Achieving Comparability with IFCC Reference Method for the Measurement of Hemoglobin A\textsubscript{1c} by Use of An Improved IDMS Method

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Outline

- Importance of HbA$_{1c}$ measurement and its standardisation.
- Principle and procedure of IDMS method for HbA1c measurement.
- Traceability of IDMS procedure.
- Key steps to ensure the accuracy and traceability of IDMS method.
- HSA External Quality Assessment (EQA) Programme and Certified Reference Materials for HbA$_{1c}$ measurement.
- Conclusion
Importance of HbA$_{1c}$ Measurement

- Haemoglobin A$_{1c}$ (HbA$_{1c}$) is an important biomarker for the diagnosis of diabetes mellitus.
  
  WHO recommendation: a HbA$_{1c}$ level of 6.5% as the cut point for diagnosing diabetes.

- HbA$_{1c}$ is an effective biomarker for monitoring the long term blood glucose level in diabetic patients to ensure proper treatment.

  In Singapore, one in nine (11.3%) residents aged 18 to 69 has been diagnosed with diabetes mellitus. HbA$_{1c}$ measurement is used to monitor glycemic control to ensure proper treatment and management of diabetes.

  - HbA$_{1c}$ < 7%: optimal glycemic control
    Treatment: mainly nutrition therapy and exercise,
  
  - HbA$_{1c}$ 7 – 9%: “sub-optimal” glycemic control,
    Treatment: mainly metformin therapy
  
  - HbA$_{1c}$ > 9%: “poor” glycemic control
    Treatment: metformin therapy, alternatively insulin therapy
Standardisation of HbA₁c Measurement

- Different reference systems with insufficient consistency with one another:
  - US [National Glycohemoglobin Standardization Program (NGSP)]
  - Japan [Japanese Diabetes Society (JDS)/Japanese Society of Clinical Chemistry (JSCC)]
  - Sweden

  - Purified HbA₀ and HbA₁c as the calibration standards
  - Purity of calibration standards determined by ion exchange chromatography

- Significant biases were found between IFCC and other reference systems.
  - Master equations are used for conversion, for example:
    \[ \text{NGSP} \% = 0.09148 \times \text{IFCC (mmol/mol)} + 2.152 \]

- It would be desirable to have an alternative accuracy-based reference method as an independent support for the accuracy of IFCC reference method.
IDMS Method for HbA$_{1c}$ Measurement

- Based on proteolysis of HbA$_{0}$ and HbA$_{1c}$, using endoproteinase Glu-C.

- Separate quantification of HbA$_{0}$ and HbA$_{1c}$ by IDMS method, using two signature hexapeptides as the calibration standards. 
  
  \[ \text{HbA}_{1c} \text{ Level} = \frac{\text{HbA}_{1c}}{\text{HbA}_{1c} + \text{HbA}_{0}} \]

- Purities of the hexapeptides as calibration standards were determined by another step of IDMS measurement using amino acid CRMs as the calibration standards.
IDMS Procedure for HbA1c Measurement

β chain N-terminal of HbA₀

Spiking of isotope labelled hexapeptides

Glu-C

β chain N-terminal of HbA₁c

Proteolysis using Glu-C

LC-MS/MS measurement of the peptides using custom-synthesised VE and GE as calibration standards
IDMS Procedure for Determination of the Purity of Hexapaptides as Calibration Standards (VEc and GEc)

Custom-synthesised VE as calibration standard for HbA₀ (VEc)

Custom-synthesised VE as calibration standard for HbA₁c (GEc)

Spiking of isotope-labelled L-proline and L-leucine

Hydrolysis using 6 N HCl

LC-MS/MS measurement of amino acids using amino acid CRMs as calibration standards
Traceability of IDMS Method

Key steps:
1. proteolysis of HbA₀ and HbA₁c
2. hydrolysis of VEc and GEc
Complete Proteolysis of HbA₀ and HbA₁c

Optimisation of the amount of endoproteinase Glu-C. The error bar of each point was estimated using the pooled CV of VE or GE results in haemolysate samples.
Identification of Impurities in Hexapeptides Calibration Standards (VEc and GEc)

- VE was found in GEc as a major impurity.
- Another step of IDMS measurement was performed to quantify the amount of VE in Gec using VEc as calibration standard.
- The purity value of GEc from IDMS measurement for amino acids was corrected accordingly.

- The purity of VEc was found to be satisfactory.
- The purity value of VEc from IDMS measurement for amino acids was used without correction.
Participation in RELA Comparisons for HbA$_{1c}$

- Except our laboratory, all other laboratories used IFCC reference method.
- Relative expanded uncertainties of IDMS method: 2.6 – 2.8% (IFCC) or 1.6 – 2.2% (NGSP).
- Inter-laboratory CV in RELA 2013 and 2014: 1.6 – 3.2% (IFCC) or 1.2 – 1.9% (NGSP).
- Desirable CVs of HbA1c measurement (NGSP): 2% (intra-laboratory) and 3.5% (Inter-laboratory).
- IDMS method is comparable with IFCC reference method.
HSA External Quality Assessment (EQA) and Certified Reference Material for HbA$_{1c}$

- HSA organises an accuracy-based EQA programme (including HbA$_{1c}$) for the local clinical laboratories.

- The main objective of the programme is to provide metrologically traceable assigned values to evaluate the results of the participating clinical laboratories.

- All target values (including HbA$_{1c}$) are independently determined by HSA using high accuracy methods (IDMS or standard addition methods).

- The materials for HbA$_{1c}$ in 2015 HSA Programme have been developed as Certified Reference Materials.

  HRM-3003A HbA$_{1c}$ in Frozen Human Blood (two concentration levels).
Conclusion

- An alternative reference measurement method for HbA\textsubscript{1c} based on IDMS was developed.
- The developed IDMS method has clear traceability to the SI by use of amino acid CRMs.
- It gives results which are comparable with those of the IFCC reference method.
- The IDMS method can be regarded as an alternative accuracy-based reference method for HbA\textsubscript{1c} measurement, which provides an independent support for the accuracy of the IFCC reference method.
- The IDMS method has been used to provide the assigned/certified values for the HSA EQA Programmes and CRMs for HbA\textsubscript{1c}.
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