Spotlight on hematology

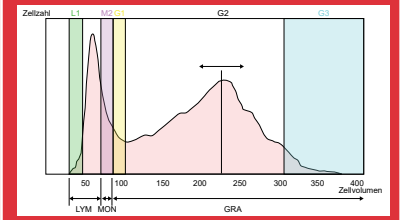
Correction formula for total leukocyte count with evidence of Erythroblasts

At > 5 Erythroblasts per 100 Leukocytes, the POCT machines must have the leukocyte count corrected manually as follows:

$$\frac{\text{100 x Lc by machine}}{\text{100 + Ec per 100 Leukocytes (Mic)}}$$

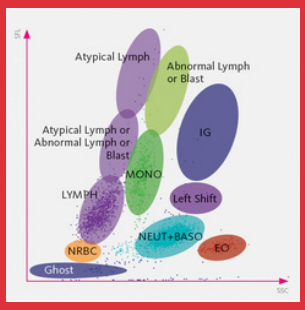
Example:
Leukocytes by machine 5.60 x109/l and 15 Erythroblasts per 100 Leukocytes (microscopically) yields a corrected leukocyte count of 4.87 x109/l.

WBC-Distribution curve: Microsemi®

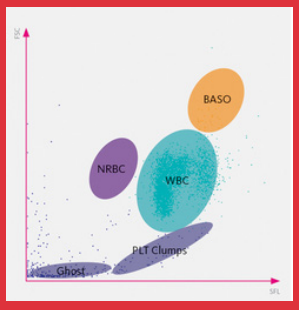


Microsemi®:
The „L1“ flag indicates the abnormal presence of small cells in comparison to the lymphocytes in the region between 30 fL and 70 fL. These may be platelet aggregates, erythroblasts, or abnormal lymphocytes.
(Source: Microsemi® Manual)

Sysmex XN-Series®



NRBC position in the scattergram of the WDF channel



NRBC population, position in the scattergram of the WNR channel (Quelle: Sysmex, Measurement technology and scattergram)

Detection of erythroblasts with automated hematology analyzers

MQZH 2020-01

Introduction

Automated hematology analyzers of various sizes have been basic equipment in medical practices, hospitals, and commercial laboratories for many years. These machines have so many advantages that the previous manual methods have almost all lost their former importance. The detection of interference and confounding factors remains a significant challenge in the field of automated hematologic analysis. In this regard, we wish to use the case of our patient 2020-01 H3b to compare the capabilities of various analyzers to detect nucleated red cell precursors, the erythroblasts (NRBC = nucleated red blood cells).

Measurement methods of 3-part differential hematology systems

3-part differential hematology systems use a two-chamber measurement system and the introduction of various reagents to count and sort cells by means of impedance measurements. The impedance measurement involves passing individual particles through a measurement aperture to which a dc voltage is applied. Each cell which passes through causes an electrical impulse. Each impulse corresponds to a measurement event, and the height of the pulse is proportional to the cell volume. The borderlines between individual populations are determined by discriminators which, depending upon the machine, are fixed or may be moved to a degree, as in the Sysmex machines.

Flags, warning messages, and error reports are generated by algorithms in the machines which may, on the one hand, be generated by deviations from the discriminators, or on the other by the presence or absence of features in certain histogram regions.

Erythroblasts interfere in the lowest lymphocyte region of the leukocyte channel due to their cell nucleus. They lead to a falsely high lymphocyte measurement or leukocyte count. The only way to clarify this is to perform a manual blood picture assessment to evaluate the erythroblast proportion and then to manually correct the total leukocyte value.

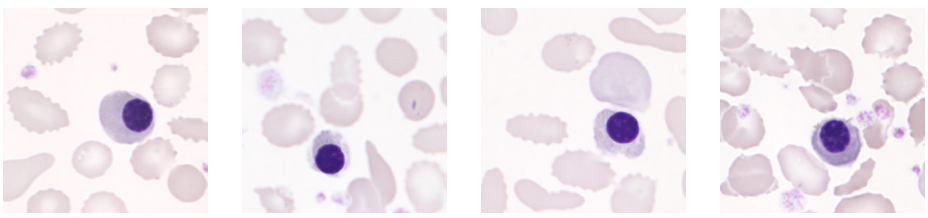
Measurement methods of 5-part differential hematology systems

These more complex hematology analyzers can make use of additional measurement techniques and evaluation options to better recognize and delimit interfering factors. Our interlaboratory tests are always analyzed with either a Sysmex XN-20 or an ADVIA 2120. In the following, we will, therefore, examine the measurement methods of these two machines more closely.

Sysmex XN-series

In these machines, an impedance method is used to count the epithelial cells and thrombocytes. Leukocytes are analyzed by flow cytometry following the addition of a specific lytic agent and staining with a fluorescent dye. For this, the cells pass through a narrow flow cell in which they are illuminated by light from a semiconductor laser (wavelength 633 nm). The cells scatter the laser light through various angles and at various intensities depending upon their cell size and degree of fluorescence. The forward scattered light (FSC) provides information about the cell size, the sideways scattered light (SFL) about the cellular contents (e.g. nuclear size). Leukocyte differentiation by flow cytometry occurs in three separate channels. Depending upon the channel, the measured light scattering is juxtaposed in various ways in the x- and y-axes of a so-called scattergram. The number of erythroblasts (NRBC%, NRBC#) is determined in the WNR channel (white cell nucleated channel). Correction of the leukocyte and lymphocyte counts occurs automatically prior to releasing the results.

Nevertheless, if more than 5 erythroblasts/100 leucocytes are detected in a patient for the first time, it is recommended to confirm the findings under the microscope.



Microscopic image of the erythroblasts of the sample MQ 2020-1 H3B

Clinical relevance of the demonstration of erythroblasts (NRBC)

In neonates, erythroblasts can be detected in the peripheral blood in the first weeks of life. If the erythroblast count is above the established reference range for neonates, this may be due to chronic or post-natal oxygen deficiency, anemia, maternal diabetes, stress, or even infectious diseases.

In adults, there are generally no erythroblasts in the peripheral blood. These may occur in increased numbers in e.g. severe anemias (especially those with ineffective erythropoiesis such as thalassemia major), leukemias, and other hematologic disorders such as myelodysplastic syndrome.

Additionally, various studies describe the NRBC content of peripheral blood as a prognostic factor in stem cell transplantations or in patients being treated in an intensive care unit. Patients with high NRBC counts seem to show a higher transplant rejection rate or higher mortality.

Literature

1. Da Rin, G. Performance evaluation of the automated nucleated red blood cell count of five commercial hematological analyzers. International Journal of Laboratory Hematology, 39(6), 663-670.

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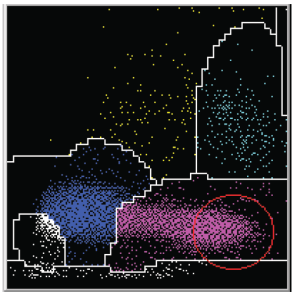
ADVIA 2120

In this machine, two different techniques are used. In the Baso-channel, the cytoplasm of all cells except the basophils is removed. Following this, the leukocyte can be separated from the basophils based on their shape and nuclear complexity and distinguished into mono- and multinucleated cells. In a supplementary peroxidation reaction, the leukocytes are fixed and stained with peroxidase. Neutrophils, eosinophils, and monocytes are stained by peroxidase, and lymphocytes, basophils, and large peroxidase-negative cells (LUC = large unstained cells) can thereby be separated into different regions. The light absorption, which is proportional to peroxidase content, is displayed on the y-axis. The x-axis shows the scattered light from the cells which indicates their size. Erythroblasts appear in the Baso-channel in the region of the polymorphonuclear cells (PMN), but their count can be obtained from the data in the peroxidase cytogram, where they appear in the bottom left corner due to their small size and absent peroxidase staining. The values obtained for NRBC (% und #NORMO) lead to automated correction of the leukocyte count.

Nevertheless, a microscopic analysis is necessary when first detected.



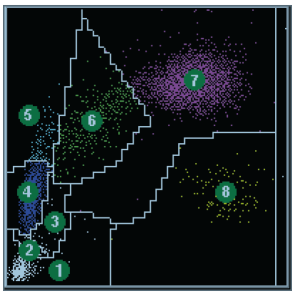
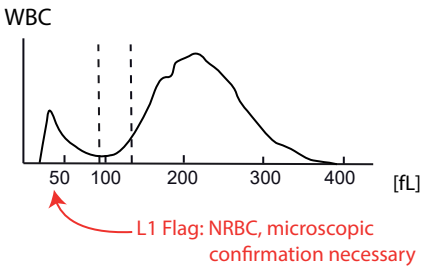


Fig. left: Position of the NRBC in the head of the PMN population in the Baso- channel.

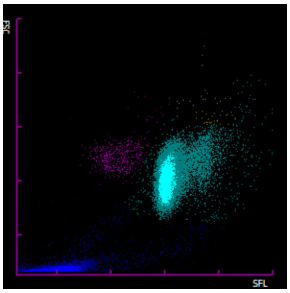
Fig. right: Position of the NRBC in section 2 of the peroxidase cytogram.

1 = Noise
2 = Erythroblasts
3 = Thrombocyte aggregates
4 = Lymphocytes/Basophils
5 = LUC- large unstained cells
6 = Monocytes
7 = Neutrophils
8 = Eosinophils

Measurement results of MQ 2020-1 H3B



Microsemi®: correct flagging in the WBC channel



Sysmex XN-20: Erythroblast population in the WNR channel

Our proficiency testing survey contained microscopically 3 erythroblasts per 100 Leukocytes corresponding to an absolute value of around 0.57 x109/l. In addition, there was a marked aniso-/poikilocytosis with many ovalocytes, few acanthocytes, and dacrocytes. The 3-part differential hematology systems all had broadened Ec-(epithelial cells-)curves in their RBC histograms due to the anisocytosis. Mythic and Sysmex XP300 show a pronounced shoulder in the lowest region of their Ec-curves, most likely caused by small ovalocytes. This leads in the Mythic to the FR1 report for „microcytes“ and in the Sysmex to the RL flag which indicates an abnormal frequency of events at the lower RBC discriminator. Only the Microsemi CRP machine produces a report of suspected NRBCs due to an atypical leukocyte curve in the lowest lymphocyte region. For these machines, it was necessary to establish the NRBC count under the microscope with subsequent manual correction of the leukocyte count.

With the 5-part differential hematology systems, the Sysmex XN20 showed an NRBC measurement of 2.9% or 0.56 x109/l, the ADVIA 2120 showed no NRBCs. The leukocyte count was correspondingly automatically corrected by the Sysmex. In our example, the small proportion of erythroblasts influences the total leukocyte count only minimally. In leukopenic samples with a high proportion of NRBCs, the difference between uncorrected and corrected leukocyte counts may, however, be relevant. It would lead, amongst other things, to a falsely favorable assessment of the total neutrophil count (when trying to establish agranulocytosis). The observed difference in sensitivity to detecting erythroblasts between the Sysmex XN and ADVIA machines was described by e.g. Da Rin et al 2017 in the International Journal of Laboratory Hematology. They measured a detection limit of >0.019 x109/l and >0.167 x109/l respectively for the Sysmex and ADVIA.