Hematology Controls
Best practices for reliable results

What are hematology controls?

Hematology controls help assess the precision of hematology analyzers, ensuring valid patient results and meeting accreditation requirements. For intra-instrument quality control, the daily measurement of quality control samples is obligatory.

Usually, instrument manufacturers supply hematology controls with two or three levels designed to closely resemble patient samples.[1] Controls with assayed values for three-part and five-part differential analysis as well as erythrocyte sedimentation controls are available. They cover multiple blood parameters, including for example erythrocytes, platelets, granulocytes, lymphocytes, monocytes, hematocrit, hemoglobin and many more.

Why is quality control in hematology important?

Quality control should be performed routinely to ensure the consistent reliability of sample results.[2]
Each laboratory should establish their own QC program complying with accreditation guidelines.
There are four main purposes of internal QC:

1. Monitoring of the analytical process.
2. Detection of errors that occur due to failure of the system, adverse environmental conditions or operator performance.
3. Monitoring of long-term test performance.
4. Provide a proof of adequate long-term quality level and comply with regulatory requirements.

With the use of controls, accuracy and precision of the patient results can be guaranteed.

What do accuracy and precision tell us in terms of hematology controls?

In hematology as in other in-vitro diagnostic areas, the main goal of QC is to control accuracy and precision of the measurements.[3] Accuracy is the ability to achieve the right result, while precision is the ability to achieve the same result over and over again. Looking more closely at accuracy and precision, different scenarios can occur where the control either falls within the acceptable range or fails. Knowing the root cause of each situation makes it easier to take the proper corrective action when controls do not meet specifications.

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Hematology Controls
Importance of accuracy and precision

Accurate and precise

The first situation is the ideal scenario showing accurate and precise values (figure 1).

The individual values are very close to the target (accurate) and close together with only minor variations (precise).

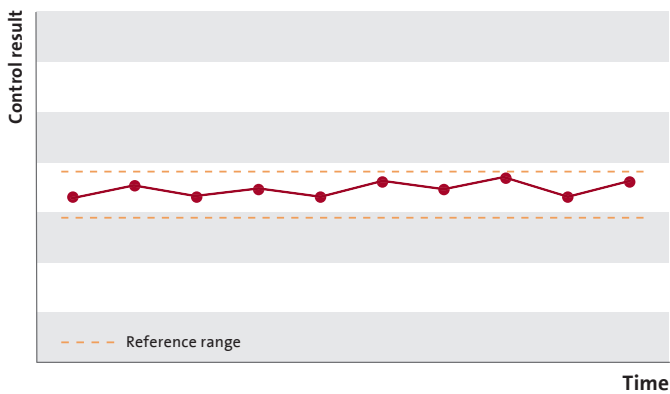The Levey-Jennings Diagram, which shows the single values over time, displays a constant line around the mean.

Figure 1: Levey-Jennings plot showing accurate and precise data

Not accurate and precise (trend)

The second situation shows neither precise nor accurate values. The associated Levey-Jennings curve shows a trend: the values are increasing slowly over time (figure 2).
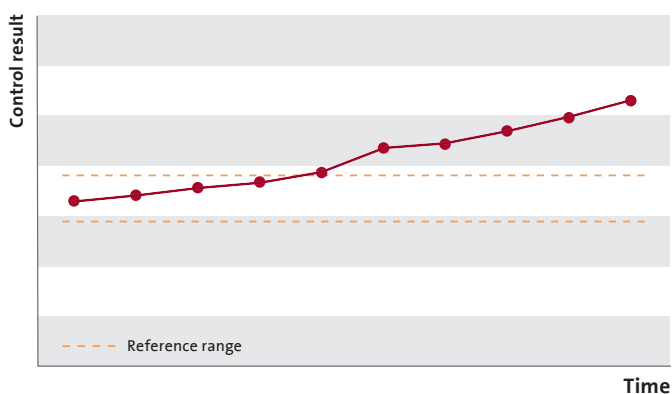

Figure 2: Levey-Jennings plot showing a trend

Trends are typically caused by a drift of the instrument.

Some variables that may contribute to a trend are:

– Controls or reagents have not been used according to the manufacturer's instructions.

– Controls or reagents may be expired.

– The next date for calibration of the system might be approaching.

– Preventative maintenance may need to be performed.

Not accurate but precise (shift)

The third situation shows a sudden shift of the values. A clear shift can be seen starting with measuring point 5. After that, the measurements are still precise, as they show only minimal deviations, but they are no longer accurate, as the correct target value is not recovered (figure 3).
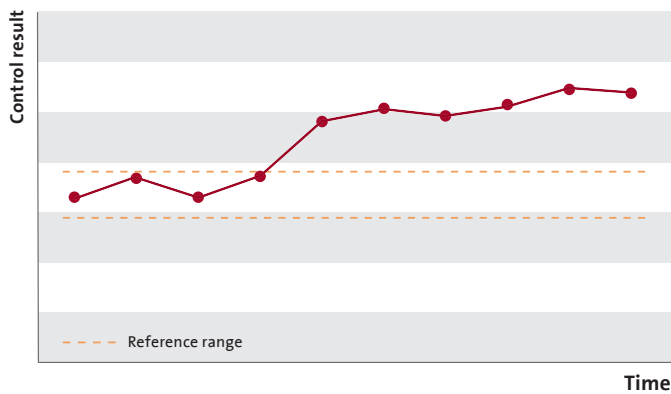

Figure 3: Levey-Jennings plot showing a shift

A shift may indicate that some variable has suddenly changed in the system. Reagents or the environment can cause the shift. The user should verify whether there was a part change on the instrument, if a lot number of reagents or controls has changed, or if there have been sudden environmental changes (e.g. temperature) in the lab.

Accurate but not precise (imprecision)

The last situation shows accurate but unprecise data (figure 4). Even though the mean value is correct, the measurement values are varying a lot between the single measurements. This is called imprecision. Several different conditions can cause this result. Very often, however, imprecision is caused by poor mixing of the control material.
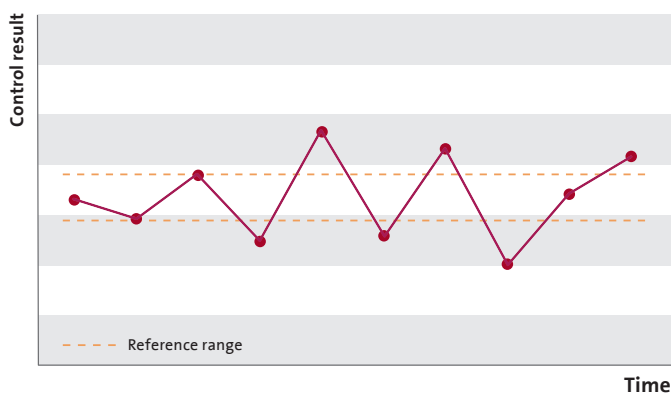

Figure 4: Levey-Jennings Plot showing imprecision

Hematology controls
Proper storage and handling – best practices

What is special about hematology controls?

The best reference material currently available is the calibration and control material consisting of stabilized, but still intact cells. Human and mammalian blood cells are used to manufacture control materials. Due to the limited lifespan of intact cells, the shelf life of the controls is limited. In vivo, red blood cells have a lifespan of ~ 120 days, platelets of ~ 7–10 days and white blood cells of ~ 13 - 20 days.

Once removed from the body, the blood cells will decompose rapidly. Stabilization can prolong the shelf life of the control products to a certain extent. However, preservation must be balanced against maintaining the physical properties of the cells and responsiveness to the lysis reagent to generate valid QC material. Therefore, the shelf life of hematology control material will always be within certain limits. Some cell types show a loss of their size, shape and function after preservation and, therefore some manufacturers use artificial substitutes which can resemble certain cell types.

The compatibility of alternate substitutes as well as the applied stabilization procedure vary between different analyzers and manufacturers. This is dependent on the technology and the reagent composition used by each system. Each control material is dedicated and optimized for a certain type of hematology system (instrument plus reagents) and results may not be comparable, if third-party materials are used. The target values only apply to the specific setting and provide reliable results only in this combination. They cannot be used with instruments from other manufacturers because each analyzer operates differently and uses different amounts and compositions of reagents.[4] Thus, only the manufacturer's control can demonstrate the full functionality with all parameters recorded by the system.

Storage and handling of hematology control material

Many problematic QC results in hematology derive from incorrect handling or inappropriate storage of the material. Storage of HUMAN´s hematology controls at 2 - 8°C is required to ensure stability and proper performance due to the sensitive primary material. Also, during transportation it is essential to keep the temperature constant at a low level.

To ensure constant transport temperature, HUMAN uses established transport conditions with Styrofoam boxes and cold packs. Expired materials or vials with too little remaining volume can lead to erroneous results.

Furthermore, controls require accurate preparation before measurement as the blood cells need to be homogenized carefully. The material must be brought to room temperature before use. Controls need to be mixed vigorously, but carefully to avoid foaming and destruction of the material. The exact procedure is explained in detail in the respective IFUs and may differ slightly according to the type of control material.

Overheating – possible error source

Exposure to temperatures at or above 20°C for prolonged periods may affect control product performance. Overheating may result in elevated mean cell volume (MCV) of red cells accompanied by possible red cell fragments and hemolysis. Furthermore, overheating will lead to denaturation of proteins. Denatured proteins are recognized by the hematology analyzer as small particles and may be counted as PLT. Additionally, an impaired measurement of HGB due to increased turbidimetry of the sample can be observed. It is important to note that damage to the control material due to overheating, while not immediately obvious, may result in a shortened shelf life of the product.

Overheating	Freezing
PLT ↑↑↑	RBC ↓
MCV ↑	MCV ↑
MCH ↑	MCH ↑
HGB ↑	RDW-SD, RDW-CV ↑
	PCT, PDW, MPV, P-LCR ↑

Table 1: Influence of overheating and freezing of control material on hematology parameters

Freezing – possible error source

Refrigeration storage outside the manufacturer's recommended storage range may destroy the control material. In any case, it must be avoided that the control freezes. Freezing causes the cells to burst and irreparably destroys the material. Storing control material at the back side of the refrigerator should be avoided as there is a risk that the back wall ices up and the material will freeze more easily. Once the control material is frozen, hemolysis occurs due to damage of RBCs during freezing. This process can already happen with storage temperatures below 2°C. Hemolysis becomes obvious by an untypical dark red color of the control together with red discoloration of the supernatant. Several parameters will be impaired, which becomes immediately obvious when measuring the control material. White blood cell parameters and HGB might still look normal, but RBC and PLT parameters are usually not recovered correctly anymore. The RBC count decreases due to the destruction of the cells. The PLT count and the associated parameters get disturbed by particles resulting from burst RBCs. A control that has frozen cannot be used anymore.
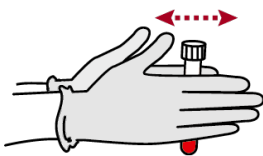

Figure 5: Hemolysis of control material

Handling instructions:

Following these simple instructions will ensure obtaining accurate QC results and extending the life of the control.

1. Remove tubes from the refrigerator and bring to room temperature (15 to 30°C) for 15 minutes before mixing.


a) Hold the vial vertically and roll each vial between the palms of the hands for 20 - 30 seconds.
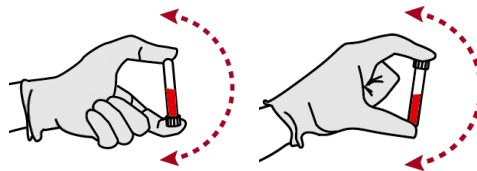
b) Continue to mix by rapidly inverting the vial end-over-end using a very quick turning motion of the wrist. Continue to mix in this manner until the red cells are completely suspended. Mix vigorously, but do not shake. Tubes stored for a long time may require extra mixing.
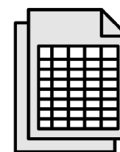
c) Analyze immediately after mixing. If this is not possible, invert the tube 8 - 10 times again before sampling.
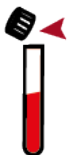
d) After analysis, clean the cap and tube rim with a lint-free tissue. Replace the cap tightly.

e) Return tubes to refrigerator within 30 minutes of use.

f) Compare obtained values with expected results from the assay sheet.

- 95% of the recovered values should fall within the expected range.
- No more than three consecutive values should exceed the expected range.
- Recovered values should not trend outside the expected range.

Hematology Controls
Proper storage and handling – best practices

Temperature changes during storage

Storing calibrators and controls in the refrigerator door should be avoided due to the associated temperature changes during opening and closing of the fridge's door.

Failures associated with impaired stability

Expired control material should no longer be used, as its correct performance cannot be guaranteed. Despite stabilization of the material, some properties of the primary cells will change over time. The target value ranges are adjusted for these changes, but if the material is used beyond the specified shelf life, certain parameters will be out of specification. The same applies for the use beyond the open vial stability date.
Another potential source of error is the use of the control material, if it is below the required minimum tube volume. In this case, there is a risk that insufficient analysis volume will be aspirated by the analyzer and results will be lower than expected.

Failures associated with mixing procedure

Inadequate mixing or resuspension of the control material will lead to invalid results. Undermixing can lead to certain values being lower than the target.
Figure 6 shows an exemplary Levey-Jennings plot for the PLT parameter using a control which was correctly mixed or undermixed. If the control is correctly and thoroughly mixed, stable data points close to the control target value (red line) are received. If, on the other hand, the control is undermixed, the PLT value shown here as an example will be too low and will fluctuate greatly between measurement points.
In an undermixed control, aspiration from the sediment can lead to increased measurement of RBCs, but underrepresented PLTs and WBCs.
In contrast, in an overmixed control, red blood cells may be destroyed and cell debris are counted as PLT due to smaller size. Therefore, the PLT parameter is expected to be elevated.
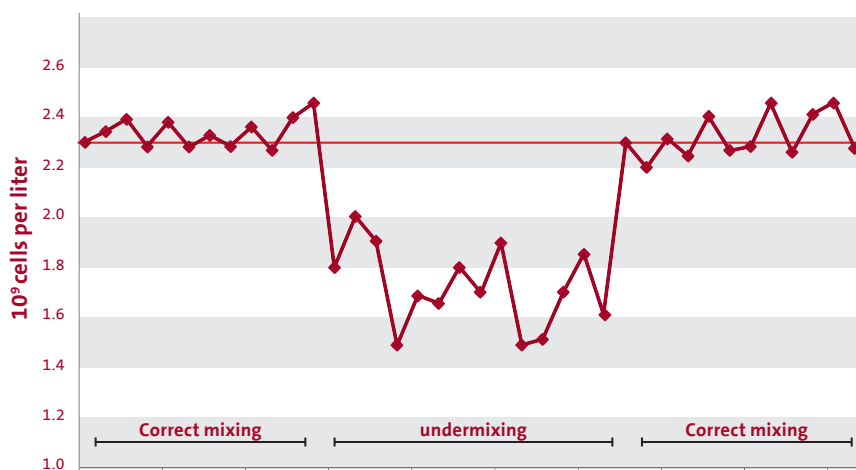

Figure 6: Levey-Jennings Plot showing the influence of the mixing procedure in platelet results

Undermixed	Overmixed
WBC ↓	WBC ↑
RBC, HGB, HCT ↑↑	RBC, HGB, HCT *not markedly influenced*
PLT ↓	PLT ↑↑

Incorrect mixing procedures lead to increased CV values for most parameters!

Table 2: Influence of undermixing and overmixing of control material on hematology parameters

Take-home message

The main objective of a laboratory is to provide reliable, timely and accurate test results. Therefore, the regular processing of internal quality control using control material that is appropriate for the specific analyzer and the consistent monitoring of the performance of each parameter is an important requirement. Only then the expected standards of the laboratory can be met and physicians can make meaningful and safe clinical decisions for all their patients.

Reference to HUMAN control products:

REF	Product	Description	Shelf life	Open vial stability
17400/40	HC-Control (3 levels) 3 x 2.5ml	For use with HUMAN 3-Part instruments: HumaCount 30/60/80[TS]	190 days	30 days
17400/50	HC-Calibrator 1 x 2ml	For use with HUMAN hematology analyzer: HumaCount 30/60/80[TS], HumaCount 5L, HumaCount 5D, HumaCount 5D[CRP]	45 days	7 days
16430/50	HC5L-Control (3 levels) 2 x 3 x 3ml	For use with HumaCount 5L	105 days	21 days
16450/40	HC5D-Control (3 levels) 2 x 3 x 3ml	For use with HumaCount 5D and HumaCount 5D[CRP]	105 days	21 days
15024/40	HSRate-Control 2 x 2ml	For use with HumaSRate 24[PT]	6 months	30 days

References

1) Gulati GL, Hyun BH. Quality control in hematology. Clin Lab Med. 1986 Dec;6(4):675-88. PMID: 3539479.
2) Lewis, Shirley Mitchell & World Health Organization. Health Laboratory Technology and Blood Safety Unit. (1998).
   Quality assurance in haematology / by S. M. Lewis. World Health Organization. https://apps.who.int/iris/handle/10665/60141
3) BS ISO 5725-1: „Accuracy (trueness and precision) of measurement methods and results - Part 1: General principles and definitions.", p.1 (1994)
4) HUMAN Fact Sheet: Why standardization in hematology is so special, 2019
5) Vidali M, Carobene A, Apassiti Esposito S, Napolitano G, Caracciolo A, Seghezzi M, Previtali G, Lippi G, Buoro S. Standardization and harmonization in hematology: Instrument alignment, quality control materials, and commutability issue. Int J Lab Hematol. 2021 Jun;43(3):364-371. doi: 10.1111/ijlh.13379. Epub 2020 Nov 10. PMID: 33174358.
6) Vis JY, Huisman A. Verification and quality control of routine hematology analyzers. Int J Lab Hematol. 2016 May;38 Suppl 1:100-9. doi: 10.1111/ijlh.12503. Epub 2016 May 9. PMID: 27161194.

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