White Blood Cell Counts
Reference Methodology

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KEYWORDS

• Automated hematology analyzer • Methodology • Leukocyte
• White blood cell count • Differential count • Laboratory instrumentation

KEY POINTS

- Numerous technologies are used to generate the white blood cell (WBC) differential including electrical impedance, radiofrequency (RF) conductivity, light scatter, cytochemistry, fluorescent labeling, monoclonal antibodies, and automated differential cell counters.
- Most current analyzers report at least a 5-part WBC differential including neutrophils, monocytes, lymphocytes, eosinophils, and basophils.
- It is important to recognize common sources of error in the automated differential, including low cell count, nucleated red blood cells (nRBCs), platelet clumps, and clotted specimens; basophil counts in particular are prone to error.
- Hematology analyzers generate flags on specimens with abnormalities requiring further investigation; these flags are based on criteria, which must be validated by individual laboratories.
- Depending on the specific analyzer, numerous WBC parameters may be reported including nRBC ratio, hematopoietic progenitor cells (HPCs), immature granulocytes (IGs), granularity index, and lymph index.

INTRODUCTION

Historically, WBC counts were performed manually. Skilled technologists are necessary to perform this labor-intensive evaluation. Although manual cell counts are still performed in some situations, modern hematology laboratories use automated hematology analyzers to perform cell counts. These instruments provide accurate, precise, low-cost differential counts with fast turnaround times. Technologies commonly used include electrical impedance volume, RF conductivity, laser light

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Abbreviations

FITC	Fluorescein isothiocyanate
HPC	Hematopoietic progenitor cell
IG	Immature granulocyte
LUC	Large unstained cells
nRBC	Nucleated red blood cell
PE	Phycoerythrin
PE-Cy5	Phycoerythrin-cyanine 5
RF	Radiofrequency
WBC	White blood cell

scattering, and cytochemistry. In addition to the traditional 5-part differential of neutrophils, eosinophils, basophils, lymphocytes, and monocytes, with flags for abnormal cells, newer analyzers are better able to quantify abnormal and immature cell types including reactive lymphocytes, IGs, and nRBCs. Some instruments also report a granularity index, indicating toxic granulation of neutrophils. This article reviews the principles of these methodologies and possible sources of error, provides guidance for selecting flagging criteria, and discusses novel, clinically relevant WBC parameters provided by new instruments, including IG count and granularity index and the lymphocyte index.

THE WHITE BLOOD CELL COUNT

WBC count is the number of neutrophils, lymphocytes, monocytes, eosinophils, basophils, and immature or atypical cells present in 1 μL of blood. Leukocytosis, or elevation of the WBC, can be seen in a broad range of conditions, including both benign and malignant conditions. Elevation of the WBC requires accurate differential count and morphologic evaluation of the peripheral blood smear along with clinical information to determine the cause.[1,2] Leukopenia, or decrease of the WBC, can also be caused by several conditions and requires accurate differential and morphologic examination to determine which cell line is decreased and to assess whether rare atypical or abnormal cells are present.[3]

METHODS

Historically, the WBC and differential count were determined manually. Cell counts were typically performed using a hemocytometer, a ruled counting chamber. This technique is still routinely used for assessment of cerebrospinal fluid and body fluid specimens and may be available as a backup method or for validating or calibrating automated analyzers. The specimen is diluted in a solution that lyses erythrocytes. The diluted specimen is added to the hemocytometer, a glass slide with ruled chambers of known volume. The technologist then counts the cells on a microscope with a low-power lens. The types of cells are differentiated, and nRBCs are included in the count. The hemocytometer method is prone to error, and skilled technologists are required. Manual differential counts require examination of a stained peripheral blood smear, with enumeration of 100 to 200 WBCs by category. This method is operator dependent and relies on the ability of the technologist to accurately classify cells. In addition, cells may be unequally distributed on the slide. When large cells are pulled to the feather edge of the smear, they may be underrepresented in the area counted by the technologist. There is also an inherent statistical error because the total number of cells analyzed is low.[4]