

SEED HAEMATOLOGY



Quality control materials: the better you treat them, the more you can trust your haematology results

One vital part of quality assurance is the internal quality control (IQC), which is used to ensure day-to-day consistency of an analytical process, helping to determine whether patient results are reliable enough to be released. Performing IQC also enables the laboratory to monitor and document the quality of its work. In most of the countries it is required to perform IQC by national regulations.

There are four main purposes of IQC:

1. To monitor the complete analytical process.
2. To detect immediate errors that occur due to
 - a) a failure of the analytical system,
 - b) adverse environmental conditions, or
 - c) operator performance; for example maintenance procedures being carried out differently because of a change of operator, etc.
3. To monitor the long-term test performance that may be influenced by changes in the performance of the
 - a) analytical system,
 - b) environmental conditions and
 - c) inter-operator variance.
4. To provide proof of an adequate long-term quality level and to comply with regulatory requirements.

IQC is conducted by running one or more control materials on the analysis system that is to be checked. The control materials undergo an analytical procedure identical to that applied to patient samples. The results are plotted on control charts as described by Levey-Jennings, and those charts are interpreted in the usual fashion.

Sounds simple? Well, it's not entirely simple. There are factors that need careful consideration if the IQC system is to represent a lab's routine analytical operation adequately.

Requirements QC materials have to meet

Controls are materials that contain an established amount of the substance to be tested – the analyte. Controls have to be tested at the same time and in the same way as patient samples. The purpose of the control is to validate the reliability of the analysis system and to evaluate operator performance and environmental conditions that might have an impact on the results. It is particularly critical to select appropriate control materials.

The best materials for IQC are typical samples of the routine test materials, assuming that they are sufficiently stable for the purpose. Table 1 lists recommended properties of quality

control materials as per recommendations of the Hong Kong Association of Medical Laboratories (HKAML) [1]. Additionally, it has to be taken into consideration that control materials need to be different from calibrator materials [2].

Table 1 Recommended properties of a QC material

1. It should resemble a human sample (blood, plasma, serum, CSF, etc.).
2. The analyte concentration should be at medically significant levels. It should span the clinically important range of an analyte's concentration.
3. The material matrix should be as much like the human sample as possible.
4. Constituents should be stable for a long period of time.
5. After the vial has been opened and material prepared it should be stable during the period of use.
6. The control material should be ready to use and require minimum preparation.
7. Convenient sizes of aliquots/vials can be prepared and vial-to-vial variability should be low.
8. It should be reasonably priced (optional).
9. The control material should be tested in the same manner as patient specimens.

QC materials from Sysmex

Manufacturing quality control materials for haematology is a challenge compared with controls for clinical chemistry if all the points mentioned above shall be covered. Native cells naturally have a very limited survival rate. To extend a cell's life to a longer period, efficient stabilisation is needed. Due to this, haematology quality control material is different to freshly collected patient samples. This means care must be taken to ensure the material has been used correctly when interpreting quality control results.

Sysmex control materials include stabilised human red blood cells (RBC), white blood cells (WBC), and a platelet (PLT) and nucleated red blood cell (NRBC) component in a preservative medium. They are free from any artificial components that simulate cells, such as latex particles. However, a microscopic white blood cell differential cannot be accomplished with this material, as the white blood cells have been treated to enhance their stability. This means they will not stain to demonstrate the typical cell morphology known from May-Gruenwald-Giemsa staining. But regarding lysing and staining behaviour with the analyser reagents, the stabilised cells only show small differences to fresh blood cells. These differences are the reason why the analyser uses a QC mode to produce and display the measurement results.

Sysmex control materials are ready to use. All controls are delivered in vials with sufficient material for the control period and are measured with the same measuring principle as patient samples.

For all diagnostic whole blood parameters Sysmex delivers controls in the low, normal and high analytical range and in order to check body fluid parameters, we provide two control levels.

For the manufacturing of our haematology control materials, we trust the well-known producer Streck, Inc. (Nebraska, USA), who is recognised worldwide as the leader in cell stabilisation. Streck's core competence is the development of quality control materials that are tailored to the customers' needs.

Transportation, storage conditions and shelf life

Sysmex haematology control materials are to be stored with a closed cap at 2–8°C. A short-term increase in temperature, which may occur during transportation, does not affect the quality of the product. All haematology control materials must be protected from freezing. When handled in this manner, the products are guaranteed stable until the expiration date stated on the package and vials. Once the vials have been opened or sampled by cap piercing, they will retain stability – depending on the product type – as stated in Table 2.

Table 2 Stability of Sysmex haematology controls

Control material	Period of use	Open vial stability
Eightcheck-3WP	84 days	7 days
e-Check (XS)	56 days	14 days
e-Check (XE)	56 days	7 days
XN-L Check	84 days	15 days
XN Check	56 days	7 days
XN Check BF	56 days	30 days

Preparation of XN-Class QC materials before measurement

When storing and preparing quality control materials it is important to carefully adhere to the manufacturer's instructions for storage and equilibrating.

1. Remove the vial from the refrigerator and equilibrate with room temperature (15–30°C) for at least 15 minutes before use.
2. Roll each vial between the palms of your hands for 15 seconds (see Fig. 1).
3. Holding the vial from end to end between the thumb and forefinger, invert the vial 20 times using a very quick turning motion of your wrist during mixing. Details can be seen in a video on the CD-ROM or USB stick accompanying each QC lot (see Fig. 2).
4. Analyse the QC material on the instrument according to the Instructions for Use. The pierceable septum in the vial cap allows sampler analysis.
5. Subsequent analyses during this test period may be performed by inverting the vial 5 times prior to instrument analysis.
6. Return the vial to the refrigerator (2–8°C) for storage.

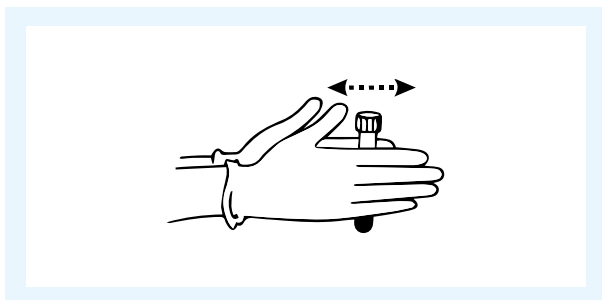


Fig. 1 Roll the QC vial for 15 seconds between the palms of your hands.

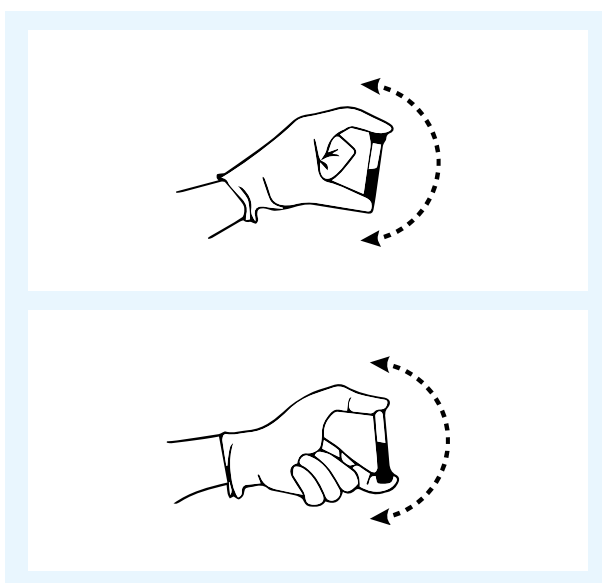


Fig. 2 Invert the vial 20 times holding it from end to end, using a very quick turning motion of your wrist during mixing.

You can also watch a movie about the mixing of the quality control vials on our webpage:

<http://www.sysmex-europe.com/media-center/sysmex-qc-material-preparation-26847.html>

A simple procedure with a great impact

When the result of a QC measurement falls outside its limits, analysis should be stopped, patient results held, and the analysis system investigated. As soon as error sources have been identified and corrections made, the measurement of control material should be repeated. If the results read correctly, then patient samples (those from the period of the last correct QC measurement until the QC error has been discovered and solved), along with another quality control specimen, should be repeated. Do not simply repeat the testing without looking for sources of error and taking corrective action.

According to the World Health Organisation (WHO), possible problems to consider include [3]:

- Degradation of reagents or kits
- Control material degradation
- Operator error
- Failure to follow manufacturer's instructions
- An outdated procedure manual
- Equipment failure
- Calibration error

In haematology it can be generally observed that a lot of problematic QC results derive from incorrect handling or inappropriate storage of the material. Also using outdated materials or vials with too little remaining volume leads to erroneous results. Especially haematology controls deserve accurate treatment before measurement as they contain blood cells that need to be homogenized before measurement.

Below, some examples are shown where the results have been out of their range due to mistreatment of the QC material. Changes in the numerical results, particularly of the complete blood count (CBC) and reticulocyte parameters, can be observed. Checking the cell distributions in histograms and scattergrams can also help to reveal differences to results obtained from correctly treated material. Measurement examples and scattergram images of all control levels can be found on the CD-ROM or USB stick that always accompanies the QC material for comparison purposes.

Incorrect mixing and its effects

Identifying mixing problems is generally difficult as it depends on both the intensity and duration of mixing. However, taking a closer look at the two extremes (insufficient vs. overmixing) reveals that numerical results of WBC, RBC and PLT can be distorted due to the use of an incorrect mixing procedure.

An overmixed sample is generally more difficult to identify and occurs more rarely. If the sample is mixed for too long a time with high speed, slightly elevated WBC counts together with markedly increased PLT counts can be observed. Never mix the haematology QC vials on an automated roller or mixing device!

In contrast to that, samples that are not mixed sufficiently can be identified by markedly increased RBC counts and related parameters (HGB, HCT) and decreased WBC counts and low counts of PLT-I especially in Level 1 with increased coefficient of variation (CV) values. Table 3 compares the results of improperly mixed QC samples.

Table 3 Results of improperly mixed QC samples

Not mixed	Overmixed
WBC ↓	WBC (↑)
RBC, HGB, HCT ↑↑	RBC, HGB, HCT not markedly influenced
PLT-I (Level 1) ↓	PLT-I ↑↑
Aspirated from the sediment, more red blood cells are measured, but white cells are underrepresented or missing.	Red blood cells are destroyed and fall by size into the PLT area, where they are then counted as platelets.

Applying an incorrect mixing procedure leads to increased CV values for most parameters as shown in Fig. 3 and Table 4. This was done as an experiment to demonstrate the influence of the mixing procedure on platelet results. Two different mixing procedures were used. For the measurements of phase 1 and 3 (○) the correct mixing procedure was applied, whereas in phase 2 (○) the QC material was less intensively mixed than described in the package insert. Platelet results were more imprecise and significantly lower than the assay mean value.

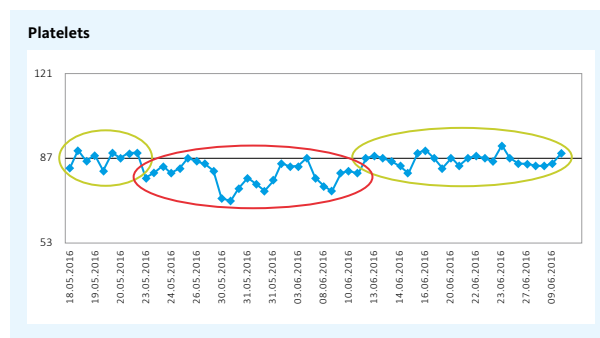


Fig. 3 Experiment results using XN Check Level 1 with two different mixing procedures

Table 4 Quantitative analysis of the experiment's results

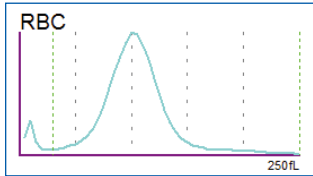
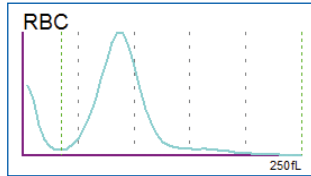
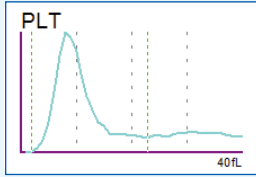
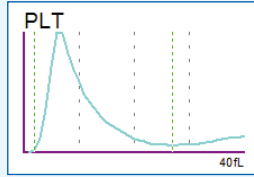
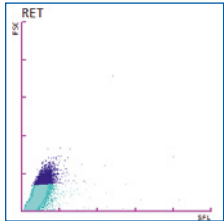
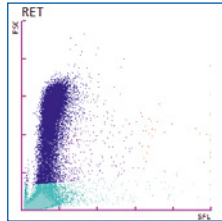
	Phase 1 (correct mixing)	Phase 2 (insufficient mixing)	Phase 3 (correct mixing)
Assay mean for platelets	87	87	87
Mean	87.0	80.2	86.3
Deviation (%) from assay mean	0.0	-7.8	-0.8
Standard deviation (SD)	2.8	4.7	2.4
CV (%)	3.3	5.9	2.8

The effect of incorrect temperatures on quality control material

Not only mixing but also temperature has an influence on the results obtained from quality control materials. Haematology controls must be stored refrigerated, but may only be used after equilibration with room temperature for at least 15 minutes. Storing QC materials below 2°C, even short-term, has an immediate impact on the cells and results in haemolysis, as shown in Table 5.

A haemolysed QC material can be recognised by a colour change from red to brownish black inside the vial, as shown in Fig. 4.

Table 5 Results of QC samples stored at incorrect temperatures

Frozen		Overheated	
RBC ↓, MCV ↑, MCH ↑, RDW-SD ↑, RDW-CV ↑		HGB ↑, MCV (↑), MCH ↑	
PDW, MPV (↑), P-LCR (↑), PCT ↑		PLT ↑↑↑	
Abnormal platelet distribution visible		No abnormal platelet distribution visible	
RET ↓↓↓↓, RET-H _e ↓↓↓↓, RBC-H _e ↓↓↓↓		RET ↓	

The membranes of the red blood cells are damaged during freezing, which results in haemolysis and therefore in lower counts plus disturbance of the red cell parameters. Although the PLT histogram is clearly disturbed by larger particles resulting from burst RBC, the PLT count may still lie within the assay range. HGB remains stable since for the haemoglobin measurement the red blood cells need to be lysed anyway. Reticulocyte measurement is totally disturbed and RET-H_e and RBC-H_e results are far too low.

Due to overheating the proteins in the sample are denatured and are recognised by the analyser as small particles, getting included in the measurement of platelets. Plus the turbidity of the sample increases, which also disturbs the photometric measurement of HGB.

The increased debris can also be observed in the reticulocyte scattergram. The entire population shifts down.

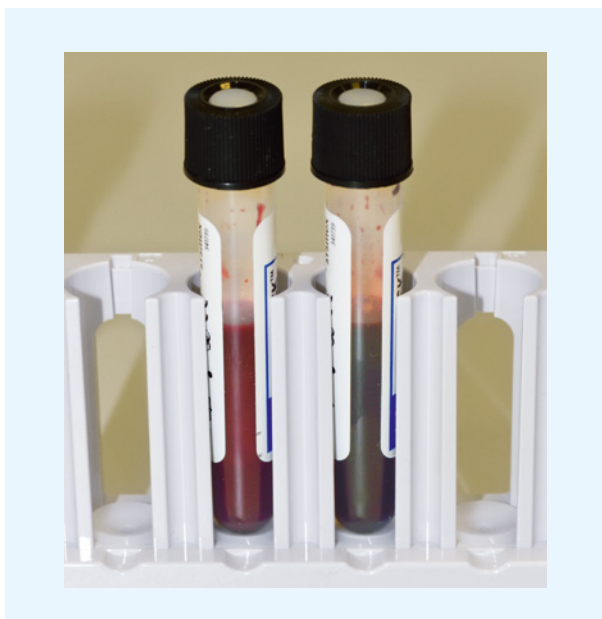


Fig. 4 Comparison of a non-haemolysed QC sample (left) with a haemolysed QC sample (right)

Conclusion – why should one bother

Small things can have a great impact. So has the mixing procedure of QC materials in haematology! As could be seen above, it greatly influences the QC measurement results. Damages that occur to the vial neither reflect a defect of the analyser nor the necessity for calibration. Therefore, it is of utmost importance to store and treat the quality control materials in the way the manufacturer describes.

The main objective of a laboratory is to provide reliable, timely and accurate test results to the requesting persons. To continuously achieve this day in, day out, you need consistent monitoring and evaluation of the laboratory's performance, and – in case of non-conformance to the procedures – the implementation and follow-up of corrective actions. A poor approach can lead to the validation of incorrect patient results, potentially setting off a spectrum of undesirable scenarios and legal actions in the worst case scenario.

References

- [1] **Phang R et al.** (2009): *A Practical Guide to Internal Quality Control (IQC) for Quantitative Tests in Medical Laboratories. Proposed Guidelines.*
- [2] **CLSI C24 – A3** (2006): *Statistical Quality Control for Quantitative Measurements: Principles and definitions – Approved Guideline.*
- [3] **WHO Content Sheet 7 – 1: Overview of Quality Control for Quantitative Tests.**
http://www.who.int/ihr/training/laboratory_quality/7_b_content_quant_qc.pdf