Reference Material Institute for Clinical Chemistry Standards (ReCCS)

Certified Reference Material for Measurement of HbA_{1c}

JCCRM 411-4

ASNITE 0006 R

Certificate of Analysis

This Certified Reference Material (CRM) was prepared and certified according to ISO/IEC 17025, ISO 15195 and ISO GUIDE 34. The preparation, standardization and measurement processes were same as for JCCRM 411-3 (JDS Lot 5), and NGSP and IFCC certified values were assigned. The JDS values were assigned based on JCCRM 411-3 (JDS lot 5) values and this lot is equivalent to JDS Lot 6.

■ Instruction for use

1. Take out a plastic vial containing this CRM and allow it to stand at room temperature for about 10 minutes until it naturally thaws.
2. Mix the content of the vial using such devices as a Vortex mixer.
3. Collect the content at the bottom of the vial, and take the necessary amount using such devices as a micro syringe or a micropipette. If the entire content of the vial needs to be collected at the bottom, centrifuge the vial at 1,000 rpm for about 30 seconds.
4. The total Hb concentration of each level is approx. 130～145g/L.

Note 1) After thawing, do not allow the vial to stand at room temperature for an extended period of time. Also, once thawed, this reference material cannot be refrozen to be used again.

■ Precautions for use In-Vitro Use only

This CRM is intended for in-vitro diagnostic use only. This is a human source material. Handle this product as a biohazardous material capable of transmitting infectious disease.
However, no known test method can offer complete assurance that hepatitis B virus, hepatitis C virus, HIV, or other infectious agents are absent from this material. This product should be handled at the Biosafety Level 2 or higher as recommended for any potentially infectious human serum or blood specimen in the Center for Disease Control (CDC)/ National Institutes of Health (NIH) Manual.

■ Preparation

This CRM was prepared as follows: Erythrocytes were separated by centrifuging human whole blood free of abnormal hemoglobin, and then washed several times, labile eliminated, and hemolysed. Next, through the use of a high-speed centrifuge, erythrocyte ghost membranes were removed, and after adding a physiological solution, containing sodium hydrogen carbonate (for pH stabilization), the resulting solution was dialyzed. The dialyzed solution was divided into smaller portions and stored in liquid nitrogen (As a result, this reference material does not contain plasma components). In order to avoid the use of preservatives, only sterilized tools were used, and to ensure storage stability, the reagents used were sterilized by filtration.

■ Expiration date

This CRM must be received with dry ice remaining and stored immediately at a temperature below –70°C. In this condition this CRM shelf life is 12 months after the date of your (end user's) receipt.

■ Specifications

Configuration:	Frozen liquid
HbA1c levels:	Five concentration levels
Contents of a set:	0.1 ml/vial, one vial for each levels, total 5 vials

■ Storage after purchasing

1. This CRM is shipped in frozen condition on dry ice. On receipt, some dry ice must still remain in the shipping box. This CRM is unusable if no dry ice is left upon receipt.
2. Upon receipt, a case containing this CRM is taken out and immediately placed in a deep freezer (< –70˚C) where temperature variation is minimal (at the bottom of a freezer).
   Note: When intending to use this CRM on the day when it is received, store it in a refrigerator till use.

■ Date of certification: September 26 , 2018

■ Intended use

This CRM is primarily intended for use in the calibration of routine methods (HPLC, Immunoassays, Enzymatic methods, etc.), also in evaluating the accuracy of clinical procedures for the determination of HbA1c.

■ Certified values

1) HbA1c (NGSP)

	HbA1c (NGSP) %	Expanded uncertainty %
Level 1	**5.08**	**0.20**
Level 2	**5.80**	**0.16**
Level 3	**7.43**	**0.17**
Level 4	**9.58**	**0.21**
Level 5	**12.02**	**0.29**

The expanded uncertainties (calculated with coverage factor k=2.6, corresponding to 95% confidence level) were calculated according to the ISO GUM.

The NGSP values (%) were determined, based on NGSP ASRL#1 values using CPRL reference panel (100 specimens, each having a CPRL adj. value) as a calibrator for KO500 HPLC.

The NGSP certified values agree well with the following values, determined by the NGSP Laboratory Network SRLs (ASRL#1, SRL#9, SRL#10, SRL#11, ESRL#10, ESRL#11, ESRL#12, ESRL#13, ESRL#14): 5.07, 5.74, 7.39, 9.60, 12.13.

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2) HbA1c (IFCC)

	HbA1c (IFCC) mmol/mol	Expanded uncertainty mmol/mol
Level 1	**30.5**	**1.0**
Level 2	**37.2**	**1.9**
Level 3	**53.9**	**2.3**
Level 4	**76.7**	**2.4**
Level 5	**102.5**	**3.5**

The expanded uncertainties (calculated with coverage factor k=2.3 (levels 1,4,5), k=2.4 (levels 2,3), corresponding to 95% confidence level) were calculated according to the ISO GUM (1) (2).

The above HbA1c (IFCC) values were measured by the following IFCC HbA1c Network Laboratories: ReCCS (LC-MS), IBM (LC-MS), Keio Univ. Hospital (LC-MS), Instand e.v. (LC-MS), Isala Klieneken (CE), Queen Beatrix Hospital (CE).The HbA1c concentrations were quantified according to the reference measurement procedure of the IFCC reference method using the primary calibrators pcal (supplied by the IFCC HbA1c WG).

■ Characteristics (reference value)

Item	Specification	Results	Measurement method
Material	Human whole blood (n>20)	Human whole blood (n=20～30)	
Additives	None	None	
HbF	<1%	<1%	KO500 method
MetHb	<6%	2～3%	spectrometry
Glutathione adduct	<0.5%	0～0.3%	KO500 method
Abnormal Hb	None	None	KO500 method
Plasma components	None	None	

Specifications were established by the Committee on Standardization of Laboratory Testing Related to Diabetes Mellitus of Japan Diabetes Society (JDS).

■ **HbA1c (JDS) (reference value)**

	HbA1c (JDS) %	Expanded uncertainty %
Level 1	4.71	0.13
Level 2	5.34	0.13
Level 3	6.96	0.15
Level 4	9.12	0.17
Level 5	11.56	0.25

The JDS values were measured by the JSCC/JDS Designated Comparison Method (KO500 method), which is a high-resolution HPLC using as a calibrator JCCRM 411-3 (JDS Lot 5). Assays were performed by the following reference laboratories approved by the Japan Reference Measurement Institute (JRMI) on behalf of JSCC: ReCCS , Institute of Biopathological Medicine and Hoshi University.

■ **Comparison between JSCC/JDS and NGSP**

Direct Comparison between JSCC/JDS DCM and NGSP CPRL (Central Primary Reference Laboratory) was made, and a relation NGSP=1.02×JDS+0.25, has been established in 2011. The repeated measurement in 2018 has confirmed this equation. Monitoring tests for NGSP SRLs also show a good agreement with this equation.

■ **Reference**

1) Guide to the expression of uncertainty in measurement, ISBN 92-67-10188-9,1st Ed. ISO, Geneva, Switzerland (corrected and reprinted, 1995)
2) Konnert A, et al. Uncertainty calculation for calibrators of the IFCC HbA1c standardization network. Accred Qual Assur 11(7):319-328, 2006.
3) Jeppsson, JO, et al. Approved IFCC reference method for the measurement of HbA1c in human blood. Clin Chem Lab Med 40:78-89, 2002.
4) Andreas Finke, et al. Preparation of a candidate primary reference material for the international standardisation of HbA1c determinations. Clin Chem Lab Med 36(5):299-308, 1998.
5) Tominaga M, Rinsyo byouri 49:71199-1204, 2001.
6) Tominaga M, et al. (Committee on Standardization of Laboratory Testing Related to Diabetes Mellitus of Japan Diabetes Society), Japanese standard reference material for JDS Lot 2 haemoglobin A1c.I:comparison of Japan Diabetes Society-assigned values to those obtained by the Japanese and USA domestic standardization programmes and by the International Federation of Clinical Chemistry reference laboratories. Ann Clin Biochem 42:41-46, 2005.
7) Rinsyokagaku 38:163-176, 2009.

Supply and Certification

Reference Material Institute for Clinical Chemistry Standards (ReCCS)
1-3-3, Azaminominami, Aoba-ku, Yokohama , 225-0012 Japan
TEL: +81-45-507-6145
FAX:+81-45-530-9036
E-mail: ando@reccs.net.
http://www.reccs.or.jp/

Hirohito Umemoto Ph. D.

Hirohito Umemoto, Ph.D.