WBC Differentials, Manual

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.

Safety

All specimens, reagents and controls should be handled as though capable of transmitting infectious diseases. Wear appropriate personal protective equipment when running patient samples or performing scheduled maintenance. Refer to: Policy and Procedures Safety Manual Infection Control and Procedures 11-085-01.

Reagents

Hematek Stainer
Hematek Stain Pak
Slides
Methanol-Anhydrous
Sysmex SP-10
Phosphate Buffer (pH 6.6-7.2)
Harleco-Wright's 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 SP10 procedure (see Hematology P&P HEM.03-0010). Or prepare a blood smear as described in the policy for Preparation of Blood Smears (see Hematology P&P HEM.03-0090) and stain the smear with Wright's Stain using the Hematek Slide Stainer.
2	Perform a White Blood Cell Differential Count using the CellaVision (see Hematology P&P HEM.03-0030) or by regular microscope. • WBC differentials should always be reported in per cent. 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. • 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.

Procedure, continued

Follow steps below:

Step	Action
2	• 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 per cent. Atypical lymphocytes should be reported as a % of the total lymphocyte count. This per cent is calculated: Atypical lymphocytes / total lymphocytes = % **Blast:** Large, round to oval cells, 10-20 um in diameter and nuclear to cytoplasmic ratio is high varying from 7:1 to1:1. There are 3 types of blast cells. All of them have central nuclei with fine, uncondensed chromatin and prominent nucleoli. • Type1 blast= lack cytoplasmic granules • Type 2 blast= have small number of primary (azurophilic) granules.

Procedure, continued

Step	Action
3	• Type 3 blast= are similar to type 1 with more abundant azurophilic granules. **<u>All smears with blasts (unknown cases) or unidentifiable cells must be sent to the Pathologist for review.</u> The pathologist will review the next workday or immediately if paged by the provider.** **<u>All Other Types:</u>** All other types of leukocytes should be differentiated and reported as described in: "The Morphology of Human Blood Cells" L.W. Diggs, MD, Dorothy Strum, and Ann Bell. Abbott Laboratories. **NOTE:** Cell 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.
4	**Microscopic procedure**: 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.
5	**WBC abnormalities:** Any WBC abnormalities seen should be reported. These should include toxic granulation, hypersegmentation and Dohle bodies.
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: <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
7	**RBC morphology**: RBC morphology should be examined and reported as normal or if abnormal the type of abnormality should be reported qualitatively. <u>Abnormalities in size</u>: Anisocytosis, Macrocytosis, Microcytosis <u>Abnormalities in shape</u>: Acanthocyte, Biconcave disk, Burr cell, Crenated cell, Codocyte, Dacryocyte, Discocyte, Drepanocyte, Echinocyte, Elliptocyte, Ovalocyte, Schistocyte, Sickle cell, Stomatocyte, Target cell, Teardrop cell

Procedure, continued

Step	Action
7	Inclusions: Basophilic Stippling, Cabot Rings, Howell-jolly Bodies, Heinz bodies, Hyperchromasia, Hypochromasia, Polychromasia, Siderotic Granules The following terms should be used when reporting RBC morphology: Slight 2-5cells/100x Few 5-10 cells/100x Moderate 10-15 cells/100x Marked >15 cells/100x
8	**Platelets**: 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 Hematology P&P HEM.3-0100 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/l00X or <130,000
9	Refer to Hematology P&P HEM.01-0040 for slides that need to be referred to a pathologist.

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. Pediatric Reference Ranges, 1995, S. Soldin, J. Hicks, Editor, AACC Press.

Document History Page

Change type: New, Major, Minor etc.	Changes Made to SOP – describe	Name of responsible person/date	Med. Dir. Reviewed/ Date	Lab Manager reviewed/ date	Date change Implemented
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