**Chemical Metrology Division**
**Applied Sciences Group**
**Health Sciences Authority**
1 Science Park Road, #01-05/06,
The Capricorn, Singapore Science Park II,
Singapore 117528
Tel: 65 6775 1605 Fax: 65 6775 1398
Website: www.hsa.gov.sg
Email: HSA_CML@hsa.gov.sg

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# *Certificate of Analysis*

## CERTIFIED REFERENCE MATERIAL
## HRM-3003B

## Haemoglobin $A_{1c}$ in Human Blood

### Batch Number
STY-0018-045
STY-0018-046
STY-0018-062

### Foreword
A unit of the certified reference material (CRM) HRM-3003B consists of three vials of frozen human blood sample with different haemoglobin $A_{1c}$ ($HbA_{1c}$) levels. Each vial contains 0.5 mL of frozen human blood. The blood materials appear as a red liquid after thawing.

The CRM is produced with reference to the requirements set out in ISO/IEC 17025:2017 [1], ISO 17034:2016 [2] and ISO Guide 35:2017 [3].

### Certified Values
The certified values for $HbA_{1c}$ in HRM-3003B are provided in Table 1 with the unit of mmol/mol (IFCC) and in Table 2 with the unit of % (NGSP). The certified values listed in Table 2 were obtained by converting the certified values in mmol/mol using the equation below [4]:

$$NGSP(\%) = 0.09148 \times IFCC(mmol/mol) + 2.152$$

**Table 1. Certified Values of $HbA_{1c}$ in HRM-3003B**

| | STY-0018-045 | STY-0018-046 | STY-0018-062 |
|---|---|---|---|
| **Analyte** | **(mmol/mol)** | **(mmol/mol)** | **(mmol/mol)** |
| **$HbA_{1c}$** | 35.1 ± 2.0 | 50.3 ± 1.9 | 65.8 ± 2.6 |

**Table 2. Certified Values of $HbA_{1c}$ in HRM-3003B**

| | STY-0018-045 | STY-0018-046 | STY-0018-062 |
|---|---|---|---|
| **Analyte** | **(%)** | **(%)** | **(%)** |
| **$HbA_{1c}$** | 5.36 ± 0.19 | 6.75 ± 0.18 | 8.17 ± 0.24 |

Each certified value is the mean of measurements of at least eight samples taken from a minimum of four different bottles. The certified values for HRM-3003B were determined using liquid

chromatography - isotope dilution tandem mass spectrometry (LC-IDMS/MS). A four-point calibration curve was used in the measurements.

The associated measurement uncertainty of each certified value was estimated in accordance with ISO/IEC Guide 98-3:2008 [5]. The expanded uncertainty (coverage factor of 2) corresponded to a level of confidence of about 95%.

**Validity**

The certified values of HRM-3003B are valid within the specified measurement uncertainty until **19 May 2026**. The validity of HRM-3003B will be extended if it is tested to be sufficiently stable for continuous use. The certified values of HRM-3003B are invalid when the blood material has deteriorated or is mishandled.

**Source of Materials**

The blood materials were obtained from Aalto Scientific Ltd. based in the United States.

**Commutability**

Commutability study of HRM-3003B was conducted by analysing 25 patient specimens and the CRM by both LC-IDMS/MS method and the routine methods using various analysers, which included Bio-Rad D10, Bio-Rad Variant II, Roche Cobas 6000 c501, Roche Cobas 8000 c502, Abbott Architect c8000, and Beckman Coulter UniCel DxC 800. The results demonstrated satisfactory commutability of HRM-3003B for Bio-Rad D10, Bio-Rad Variant II, Roche Cobas 6000 c501, and Roche Cobas 8000 c502. For Abbott Architect c8000, two levels of HRM-3003B (STY-0018-045 and STY-0018-046) were found to be commutable; while for Beckman Coulter UniCel DxC 800, all three levels of HRM-3003B were found to be non-commutable. This suggested that although the commutability of HRM-3003B is satisfactory for most of the clinical analysers, it may not be satisfactory for all clinical analysers. For some individual clinical analysers such as Beckman Coulter UniCel DxC 800, there may be some deviations between the results from routine testing methods and the certified values of HRM-3003B.

**Homogeneity**

Homogeneity testing on $HbA_{1c}$ was performed on two subsamples taken from eleven vials. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used. The sample size taken for homogeneity testing was about 30 µL. No significant differences in the between and within-vial variances were found using *F*-test (ANOVA) at 95 % confidence level. The $u_{bb}$ was evaluated from the uncertainty due to between-vial inhomogeneity.

**Stability**

Evidence on the stability of $HbA_{1c}$ in HRM-3003B is based on prior experience of stability from two other batches of closely similar human blood materials. The materials were also characterised for $HbA_{1c}$, produced by the same supplier, contained in the same packaging and stored under the same condition. Their stability was assessed on four occasions over a period of up to 280 days. The results showed that the $HbA_{1c}$ in all batches were stable when stored at below – 70 °C over the study period [3]. Using the experimental evidence from prior experience, the standard error of the slope of the results was used for the estimation of $u_{stab}$ for all batches of materials for HRM-3003B.

**Analytical Methods**

A fully validated LC-IDMS/MS method was used to determine the certified values of $HbA_{1c}$ in HRM-3003B [6,7]. The method involved proteolysis of $HbA_{1c}$ and $HbA_0$ using endoproteinase Glu-C, separation using an Agilent Zorbax Eclipse Plus C18 column, followed by tandem mass spectrometric measurements of the signature peptides of $HbA_{1c}$ and $HbA_0$. The purity of the signature peptides used as the calibration standards were determined by amino acid analysis (L-proline and L-leucine) using a LC-IDMS/MS method.

**Metrological Traceability**

The certified values are traceable to the International System of Units (SI) through the use of L-proline and L-leucine CRMs (HRM-1007A and HRM-1008A, respectively) from Health Sciences Authority, Singapore.

**Intended Use**

HRM-3003B is intended for use in the validation of methods or as quality control materials for the determination of $HbA_{1c}$ in human blood. Users may refer to ISO Guide 33:2015 [8] for the recommended statistical treatment of the certified reference value and the associated uncertainty of the CRM as control materials.

**Warning and Safety Precautions for Users**

**HRM-3003B is intended for in-vitro use only and shall be handled as a biohazardous material with the potential of transmitting infectious disease. Hence, this material shall be handled using biosafety level 2 (or higher) practices, equipment, and facility** [9].

**Instructions for Use**

**HRM-3003B should be treated the same as patient specimens. Accordingly, these materials should be handled and disposed according to associated regional, national and local legislation and regulations for any potentially infectious human or blood specimen.**

Prior to use, the CRM should be thawed at room temperature (between 18 $^{o}$C to 25 $^{o}$C), then analysed immediately. The materials should be mixed well by gentle swirling before withdrawing any aliquots. The certified values may not be valid for re-thawed and opened bottles as the stability of $HbA_{1c}$ subjected to such conditions has not been investigated.

The recommended minimum sample size of HRM-3003B is 30 µL. The certified values may not be valid if smaller amounts are taken.

**Transport and Storage**

The CRM is transported in frozen state (in dry ice). Upon receipt, it should be stored at below – 70 $^{o}$C. The CRM should not be exposed to sunlight or ultraviolet radiation. Storage of the thawed material at room temperature or in the refrigerator may result in changes in the certified values of the $HbA_{1c}$.

**Further Information**

Please direct all enquiries regarding this CRM to the contact in this Certificate.

**References**


[1] ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.
[2] ISO 17034:2016 General requirements for the competence of reference material producers.
[3] ISO Guide 35:2017 Reference materials – General and statistical principles for certification.
[4] IFCC Standardization of HbA1c, http://www.ngsp.org/ifccngsp.asp, accessed 25 Jul 2017.
[5] ISO/IEC Guide 98-3:2008 Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM: 1995).
[6] Wong, L.; Liu, H.; Yong, S.; Liu, Q.; Lee, T.K.; Clin. Chem., 2015, 61, 435-436.
[7] Liu, H.; Wong, L.; Yong, S.; Liu, Q.; Lee, T.K.; Anal. Bioanal. Chem., 2015, 407, 7579-7587.
[8] ISO Guide 33:2015 Reference materials – Good practice in using reference materials.
[9] U.S Department of Health and Human Services; Biosafety in Microbiological and Biomedical Laboratories, 5th ed.; HHS Publication No. (CDC) 21-1112.

**Note**

*HSA does not assume any liability with respect to any loss caused by improper use and/or storage of the CRM by the customer.*

**Certificate Revision Records**

Certificate of Analysis CML-HRM-J3003B/02 replaces Certificate of Analysis CML-HRM-J3003B/01 issued on 28 Jul 2017.

Certificate of Analysis CML-HRM-J3003B/03 replaces Certificate of Analysis CML-HRM-J3003B/02 issued on 18 May 2018.

Certificate of Analysis CML-HRM-J3003B/04 replaces Certificate of Analysis CML-HRM-J3003B/03 issued on 04 Apr 2019.

Certificate of Analysis CML-HRM-J3003B/05 replaces Certificate of Analysis CML-HRM-J3003B/04 issued on 08 Apr 2020.

Certificate of Analysis CML-HRM-J3003B/06 replaces Certificate of Analysis CML-HRM-J3003B/05 issued on 17 May 2021.

Dr Teo Tang Lin
Division Director
Chemical Metrology Laboratory
Chemical Metrology Division