The IFCC HbA$_2$ standardization program

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2nd European Hemoglobinopathy Forum
Insights on the diagnosis of hemoglobin disorders

November 29th, 2011 – Madrid, Eurostars Madrid Tower Hotel
agenda

- Terms of reference and metrological traceability
- Primary and secondary reference measurement procedure
- Primary and secondary reference materials
- WHO and ICSH interactions
- How accurate should we be?
8.3.35. Standardisation of Hemoglobin A2 (WG-HbA2)

Membership

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Country</th>
<th>Term</th>
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<tr>
<td>R. Paleari</td>
<td>Chair</td>
<td>IT</td>
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<td>E. Bissé</td>
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<td>D. Caruso</td>
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<td>P. Kaiser</td>
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<td>A. Mosca</td>
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<td>H. Reinauer</td>
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<td>C. Schaeffer</td>
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<td>A. Van Dorsselaer</td>
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<td>B. Wild</td>
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Terms of Reference

- To promote the standardisation of hemoglobin A2 measurement through the definition of an international reference system, including a reference measurement procedure and primary and secondary reference materials.

Current Projects

- Definition of a reference measurement procedure using mass spectrometry associated with proteolytic degradation.
- Preparation of a secondary reference material for hemoglobin A2 (in cooperation with IRMM).

WG-HbA2 Corresponding Members

Committee Chair’s contact
new SOP (under development)

- **blood**
  - **erythrocytes**
  - **hemolysate**
    - enzymatic cleavage with trypsin
      - + IS$\delta$ – 5$^{13}$C
      - + IS$\alpha$ – 5$^{13}$C
  - HPLC - Mass spectrometry
  - quantification of specific peptides
    - $\delta$T2 (TAVNALWGK) and $\alpha$T11 (VDPVNFK)
P. Kaiser and H. Reinauer are going to develop the same approach for HbA2

Next report: meeting in January 2012
The δ chain/α chain ratio as a surrogate biomarker for HbA₂

\[ y = 0.9881x - 0.03 \]
\[ r = 0.9940 \]
\[ n = 30 \]
Secondary measurement procedure for HbA$_2$ based on quantification of tryptic peptides

R Neil Dalton, Yvonne Daniel, Charles Turner
WellChild Laboratory
King’s College London/Evelina Children’s Hospital

IFCC Working Group on Standardisation of HbA$_2$
Meeting IFCC Euromedlab 2011, Berlin
Secondary measurement procedure for HbA₂ based on quantification of tryptic peptides

The delta chain/beta chain ratio as a surrogate biomarker for HbA₂

Results: Within- and between-assay imprecision values (CVs) were <6.1% and <8.4%, respectively, for the δ:β peptide ratios. Digests were stable at 10°C for 6 days.


News, 19 September 2011: the method is ready to be transferred to the other MS labs of the WG wishing to test it, providing a license and coverage of the costs for the labeled peptides
Second batch prepared at the IRMM on July 2011 (from materials sent to them on November 2010)

• Call for tenders under development:
  – Testing Methemoglobin formation during storage over one year
  – Minor Hbs analysis by capillary electrophoresis or HPLC
  – Testing total Hb content
Issues about the WHO material:

a) Just one level of HbA$_2$ (5.3 %)
b) Title assigned by LC (minicolumns?)
c) No data on the commutability
d) No data on stability
e) No information about how the manufacturers are using it
f) Usually handled differently from blood samples
NIBSC-WHO material

Bio-Rad Variant II

- F Concentration = 1.4 %
- HbA1c Concentration = 6.0 %
- A2 Concentration = 5.3 %

Analysis comments:

Tosoh G8

- 2011/04/15 15:16
- TOSOH CORPORATION V05.01
- NO: 0006 SL 0001 - 06
- ID: 0001 - 06
- CALIB: F Y = 0.9838X
- A2 Y = 1.2505X
- TP 1701

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<td>1.4</td>
<td>0.73</td>
<td>50.08</td>
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<td>A1c</td>
<td>75.7</td>
<td>2.28</td>
<td>2562.07</td>
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<tr>
<td>A2</td>
<td>5.3</td>
<td>2.94</td>
<td>143.60</td>
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<tr>
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<td>0.00</td>
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<tr>
<td>S+</td>
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<tr>
<td>C+</td>
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Fresh whole blood

Bio-Rad Variant II

F Concentration = 2.5%<sup>*</sup>
A1c Concentration = 5.6%<sup>*</sup>
A2 Concentration = 5.2%<sup>*</sup>

Analysis comments:

Tosoh G8

2011/04/15 16:40
TOSOH CORPORATION V05.01
NO: 0020 SL 0003 - 05
ID: 0003 - 05
CALIB F Y = 0.9636X
A2 Y = 1.2505X

TF 1854

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<td>A0</td>
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<td>A2</td>
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<tr>
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<tr>
<td>Ct</td>
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AREA TOTALE 3417.19

F: 2.3%
A2: 5.7%
Experimental protocol

• N = 18 healthy subjects
  – N = 9 Men
  – N = 9 Women
  – Age: 26 – 52 y
• Five blood samples (every 2 weeks for 2 months)
• Parameters:
  – HbA$_{1c}$, glycated albumin, fructosamine, HbA$_2$
  – RBC, WBC, PLT, Hb, MCH, MCHC, MCV, RDW
• Measurements on fresh blood samples and storage at -80 °C
• Analysis by HPLC, NGSP calibrated (HbA$_{1c}$)
Data analysis

$$\sigma^2_{\text{total}} = \sigma^2_{\text{anal}} + \sigma^2_I + \sigma^2_G$$

• Analytical variation: from the duplicate results for each specimen or from internal QC (whole blood cell count)
• Intra-individual variation: from the serial results for each subject
• Inter-individual variation: from the total variance of data, minus the analytical and intra-individual components
Raw data, HbA₂

![Graph showing raw data for HbA₂ with markers for females and males.](image)
<table>
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<th>Analytical goal</th>
<th>Quality level</th>
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<tr>
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<td>Minimal</td>
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<td>Imprecision, %</td>
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<td>Bias, %</td>
<td>2.8</td>
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<td>Total error, %</td>
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HbA$_2$
Take-home message

- The biological variability of glycated Hb is small, that of HbA₂ is even smaller
- CV₁ is < than CV₆: limit to the use of reference intervals based on populations
- The analytical goal for CVₐ is very stringent
- EQAS and IQC are essential in order to keep under strict control the HbA₂ methods
Aknowledgments

Renata Paleari (*University of Milano, Milano*)
(HPLC analysis, data elaboration)

Martina Montagnana, Giancesare Guidi (*Verona University Hospital, Verona*)
(protocol, subjects)
Looking forward to meeting you at

EUROMEDLAB Milano 2013
20th IFCC-EFCC European Congress of Clinical Chemistry and Laboratory Medicine

www.milan2013.org
2nd European Hemoglobinopathy Forum

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