

SEED Haematology

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Internal quality control in haematology – for the sake of the patient.

What is quality assurance?

The laboratory plays a pivotal role in medical practice as test results have major influence on clinical diagnosis and patient management. The laboratory therefore has an ethical obligation to produce reliable and reproducible test results and to provide clinicians with unambiguous meaningful reports that are relevant for the clinical problem being investigated. Quality assurance is the sum of all activities that a laboratory must undertake to ensure that results generated are reliable and correct. By strict adherence to quality assurance processes any mistakes should be found and corrected before patient results are released thereby avoiding any adverse outcomes. A key element of any quality assurance programme is the fact that the laboratory must aim to strive for continuing improvement through constant feedback and corrective action.

The major activities of a typical quality assurance process can be divided into three main phases, namely preventative, assessment and corrective action as shown in Tab. 1.

What is internal quality control?

The phrase 'quality control' is used to refer to the component of quality assurance that constitutes the assessment or analytical phase of testing. It includes the repeated measurement

of specially prepared control blood materials on the same haematology analysers that are used to test patient samples. It also includes day by day monitoring of these measurements to ensure that the values obtained are within predetermined limits. This process is referred to as 'internal quality control' as it constitutes a continuous self-evaluation of the reliability of the results generated by the laboratory before reports are issued.

An additional quality control activity is the participation in external quality assessment schemes (EQAS). This process is referred to as 'external quality control'. The details of this are beyond the scope of this edition.

Why is quality control important?

It is of critical importance that doctors and other healthcare workers are able to confidently rely on laboratory test results in order to make meaningful and safe decisions about the diagnosis and treatment of patients entrusted into their care. If results are produced in a quality controlled manner then a doctor can safely assume that any deviation from normal or any change from a previous result is solely due to the patient's clinical condition and not due to any technical issue within the laboratory.

1) Preventative (activities performed prior to specimen testing)	2) Assessment (specimen analysis)	3) Corrective action
<ul style="list-style-type: none"> ■ Verification of sample quality ■ Establish readiness of analytical system <ul style="list-style-type: none"> a) Calibration b) Staff training c) Instrument maintenance 	<ul style="list-style-type: none"> ■ Run internal quality control material ■ Monitor performance ■ Run external quality control material 	<ul style="list-style-type: none"> ■ Troubleshooting

Tab. 1 The phases and major activities of a typical laboratory quality assurance process

The role of the Complete Blood Count in clinical decision-making

In order for doctors to ascertain what is wrong with a patient and decide on what treatment to initiate, they go through a process of asking a series of questions (taking a history) and then performing a clinical examination. Invariably however, further investigations are usually required before a diagnosis can be made with certainty. As science and technology progresses, the list of possible investigations gets longer and longer, however, laboratory tests continue to make up 70% of investigations that doctors rely on to make diagnoses. Of all laboratory investigations the complete blood count (CBC) is still one of the most commonly requested laboratory tests. The CBC is thus at the core of almost all clinical management decisions. In this context, it is of paramount importance that stringent CBC quality control procedures are consistently adhered to and that any deviations are investigated and rectified immediately. The value of a CBC is only as good as its quality control.

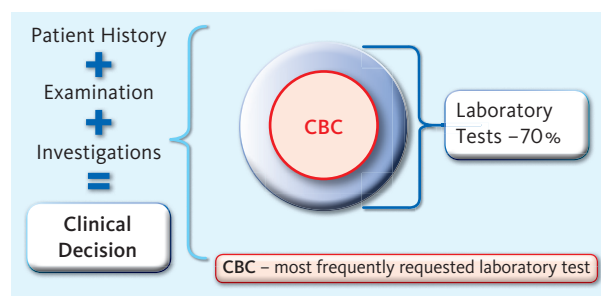


Fig. 1 CBC – one of the most frequently requested laboratory tests

What factors can influence the results?

It is important to be aware of the fact that there are several pre-analytical factors that can give rise to erroneous test results as these factors are sample specific and will not be detected by the standard internal quality control procedures that will be described later in this text. The main points to consider here are the fill volume of the collection tube and the time delay that has taken place between phlebotomy

and actual analysis. Blood for haematological tests is collected into tubes that are prefilled with the anticoagulant EDTA. Under-filling of tubes and old samples both tend to give erroneous results that follow a similar pattern. As the quality control material that is designed for use on specific automated haematology analysers is supplied ready to use any such pre-analytical variables cannot be detected through routine quality control measures. The laboratory must institute a standard operating procedure which requires the fill volume and time of collection to be assessed prior to analysis, and where necessary, patient samples may need to be rejected. The temptation to process such specimens in the belief that the laboratory staff are being kind by sparing doctors the additional work and patients the discomfort of repeating blood sampling must be avoided at all costs as the processing of inadequate specimens may in actual fact be harmful as test results are no longer reliable.

Establishing the readiness of the haematology analyser

In order to ensure that the haematology analyser is always in a state of readiness to perform patient sample analysis, certain core activities need to be adhered to:

- a. Calibration:** The great advantage of Sysmex haematology analysers is that no end-user calibration is required. It is the duty of the Sysmex technical representative to ensure that the analyser is correctly calibrated at installation and that this is checked after every breakdown service intervention.
- b. Staff training:** Staff must be properly trained on the basic operations of the haematology analyser.
- c. Maintenance:** All maintenance must be conducted at regular times as prescribed in the official instrument operator manual or as advised by an official Sysmex representative.

What is meant by an ‘analytical system’?

The analytical system is defined as the Sysmex haematology analyser plus Sysmex reagents. Quality control samples with known values are used to test the analytical system. The purpose of running quality control samples is to check the reliability of performance of the haematology analytical system. It therefore follows that only Sysmex quality control blood designed for this purpose should be used. The complete Sysmex analytical package is thus comprised of the Sysmex analyser, Sysmex reagents, Sysmex quality control bloods and Sysmex certified service support.





Internal quality control using Sysmex quality control bloods

The primary purpose of quality control is to detect any systematic errors within the analytical system that may cause a wrong patient result to be issued, and consequently a wrong clinical action to be taken. To ensure reliability of results, continuous monitoring of the analyser is an absolute requirement. In order to do this effectively, the performance of quality control using Sysmex quality control bloods specific for each class of analyser must be incorporated into daily routine practice. The Sysmex control bloods listed in Tab. 2 have been specifically designed for each corresponding instrument in order to thoroughly check the reagent system and technical system as it pertains to each particular instrument model.

Why third party reagents and control material cannot be used as substitutes for Sysmex control bloods

It is of paramount importance to strictly adhere to the concept of an analytical system as the technology of measurement is designed and validated based on the combination of Sysmex hardware and Sysmex reagents. Although all haematology analysers will generate the same basic results, the technology used to perform the measurement may differ substantially, e.g. Sysmex X-Class analysers utilise fluorescence flow cytometry to perform a differential count whereas all competitor systems do not. If a third party quality control is used on an X-Class analyser it is highly likely that the differential count may not be reliably measured as the material would not have been designed and validated

for this detection principle. Furthermore, haematology analysis involves the measurement of live blood cells (in contrast to chemistry which primarily involves the measurement of inert chemicals). Normal blood cells have a limited lifespan in vivo; red blood cells ~ 120 days, platelets ~ 7–10 days and white blood cells ~ 36 hours although memory lymphocytes may last for several years. However once removed from the body, blood cells will disintegrate very rapidly hence the need to test patient samples within hours after collection. Blood cells within quality control material are therefore stabilised to prevent disintegration with time. Not all cells can however be stabilised without unacceptable loss of function and therefore sometimes artificial substitutes are used. The compatibility of such alternate substitutes will vary from analyser to analyser depending on the technology used by each system. There will be a good chance that results may not be comparable if third party materials are used. Moreover, if target values are supplied with third party materials there is no guarantee that the results have been thoroughly validated on each specific analyser model for each lot number. It should also be noted that the use of third party reagents on a Sysmex analyser invalidates the manufacturer’s performance claims. What this means is that the laboratory will carry full liability in the event of any medico-legal claim arising from an erroneous test result having been issued.

	Control Blood	Analyser
	XN CHECK	XN-Series analysers
	e-CHECK (XE)	XE-, XT- and XS-Series analysers
	e-CHECK (XS)	XS-Series analysers
	EIGHTCHECK-3WP	XP-300, pocH-100i and K-Series analysers

Tab. 2 Sysmex quality control bloods

Sysmex quality control blood

As indicated in Tab. 2, Sysmex quality control material is specific to the class of analyser. Three levels of control, namely low (level 1 – red top), normal (level 2 – white top) and high (level 3 – black top) are produced per lot number. The use of all three levels is recommended to ensure that the performance of the analyser is validated across the range of expected patient results. Sysmex quality control bloods are supplied together with assay data sheets which indicate the assay mean value and upper and lower limits which determine the assay ranges for each parameter (Fig. 2). The assay mean values are established independently for each lot number produced and the assay range is calculated using the limit (%) values that have been predetermined by the R&D Division of Sysmex Corporation Japan based on the results of replicate measurements performed on multiple standard analysers and for multiple control blood lots. The limit (%) values are specific for the control blood, analyser type, concentration level, measurement mode and parameter. These values are used consistently for the calculation of assay ranges for all lots of a specific control blood. The assay data is supplied as a specific data sheet which is included in the clam shell packaging and/or electronically on a CD-ROM. The benefit of using the electronic data is that it eliminates any transcription errors when uploading new quality control lot numbers onto an analyser.

Which quality control data indicates satisfactory analyser performance?

The assay ranges (upper and lower limit) represent the interval of acceptable values. Individual QC results that are consistently located in a stable pattern between the upper and lower limits are indicative of satisfactory performance of the analyser. A stable pattern refers to the absence of trends or shifts of data and that the daily variation between individual QC measurements is low. It is a common misconception that QC results must be located on or around the assay mean. This is not a prerequisite as the assay mean is a reference only and should not be interpreted as the 'true value'. QC data that is consistently either above or below the assay mean, but within the target range is judged as good QC performance. The QC data for each parameter is automatically charted on the analyser per lot number, mode and level.

Any sporadic outliers, shifts or trends* should be investigated in accordance with the analyser operator manual, QC package insert, end-user training received and the laboratories own troubleshooting standard operating procedures. In the event of uncertainty please contact your local Sysmex representative. The details of this are beyond the scope of this newsletter.

<div> <div>CE</div> <div>EIGHTCHECK-3WP Assay Sheet</div> </div>												
Low Level												
		LOT		3095		0821						
								13-July-2013				
								Temperature during Assay				
								25°C				
Model		WBC	RBC	HGB		HCT	MCV	MCH		MCHC		PLT
		10e3/ μ l	10e6/ μ l	g/dl	mmol/l	%	fl	pg	fmol	g/dl	mmol/l	10e3/ μ l
KX-21 XP-Series ¹⁾	Range	3.5	2.45	6.7	4.2	19.1	80.0	29.2	1809	39.3	24.4	70
		2.7	2.21	6.1	3.8	16.1	71.0	25.8	1605	33.5	20.8	38
	Mean	3.1	2.33	6.4	4.0	17.6	75.5	27.5	1707	36.4	22.6	54
	Limit %	12.0	5.0	5.0	5.0	8.5	6.0	6.0	6.0	8.0	8.0	30
	Limit #	0.4	0.12	0.3	0.2	1.5	4.5	1.7	102	2.9	1.8	16

¹⁾ MCH value in amol and not in fmol

Fig. 2 Example of a Sysmex control blood assay data sheet.

*For a few parameters a slight trend over the life time of the control blood is expected. This applies mainly for the parameters that are influenced by the cell volume of the red blood cells. It is a natural process that red blood cells in blood samples swell over time and this also occurs in control bloods although to a much smaller extent because the cells in control bloods are stabilised. Therefore the haematocrit (HCT) and the mean cell volume (MCV) very slightly increase over the life time of the control blood while the mean corpuscular haemoglobin concentration (MCHC) decreases very slightly. As this effect is influenced by the temperature a proper storage of the control blood according to the package insert is important to minimise its extent.

When should internal quality control be performed?

When and for how many levels internal QC should be performed may be subject to national regulations. The following gives some general recommendations. In order to cover the full clinical spectrum of patient samples that may be encountered it is recommended to process all three levels of QC material on each haematology analyser. If this is not applied at least two levels of QC material should be used. The frequency of QC measurements may vary between once per day (e.g. for one working shift of 8 hours per day) to two or three times per day (e.g. for 24 hours operation). If an analyser has both an open and a closed mode (e.g. XT- and XE-Series analysers) and both modes are used to test patient samples QC must be performed on both modes. Whenever any intervention has occurred on the analytical system such as a service intervention, or when a technical problem is suspected and after it has been rectified, additional QC measurements are required.

Expiry dates and open vial stability of Sysmex quality control bloods

It is important to adhere to the expiry date and open vial stability of Sysmex quality control bloods. The expiry date of each lot number is clearly printed on each vial and on each assay data sheet. Although lifespan of the blood cells within the QC material has been extended through stabilisation, this is not indefinite and therefore the material can no longer be guaranteed to perform predictably beyond the expiry date. Likewise, it is important to understand the concept of open vial stability. Once a QC vial has been pierced by the analyser needle or has been decapped and exposed to air, the material starts to slowly deteriorate and performance is only guaranteed if the open vial stability is adhered to. Open vial stability is 7 days for XN Check, e-Check (XE) and Eightcheck-3WP and 14 days for e-Check (XS).

Handling of Sysmex quality control bloods

It is important to handle Sysmex quality control bloods in accordance with the QC package insert and the end-user training received because incorrect handling can lead to erroneous QC results and impairs the quality of your internal QC. After taking the QC vial out of the refrigerator the first step is to bring it to room temperature by letting it stand for the

time specified in the package insert. The cold QC vial must not be warmed, e.g. within the hands, because the blood cells in the QC material can be damaged if they are exposed to a high temperature difference. The second step is to mix the QC vial according to the procedure specified for the particular control blood product by end-to-end inversion or by rolling between the palms until all cells are resuspended. When first opening a new vial it is particularly important to ensure sufficient mixing to resuspend all cells. It is important to understand that the stabilisation of the blood cells within the QC material influences their properties and therefore the QC material needs to be mixed in a different way than patient samples and must not be mixed using automatic mixers.

What are the consequences of not performing appropriate internal quality control on haematology analysers?

The consequences of choosing not to run QC at all, or by using third party products, or by not rectifying QC errors that occur are serious as it means that the laboratory will have no means of ensuring that patient results produced during this time are accurate. The patient results may still be accurate but on the other hand they may not be. In the absence of an objective QC check with rigorous attention to troubleshoot and rectify any errors, we can not be sure. Laboratory professionals have an ethical obligation to ensure that every laboratory result issued has been produced under rigorous quality control conditions. Doctors consequently are justified in their expectation that all laboratory results that have been authorised and released by the laboratory are true. They in turn have an ethical obligation to treat their patients taking all available information under consideration. The laboratory, and not the doctor, will therefore be liable in the event that any deleterious consequences follow on a clinical decision that was taken in good faith based on an incorrect laboratory result that was duly authorised by the laboratory. As it is understandably difficult for laboratory staff to feel the same connection to patients as there is little of not direct interaction, this message is best illustrated through some clinical case studies.

a) Clinical case study 1: a 4 year old child with acute leukaemia, currently receiving a cycle of chemotherapy every 3 weeks

- CBC+DIFF reveals NEUT# of $0.8 \times 10^9/L$
- Clinical decision – withhold chemotherapy until NEUT# rises to above $1 \times 10^9/L$
- BUT True NEUT# was $1.2 \times 10^9/L$

The consequence is that by unnecessarily delaying chemotherapy the chances of remission and possible cure for the child are significantly reduced.

b) Clinical case study 2: female patient with autoimmune haemolytic anaemia

- CBC reveals a HGB of 8 g/dL
- Clinical decision – blood transfusion not indicated
- BUT True HGB is actually 6 g/dL

Consequence of erroneously withholding blood transfusion is that the patient may become seriously compromised and develop multi-organ failure needing intensive care unit admission at major cost!

c) Clinical case study 3: a 2 year old child with mild fever and earache

- CBC+DIFF reveals a normal WBC and normal NEUT#
- Clinical decision – infection probably viral, send child home
- BUT True WBC and NEUT# is actually elevated suggesting bacterial infection

Consequence is that antibiotics are erroneously withheld. The risk is that an untreated bacterial ear infection in a young child can rapidly spread and become meningitis which in turn carries a high risk of possible brain damage or even death.

Take home message

Regular processing of internal quality control using the Sysmex quality control bloods that are appropriate for each specific Sysmex analyser and conscientious monitoring of the performance of each parameter, on all modes (if appropriate) using all levels (or at least 2 of the 3) is an absolute non-negotiable requirement for any laboratory to be able to issue results in order to enable doctors to make meaningful and safe clinical decisions for all patients entrusted into their care.