



# Automated Differentiation of Leukocyte Subtypes

## Effects of the lysis reagent on leukocytes

For differentiation of leukocytes, cells are treated with leukocyte lysis reagents. This leads to lysis of erythrocytes and shrinkage of platelets. However, various lysis reagents used by manufacturers have different effects on individual cell types (leukocyte subtypes). This results in the cells falling on different positions in various histogram instruments. To correctly interpret a histogram, the user must therefore be knowledgeable about the type of method used.

The result of the leukocyte's cytoplasmic membrane reaction to the lysis reagent is loss of cytoplasm. The remaining membrane shrinks and moves closer to the nucleus. The extent to which cells change under the influence of the lysis reagent is dependent on the cell type and the lysis reagent used. Because of the lysis-mediated effect on cells, the sizes of the individual leukocyte subtypes do not correlate with the cell sizes as observed in microscopy.

## Changes in cell size

Examples of lymphocytes, monocytes, and neutrophil granulocytes.

	Size before lysing agent	Size after lysing agent
Lymphocyte		
Neutrophil Granulocyte		
Monocyte		

The size before lysis corresponds to the size observed in microscopy.

The size after lysis corresponds to the differentiation of cell types in the instrument histogram.

## Introduction

Compact hematology analyzers measure up to 18 different hematology parameters. These include – in addition to cell counts, hemoglobin concentration, and many other parameters – the differentiation into three groups of leukocyte subtypes.

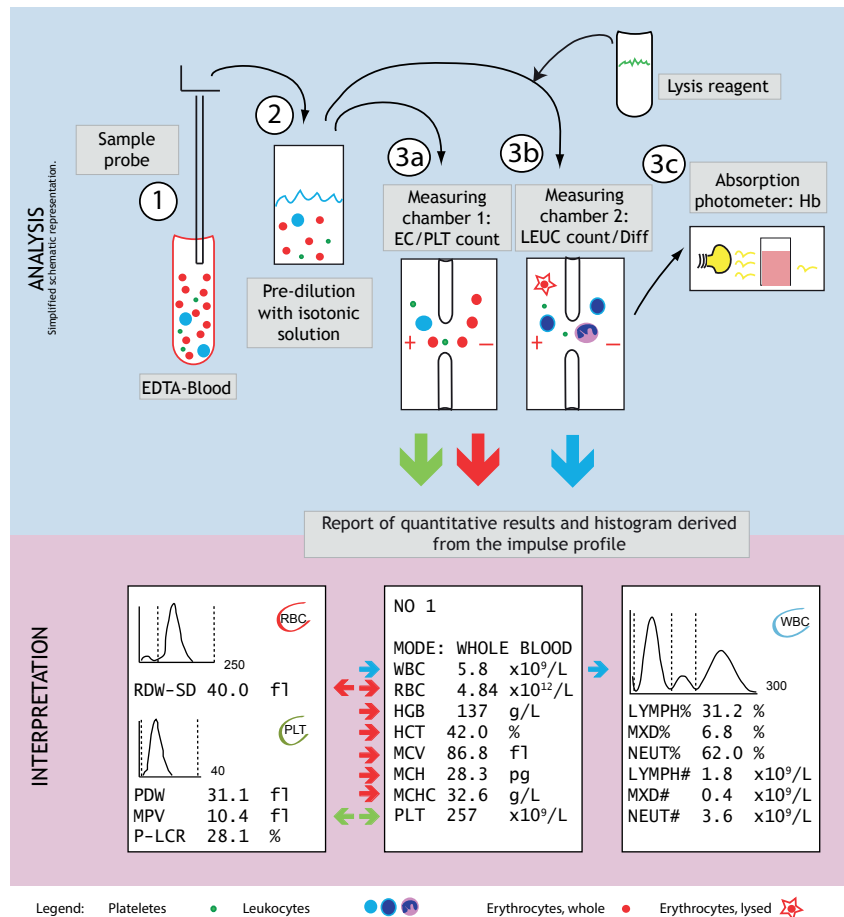
Results of platelet differentiation are reported as percentages and as absolute numeric values (quantitative) as well as by graphical representation (histogram). Additional warnings–“flags”– indicate potential technical problems or abnormal findings in patients.

The instrument operator (biomedical analyst/medical practice assistant) is responsible for the technical validation of the test results. They must interpret the results correctly, possibly follow up with a microscopic blood examination, and/or communicate findings to the physician who ordered the tests. For this, an understanding of the measurement technique and interpretation of the histogram are essential.

## Measurement of hematology parameters using the impedance technique

For analysis, blood is diluted in a conductive, isotonic solution and then transferred to various counting chambers where cells traverse individually through an aperture to which direct current is applied. Since cells are poor electrical conductors, each cell that traverses through the aperture causes an increase in electrical resistance. When constant electrical current is applied, this results in an increase of voltage between the electrodes that is reported as an electrical signal, whereby every signal corresponds to one cell and the height of the impulse provides information about the cell's size. The image resulting from these signals is converted into a graphical representation, a histogram, which is printed together with the quantitative measurements. Cell counts are made in two different test chambers (EC/PLT- and LEUC-chamber).

The measurement in the platelet chamber is made in the presence of a lysis reagent, which causes erythrocytes to lyse and platelets to shrink. In parallel, the hemoglobin content of the sample is quantified by transferring part of the lysed sample to a separate measuring chamber where analysis is performed by absorption photometry.





## Spotlight on hematology

### «Flags»

#### Possible problems with measurement

Interference in the region of the lower discriminator, e.g.,

- Large platelet aggregates  
→ verify with blood smear  
(EDTA-induced pseudo-PLT-penia?)
- Giant platelets  
→ verify with blood smear
- Erythroblasts  
→ verify with blood smear  
with >5 EB/100 LEUC,  
correct LEUC manually.

- Lysis-resistant erythrocytes  
→ *potentially dilute sample*

Interference in the region of the upper discriminator, e.g.,

- Immature cells, blasts  
→ *verify with blood smear*
- Extremely high LEUC counts  
→ *pre-dilute sample*

#### Possible abnormal findings in patients

- Suspicion of pre-stages of granulopoiesis
- Suspicion of atypical lymphocytes
- Suspicion of immature cells (blasts)
- Suspicion of eosinophilia/basophilia
- Suspicion of monocytosis

→ *verify with blood smear*

#### Report on quantitative differentiation findings

%CELLTYPE: percentage of 100 leukocytes

# CELLTYPE: absolute number (proportion of total LEUC number\*)

\* Calculation of the absolute values:  
(Total number of LEUC/100) x % CELLTYPE

#### About

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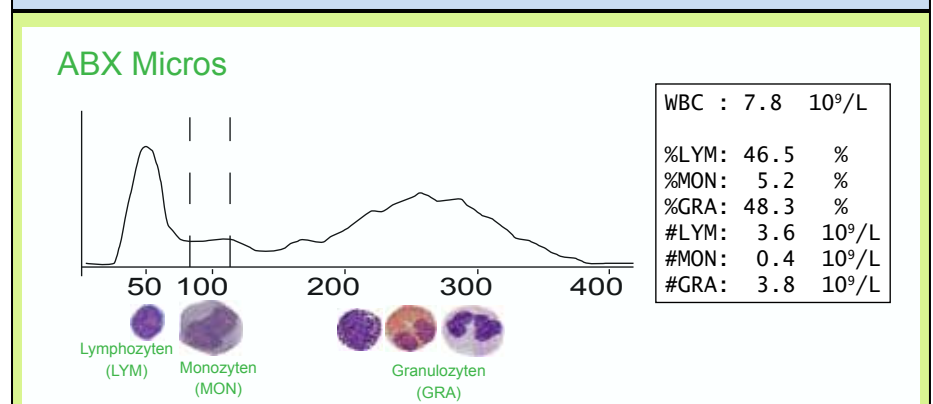
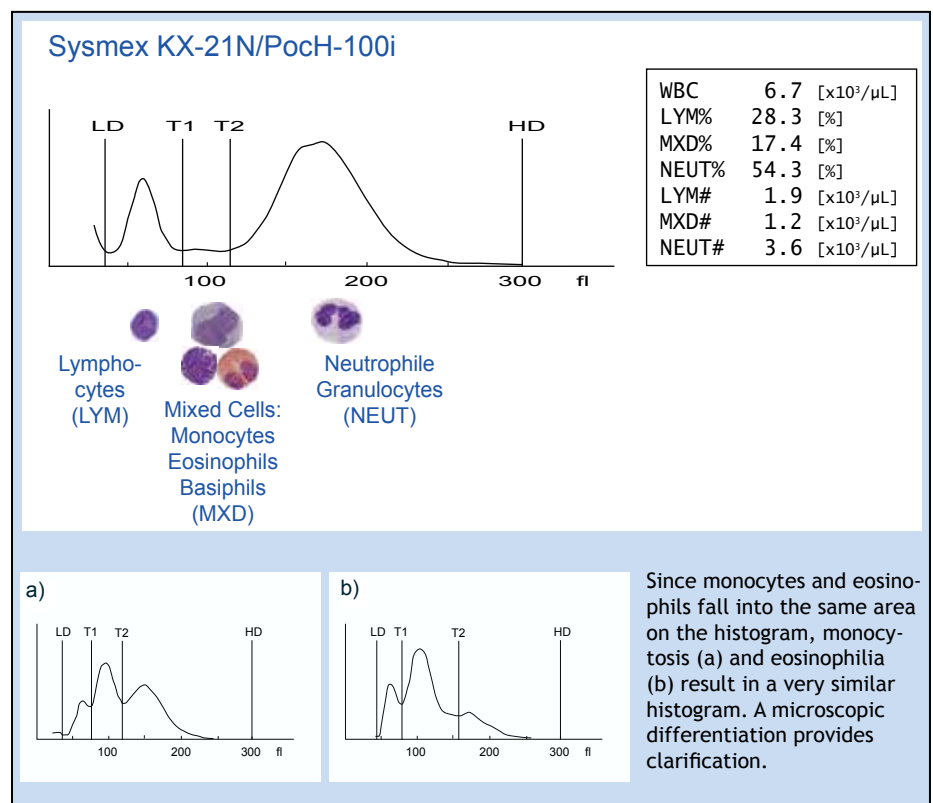
#### Advisory

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## Differential classification of leukocyte subpopulations with ABX Micros and Sysmex KX Series/Poch-i as examples

The upper and lower ranges of detection for leukocytes in the WBC (white blood cell) histogram are fixed by the instrument (for example, by the fixed discriminators LD and UD in Sysmex instruments). The entire leukocyte histogram must fall within this range. Two additional discriminators (such as T1 and T2 in Sysmex) distinguish the three cell populations from one another. Issues with measurement (interference factors) or abnormal findings may result in the discriminators no longer being able to accurately fix the separation lines. The instrument will then tag the results with a corresponding „flag“ (with ABX Micros e.g., G1: „suspicion of eosinophilia/myelocytes/hyper-segmented neutrophils“). These flags are instrument-specific and are listed in the manual or in the device manufacturer's corresponding training materials. Often, when directly importing the results of measurements to electronic systems, the „flagging“ information is not transmitted. It is therefore particularly important that the user of the device performs an accurate evaluation of the technical results and communicates important information to the physician who ordered the hematogram.



Example of an ABX Micros histogram: Survey specimen 2013-03 H3b, acute leukemia AML-M0

