

**Accurate Results for Patient Care Workshop 2019**  
A JCTLM Members' and Stakeholders' meeting  
2-3 December 2019, BIPM

 Accurate results for patient care

**\* Implementation of the reference system for hemoglobin A<sub>2</sub>: what impact on thalassemia screening and diagnosis**

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Chair, IFCC WG Standardization of HbA<sub>2</sub>  
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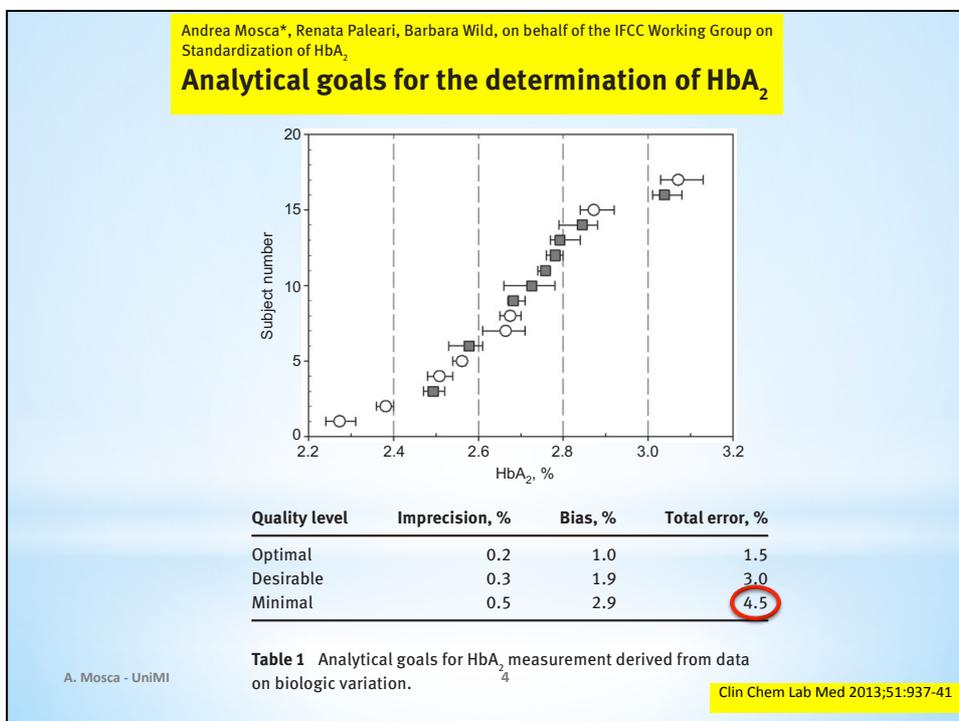
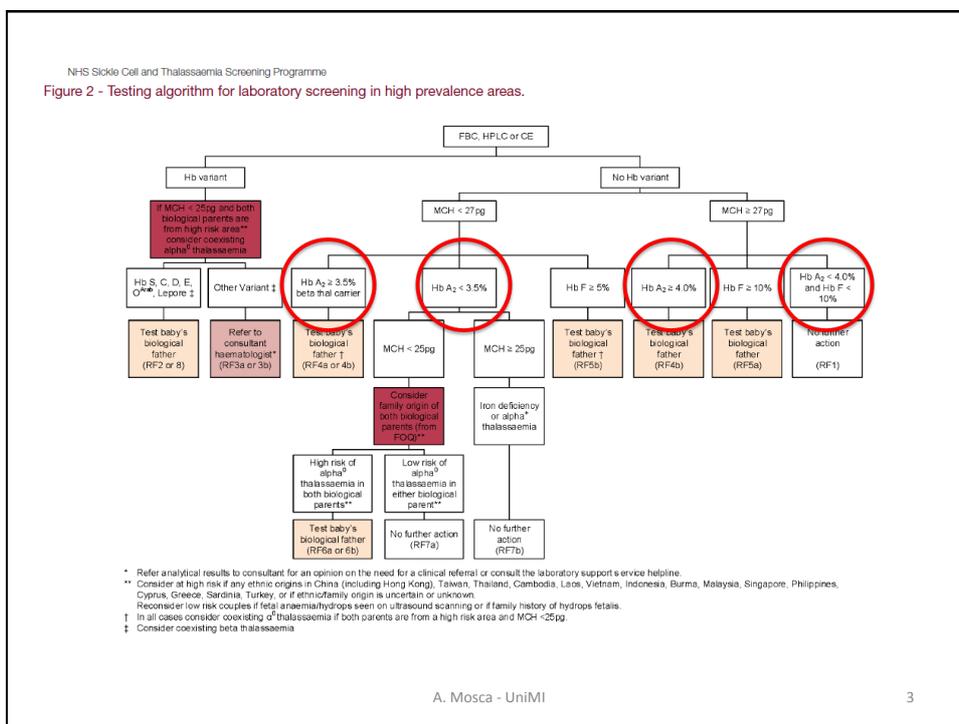
 

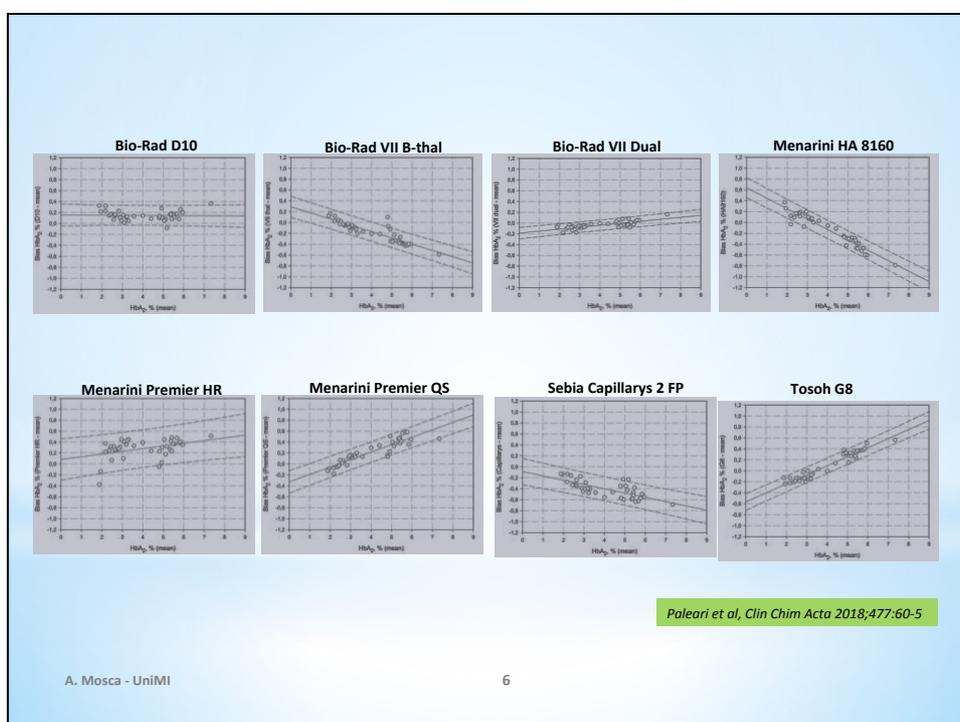
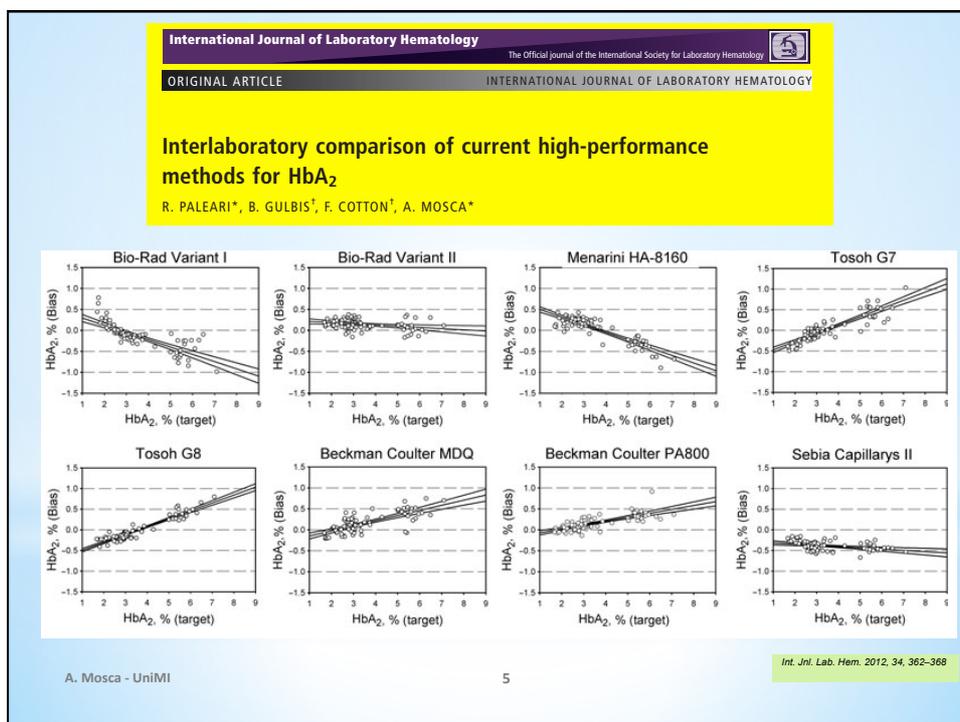
**NHS Sickle Cell and Thalassaemia Screening Programme**  
Handbook for antenatal laboratories

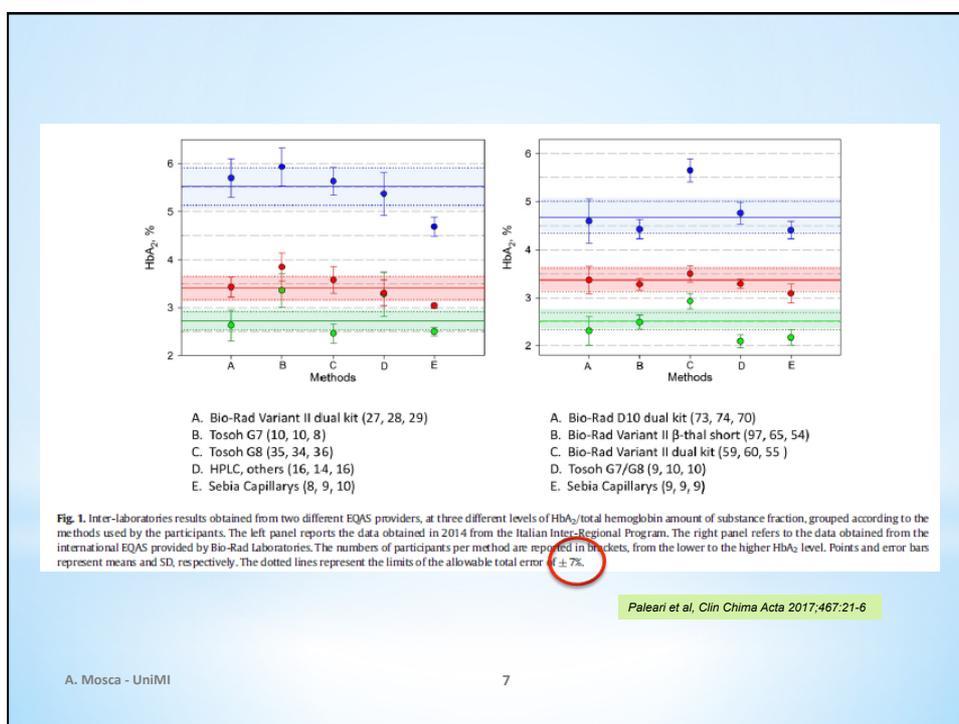


October 2017  
Public Health England leads the NHS Screening Programmes

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| topic   | notes   | Availability date | Implementation |
|---|---|-------------------|----------------|
| Units of measurement                                | %<br>(fraction of absolute amount<br>of HbA <sub>2</sub> and HbA total)                     | (already in use)  | --             |
| Primary calibrators                                 | rHbA, rHbA <sub>2</sub><br>(Trenzyme GbH)   | September 2019    | JRC-EU         |
| Candidate Reference<br>measurement<br>procedure (1) | ID LC-MS/MS<br>(Clin Chim Acta<br>2018;487:318-24)  | December 2018     | IFCC-WG        |
| Reference<br>measurement<br>procedure (RMP) (2)     | ID LC-MS/MS   | 2020 (?)          | IFCC (ballot)  |
| Certified Reference<br>Materials (CRMs)             | Lyophilized, 2 levels<br>(tested for homogeneity,<br>stability, title with U <sub>c</sub> ) | 2020 (?)          | JRC-EU         |

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Clinica Chimica Acta 487 (2018) 318–324

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**Clinica Chimica Acta**

journal homepage: [www.elsevier.com/locate/cca](http://www.elsevier.com/locate/cca)

**Determination of HbA<sub>2</sub> by quantitative bottom-up proteomics and isotope dilution mass spectrometry**

Cristian Gabriel Arsene<sup>a</sup>, Patricia Kaiser<sup>b</sup>, Renata Paleari<sup>c</sup>, André Henrion<sup>b</sup>, Michael Spannagl<sup>d</sup>, Andrea Mosca<sup>e,\*</sup>, on behalf of the IFCC Working Group on Standardisation of Hemoglobin A2 (WG-HbA2)

**Table 3**  
Estimated measurement uncertainty by combining individual contributions. Within-laboratory precisions are those obtained from the repeatability-experiment (see Table 1). Uncertainties were combined as root from sum of squares of individual contributions; expanded uncertainties were estimated applying a coverage factor  $k = 2$ , corresponding to a 95% confidence level.

|   | Normal HbA <sub>2</sub><br>fraction (%) | Elevated HbA <sub>2</sub><br>fraction (%) |
|---|---|---|
| Within-lab. precision   | 1.68                                    | 1.84                                      |
| Standard uncertainty ( $u$ ) HbA <sub>0</sub> -<br>stock sol. | 1.50                                    | 1.50                                      |
| Standard uncertainty ( $u$ ) HbA <sub>2</sub> -<br>stock sol. | 1.25                                    | 1.25                                      |
| Combined standard uncertainty<br>( $u_c$ )                    | 2.6                                     | 2.7                                       |
| Expanded uncertainty ( $U$ ), $k = 2$                         | 5.2                                     | 5.4                                       |

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**Development of a candidate  
certified reference material (CRM)**

**First pilot batch (April 2008)**

- homogeneity
- total Hb content
- MetHb
- stability at +4°/-20 °C
- commutability



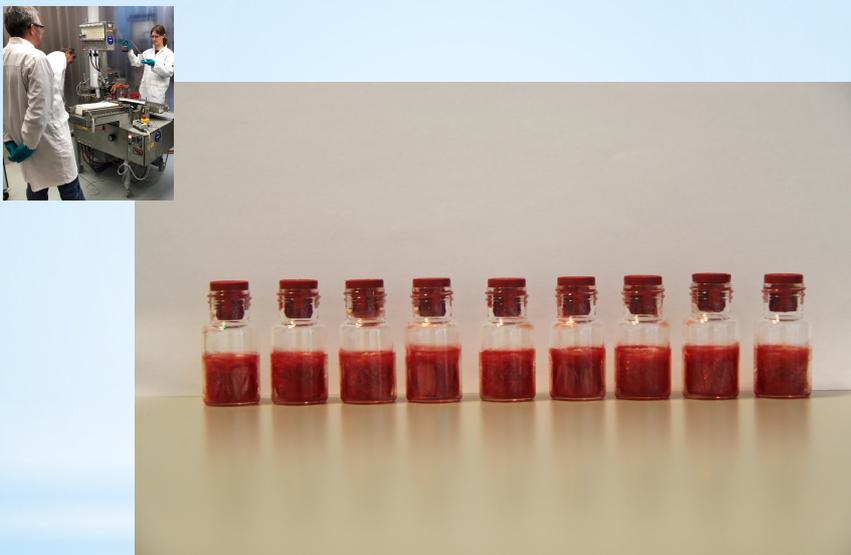
**Second batch (November 2010)**

- Storage without O<sub>2</sub>  
to limit oxydation
- accelerated degradation  
experiments
- Long term stability



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JRC, Geel (April 2019)

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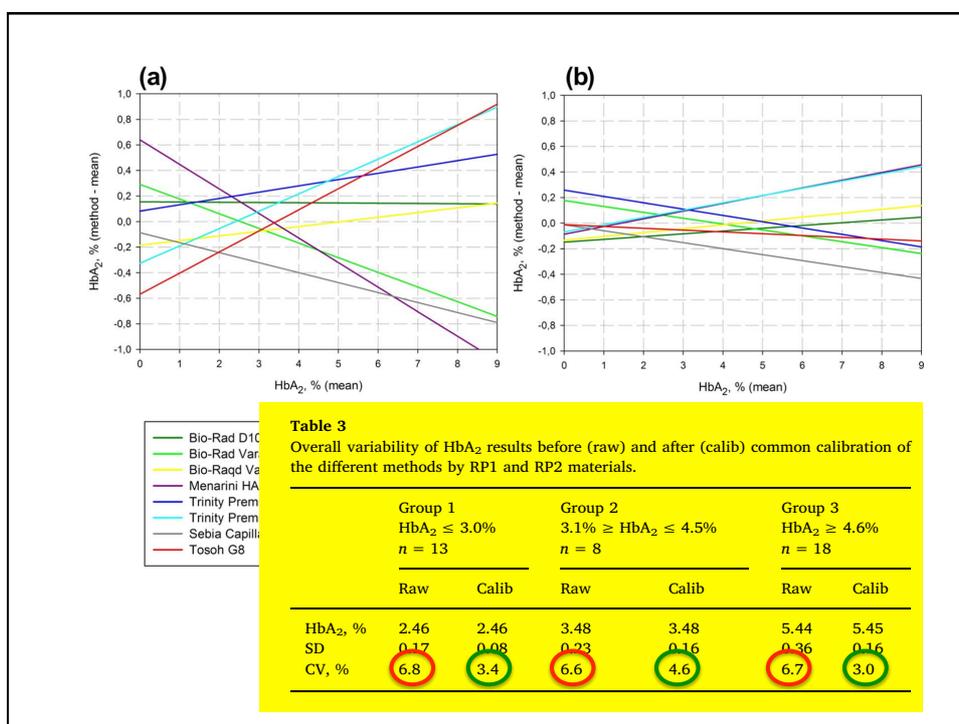
journal homepage: [www.elsevier.com/locate/cca](http://www.elsevier.com/locate/cca)

**Calibration by commutable control materials is able to reduce inter-method differences of current high-performance methods for HbA<sub>2c</sub><sup>†</sup>**

| WHO | Lyphocheck 1 | Lyphocheck 2 |
|-----|--------------|--------------|
| 1   | 1            | 1            |
| 2   | 2            | 2            |
| 3   | 3            | 3            |
| 4   | 4            | 4            |
| 5   | 5            | 5            |
| 6   | 6            | 6            |
| 7   | 7            | 7            |
| 8   | 8            | 8            |
| 1   | 1            | 1            |
| 2   | 2            | 2            |
| 3   | 3            | 3            |
| 4   | 4            | 4            |
| 5   | 5            | 5            |
| 6   | 6            | 6            |
| 7   | 7            | 7            |
| 8   | 8            | 8            |

| RP 1 | RP 2 | RP 3 |
|------|------|------|
| 1    | 1    | 1    |
| 2    | 2    | 2    |
| 3    | 3    | 3    |
| 4    | 4    | 4    |
| 5    | 5    | 5    |
| 6    | 6    | 6    |
| 7    | 7    | 7    |
| 8    | 8    | 8    |
| 1    | 1    | 1    |
| 2    | 2    | 2    |
| 3    | 3    | 3    |
| 4    | 4    | 4    |
| 5    | 5    | 5    |
| 6    | 6    | 6    |
| 7    | 7    | 7    |
| 8    | 8    | 8    |

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- \* Reference measurement procedure has been defined
- \* Certified reference material
  - \* Defined the optimal condition for sample preparation and lyophilization
  - \* Composition in Hb similar to that of blood (Hbtot, MetHb)
  - \* Good commutability (for the methods tested)
  - \* HbA<sub>2</sub> stable at least for 7 years at +4°C or -20°C (lyophilized form)
  - \* First pilot batch produced at the JRC in April 2019

\* **Conclusions**

- \* **Reference measurement procedure (RMP):**
  - \* to be finalized for some aspects (linearity, recovery)
  - \* approved by IFCC (ballot)
- \* **Certified reference materials (CRMs):**
  - \* to be prepared in at least one large batch
  - \* to be characterized (homogeneity, stability, title..)
  - \* to be distributed and used (manufacturers)
- \* **Definition of a roadmap for the standardization of HbA<sub>2</sub> as a support to the optimal use of clinical guidelines**
- \* **Outcome: screening procedures to be optimized, more careful requirements for molecular analysis**

\* **Next steps**

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## \* Standardization of Hb A<sub>2</sub> measurement Joint Working Group of the IFCC & ICSH

### IFCC

A. Mosca (IFCC WG, IT), I. Young (JCTLM, UK), S. Sandberg (WHO liaison, head of laboratory, NO), F. Ceriotti (laboratory professional, IT), Christa Cobbaert (head of laboratory, NL)

### ICSH

A. Stephens (haematologist, UK), B. Wild (laboratory professional, UK), B. De La Salle (EQAS provider, UK), G. Zini (WHO liaison, IT), K. Hartevelde (laboratory professional, NL), S. Machin (ICSH President, UK)

