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Manual Differential Count in Peripheral Blood

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, morphologic findings, platelet estimate, and suspected microogranisms (if seen).

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. ACRONYMS:

A. White Blood Cell (WBC)
B. Nucleated Red Blood Cell (NRBC)
C. Red Blood Cell (RBC)
D. Immature Granulocyte (IG)
E. Complete Blood Count (CBC)
F. Myelodysplastic Syndrome (MDS)
G. Laboratory Information System (LIS)
H. Complete Blood Count with Differential (CBCWD)

I. Complete Blood Count without Differential (CBCND)

J. Standard Operating Procedure (SOP)

K. Mean Corpuscular Volume (MCV)

L. Platelet (PLT)

IV. SPECIMEN COLLECTION AND HANDLING:

Peripheral blood smear made by the wedge technique and stained either by an automated stainer or manually.

V. SUPPLIES:

A. Microscope Slides

B. Diff-Safe adapter or wooden sticks

VI. REAGENTS:

Refer to site specific instructions for staining slides.

VII. CALIBRATION:

Refer to the Microscope Maintenance procedure for the microscope adjustment (Köehler illumination) process. Microscope adjustment should be performed on each day of use.

VIII. QUALITY CONTROL:

Quality control consists of a daily check for visual examination of the smear for quality appearance and successful staining. Refer to site specific instructions for staining slides and proper documentation of quality control.

IX. WBC < 0.4 BIL/L:

All CBCWD orders with a WBC ≥ 0.4 bil/L will have a differential resulted. If the tech agrees with the automated differential, the analyzer count may be resulted. If the tech does not agree with the automated differential or if the analyzer is unable to report any differential parameters, a manual differential should be performed. Count as many WBCs as possible (up to 100) from one to two peripheral smears. If unable to count 100 cells, report the differential from as many WBCs as you can. The middleware will automatically convert the differential to 100%. Add comment to the WBC result, *"Differential performed on "X" cells"*.

All CBCWD orders with a WBC < 0.4 bil/L will not have a differential resulted. See steps below for how to report:

A. Confirm in middleware a WBC < 0.4 bil/L on a CBCWD order.

    1. If pathologist review needed based on the smear review checklist (ex. first time

neutrophils < 0.5), keep the CBCWD order, perform manual slide review per SOP, add path review in the middleware per SOP, and send for pathologist review.

2. If pathologist review is not needed based on the smear review checklist, continue with step B.

B. In the LIS, go to Specimen Inquiry by Specimen and scan the patient label.

C. Select Specimen Update.

D. Select Add On.

E. Add a CBCND order if the original order was for a CBCWD.

1. Add a CBCND chemo order if the original order was for a CBCWD chemo.

F. Go to the outstanding list. You will notice a CBCWD and CBCND order for the patient.

G. Cancel the CBCWD order. Reason "per policy", add comment "Differential not performed when WBC < 0.4."

H. Select the CBCND order, click the Actions button, and select Instrument Trigger.

I. Open the updated order for the CBCND in the middleware.

J. Enter internal comments as needed.

K. Add the HE10 comment to the WBC result, stating "Differential not performed when WBC < 0.4."

L. Cancel smear as needed.

M. Cancel second level review.

N. Verify all results.

X. 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.

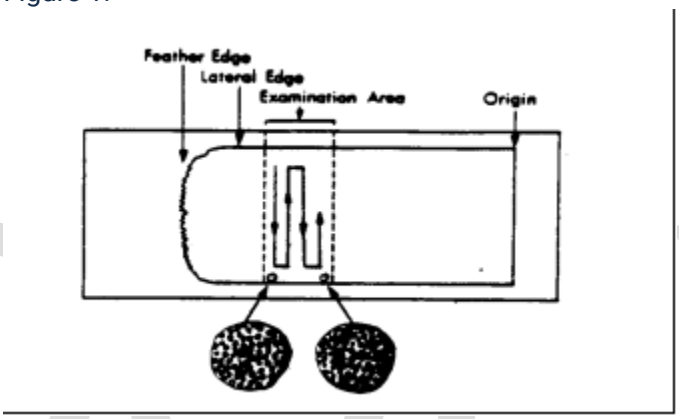
1. Result the Instrument Flags field in the middleware.

B. Scan slide on low power objective 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 or average # WBC @ 50X x 3= WBC bil/L ± 25%).
    a. Result the WBC estimate field in the middleware.
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 IX. Procedure, Step H).
7. Platelet clumps (see IX. Procedure, Step E).
8. RBC distribution (e.g., rouleaux, agglutination, anemia/polycythemia) and RBC morphology (e.g., dual population, hypochromasia, polychromasia, poikilocytosis, nucleated RBCs, target cells): roughly three fields inward from the lacey area of the feathered edge.
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.



Reprinted with permission from Lab Med, 11:371-375, 1980
Figure 1

    2. Identify WBCs first by chromatin pattern and second by cytoplasm. See XI. Notes, Step A.
    3. Count each WBC seen and tabulate on the middleware diffpad.
    4. If there is a significant disagreement (+/- 10) between the number of NRBCs seen on the smear and the number of NRBCs counted by the analyzer, count the NRBCs while performing the manual differential. Report the manual NRBC count and correct the WBC count manually. Enter the corrected WBC into the middleware. Refer to Hematology Caresphere Operation, Attachment A-Caresphere Workflow and XN CBC Corrections procedures.
        a. **NOTE:** Avoid studying cells too far into the thick or thin areas as cells will be distorted and not show characteristic morphologic features.
        b. **NOTE**: For XN-L analyzers, refer to the XN-L analyzer attachment in the XN CBC Corrections procedure.
    5. Note and report any WBC inclusions or abnormalities (e.g., Döhle bodies, vacuoles,

toxic granulation, bacteria, hypersegmentation, etc.).

a. **Neutrophils**:

i.

Toxic granules	Present
DÖhle bodies	Present
Vacuolization	Present (look for bacteria)
Intracellular organisms	Present (send directly to Microbiology for confirmation)
Hypersegmented (6+ lobes)	Present (check MCV)
Pelgeroid	Present

b. NOTE:

i. If greater than 40% cells with toxic granules, consider either bad stain or "Alder-Reilly anomaly."

ii. If greater than 30% cells with DÖhle bodies, consider May-Hegglin and check for large/giant platelets.

c. **Lymphocytes:**

i. Reactive lymphocytes: If greater than 10% reactive lymphocytes are observed on scanning, a manual differential should be performed. These reactive lymphs should then be enumerated separately from the general lymph population. If reactive lymphocyte absolute number is >1.0 bill/L, add comment when applicable, “Peripheral blood smear review identified reactive lymphocytes. If clinical signs and symptoms are consistent with infectious mononucleosis, recommend additional testing (i.e. Monospot) if clinically indicated.”

D. Examine the red blood cell morphology at 100x oil immersion in a thin area of the smear where the RBCs slightly overlap. Report the RBC Morphology field in the middleware.

1. Enter "Unremarkable", if observed changes in RBC morphology are less than the guidelines listed below.
2. Reportable RBC morphology guidelines (Must be present in ALL fields).

a.

Morphologic Finding	Quantity Noted	Grading
Hypochromasia	11-20 %	2+
	>20%	3+
Acanthocytes Echinocytes Ovalocytes Polychromasia Spherocytes	5-10%	1+
	11-20%	2+
	>20%	3+

Stomatocytes Target Cells (Codocytes) Teardrops (Dacrocytes)		
Schistocytes/Bite Cells/Blister Cells	<1/hpf 1-2/hpf 3-5/hpf 6-10/hpf 11-20/hpf >20/hpf	
Sickle Cells Pappenheimer Bodies CC/SC Crystals Howell-Jolly Bodies Basophilic Stippling	Any seen	Present

3. Additional RBC Findings:
    a. RBC rouleaux present.
    b. RBC agglutinates present.
    c. Dimorphic RBC population present.
        i. If morphology is in agreement with MCV, no notation is necessary.
        ii. If microcytes or macrocytes are noted and the MCV is in the reference range, check RBC histogram for dual population.

E. Examine the number and morphology of platelets at 100x oil immersion in the same area used for the red blood cell evaluation. Report the PLT Estimate field in the middleware.
    1. A smear should be performed when the Sysmex hematology analyzer results the following:
        a. First occurrence: PLT result less than 75 bil/L or greater than 1000 bil/L.
        b. PLT CLUMPS flag on second run.
        c. Fragments? flag.
        d. NOTE: Always check for a clot when PLT<75, regardless if patient has been running that way or not. Document as an internal comment in the middleware.
        e. NOTE: If there is a platelet delta check (decrease only), check for clot and document as an internal comment in the middleware. The smear may need to be reviewed depending on patient history.
    2. **Number**: Estimate decreased, adequate, or increased.
        a. 15-40 PLT/100X approximates an estimate of "adequate" for adult patients (normal range of 150-400x10^9/L), and 15-45 PLT/100X approximates an

estimate of "adequate" for patients less than 18 years (normal range of 150-450x10^9/L).

b. Platelet number @ 100X x 10 should approximate the platelet count. PLT estimate should agree ± 25% with the analyzer PLT if no interferences are present. If the PLT estimate agrees with the hematology analyzer result, report the hematology analyzer results.

c. **NOTE: Before reporting a decreased platelet estimate, scan smear along feathered/lateral edges for clumps. Check tube of blood for clot.**

i. If there is instrument/ smear disagreement:

a. Check for clot.

b. Remake the slide.

c. If the instrument/ smear disagreement cannot be resolved by making/ staining a new slide, enter the following comment: *"Platelet interference observed; appear ___ (adq, inc, dec) on smear"*.

3. **Size**: Giant platelets: report *"giant plts present"* when larger than normal RBC and at least 2 per each field (100X).

4. **Clumps:** Report when greater than 5 clumps (containing 5 or more platelets per clump) seen in smear made from EDTA. Check PLT/WBC histograms / scattergrams. Remove the platelet result from the analyzer. Report the following comment in the platelet result field and the platelet comment field: *"Clumped in EDTA; appear (adequate/decreased/increased) on smear."*

a. If platelet clumps are present but are insignificant, report instrument count and add internal middleware comment, "*Insignificant platelet clumps present*."

5. **Satellitosis:** Report when platelets adhering to neutrophils. Remove the platelet result from the analyzer. Report the following comment in the platelet result field and the platelet comment field: *"Platelet satellitosis present, appear (adequate/ decreased/increased) 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 and Body Fluid 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 site specific location.

G. Report any unusual findings in the differential comment (diff comment) field in the middleware.

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

Finding	Procedure
>10% smudge cells	Albumin smear *
Sickle cells	Sickle Screen (unknown patient)
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

1. * See Albumin Smear for Smudge Cells procedure.
    a. NOTE: If an albumin smear is made, perform the RBC morphology and PLT estimate on the non-albumin smear.

XI. REPORTING SUSPECTED PERIPHERAL BLOOD INTRACELLULAR MICROORGANISMS:

A. Result "See Below" in the "Intracellular Bacteria" field in the middleware. Refer to the Hematology Caresphere Operation procedure, Attachment A - Caresphere Workflow.

B. After validating all, an order for a gram stain will reflex for the sample in the LIS. The gram stain reflex will appear on the Scanned Outstanding List and a specimen ID for Microbiology will be generated as an alternate specimen ID in addition to the Hematology specimen ID.

C. Microbiology receives a notification to perform a gram stain.

D. Clean at least 2 microscope slides by dipping them in methanol. Allow slides to air dry.

E. Use the slides to prepare 2 push smears. Allow to air dry.

F. Fix prepared slides by immersing 30-45 seconds in methanol.

G. Send fixed slides to Microbiology per site specific instructions.
    1. NOTE: If sending to micriobiology for suspected parasites, send tube of blood with fixed slides.

H. Notify Microbiology and track slides per site specific instructions.

XII. CALCULATIONS:

A. Correction for Nucleated Red Blood Cells

$$\text{Corrected WBC} = \frac{\text{Uncorrected WBC} \times 100}{100 + \text{NRBC (Counted/100 WBC)}}$$

B. Calculation of the Absolute Differential Count

$$\text{Absolute \#} = \frac{\text{\% of cells} \times \text{WBC count}}{100}$$

XIII. EXPECTED VALUES:

A. WBC Differential

Parameter (bil/L)	Female	Male
Neutrophils	1.6 - 7.2	1.6 - 7.2
Lymphocytes	1.1 - 4.0	1.1 - 4.0
Monocytes	0.0 - 0.8	0.0 - 0.9
Eosinophils	0.0 - 0.5	0.0 - 0.4
Basophils	0.0 - 0.1	0.0 - 0.1

B. RBC Morphology: Unremarkable

C. Platelet Estimate: Adequate

D. WBC Estimate: Confirmed

XIV. NOTES:

A. For detailed definitions of normal and abnormal cells, according to the College of American Pathologists (CAP), refer to references available on the bench at each site and attachment A.

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. It is important to document each morphology check parameter (WBC estimate, Instrument flags, RBC Morphology, and PLT estimate) as the technologist progresses through the slide review (rather than all at once before you take the slide off the microscope stage) to ensure no findings are missed or overlooked if the technologist experiences any interruptions.

D. A **manual** differential must be performed by a medical technologist if:

1. Any blasts are seen.
2. Disagreement between IG% and combined metas/myelos/promyelos seen on scan.
3. 10% or more reactive lymphs are seen.
4. Scattergram has "----" for any diff parameter except basos.
5. Scan does not agree with hematology analyzer.
6. 5 or more plasma cells are seen.
7. *Exception when WBC < 0.4 bil/L. Follow the WBC < 0.4 bil/L steps above.

E. If pancytopenia in unknown patient, suspect MDS/leukemia.

F. If the manual differential is performed and agrees with the hematology analyzer, accept the analyzer diff.

G. Report the PLT estimate on all slides reviewed.

H. RBC morphology must be reported, even when normal. Use the comment "Unremarkable" when normal RBC morphology is seen.

XV. REFERENCES:

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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.

D. William Beaumont Hospital. Platelet Estimate Verification Study, 2020. Platelet estimates were performed during a study including patients with decreased, adequate, and increased platelet values and used to establish the multiplier for the semi-quantitative platelet estimate. William Beaumont Hospital, Royal Oak, MI 48073.

E. CAP Commission on Laboratory Accreditation Inspection Checklist, Hematology, Question 02.2560, 1998.0 Edition.

F. Cembrowski, GS and Cornbleet, PJ. Calibration Verification in Hematology: What's Necessary and What's Not. CAP Today. Spring, 1996.

G. XN-Series Automated Hematology Systems Flagging Interpretation Guide, Document 1166-LSS Rev. 5, February 2019.

H. CAP Hematology and Clinical Microscopy Glossary: Blood Cell Identification, 2023; p.1-24.

Attachments

Attachment A - White Blood Cell Morphology.pdf

Approval Signatures

Step Description	Approver	Date
	Ann Marie Blenc: System Med Dir, Hematopath	4/23/2024
	Muhammad Arshad: Chief, Pathology	4/11/2024
	Jeremy Powers: Chief, Pathology	4/9/2024
	Ryan Johnson: OUWB Clinical Faculty	4/5/2024
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	Masood Siddiqui: Staff Pathologist	4/5/2024
	John Pui: Chief, Pathology	4/5/2024
Policy and Forms Steering Committee Approval (if needed)	Megan Masakowski: Mgr, Division Laboratory	4/5/2024
	Udayasree Bartley: Supv, Laboratory	3/26/2024
	Ashley Beesley: Mgr, Laboratory	3/14/2024
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	Kristen DiCicco: Mgr, Laboratory	2/28/2024
	Katherine Persinger: Mgr, Laboratory	2/27/2024
	Christopher Ferguson: Mgr, Laboratory	2/22/2024
	Jennifer Yaker: Mgr, Laboratory	2/19/2024
	Megan Masakowski: Mgr, Division Laboratory	2/12/2024

Applicability

Dearborn, Farmington Hills, Grosse Pointe, Royal Oak, Taylor, Trenton, Troy, Wayne