

## Manual WBC Differential & Smear Review

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**Principle** A blood smear is stained with Wright's Stain and then examined microscopically. One hundred white blood cells are counted and differentiated as to classification. White cell, red cells and platelets are examined, and any abnormal findings are noted and reported.

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**Policy** Our smear review criteria are built into the XN 550 flagging rules. When a prompt for manual review rule is triggered, XN 550 flags and messages referred to as Interpretative Program (IP) messages will alert the CLS if a smear review is needed for WBC, RBC and/or Platelets.

The **IP messages** 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 IP Messages 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 *HEM.03.0100 Resolving Pre-Analytical CBC Sample Problems Procedure*.

Peripheral blood smears are saved for 1 month.

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**Safety** ***Refer to the safety manual for general safety requirements.***

## Manual WBC Differentials & Smear Review, Continued

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**Reagents**      Hematek Stainer  
                     Hematek Stain Pak  
                     Slides

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

## Manual WBC Differentials & Smear Review, Continued

**Procedure** Follow steps below:

Step	Action
1	Prepare a stained blood smear using policy for Preparation of Blood Smear. (HEM.MOB. 03-0040)
2	<p>Perform a White Blood Cell Differential Count by regular microscope</p> <ul style="list-style-type: none"> <li>• Manual WBC differentials should always be reported in per cent. The total reported should always equal 100%.</li> <li>• If the total WBC is 1,000 or greater, 100 cells should be counted. The number counted should be reported directly as percent.</li> <li>• 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.</li> <li>• 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.</li> <li>• 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.</li> </ul>
3	<p>Differentiation of white blood cells.</p> <p><b><u>Polymorphonuclear neutrophils (Segs):</u></b> 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.</p> <ul style="list-style-type: none"> <li>• <b>NOTE:</b> 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.</li> </ul> <p><b><u>Band:</u></b> 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</p>

## Manual WBC Differentials & Smear Review, Continued

Procedure,  
 continued

Follow steps below:

Step	Action
3	<ul style="list-style-type: none"> <li>• <b>NOTE:</b> Normal range for Band Cells using the above criteria is 0-6% of the total white blood cell count.</li> </ul> <p><b><u>Metamyelocyte:</u></b> 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.</p> <p><b><u>Myelocyte:</u></b> A typical myelocyte has a nucleus which is somewhat eccentric, lack nucleolus and begin to demonstrate chromatin clumping. The cytoplasm is relatively more abundant than the earlier stages and is amphophilic. Both primary and specific granules are present.</p> <p><b><u>Promyelocyte:</u></b> A cell is considered a promyelocyte when it develops distinct primary granules in the cytoplasm. They still have a high N:C and their nuclei have fine chromatin.</p> <p><b><u>Lymphocyte:</u></b> All normal and atypical lymphocytes should be reported as the total lymphocyte percent. Atypical lymphocytes will be reported as <b>FEW, MOD or MANY</b> in the morphology section.</p> <p><b><u>Blast:</u></b> Large, round to oval cells, 10-20 um in diameter and nuclear to cytoplasmic ratio is high varying from 7:1 to 1:1. Blast have a central nucleus with fine, uncondensed chromatin and prominent nucleoli.</p> <p><b><u>All smears with blasts (unknown cases) or unidentifiable cells must be sent to the Pathologist for review. The pathologist will review the next workday or immediately if paged by the provider.</u></b></p> <p><b><u>All Other Types:</u></b> All other types of leukocytes should be differentiated and reported.</p> <p><b><u>WBC abnormalities:</u></b> Any WBC abnormalities seen should be reported. These should include toxic granulation, hypersegmentation, Auer Rods, Dohle bodies, etc.</p> <p>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.</p> <p>Refer to <b><i>Hematology P&amp;P HEM.03.0100 Slide Request for Pathology Review</i></b>, for slides that need to be referred to a pathologist.</p>
4	<p><b>Microscopic procedure:</b> (if performing in regular scope):</p> <p>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.</p> <p>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.</p>

## Manual WBC Differentials & Smear Review, Continued

**Procedure,**  
 continued

5	<p><b>WBC abnormalities:</b>          Any WBC abnormalities seen should be reported. These should include toxic granulation, hypersegmentation and Dohle bodies</p>										
6	<p><b>WBC estimation:</b> An estimation of the total WBC count should be made from the smear and compared to instrument or manual count as follows:</p> <table border="1" style="margin-left: 40px;"> <thead> <tr> <th style="text-align: center;"><u>No/High-Power Field</u></th> <th style="text-align: center;"><u>Estimated Count</u></th> </tr> </thead> <tbody> <tr> <td style="text-align: center;">2 - 4</td> <td style="text-align: center;">4,000 - 7,000</td> </tr> <tr> <td style="text-align: center;">4 - 6</td> <td style="text-align: center;">7,000 - 10,000</td> </tr> <tr> <td style="text-align: center;">6 - 10</td> <td style="text-align: center;">10,000 - 13,000</td> </tr> <tr> <td style="text-align: center;">10 - 20</td> <td style="text-align: center;">13,000 - 18,000</td> </tr> </tbody> </table>	<u>No/High-Power Field</u>	<u>Estimated Count</u>	2 - 4	4,000 - 7,000	4 - 6	7,000 - 10,000	6 - 10	10,000 - 13,000	10 - 20	13,000 - 18,000
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7	<p><b>RBC morphology:</b> RBC morphology should be examined and reported as normal or if abnormal the type of abnormality should be reported qualitatively.</p> <p><b>Abnormalities in size:</b> Anisocytosis, Macrocytosis, Microcytosis</p> <p><b>Abnormalities in shape:</b> 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.</p> <p><b>Other:</b> Basophilic Stippling, Cabot Rings, Howell-Jolly Bodies, Pappenheimer Bodies/Siderocytes, Polychromasia, Rouleaux, Dimorphic Cell population.</p> <p>Refer to <i>Hematology P&amp;P HEM.03-0050 Resolving Pre-Analytical CBC Sample Problems</i> for proper analysis and reporting of RBC results.</p>										

## Manual WBC Differentials & Smear Review, Continued

**Procedure,  
 continued**

<b>8</b>	<p><b>Reporting threshold:</b>                  These are the only ones that we will be reporting for RBC morphology and it would have to be <math>\geq</math> to the threshold on the table below.</p> <table border="1"> <tr><td>Spur Cell / Acanthocyte</td><td>5 – 20% (2+)</td></tr> <tr><td>Burr Cell/ Echinocyte</td><td>5 – 20% (2+)</td></tr> <tr><td>Tear Drop Cell/Dacryocyte</td><td>5 – 20% (2+)</td></tr> <tr><td>Elliptocyte</td><td>5 – 20% (2+)</td></tr> <tr><td>Ovalocyte</td><td>5 – 20% (2+)</td></tr> <tr><td>Schistocyte (includes Helmet Cells)</td><td>0.5% (1+)</td></tr> <tr><td>Sickle Cell</td><td>1 – 2% (2+)</td></tr> <tr><td>Spherocyte</td><td>5 – 20% (2+)</td></tr> <tr><td>Stomatocyte</td><td>5 – 20% (2+)</td></tr> <tr><td>Target cell</td><td>5 – 20% (2+)</td></tr> <tr><td>Bite Cell</td><td>5 – 20% (2+)</td></tr> <tr><td>Blister Cell</td><td>5 – 20% (2+)</td></tr> <tr><td>Basophilic Stippling</td><td>5 – 20% (2+)</td></tr> <tr><td>Cabot Rings</td><td>0.5%</td></tr> <tr><td>Howell-Jolly Bodies</td><td>2 – 3% (2+)</td></tr> <tr><td>Pappenheimer Bodies / Siderocytes</td><td>5 – 20% (2+)</td></tr> <tr><td>Polychromasia</td><td>5 – 20% (2+)</td></tr> <tr><td>Rouleaux</td><td>0.5% (1+)</td></tr> <tr><td>Dimorphic Cell population</td><td>20%</td></tr> </table>	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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<b>9</b>	<p><b>Platelets:</b> Platelets should be estimated and reported IF the instrument does not print out a platelet result.                  If a result is printed, 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 <i>Hematology P&amp;P HEM.03-0050 Resolving Pre-Analytical CBC Sample Problems</i>, for proper analysis and reporting of PLTs with clumps.</p> <p><b>The platelet estimate should be reported as follows:</b></p> <table border="1"> <tr><td>Increased</td><td>More than 25 platelets/100X or &gt;400,000</td></tr> <tr><td>Adequate</td><td>Less than 25 platelets/100X but greater than 7 platelets/100X or 130-400,000</td></tr> <tr><td>Decreased</td><td>Less than 7 platelets/100X or &lt;130,000</td></tr> </table>	Increased	More than 25 platelets/100X or >400,000	Adequate	Less than 25 platelets/100X but greater than 7 platelets/100X or 130-400,000	Decreased	Less than 7 platelets/100X or <130,000																																
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## Manual WBC Differentials & Smear Review, Continued

### Controlled Documents

The following controlled documents support this procedure

Reference
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. Pediatric Reference Ranges,_1995, S. Soldin, J. Hicks, Editor, AACCC Press.

Related Documents
1. HEM.03.0100 Resolving Pre-Analytical CBC Sample Problems Procedure
2. HEM.03-0010 Sysmex XN-9000 Procedure
3. HEM.03-0090 Blood Smear Preparation
4. HEM.03-0030 Sysmex DI-60 Cellavision
5. HEM.03.0250 Slide Request for Pathology Review
6. HEM.3-0100 Resolving Pre-Analytical CBC Sample Problems

## Manual WBC Differentials & Smear Review, Continued

### Document History Page

Change type: New, Major, Minor etc.	Changes Made to SOP – describe	Name of responsible person/date	Med. Dir. Reviewed/ Date	Operations Dir. Manager reviewed/ date	Date change Implemented
Major	1. Updated format. 2. Updated Policy # and title 3. Added Safety	Yvette Lingat 3/20/2020		Mary Lou Beaumont	4/28/2020