Manual WBC Differentials and Smear Review

Policy

Our smear review criteria are built into the WAM rules. When a prompt for manual review rule is triggered WAM will alert the CLS if a smear review is needed for WBC, RBC, or platelets.

The **WAM OP ALERT** will state the issue and specify what is needed like:

- WBC – Scan slide, do ManDiff if indicated.
- RBC – Scan slide, follow protocol.
- PLT – Scan slide, rerun PLTF, follow protocol.

Follow the OP Alert instructions.

Please note that we will perform **ManDiff** if it is indicated **ONLY**.

- Perform a Manual Diff when scanning shows the presence of **ANY** abnormal cells, immature myeloid (more than 10 bands or more than 1 metamyelocyte) or any myelocyte, promyelocyte or any blasts.
- Release/verify manual differential when performed, otherwise keep, and release the automated differential results.

For **RBC and PLT issues**, follow protocol as stated in the Resolving Pre-Analytical CBC Sample Problems Procedure.

Peripheral blood smears are saved for 1 month.

Reagents

Sysmex SP-50
Slides
Methanol-Anhydrous
Conc. Phosphate Buffer (pH 6.8)
ColorWright Stain
De-ionized water

NOTE: Waste from these reagents are hazardous and should be accumulated in the appropriate container (Stains, Alcohols).

Procedure

Follow steps below:

Step	Action
1	Prepare a stained blood smear using Sysmex SP50 procedure (see Sysmex XN-3100 Procedure).
2	Perform a White Blood Cell Differential Count using the Cellavision or by regular microscope (if necessary). • Manual WBC differentials will always be reported in percent. The total reported should always equal 100%. • If the total WBC is 1,000 or greater, 100 cells should be counted. The number counted should be reported directly as percent.

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Procedure, continued

Step	Action
2	• If the total WBC is less than 1,000 but greater than 500, 50 cells should be counted. The cells counted should be multiplied by 2 and reported as percent. It should be noted in the comment section that the differential is based on 50 cells. • If the total WBC is 500 or less, 25 cells should be counted. The cells counted should be multiplied by 4 and reported as per cent. It should be noted in the comment section that the differential is based on 25 cells. • If the required number of cells cannot be found, a second blood smear should be made and the WBC's counted until the required number is found.
3	Differentiation of white blood cells. **Polymorphonuclear neutrophils (Segs):** These cells are recognized by the presence of a thin filament connecting at least two lobes of nuclear material. The filament is composed of apposition of two layers of nuclear membrane. No recognizable chromatin is present in the filament. • **NOTE**: Folded cells and cells with nuclei folded upon themselves so that the entire nuclear outline is not visible should be identified as a "poly" so long as the cytoplasmic criteria for neutrophilic granulocytes are fulfilled. **Band:** A typical band or "stab" cell has a recognizable nuclear indentation more than one-half the diameter of the theoretical circular nucleus. Recognizable material is present in the connecting bridge and there can be variable extent of parallel margins of nuclear membrane. This means that any granulocyte having an indentation greater than one-half the diameter of the nucleus with some chromatin present causing a thickened connecting strand should be identified as a band cell. • **NOTE**: Normal range for Band Cells using the above criteria is 0-6% of the total white blood cell count. **Metamyelocyte:** A granulocyte is considered a metamyelocyte if nuclear indentation is less than one-half the diameter of the nucleus or parallelism of the constricted side is not present. **Lymphocyte:** All normal and atypical lymphocytes should be reported as the total lymphocyte percent. Atypical lymphocytes will be reported as **FEW, MOD or MANY**.

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Procedure, continued

Step	Action
3	**Blast:** Large, round to oval cells, 10-20 um in diameter and nuclear to cytoplasmic ratio is high varying from 7:1 to1:1. Blast have central nuclei with fine, uncondensed chromatin and prominent nucleoli. **All smears with blasts (unknown cases) or unidentifiable cells must be sent to the Pathologist for review.** **All Other Types:** All other types of leukocytes should be differentiated and reported. **WBC abnormalities:** Any WBC abnormalities seen should be reported. These should include toxic granulation, hypersegmentation, Auer Rods, Dohle bodies, etc. Cells and parasite identification can be difficult. If you have any doubt of the correct identification, you may consult your co-worker, supervisor, or pathologist for assistance. Refer to **Procedure for Pathologist's Smear Review** for slides that need to be referred to a pathologist.
4	**Smudge Cells:** If there are more than twenty (20) smudge cells present, do not report the Cellavision differential. • Make an Albumin slide 1. In a12x75 tube, add 4 drops of blood to 1 drop of albumin (1:5 ratio). 2. Make a push slide and allow to air dry thoroughly. 3. Label the slide with patient identifier and write “albumin” on the frosted edge of the slide. 4. Stain the slide on the SP-50. • Perform a manual differential on the albumin slide using the regular microscope (not Cellavision). Enter results manually in WAM and click **[SAVE]**. • Perform RBC morphology and PLT estimate on the non-albumin slide. Enter results manually in WAM and click **[SAVE]**.
5	**Microscopic procedure** (if performing in regular scope)**:** Inspect smear under low power. Observe the distribution of leukocytes and choose that portion of the smear, usually near the thin end, where there is no overlapping of erythrocytes. Apply a layer of oil to slide. Shift to 40X or 50X objective. Move the slide from the extreme upper edge of the smear to the extreme lower edge, counting and classifying each leukocyte in the successive fields. Shift over one field and proceed to the upper edge, still classifying each leukocyte. Continue in this fashion until the required number of cells is counted.

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Procedure, continued

Step	Action
6	**WBC estimation**: An estimation of the total WBC count should be made from the smear and compared to instrument or manual count as follows: No/High-Power Field — Estimated Count 2 - 4 — 4,000 - 7,000 4 - 6 — 7,000 - 10,000 6 - 10 — 10,000 - 13,000 10 - 20 — 13,000 - 18,000
7	**RBC morphology**: RBC morphology should be examined and reported as normal or if abnormal the type of abnormality should be reported qualitatively. Scan and review RBC morphology and inclusions using 50x and 100x objective. **Abnormalities in shape**: Spur Cell/Acanthocyte, Burr Cell/ Echinocyte, Tear Drop Cell/Dacryocyte, Elliptocyte, Ovalocyte, Schistocyte (includes Helmet Cells), Sickle Cell, Spherocyte, Stomatocyte, Target cell, Bite Cell, Blister Cell. **Other**: Basophilic Stippling, Cabot Rings, Howell-Jolly Bodies, Pappenheimer Bodies/Siderocytes, Polychromasia, Rouleaux, Dimorphic Cell population. Refer to Hematology P&P Resolving Pre-Analytical CBC Sample Problems for proper analysis and reporting of RBC results. **Reporting threshold:** These are the only ones that we will be reporting for RBC morphology and it would have to be ≥ to the threshold on the table below.

Spur Cell / Acanthocyte	5 – 20% (2+)
Burr Cell/ Echinocyte	5 – 20% (2+)
Tear Drop Cell/Dacryocyte	5 – 20% (2+)
Elliptocyte	5 – 20% (2+)
Ovalocyte	5 – 20% (2+)
Schistocyte (includes Helmet Cells)	0.5% (1+)
Sickle Cell	1 – 2% (2+)
Spherocyte	5 – 20% (2+)
Stomatocyte	5 – 20% (2+)
Target cell	5 – 20% (2+)
Bite Cell	5 – 20% (2+)
Blister Cell	5 – 20% (2+)
Basophilic Stippling	5 – 20% (2+)
Cabot Rings	0.5%
Howell-Jolly Bodies	2 – 3% (2+)
Pappenheimer Bodies / Siderocytes	5 – 20% (2+)
Polychromasia	5 – 20% (2+)
Rouleaux	0.5% (1+)
Dimorphic Cell population	20%

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Procedure, continued

Step	Action
8	**Platelets**: Platelets should be estimated and reported **IF** the instrument does not provide a platelet result. If a result is provided, it should be verified by the slide estimate. Abnormal platelets should be reported semi-qualitatively. If slide estimate does not agree with automated result, platelet clumping in EDTA is suspected. Refer to Hematology P&P Resolving Pre-Analytical CBC Sample Problems for proper analysis and reporting of PLTs with clumps. **The platelet estimate should be reported as follows**:

Increased	More than 25 platelets/100X or >400,000
Adequate	Less than 25 platelets/100X but greater than 7 platelets/l00X or 130-400,000
Decreased	Less than 7 platelets/100X or <130,000

References:

1. Technical Hematology Arthur Simmons, 2nd edition, J.B. Lippencott Company, Philadelphia. p.103.
2. Laboratory Medicine Hematology, John B. Miale, 6th edition, O.V. Mosby Company, St. Louis. p.475, 869.
3. American Journal of Clinical Pathology, Committee for Clarification of the nomenclature of Cells and Diseases of the Blood and Blood Forming Organs: second report, 56:19 (1949).
4. Technical Improvement Service, “What is a Band”, Thomas F. Deutcher, MD., Commission on Continuing Education of the Society of Clinical Pathologists, No. 15 (1973) pg. 10-19.
5. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features, L. Palmer et al. International Journal. Lab. Hem. 2015, 37, 287–303