Traceability and Measurement Uncertainty of Hematology Analyzer

August 7, 2015
Scientific Affairs, Sysmex Corporation
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Contents

1. How to ensure a measured value is correct?
2. Traceability
3. Measurement Uncertainty
4. Data Assurance System in Sysmex
Contents

1. How to ensure a measured value is correct?
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Question

How do we ensure a measured value is correct?

WBC 6.88 \times 10^3 / \text{uL}
RBC 4.60 \times 10^6 / \text{uL}
HGB 13.5 \text{ g/dL}
HCT 37.6 %
PLT 24.3 \times 10^4 / \text{uL}

Reliable Data?

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What is expected of Clinical Laboratories

We have to get the same result whenever and wherever we measure a sample!

Assessment of Patients
- Medical Interview
  - In Vitro Diagnostics
- In Vivo Diagnostics
  - Radiology Services
  - Diagnostic Imaging Services

Care of Patients
- Care Delivery
- Recognition of Changes to Patient Condition
- Food and Nutrition Therapy
- Pain Management
- Anesthesia and Surgical Care
- Medication

Outcome
- End-of-Life Care
- Transfer of Patients
What Is Your Goal?

- **Precision:** ×
- **Trueness:** ×

- **Precision:** ○
- **Trueness:** ○

GOAL
For Data Assurance

Traceability and Measurement Uncertainty

Internal Quality Control
To check variance of intra-lab (between instruments or methods)

External Quality Assessment
To check bias from other labs

To confirm a measured value correct
Steps for getting a reliable measured value

**STEP 1. Verify Traceability**
- Calibration and estimating Measurement Uncertainty

**STEP 2. Internal Quality Control: IQC**
- Running QC material daily and check variance of a method in Lab.

**STEP 3. External Quality Assessment: EQA**
- Running EQA material and check bias from other labs
ISO 15189:2012

Pre-examination Process
• Sample collection manual
• Request form
• Primary sample collection and handling

Examination Process
• Standard Operating Procedure (SOP)
• Verification
  ➢ Traceability
  ➢ Measurement Uncertainty
• Quality Control
  ➢ IQC
  ➢ EQA
• Comparability

Post-examination Process
• Review of results
• Reporting of results
• Release of results

*ISO 15189:2012 Medical laboratories — Requirements for quality and competence
1. How to ensure a measured value is correct?
2. Traceability
3. Measurement Uncertainty
4. Data Assurance System in Sysmex
Before thinking about Traceability...

How do you prove a measured value correct?

Trueness : ×  
Trueness : ○

GOOD!
How do you get a measured value?

**Example**

- **Fresh Whole Blood**
- **ICSH Reference Method**
- **Primary Standard**
- **Manufacturer’s Standard Analyzer**
- **Value Assignment**
- **Calibration**
- **Hematology Analyzer in Laboratory**

**Traceability**

**Traceability is to show the basis of the measured value!**

- **Patient Sample**
- **RBC 4.502 *10^{12}/L**

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Term Definition

**Metrological Traceability**

“property of a measurement result whereby the result can be related to a reference through a **documented unbroken chain** of calibrations, each contributing to the measurement uncertainty”

[VIM 2012 (2.41)]

*International vocabulary of metrology*
- Basic and general concepts and associated terms, 3rd edition
Meaning of Establishing Traceability

When the source of Traceability is the same and establishing Traceability Chain by an appropriate way, a measured value is ensured universality and comparability.
ISO 17511:2003

* ISO 17511:2003 In vitro diagnostic medical devices
- Measurement of quantities in biological samples- Metrological traceability of values assigned to calibrators and control materials -

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>CALIBRATION VALUE ASSIGNMENT</th>
<th>PROCEDURE</th>
<th>IMPLEMENTATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a) definition of SI unit by CGPM</td>
<td></td>
<td>BiPM, NMI, ARML</td>
</tr>
<tr>
<td></td>
<td>c) primary calibrator</td>
<td>b) primary reference measurement procedure</td>
<td>BiPM, NMI</td>
</tr>
<tr>
<td></td>
<td>e) secondary calibrator</td>
<td>d) secondary reference measurement procedure</td>
<td>NMI, ARML</td>
</tr>
<tr>
<td></td>
<td>g) manufacturer’s working calibrator</td>
<td>f) manufacturer’s selected measurement procedure</td>
<td>NMI, ARML, ML</td>
</tr>
<tr>
<td></td>
<td>i) manufacturer’s product calibrator</td>
<td>h) manufacturer’s standing measurement procedure</td>
<td>ML</td>
</tr>
<tr>
<td></td>
<td>routine sample</td>
<td>i) end-user’s routine measurement procedure</td>
<td>ML</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>manufactured and/or end-user</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>end-user</td>
</tr>
</tbody>
</table>

RESULT
According to ISO 17511*, 6 typical upper ends of the metrological traceability chain can be identified:

<table>
<thead>
<tr>
<th>Quantities for which results of measurements are</th>
<th>Reference Measurement Procedure</th>
<th>Reference Materials</th>
<th>Test Items / examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Metrologically traceable to SI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Available</td>
<td>Available</td>
<td>25 – 30 items</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Electrolytes, metabolites, steroid hormones</td>
</tr>
<tr>
<td>b) NOT Metrologically traceable to SI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Available</td>
<td>Available</td>
<td>HbA1c, Hb</td>
</tr>
<tr>
<td>3</td>
<td>Available</td>
<td><strong>Not Available</strong></td>
<td><strong>About 30 items</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Haemostatic factors, <strong>WBC, RBC</strong></td>
</tr>
<tr>
<td>4</td>
<td><strong>Not Available</strong></td>
<td>Available</td>
<td>&gt; 300 items</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Protein hormones, some antibodies &amp; tumor markers (WHO’s Intl. Standards)</td>
</tr>
<tr>
<td>5</td>
<td><strong>Not Available</strong></td>
<td><strong>Not Available</strong></td>
<td><strong>About 300 items</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tumor markers and antibodies</td>
</tr>
</tbody>
</table>

*ISO 17511:2003 In vitro diagnostic medical devices*
- Measurement of quantities in biological samples- Metrological traceability of values assigned to calibrators and control materials -
Traceability Chain of CBC

The primary standard of CBC is **Reference Method**.
Ref.) Traceability Chain in Biochemistry

For some parameters, the primary standard in Biochemistry is **Certified Reference Material**.
Calibrate your analyzer periodically using Calibrator from Sysmex to maintain the traceability!
Contents

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Before thinking about Measurement Uncertainty

Manufacturer’s Calibrator

Patient Sample

RBC: 4.456 *10^{12}/L

How reliable is your measured value?
“property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty”

[VIM 2012 (2.41)]

*International vocabulary of metrology - Basic and general concepts and associated terms, 3rd edition
Term Definition

Measurement Uncertainty

“non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used”

Note2) The parameter may be, for example, a standard deviation (SD) ...

Measurement Uncertainty is a concrete expression of the reliability of the traceability chain.
Meaning of Measurement Uncertainty

For example

When you measure a sample many times, the measurement has some variance. These variance is also called **Measurement Uncertainty (MU)**.

![Diagram showing variation and measurement uncertainty]

- **Variation**: Large
  - **MU**: Large
- **Variation**: Small
  - **MU**: Small
Traceability and Measurement Uncertainty

- Fresh Whole Blood
- Manufacturer’s Calibrator
- Patient Sample
- Reference Method
- Manufacturer’s Standard Analyzer
- Hematology Analyzer in Laboratory
- Result (RBC)
Factors of Measurement Uncertainty are considered through a measurement process.

- Storage Condition (Temperature etc…)
- Storage term etc…
- MU of calibration
- Pipette
- Dilution etc…
- Medicine
- Intra-individual variability etc…
- Rounding off the decimal point etc…

Example
A measured value of RBC is

\[4.62 \pm 0.042 \times 10^{12}/\text{L}\], \((k=2)\)

This means “A measured value of RBC is in the range of
\[4.62 \pm 0.042 \times 10^{12}/\text{L}\]
with about 95% confidence interval.”
MU estimation process (GUM method)

**Step 1.** Identify all important components of Measurement Uncertainty

**Step 2.** Calculate the **Standard Uncertainty** of each component of Measurement Uncertainty

**Step 3.** Calculate the “**Combined Standard Uncertainty**”

\[ u_c = \sqrt{u_1^2 + u_2^2 + \cdots + u_n^2} \]

**Step 4.** Calculate the “**Expanded Uncertainty**”

\[ U = k \cdot u_c \text{ (coverage factor; } k=2) \]

**Step 5.** State the measurement result

\[ Y = y \pm U, \ (k=2) \]
Estimation of Measurement Uncertainty for a routine sample

Manufacturer’s Responsibility

- Value Assignment for fresh blood
- Value Assignment for Calibrator

- Uncertainty Assignment of Calibrator’s Assay

- Uncertainty of Calibration

Laboratory’s Responsibility

- MU of Calibrator

- Estimating MU for individual patient samples

Not Realistic!

Uncertainty of Calibrator’s Assay

Internal QC

- Standard Deviation (SD); Reflecting all possible uncertainty sources

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Estimation of Measurement Uncertainty for a routine sample

Reference measurement procedure

Human blood

Working standard analyzer

Product calibrator

An analyzer in a laboratory

A sample

Standard uncertainty of an assigned value of a calibrator

Step1

Step2

Combined standard uncertainty of a test result of a sample

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Steps for Estimating MU of a routine sample

Step 1: Estimating MU of calibration on an analyzer
   i. MU of calibrator
   ii. MU of repeatability by running a calibrator

Step 2: Estimating MU of the routine sample
   i. MU of calibration on an analyzer
   ii. MU of reproducibility from IQC
1. How to ensure a measured value is correct?
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Responsibility of Sysmex

Manufacture’s responsibility

- Fresh Whole Blood
- Manufacturer’s Calibrator
- Patient Sample
- Result (RBC)

Reference Method

- Manufacturer’s Standard Analyzer
- Hematology Analyzer in Laboratory

Traceability
(Ref.) Scientific Lab in Japan
(Ref.) Reference Method Room

- A special room for CBC reference methods
- Temperature: between 18 ℃ – 22 ℃
- Place equipment for reference method including Reference Counter for RBC and WBC
- Measure fresh blood samples by CBC reference method every 3 months.
(Ref.) Standard Analyzers

- XN-2000, XN-350, XE-5000, XT-4000i, XS-1000i, K-4500, XP-300, KX-21, KX-21N, pocH-100i, F-820

(As of July 31, 2015)
Whole Blood Calibration for standard analyzers 1/3

- Parameters
  CBC (RBC, WBC, PLT, HGB, HCT)

- Reference method
  ICSH (INTERNATIONAL COUNCIL FOR STANDARDIZATION IN HAEMATOLOGY)
  CLSI (CLINICAL AND LABORATORY STANDARDS INSTITUTE)

- Environment
  Room temperature: 18 - 22°C (following ICSH)

- Sample
  Fresh venous bloods drawn from healthy donors
  (No visible haemolysis, no microclots)

- Frequency
  Every 3 months
1-1. Preparing Whole Blood Samples

a) Collect Healthy Donors’ blood in EDTA-2K tubes.
b) Pool blood samples into one tube and mix them well.
c) Take aliquot volume into several tubes.

Ready for measurement.


a) Prepare “Macro-dilution”.
b) Measurement with Reference Counter.

Assign Values of RBC for whole blood samples.
Whole Blood Calibration for standard analyzers 3/3

1-3. Measurement on Standard Analyzers

At the same time, all whole blood samples are measured on manufacturer’s Standard Analyzers.

1-4. Calibration of Standard Analyzers

After all measurements, calculate the difference between reference method’s data and standard analyzers’ data. If the difference is out of allowable limit, calibrate standard analyzer’s setting.
Reference Method: RBC and WBC

RBC and WBC

Reference method for the enumeration of erythrocytes and leucocytes
International Council for Standardization in Haematology; Prepared by
the Expert Panel on Cytometry - Clinical Laboratory Haematology, 16,
131-138 (1994)
Reference Method: HCT

HCT


Blood specimen

Capillary tube

Aspirating sample

Length of Red Cell Column (b) (mm) × 100 = ?
Length of Cells Plus Plasma Column (a) (mm)

Centrifugation

Sealing capillary tube 12,000 rpm, 5min

Measuring

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Reference Method: PLT

Platelet Counting by the RBC/Platelet Ratio Method - A Reference Method

Earn RBC/PLT ratio
Traceability Chain based on ISO in Sysmex

Responsibility

Manufacturer (Sysmex)

ICSH/CLSI-recommended Reference Method

Material / Sample

Fresh Whole Blood Samples

Product Calibrator

Method / Device

In-house Standard Analyzers

End-user’s Analyzer

Value Assignment Calibration

ISO15195

ISO17025

Laboratory

Routine Test Sample

Measurement Results (RBC, WBC, HGB, HCT, PLT)

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Calibration Service accredited to ISO/IEC 17025

What is ISO/IEC 17025?

An international standard for Testing and Calibration laboratory

“Sysmex Corporation Calibration Services” is internationally acknowledged as having the capability of calibration laboratories.

Merits for Customer

- Calibration Certificates will be issued as an official certificate acknowledged by ISO.
- Calibration Certificates are compliant to the requirements of ISO 15189.
- Calibration Certificates are issued as an official certificate acknowledged by ISO.

※This service is provided only in Japan so far.
Data Assurance based on ISO in Sysmex

- Primary Standard
- Secondary Standard
- Working Standard

Reference Method
- In-house Standard Analyzers
- Product Calibrator
- Laboratory’s Analyzer

ISO/IEC17025
ISO 15195
ISO/IEC17043

Measurement uncertainty based on Traceability Chain
EQA scheme

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SNCS  (Sysmex Network Communication Systems)

A service which analyzers and call center are connected by network. (Began in 1999)
Online QC

Integration of IQC and EQA

**EQA**
- Periodical measurement of QC material
- Ensure accuracy of QC data by comparing with other analyzers

**IQC**
- Daily measurement of control material
- Ensure precision of daily QC data by comparing with assay limit & monitoring the trend

**QC in real-time. Error will be informed by email or phone call.**

**IQC and EQA can be done simultaneously**

**Data will be updated approximately every 10 minutes.**
SNCS/eQAPi in the world

Europe

China

Japan

Americas

AP

June 2015 Total: 33,004

Europe: 4,050
China: 3,181
Japan: 9,978
AP: 1,096
Americas: 14,699
SNCS EQA accredited to ISO/IEC 17043
※This service is provided only in Japan, EU and Taiwan so far.

What is ISO/IEC 17043?
An international standard for Proficiency Testing provider

“Sysmex Corporation Quality Control Services” is internationally acknowledged as having the capability of providing proficiency testing.

Merits for Customer
・QC reports will be issued as an official certificate acknowledged by ISO.
・QC reports are compliant to the requirements of ISO 15189.
・QC reports are issued as an official certificate acknowledged by ISO.
Data Assurance based on ISO in Sysmex

Measurement uncertainty based on Traceability Chain

Reference Method

In-house Standard

Analyzers

Product Calibrator

Laboratory's Analyzer

Measurement Uncertainty

Primary Standard

Secondary Standard

Working Standard

Laboratory

ISO 15195

ISO/IEC17025

ISO/IEC17043

EQA scheme

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Thank you for your attention!

Terima kasih banyak!