

Reference Material Institute for Clinical Chemistry Standards (ReCCS)

Certified Reference Material for Measurement of HbA_{1c}

JCCRM 411-3 (JDS Lot 5)

Certificate of Analysis

Intended use

JCCRM411-3 (JDS Lot5) is primarily intended for use in the calibration of routine methods(HPLC,Immunoassays, Enzymatic methods, etc.),standardization of HbA_{1c} measurements using well-designed reference materials. Measurement of HbA_{1c} with IFCC (mmol/mol), NGSP (%) and JDS numbers(%).And can be used for a test material (IFCC and for checking your measurement procedure of the IFCC reference method. In Japan, the JDS-JSCC reference system is established, based on a Designated Comparison Method, a high resolution HPLC separating HbA_{1c} as a single peak:KO500.The Laboratory of ReCCS is NGSP network laboratory implementing KO500.

The NGSP values in this material, JCCRM411-3 were assayed by ASRL#1 using NGSP CPRL reference panel and other NGSP SRLs. The IFCC values were assayed by principal laboratories of the IFCC HbA_{1c} Laboratory Network. The JDS values were measured by JDS-JSCC Network Laboratories using KO500.

Preparation

JCCRM411-3 was prepared as follows: Erythrocytes were separated by centrifuging human whole blood free of abnormal hemoglobin, and then washed and hemolysed. Next, through the use of a high-speed centrifuge, erythrocyte ghost membranes were removed, and after adding a carbonate buffer solution, 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.

Specifications

Configuration: Frozen liquid
HbA_{1c} levels: Five concentration levels ranging from 4 to 12%
Contents: A single set of JCCRM411-3 consists of five vials (one vial for each of the five concentration levels, and each vial contains 0.1 ml of liquid containing certified reference material for HbA_{1c}).

Storage after purchasing

1. JCCRM411-3 is shipped frozen on dry ice. On receipt, some dry ice must still remain in the shipping box. JCCRM411-3 is unusable if no dry ice is left upon receipt.
2. Upon receipt, a case containing JCCRM411-3 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 JCCRM411-3 on the day received, store it in a refrigerator till use.

Expiration date

The material must be received with dry ice remained and stored immediately at a temperature below -70°C. When this condition JCCRM411-3 expiring date is 12 months after the date of your (end user's) receipt.

Instruction for use

1. Take out a plastic vial containing JCCRM411-3 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 need 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. 130~140 g/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

JCCRM411-3 is intended for in-vitro diagnostic use only. This is a human source material. Handle 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(2).

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**Certificate of Analysis
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Certified values and Expanded uncertainties

(1)The IFCC values (IFCC unit: mmol/mol) of JCCRM411-3 (JDS Lot 5) are as follows:

Level	HbA1c(IFCC) mmol/mol and expanded uncertainty mmol/mol
Level 1	32.0 ± 1.0
Level 2**	38.6 ± 1.1
Level 3	56.9 ± 1.5
Level 4	80.0 ± 2.0
Level 5	106.5 ± 2.6

* These values represent the concentrations of stable HbA1c only.

** The concentration and the expanded uncertainty of total hemoglobin is 139.3 ± 1.6 g/L.

Laboratory of ReCCS,(LC-MS), IBM (LC-MS,CE), Instand e.v.(LC-MS), Isala Klienenken(CE)
, Queen Beatrix Hospital (CE)

The expanded uncertainties (calculated with coverage factor $k=2$, corresponding to 95% confidence level) were determined according to the ISO GUM (1)(2).

Measurement of Certified Values

The above IFCC values were measured by 6 reference laboratories of the IFCC HbA1c network of laboratories.

The HbA1c concentrations were quantified according to the reference measurement procedure of the IFCC reference method using the primary calibrators pcal 2010 (supplied by the IFCC HbA1c WG). Analysis was performed by the following IFCC HbA1c network laboratories: Laboratory of ReCCS,(LC-MS), IBM (LC-MS,CE), Instand e.v.(LC-MS), Isala Klienenken(CE), Queen Beatrix Hospital (CE)

(2)NGSP values (%) of JCCRM411-3 (JDS Lot 5) are as follows:

Level	HbA1c (NGSP) % and expanded uncertainty %	4 NGSP SRLs
Level 1	5.10 ± 0.13	
Level 2	5.77 ± 0.14	
Level 3	7.39 ± 0.19	
Level 4	9.60 ± 0.23	
Level 5	11.98 ± 0.28	

The NGSP values(%)were determined , based on NGSP ASRL#1 values and 4 NGSP SRL values. The values obtained from this NGSP-JDS equation agree well with the above NGSP Laboratory Network values in parenthesis.

(3)The JDS values (%) of JCCRM411-3 (JDS Lot 5) are as follows:

Level	HbA1c (JDS) %* and expanded uncertainty**(%)
Level 1	4.70 ± 0.13
Level 2	5.34 ± 0.13
Level 3	6.94 ± 0.16
Level 4	9.12 ± 0.17
Level 5	11.51 ± 0.21

* These values represent the concentrations of stable HbA1c only.

The JDS values were measured by the JSCC/JDS Designated Comparison Method (KO500 method), which is a high-resolution HPLC using JDS Lot 4 traceable to JDS Lot 2 as a calibrator. Assays were performed by the following reference laboratories approved by the Japan Reference Measurement Institute (JRMI) on behalf of JSCC: Laboratory of ReCCS (Masao Umamoto), Nihon University (Tetsuo Miyashita) and Institute of Biopathological Medicine (Tadao Hoshino).

Characteristics

The characteristics of JCCRM411-3(JDS Lot5) are tabulated below:

Item	Specification	Results	Measurement method
Material	Human whole blood (n>20)	Human whole blood (n=20~30)	ICSH method KO500 method Van Assendelft method KO500 method KO500 method
Additives	None	None	
Total Hb concentration	140 ± 10 g/l	130~145 g/l	
HbF	<1%	<1%	
MetHb	<6%	2~5%	
Glutathione adduct	<0.5%	0~0.2%	
Abnormal Hb	None	None	
Plasma components	None	None	

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

Information on International Standardization (IFCC, NGSP, and JDS reference values):

IFCC values of JDS Lot 5 assayed by the IFCC network of HbA1c reference laboratories using the IFCC reference method (3) are shown below .

Level	IFCC values and uncertainties	
1	32.0	± 1.0
2	38.6	± 1.1
3	56.9	± 1.5
4	80.0	± 2.0
5	106.5	± 2.6

The IFCC values are the means of the measurements made by 6 reference laboratories in 2013. The above expanded uncertainties were calculated from $U = ku$, where u is the combined uncertainty of measurement and primary calibrators (4)(5), and k is a coverage factor (95% level of interval), 2.23, for 10 degrees of freedom.

The relationship between the IFCC and the other (NGSP, JDS and Sweden) reference systems has been investigated(6). In 2004 the relations were calculated on the basis of the IFCC HbA1c WG studies, and published (7). The relation between IFCC and JDS values is: $JDS = 0.0927 IFCC + 1.724$. The JDS values given by this Master Equation are close (being in the range of uncertainties) to the certified JDS values as seen from the comparison between the below values and the certified JDS values.

Level	derived JDS values by Master Equation	Measured by JDS Network (%)
1	4.69	4.70
2	5.30	5.34
3	7.00	6.94
4	9.14	9.12
5	11.60	11.50

*Comparison between IFCC-derived JDS and Measured JDS

Recently, the IFCC working group on HbA1c standardization has offered an accuracy-based system comprised of the IFCC reference method, which means “traceable to SI units” and it is almost agreed that the IFCC reference system should be the anchor for worldwide standardization of hemoglobin A1C. The IFCC reference method is characterized as a greater specific method to HbA1c (Note that HbA₂ is the only interference) through the measurement of hexapeptide and glycated-hexapeptide digested by Glu-C peptidase using LC-MS or CE. Based on results from long-term comparison studies between NGSP, JDS, Sweden DCM (designated comparison method) values and IFCC values, each correlation is proved to be stable (7), but the numbers (HbA1c %) are different and each relationship is not parallel. Thus changing to the IFCC number would involve adoption of new reference ranges, translation of clinical data and modification of standards of treatment for diabetes .

ADA/EASD/IDF/IFCC have recently issued a consensus statement on the worldwide standardization of the hemoglobin A1C measurement (8), which is summarized as far as HbA1c standardization is concerned: 1) A1C results are to be reported world-wide in IFCC units (mmol/mol) and derived NGSP units (%), using the IFCC-NGSP Master Equation; 2) The IFCC reference system(units:mmol/mol) represents the only valid anchor to implement standardization of the measurement.

Since the EU Directive for *In vitro* Diagnostics requires that *In vitro* Diagnostics should be traceable to a higher-order reference material, HbA1c reagents and instruments to be used in Europe should documents their traceability to the IFCC reference system. Combining the Directive and the consensus statement on hemoglobin A1C, HbA1c reagent and instrument manufactures are to document their traceability to the IFCC reference system with both the IFCC units (mmol/mol) and derived NGSP units (%). Since the IFCC-NGSP Master Equation is $NGSP (\%) = 0.09148 \text{ IFCC (mmol/mol)} + 2.152$, it comes that the derived NGSP (%) of the JDS Lot5 is as follows:

Table 5 *

Level	IFCC-derived NGSP values (%)	Measured (%)
1	5.08	5.10
2	5.68	5.77
3	7.36	7.39
4	9.47	9.60
5	11.90	11.98

*Comparison between IFCC-derived NGSP and Measured NGSP

Direct Comparisons between JSCC/JDS DCM and NGSP CPRL (Central Primary Reference Laboratory) were made in 2011, and a relation, $NGSP=1.02 \times JDS+0.25$, has been established. Monitoring tests for NGSP SRLs also show a good agreement with this Equation. NGSP- derived JDS values are tabulated in Table6.

Table 6 *

Level	NGSP- derived JDS values (%)	Measured JDS values(%)
1	4.75	4.70
2	5.41	5.34
3	7.00	6.94
4	9.17	9.12
5	11.50	11.50

* Comparison between NGSP derived JDS and Measured JDS

Certification body

JCCRM411-3 was certified by the Reference Material Institute for Clinical Chemistry Standards (Laboratory of ReCCS).

For certification, a certification committee was organized.

Date of certification: March 18,2013

References

- 1) Guide to the Expression of Uncertainty in Measurement, ISBN 92-67-10188-9,1st Ed. ISO., Geneva, Switzerland (1993).
- 2) Andrea Konnert, et al. Uncertainty calculation for calibrators of the IFCC HbA1c standardization network. *Accred Qual Assu* **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) European Commission – Joint Research Centre Institute for Reference Materials and Measurements (IRMM), Certified reference material IRMM/IFCC-466, 2007
- 5) European Commission – Joint Research Centre Institute for Reference Materials and Measurements (IRMM), Certified reference material IRMM/IFCC-467, 2007
- 6) Andrea Geistanger, et al. Statistical Methods for Monitoring the Relationship between the IFCC Reference Measurement Procedure for Hemoglobin A1c and the Designated Comparison Methods in the United States, Japan and Sweden. *Clin Chem* **54**:1379-1385, 2008.
- 7) Wieland Hoelzel, et al. IFCC Reference System for Measurement of Hemoglobin A1c in Human Blood and the National Standardization Schemes in the United States, Japan, and Sweden: A Method-Comparison Study. *Clin Chem* **50**:166-174, 2004.
- 8) The American Diabetes Association, European Association for the Study of Diabetes, International Federation of Clinical Laboratory and Laboratory Medicine, and the International Diabetes Federation, Consensus statement on the worldwide standardization of the hemoglobin A1c measurement. *Diabetes Care*. **30**:2399-2400(2007).
- 9) 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: 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.
- 10) International Committee for Standardization in Haematology, Recommendations for reference method for haemoglobinometry in human blood (ICSH Standard EP6/2:1977) and specifications for international haemoglobincyanide reference preparation (ICSH Standard EP 6/3:1977). *Journal of Pathology* **41**:139-143, 1978.

Provider of JCCRM411-3 (JDS Lot 5)

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Reference Material Institute for Clinical Chemistry Standards (ReCCS)

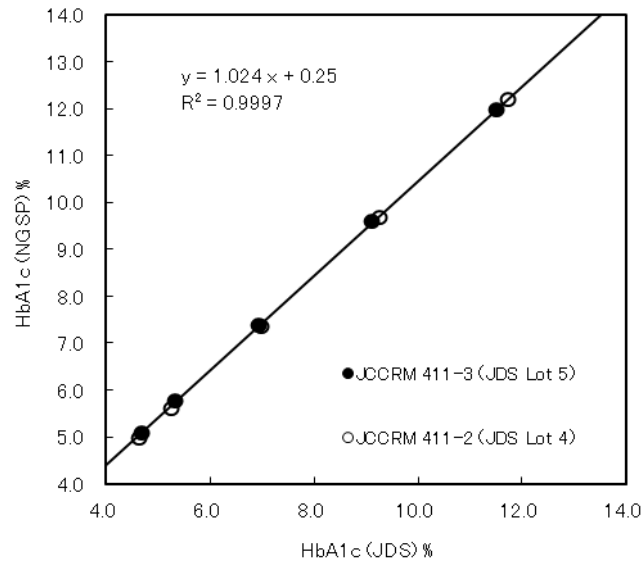
Kanagawa Science Park A205, 3-2-1 Sakado, Takatsu-ku, Kawasaki-shi, 213-0012

TEL: 044-850-3140

FAX: 044-850-3141

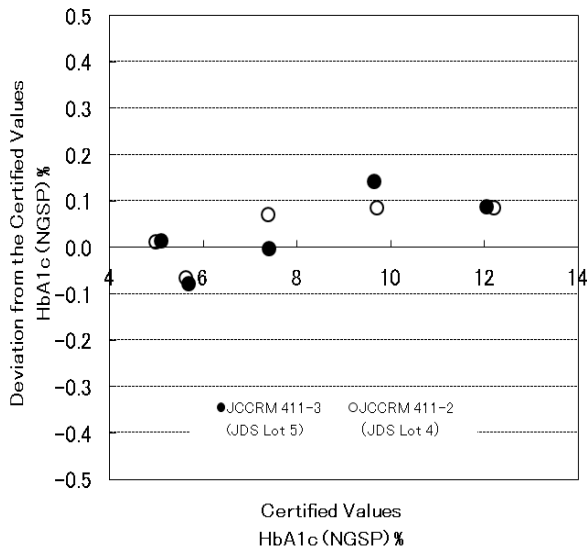
E-mail: ando@reccs.net.

<http://www.reccs.or.jp/>

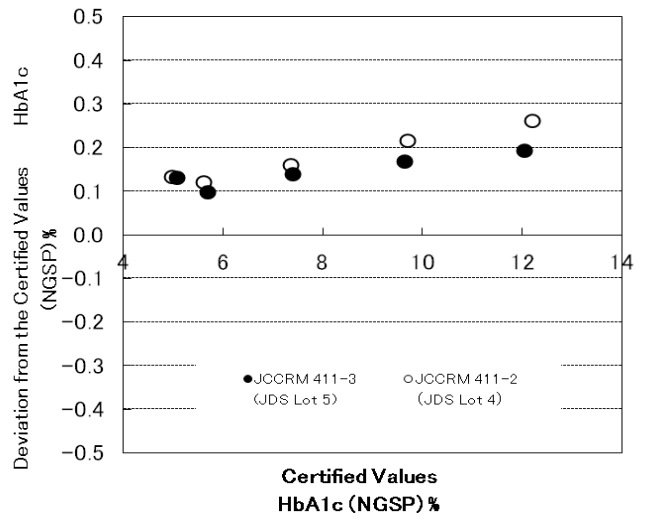


JCCRM 411-3 (JDS Lot 5)

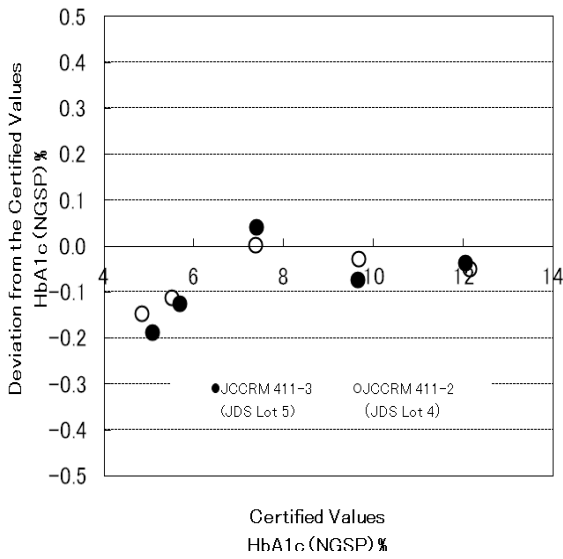
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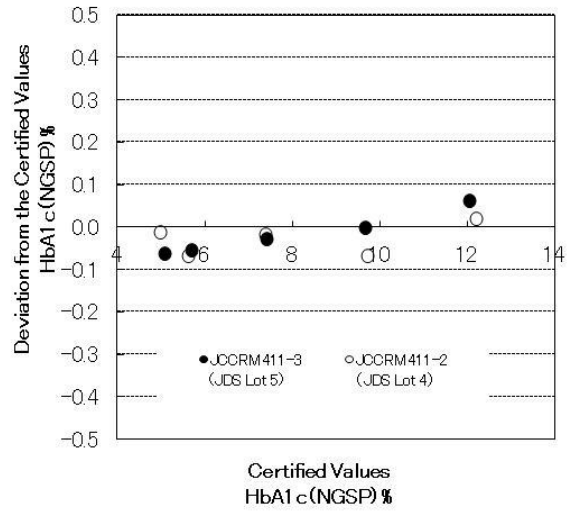
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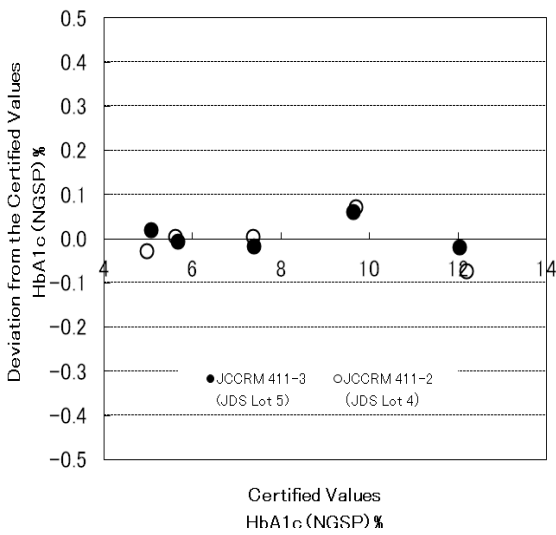
Immune Method A



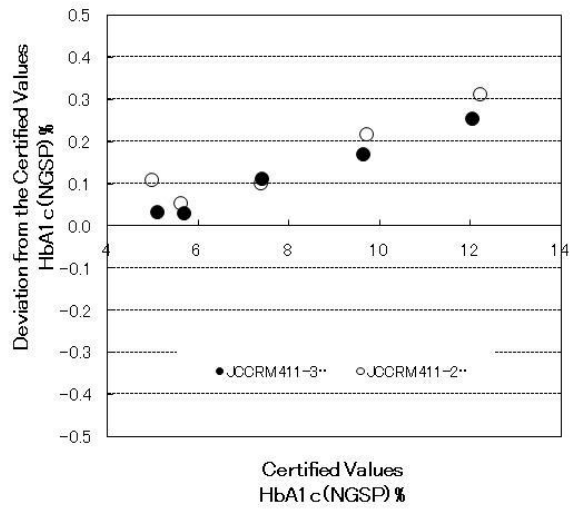
Enzymatic Method A



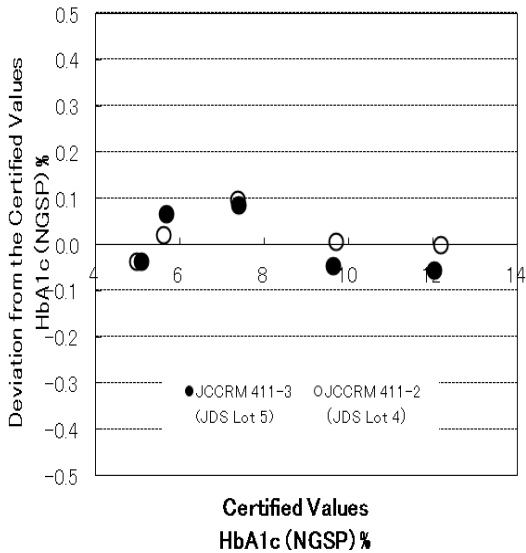
Immune Method B



Enzymatic Method B



Immune Method C



Enzymatic Method C

