**HEM.SMEARS.4.0 BLOOD SMEAR REVIEW AND WBC DIFFERENTIAL**

**PRINCIPLE**

The blood smear review is a quantitative and qualitative study of stained blood film for RBCs, WBCs and platelets. The WBC differential is a means of reporting relative numbers of each type of white cell in the peripheral blood. WBC, RBC and platelet morphology is studied and WBC and platelet count are estimated.

**OWNERS**

Manager, Regional Hematology

**SPECIMEN COLLECTION**

A. Wright’s stained blood smear. A satisfactory blood smear is free of precipitates, properly stained and has even cell distribution.

B. Cell profile results from automated analyzer.

**REAGENTS / EQUIPMENT**

A. Microscope

B. Immersion oil

C. Computer

**QUALITY CONTROL**

A. Slides should be checked for adequate staining. The quality of the stain should be recorded. An optimally stained smear has the following characteristics:

1. The red blood cells should be pink to salmon in color.

2. Nuclei are dark blue to purple.

3. Cytoplasmic granules of neutrophils are lilac.

4. Cytoplasmic granules of basophils are dark blue to black.

5. Cytoplasmic granules of eosinophils are red to orange.

6. The area between the cells should be clean and free of precipitated stain.

B. A well-stained slide is necessary for accurate interpretation of cellular morphology. If any of the above characteristics are not adequate, follow troubleshooting procedures for the current staining method in place (using the individual procedure or operator’s manual).

**PROCEDURE**

A. Examine the blood smear using 50X or 100X oil objective.

1. WBCs should be evenly distributed over the smear.

2. Examine thin peripheral edge of smear. Smear should be discarded and new one made if there is poor distribution of WBC types, excess WBCs or platelet clumping.

3. Scan smear to note anything unusual or irregular.

4. RBCs should only slightly overlap.

B. Perform WBC differential and examine WBC morphology.

1. WBC counting may be done using the 50X or 100X oil objective.

2. Begin in the thin area of slide with RBCs slightly overlapping. Gradually move the slide. Count each WBC seen, and record on computer differential cell counter, until 100 WBCs have been counted. NRBCs are recorded on computer and correction to the WBC count is automatically made for NRBC.

3. Note WBC abnormalities and record as present in the computer. Do not differentiate in very thin or very thick areas. Very thin areas often show cellular distortion, very thick areas make distinguishing morphology difficult. See exception for handling old bloods in procedure notes.

C. Examine RBC Morphology:

1. RBCs should be adjacent, but not overlap.

2. The very thin edge of smear should not be used. Cells in this area are generally distorted and appear filled with Hgb, showing no area of central pallor.

3. Note any variations from normal and semi-quantitate as rare, slight, moderate, marked, or record as present. RBCs are examined for size, shape, relative Hgb concentration, polychromatophilia, inclusions, rouleaux, and agglutination.

4. Upon review of slide if morphology is normal, modify CMOR from "Hide" to Normal, "Norm"

D. Examine platelets for morphology using the same fields as for RBC morphology. Note abnormal morphology or clumping and record as present. If EDTA clumped platelets are present, follow procedure on "Complete Blood Count", platelet section.

**GUIDELINES FOR RECOGNITION AND GRADING OF WBC, PLATELET AND RBC ABNORMALITIES:**

Present: All WBC abnormalities, all platelet abnormalities, rouleaux, agglutination, Howell-Jolly bodies, cabot rings, basophilic stippling and Pappenheimer bodies will be recorded as present.

NOTE: NRBCs and megakaryocytic cells will be enumerated and WBC count corrected. Smudge cells are counted as part of the WBC differential.

For grading, determine average number of cells in 10 oil immersion fields.

Anisocytosis, microcytosis, macrocytosis, and hypochromia are graded as:

|  |  |  |
| --- | --- | --- |
| **GRADE** | **AVERAGE # OF CELLS/100X** | **AVERAGE # OF CELLS/50X** |
| Slight | 6 - 15 | 11 - 30 |
| Moderate | 16 - 30 | 31 - 60 |
| Marked | > 30 | > 60 |

All RBC abnormalities not listed above and polychromasia are graded:

|  |  |  |
| --- | --- | --- |
| **GRADE** | **AVERAGE # OF CELLS/100X** | **AVERAGE # OF CELLS/50X** |
| Rare | 0 - 1 | 2 - 3 |
| Slight | 2 - 5 | 4 - 10 |
| Moderate | 6 - 15 | 11 - 30 |
| Marked | > 15 | > 30 |

A. RBC Morphology

1. Normal = Cells are evenly distributed and appear as circular homogeneous discs that are uniform in size. Size can range from 6-8 microns in diameter. The size of a small mature lymphocyte can be used as a reference, the nucleus is 7-9 microns.

2. Abnormal RBC size:

a. Microcytes - RBCs smaller than 6 microns when compared to the nucleus of a small lymphocyte that is 7-9 microns.

b. Macrocytes - RBCs larger than 9 microns.

c. Anisocytosis - presence of more than one easily distinguishable cell line. The overall amount of anisocytosis is determined from the total of the average numbers of microcytes and/or macrocytes present per oil immersion field.

3. Abnormal RBC color: Variations in Hgb concentration correspond to the size of the area of central pallor. These variations may show corresponding changes in the MCHC and MCH.

a. Hypochromia - the area of central pallor is greater than 1/3 of the area of the cell.

b. Polychromasia - RBCs show increase bluish tinge with Wright’s stain due to presence of RNA.

4. Abnormal RBC shape: Variations from the normal biconcave disc of the mature RBC (poikilocytosis).

a. Spherocytes - round cells lacking central pallor, showing increased staining intensity and having a smaller volume than a normal cell.

b. Elliptocytes - RBCs that have an oval or elliptical shape. These cells result from hereditary or acquired conditions and range from egg shaped or slightly oval to sausage, rod or pencil forms.

c. Burr cell (echinocyte) - RBCs with long, sharp irregularly spaced projections due to an abnormal environment; to be differentiated from crenated cells, which have regular, short blunt projections. Crenated cells are an artifact and should not be reported.

d. Target cells - RBCs that have a central area of hemoglobin surrounded by a relatively colorless ring and a peripheral ring of hemoglobin, due to a cytoplasmic defect.

e. Teardrops - oval cells with one end elongated as a result of membrane injury.

f. Fragments (schistocytes) - result from localized membrane damage.

g. Helmet cells - RBCs where the membrane develops a vacuole that enlarges causing the red cell membrane to rupture, leaving two horns which give the cell a half moon shape with a central crater effect.

h. Stomatocytes - RBCs that appear on a fixed and stained film to have an elongated or slitlike area of central pallor instead of the circular form.

i. Acanthocyte - RBCs similar to the burr cell, but more like a spiny spherocyte due to irreversible cell membrane alteration.

j. Sickle cells - elongated RBC with sharp double points in U, L or S shapes due to an abnormal hemoglobin.

5. Abnormal RBC aggregation:

a. Rouleaux - RBCs that are stacked like coins due to increased numbers of large asymmetrical proteins. Examine several high power fields in the thinner area of the smear to confirm its presence.

b. Autoagglutination - RBCs that are irregularly clumped due to circulating antibodies. Examine several high power fields in the thinner area of the smear to confirm its presence.

6. Red cell inclusions:

a. Basophilic stippling - aggregates of multiple bluish dots or granules of varying size due to the presence of RNA.

b. Cabot ring - delicately looped purplish ring or rings concentric to the outer margin of the red cell.

c. Pappenheimer bodies - ferric iron aggregates, usually multiple, appearing as faint blue granules.

d. Howell-jolly bodies - small spherical basophilic bodies usually no larger than one micron.

B. WBC abnormalities:

1. Toxic granulation - the presence of large, coarse, dark-purple, cytoplasmic granules in neutrophilic metamyelocytes, band or segmented forms. True toxic granulation is distinguishable from artifactual granulation (overstaining) by the tendency of the toxic granules to cluster in one area of the cytoplasm and by the fact that not all cells are affected equally.

2. Dohle bodies - light blue oval or spindle shaped cytoplasmic inclusions in neutrophilic bands or segmented cells. Remnants of immature cytoplasm, Dohle bodies result from disturbance of normal cytoplasmic maturation due to a variety of toxic conditions.

3. Auer rod - elongated, red staining, peroxidase-positive rod in the cytoplasm of early non-lymphocytic cells. Usually appearing singly, but can be multiple, the rods are thought to be an abnormal coalescing of lysozomes.

4. Pelger-Huet cells - hypolobulated cells of the neutrophilic series. Nuclei with "dumbbell", "peanut", or bilobed with attaching filament shapes are common. Non-segmented forms are mature but classified as bands. Use text code PLGR.

5. Hypersegmented neutrophils - a segmented nucleus containing more than 5 lobes resulting from a defect in maturation and delivery of the cells.

6. Smudge cells - disintegrating bare nucleus of a ruptured WBC with an enlarged amorphous shape, reddish purple staining and no chromatic structure. If > 10 / 100 WBC, diff must be performed on albuminized specimen (follow Smudge Cell Procedure).

7. Plasma cell - a WBC which has an eccentrically placed nucleus containing coarsely granular blue-purple chromatin. Adjacent to the nucleus is a perinuclear clear zone. The one-sided cytoplasm is basophilic and often contains small white vacuoles near the periphery. Plasma cells are not usually seen in the peripheral blood except in viral disease, immunological reactions, and multiple myeloma patients.

8. Vacuolated neutrophil - results when degenerating cytoplasm begins to acquire holes or as the result of active phagocytosis (may reflect increased lysosomal activity). This condition may be found in septicemia and severe infection.

9. Reactive Lymphocyte: A benign reactive change in the morphologic appearance of the lymphocyte frequently secondary to viral disease. There is a 2:1 or 1:1 nuclear:cytoplasmic ratio due to enlarged nucleus with mature chromatin and an increased cytoplasmic complement with increased basophilia and frequently the presence of nucleoli.

10. Hairy Cell – round to ovoid lymphocyte with moderate to abundant pale to grayish blue cytoplasm. Cell border of fine (hairy) cytoplasmic projections, which may be thick, blunted, smudged, serrated, or short. Cells are seen in Hairy Cell Leukemia. Count the cells as unclassified and comment ‘possible hairy cell’ and send the smear along with the CBCD report to a pathologist.

C. Abnormal platelets:

1. Giant platelets - platelets that are abnormally large but have normal morphology. Giant platelets are defined as equal to or greater than the size of a normal RBC.

2. Clumped platelets - aggregates consisting of two or more platelets per mass.

If quantitative count cannot be reported due to clumping, a statement of platelet adequacy should be reported; PAD - Platelets appear decreased on smear but clumped in EDTA; recollect in citrate. PAQ - Platelets appear adequate on smear but clumped in EDTA; recollect in citrate. PAI – Platelets appear increased on smear but clumped in EDTA; recollect in citrate.

3. Platelet satellitism – platelets resetting around PNS in blood collected in EDTA. If count appears adequate, comment with PLSAT – “Platelet satellitism in EDTA, but count appears adequate on smear. Recollect in citrate for accurate count.” If count does not appear adequate, comment with PAD.

D. Microorganisms

1. Make a comment that the microorganism is present, and whether it is intracellular or extracellular.

 2. Refer the smear and CBCD report to a pathologist.

 3. Examples: babesia, malaria, other RBC parasites; bacteria; fungi

**CALCULATIONS**

A. WBC count estimate

Perform estimate in area of smear where RBCs barely touch. Average the number of WBCs counted in 5 fields.

1. Method 1:

|  |  |
| --- | --- |
| **WBCs/HPF (50X obj)** | **Count X 1000/mm3** |
| 1 | 1.5 |
| 2 - 4 | 2 - 6 |
| 4 - 6 | 7 - 10 |
| 6 - 10 | 10 - 12 |
| 10 – 20 | 13 - 18 |

2. Method 2:

WBCs/LPF x 1000/mm3 to WBCs/LPF x 1000/mm3 = estimated WBC range

4 3

Example:

200 WBC counted in 5 LP fields (10X objective)

Average WBC/LPF = 40 WBC/LPF

40 to 40 or 10,000 to 13,000 WBC/mm3

 4 3

3. Method 3:

WBC = (Average WBC/40X field) x (2 x 1000/mm3)

Example: Average WBCs/40X field = 5.2

5.2 x 2 = 10,400 WBCs/mm3

WBC = (Average WBC/50X field) x (3 x 1000/mm3)

Example: Average WBCs/50X field = 5.2

5.2 x 3 = 15,600 WBCs/mm3

If estimate does not agree within 20% of automated count, then a manual count must be performed.

B. Platelet count estimate:

Perform estimate in area of smear where RBCs barely touch. Average the number of platelets counted in 10 oil immersion fields (100X obj).

Average number platelets 100X oil x 10,000 if low RBC count

x 15,000 if normal RBC count

x 20,000 if high RBC count

Example: 170 platelets on 10 oil immersion fields with normal RBC count

170

 10 x 15,000 = 255,000 platelets

C. WBC correction for NRBC or megakaryocyte cells:

Computer automatically adjusts WBC count for NRBCs in the differential mode of computer. If manual correction is needed, follow calculation below, and use text code ADJ (adjusted for nucleated RBCs). Manual correction should be done for any nRBC or megakaryocyte seen.

100 x uncorrected WBC count

100 + #NRBCs or megakaryocytes = Corrected WBC count

Example: # of WBCs counted = 100

# of NRBCs = 18

Uncorrected WBC count = 22.0 x 103 / uL

Corrected WBC count = 100 x 22.0

 100 + 18 = 18.6 x 103 / uL

D. Absolute cell count = Total WBC count x % cell type x 10

Example: Total WBC count = 10.3 x 103 / uL

% cell type = 1.3% Eos

Absolute Cell Count = 10.3 x 1.3 x 10 = 133.9 EOS or 0.13 x 103 / uL

**REPORTING RESULTS**

A. Normal Absolute Values:

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **0-14 days** | **15-30 days** | **31-60 days** | **61-180 days** | **0.5 -2 Yrs.** | **2-10 Years** | **10-18 Years** | **>18 Years** |
| **SEGSA-M** | 2.1-7.5 | 1.7-4.1 | 1.1-3.2 | 1.4-6.7 | 1.8-9.4 | 1.2-6.0 | 1.3-6.0 | 1.3-6.0 |
| **SEGSA - F** | 2.2-4.1 | 1.8-2.9 | 1.3-3.8 | 1.7-7.9 | 1.9-9.6 | 1.2-6.0 | 1.3-6.0 | 1.3-6.0 |
| **LYMPA -M** | 3.9-7.2 | 3.1-7.0 | 2.6-6.8 | 2.5-7.8 | 1.7-6.5 | 1.0-5.5 | 1.0-3.5 | 1.5-3.5 |
| **LYMPA-F** | 3.0-6.5 | 3.2-7.8 | 2.2-7.1 | 2.3-7.7 | 1.7-6.7 | 1.0-5.5 | 1.0-3.5 | 1.5-3.5 |
| **MONOA-M** | 0.7-2.1 | 0.6-1.8 | 0.4-1.9 | 0.3-1.7 | 0.2-1.5 | 0.0-1.0 | 0.0-1.0 | 0.0-1.0 |
| **MONOA-F** | 0.6-2.1 | 0.4-2.0 | 0.4-1.7 | 0.3-1.8 | 0.2-1.4 | 0.0-1.0 | 0.0-1.0 | 0.0-1.0 |
| **EOSA-M** | 0.0-0.7 | 0.0-1.0 | 0.0-0.6 | 0.0-0.4 | 0.0-0.4 | 0.0-0.7 | 0.0-0.7 | 0.0-0.7 |
| **EOSA-F** | 0.0-0.6 | 0.0-1.0 | 0.0-0.5 | 0.0-0.4 | 0.0-0.3 | 0.0-0.7 | 0.0-0.7 | 0.0-0.7 |
| **BASOA-M** | 0.0-0.2 | 0.0-0.1 | 0.0-0.1 | 0.0-0.2 | 0.0-0.2 | 0.0-0.1 | 0.0-0.1 | 0.0-0.1 |
| **BASOA-F** | 0.0-0.3 | 0.0-0.1 | 0.0-0.1 | 0.0-0.2 | 0.0-0.2 | 0.0-0.1 | 0.0-0.1 | 0.0-0.1 |
| **BANDA-M** | 0.0-3.5 | 0.0-3.5 | 0.0-1.0 | 0.0-1.0 | 0.0-1.0 | 0.0-1.0 | 0.0-1.0 | 0.0-1.0 |
| **BANDA-F** | 0.0-3.5 | 0.0-3.5 | 0.0-1.0 | 0.0-1.0 | 0.0-1.0 | 0.0-1.0 | 0.0-1.0 | 0.0-1.0 |
| **METAA-F** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **METAA-M** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **MYELA-F** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **MYELA-M** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **PRMYA-F** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **PRMYA-M** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **BLSTA-F** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **BLSTA-M** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

C. Critical value: SEGSA < 0.5

 BLST > 0.0

D. How to Use Differential function of Sunquest GUI:

1. Open Sunquest GUI by double-clicking the icon and select Work Area:

 a. SV Evansville users: CIS

 b. All other users: CII-A

2. A log in box will appear. Enter your Sunquest username , password, and site specific DLL location.

3. Open the **Differential Result Entry** Menu.

4. An information dialog box will appear with a dropdown list of available keyboards:

a. **SYSXE**: for Sysmex XE5000 and XE2100 users; WBCs are adjusted for NRBCs prior to manual differential.

b. **DIFF**: for all other users; WBCs are adjusted only at the time of a manual differential

c.  **KXPOX**: for SVAN users; a 3-part differential

5. **Differential Result Entry Menu** will open.

6. Under the “HISTORY” tab, enter the accession number in the highlighted (yellow) box in the upper left corner.

NOTE: To correct already released reports, simply enter the accession number. It is no longer necessary to add “M-“before the accession number.

 Press RETURN. Previous accessions for that patient will display. To view comments indicated by “…”: select the “…” and click on the green “Comment” button at the bottom of the screen.

 NOTE: To open a generic keyboard: click on Options (top left corner) -> Offline Counter.

7. Proceed to the “Count” tab. Several options are available:

a. **New Count:** select when you wish to clear a previous count and start a new differential. This differential will be completed at 100 cells. If at this point it’s realized that 100 cells will not be available, click on TERM (the = sign on the keyboard) and the relative cell differential will be adjusted up to 100 cells (only evident under the “QA Review” tab, the Total number under the “Count” tab will not change). To delete a cell from the count, click “Ctrl” + the corresponding key of the cell you wish to delete.

b. **Set Count:** select if the WBC count is really low and 100 cells will not be available. This function offers a choice between 10, 25, 50, 100 and 200 cells.

c. **Temp Save:** select if you wish to temporarily save a differential on a slide, and you wish to perform another differential on the same slide. After each temporary save all of the completed differentials will be displayed and the technologist will be able to consolidate them into one by holding the CTRL key, selecting the differentials and clicking “Average.”

d. **Count:** no need to select it; it is automatically highlighted when Set Count or New Count are in use.

e. **Manual:** select if the differential was previously performed and you wish to type in the percentages manually. Click on each cell at the top and enter the corresponding numbers.

f. **Comment:** use only when documenting a critical BLAST or SEGSA result. No other comments should be entered under the “Count” tab. If a critical absolute neutrophil (SEGSA) needs to be called and documented, click on the SEGS cell, then on the green “Comment” button and choose either absolute or relative value from the pop-up box. For SEGSA select absolute, for blasts both Relative and Absolute values must be documented. Even though there are many comment codes available under the Text code dropdown box, only the following will be used here for the purposes of a critical documentation: CRR (Critical Result Noted. Specimen appearance and label verified. Suggest repeat if questionable. Results called to), RBV (Read Back Verification) or NRBV (No Read Back Verification). Click “Add”. Free text the name of the person called (first and last) as well as the time of the call in the box at the bottom. Click “Add to List” and then “OK”.

8. Proceed to the “Morphology” tab. Select a DTYP if manual differential was performed: for CBCAD orders, select the “=” on the keyboard; for CBCAM orders the “]” key should be selected (EDIF should have automatically crossed over if the sample has been run on an analyzer). Enter your morphology either by clicking on the screen or by using the physical keyboard. The following comments are not displayed as keys from the keyboard:

 - RBC AUTOAGGLUTINATION

 - DIFFERENTIAL PERFORMED ON ALBUMINZED SLIDE

 - AUER RODS

 - CABOT RINGS PRESENT

 - EXTRACELLULAR MICROORGANISMS PRESENT

 - POSSIBLE HAIRY CELLS

 - INTRACELLULAR MICROORGANISMS PRESENT

 - POSSIBLE PLASMA CELLS

 - PELGER-HUET LIKE CELLS

 If the above comments need to be selected, click on the “/” key (called COM2). Then click on the green “Edit/Comment” button and a dialogue box will display. In the text code dropdown box all of the above comments will be available. Select and click “Add” to the right. Then, click OK.

 NOTE: If the differential was performed on less than 100 cells, this needs to be stated in the freetext box, located on the bottom of the Comment pop up box.

9. Proceed to the “QA Review” tab. Review all of the results. Critical results will be indicated by the “FAILED VERIFY” flag.

NOTE: Do not make any comments under this tab. If a critical is present, go back to the “Count” tab and enter proper documentation there.

To release your results, click “Save”. To reject your modifications, click “Reject” – if accession has been previously filed, this option will only reject the most recent modifications and not the already filed data. The “Close” button will close the Differential Result Entry menu.

**PROCEDURE NOTES**

A. If a significant number of microcytic RBCs or fragments are seen, the platelet count should be verified by a smear estimate. If estimate from smear doesn’t agree with automated count, a manual platelet count must be performed.

B. All smears are to be kept for a minimum of one week.

D. The technical error in the differential cell count has been described as ± 15%.

E. When a patient has a very low hemoglobin, the relative number of platelets on the smear will normally show a slight increase, while actually being present in the blood in normal numbers.

F. Old blood specimens which have degenerating cells should be scanned on a microscope to see if instrument flags such as LS (left shift), blasts, reactive lymphs, IG (immature granulocytes) truly exist. If blasts, reactive lymphs, metamyelocytes, or myelocytes are present, a manual diff must be performed. If slide agrees with automated differential, accept electronic diff. Do not perform a manual diff if only bands are seen. These may be degenerated cells. Albuminized blood smear can enhance cellular integrity. (See HEM.SMEARS.5.0 HANDLING SMUDGE CELLS ON BLOOD SMEARS for making albuminized slides.)

G. For any CBCAD slide viewed microscopically, CMOR must be answered. If no abnormality is noted, enter normal for CMOR (#8 on diff keyboard).

H. The analyzer may flag lymphocytes as atypical lymphs. Our current system for reporting these atypicals are as reactive lymphocytes.

**REFERENCES**

A. O’Connor, Barbara, *A Color Atlas and Instruction Manual of Peripheral Blood Cell Morphology*, 1984, pp. 20-35.

B. Lotspeich-Steininger, Cheryl, *Clinical Hematology*, 1992, pp. 88-105, 323-329.

C. Carr, Jacqueline H. and Rodak, Bernadette F. *Clinical Hematology Atlas*, 2009, pp. 5