Spotlight on hematology

Error sources in analysis

Technical problems

- Technical defects of device components (e.g. leaking hose systems, defective photometer lamp, defective pumps)
- Exceeding the linearity of a parameter (sample dilution required)
- Contamination in the system, such as protein residues, bacterial contamination (high blank measurement, blockages)

Error sources in the pre-analysis

e.g. in vitro haemolysis

Blood collection

- Compressing for too long
- «pumping» with the fist
- poking around in the vein
- Applying strong presses during capillary blood collection
- Tubes with anticoagulant not sufficiently mixed, blood clots in the tube.
- Under-filled, over-filled tubes
- Collection sequence not observed

Transport and storage

- EDTA blood exposed to too low, too high temperatures (frozen, overheated)

Comparison with initial values

Parameters	KD	RCV
Hämoglobin	4.8 %	6.4 %
Hämatokrit	6.5 %	7.1 %
Erythrozyten	8.1 %	8.5 %
Leukozyten	10.7 %	22.6 %
Thrombozyten	12.6 %	19.9 %

CD=Critical Difference, RCV=Reference Change Value
Example per Fried, pipette 11-2011 (www.sulm.ch)

Alarm Values

Alarm values indicate a life-threatening situation, which must be communicated to the doctor immediately.

Parameter	LL	UL
Leukocytes*	2.0	40.0
Neutrophils**	1	
Hemoglobin*	69	200
Platletes*	50	1000

LL=lower limit, UL= upper limit
* McFarnale, A. et al, Int. J. Lab Hemat, 2015
** Universitätsspital Zürich

Plausibility Control in Hematology

MQZH 2015-4

Introduction

A series of quality assurance measures are required to reliably determinate hematological parameters on a hematology analyzer. In addition to the proper performance of internal and external quality control according to Qualab, plausibility controls are also required, which detect not only analytical but also preanalytical problems.

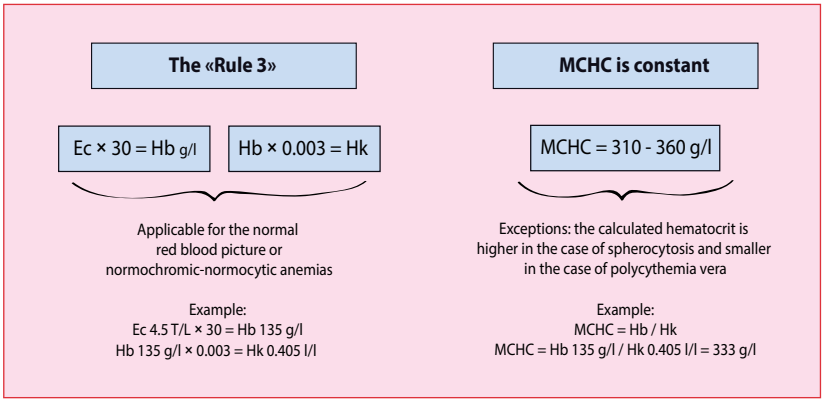
1. Device Warnings

Modern Hematology machines are able to very precisely quantify normal cell populations. However, if the cells are abnormally changed or if additional special population appear, they are not reliably detected. However, if the number of the special cells is large enough, the device recognizes that something is not right and warns the user with a «flag». These type of samples should be additionally examined with the microscope. The exact meaning of the different flags can be found in the device manual.

2. Constellation

Question: Are the hematological laboratory results plausible in constellation with each other or with other performed analyzes (e.g. clinical chemistry parameters)?

Example: To check the constellation within the red blood cell parameters, the so-called rule 3 and the MCHC control can be applied.



3. Initial Values

Question: Are laboratory results possible during the temporal / therapeutic course?

If initial values are available and the MCV has changed by more than 4 fl, a sample mix-up could have occurred.

Since each measuring device a random scatter, two measured values are only then secured as different if they differ by the «critical difference».

If the initial values are not from the same sample, the intra-individual scatter of the patient must also be taken into account. In this case, one speaks of <<Reference Change Value>> (RCV).

Changes that are greater than the specified values are significant. Before the report is submitted, it must be ensured that these values are not caused by analytical or preanalytical problems.

4. Extreme Values

Measurement values that are far outside the reference range are called extreme values. Sometimes they are so high or low that the values cannot originate from a living human being.

Values that are still possible, but are potentially life threatening for the patient, are referred to as alarm values. If such values unexpectedly appear and preanalytical and analytical problems can be excluded, they must be immediately reported to the doctor.

5. Expectations

Question: Are the hematological laboratory results compatible with the clinical picture / disease picture of the patient?

Patient-related factors

- High triglyceride levels after high fat meal - lipemic plasma
- Disease-related - hemolytic (in vivo haemolysis)
- Time of the day blood was drawn

Cold agglutinins

Cold agglutinins are antibodies, which cause erythrocyte agglutination when the blood is cooled. Because agglutination is reversible at increasing temperatures, the blood samples can be incubated for 30 minutes at 37°C before re-measuring. Cold agglutinins appear idiopathically, post-infectious (e.g. with mycoplasma or EBV infections) or with lymphoproliferative disorders.

Cryo-globulins

Cryo-globulins are immunoglobulins, which become insoluble and form aggregates when the blood is cooled. As with the cold agglutinins, the measurement is repeated after a 30-minute sample incubation at 37°C. Causes are haematologic systemic diseases, autoimmune diseases or viral infections (more frequently with hepatitis C).

Platelet aggregates

The cause of platelet aggregates can be errors in blood collection (especially capillary). In approx. 1% of patients, EDTA-induced aggregate formation occurs. By switching to an alternative anticoagulant (lithium heparin or citrate), an accurate platelet count can be determined.

Predilute sample with 0.9% NaCl

A 1:2 dilution (1 part EDTA blood + 1 part NaCl) is generally sufficient.
The measured results must be multiplied by the factor of 2 (except erythrocyte-indices).

About
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Example MQ 2015-4 H3B

Warnings
In the ABX Micros System, «PLT Flags: SCL MIC» appears
SCL means that a remarkable number of very small cells (2-3 fl) appear, MIC means that the distinction between platelets and red blood cells is uncertain.
In accordance with the manual, in this case the microscopic blood picture must be examined.

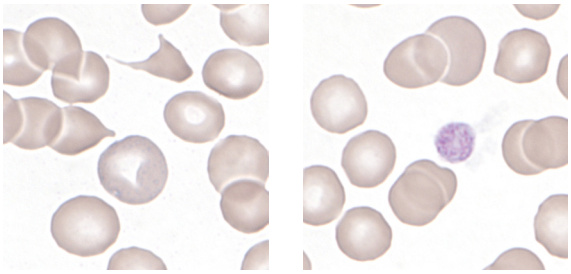
Constellation
The hemoglobin concentration is 108 g/l.
The calculated hematocrit is 108×0.003=0.324 l/l, the measured value is 0.337 l/l.
With 321 g/L, the MCHC is within the reference range.
Therefore, the values of the red blood cells are plausible.

Initial Values
Not Specified

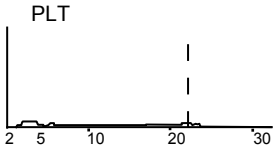
Extreme Values
The number of platelets is 3 g/l. With unexpectedly low platelet numbers, the microscopic blood picture must be used to check whether platelet aggregates are present.
If, as in this case, platelet aggregates are not present, the value is an alarm value that must be reported immediately.

Expectations
The values are plausible in the context of immune thrombocytopenia (ITP).

Summary
Anisocytosis with very small erythrocytes and very few platelets, practically no signal in the PLT-channel. The low platelet value is plausible. The MCHC is within the normal range, but the RDW as a measure for the size distribution is increased with 16%.



MQ2015-4 H3B: Erythrocytes and a large platelet



ABX Micros, PLT histogram

Problems	expected value change	further progress
Exceeding the measurement range (linearity)	all, no result output by the device	Predilute sample 1:2 with 0.9 % NaCl
Erythroblasts	Lc ↑	Count erythroblasts in the blood smear, perform correction calculation
Cold agglutinins (erythrocyte agglutination)	↓ Ec, ↑ MCV, MCH, MCHC	Incubate sample at 37 °C
Cryo-globulins	↑ TC, LC, ev. Messprobleme Lc-Diff, Ec, Hb	Incubate sample at 37 °C
Leukocytes values > 100 G/l	↑ Ec	Predilute sample with 0.9 % NaCl
Giant platelets	↓ Tc, ↑ Ec	potential chamber count or flow cytometric measurement
Platelet aggregates Micro-blood clots	↓ Tc, Lc ↑	redraw blood, with suspicion of EDTA-induced thrombocytopenia with alternative anticoagulant
Fragmentocytes	↓ Ec, ↑ Tc	Verification in differential blood count. Submit results with the corresponding information.
Haemolysis	↓ Ec, ↑ MCV, MCH, MCHC	Redraw blood in the case of in vitro haemolysis
Lipemia	↑ Ec, ↑ Hb, ↓Hk, ↑ MCV, MCH, MCHC, ↑ Lc, Tc	Repeat with fasting blood draw or predilute with 0.9% NaCl. (Or correct according to the manual)
Bacteria and fungi	↑ Tc	Exclude external sample contamination.