*Reference Material Institute for Clinical Chemistry Standards (ReCCS)*

## Certified Reference Material for Measurement of HbA1c

# JCCRM 411-5
# Certificate of Analysis

Accredited
ASNITE 0006 RMP

## ■ Intended use

This Certified Reference Material (CRM) was manufactured and certified by ReCCS according to ISO 17034 (general requirements regarding the ability of reference material producers), ISO/IEC17025 (requirements of calibration laboratory) and ISO15195 (requirements of clinical laboratory standard measurement institutes).

This CRM is intended primarily for use in calibrating and evaluating the accuracy of routine methods.

## ■ Certified concentration values and uncertainties for HbA1c (NGSP values)

The certified values and uncertainties at 25℃ of concentrations are shown below. The values in Table 1 are traceable to the DCCT values which maintained by the NGSP Central Primary Reference Laboratory (CPRL).

**Table 1. Certified concentration values and uncertainties for HbA1c (NGSP values)**

Unit: %

| | Certified value | Expanded uncertainty |
|---|---|---|
| JCCRM 411-5 Level 1 | 5.16 | 0.18 |
| JCCRM 411-5 Level 2 | 5.80 | 0.20 |
| JCCRM 411-5 Level 3 | 7.47 | 0.25 |
| JCCRM 411-5 Level 4 | 9.62 | 0.34 |
| JCCRM 411-5 Level 5 | 11.92 | 0.42 |

The expanded uncertainty U was obtained according to the ISO guideline (GUM: Guide to the Expression of Uncertainty in Measurement)[1)] by multiplying the combined standard uncertainty Uc which is a composite of measurement uncertainty, homogeneity and stability uncertainty, by a coverage factor *k*. The coverage factor *k* is 2.0.

## ■ Certified concentration values and uncertainties for HbA1c (IFCC values)

The certified values and uncertainties at 25℃ for HbA1c (NGSP) are shown below.
These values are determined in accordance with ISO/IEC 17025 and ISO 15195, and are accepted internationally via ILAC/APLAC MRA.

ilac-MRA

Accredited
ASNITE 0006 Calibration

**Table 1. Certified concentration values and uncertainties for HbA1c (IFCC values)**

Unit: mmol/mol

| | Certified value | Expanded uncertainty |
|---|---|---|
| JCCRM 411-5 Level 1 | 31.1 | 1.1 |
| JCCRM 411-5 Level 2 | 37.4 | 1.4 |
| JCCRM 411-5 Level 3 | 54.2 | 1.6 |
| JCCRM 411-5 Level 4 | 77.0 | 2.3 |
| JCCRM 411-5 Level 5 | 102.6 | 3.0 |

The expanded uncertainty U was obtained according to the ISO guideline (GUM: Guide to the Expression of Uncertainty in Measurement)[1)] by multiplying the combined standard uncertainty Uc which is a composite of measurement uncertainty, homogeneity and stability uncertainty, by a coverage factor *k*. The coverage factor *k* is 2.0.

Sample

## ■ Measurement methods for certified values and Traceability

The NGSP values for HbA1c of this CRM were measured by Reference Material Institute for Clinical Chemistry Standards (ReCCS, ASRL#1) using HPLC method [2,3]. ReCCS is a secondary reference laboratory (ASRL#1) of the NGSP Network Laboratory, and the agreement of the measured values with the CPRL values is confirmed through monthly monitoring comparison.

The IFCC values for HbA1c of this CRM were measured by the IFCC reference method [4]. The measurements were performed at two Reference Laboratories at IFCC HbA1c Network, ReCCS (LC-MS method) and Keio University School of Medicine (LC-MS method). The IFCC primary calibrator (Network primary calibrator, Pcal 2023) [5,6] was used as the calibrator for measurement and were measured by the IFCC reference method.

## ■ Instructions for use

Remove a glass vial containing this CRM from the bag and stand it by placing the vial upright. Leave the vial to stand for about 10 minutes to bring the temperature of the content to room temperature. Mix the content of the vial using such devices as a Vortex mixer. 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. This CRM is diluted and used according to the instructions of the HbA1c measuring device or reagent to be used.

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 was prepared from human whole blood and shown to be negative to the HBs antigens, HCV antibodies and HIV antibodies. However, this does not completely rule out its infectivity, and when handling this CRM, exercise the same caution used for any other patient specimens.

## ■ 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, using 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.

## ■ Storage and expiration

This CRM is shipped in frozen condition on dry ice. Store this CRM in a deep freezer immediately after receiving it. Confirm that dry ice remains on arrival. If no dry ice remains, the CRM cannot be used.

The expiration date from the shipping date is as bellow:

| | |
|---|---|
| Below −70 ℃ | 6 months |

The Shipping date is labeled at the outer label.

## ■ Product specifications

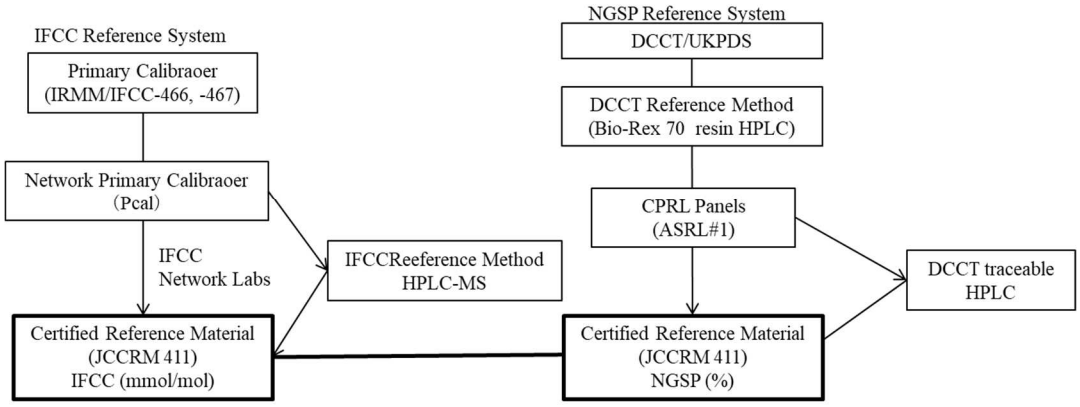
| | |
|---|---|
| Configuration: | Frozen liquid |
| HbA1c levels: | Five concentration levels |
| Contents of a set: | 0.1 ml/vial, one vial for each level, total 5 vials |

## ■ Date of Certification

July 3, 2025

Sample

## ■ Measurement System




## ■ Characteristics of This CRM

The characteristics of this CRM are as follows. Specifications were established by the Committee on Standardization of Laboratory Testing Related to Diabetes Mellitus of Japan Diabetes Society (JDS) [7,8].

| Item | Specification | Results | Method |
|---|---|---|---|
| Material | Human whole blood (n>20) | Human whole blood (n>20) | - |
| Additives | None | None | - |
| tHb (g/L) | 140±10 | 144 | spectrometry |
| HbF (%) | < 1 | 0.1～0.6 | KO500 method |
| Glutathione adducts (%) | < 0.5 | 0.3～0.5 | KO500 method |
| Abnormal Hb | None | None | KO500 method |
| Plasma components | None | None | - |

## ■ References


1) Evaluation of measurement data - Guide to the expression of uncertainty in measurement. ISO/IEC Guide 98-3 (JCGM 100:2008).
2) Project team for establishment of hemoglobin A1c measurement, Committee on Diabetes Mellitus Indices, Japan Society of Clinical Chemistry. JSCC/JDS Standard Operating Procedure for HbA1c Measurement. Rinsho Kagaku 38(2):163-176, 2009.
3) Umemoto M, Raneva V, Tominaga M, Kuwa K, Abel G, Takei I, Ueki K, Kashiwagi A. Relation between NGSP and JDS HbA1c numbers. Diabetol Int. 6(1):77-81, 2015.
4) Jeppsson JO, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, Miedema K, Mosca A, Mauri P, Paroni R, Thienpont L, Umemoto M, Weykamp C. Approved IFCC Reference Method for the Measurement of HbA1c in Human Blood. Clin Chem Lab Med. 40(1):78–89, 2002.
5) Finke A, Kobold U, Hoelzel W, Weykamp C, Miedema K, Jeppsson JO. Preparation of a candidate primary reference material for the international standardisation of HbA1c determinations. Clin Chem Lab Med. 36(5):299–308, 1998.
6) Konnert A, Arends S, Schubert S, Berding C, Weykamp C, Siebelder C. Uncertainty calculation for calibrators of the IFCC HbA1c standardization network. Accred Qual Assur. 11(7):319-328, 2006.
7) Tominaga M. Standard Material for Measurement of Hemoglobin A1c. Rinsho Byori 49(12):1199-1204, 2001.
8) Tominaga M, Makino H, Yoshino G, Kuwa K, Takei I, Aono Y, Hoshino T, Umemoto M, Shimatsu A, Sanke T, Kuwashima M, Taminato T, Ono J. 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(1):41-46, 2005.


## ■ Provider of JCCRM 411-5

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R0 2025.7.3 Original certificate issue date

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