Basic Haematology
The purpose of this newsletter is to provide an overview of the critical importance of quality assurance in full blood count analysis.

Key words:
FBC, Quality Control, e-Check (XE), e-Check (XS), Eightcheck-3WP

What is accreditation?
Accreditation is the process whereby an independent external body certifies that a laboratory is operating in accordance with a particular set of standards. The purpose of this is to provide an independent stamp of approval which assures users of a particular laboratory that the test results that are being issued have been generated in a quality controlled environment and can therefore be trusted.

Why the focus on accreditation?
There is growing recognition that laboratories play a pivotal role in healthcare. In line with this, donor organisations have realised that the full benefit of investment into healthcare in Africa can only be realised if the laboratory results are of a good quality. In this regard major emphasis is being placed on the strengthening of laboratory networks. As a laboratory has to demonstrate that it is operating in accordance with “Good Laboratory Practice” principles, which in turn require the laboratory to implement a quality assurance programme in order to be accredited, the goal of accreditation has been selected by donor organisations, the WHO and Ministries of Health to achieve wide scale healthcare improvements in Africa.

What is quality assurance?
The laboratory plays a pivotal 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 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 table 1.

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

<table>
<thead>
<tr>
<th>1) Preventative (activities performed prior to specimen testing)</th>
<th>2) Assessment (specimen analysis)</th>
<th>3) Corrective action</th>
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<td>c) Staff training</td>
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Quality control in full blood count analysis – for the sake of the patient.
What is 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 often 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.

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 for them 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.

The role of the Full 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 full blood count (FBC) is still the single most commonly requested laboratory test. The FBC is thus at the core of almost all clinical management decisions. In this context, it is of paramount importance that stringent FBC quality control procedures are consistently adhered to and that any deviations are investigated and rectified immediately. The value of an FBC is only as good as its quality control.

What factors can influence the FBC result?
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 on 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 FBC analysis is collected into tubes that are prefilled with the anticoagulant EDTA. Underfilling 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 preventative or 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 table 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, FBC 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. Not only will there be a good chance that results may not be comparable but also the third party material may not be sold together with target values that are specific for Sysmex analysers. Moreover, if target values are indeed supplied 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.

**Table 2**  Sysmex quality control bloods

<table>
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<th>Control Blood</th>
<th>Analyser</th>
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<tr>
<td>e-CHECK (XE)</td>
<td>XE, XT and XS analysers</td>
</tr>
<tr>
<td>e-CHECK (XS)</td>
<td>XS analysers</td>
</tr>
<tr>
<td>EIGHTCHECK-3WP</td>
<td>KX-21N, PocH-100i and K series analysers</td>
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Sysmex quality control blood
As indicated in table 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 (see figure 2). The mean assay 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. 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 both as a specific data sheet which is included in the clamshell packaging as well as electronically on a CD-ROM/floppy disk. 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, trends or shifts should be investigated in accordance with the analyser operator manual, QC package insert, end-user training received and the laboratory’s 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.

**Figure 2** Example of a Sysmex control blood assay data sheet.
When should internal quality control be performed?
All three levels of QC material should be processed on each haematology analyser, failing which at least two different levels should be performed at least once per working shift in accordance with international guidelines. If the latter approach is adopted then it is recommended to process the normal level each time and to alternate between the low and high levels as far as possible in order to cover the full clinical spectrum of patient samples that may be encountered. If an analyser has both an open and a closed mode (e.g. XT and XE analysers) QC must be performed on both modes. Whenever any intervention has occurred on the analytical system such as a service intervention or change of reagents, 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. As a safeguard, the analyser will warn the user that the QC material has expired, provided that the QC material is subject to the analyser’s barcode reader. If a QC sample is manually entered, this will not occur. 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 Eightcheck-3WP and e-Check (XE) and 14 days for e-Check (XS). The only safeguard here is for the staff to be vigilant by signing and dating each vial as it is opened.

Why is it necessary to order Sysmex control bloods well in advance?
As previously alluded to, FBC controls have to fulfil the criteria of being stable enough to be used repeatedly over a prolonged period of time but still retain the biological structural and functional characteristics that the various detection channels of each specific analyser depend on for accurate measurement of individual parameters. By their very nature quality control bloods for use on automated haematology systems therefore have very tight fixed expiry periods. In order to guarantee the required high level of stable performance and an adequate supply to all customers, the quantity of material produced is determined by actual confirmed customer orders. As each new batch (lot number) has to go through several rigorous controls to ensure quality and the assignment of control values whilst still having sufficient residual shelf life to accommodate shipping logistics and still be practical for the customer to use for an extended period of time, no ad hoc orders can be accommodated. As patient care depends on the conscientious performance of QC one has to ensure an uninterrupted supply of sufficient quantities of Sysmex control bloods. Therefore customers should as far as possible place standing orders for 12 months supply in advance. The absolute minimum lead time that can be accommodated is 3 months in advance if ordering individually per QC lot number cycle. The QC lot number ordering cut-off dates are published 12 months in advance to facilitate planning. Sysmex cannot guarantee, for reasons stated above, that any order received after the cut-off date will be accommodated.

What are the consequences of not performing appropriate internal quality control on haematology analysers?
The consequences of failing to meet the cut-off date for QC ordering, or 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 authorized 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
   - FBC reveals NEUT# of “0.8 x 10^9/L”
   - Clinical decision – withhold chemotherapy until NEUT# rises to above 1 x 10^9/L
   - BUT TRUE NEUT# was 1.2 x 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
   - FBC reveals a HGB of “8g/dl”
   - Clinical decision – blood transfusion not indicated
   - BUT True HGB is actually 6g/dl
   - Consequence of erroneously withholding blood transfusion is that the patient may become seriously compromised and develop multi-organ failure needing ICU admission at major cost!

c) Clinical case study 3 - 2 year old child with mild fever and earache
   - FBC reveals a normal WCC and normal NEUT#.
   - Clinical decision – infection probably viral, send child home
   - BUT True WCC 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 the very least 2 of the 3) is an absolute non negotiable requirement for any laboratory to be able to issue FBC results in order to enable doctors to make meaningful and safe clinical decisions for all patients entrusted into their care.

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