

**Reference Material Institute for Clinical Chemistry Standards
(ReCCS)
Certificate of Analysis
Certified Reference Material for Measurement of HbA1c
JDS HbA1c Lot 2**

JDS HbA1c Lot 2 is a certified reference material for measurement of HbA1c which is traceable to SI units. This material was prepared by the Reference Material Institute for Clinical Chemistry Standards (ReCCS), and its JDS HbA1c values was certified by the Committee on Standardization of Laboratory Testing of the Japan Diabetes Society (JDS).

In the Kyoto 2 Study made in 2002 by the IFCC HbA1c Working Group (chaired by Dr. K. Miedema), its IFCC reference method values were measured by its 11 network laboratories, and then were certified by ReCCS in 2012.

Intended use:

JDS HbA1c Lot 2 is primarily intended for use in the calibration and validation of procedures and methods for measurement of HbA1c employed in clinical analysis, and is intended to be used for validation of and securing traceability of working, or secondary reference materials. Information on international standardization is also described in this Certificate.

Specification:

The specification of JDS HbA1c Lot 2 (10) (11) is tabulated below.

Item	Specification	Results	Measurement method
Material	Human whole blood (n>20)	Human whole blood (n=about 200)	
Additives	None	None	
Total Hb concentration	140 ± 10 g/l	140 ± 10 g/l	ICSH method (1)
Labile HbA1c	None	None	KO500 method (4)
HbF	<1%	<1%	KO500 method
Methemoglobin	<6%	<6%	Van Assendelft method (5)
Glutathione adduct	<0.5%	<0.1%	KO500 method (4)
Abnormal Hb	None	None	KO500 method (4)
Plasma components	None	None	

This specifications was established by Committee on Standardization of Laboratory Testing Related to Diabetes Mellitus of the Japan Diabetes Society.

Preparation:

The source of this material met the specification in Table1. The preparation of JDS HbA1c Lot 2 was as follows: Erythrocytes in human whole blood which was free of abnormal hemoglobin was separated by centrifigation, and then washed and hemolysed by freezing. Using a high -speed centrifuge, erythrocyte ghost membranes were removed, and after adding a carbonate buffer solution, the resulting solution was dialyzed. (As a result, this reference material does not contain plasma components.) The dialyzed solution was incubated at 37°C for 2 hours and then divided into vials. Finally the vials were stored in liquid nitrogen In order to avoid the use of preservatives, only sterilized tools were used, and to ensure storage stability, the carbonate buffer solution was sterilized by filtration before use.

Certified HbA1c values:

The certified HbA1c values were determined at ReCCS on the basis of the results of the Kyoto 1 Study of the IFCC HbA1c WG made in 2002. The measurement methods were HPLC-separation followed by capillary electrophoresis and/or HPLC-separation followed by LC/MS.

Products:

JDS HbA1c Lot 2 is a frozen liquid material.

A single set of JDS HbA1c Lot 2 consists of five vials (one vial for each of the five concentration levels ranging from 4 to 13%, and each vial contains 0.1 ml of liquid).

Storage:

- (1) JDS HbA1c Lot 2 is shipped with dry ice. On arrival, some dry ice still must remain in the shipping box. JDS HbA1c Lot 2 should not be used if no dry ice is left upon receipt.
- (2) Upon receipt, the case containing JDS HbA1c Lot 2 should be taken out, and immediately placed in a section of a deep freezer (< -70°C) where temperature fluctuations are minimal (either in the bottom or back section of the freezer).

Expiration date:

When stored at a temperature below -70°C , the expiration date of JDS HbA1c Lot 2 is 6 months from the date of shipping indicated on the label.

Instruction for use

1. Remove a plastic vial containing JDS HbA1c Lot 2 from a deep-freezer and allow it to stand at room temperature for about 15 minutes until it naturally thaws.
2. Mix the content of the vial by turning up-side down over 20 times or
3. Collect the content at the bottom of the vial, and take an necessary amount using a micro syringe or a micropipette. Also, when the entire content of the vial need to be collected at the bottom, centrifuge the vial at 1,000 r.p.m. for about 30 seconds.

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 should not be refrozen to be used later.

Note 2) The total Hb concentration of this reference material varies for each level as shown in the table below. When it is necessary to measure under a constant total Hb concentration, use the table below to check total Hb concentrations and determine an appropriate sampling volume for dilution referring to the instruction manual of an HbA1c analyzer.

Table. Total Hb concentration of JDS HbA1c Lot 2

Measurement method: ICSH method (cyanmethemoglobin method) (1)

Level	Total Hb concentration (g/l)
Level 1	135
Level 2	145
Level 3	145
Level 4	137
Level 5	132

The use of the above total Hb concentrations should be strictly limited for referencing purposes, not for evaluating accuracy.

Precautions for use and warning

JDS HbA1c Lot 2 is intended for *in vitro* diagnostic use only. JDS HbA1c Lot 2 was tested and shown to be negative for HBs antigen, HCV antibody and HIV-1 antibody and shown to be. However, no known test method can offer complete assurance that any infectious agents including hepatitis B virus, hepatitis C virus and HIV are absent from this material, as is the case with any other biological material, handle JDS HbA1c Lot 2 as an infectious and bio-hazardous material.

Certified values and uncertainties

Certified HbA1c values and uncertainties in IFCC units (mmol/mol) of JDS HbA1c Lot 2 are as follows:

Level	HbA1c and uncertainties (IFCC units: mmol/mol)
1	26.6 ± 0.6
2	40.0 ± 0.8
3	59.9 ± 1.2
4	87.3 ± 1.7
5	118.4 ± 1.8

*Not-for-use of this level 1 is recommended because of too low to be precisely determined by IFCC method.

The IFCC values of JDS Lot 2 below were assayed by the IFCC HbA1c network laboratories using the IFCC reference method for HbA1c (6) in 2002, where the IFCC reference method was calibrated with primary calibrating prepared according to the IFCC HbA1c reference measurement procedure using IRMM/IFCC-466 and 467 as primary standard. Each certified value is the mean of the measurements made by 11 reference laboratories. The above expanded uncertainties were calculated from $U = k u$, where u is the combined uncertainty of measurement and primary calibrators (8), and k is a coverage factor (95% level of interval), 2.23, for 10 degrees of freedom.

Analyses:

The overall and coordination of the analyses by the IFCC network laboratories were done by Dr. C. Weykamp (MCA Laboratory).

The overall direction and coordination of the analyses by the JDS/JSCC network laboratories were made by Prof. M. Tominaga (chair of the JDS Committee on Standardization). The overall direction and coordination of the analyses by NGSP CPRL and NGSP SRLs 3 and 9 were done by Dr. R. Little (the Missouri Medical School of Medicine).

Certification:

JDS HbA1c values in JDS HbA1c Lot 2 was certified on Feb. 10, 2001 by Committee on Standardization of Laboratory Testing Related to Diabetes Mellitus Chaired by Makoto Tominaga: (Professor, Yamagata University School of Medicine).

The certification Committee for HbA1c values by the IFCC HbA1c reference method at ReCCS of which members were Masao Umemoto Ph.D., Raneva Violetta Ph.D., Wataru Tani, Katsuhiko Kuwa (Professor, Tsukuba University), Tadao Hoshino (MD.Ph.D., IBM)

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

The relationship between the IFCC and the other (NGSP, JDS and Sweden) reference systems has been investigated. In 2004 the relations were calculated on the basis of the IFCC HbA1c WG Studies, and published (9) (13). The relation between IFCC and JDS values is: $JDS = 0.0927 IFCC + 1.724$. JDS values given by this Master Equation are close (being in the range of uncertainties) to the measured mean JDS values as seen in the comparison table below. The differences are less than 0.1 HbA1c (%) except Level 1.

Level	derived JDS values by Master Equation	Measured mean*	△
1	4.20	4.04	+0.16
2	5.43	5.38	+0.05
3	7.28	7.32	-0.04
4	9.82	9.88	-0.06
5	12.70	12.63	+0.07

△ difference

The JDS HbA1c measured means in Table4 were measured by the KO500 method (4), which is a high-resolution high-performance liquid chromatography technique using JDS HbA1c Lot 1 (3) (established by the Japan Diabetes Society's Committee for Standardization of Glycohemoglobin in 1994). Analysis were performed by the following reference laboratories approved by Committee on Standardization of Laboratory Testing Related to Diabetes Mellitus: Keio University School of Medicine (Izumi Takei), Standard Reference Center (Masao Umemoto), and Pathology Laboratory (Tadao Hoshino).

The significant difference at Level 1 was originated from a large between lab variation for Level 1 .

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 (9), 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 (12).

ADA/EASD/IDF/IFCC have recently issued a consensus statement on the worldwide standardization of the hemoglobin A1C measurement (14), 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 $NGSP (\%) = 0.09148 IFCC (mmol/mol) + 2.152$, it comes that the derived NGSP (%) of the JDS Lot2 is as follows:

Level	derived NGSP values (%)	Measured by NGSP SRLs
1	4.59	4.3
2	5.81	5.7
3	7.63	7.6
4	10.14	10.2
5	13.00	12.9

NGSP SRLs: SRL #3 and SRL #9

Comparison between the derived-NGSP values and NGSP values measured by SRL #3 and #9 shows a good agreement (less than 0.1%) except Level 1..

Direct Comparisons between JSCC/JDS DCM and NGSP CPRL (Central Primary Reference Laboratory) were made in 2011, and a relation, $NGSP(JDS)=1.02 \times JDS+0.25$, has been established. Monitoring tests for NGSP SRLs conducted by NGSP NETCORE also show a good agreement with this Equation. Giving derived JDS values in the Equation produces NGSP values: 4.52, 5.79, 7.68, 10.27, 13.20. These values NGSP (JDS) are tabulated in Table6, showing a good agreement between the two.

Level	derived NGSP values (%)	NGSP (JDS) (%)	Δ
1	4.59	4.53	0.06
2	5.81	5.79	0.02
3	7.63	7.68	-0.05
4	10.14	10.27	-0.13
5	13.00	13.20	-0.20

References:

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- (2) Guide to the Expression of Uncertainty in Measurement, ISBN 92–67–10188–9, 1st Ed., ISO. Geneva, Switzerland (1993)
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- (9) W. Hoelzel et.al.: IFCC Reference System for the measurement of HbA1c in human blood and the national standardization schemes in the USA, Japan and Sweden – a method comparison study, Clin. Chem. 50, 166–174 (2004).
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- (11) M. Tominaga, et.al.: HbA1C value of JDS Lot2 Determined by a NGSP lab, J. Japan Diab. Soc. 45 (5), 385–388 (2002).
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(13) http://www.ifcchba1c.net/IFCC_08.asp

(14) M. Panteghini et. al.: Implementation of hemoglobin A1c results traceable to the IFCC reference system: the way forward. Clin. Chem. Lab. Med. 2007; 45:942-944

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