Traceability of HbA1c measurements: weak and strong points

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University of Milano, Milano (IT)

prologue
Analytical Bias - Therapeutic Consequence

Rate of Progression of Retinopathy (100 patient years)

Measured HbA1c = 6.5%

HbA1c
Insulin-dependent patients

P. Hyltoft Petersen et al 1997

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Programme

10:10 Meeting/inspiration
National Authorities

Morning session
Chairperson: W. Mallet, P. Cottini

10.10 The Centre for Metabolism Therapies in Laboratory Medicine (CIRM): scope and activities
W. Mallet (Milano, IT)

11.00 Defining Hba1c: the indispensable element to approach measurement standardization
J.O. Jorgensen (Hauterive, SE)

11.30 The Hba1c network: structure, performance and rules
P. Fromherz (Milano, IT)

12.30 Implementation of the IFCC reference system for Hba1c in clinical practice: how to educate clinicians
S. John (Krefeld, DE)

13.30 Discussion

14.00 Break

Afternoon session
Chairperson: J. Holle, A. Pliniani

14.00 Appropriate ways of adapting the Hba1c standardization at clinical practice level
E. Francisco (Paris, IT)

14.30 Why we need traceability in Laboratory Medicine
M.M. Mainval-Otten (Milano, IT)

15.00 Standardization of Hemoglobin A1c: does Multi-Laboratory report work?
A. Mosca (Milano, IT)

15.30 Presentation

16.00 Meeting/conclusions
J. Holle (Washington, DC, US)
agenda

- IFCC reference system for HbA\textsubscript{1c}
  - Top of the chain
  - Middle of the chain
  - Bottom of the chain
- Conclusions and future activities
Reference System for HbA\textsubscript{1c}

**Measurand**

- HbA\textsubscript{1c} - N-(1-deoxyfructosyl)-haemoglobin-β-chain in blood
  - SI-Unit: mmol/mol

**Primary reference materials** (IRMM 466 and 467)

**IFCC Network**

- IFCC reference measurement procedure (HPLC-CE or HPLC-MS)

**Secondary reference materials** (blood panels)

**Manufacturer**

- Manufacturer’s internal reference measurement procedure

- Manufacturer’s standing measurement procedure

**Manufacturer’s internal reference measurement procedure**

**Manufacturer’s standing measurement procedure**

**Manufacturer’s product calibrator**

**Routine measurement procedure**

**Patient Sample**

**Glycation sites of Hemoglobin**

- β-Chains: Yellow
- α-Chains: Blue

- N-terminal
  - Valine: red
  - Lysine: green
The Analytical Challenge

Proteolytic cleavage of β-chain (146 amino acids)

HbAo-peptide → Glu-C ↓
Val Val His Leu Thr Pro Glu Glu Lys Ser . . . . . . .

HbA1c-peptide → Glu-C ↓
Glu Val His Leu Thr Pro Glu Glu Lys Ser . . . . . . .

top of the chain
Photometric detection of peptides

Flow Chart for Reference Methods

- Hemolysate
- Enzymatic Cleavage
- HPLC-Capillary Electrophoresis
- HPLC-Mass Spectrometry

Approved by IFCC 2001
ESI mass spectra of nonglycated and glycated βN-terminal hexapeptides

Two-dimensional separation of N-terminal hexapeptides of hemoglobin
Preparation of calibrators

Hemoglobin

\[ \downarrow \]

Cation exchange chromatography

SP Sepharose HPLC, pH 6.2

\[ \downarrow \downarrow \]

HbA1c (crude)      HbA0 (crude)

\[ \downarrow \]

Affinity chromatography

Glycogel Boronate, pH 8.0

\[ \downarrow \]

Cation exchange chromatography

SP Sepharose HPLC, pH 6.2

\[ \downarrow \downarrow \]

HbA1c (pure)      HbA0 (pure)


A. Mosca - UniMi
The material is only to be used together with IFCC HbA1c Network pure HbA1c. In the primary calibration, the IFCC HbA1c Network Reference Materials, and the IFCC HbA1c Reference Measurement Procedure.

Product Description:
The material is prepared from human whole blood obtained from non-diseased volunteers. The material is deep frozen into 1°C and stored for 10 minutes. The materials stored at 4°C are stored for 10°C and the primary measurement is performed.

IFCC HbA1c, NETWORK PRIMARY CALIBRATORS

<table>
<thead>
<tr>
<th>Product Certificate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration set IFCC HbA1c, Reference Measurement Procedure</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Material</th>
<th>Value</th>
<th>Value</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFCC HbA1c, Network pure HbA1c</td>
<td>0.00001</td>
<td>0.00001</td>
<td>0.00001</td>
</tr>
<tr>
<td>IFCC HbA1c, Network Reference Material</td>
<td>0.00001</td>
<td>0.00001</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

Uncertainties:
- \( u_c = 0.27\% \)
- \( u_c = 0.26\% \)
REPORT
Intercomparison Studies Shanghai-1 and Shanghai-2
of the IFCC Network on Standardisation of HbA1c

<table>
<thead>
<tr>
<th>Lab</th>
<th>Coefficients Bias (mmol/mol)</th>
<th>Coefficients Bias (mmol/mol)</th>
<th>Coefficients Standard Deviation (mmol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lab_02</td>
<td>-0.06</td>
<td>0.320</td>
<td>0.1</td>
</tr>
<tr>
<td>lab_03</td>
<td>1.03</td>
<td>0.015</td>
<td>0.2</td>
</tr>
<tr>
<td>lab_04</td>
<td>0.70</td>
<td>-0.30</td>
<td>0.8</td>
</tr>
<tr>
<td>lab_06</td>
<td>-0.98</td>
<td>-0.02</td>
<td>1.5</td>
</tr>
<tr>
<td>lab_07</td>
<td>-0.87</td>
<td>0.016</td>
<td>1.9</td>
</tr>
<tr>
<td>lab_09</td>
<td>-0.10</td>
<td>0.204</td>
<td>0.2</td>
</tr>
<tr>
<td>lab_10</td>
<td>-0.25</td>
<td>0.015</td>
<td>2.1</td>
</tr>
<tr>
<td>lab_11</td>
<td>0.43</td>
<td>0.012</td>
<td>1.7</td>
</tr>
<tr>
<td>lab_12</td>
<td>0.54</td>
<td>-0.30</td>
<td>2.5</td>
</tr>
<tr>
<td>lab_14</td>
<td>0.82</td>
<td>-0.31</td>
<td>1.9</td>
</tr>
<tr>
<td>lab_16</td>
<td>-1.15</td>
<td>-0.320</td>
<td></td>
</tr>
<tr>
<td>lab_18</td>
<td>1.12</td>
<td>-0.20</td>
<td>3.8</td>
</tr>
<tr>
<td>lab_20</td>
<td>-0.45</td>
<td>0.200</td>
<td>0.3</td>
</tr>
<tr>
<td>lab_21</td>
<td>-1.36</td>
<td>0.351</td>
<td>6.7</td>
</tr>
<tr>
<td>lab_23</td>
<td>0.43</td>
<td>0.050</td>
<td>2.5</td>
</tr>
<tr>
<td>lab_24</td>
<td>0.30</td>
<td>0.010</td>
<td>1.5</td>
</tr>
<tr>
<td>lab_26</td>
<td>-0.41</td>
<td>0.016</td>
<td>1.7</td>
</tr>
<tr>
<td>lab_28</td>
<td>-0.42</td>
<td>0.020</td>
<td>6.4</td>
</tr>
<tr>
<td>lab_29</td>
<td>-1.39</td>
<td>0.020</td>
<td>4.4</td>
</tr>
</tbody>
</table>
4.2.1.4 Monitoring Programme

To prove traceability (especially in respect to the IVD directive), manufacturers appreciate the availability of an independent monitoring programme. This programme is available from the IFCC network. In summary, participating manufacturers receive 24 frozen whole blood samples once a year. Following the list with deadlines, every two weeks a specimen is assayed. Results are submitted via the interactive website of the IFCC RMN Network immediately after the deadline for submission is passed the report can be requested from the website. The report shows the submitted result in comparison to the target value. Additionally trend reports (either related to time or to HbA1c content) can be requested and once the annual cycle is completed the annual report deals with precision and linearity. Results are strictly confidential: only the manufacturer has access to his results but he is free to use the results. Manufacturers get a certificate of traceability on which their performance in terms of deviation from the IFCC target, precision and linearity are specified. Details and certificate instructions can be downloaded from the website.

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Proceedings Manual - IFCC Network of Reference Laboratories for HbA1c

Version 10.05.2011

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Monitoring Programme Manufacturers 2013

Monitoring Programme Manufacturers 2015
middle of the chain

What information should be provided

- which kind of higher-order materials and methods to assign traceable values to the calibrators
- which kind of internal calibration hierarchy
- \( u_c \) of commercial calibrators
Table 1

<table>
<thead>
<tr>
<th>Company</th>
<th>Platform</th>
<th>Principle of commercial method</th>
<th>Calibrator</th>
<th>Declared standard uncertainty*</th>
<th>Higher-order reference employed</th>
<th>Type of traceability chain used</th>
<th>Combined standard uncertainty associated with the used chain [u]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott</td>
<td>Architec</td>
<td>ND</td>
<td>Multicomponent calibrator</td>
<td>2.70%</td>
<td>IDMS NIST 8985 A</td>
<td>A</td>
<td>1.22-1.40 μV</td>
</tr>
<tr>
<td>Beckman</td>
<td>AU</td>
<td>ND</td>
<td>System calibrator</td>
<td>ND</td>
<td>IDMS NIST 8985 A</td>
<td>A</td>
<td>1.22-1.40 μV</td>
</tr>
<tr>
<td>Roche</td>
<td>Cobas e</td>
<td>ND</td>
<td>Multicomponent calibrator</td>
<td>0.84%</td>
<td>IDMS NIST 8985 A</td>
<td>A</td>
<td>1.22-1.40 μV</td>
</tr>
<tr>
<td>Siemens</td>
<td>Advia</td>
<td>ND</td>
<td>Chemistry calibrator</td>
<td>0.80%</td>
<td>IDMS NIST 8985 A</td>
<td>A</td>
<td>1.22-1.40 μV</td>
</tr>
</tbody>
</table>

Verification of in vitro medical diagnostics (IVD) metrological traceability: Responsibilities and strategies

Federica Braga, Mauro Panteghini

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Certificate

Traceability of Manufacturers to the IFCC Reference Measurement Procedure for HbA1c

This certificate demonstrates that the participating laboratory, A. Mosca - UniMi, in the Monitoring Programme for HbA1c on December 4th, 2015, obtained the following performance:

- Deviation from IFCC-target: 0.1 mmol/mol
- Reproducibility, coefficient of variation: 0.42%
- Linearity, correlation coefficient: 0.9995

Date of Issue: December 4, 2015
Certification expires: December 31, 2016

A. Mosca - UniMi
Product certificate HbA1c

Product name

Product number 4755

Product code

<table>
<thead>
<tr>
<th>Level</th>
<th>Product code</th>
<th>Colour cap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Level 1</td>
<td>Green</td>
</tr>
<tr>
<td>High</td>
<td>Level 2</td>
<td>Red</td>
</tr>
</tbody>
</table>

Batch number and Expiry date

<table>
<thead>
<tr>
<th>Level</th>
<th>Batch number</th>
<th>Exp. Date stored at 20°C / -15°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>NT 05046/01</td>
<td>2023/03</td>
</tr>
<tr>
<td>High</td>
<td>NT 06046/01</td>
<td>2023/03</td>
</tr>
</tbody>
</table>

Reconstitution volume 0.6 ml with a precision of ± 1.0%

Haemoglobin

<table>
<thead>
<tr>
<th>Level</th>
<th>Hb [mmol/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>7.1</td>
</tr>
<tr>
<td>High</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Assigned Values*

<table>
<thead>
<tr>
<th>Level</th>
<th>Certified Value (Expanded Uncertainty, k=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FCC mmol/mol</td>
</tr>
<tr>
<td>Low</td>
<td>36.8 (0.7)</td>
</tr>
<tr>
<td>High</td>
<td>86.8 (1.5)</td>
</tr>
</tbody>
</table>

bottom of the chain
What should be done to prove the metrological traceability - uncertainties -

- The use of a traceable method
- The uncertainty of the commercial calibrators
- The long-term imprecision of the routine method
Other factors contributing to the uncertainty

- Bias
- Biological variation

\[ u_{\text{(composite)}} = (u^2_{\text{IQC}} + u^2_{\text{EQC}} + u^2_{\text{intra-individual}})^{1/2} \]
What should be done to guarantee the long-term performance

- analytical goals
- EQAS

Weykamp et al, Clin Chem 2015;61:752
Reevaluation of biological variation of glycated hemoglobin (HbA1c) using an accurately designed protocol and an assay traceable to the IFCC reference system

Federica Braga a, b, c, Alberto Doki b, Martina Montagnana c, Franca Pagani d, Renata Palaei a, Gian Cesare Guidi c, Andrea Mosca a, Mauro Panteghini a, b

Table 3
Analytical goals for HbA1c measurement derived from data on biological variation.

<table>
<thead>
<tr>
<th>Quality level</th>
<th>Imprecision, %</th>
<th>Bias, %</th>
<th>Total error, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal</td>
<td>≤ 0.6</td>
<td>≤ ±0.9</td>
<td>≤ ±2.0</td>
</tr>
<tr>
<td>Desirable</td>
<td>≤ 1.3</td>
<td>≤ ±1.9</td>
<td>≤ ±3.9</td>
</tr>
<tr>
<td>Minimal</td>
<td>≤ 1.9</td>
<td>≤ ±2.8</td>
<td>≤ ±5.9</td>
</tr>
</tbody>
</table>

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Università degli Studi
di Milano

A. Mosca - UniMi

20/06/17
Centro di Riferimento Sicurezza e Qualità
Valutazione esterna di qualità
HbA1c - Ciclo 2016

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Campioni 43
Standardization of HbA1c: are all the pieces in place?

- Top of the chain: YES (IFCC Network)
- Middle: probably YES (information not uniform and difficult to be released)
- Bottom: difficult to draw objective evidences (depending on country and rules); more efforts needed to achieve standardization overall
WHAT NEXT?

- Consensus on uncertainty
- Improving EQAS (EurA1c)
- Education and communication

AKNOWLEDGMENTS

- The IFCC Network laboratories
- Manufacturers
- Mauro Panteghini and CIRME staff
CIRME
Centro Interdipartimentale per la
RiferibilitàMetrologica in Medicina di
Laboratorio (CIRME)

under the auspices of

11th International Scientific Meeting
MEASUREMENT UNCERTAINTY
IN MEDICAL LABORATORIES:
FRIEND OR FOE?

MILANO, ITALY
November 30th, 2017
AULA MAGNA - LIFAB SGRATE
Via Fratelli Cervi, 93 - Segrate, Milano