quality control systems have been developed to guarantee the reliability of measurements and define the significance of patient changes. QC is based on utilizing control samples with *known* values to verify the proper operation of a testing system. The control material has allowable limits of variation established. When values deviate outside the predictable limits, there is strong reason to doubt the validity of results. Besides confirming the accuracy of results, quality control is a process that evaluates all parts of the actual testing process including reagents, equipment/instrument function and individual testing technique.

Terms:

- **Accuracy** – closeness of the measured value to the true value.
- **Precision** – reproducibility of the result, i.e., the spread between replicate determinations.
- **Reliability** – ability to maintain both accuracy and precision.
- **Mean** – average of all observations (X)….determined by assay.
- **Confidence limits/intervals** – range of values from acceptable low to acceptable high limits…usually established at two standard deviations (SD).
- **Gaussian curve** – used to derive standard deviation, which is the dispersion of values around the mean……± 2 standard deviations should include about 95% of results.
- **Levey-Jennings chart** – gives rapid, visual display of quality control data and aids in the detection of "out of control" situations. The chart displays the assay value of controls versus time and shows the control mean value with the range of acceptable control limits.



Levey-Jennings Chart



Standards:

- Pure substance of known composition that is used to standardize/calibrate an instrument or testing procedure**.**

Controls:

- Material whose composition closely resembles unknown patient samples and is tested in conjunction with unknown samples. Purchased from manufacturer. The control material is assayed to determine a known mean or target value.
- Testing often utilizes a control level with normal analyte concentrations and abnormal low or high control levels. In certain tests, known positive and negative control samples are run in conjunction with unknown patient samples to assure valid results. Note: If only 1 control level is run, the control value must fall within 2 SD of the mean to be acceptable.

►A control that 'reads' is "in control" and falls within the acceptable value range of ± 2 standard deviations of the mean **or** shows the correct positive & negative results…the results of unknown patient samples are assumed to be correct and can be reported.

►If the control value is not within 2 standard deviations of the mean (i.e., does not 'read') **or** the control fails to show the correct positive & negative results, the results of unknown patient samples are doubtful and cannot be reported.

Summary: Laboratory quality control employs control materials to confirm accuracy, monitor precision, detect analytical errors, and check reliability of results. Controls do not detect pre-analytical errors.

PROCEDURE LIST

Refer to the NORMALS sheet for procedures in which the reference ranges are not listed.

Important NOTE:

For ALL procedures performed, important steps are:

1. Assuring that the sample to be analyzed is properly labeled AND meets the specimen requirements for the test being performed….detect pre-analytical errors.

2. Verifying the lot# and/or expiration date of all reagents AND control levels….prevent analytical errors.

3. Checking that your unknown test results are realistic and reported in the proper units….prevent post-analytical errors. **A number without units is meaningless**.

ESSENTIAL COMPONENTS OF A TEST PROCEDURE (CLSI STANDARD FORMAT GUIDELINES)

1. Principle of test (including methodology and significance)
2. Specimen requirements
3. Reagents, supplies and equipment (including safety)
4. Quality control
5. Procedure (including calculations if applicable)
6. Procedural limitations (including sources of error, any interfering substances, linearity limits and sensitivity, or other special considerations)
7. Reporting results (including units, reference ranges, critical values)
8. References

CAPILLARY BLOOD COLLECTION[1]

A. **EQUIPMENT (Have equipment ready!):**
1. PPE, cotton/gauze, alcohol (70%) wipes, skin puncture device, sharps container, marker, band-aid, sticky label.
2. MICROTAINER brand microtainers - lavender cap with K_2EDTA coating.
3. Treated hematocrit tubes (heparin) if drawing for a spun HCT or slides if making smears.

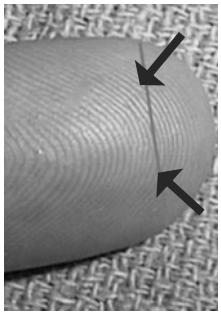
B. **FOLLOW STEPS** as listed on the capillary blood collection COMPETENCY CHECKLIST and refer to the information provided regarding capillary draws in the Phlebotomy CLS424 packet.

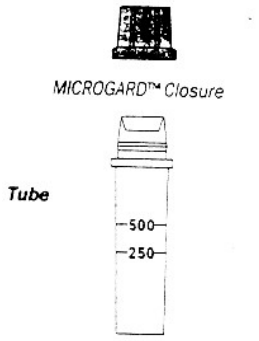
C. **TECHNIQUE:**
1. On children and adults use **3rd** or 4th finger...area just to side of ball of fingertip, <u>not</u> close to the nail/knuckle or in the center of the fingertip. Do not use cyanotic or edematous tissue as a puncture site. Cold extremities may be warmed ~ 3 minutes in warm water.

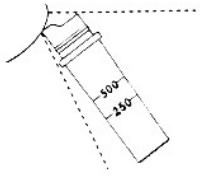
2. Cleanse area with 70% alcohol and let **completely dry**. A moist surface will not allow formation of a rounded drop. Twist & remove cap (Microgard closure) of the microtainer.

3. Gently massage prior to puncture; hold finger firmly. Place the puncture device at an angle and just to the right (or left) side of the fingertip as shown in the picture below. Press down on the top of the device to make the puncture (you will hear a click). **Immediately discard the puncture device in a sharps container.**

4. The ***first drop of blood should be wiped away** with dry cotton, as it is diluted with tissue fluid. After a drop of blood has formed at puncture site, hold microtainer at an angle below site and <u>touch collector end</u> <u>to the drop of blood</u>. The blood will flow down wall to bottom of tube by capillary action. Avoid 'scooping' the blood off the finger as this can result in blood flow that "resists" going into the microtainer.







5. <u>Slight pressure</u> may be applied a short distance from the wound. <u>Gentle</u> "milking" is acceptable but excessive squeezing is not. Squeezing dilutes the blood with tissue juice and invalidates the tests to be performed.

6. To stimulate a better blood flow, you can wipe the blood away and start with a fresh drop. When collection is complete, replace cap. Apply pressure to the site.

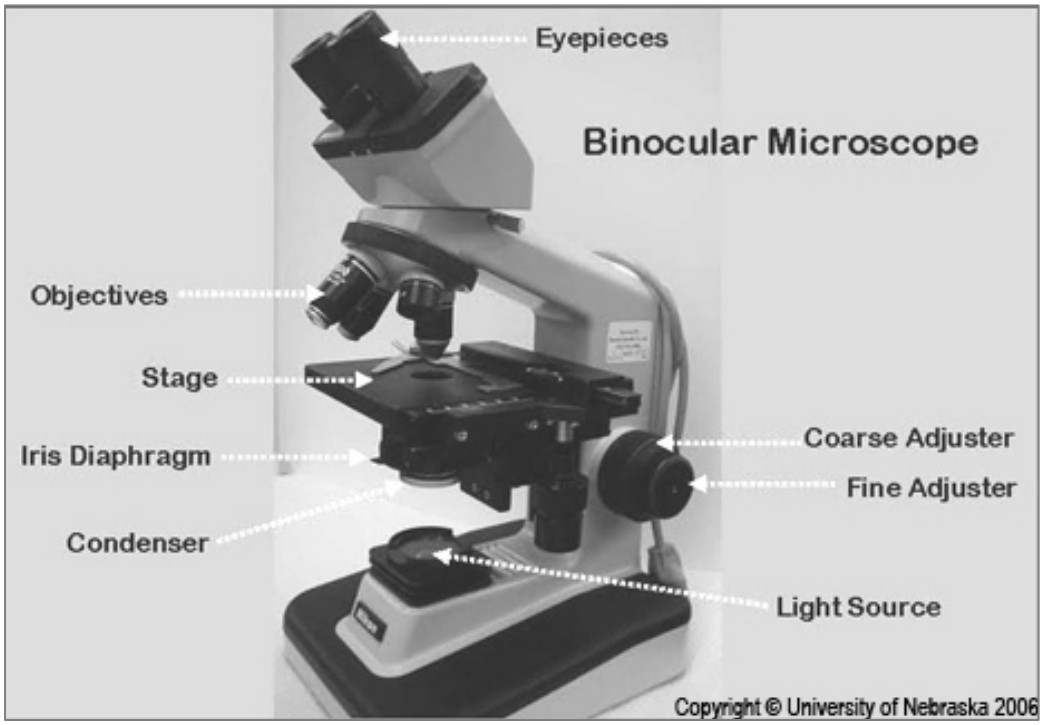
7. Immediately ***MIX** microtainer by inversion a minimum of 10 times and **LABEL**. Properly dispose of all contaminated equipment.

8. ●Minimum draw is 500 ul - **2nd line** on microtainer. Over or underfilling of tube may result in erroneous results due to alterations of the blood to anticoagulant ratio.

D. **SOURCES OF ERROR:**
1. Failure to wipe away the first drop of blood and/or excessive squeezing.
2. Clotting of the sample (e.g., micro-clots) due to inadequate mixing of the microtainer, difficulty obtaining the blood or overfilling the microtainer (making it hard to properly mix).

MICROSCOPE USE and CARE [2]

A. Major COMPONENTS of the microscope and their function:



1. **Eyepiece (ocular)**: produces the secondary image magnification (x10) of the specimen
2. **Objectives**: produces the primary image of the specimen; directs image to the eyepiece
3. **Stage**: adjustable component that holds the specimen slide (front to back, side to side)
4. **Iris diaphragm**: controls the amount of light passing through the condenser to specimen
5. **Condenser**: gathers and focuses the illumination light onto the specimen
6. **Coarse and fine adjustment knobs**: focuses the image for optimal viewing
7. **Light source**: provides illumination light

B. MAGNIFICATION

Total magnification of the lens system is the magnification of the eyepiece (10x) times the magnification of the objective lens; each lens system is used for a specific task:

1. **Low power** = 10x objective (total magnification = 100x):
   a. Use for manual WBC counts (hemocytometer).
   a. Good for scanning stained blood (or cytospin) slides

2. **High dry power** = 40x objective (total magnification = 400x):
   a. Use for manual PLT counts (hemocytometer) and WBC estimates from a stained slide.
   b. The fields seen using this lens are called high power fields (HPFs).

3. **Oil immersion** = 100x objective (total magnification = 1000x):
   a. Use for WBC differentials, evaluation of RBC/WBC/PLT morphology, PLT estimates and Retic counts from stained slides.
   b. The fields seen using this lens are called oil immersion fields (OIFs).

C. FIELD of VIEW

Adjust the binocular eyepiece for maximal viewing by adjusting the interpupillary distance so that you see only one circle (field of view) through the eyepiece when viewing with both eyes open. If you can only see the field of view with one eye open, check that eyepieces are the same height and adjust if necessary.

D. USE and ADJUSTMENT of the microscope

1. Place microscope on a level, vibration-free surface, plug it in and turn on light source (adjust light intensity to at least 2/3 maximum with rheostat). To avoid scratching or damaging the objective lens, move the stage to its lowest position using the coarse adjustment knob before placing a specimen onto the stage or rotating objectives into position. Rotate the low power (10x) objective into the light path until it 'clicks'.

Adjust light by raising or lowering condenser AND opening or closing the iris diaphragm.

2. The height of the condenser is controlled by a knob located below the stage and towards the rear of the scope. Move the condenser to the position stated below for optimal viewing with:
   a. Low power (10x) objective = the condenser should be low
   b. High dry power (40x) objective = the condenser should be high
   c. Oil immersion (100x oil) = the condenser should be at its highest point

3. Adjust the iris/aperture diaphragm of the condenser from one-third open (10x) to fully open (100x), depending on the objective lens to be used.
   a. **Generally, more light is needed as a higher objective lens is used.**
   b. The iris diaphragm is adjusted using a small lever or rotating ring on the condenser.

Focusing

4. Place a stained blood slide (or hemocytometer) on the stage using holder clips to secure.
   a. Using the stage adjustment knobs, move the slide directly under the low power (10x) objective and over the central opening for the light source (light path).
   b. Check that you have moved the slide to the correct area for viewing cells on a blood smear. You should be in the *rainbow area* of the slide, NOT in a thick area by the label.

5. With the **low power (10x) objective** in place, slowly raise the stage while looking through the eyepiece with both eyes (the objective should NEVER touch the slide).
   a. Slowly turn the coarse adjustment knob towards you until you see the object(s) on the slide (e.g., WBCs and RBCs). Then use the fine adjustment knob to focus the object(s).
   b. Less light is needed so the condenser should be low and iris diaphragm ~one-third open.

6. Next, move the **high dry (40x) objective** into place if needed (e.g., WBC estimate). Do NOT use the coarse adjustment knob. You should only use the fine adjustment knob to bring the object(s) into sharper focus. The condenser should be high and iris diaphragm ~half open.

7. Oil must be added to the slide to use the **oil immersion (100x) objective**. Move the 40x objective aside and place a drop of oil on the slide directly over the circle of the light path. Be careful not to get oil on the high dry (40x) objective lens.
   a. Next, move the oil immersion (100x) objective into place and use ONLY the fine adjustment knob to focus. Adjustment should be minimal.
   b. High light is needed so the condenser should be fully raised and diaphragm fully open.

E. MAINTENANCE

1. **After each use**, CLEAN and thoroughly dry the eyepieces, all objectives, the stage and condenser lens using ONLY lens cleaner and lens paper.
   a. Lower the stage, check that stage is empty, and store with low power objective in place.
   b. Carefully clean coverslipped slides with lens cleaner after use. For slides without a coverslip, do NOT use lens cleaner but *gently* clean off oil with a tissue.

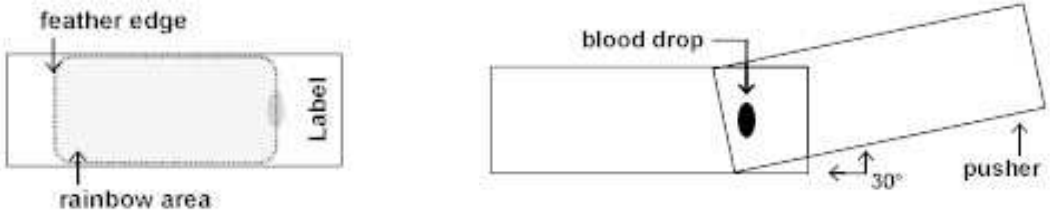
   Note: If you are not going to use the microscope for a period of time, NEVER leave the scope with a slide under the oil objective and always turn off the light source.

2. If you can't focus and/or 'everything' looks hazy, you may need to clean eyepieces and/or objectives (most likely an oil problem). If the eyepiece is dirty, dirt will rotate when the eyepiece is turned.
   a. If you focus on a hemocytometer and don't see any cells, you likely have too much light.
   b. If you can't focus on a slide, check that the blood (and coverslip) on the slide is *facing up*.

BLOOD SMEAR PREPARATION/WRIGHT'S STAIN [3]

PRINCIPLE:
The well made peripheral blood smear is essential to the morphologic evaluation of hematologic disorders. Poorly made smears are misleading and may cause erroneous findings. EDTA whole blood or capillary blood may be used. Heparinized blood is unacceptable as it causes a blue background.

The 'IDEAL SMEAR' has a straight "feather" edge and becomes gradually thicker toward the point of origin (blood drop). The feather edge should include a large rainbow area with no ridges, tails, streaks or holes.



EQUIPMENT:
Clean 3 x 1" quality slides, untreated microhematocrit tubes, plastic pipets or wooden sticks, pencil

PROCEDURE:
1. Place a small drop of blood on a clean slide at one end. The drop of blood should be small enough so that the entire drop can be spread, but large enough so the smear goes 1/2 to 1 inch from the opposite end. **The size of the blood drop will affect smear thickness and length.**

2. Take a pusher slide and place it **just in front of the drop** of blood at a 30^{o} angle from the smear slide. **The angle of the pusher slide will affect smear thickness and length.**

3. Draw the pusher slide back into the drop of blood and allow the blood to spread evenly. **Gently** push it forward with very little pressure and a moderate amount of speed across the surface of the smear slide. ●**Use a new pusher slide edge for each smear.**

2. Slides **must be properly labelled** before staining with Wright's stain. Use pencil to label (first and last name) on frosted end. ■**NEVER PRE-LABEL.**

FOUR CRITERIA of a suitable blood smear by macroscopic observation:
1. The presence of a "squared" feathered edge (with rainbow area and no tails).
2. Proper thickness of blood film.
3. Proper length of the blood smear (2/3 to 3/4 of slide).
4. Proper width of the blood smear.

SPECIAL CONSIDERATIONS:
1. EDTA tube must be **mixed** before obtaining an aliquot of blood. Tilt to mix, do not shake.

2. The blood should be spread immediately. The drop of blood should not rest on the slide for longer than **5 seconds** before spreading or the bigger cells will be carried to slide edges which may distort the WBC differential result.

3. For normal bloods, a 30^{o} angle is best along with moderate speed.
   a. The smaller the angle of the pusher to the smear slide and the slower the "push" of the pusher slide, the thinner the smear. The larger the angle of the pusher to the smear slide, the thicker the smear.
   b. ***Decrease*** the angle of the pusher slide to make a longer, thinner smear and ***increase*** the angle of the pusher slide to make a shorter, thicker smear.

4. ●**Do not apply pressure** to the pusher slide as this will break cells and/or distort the distribution and morphology of the cells. The pusher slide should literally be glided gently across the smear slide with only enough pressure to guide and hold the pusher slide over the smear slide.

5. Use clean slides only. Chipped slides cause tails and any blood on the pusher slide causes tails.

BLOOD SMEAR PREPARATION/WRIGHT'S STAIN PROCEDURE [3]

PRINCIPLE:
Wright's stain is widely used for staining peripheral blood smears. Wright's stain is a Romanowsky stain that contains Eosin and Methylene blue. Eosin (acid dye) stains basic cell structures such as hemoglobin, eosinophil granules, and primary granules a red-orange color. Methylene blue (basic dye) stains acid cell structures such as RNA in nucleolus and cytoplasm, nuclear chromatin, and basophil granules a blue color. A combination of both dyes stains neutral cell structures such as neutrophil granules a pinkish-tan color.

Prepare and **LABEL** smears that meet the criteria of a properly made blood smear.

STAINING PROCEDURE using Quick Wright's Stain:

1. Dip blood smears in stain for 15 seconds.^^
2. Dip blood smears in jar 2 for 30-60 seconds.
   ■If jar 2 appears very blue, dump and refill with deaminized water.
3. Dip blood smears in jar 3 for 10 seconds.
4. Wipe off stain on backside of smears. Let smears air dry in rack.

^^More than one slide can be stained at a time AND stain times are not exact.

STAINING PROCEDURE using Neat Stain:

1. Dip blood smears for five, one second dips into the Fixative Solution. Allow excess fixative to drain back into the cuvette, or blot the backside of the smear.^^
2. Dip blood smears for five, one second dips into Solution I. Allow excess stain to drain back into the cuvette, or blot the backside of the smear.
3. Dip blood smears for five, one second dips into Solution II. Allow excess stain to drain back into the cuvette, or blot the backside of the smear.
4. Rinse blood smears with deaminized water. Let smears air dry in rack.
   ■If jar appears very blue, dump and refill with fresh deaminized water.

^^More than one slide can be stained at a time if placed back to back. Dip times are not exact.

Staining notes: ●A properly stained smear looks pinkish-purple macroscopically; the red cells appear red-orange microscopically. For the Quick Wright's stain, methanol in the stain fixes cells to the slide; deaminized water is the buffer.

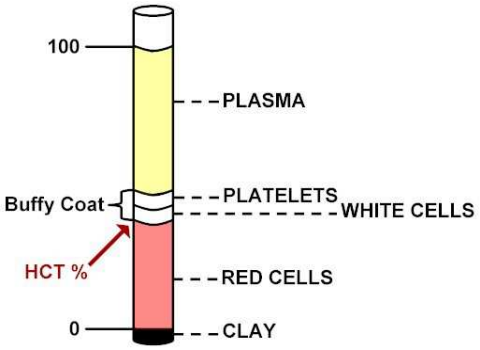
1. The RBCs may appear blue and the WBC nuclei deeply stained if:
   a. The staining time is too long.
   b. Washing is inadequate.
   c. The stain or buffer is excessively alkaline.
   d. The smear is too thick.
      ●Correct by decreasing stain time, decreasing pH of buffer, or making a thinner slide.
2. The RBCs may appear excessively red and the WBC nuclei poorly stained if:
   a. The staining time is inadequate.
   b. Washing of slide is excessive.
   c. The stain or buffer is too acidic.
      ●Correct by increasing stain time or increasing pH of the buffer.
3. Excess precipitate is caused by insufficient washing or inadequate filtering of the stain.

MANUAL SPUN HEMATOCRIT (HCT)/Packed Cell Volume (PCV) PROCEDURE [4]

A. **PRINCIPLE:**
The hematocrit measures the volume of packed red cells in a given volume of whole blood. This method uses EDTA anticoagulated whole blood or capillary blood obtained by fingerstick.

B. **PROCEDURE:**
1. **MIX** EDTA blood specimen and whole blood control vial well before taking blood sample. TILT to mix – do NOT shake.
2. Fill **TWO** microhematocrit tubes 2/3 full for each EDTA sample and/or control. Bubbles are OK. Each test is done in *duplicate*.
3. Clay the dry end of each tube and put tubes in HCT sheet holes. Label the sheet with your name and carefully transport to centrifuge area.
4. Balance tubes in centrifuge, PUT ON LID, and centrifuge **5** mins. Record the centrifuge # and groove #'s used for each tube on sheet.
5. After centrifugation, use the HCT reader (e.g. card) to set 0 and 100, then read the HCT% at the top of the packed red cells; read to the nearest 0.5%. Record the HCT% obtained for each tube on the labsheet.

Refer to the diagram below of layers present in a spun hematocrit tube.



C. **QUALITY CONTROL:**
1. Duplicate HCT tubes must agree **± 1%** (1% = 1 HCT percentage point) to accept results or another HCT tube must be centrifuged.

2. The control must be within **± 2** standard deviations of the assayed control mean to accept patient HCT results. Check that the HCT control value is within the acceptable limits given on the **HCT QC chart**. If the control does not "read", testing must be repeated^.

^If the HCT control is not within the acceptable range, first check that you are using the HCT reader properly.

D. **REPORTING:**
1. Average the final patient HCT results IF the control is acceptable and duplicate HCT tubes agree.
2. Report manual hematocrit results to the nearest **0.5**%. Do not average results if, for example, the patient HCT results are 32.5% and 33.0%, report either.

E. **LIMITATIONS:**
●Pre-analytical/blood collection errors, e.g. clotted blood, hemolysis, EDTA tube filled less than half full with blood.
●Analytical/technical errors, e.g. inadequate mixing of blood sample, insufficient centrifugation, poor duplication of results, improper use of HCT reader or including buffy coat in HCT reading.

F. **FINAL CHECK OF HGB AND HCT RESULTS:**
Check that your patient HGB and HCT results correlate.....does the HGB x 3 = HCT ± 3%?
■H&H values obtained on the control sample will NOT correlate.

HEMOCUE PHOTOMETER FOR HEMOGLOBIN MEASUREMENT 5

A. **PRINCIPLE:**

The hemoglobin concentration in a fresh capillary or anticoagulated blood sample (EDTA preferred) is determined photometrically using a dry reagent system. The red cells are lysed and hemoglobin is converted to azidemethemoglobin by sodium nitrite and sodium azide. This method of HGB measurement is a widely used point-of-care test.

B. **HEMOCUE OPERATION:**

1. Turn the Hemocue on using the switch in the back. The display screen should read "Hb". Pull the black cuvette holder out to the insertion position. After about 6 seconds, the screen should read "READY".

2. Place a cuvette into the holder and insert to the "measuring" position. The HGB results in g/dl will be displayed in 45-60 seconds (results remain on screen about 5 minutes).

C. **QUALITY CONTROL:**

1. Perform an electronic calibration check of the instrument prior to **each use** and verify that the value falls within the assigned range. The RED control cuvette ***must read within ± 0.3 gm/dl of the assigned value*** for the specific instrument to assure proper function or the instrument cannot be used. Record the Hemocue instrument you are using AND the value obtained for the RED electronic calibration cuvette on your labsheet.

2. A hemoglobin whole blood control sample (normal level) must be run ***once per day*** on each Hemocue instrument. The control HGB value must read within the assayed range to accept patient hemoglobin results.

   a. **Run the HGB whole blood control ONLY IF the control has not yet been run on the Hemocue you are using**. Follow the **PROCEDURE** below and check that the HGB control value obtained reads within the limits on the **HGB QC chart.** Repeat the control if it is not within established limits. **Document** the control result on the Hemocue Daily QC Sheet for the specific Hemocue instrument you are using AND record the HGB control value on your labsheet.

   b. **If the daily HGB whole blood control has already been run on the Hemocue you are using, do NOT run**. Check that the HGB control result documented on the Daily QC Sheet for the Hemocue instrument you are using reads within limits on the **HGB QC chart** AND record the HGB control value on your labsheet.

D. **PROCEDURE:**

1. **Fill the cuvette with blood (obtained from a mixed EDTA tube or control vial) in a CONTINUOUS process WITHOUT BUBBLES**. ●The cuvette needs to be filled all at once because the chemical reaction starts immediately and any delay in filling the cuvette results in incomplete red cell lysis.
2. ●Air bubbles in the center of the cuvette require repeating.
3. Wipe any excess blood from the outside of the cuvette, being careful not to touch the curved edge...**important.** Cuvettes must be read within ten minutes of filling with blood to prevent alteration of the result by drying.
4. Place a cuvette (control or unknown) into the holder and insert to the "measuring" position. The results in g/dl will be displayed in 45-60 seconds. Record results to the nearest **tenth** on your labsheet.

E. **LIMITATIONS:**

●Pre-analytical/specimen collection errors, e.g. clotted blood or wrong patient identification.
●Analytic/technical errors, e.g. filled cuvette is not read within 10 minutes or reagent deterioration.

F. **FINAL CHECK OF HGB AND HCT RESULTS:**

Check that your patient HGB and HCT results correlate.....i.e., does the HGB x 3 = HCT ± 3%?
■H&H values obtained on the control sample will NOT correlate.

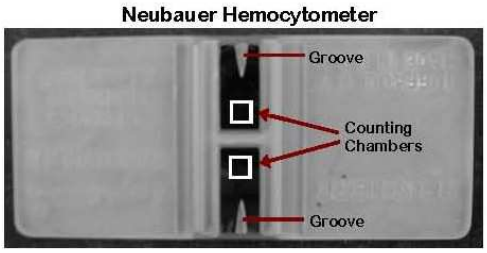
MANUAL CELL COUNTING 6

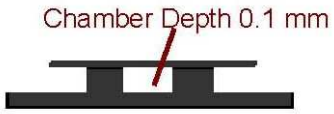
PRINCIPLE: Manual cell counts may be performed when a parameter is below the automated instrument's linearity, to verify a doubtful result "flagged" by the instrument or when smear findings don't agree with the automated result.

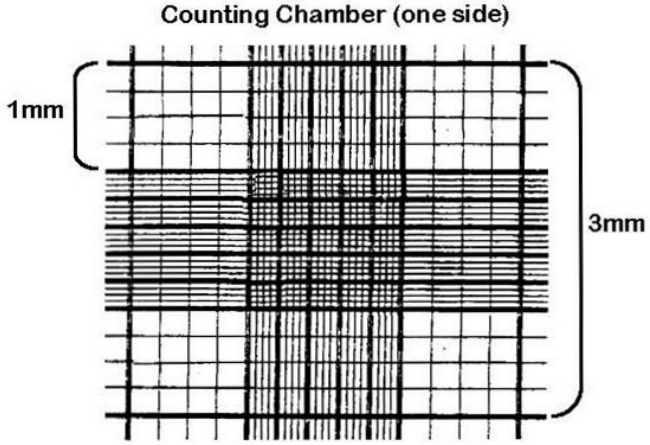
Manual cell counts are performed with the use of a hemocytometer and blood dilutions (or dilutions of body fluids such as CSF). **The principle is the same for leukocytes, erythrocytes and platelets, however, the dilution, diluting fluid and area counted can VARY**. **Dilutions are often made with Unopettes.**

A. **Neubauer hemocytometer - dimensions**

1. Each hemocytometer side has a counting chamber (two). Each counting chamber is 3mm x 3mm (area of $9mm^2$).
2. Each chamber is divided into 9 squares - each square is 1mm x 1mm (area of $1mm^2$).
3. Each of four corner squares are divided into 16 smaller squares.
4. The center square (1 mm^2) is divided into 25 smaller squares each bordered by double-ruled lines.
   a. Each of the 25 small squares in center square is 1/5mm per side (area of $1/25mm^2$).
   b. Each of these 25 small squares is further subdivided into 16 smaller squares.
   c. Divisions (smaller squares within squares) help counting navigation.
5. 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.







6. Each chamber has a total area of $9mm^2$ (3mm x 3mm).
7. Each chamber has a total volume of $0.9mm^3$.
   ■**Chamber DIMENSIONS** - **3mm x 3mm x 0.1mm**.
8. The area (# of squares) to count and/or the blood dilution to make is determined by the number of cells present. 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. **STANDARD FORMULA** to obtain # of cells/mm^3 (cmm):

1. Must correct for dilution used and area (squares) counted, **VARIES**.
2. Standard formula:

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

OR

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

   a. Dilution factor - invert dilution used (if 1:100 use x100).
   b. Depth - 0.1 mm - use this to obtain # of cells/cmm (a volume) rather than # of cells/mm^2 (an area).
   c. Often see depth factor of "x10" in numerator obtained by inversion of 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 that we can see the cells we want to count.
3. **PLT/WBC unopettes** can be used for manual PLT or manual WBC counts and contain 1% ammonium oxalate (diluent); red cells will lyse but platelets and WBCs are left intact; may occasionally see intact red cells or nucleated red cells (if present).

D. **MANUAL WBC Counts:**

1. Manual WBC counts are performed using a hemocytometer and a blood dilution 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 & PLTs.

2. **PROCEDURE:**

Diluting and plating

a. Puncture a PLT/WBC unopette reservoir and remove shield from pipet.
b. MIX EDTA whole blood specimen before obtaining aliquot of blood (tilt to mix). The pipet will fill with blood by capillary action. It is IMPORTANT that there are NO BUBBLES in pipet and that any excess blood is wiped from outside of pipet.
c. Completely rinse blood from pipet into unopette reservoir and mix blood with diluent. LABEL; let unopette stand 10 minutes to allow for complete RBC lysis.
d. CLEAN hemocytometer with alcohol and dry well; place coverslip on top of the hemocytometer.
e. After 10 minutes, invert unopette several times to mix blood with diluent before converting to dropper assembly and plating dilution on the hemocytometer.
f. Discard a few drops and plate/load the unopette dilution by placing the pipet in groove and slowly squeezing. Smoothly fill the entire area under the coverslip on *each side* of the hemocytometer without bubbles; be careful not to overfill.
g. Allow cells to settle about 3 minutes in a petri dish with a damp cotton ball before placing hemocytometer on the microscope stage for counting.

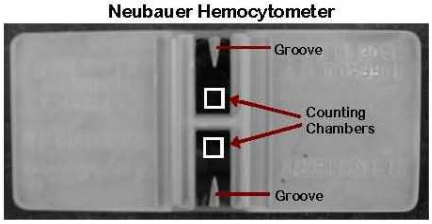
Counting

h. ●Using 10x (low power) objective and **LOW** light, **focus on hemocytometer groove, then move to the counting area.** Check for even cell distribution in the counting chamber squares before counting.
i. For consistency in counting – count dark cells which touch the top and left boundary lines; exclude light cells touching bottom and right lines…see diagram @ bottom right which also has arrows showing a systematic way to count cells.
j. ●Count 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. You can use 40x (high power) if there is difficulty distinguishing cells from debris.
k. Record the # of cells counted in each counting chamber side (and the total #) on the chambercount worksheets on the labsheet.
l. The # of cells counted between sides must agree **±20%** to accept (or replate).
m. Calculations:

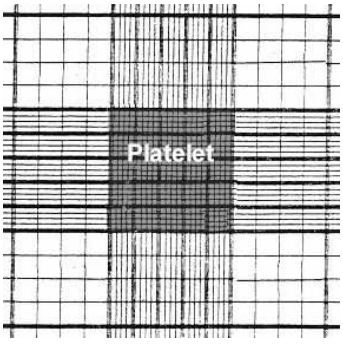
$$\text{WBC/cmm} = \frac{\#\text{ cells (both sides)} \times 100}{0.1\text{ mm} \times 18\text{ sqmm}} \quad \textbf{or} \quad \frac{\#\text{ cells} \times 100 \times 10\text{ mm}}{18\text{ sqmm}}$$

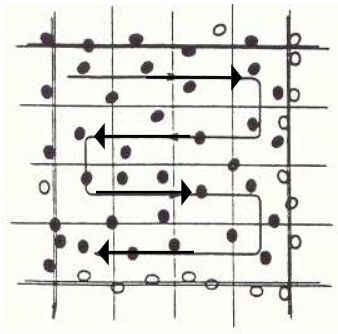
n. ■Report WBC counts to nearest hundred if no decimal or nearest tenth if a decimal. See Normals & Calculation sheets.

Neubauer Hemocytometer



Counting chamber (one side)





3. **SOURCES OF ERROR:**

●Pre-analytical/specimen collection errors, e.g. clotted blood.

●Analytical/technicial errors:

- Failure to mix blood specimen before taking blood aliquot or not remixing unopette before plating.
- Improperly filled chamber - sides won't agree due to poor cell distribution. ■**MUST repeat** if sides are not within acceptable duplication. If replating, first remix unopette, then discard 1 drop and plate.
- Wrong dilution or calculation; <u>always recheck math</u> and check for correct units.
- Hemocytometer was not cleaned well, sat too long and/or dried up.
- Counting artifact as cells, counting cells in wrong area, or wrong counting due to incorrect light adjustment^^.
  ^^If you focus on the chamber counting area and cannot see any cells, you likely have too much light…change condenser and/or diaphragm.

E. **MANUAL PLT Counts**

1. Manual PLT counts are performed using a hemocytometer and a blood dilution made with a PLT/WBC unopette. The reservoir contains 1.98 ml of 1% ammonium oxalate (diluent) and 20 ul of blood is added → <u>1:100 dilution</u>. RBCs lyse leaving WBCs & PLTs.

2. **PROCEDURE:**

Diluting and plating

a. Puncture a PLT/WBC unopette reservoir and remove shield from pipet.
b. MIX EDTA whole blood specimen before obtaining aliquot of blood (tilt to mix). The pipet will fill with blood by capillary action. It is IMPORTANT that there are <u>NO BUBBLES</u> in pipet and that any excess blood is wiped from outside of pipet.
c. Completely rinse blood from pipet into unopette reservoir and <u>mix</u> blood with diluent. LABEL; let unopette stand 10 minutes to allow for complete RBC lysis.
d. <u>CLEAN</u> hemocytometer WELL with alcohol (to avoid confusing junk with platelets when counting) and dry well; place coverslip on top of hemocytometer.
e. After 10 minutes, invert unopette several times to mix blood with diluent before converting to dropper assembly and plating dilution on the hemocytometer.
f. Discard a few drops and plate/load the unopette dilution by placing the pipet in groove and slowly squeezing. Smoothly fill the entire area under the coverslip on <u>*each side*</u> of the hemocytometer without bubbles; be careful not to overfill.
g. Allow cells to settle 10 minutes in a petri dish with a damp cotton ball before placing hemocytometer on the microscope stage for counting.

Counting

h. Focus on hemocytometer groove using 10x (low power), then move to counting area. ●Using 40x (high dry power) objective and **<u>LOW</u>** light (adjust light for best contrast), check for even cell distribution in the counting chamber squares.
i. For consistency - count dark cells which touch the top and left boundary lines; exclude light cells touching bottom and right lines...see diagrams on page 13.
j. ●Count the <u>center 1 sq mm</u> on <u>each side</u> of hemocytometer (divided into 25 squares)...total area counted = **2 mm^2**. Use 2 counter keys…one key for each side. **Focus up & down while counting; platelets are greenish, <u>NOT</u> shiny.**
k. Record the # of cells counted in each counting chamber side on the chambercount worksheets.
l. The # of cells counted between sides must agree **<u>±</u>20%** to accept (or replate).
m. Calculations:

$$\text{PLT/cmm} = \frac{\text{\# cells (both sides)} \times 100}{0.1 \text{ mm} \times 2 \text{ sqmm}} \quad \textbf{or} \quad \frac{\text{\# cells} \times 100 \times 10 \text{ mm}}{2 \text{ sqmm}}$$

n. ■Report PLT counts to nearest thousand. See Normals & Calculations sheets.
o. The manual platelet count must agree with the platelet estimate from the blood smear <u>±</u> 20% if the PLT count is <u>≥</u> 50,000/cmm and <u>±</u> 10,000/cmm if the PLT count is < 50,000/cmm to accept. If agreement is acceptable, the platelet count is reported, NOT the estimate from the blood smear.

3. **SOURCES OF ERROR: See those listed for manual WBC counts.**

SMEAR PROCEDURES - DIFFERENTIAL, PLT & WBC ESTIMATES 7

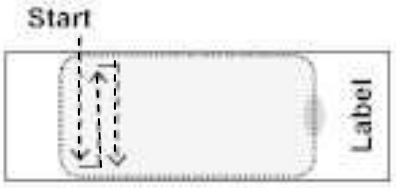
●**REMEMBER to adjust light** (raise condenser & open diaphragm) when changing from manual cell counting (low light) to looking at stained blood smears (oil immersion and high light). ■Scan the smear on low power (10x) to check cell distribution and smear quality (or "BAD" cells) before going to oil immersion.

A. Manual DIFFERENTIALS:

Performed on a properly prepared Wright's stained blood smear using **OIL** immersion (100x) and **HIGH** light in the "rainbow" area where the **red cells just touch**.

The leukocyte differential is done to classify the type of leukocytes present in the blood. The slide is scanned from side to side counting & identifying 100 consecutive WBC's using counter keys = **relative (%)**.

USUAL Diff Sequence: Segs %-Bands %-Lymphs %-Monos %-Eos %-Basos %



Eos & Basos	Monos	Lymphs	Band Neutrophils	Segmented Neutrophils	Counter TOTAL
# = %	# = %	# = %	# = %	# = %	100

NOTE: IF SEEN, Nucleated RBC's are reported # per diff & immature WBCs are included in 100 cell diff.

B. **Cell MORPHOLOGY:**

The white cells, red cells and platelets are observed for normal size, shape, inclusions and/or granulation. Any variations from normal are quantitated or noted per institution policy. **SEE GUIDELINES for GRADING ABNORMAL MORPHOLOGY (pg 16).**

C. **PLATELET ESTIMATE:**

The number of platelets are estimated from the Wright's stained smear where the **red cells just touch** by the following method:

1. Count the # of platelets in each of 10 fields using **OIL** immersion (OIF's)...use counter keys when counting platelets. Find the average (don't round) **= Avg # PLTS per OIF.**

   ●Take 1st 10 PLTS (from Avg # PLTs/OIF) x **20,000 each** (or 200,000/cmm) **PLUS** each additional PLT (above 10) x **10,000/cmm each** = PLT estimate/cmm.

2. The platelet estimate should agree with the PLT count and corresponds to a platelet range.
   a. Should agree ± 20% if the PLT count is ≥ 50,000/cmm.
   b. Should agree ± 10,000 if the PLT count is < 50,000/cmm.
   c. The platelet estimate corresponds to a platelet comment range, as listed on the bottom of page 16.

D. **WBC ESTIMATE:**

The number of WBC's are estimated from the stained smear using the high power lens and in an area where the **red cells are slightly overlapping**.

1. Count the # of WBC's in 10 **HIGH (40x) power** fields (HPFs); find average.
2. The average # WBC'S per HPF x 2,000 = WBC estimate/cmm.
3. The estimate should agree ± 20% with the WBC count.

E. **PLT/WBC ESTIMATE NOTES:**

■Platelet and WBC estimates include those fields with no cells counted.
■WBC estimates include smudge or broken cells.
■PLT/WBC estimates are used to check the validity of automated/manual cell counts; estimates are NOT 'reported'.
■If the estimate does not 'agree', check estimate procedure, e.g., calculation, objective, area.

GUIDELINES FOR GRADING ABNORMAL MORPHOLOGY - Vary per institution policy

You will be using the following criteria for grading/quantitating abnormal cell morphology seen on the blood smear. Memorization is not necessary. Always follow your institution established policy.

Anisocytosis, Microcytosis, Macrocytosis Or **General** Poikilocytosis: Correlate your grading of anisocytosis, microcytosis and macrocytosis with RBC indices if values are available. Anisocytosis with microcytosis and/or macrocytosis may be reported; only anisocytosis is reported if the red cells are normocytic.

Slight:	5-15% RBC's
Moderate:	15-40%
Marked:	greater than 40%

Specific Poik - Schistocytes, Spherocytes, Acanthocytes, Echinocytes (Crenated/Burr cells), Target cells, Teardrops, Ovalocytes, Pencil cells (ONLY reported if micro/hypo red cells), Stomatocytes:

Occ:	1-5% -- ***Use for schistocytes and spherocytes ONLY***
Few:	5-10%
Several:	10-25%
Many:	greater than 25%

Hypochromia: By definition, this means the central pallor area of the RBC is greater than 1/3 the cell diameter. If the central pallor area is greater than 3/4 the red cell diameter, increase the hypochromia category one degree from that used to quantitate the number of hypochromic red cells seen.

Slight:	5-15%
Moderate:	15-40%
Marked:	greater than 40%

Polychromasia: Try to exempt polychromatophilic red cells (which are larger than mature red cells) from your sizing judgement of macrocytosis.

Slight:	2-3%
Moderate:	3-5%
Marked:	greater than 5%

Hypersegmented Neutrophils:

Occasional:	On scan to 1%
Few:	1-2%
Several:	3-5%
Many:	greater than 5%

Reactive (Atypical) Lymphocytes:

Few:	25% lymphocytes involved
Several:	50% lymphocytes involved
Many:	75% lymphocytes involved

Items Noted but not quantitated:

Basophilic stippling, Howell-Jolly bodies, or Pappenheimer bodies
Sickle cells, SC crystals, or C crystals
Rouleaux tendency or agglutination of RBC's
Toxic granulation, Dohle bodies, or vacuolization of neutrophils
Plasmacytoid lymphs are noted or quantitated like reactive lymphocytes
Giant platelets (platelet size is larger than normal RBC, ~7u) or platelet satellitism
Certain findings noted require a pathologist review: Auer rods, hyperclumped lymphs, ingested bacteria, hyposegmented (Pelgeroid tendency) or hypogranular neutrophils, hypogranular platelets

Reported # per Differential (100 WBC's)

Nucleated red cells (#NRBC/diff); smudge cells (#smudge cells/diff) ***only if CLL***

Platelet Estimate/Comment Ranges:

150,000-450,000	adequate	450,000-500,000	slight increased
100,000-150,000	slight decrease	500,000-650,000	increased
50,000-100,000	decreased	>650,000	marked increase
<50,000	marked decrease		

8

MANUAL RETICULOCYTE COUNT PROCEDURE

A. **PRINCIPLE:** The reticulocyte is a non-nucleated immature red cell containing residual RNA. A supravital stain, **new methylene blue**, is used to precipitate the RNA into dark-blue filaments or granules to identify retics.

B. **SPECIMEN and REAGENTS**: EDTA whole blood is the preferred anticoagulant; New Methylene Blue Staining solution, 12x75mm tubes, pipets, glass slides.

C. **PROCEDURE:**

1. Put 2 drops of new methylene blue in the bottom of a 12x75mm tube. Using a pipette, add 2 drops of well-mixed EDTA blood to the tube.
2. Mix blood/stain mixture. The mixture color should be smoky-gray. Adjust if needed, i.e., add more blood if mixture is too blue.
3. Incubate mixture at least 5 minutes but no longer than 10 minutes.
4. **MIX** solution again….important! Prepare 2-4 good smears, *LABEL* and let dry.
5. **Counting:** Using **oil/100x** power, count 500 total red cells separating mature RBC's from retics (use two counter keys). Retics are greenish with blue precipitates of RNA. **Two "dots" or more is a retic.** Go from feather edge to body of smear, making sure you are not counting too thick.
6. Two techs count 500 RBC's on different retic smears for a total of 1000 RBC's counted.
7. **Quality control:** The number of retics/500 RBC's must agree $\pm$ 2 retics between techs to accept results or another slide is counted. Controls must read within the assayed range to accept results.
8. Both a relative percent retic and an absolute retic are reported:
   a. Relative number - # of retics in total of 1000 RBC's = **percent (%)**.
   b. Absolute number - retic % x the RBC count/cmm = **thousands/cmm**.

D. **CALCULATIONS:**

1. The relative reticulocyte count uses the sum of the two techs answers and the percent is reported to the nearest tenth (one decimal):

$$\frac{\text{\# retics in 1000 RBCs}}{10} = \textbf{\%} \qquad \textbf{OR} \qquad \frac{\text{\# retics}}{1000\ \text{RBCs}} \times 100 = \textbf{\%.}$$

2. The absolute reticulocyte count is reported to the nearest thousand/cmm using the following calculation:

$$\text{\# retics/cmm} = \frac{\text{\# retics}}{1000\ \text{RBCs}} \times \text{RBC millions/cmm} \qquad \textbf{OR} \qquad \frac{\text{retic \%}}{100} \times \text{RBC/cmm}$$

E. **SOURCES OF ERROR:**

1. Inadequate mixing before making smears
2. Counting artifact or other inclusions as retics……black/shiny inclusions are "junk".
3. Improper ratio of blood to stain.
4. Not counting all of the retics…..two blue "dots" or more is a retic.
5. Wrong calculation

HGB S SCREENING PROCEDURE - Sickle Cell Prep/Tube Solubility Test **9**

A. **PRINCIPLE:** The HGB S prep is a screening test based on the **insolubility** of HGB S when oxygen is removed as compared to other hemoglobins. Blood is added to the reagent which causes red cell lysis, hemoglobin release, and deoxygenation. **Blood containing HGB S will form a cloudy, turbid suspension while other hemoglobins (A,F,C) are soluble in the reagent**.

B. **REAGENT SOLUTION (Sickledex/Sickle-Sol) contains**:
1. Saponin - causes red cell lysis and release of hemoglobin.
2. Sodium hydrosulfite/dithionite - a reducing agent which removes oxygen (i.e. deoxygenates the blood).

C. **CONTROLS:** Positive (AS) and Negative (AA) controls are done with each patient run to check the reagent.

D. **SPECIMEN:** EDTA, heparinized, partially clotted or hemolyzed blood.

E. **PROCEDURE:**
1. Make a 2ml mark on 12x75mm tubes for each control or patient to be run and LABEL. Dispense 2mL of reagent solution into marked tubes. The solution should be at room temperature before adding the blood.
2. Add 20 ul (0.02 mL) of mixed control or patient blood to the tube using a pipet. Mix tubes by inversion using parafilm or by capping.
3. Allow each tube to stand in test tube holder for 10 minutes. Test interpretation can be done between 10 and 20 minutes. The reaction is read macroscopically by looking through the tube at ruled lines.

F. **INTERPRETATION:**
- Solution cloudy (lines not visible) - POSITIVE for HGB S.
- Solution transparent (lines visible) - NEGATIVE for HGB S.
- Report patient as POSITIVE or NEGATIVE for HGB S **ONLY IF** controls read.

G. **SIGNIFICANCE:**
1. Positive S preps occur for individuals with Hgb SS, SA, SC.
2. Negative S preps occur for individuals with Hgb AA, AC, CC or other non-sickling hemoglobins.

H. **LIMITATIONS:**
1. False positives may occur if the test sample is lipemic or contains high levels of globulins, presence of Hgb C-Harlem or Polycythemia (too much blood/reagent).
2. False negatives may occur if the Hgb S level is below 10% (e.g., infants <3 months old or recent transfusion), reagent deterioration or severe anemia (too little blood/reagent).

NOTE: The HGB S prep is sensitive to HGB S levels as low as 10%. **All** positive screens should be followed by a hemoglobin electrophoresis to determine disease versus trait condition (i.e., quantitate the amount of Hgb S).

TEST FOR INFECTIOUS MONONUCLEOSIS (IM) - Seradyn Mononucleosis Test **10**

A. **PRINCIPLE:** The heterophile antibodies of infectious mononucleosis may appear by the 4th day but almost always by the 21st day of illness persisting for several months. The SERADYN method uses horse erythrocytes (Reagent B) to detect the heterophile antibodies (IgM class) associated with IM and guinea pig kidney antigen (Reagent A) to absorb out interfering antibodies (Forssman or serum sickness antibodies). The etiologic agent of infectious mononucleosis (IM) is usually the Epstein-Barr virus (EBV) via infectious secretions.

B. **SPECIMEN:** Serum is the preferred patient sample but plasma can be used.

C. **REAGENTS:** Seradyn Reagent A and Reagent B, test cards, pipets

D. **QUALITY CONTROL:** Run the Positive and Negative Control sera whenever a patient is tested. Controls are run exactly like a patient. BOTH controls must read correctly. If controls do not read, the kit may be defective or there was an error in technique.

E. **PROCEDURE:** Bring reagents to room temperature. One test circle is required for each control or patient sample to be tested...you will need one test card. Pipets and reagent droppers should always be held VERTICALLY when delivering drops.

1. Gently mix Reagent A; add one drop to left side of each test circle.
2. Invert Reagent B several times to mix; add one drop to the right side of each test circle. *Note that reagents are added to all testing circles at each step.*
3. Using pipets provided, add one drop of a control serum or patient's serum next to Reagent A on left side of each test circle.
4. Invert pipet and use the "paddle" portion to thoroughly mix Reagent A and serums (controls or patient). Then gradually mix this solution into Reagent B (reddish-brown) while covering entire test circle.
5. Rock card slowly and gently for <u>exactly</u> one minute.
6. Read results immediately.

F. **INTERPRETATION:**

■A POSITIVE IM reaction will have dark agglutinins (clumps).
■A NEGATIVE reaction will have no agglutination or may have fine granules.
■Report the patient as POSITIVE or NEGATIVE for infectious mononucleosis ONLY IF the Positive and Negative controls read correctly.

G. **LIMITATIONS**:

1. If testing too early, a low titer results in a negative test. Repeat later. About 10% of young adults and 50% of children under age 4 do not produce detectable IM heterophile antibodies.

2. ●**The test result should be correlated** with the patient's blood count findings and clinical symptoms to make a diagnosis of infectious mononucleosis…false positive and negative results can occur. Cytomegalovirus, toxoplasmosis, viral hepatitis or HIV may show a similar blood picture. Positive heterophile tests have been rarely reported in certain pathologic conditions such as hepatitis, rubella, leukemia, rheumatoid arthritis and Burkitt's lymphoma.

3. If indicated, can test for EBV-specific antibodies in heterophile negative types.

Manual Westergren ERYTHROCYTE SEDIMENTATION RATE (ESR) PROCEDURE 11

A. **PRINCIPLE:** The 'sed rate' is a **non-specific** indicator of disease and is commonly performed. ESR refers to the rate red cells settle in a vertical tube and is expressed as the distance the red cells fall in mm/time. This test is primarily used to monitor patients with inflammatory disease particularly rheumatoid arthritis.

B. **SPECIMEN:** EDTA anticoagulated whole blood that is less than 4 hours old; the EDTA tube must be at least half full.

C. **EQUIPMENT:** Sediplast system including sedivials (with 0.2 ml sodium citrate), autozero Westergren tubes and Sediplast rack; plastic pipets.

D. **CONTROLS:** Both normal and abnormal ESR control samples are run daily and must fall within established limits.

E. **PROCEDURE:**

1. Mix EDTA whole blood sample or control vial. LABEL sedivial and remove sedivial cap. Using a transfer pipet, obtain an aliquot of blood and fill the sedivial with blood (0.8 ml) to the fill line. Recap and mix thoroughly.
2. Place sedivial in rack on a level surface. Insert the autozero tube into the sedivial (either with cap removed or directly through cap). Continue inserting until the tube rests at the bottom of sedivial.
3. **Verify that the blood is at the zero mark and that there are no bubbles in the tube.**
4. Allow the sample to stand undisturbed for exactly one hour and then read the results of the sed rate in millimeters (distance the red cells have fallen in one hour).

F. **SIGNIFICANCE:**

■**In normal individuals, sedimentation or falling of the red cells is slow.**

NORMAL Reference Ranges:	Males 0-10 mm/hr	>50yo 0-20 mm/hr
	Females 0-20 mm/hr	>50yo 0-30 mm/hr

■**In conditions with increased concentrations of certain plasma proteins that promote rouleaux (such as FIBRINOGEN,** an acute phase reactant), red cell falling is accelerated causing an abnormal (increased) ESR.

■**Abnormal/increased** ESR results are seen in acute and chronic infections, chronic inflammatory disorders (RA), malignancies especially multiple myeloma, tissue necrosis and pregnancy.

G. **SOURCES OF ERROR:** Erroneous results occur if the blood is clotted or over four hours old; if the blood tube is not at least 1/2 full; if the tube is slanted or there are bubbles in the tube.

WHOLE BLOOD CLOTTING TIME (WBCT) and CLOT RETRACTION PROCEDURES 12

Obtain blood by venipuncture using a syringe. Draw 5 ml of blood and **slowly** place 1 ml of blood into each of two 12x75mm glass tubes; parafilm each tube. Put the other 3 ml of blood into an EDTA tube, mix and label [for use later].

A. **Whole blood clotting time (WBCT) -** Tilt one glass tube every 30 secs until the blood clots = WBCT. The glass contact activates coagulation; it will take longer for the other tube to clot. After the blood in both tubes has clotted, place the tubes in a 37^{o}C waterbath for the clot retraction test.

B. **Clot Retraction** - Evaluate tubes at 1 hour for retraction of the clot from the sides of the tubes. Although rarely performed, the clot retraction test measures the ability of platelet contractile proteins (actomyosin, thrombasthenin) to reduce clot size.

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TEMPLATE BLEEDING TIME

A. **PRINCIPLE:** The bleeding time is an ***in vivo*** measure of platelet plug formation following capillary injury. It is a screening test for disorders of platelet function and may be affected by vessel defects or low platelet numbers (e.g., PLT count below 100,000/cmm).

B. **SPECIMEN:** This procedure is performed at the bedside. No special patient prep is required; the patient's arm must not have IVs.

C. **SUPPLIES/EQUIPMENT**: Surgicutt template device for making standardized incision, stopwatch, sphygmomanometer, filter paper, alcohol, butterfly bandage.

D. **PROCEDURE:**

1. Place the patient's arm on a supportive surface with palm up. Cleanse the area selected with alcohol being careful to avoid surface veins.

2. Place blood pressure cuff on patient's forearm and inflate cuff to 40 mm Hg. This pressure should be frequently monitored during the procedure.

3. Remove Surgicutt device from blister pack and remove safety clip. Rest the device lightly on the forearm so that the cut is made parallel to the fold in the elbow.

4. Push the trigger and simultaneously start the stopwatch. Remove the device and dispose of in a biohazard container.

5. Wick the flow of blood with filter paper every 30 seconds. **DO NOT TOUCH** the paper directly to the incision to avoid disturbing the formation of the platelet plug.

6. Continue blotting until blood no longer stains the filter paper. Stop the stopwatch and record time to nearest 30 seconds. Discontinue procedure if bleeding does not stop within 15 minutes.

7. Remove cuff, clean arm and apply butterfly bandage across the incision.

E. **NORMAL**: $\leq$ 8 minutes

F. **SOURCES OF ERROR:**

1. Failure to maintain pressure cuff at 40 mm Hg.
2. Touching the wound site when wicking away the blood.

G. **PRECAUTIONS**:

1. A bleeding time ordered on a patient with a platelet count below 40,000/cmm must be approved before doing the bleeding time.
2. Recent aspirin ingestion may prolong the BT result.

H. **DISADVANTAGES:** This procedure may leave scars.

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FIBROMETER PT AND APTT PROCEDURES

A. **PRINCIPLE:** The PT and APTT tests measure certain plasma proteins which participate in clot formation. The time for clot formation is detected by completion of an electrical circuit by a fibrin strand (endpoint). *Neither test measures platelets, calcium, or factor XIII.*

B. **SPECIMEN:** The specimen of choice is citrated platelet-poor plasma that is collected atraumatically in 3.2% sodium citrate. **A full tube is required**. *Tissue factor should not be present if the blood draw is trauma-free.*

C. **PROCEDURE:**

1. **Prothrombin time/PT** - Measures factors in the extrinsic and common pathways.
   a. Reagent: Tissue thromboplastin (a tissue phospholipid source, i.e., TF) with calcium.
   b.. Add 0.1 ml patient citrated plasma (prewarmed at 37^{o}) to 0.2 ml reagent (prewarmed at 37^{o}) and time for clot formation.
   c. Normal range: 11.0-13.4 seconds.

2. **Activated Partial Thromboplastin time/APTT** - Measures factors in the intrinsic and common pathways.
   a. Reagents: A platelet phospholipid source with surface activator and calcium.
   b. Add 0.1 ml reagent (prewarmed at 37^{o}) to 0.1 ml patient citrated plasma and incubate together for 3 minutes.
   c. At 3 minutes, add 0.1 ml calcium chloride and time for clot formation.
   d. Normal range: 22.0-37.0 seconds.

D. **CONTROLS:** Both normal and abnormal control levels are run and must "read" to report patient results.

E. **SOURCES OF ERROR:** 'Short draw' (i.e., citrate tube not full), clotted sample, heparin contamination, traumatic draw, hemolyzed sample.