HealthPartners®

Hemogram: Manual Differential, Cell Morphology, and Platelet Estimate Procedure (Wright Stain)	**Attachments** ☒ Yes ☐ No
Key words Diff, Cell count, cell stains, smear, morphology	**Number** **GHP-PC-CLINIC LAB-Procedures-Differential v. 03-2010**
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Manual Clinic Laboratory Procedure Manual	**Last Review Date** **January 2015**
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Applicable Clinic Laboratory Staff	**Origination Date** **September 1985**
	Retired Date
Level of Complexity Non-waived	**Approved Date** **September 1985**
Review Responsibility Laboratory Technical Consultants	**Contact** Laboratory Technical Consultants
APPROVAL(S) Laboratory Medical Director	

Differentials – Morphology and Platelet Estimate-Wright Stain
Clinic Lab Procedure (Pages 1-10)
Result entry (Refer to Computer entry procedure)

PURPOSE/PRINCIPLE

Dried blood smears are fixed in methanol and stained with Wright Stain. Stained smears are used to establish the relative frequency of each WBC type and to assess blood cell morphology.

Note: If a differential is ordered, a WBC or hemogram must also be ordered on the same access number.
If a provider wants NRBCs only, it is not necessary to do a differential.
See the Differentials procedure in the Computer Test Manual. These are sent to Central Lab.

POLICY

Laboratory Staff will follow the approved techniques for white cell count differentials outlined in this procedure.

Specimen

1. EDTA (K+) whole blood. **Slides should be made the same day the specimen is collected; within 4 hours is preferred.** As the blood ages a number of changes occur: disintegration of WBCs, clumping of WBCs, platelet satelliting, swelling of WBCs and RBCs, vacuolization of WBCs, burring of RBCs.

2. Tube must be at least 1/3 full. Before testing, allow the EDTA anticoagulant and blood to equilibrate for at least 15 minutes before testing. Specimens should be well mixed prior to testing and *check for clots with applicator sticks*.
3. Specimens cannot be tested if tube is not adequately filled, a clot is present, or grossly hemolyzed and will need to be redrawn.
4. Slides should be made as soon as possible after collection. As blood ages, a number of changes may occur: disintegration of WBCs, clumping of WBCs, platelet satelliting, swelling of WBCs and RBCs, vacuolization of WBCs, burring of RBCs. Cut off time for making slides is 24 hours, within 4 hours is preferred. Take time difference into account if abnormal morphology.

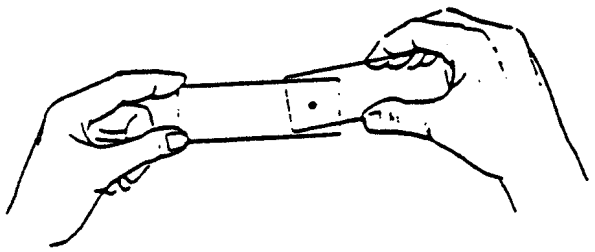


Fig. 116. Method of holding slides for preparation of blood smear.

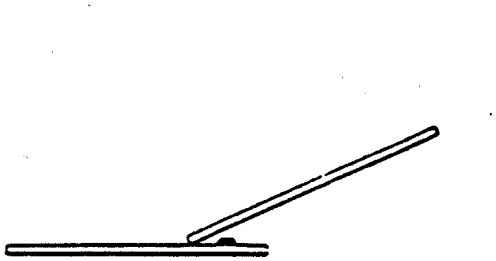


Fig. 117. Proper 25° angle for spreader slide.

5. Fingerstick: A free flowing puncture is needed.
6. Smears should be of good quality: smear should take up ½ to ¾ of slide, feather edge should be even with no tails or streaks.
7. The feather edge should be towards the frosted end of the slide.

Quality Control and Documentation

Blood smears stained with Wright stain look pink-purple when viewed with the naked eye. Microscopically, the red cells should be pink, not reddish orange or blue green. There should be no precipitate and the color of the film should be uniform. The nuclei of leukocytes are blue to purple, the chromatin and parachromatin clearly differentiated. Neutrophilic granules should be tan-pink in color, eosinophilic granules red-orange in color, and basophilic granules blue-purple in color. Platelets should have blue-purple granules. Cells should be evenly distributed.

If smear does NOT meet these criteria, it fails QC specifications. Reject and make new slides immediately.

Document if stain is OK on heme daily startup worksheet.

Document slide check specifics on heme print out (WBC and Plt checks, MCV if macro, micro, etc.)

If WBC is outside of normal range and diff is not ordered, please make and fix “just in case” slides.

If Diff “if” is ordered and diff is not indicated, please make and fix “just in case” slides.

Wright stain

Methanol (store at room temperature)
Glass slides
Microscope
Distilled Water
Three Step Stain Set (store at room temperature)

a. Fixative (light blue) solution. Methyl alcohol can be used as an alternative.
b. Solution I: Eosin Y (orange liquid)
c. Solution II: Azure A (purple liquid)

Three Step Stain Set working reagents should be changed and coplin jars cleaned monthly or more often as needed. Discard reagents if any precipitate or bacterial contamination problems.

Distilled water should be changed every day of use and more often if needed.

Coplin jars must be kept covered to minimize vapors and hydration of alcohol. Use away from all sources of heat. Lab coats and gloves should be worn during staining and pouring of reagents to prevent skin contact with reagents. Use care to prevent splashing, especially eye contact.

PROCEDURES

THREE-STEP STAIN PROCEDURE

1) Dry slide promptly and thoroughly. Fix in methanol (Fixative Solution) immediately, even if not stained immediately (Step b.). To prevent the plasma background of the film from staining blue, blood films should be stained within a few hours of preparation.

2) Dip slide in Fixative (light blue) solution 10-15 seconds. Allow excess to drain.

3) Dip slide in Solution I (orange liquid) 10-15 seconds. Allow excess to drain.

4) Dip slide in Solution II (purple liquid) 10-15 seconds. Allow excess to drain.

5) Rinse slide in distilled water 10-15 seconds.

Troubleshooting:

- If more intense overall stain is desired, increase the time the slide is in Solutions I and II.
- If a paler stain is desired, decrease the time the slide is in Solutions I and II.
- If color is too blue: RBCs appear blue or green, nuclear chromatin blue-black, eosin granules may be deep gray or blue, or neutrophil granules intensely overstain and may appear larger than normal thereby mimicking “toxic granulation”. Consider:
    a. Blood smear may be too thick.
    b. Staining time too long
    c. Increase the time the slide is in Solution I, or decrease the time in Solution II, or both. If this does not work, increase the rinse time.
- If color is too pink: RBCs appear bright red or orange, nuclear chromatin pale blue. Consider:
    a. Staining time is insufficient
    b. Decrease the time in Solution I, or increase the time in Solution II, or both. If this does not work, decrease rinse time
- Possible causes for precipitate on slide:
    a. Dirty slides
    b. Inadequate washing of slide; agitate more in rinse step.
    c. Dirty distilled water.
    d. Contaminated solutions.

DIFFERENTIAL PROCEDURE

A. WBC and Platelet Estimates

1. Evaluate the smear under low power (10x). Check cell distribution. If needed, make and stain a NEW slide for better stain quality or cell distribution.

a. Scan feather edge. Note the WBC and platelet distribution. Cells should be evenly distributed and not aggregated at sides of feather edge of slide.

b. Perform a WBC estimate. See below for calculations. Count the number of leukocytes in several fields of the examining area (red cells touching but not overlapping). **Reliability of the WBC estimate depends on counting in the proper area of the slide, and on the uniform distribution of the cells on the slide.** All nucleated cells, including damaged cells, should be included in the estimate.

Estimate WBC count using either formula below:

- WBCs/lpf x 200 = WBC / uL
  (same as WBC / 5 x 1000)

- 20-40 WBCs/lpf = 4,000 – 8,000 WBC / uL
  40-60 WBCs/lpf = 8,001 – 12,000 WBC / uL
  60-100 WBCs/lpf = 12,001 – 20,000 WBC / uL
  100-200 WBCs/lpf = 20,001 – 40,000 WBC / uL

The estimate should agree with ± 25% of the instrument WBC count. If the count and slide do not agree, check labeling, make new slide and rerun specimen through ABX. If problems persist, consult with the technical consultants.

2. Switch to 100x oil.
   a. Scan feather edge. If any immature cells are seen on feather edge, continue but consider making a new slide and consult with a lab technical consultant if only mature cells are seen on later differential.
   b. Perform a platelet estimate, using either formula below:

- # of platelets / oil field x 15,000 = count

- 0-3 plts / oil field — marked decrease
  4-6 plts / oil field — moderate decrease
  7-9 plts / oil field — slight decrease
  10-30 plts / oil field — adequate
  31-40 plts / oil field — slight increase
  41-50 plts / oil field — moderate increase
  >50 plts / oil field — marked increase

Note: If the patient's RBC count (or HGB) is normal, the above guidelines apply. If the RBC count (or HGB) is low, platelets may appear falsely increased. Therefore, the above guidelines will not apply.

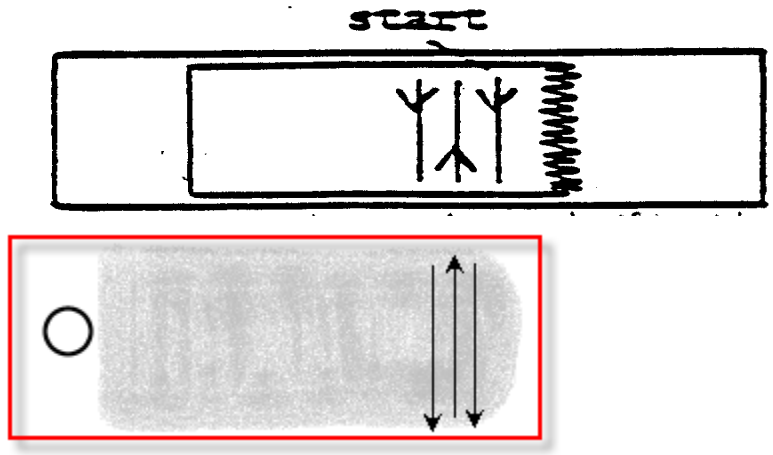
B. WBC Differential

Follow ABX Hematology Verification Guidelines and ABX flow sheets for instructions regarding ABX differential actions that can lead to the need for a manual differential.

C. Perform a 100 cell differential (50 cell diffs are **not** acceptable).

- The differential should be performed where the red cells are just touching, but not overlapping. Larger cells tend to congregate at the edge of the smear, so a false increase in neutrophils or monocytes may be reported if performing the diff in too thin an area. In too thick an area, a false increase in lymphocytes may be reported. In addition, WBCs are often distorted in this area.
- Multiple slides should be used to perform 100 cell diffs on low WBC counts. Do **not** count in too thick an area.

- The following drawing illustrates the pattern in counting a differential:



- The ABX and manual cell check results should match within the following guidelines:
  - Neutrophils - 10 cells
  - Lymphocytes - 10 cells
  - Monocytes - 8 cells
  - Eosinophils - 5 cells
  - Basophils - 2 cells

  If the results match within these guidelines:
  Be sure to reject the manual differential and accept the ABX results.
  If the results do not match within these guidelines:
  Perform a 200 cell count manual differential on the GUI keyboard
  (see computer entry procedure)
  The second 100 cells should be counted on a second slide and if possible, by a 2nd tech.
  Before accepting diff results calculate the absolute band count.

  $$\text{Formula: } \frac{\%\ \text{cells}}{100} \times \text{WBC} = \text{absolute count}$$

  $$\text{Example: Band \% = 12, WBC = 5.9 } \quad \frac{12}{100} \times 5.9 = 0.71 \text{ absolute bands.}$$

- **If absolute band count is > 1.0**, then add LFTs by pressing [LFT key.
- **Review the reactive lymph %**. If it's >10% of total lymphs, be sure to include]RL key.
- **Add the RBC morphology and platelet estimate** and accept manual results.
- **NRBC's:** If nucleated RBC's are seen, report their number per 100 WBC. Sunquest will calculate corrected WBC if the differential (including NRBCS) is accepted AFTER the CBC results have been accepted. The following formula may be used to manually correct the WBC for NRBC's when Sunquest is unavailable

  $$\frac{\text{Uncorrected WBC} \times 100}{100 + \text{NRBC's/100 WBC}} = \text{CORRECTED WBC}$$

- **Differential is ordered and WBC is <2.0.**
  - If patient WBC has not been <2.0 in the past 6 months, perform 100 cell differential.
  - If patient has had <2.0 WBC in last 6 months, do **not** automatically perform differential. Verify that the provider really does want the differential when the critical WBC Is being called. If provider does not want differential credit with code LWBC (WBC <2.0 Diff Cancelled by Provider).

- **Absolute counts** will be calculated by Sunquest when using the DF function described in the Computer Entry for Hematology Procedure in the Hematology Procedure Manual. To manually calculate absolute counts, multiply the WBC by the cell type %. The absolute neutrophil count includes both segmented and band neutrophils. The Absolute lymphocyte count are includes both normal and reactive lymphs.

  Example:
  WBC = 8.4
  PMN's = 40%
  Bands = 7%
  Lymphs = 35%
  Reactive Lymphs = 10%
  Monos = 5%
  Eos = 3%

  Absolute neutrophils = 8.4 x (0.40 + 0.07) = 3.9
  Absolute lymphocytes = 8.4 x (0.35 + 0.10) = 3.7
  Absolute monocytes = 8.4 x 0.05 = 0.4
  Absolute eosinophils = 8.4 x 0.03 = 0.2
  Absolute basophils = 8.4 x 0.00 = 0.0

Notes:

- **While performing a manual differential, if any immature or unidentifiable cells are seen, and you don't feel confident in identifying these cells, stop immediately and send sample and slides to Central Lab. Follow preliminary process if provider requests. Immature cells are defined as:**
  - *Metamyelocyte*
  - *Myelocytes*
  - *Promyeloctyes*
  - *Blasts*
  - *NRBCs*
  - Plasma Cells
- **In any case of questionable hematology results, make and fix multiple slides ASAP for possible review.**
- **If uncomfortable with entering diff results into computer, consult with the technical consultants, Central Lab hematology and/or provider. Follow Preliminary Process if appropriate.**
- **Any blood smear with immature or unidentifiable cells must be sent to Central Lab if you don't feel confident in identifying these cells or if it is a new patient without previous history. Do not report out the differential.**
- **Follow Preliminary Process if appropriate.**

Differential Reference Ranges

NEUT %	16-Adult	43-72%
	12-16 yrs	35-71
	8-12 yrs	32-70
	5-8 yrs	32-64
	2-5 yrs	23-55
	10 days-2 yrs	14-45
	4-10 days	19-59
	0-4 days	32-72

LYMPH %	12-Adult	17-43%
	5-12 yrs	23-48
	2-5 yrs	35-65
	6 mo.-2 yrs	45-76
	1-6 mos.	41-71
	14 days-1 mo.	43-53
	10-14 days	36-45
	4-10 days	26-36
	0-4 days	19-29
MONOS %	All ages	4-12%
EOS %	All ages	0-8%
BASOS %	All ages	0-1%
ABS NEUT	21-Adult	1.8-7.7 k/ul
	16-21 yrs	1.8-8.0
	6-16 yrs	1.5-8.0
	1-6 yrs	1.5-8.5
ABS NEUTs	6 mo.-1 yr	1.0-8.5
	7 days - 6 mos.	1.5-10.0
	0-7 days	6.0-26.0
ABS LYMPHs	21-Adult	1.0-4.8 k/ul
	16-21 yrs	1.2-5.2
	6-16 yrs	1.5-7.0
	4-6 yrs	2.0-8.0
	1-4 yrs	4.0-10.5
	1 mo.-1 yr	2.5-16.5
	7 days-1 mo.	2.0-17.0
	0-7 days	2.0-11.0
ABS MONOs	18-Adult	0.1-0.7 k/ul
	0-18 yrs	0.0-1.0
ABS EOS	All Ages	0.0-0.5 k/ul
ABS BASOS	All Ages	0.0-0.2 k/ul

MORPHOLOGY

Evaluate RBCs under oil immersion, using fields in which red cells are just touching but not overlapping. The feather edge and deeper portions of the smear should be avoided; RBCs at the feather edge appear large, flat, and have no central pallor, while those in the deeper portions appear small with sharply delineated areas of central pallor. Examine a minimum of 10 fields. Make and stain a new slide if smear is poorly stained or smear is too thick or thin.
A normocytic (normal size and shape), normochromic (normal color) blood smear exhibits a small amount of variation in size, shape, and color randomly occurring in each oil immersion field. This is accepted as being within normal limits.
To make the system more standardized, guidelines have been established for each category. Note that the ranges for quantitation represent the **number** of abnormal cells in an oil field of approximately 150 RBCs.

Size:

The term anisocytosis will not be used. If the size is abnormal, report as few, moderate, or many microcytes or macrocytes using the following guidelines:

0-4 cells / oil field Normal or Occasional

5-15 cells / oil field	Few
16-35 cells / oil field	Moderate
>35 cells / oil field	Many

Microcyte: Less than 6 microns in an adult
Macrocyte: Greater than 8.5 microns in an adult

What is seen on the smear should correlate with MCV. If it does not, make a new smear and/or repeat counts. Check patient history and consult with a lab technical consultant if needed.

Notes:

- The cell size in children is different than adults. For example, children 6 months of age can have MCV's as low as 74. Since this is normal for them microcytes should **not** be reported.
- In some instances when the RBC's are fragile (thalassemia, spherocytosis), the RBC's seen on the smear may not correlate with the MCV. This is because you see only 2 dimensions on the smear versus 3 dimensions measured by the MCV. Thalassemic cells tend to spread out when subjected to smearing, making them appear larger and paler than they actually are. Spherocytic cells tend to round up and pull in when smeared, making them look smaller than they actually are. In these situations, the MCV should over-rule the smear in reporting morphology

Shape:

The term poikilocytosis will not be used. Quantitate each type of abnormality using the guidelines below. This will include elliptocytes/ovalocytes, target cells, spherocytes, helmet cells, sickle cells, teardrops, acanthocytes, schistocytes, burr cells, stomatocyte, etc.

Note: Correlate spherocytes with normal or increased MCHC.

0-1 cells / oil field	Normal or Occasional
2-5 cells / oil field	Few
6-15 cells/ oil field	Moderate
>15 cells / oil field	Many

Color:

Hypochromasia is defined as less than the normal amount and concentration of hemoglobin in RBCs; identified by the expanded zone of central pallor to more than 1/3 of the cell. What is seen on the smear should correlate with the MCHC. If MCHC is normal, make a new smear and/or repeat counts. Dry slides properly and promptly before staining. Look for Hypochromasia when MCHC is less than 32. As a guideline, the amount of hypochromasia seen on the smear follows the MCHC:

31-32 MCHC	Slight
30-31 MCHC	Moderate
<30.0 MCHC	Marked

Polychromasia

Polychromatophilic cells should be non-nucleated round or oval, approx 8-10 microns in diameter. Cells lack central pallor and stain homogeneously pink-gray or pale purple.

0-1 / oil field	Normal
2 / oil field	Slight
3-6 / oil field	Moderate
>6 / oil field	Marked

Inclusions:

Howell-Jolly bodies are singular, almost perfectly round, purple nuclear fragments, approximately 0.5 micron in diameter, seen within non-nucleated RBCs.

Basophilic Stippling is defined as fine, medium or coarse blue granules uniformly distributed throughout the red cell. Fine basophilic stippling is commonly seen in polychromatophilic RBCs.

1-2 / oil field	Slight
3-6 / oil field	Moderate
>6 / oil field	Marked

Distribution:

Rouleaux describes red cells (4 or more) aligned in a linear fashion, simulating a stack of coins.
Rouleaux is an artifact generally seen in the thick areas on all slides. True rouleaux is seen in thin areas of the slide, where normally the red cells barely touch one another. Before reporting rouleaux, please make another slide; dry properly and promptly before staining.
If true rouleaux is present, report as slight, moderate, or marked.

Agglutination

If agglutination (cell clumping) is present, suspect Cold Agglutinins. See Hemogram procedure, Special Procedure #1.

Artifacts:

The blood smear is one of the most valuable tools in evaluating hematologic disorders. A poorly stained or made smear can do a great disservice to that evaluation.

Smears that are not dried quickly may create such false abnormalities such as target cells, stomatocytes, rouleaux, hypochromasis, etc.

It is therefore imperative that smears be of good quality, and that they be correlated with the hemogram results.

REPORTING OF RESULTS

Clinic Labs: Refer to the Computer Entry for Hematology for Clinic Labs

REFERENCES

Miale: Laboratory Medicine Hematology.
Wintrobe: Clinical Hematology.
Davidsohn and Henry: Clinical Diagnosis by Laboratory Methods.
Densmore, CM: Eliminating Disintegrated Cells on Hematologic Smears. Lab Med, vol. 12# 10, October 1981, pp 640 – 641
Wise, K: RBC Morphology Review – Correlation with RBC Indices. GHI Paper Inservice, March 1992
Diff-Quik Package Insert
O'Connor, BH A Color Atlas and Instruction Manual of Peripheral Blood Cell Morphology
Brown: Hematology: Principles and Procedures
College of American Pathologists, 1995 EXCEL, Proficiency Testing Program. Appendix II,

RELATED DOCUMENTS

NA

APPENDIXES

NA

AUTHOR(S)/REVIEWER(S)

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IV. DEFINITIONS

V. COMPLIANCE

Failure to comply with this policy or the procedures may result in disciplinary action, up to and including termination.

OTHER RESOURCES

College of American Pathologists, 1995 EXCEL, Proficiency Testing Program. Appendix II, Glossary of Hematology
Computer Order and Result Entry
ABX Hematology Verification guidelines

VII. ENDORSEMENT

Laboratory Administration