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Leukocyte Differential Count in Peripheral Blood Manual-RO

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This procedure provides instructions for reviewing a peripheral blood smear and reporting the white blood cell (WBC) differential and morphologic findings.

II. PRINCIPLE:

The peripheral blood white blood cell differential count is performed to determine the absolute number of each type of white blood cell circulating in the peripheral blood. At the same time, a study of red blood cell, white blood cell and platelet morphology is performed.

III. SPECIMEN COLLECTION AND HANDLING:

Peripheral blood smear made by the wedge-pulled technique and stained on the Sysmex SP-1000*i* slide maker as described in the "Blood Smear: Manual Slide Preparation and Staining" procedure OR "Smear Preparation & Staining - Sysmex SP-1000*i*" procedure. Alternatively, the slide is stained on the SP-50 slide maker as described in the "Blood Slide Preparation and Staining SP-50" procedure.

IV. REAGENTS:

Refer to the "Blood Smear: Manual Slide Preparation and Staining" procedure.

V. CALIBRATION:

See Microscope Adjustment Procedure (Köehler illumination) procedure. This should be performed on a **daily** basis at minimum.

VI. QUALITY CONTROL:

Quality control consists of: (1) visual examination of the smear for quality appearance and successful staining as described in the quality control section of the "Blood Smear: Manual Slide Preparation and Staining" procedure and (2) daily comparison with the hematology analyzer automated differential.

VII. REPEAT DIFFERENTIAL WHEN WBC<1.0 BILL/L:

If an automated differential is not available due to a low WBC count, instrument flags, etc, a manual differential is performed and reported on the first ("first occurrence of a") specimen with a WBC <1.0 bill/L, based on the available cellularity, primarily to look for abnormal cells. However, repeat differentials on the same patient admission or encounter will not be performed when the WBC count is <1.0 bill/L and an automated differential cannot be obtained. Only a CBC is reported and charged in these instances.

VIII. PROCEDURE:

A. Review the hematology analyzer results for CBC parameter validity, differential percentages/ absolute values, and presence/absence of histogram/ scattergram, Interpretive (IP) and/ or definitive flags. (See IX, Note C.)

B. Scan slide at 20X dry immersion for:

1. satisfactory staining of cells
2. abnormal cells and platelet clumps along lateral edges of smear and in feathered edge
3. WBC count estimate (average # WBC @ 20X x 0.5 = WBC bill/L ± 25%)
4. even WBC distribution
5. immature cells or abnormal nucleated cells (e.g., blasts, promyelocytes, myelocytes, metamyelocytes, nucleated RBCs, etc.)
6. smudge cells and artifacts (see VIII, Step F)
7. platelet clumps (see VIII, Step F)
8. RBC distribution (e.g., rouleaux, agglutination, anemia/polycythemia) three fields inward from feathered edge RBC morphology (e.g., dual population, hypochromasia, polychromasia, poikilocytosis, nucleated RBCs, target cells)
9. selection of "differential area" where RBCs have central pallor and are in doublets/triplets but not overlapping.

C. Perform the WBC differential count at 100x or 50x oil immersion according to the following guidelines:

1. Traverse the smear thin to thick and from side to side (the "battlement" pattern). See Figure 1.

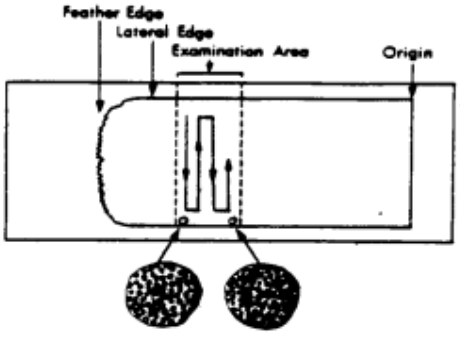

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Figure 1

2. Identify WBCs first by chromatin pattern and second by cytoplasm. See IX, Note A.
3. Count each WBC and nucleated RBC (if any) seen and tabulate on the WAM diffpad. See IX, Note B. Refer to Resulting a Manual Differential workflow.
    a. **NOTE:** Avoid studying cells too far into the thick or thin areas as cells will be distorted and not show characteristic morphologic features.
4. Note and report any WBC inclusions or abnormalities (e.g., Döhle bodies, vacuoles, toxic granulation, bacteria, hypersegmentation, etc.).
    a. **NOTE:** For WBC reporting/grading policies, refer to the "WBC & RBC Morphology Reporting" procedure.
5. If 5 or more nucleated RBCs are found, run the specimen in the differential mode on the Sysmex hematology analyzer to obtain the actual nRBC count.
    a. **NOTE:** In the event the WAM is down, count nucleated RBCs in the Cell Counting software program loaded at the morphology bench personal computers (PCs).

D. Examine the red blood cell morphology in a thin area of the smear where the RBCs slightly overlap. Report abnormalities in arrangement, size, hemoglobin content, shape or inclusions according to the "WBC & RBC Morphology Reporting procedure".

E. Examine the platelets for number and morphology in the same area used for the red blood cell evaluation. Report an estimate of either "ADQ", "DEC", or "INC".
    1. **NOTE:** The following guidelines are helpful for estimating platelet number:
        a. 7-23 PLT/100X approximates an estimate of "adequate" (normal range of 150-450x10^9/L).
        b. Platelet number @ 100X x 20 should approximate the platelet count.
    2. **NOTE: Before reporting a decreased platelet estimate, scan smear along feathered/lateral edges for clumps. Check tube of blood for clot.**
        a. If there is instrument/ smear disagreement:
            i. Check for clot.
            ii. Remake the slide.
            iii. If the instrument/ smear disagreement cannot be resolved by making/ staining a new slide, enter the following comment: *"No platelet interference observed; appear ___ (adq, inc, dec) on smear".*

F. For any questionable cell identification, seek the review of a second technologist. If morphology is still questionable, refer smear to pathologist or resident for review. (See "Criteria for Review of Peripheral Blood Smears" procedure.)
    1. **NOTE:** When saving smear for review, **BLOT** oil off onto paper towel / Kimwipe. **DO NOT RUB** oil off with Kimwipe as it scratches and removes cells from slide. Track the specimen in Beaker. Place smear for review, criteria review worksheet, and analyzer printout in designated folder at diff bench.

G. Report any unusual findings in the differential comment field **DIFFC** in the WAM.

H. Perform additional laboratory procedures stated below if the following smear findings are observed:

Finding	Procedure
>10% smudge cells	Albumin smear

Finding	Procedure
Sickle cells	Sickle Screen
Hgb crystals	Hgb Electrophoresis (after pathologist review)
PLT clumps	PLT estimate OR citrated (blue top) PLT count
PLT satellitosis	PLT estimate OR citrated (blue top) PLT count
Bacteria	Gram stain

IX. NOTES:

A. For detailed definitions of normal and abnormal cells, according to the College of American Pathologists (CAP), refer to Morphology Bench references.

B. Segmented **and** band form neutrophils are both reported as the "Neutrophil" count. Due to the introduction of the IG parameter on the Sysmex hematology analyzers, no band counts are reported for any patient population.

C. When the WBC count is less than 1.0 x 10^9/L, count as many WBCs as possible (up to 100) from two peripheral smears. If unable to count 100 cells, report the differential from as many WBCs as you can. The WAM will automatically convert the differential to 100%. Add comment, *"Differential performed on "X" cells".*

D. See Attachment A for a memorandum on the policy of reporting the WBC Differential count in absolute numbers versus relative percentages.

X. REFERENCES:

A. Dutcher, T.F. Personal communication: Peripheral blood smear examination, 1982.

B. National Committee for Clinical Laboratory Standards. Leukocyte differential counting (H20-T, Vol. 4, No. 11). Villanova, PA: NCCLS. 1984: 257-266.

C. Nelson, DA and Morris, MW. Basic methodology. In: Henry, JB (ed.). Clinical diagnosis and management by laboratory methods. 17th ed., Philadelphia: WB Saunders. 1984: 611-617.

Attachments

Attachment A - Absolute WBC Differential.pdf
Attachment B - Memo Re Phase Platelet Counts No Longer Available.pdf

Approval Signatures

Step Description	Approver	Date
	Ann Marie Blenc: System Med Dir, Hematopath	12/9/2021
Hematology Medical Director Designee	Ann Marie Blenc: System Med Dir, Hematopath	12/9/2021
Policy and Forms Steering Committee Approval (if needed)	Gail Juleff: Project Mgr Policy	12/7/2021

Step Description	Approver	Date
Policy and Forms Steering Committee Approval (if needed)	Michele Sedlak: Medical Technologist Lead	12/7/2021
System Manager	Rebecca Bacarella: Mgr Laboratory	9/23/2021
	Michele Sedlak: Medical Technologist Lead	7/20/2021

Applicability

Royal Oak