Spotlight on hematology

Effects of storage time and temperature on hematologic parameters

MQZH 2019-3

Introduction

The fresher the sample the more accurate the results. Samples for automated blood picture analysis would therefore ideally be processed as rapidly as possible after being taken, with only a short transport.

In daily practice, however there are unavoidable delays between blood sampling and analysis. In such cases, it is helpful to know what effect these delays will have on the analysis of the various parameters. In addition to the storage time, temperature and the measurement procedures of the hematological system also play a role. Pathological changes in the samples may also have an additional effect upon their stability.

With the 3-part hematology analyzers, differentiation is carried out on the basis of cell size alone. Examination of the leukocyte histogram helps to recognize storage artefacts.

Effects of storage time and temperature

How much can a value change and still remain correct? In [1] it is suggested that the coefficients of variation of the methods used be taken as the criterion. As long as the change in the values is smaller than the %age coefficient of variation, then the sample is considered to be stable. Based upon measurements using 5-part Hematology machines, the following recommendations were made.

Analyses	Stability	Remarks
Erythrocyte concentrate, Hb, MCH, red cell width	72 hrs. at 4-8°C	Also unchanged at room temperature.
MCV and Hct	24-72 hrs. at 4-8°C	Elevation due to swelling of the erythrocytes (increased Hct as a consequence). Strongly dependent upon the apparatus, already changed after 4 hours at room temperature.
Leukocytes	72 hrs. at 4-8°C	At room temperature, the value already falls after 24 hours depending upon the type of apparatus.
Automated leukocyte differential	24-72 hrs. at 4-8°C	Significant differences depending upon the machine. The monocyte count can either rise or fall using the 5-part machines depending on the measurement procedures, whilst it always rises with the 3-part machines. At room temperature the values are already changed after 4 hrs.
Thrombocyte concentrate	24-72 hrs. at 4-8°C	Continuous, mild fall in the values.

[1] Imeri F, et al. Clin Chim Acta 2008;397:68-71.

Age-related changes in differentiation using the 3-part Hematology analyzers

For which analyses is the difference greatest?
The MCV (and thereby also the Hct) increase rapidly at room temperature. The MCV is already up to 50% higher after 24 hours without cooling, and leukocyte differentiation is no longer possible with the Sysmex XP-300, since the apparatus can no longer find the „valleys“ in the histogram (leukocyte aggregation-discriminator 1 and 2).
The monocytes are represented in the Sysmex-3-part machines in the intermediate peak, the MXD-region. The granulocytes shrink as a result of storage. The individual peaks can no longer be clearly recognized. The granulocyte population is shifted to the left, and is now seen at approximately 120 fl. In fresh blood, it lies at between approximately 150 and 200 fl. In the presence of an elevated monocyte count, one must always examine the histogram and search for the monocyte peak. If this cannot be recognized, then the elevated monocyte count may be an artefact due to the size changes of the granulocyte population.

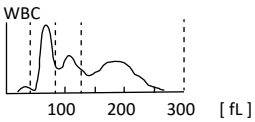


Figure 4. True monocytosis in preparation MQ 2019-3 H3b. The peak in the MXD-region is easily recognizable. Histogram from the Sysmex XP-300.

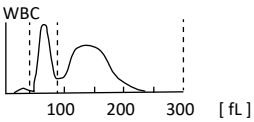
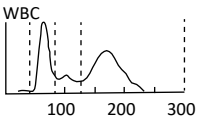


Figure 5. Storage artefact (27h at room temperature). Due to shrinkage of neutrophils, the curve is shifted into the MXD-region. Histogram from the Sysmex XP300.

Example of a blood sample which was measured with the Sysmex XP-300

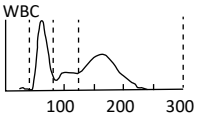
WBC	6.4 x10³/µL
RBC	- 3.66 x10⁶/µL
HGB	- 11.5 g/dL
HCT	- 34.4 %
MCV	94.0 fL
MCH	31.4 pg
MCHC	33.4 g/dL
PLT	311 x10³/µL



LYM%	32.8 %
MXD%	12.6 %
NEUT%	54.6 %
LYM#	2.1 x10³/µL
MXD#	0.8 x10³/µL
NEUT#	3.5 x10³/µL

Figure 1. Fresh sample

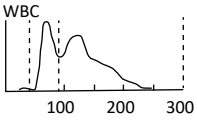
WBC	6.5 x10³/µL
RBC	- 3.69 x10⁶/µL
HGB	- 12.1 g/dL
HCT	- 34.5 %
MCV	93.5 fL
MCH	32.8 pg
MCHC	35.1 g/dL
PLT	291 x10³/µL



LYM%	32.8 %
MXD%	+ 15.2 %
NEUT%	52.0 %
LYM#	2.1 x10³/µL
MXD#	1.0 x10³/µL
NEUT#	3.4 x10³/µL

Figure 2. After 72 h at 4°C

WBC	6.4 x10³/µL
RBC	- 3.57 x10⁶/µL
HGB	- 11.7 g/dL
HCT	- 36.1 %
MCV	+ 101.0 fL
MCH	32.8 pg
MCHC	32.4 g/dL
PLT	297 x10³/µL



LYM%	F1* 32.0 %
MXD%	T2 ---.- %
NEUT%	T2 ---.- %
LYM#	F1* 2.0 x10³/µL
MXD#	T2 ---.- x10³/µL
NEUT#	T2 ---.- x10³/µL

Figure 3. After 72 h at room temperature

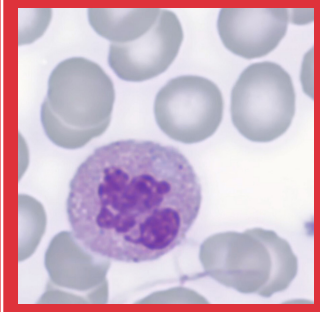


Figure 6. Fresh sample

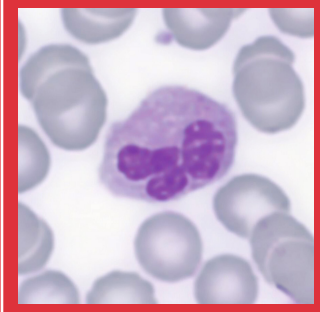


Figure 7. Sample cooled for 24 h

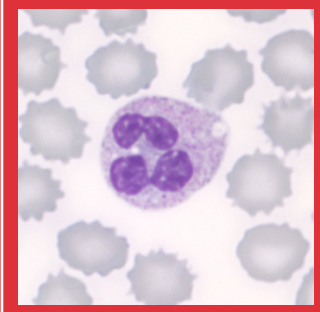


Figure 8. Sample at room temperature for 24 h

About Author *Annette Steiger*
Photography *Dr. Roman Fried*

Advisory
C. Hviid, Dr. C. Widmer, Klinik für Medizinische Onkologie und Hämatologie, Universitätsspital Zürich, Dr. J. Goede, Hämatologie, Kantonsspital Winterthur

Changes in analyzers with fixed discriminators

Other analysis systems, which use fixed discriminators, can occasionally give very high values for the monocytes in older samples. In this measurement technique, the neutrophils again shrink into the monocyte region (Figure 11). It is clearly apparent in this example that in the chilled sample this shrinkage did not occur, and thus the monocyte count is not elevated (Figure 10).

Example of a blood sample measured with the Microsemi

DIFF :		
LYM%:	33.1	%
MON%:	6.3	%
GRA%:	60.6	%
LYM#:	2.1	$10^3/\mu L$
MON#:	0.4	$10^3/\mu L$
GRA#:	4.0	$10^3/\mu L$

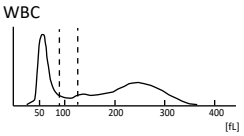


Figure 9. Fresh sample, measured with the Microsemi

DIFF :		
LYM%:	33.7	%
MON%:	6.8	%
GRA%:	59.5	%
LYM#:	2.1	$10^3/\mu L$
MON#:	0.4	$10^3/\mu L$
GRA#:	3.8	$10^3/\mu L$

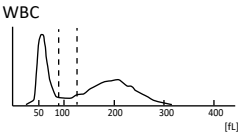


Figure 10. Sample after 72h at 4°C, measured with the Microsemi

DIFF :		
LYM%:	45.6	%
MON%:	30.4	H %
GRA%:	24.0	L %
LYM#:	2.8	$10^3/\mu L$
MON#:	1.9	H $10^3/\mu L$
GRA#:	1.4	$10^3/\mu L$

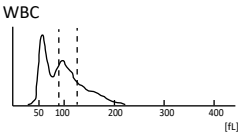


Figure 11. Sample after 72h at room temperature measured with the Microsemi

Changes in the microscopic blood picture due to storage of blood samples

Just as was seen for the automated blood picture, here it is also the case that the sooner the smear is made the better. Changes during the first 2-4 hours do not affect the results. If the sample has to be stored for longer, then the effects can be reduced by cooling.

If the EDTA-blood is first smeared, the cells are fixed on the specimen slide and are not subject to any further changes. Changes in the pigmentation can still occur artificially in this state, if smears are exposed to dust and sunlight for extended periods before staining. If a smear is not going to be stained immediately, then it should be stored in an appropriate protective slide container.

Neutrophils	Nuclei: Swelling of the nucleus , homogeneous chromatin pigmentation, loss of structure in the nuclear segments, development of multinucleation. Apoptotic forms (degenerate forms): nuclear shrinkage, chromatin condensation, dense, black chromatin masses, karyorrhexis. Cytoplasm: margins poorly delineated (ragged). Reduction of cytoplasmic structures. Mild increase in the vacuoles after 3-4 hrs., marked vacuolation after 6 hrs
Monocytes	Cytoplasm: Mild increase in the vacuoles after 4 hrs., marked vacuolation after 6 hrs..
Lymphocytes	Up to 5 hrs. no significant changes. Nucleus: Thereafter, homogeneous chromatin staining, blister-like nuclear outpouchings (nuclear budding). Cytoplasm: blister- or hair-like cytoplasmic offshoots and occasional fine vacuolation, blister-like nuclear outpouchings (nuclear budding).
Damaged cells	Increased occurrence after 24-48 hrs.
Nucleated RBCs	Erythroblasts decline within 1-2 days
Erythrocytes	Reduced membrane stability: formation of echinocytes (thorn-apple forms), spherocytes (Sphero-echinocytosis)
Thrombocytes	Swelling of the thrombocytes (increased size), which thereby appear hypogranular.