Quality control in the hematology laboratory – for the sake of the patient

Dr Marion Münster | Manager Medical & Scientific Affairs | Sysmex South Africa
Most frequently requested lab test
Usually delegated to least experienced staff
Fully automated but…..

RESULTS ONLY AS GOOD AS THE QUALITY CONTROL

QC management is therefore of paramount importance
Overview

• Basic Concepts
• What is QA?
• Pre-analytical factors
• QC System
• Sysmex QC Material
• Internal Quality Control
• EQA
• Normal Reference Ranges
• Clinically Relevant Decision Levels
• Clinical Case Studies
• Sysmex quality guidance manual
Basic Concepts – Role of the laboratory

Pivotal in medical practice as test results have major influence of clinical diagnosis and patient management

Laboratory has **ethical obligation** to produce reliable and reproducible test results

Provide clinicians with unambiguous meaningful reports that are relevant to clinical problem being investigated

Train and advance knowledge of lab staff and ensure their safety

Achieved through process of **TOTAL QUALITY MANAGEMENT**
Quality Cycle
Purpose of Quality Assurance

“to ensure reliability of test results to inform meaningful and safe medical decision-making”
Quality Management Cycle

Post-analytical

Pre-analytical

Analytical

Patient/Doctor

Sample collection

Laboratory Report

Sample Analysis

Transport
Process Flow and Quality Checks

Anticoagulant, Posture, tourniquet

Specimen Collection
Specimen Transport
Test Request ordered

Analytical process

Correct technology, Instrument Calibration

Quality Control EQA

Data Processing
Record Keeping
Laboratory Report

Transport to Clinician

Relevant Reference ranges
QA – Basic Concepts

Analytical

Post-Analytical

Pre-Analytical

Analyser Maintenance/Calibration

IQC/EQA

Corrective Action

Monitor

Lab Test

Train Staff
Preanalytical Variables

- Prolonged use of tourniquet during phlebotomy
  - Haemoconcentration impacts on quantitative measures

- Choice of anticoagulant
  - $K_2$EDTA or $K_3$EDTA?
  - $K_3$EDTA causes some shrinkage of RBCs with a reduction in 1-2% of MCV
  - $K_2$EDTA caused minimal change in fresh samples

- Underfilling of blood collection tube – excess EDTA
  - Initially results in cell shrinkage and degeneration, followed by swelling of RBCs and PLTs
  - PLTs swell, MPV goes up, then PLTs disintegrate into smaller PLT particles, so PLT count rises and MPV falls.

- Delay in analysis
  - Similar effects to tube under-filling
Most values stable for 24 hours if kept at 4°C
The longer the delay and the warmer the T° the greater the change

- **MCV & HCT**: cells start to swell so the MCV and HCT increase.
- **WBC & Platelets**: it is best to analyse within 2 hours. WCC may fall sharply after 2 hours if the tube is under-filled.
- **Reticulocytes**: counts drop after 6 hours.
- **Nucleated RBCs**: these disintegrate at room temperature after 1 to 2 days.
- **Hb**: This is a very stable parameter and remains unchanged for 2-3 days. The higher the temperature, the greater the risk of cell lysis and hence the chance that the RCC and HCT will drop, with a rise in MCH and MCHC
Qualitative Changes with delays in specimen analysis

- Most values stable for 24 hours if kept at 4°C
  - The longer the delay and the warmer the T° the greater the change
- Smear should be made as fresh as possible
- Morphological appearance of “old samples” – microscopic view
  - WBC lose nuclear detail
  - Monocytes become vacuolated – appear “activated”
  - Lymphocyte nuclei – bud
  - Hard to separate Monocytes and Lymphocytes
  - RBCs – become crenated – false diagnosis of “renal disease”
  - RBCs – become spherocytic – false diagnosis of “HS, burns, haemolysis”
- Automated differential more stable as differentiation not based on cell size.

  **SYSMEX ONLY!**
QA – major activities

Preventative
» Activities performed prior to specimen testing
» Establish readiness of analytical system
  – Instrument maintenance
  – Calibration
  – Staff training etc

Assessment
» Run QC material
» Monitor performance

Corrective Action
» troubleshooting
QA – Analytical

QA programme is comprised of

» Standardisation
  – Reference Materials and methods
  – Calibrations

» Internal Quality Control

» External Quality Assessment

Reliability assessed by measures of

» Accuracy – closeness of measured value to “truth”

» Precision – reproducibility of results

“Process Control” is cornerstone of any total quality assurance programme
“Quality Control” – assessment of Analytical Phase

- Checking the reliability of performance of Haematology Analytical System
- Analytical System = Sysmex analyser plus Sysmex reagents
  - Technology of measurement designed and validated based on combination of Sysmex hardware and Sysmex reagents
  - The use of third party reagents on a Sysmex Analyser invalidates manufacturer’s performance claims
- Quality Control Samples with known values are used to test analytical system
- Complete Sysmex Analytical Package = Sysmex analyser, Sysmex reagents, Sysmex Quality Control bloods and Sysmex certified service support.
How does one monitor the performance of Sysmex haematology analysers?

- Internal Quality Control using Sysmex analyser specific control bloods
- External Quality Assurance Schemes
- X-bar M control to automatically monitor performance of instruments using patient samples
- Calibration verification of CBC parameters and sensitivity adjustments using fresh normal human blood samples.
- Only on specific request or need: precision check using human blood samples
Monitor the **accuracy** and **precision** of the analyser.
Internal Quality Control using Sysmex Control Bloods

- Purpose of QC is to detect a **systematic error** that may cause normal patient sample to appear abnormal or an abnormality to remain undetected.

- To ensure reliability of FBC results, the stability of performance of the analyser needs to be constantly monitored.

- To efficiently monitor performance of a Sysmex haematology analyser, Sysmex control bloods specific to the class of analyser need to be used on a daily basis.
Sysmex Control Bloods

- EIGHTCHECK-3WP
- e-CHECK (XS)
- e-CHECK (XE)
- XN CHECK

- The control bloods have been specifically designed for each corresponding instrument in order to thoroughly check the reagent system and the technical function of the specific model of analyser.
- Level 1 (Abnormal Low)
- Level 2 (Normal)
- Level 3 (Abnormal High)
Haematology Quality Control Blood - challenges

• FBC Analysis involves the measurement of live blood cells (in contrast to chemistry - inert chemicals)

• Normal Blood cells have a limited lifespan in vivo
  • Red Blood Cells - ~ 120 days
  • Platelets - ~ 7-10 days
  • White Blood Cells - ~ 36 hours although memory lymphocytes ~ years

• Ex vivo, blood cells disintegrate within hours.

• Quality Control Blood must provide stable results for all measured parameters over prolonged period of time

• Blood cells need to be stabilised to prevent disintegration with time but not all cells can be stabilised without unacceptable loss of function.
  • Alternate substitutes
Quality Control Material production

- QC Bloods are made to order
- Products have a tight fixed expiry.
- Cannot order on an ad hoc basis
- Must place standing order for 12 months supply in advance as production is carefully planned based on actual orders
- At very least, need to order 3 months in advance if ordering individually per QC lot# number cycle
Control Bloods supplied together with assay data

Mean and assay ranges

<table>
<thead>
<tr>
<th>RBC (10^6/L)</th>
<th>HGB (g/dL)</th>
<th>HCT (%)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>MCV (fL)</th>
<th>MCV CV</th>
<th>HCT CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>4.07 - 4.11</td>
<td>13.2 - 12.8</td>
<td>41.2 - 39.4</td>
<td>30.8 - 30.4</td>
<td>78.7 - 75.9</td>
<td>27.9 - 17.4</td>
<td>1.4 - 0.9</td>
</tr>
<tr>
<td>Max.</td>
<td>4.11</td>
<td>12.8</td>
<td>41.2</td>
<td>30.4</td>
<td>75.9</td>
<td>17.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Min.</td>
<td>4.07</td>
<td>13.2</td>
<td>39.4</td>
<td>30.8</td>
<td>78.7</td>
<td>17.4</td>
<td>0.9</td>
</tr>
<tr>
<td>% CV</td>
<td>1.4</td>
<td>0.9</td>
<td>1.4</td>
<td>0.9</td>
<td>1.4</td>
<td>0.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Level A</td>
<td>4.07</td>
<td>13.2</td>
<td>39.4</td>
<td>30.8</td>
<td>78.7</td>
<td>17.4</td>
<td>0.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RDW-SC (%)</th>
<th>RDW-CV (%)</th>
<th>PLT (10^9/L)</th>
<th>MPV (fL)</th>
<th>P-LCR %</th>
<th>PCT (%)</th>
<th>WBC-C (10^9/L)</th>
<th>WBC-D (10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>51.1 - 37.7</td>
<td>20.0 - 23.7</td>
<td>12.4 - 8.8</td>
<td>23.9 - 7.9</td>
<td>6.27 - 5.82</td>
<td>7.30 - 7.42</td>
<td></td>
</tr>
<tr>
<td>Max.</td>
<td>51.1</td>
<td>23.7</td>
<td>12.4</td>
<td>23.9</td>
<td>6.27</td>
<td>7.30</td>
<td></td>
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<tr>
<td>Min.</td>
<td>37.7</td>
<td>20.0</td>
<td>8.8</td>
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<td>5.82</td>
<td>7.42</td>
<td></td>
</tr>
<tr>
<td>% CV</td>
<td>1.4</td>
<td>1.4</td>
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<td>23.9</td>
<td>6.27</td>
<td>7.30</td>
<td>7.42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NEUT (10^6/L)</th>
<th>LYM (10^6/L)</th>
<th>MONO (10^6/L)</th>
<th>EO (10^6/L)</th>
<th>BASO (10^6/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>3.70 - 2.02</td>
<td>1.55 - 0.66</td>
<td>0.25 - 0.09</td>
<td>0.03 - 0.00</td>
</tr>
<tr>
<td>Max.</td>
<td>3.70</td>
<td>1.55</td>
<td>0.25</td>
<td>0.03</td>
</tr>
<tr>
<td>Min.</td>
<td>2.02</td>
<td>0.66</td>
<td>0.09</td>
<td>0.00</td>
</tr>
<tr>
<td>% CV</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
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</tr>
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<td>3.70</td>
<td>1.55</td>
<td>0.25</td>
<td>0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DIFF-X (data)</th>
<th>DIFF-Y (data)</th>
<th>PDG-X (data)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>182.0 - 150.0</td>
<td>95.9 - 41.0</td>
</tr>
<tr>
<td>Max.</td>
<td>182.0</td>
<td>95.9</td>
</tr>
<tr>
<td>Min.</td>
<td>150.0</td>
<td>41.0</td>
</tr>
<tr>
<td>% CV</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Level A</td>
<td>182.0</td>
<td>95.9</td>
</tr>
</tbody>
</table>
What must we observe about Sysmex Control Blood?

- Control bloods go hand in hand with specific analysers
- Assay values are LOT Number specific
- 3 levels
- Cold Chain!
- Proper mixing
- Observe open vial stability – label tubes (7 days – 14 days)
- Observe expiry dates – QC Cycle (8 weeks or 12 weeks)

E-Check (XS)

Eightcheck – 3WP
When should internal Quality Control bloods be run?

• It is recommended that all three levels (L1, L2, L3) are analysed at least once per working shift
  • 24 Hour laboratory – at least 2 x per day
  • Day shift only – once per day

• After any service intervention, recalibration etc

• When any technical problem is suspected, and after it has been remedied.

• Open and closed (if available) mode QC must be performed
### How much QC blood is required?

#### Sample aspiration volumes – e-Check (XE) Vial Size 4.5ml:

<table>
<thead>
<tr>
<th></th>
<th>Open Mode</th>
<th>Closed Mode</th>
<th>Vol/week</th>
</tr>
</thead>
<tbody>
<tr>
<td>XT</td>
<td>85 µl</td>
<td>150 µl</td>
<td>1,645 ml</td>
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</table>

#### Sample aspiration volumes – e-Check (XS) Vial Size 1.5ml:

<table>
<thead>
<tr>
<th></th>
<th>Open Mode</th>
<th>Closed Mode</th>
<th>Vol/2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>XS</td>
<td>20 µl</td>
<td>20 µl</td>
<td>0.52 ml</td>
</tr>
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</table>

#### Sample aspiration volumes – Eightcheck 3WP Vial Size 1.5ml:

<table>
<thead>
<tr>
<th></th>
<th>Open Mode</th>
<th>Vol/week</th>
</tr>
</thead>
<tbody>
<tr>
<td>POCHi</td>
<td>15 µl</td>
<td>0.105 ml</td>
</tr>
<tr>
<td>KX21N/XP-300</td>
<td>50 µl</td>
<td>0.320 ml</td>
</tr>
</tbody>
</table>

#### Sample aspiration volumes – XN CHECK Vial; Size 3.0ml

<table>
<thead>
<tr>
<th></th>
<th>Open Mode</th>
<th>Vol/week</th>
</tr>
</thead>
<tbody>
<tr>
<td>XN series</td>
<td>88 µl</td>
<td>0.105 ml</td>
</tr>
<tr>
<td>XN-L series</td>
<td>25 µl</td>
<td>0.320 ml</td>
</tr>
</tbody>
</table>

In keeping with open vial stability
How do we monitor IQC performance

• Need to track QC results for each parameter
• QC data records are stored on the analyser
• QC file – stored per Lot #, level and mode of measurement
• Values should run within assay limits – these are graphically displayed when QC is uploaded (X-Class and XN )
  • Upper limits
  • Mean
  • Lower Limit
• XBar Mean plots
Analytical problems are highlighted as follows:

» Within assay values? Y or N?
» Trends? Significant?
» Shifts? Significant?
Daily QC Plot

Haemoglobin (g/dl)  e-Check XE
Control Level 1

Day

UL 6

LL 5.6

Haemoglobin (g/dl) e-Check XE
Control Level 1

Trend
Haemoglobin (g/dl) e-Check XE
Control Level 1

Shift
Troubleshooting

- Procedure will depend on findings
- Is it just one parameter?
- Several related parameters?
- All parameters?
- One Level only or more than 1?
- Open mode or closed mode? Or both?

Detailed in Applications training Courses
External Quality Assessment Schemes

- Analysis of blinded sample at predetermined intervals
- Samples are sent to multiple laboratories and then results compared against each other – “truth” determined by peer group.
- Results sent back to organisers within specified deadline
- Report sent back to laboratory
- SPOT CHECK!!
SNCS – Sysmex Network Communication System

• SNCS IQAS Online

XP-300
X Class
XN/XN-L

Internal QC

External QC

• Daily QC measurement of Sysmex Control Blood
Dear Customer,

This is an automatic IQAS ONLINE notification mail.
Some suspect error(s) has/have been detected for Intraday statistics when comparing the individual data displayed in the column "Your data" to the group mean (peer group: ALL / at judgement):

<table>
<thead>
<tr>
<th>Model</th>
<th>Serial number</th>
<th>Error occurrence date and time</th>
<th>Measurement time</th>
<th>Measurement number</th>
<th>Control material Lot</th>
<th>Level</th>
<th>Measurement mode</th>
<th>Parameter</th>
<th>Your data</th>
<th>Group mean</th>
<th>Error code</th>
</tr>
</thead>
<tbody>
<tr>
<td>XT-4000i</td>
<td>11296</td>
<td>05.06.2015 08:12:53</td>
<td>09:57:41</td>
<td>1</td>
<td>e-CHECK(XE)</td>
<td>6093</td>
<td>CLOSED</td>
<td>DIFF-Y</td>
<td>52.1</td>
<td>64.923</td>
<td>3SDI over</td>
</tr>
<tr>
<td>XT-4000i</td>
<td>11296</td>
<td>05.06.2015 08:13:11</td>
<td>09:58:27</td>
<td>1</td>
<td>e-CHECK(XE)</td>
<td>6093</td>
<td>CLOSED</td>
<td>DIFF-Y</td>
<td>46</td>
<td>59.76</td>
<td>3SDI over</td>
</tr>
<tr>
<td>XT-4000i</td>
<td>11296</td>
<td>05.06.2015 08:13:12</td>
<td>09:59:12</td>
<td>1</td>
<td>e-CHECK(XE)</td>
<td>6093</td>
<td>CLOSED</td>
<td>DIFF-Y</td>
<td>44.1</td>
<td>56.775</td>
<td>3SDI over</td>
</tr>
</tbody>
</table>

Please visit http://www.sysmex-europe.com/sncs and check the details of the detected error(s) for your instrument.

For data review please select the peer group "ALL / at judgement" and data type "Intraday".

If you need further advice, please contact your Sysmex service representative.

Sincerely,

Administrator
Sysmex Europe GmbH
### Online results look up

**Parameter: RBC | Condition 1: 0 | Condition 2: 01 | Model: JAE512 | Calibration: 69 | Temperature: 69 | Reagent**

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Parameter Unit</td>
<td>RBC</td>
<td>x10^9/L</td>
<td>RBC</td>
<td>x10^9/L</td>
<td>RBC</td>
<td>x10^9/L</td>
<td>RBC</td>
</tr>
<tr>
<td>Control Lot</td>
<td>#0019(L1)_CL</td>
<td>#0019(L1)_CL</td>
<td>#0019(L1)_CL</td>
<td>#0019(L1)_CL</td>
<td>#0019(L1)_CL</td>
<td>#0019(L1)_CL</td>
<td>#0019(L1)_CL</td>
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<tr>
<td>Your data</td>
<td>2.396</td>
<td>2.390</td>
<td>2.415</td>
<td>2.410</td>
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<tr>
<td>Group Mean</td>
<td>2.400</td>
<td>2.399</td>
<td>2.399</td>
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<tr>
<td>Your Cumulative Mean</td>
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<td>Your SD</td>
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<tr>
<td>Peer group Inter-SD</td>
<td></td>
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<td>Your SD</td>
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<tr>
<td>Your Cumulative SD</td>
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<td>Your PI</td>
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<tr>
<td>Peer group Intra-SD</td>
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<tr>
<td>Your N</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Group N</td>
<td></td>
<td></td>
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<tr>
<td>Judge</td>
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</tr>
</tbody>
</table>
Benefits of SNCS QC Module to labs

• Saves Time
• Automatic Charting
• Uniform high standard approach
• Real time monitoring
• Overseen by experts
• Alerts labs when QC errors require attention
• Expert assistance for troubleshooting provided
• Minimise down-time

Caveat - Requires proper IT infrastructure
## SNCS vs traditional EQA

<table>
<thead>
<tr>
<th>SNCS</th>
<th>Traditional EQA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily QC doubles up as EQA</td>
<td>Special samples - infrequently</td>
</tr>
<tr>
<td>All modes of analysis checked</td>
<td>Usually open mode only</td>
</tr>
<tr>
<td>Real time feedback</td>
<td>Feedback delayed – weeks - months</td>
</tr>
<tr>
<td>No matrix effect</td>
<td>Matrix effect or genuine outlier?</td>
</tr>
<tr>
<td>No transcription errors possible</td>
<td>Transcription errors/non-submission common cause of EQA Failure</td>
</tr>
<tr>
<td>No costs involved</td>
<td>Expensive</td>
</tr>
<tr>
<td>All parameters are monitored including service parameters</td>
<td>Only basic parameters are included</td>
</tr>
<tr>
<td>Error notification emails</td>
<td>No proactive alert system</td>
</tr>
<tr>
<td>ISO Accredited (17043)</td>
<td>May or may not be ISO accredited</td>
</tr>
</tbody>
</table>
How does a clinician judge a laboratory result?
What does it mean for an individual patient?
Compare to what is expected to be normal
Is result normal or abnormal?
If abnormal is it abnormally high or low?

Determine values for 40+ Normal People
(May need separate exercise for M and F)
Statistical evaluation
Range with a lower and upper limit
= NORMAL REFERENCE RANGE

Patient result judged in relationship to reference range
Influences clinical diagnosis and management
Clinical Decision Limits

Defined value which determines whether a patient is eligible for a particular treatment or intervention

Examples:

Platelet Count > 20 – withhold platelet transfusion
Neutrophil# Count <1 – withhold next round of chemotherapy

Accuracy of quantitative values has a direct impact on this
What happens when we either do not run QC bloods or use Non-Sysmex materials?
Clinical Case Studies - 1

- 4 year old child with 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 chemo.

Chances of remission and possible cure for child are significantly reduced
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

Patient becomes seriously compromised and develops multi-organ failure needing ICU admission – at major cost!
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

Risk that a young child with untreated bacterial ear infection can rapidly spread and become meningitis; high risk of brain damage and or death.
Clinical Cases Conclusion

• In all cases, if QC was processed daily, technical problems would be identified and wrong results would not be issued.

• 70% of clinical decisions rely on laboratory investigations, so accurate results are the cornerstone of GOOD MEDICAL PRACTICE and patient care
It’s all about the patient.....
Our commitment to quality

Our core focus has always been on the generation of reliable lab results through stringent adherence to good quality control principles.

We appreciate that high quality results can only be consistently achieved if "Good Laboratory Practice" becomes a "way of life".

Proven by Accreditation

In recognition that “accreditation” is easier said than done, Sysmex has created the “Sysmex Quality Guidance Manual”.

Committed to Quality. Committed to You.
The Sysmex Offering…

The Sysmex Quality Guidance Manual for Haematology Laboratory Services

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Thank you very much for your attention!