Achieving Comparability with IFCC Reference Method for the Measurement of Hemoglobin A_{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 HbA_{1c} 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
- IFCC reference method – the accuracy-based reference method for standardisation of HbA1c measurement.
  - Purified HbA_0 and HbA_{1c} 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:

  NGSP (%)= 0.09148 × 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.

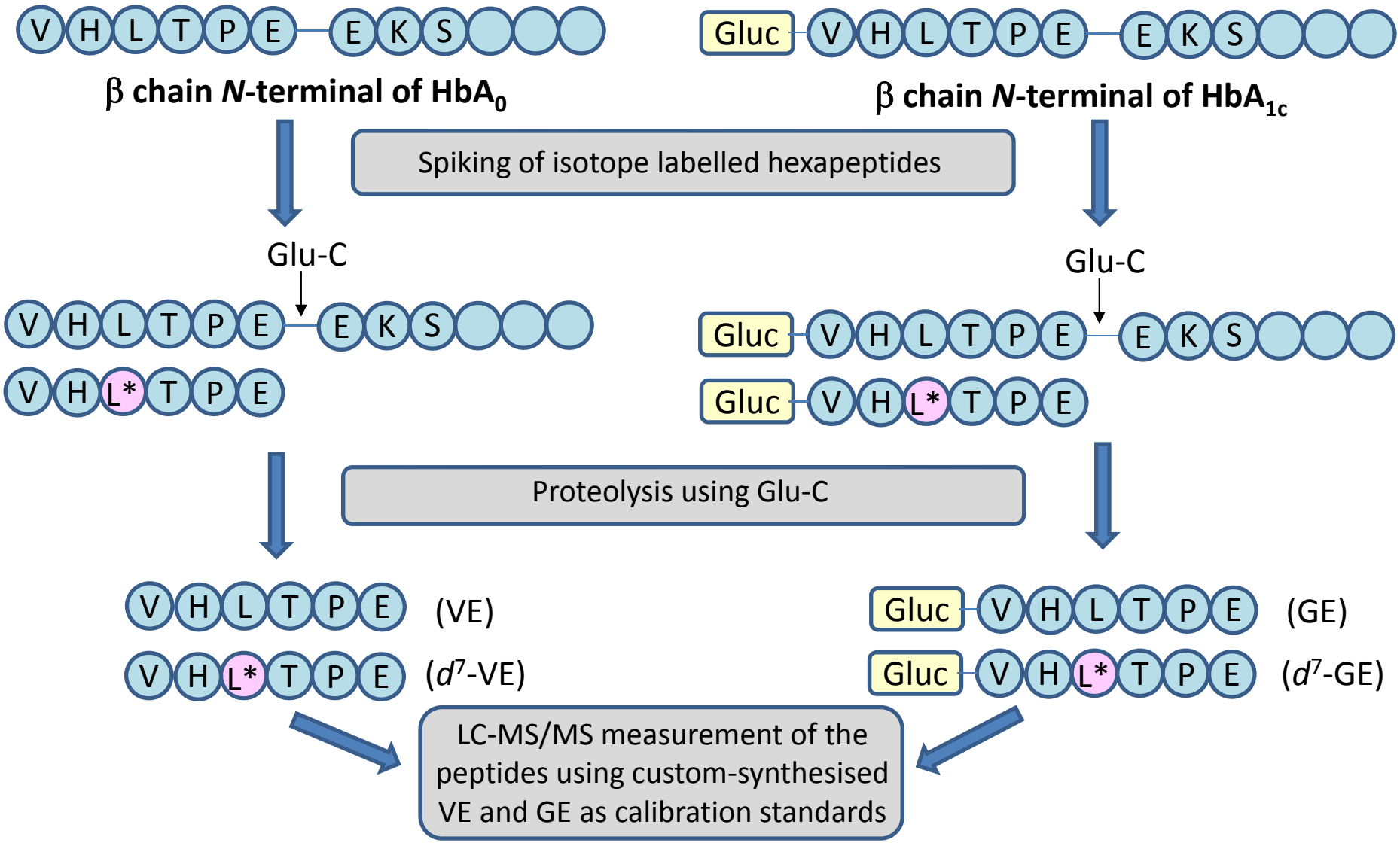
IDMS Method for HbA_{1c} Measurement

- Based on proteolysis of HbA_0 and HbA_{1c}, using endoproteinase Glu-C.

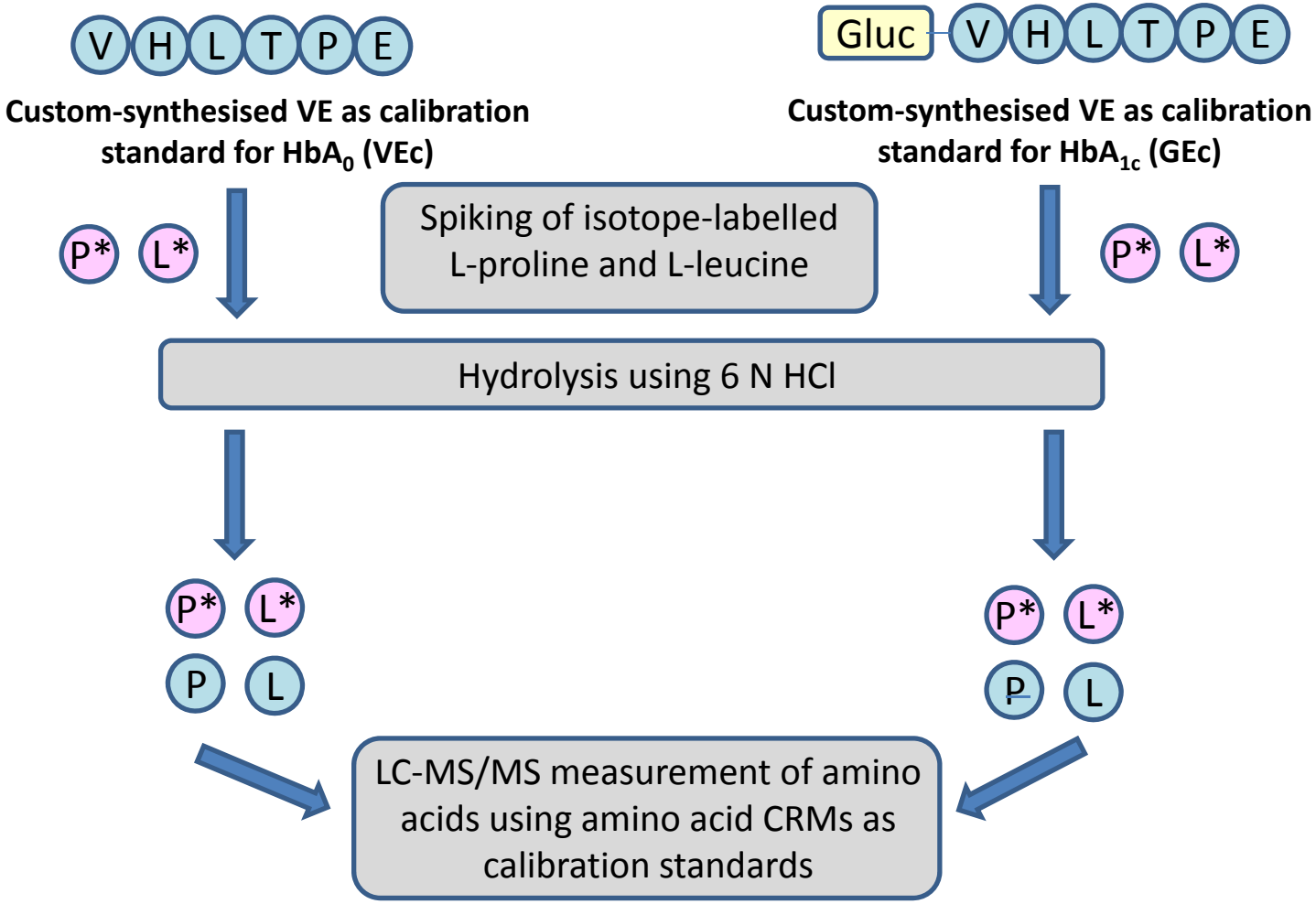
- Separate quantification of HbA_0 and HbA_{1c} by IDMS method, using two signature hexapeptides as the calibration standards.
  HbA_{1c} Level = HbA_{1c} /(HbA_{1c}+ HbA_0)

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

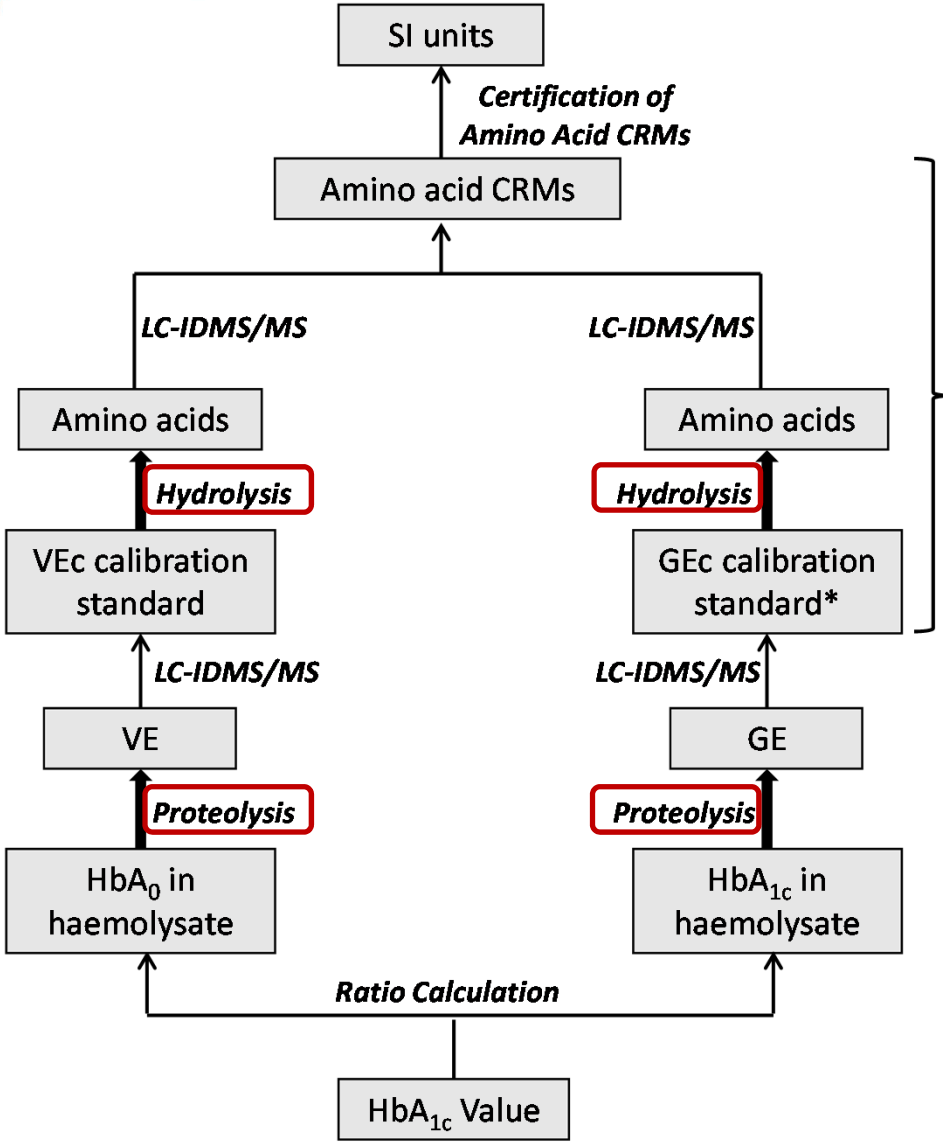
IDMS Procedure for HbA1c Measurement



IDMS Procedure for Determination of the Purity of Hexapaptides as Calibration Standards (VEc and GEc)



Traceability of IDMS Method



(A) Determination of hexapeptide purity

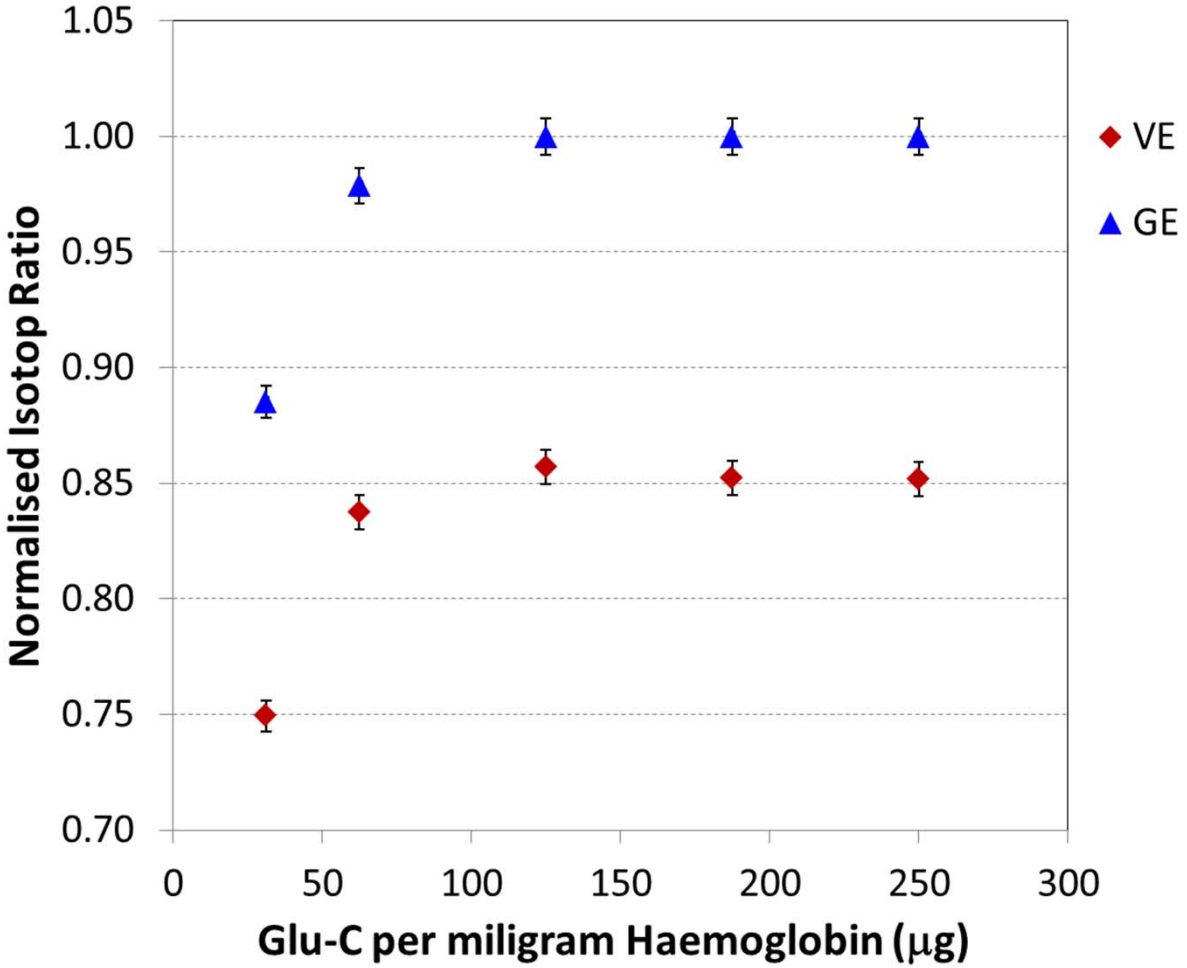
(B) Measurement of HbA_{1c} in haemolysate samples

Key steps:

(1) proteolysis of HbA_0 and HbA_{1c}

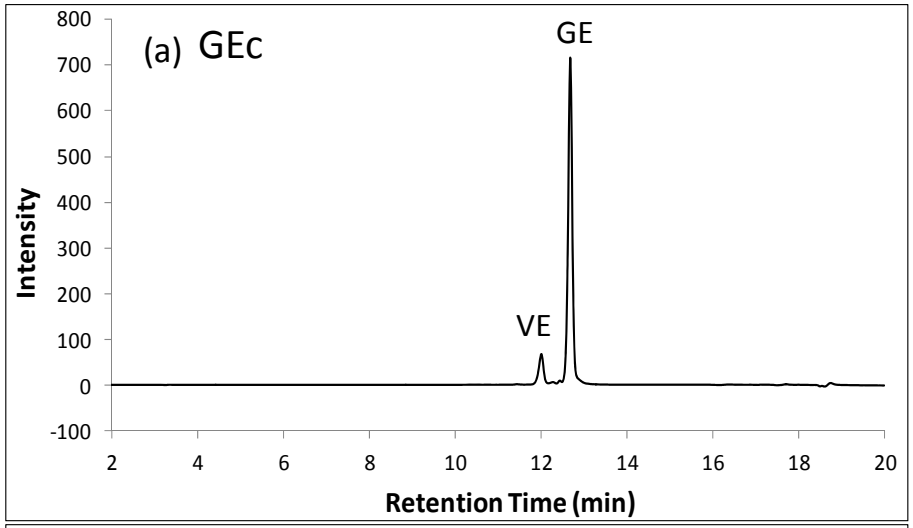
(2) hydrolysis of VEc and GEc

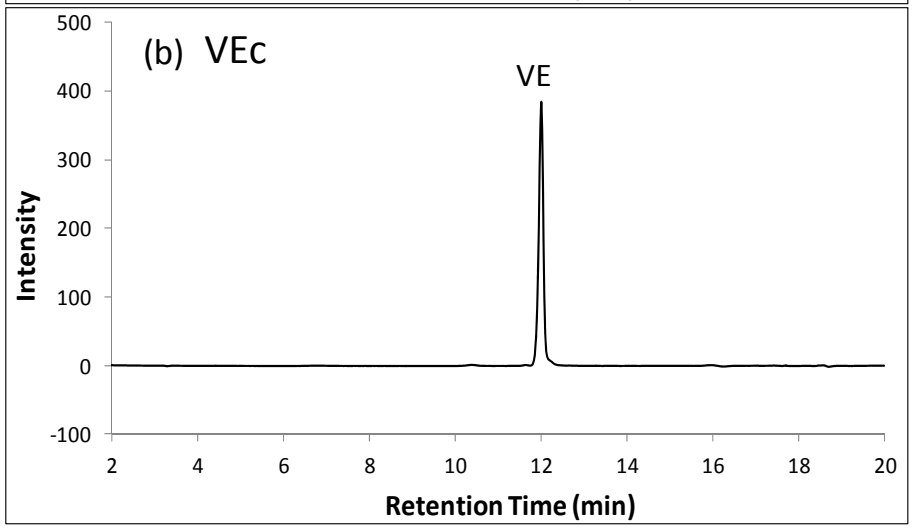
Complete Proteolysis of HbA_0 and HbA_{1c}



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)

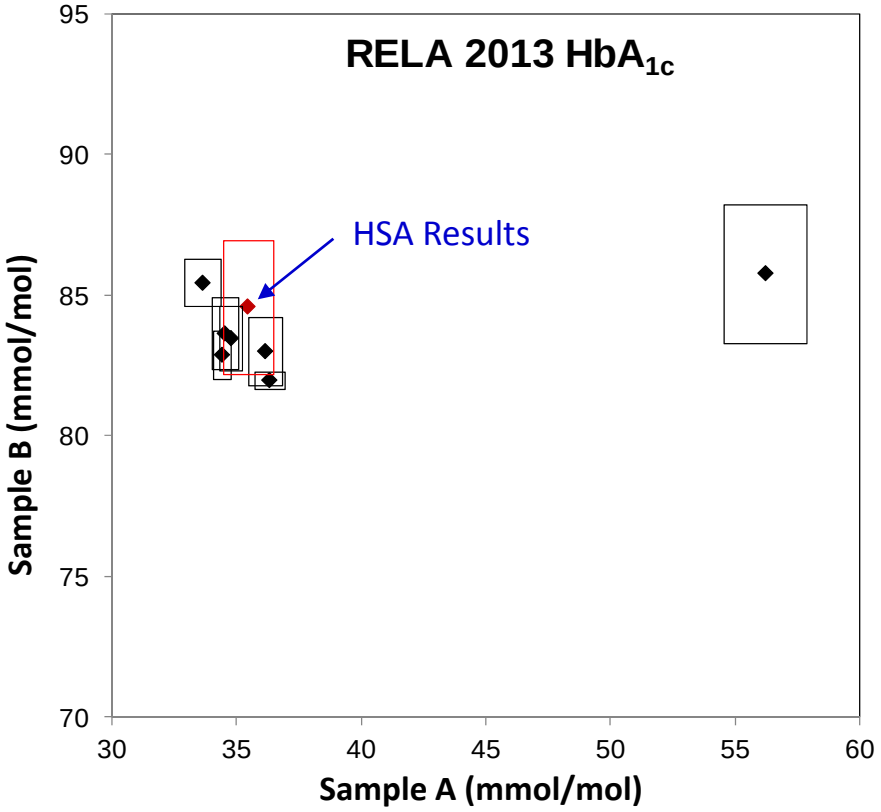


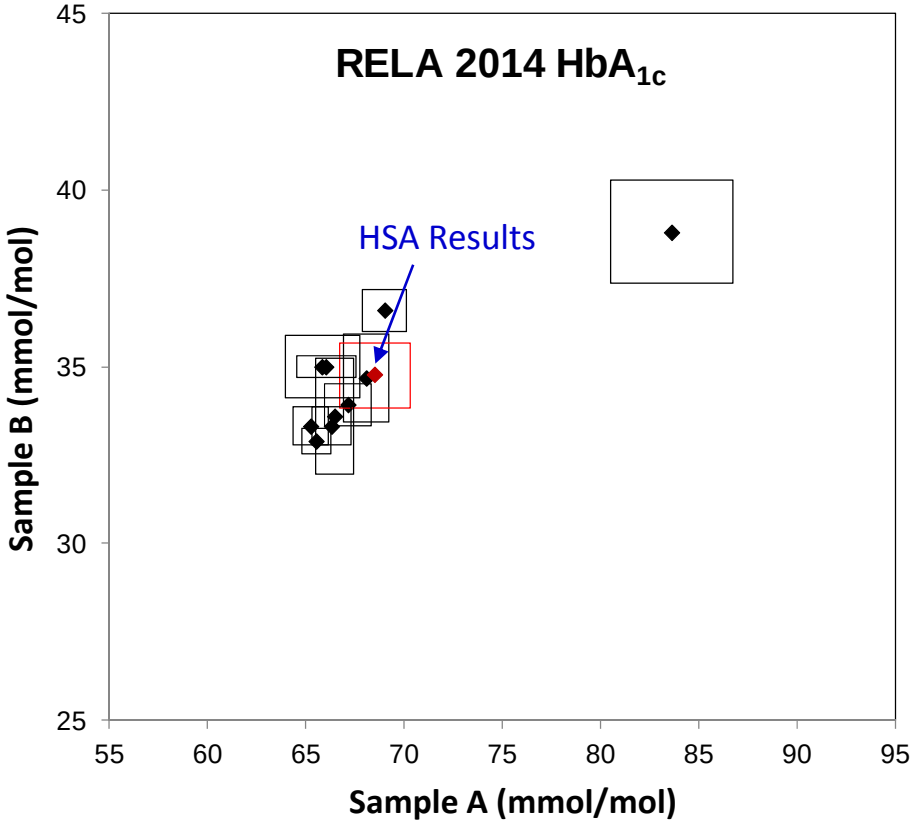


- VE was found in GEc as a major impurity.
- Another step of IDMS measurement was performed to quantify the amount of VE in Gec using VEc as calibration standard.
- The purity value of GEc from IDMS measurement for amino acids was corrected accordingly.

- 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_{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_{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_{1c}.

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Thank you