

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

Certificate of Analysis

**Certified Reference Material for Measurements of  
 Glycated Albumin in Human Serum**

**JCCRM 611-1**

**Intended use**

This certified reference material (CRM) is intended for use in evaluating the accuracy of Glycated Albumin assays and calibrating working reference materials.

**Preparation**

This CRM meets the criteria of CLSI preparation of the secondary reference material for cholesterol [2].

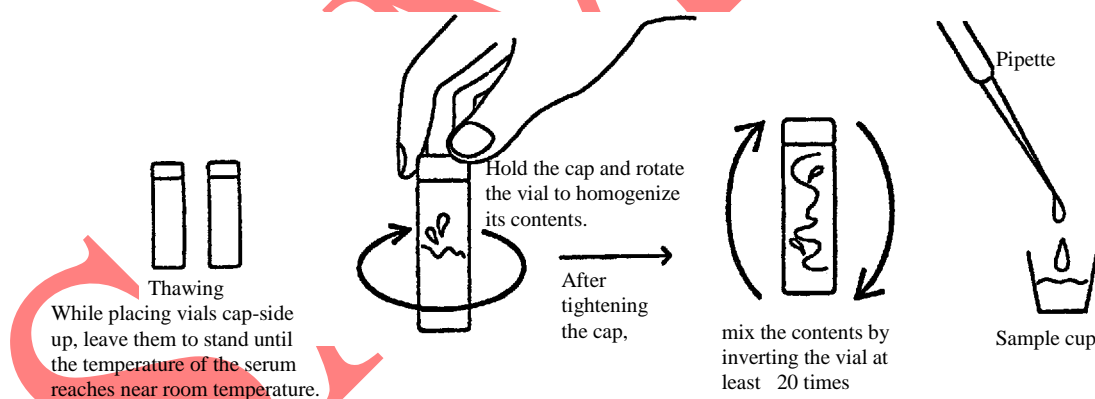
**Product specifications**

Configuration: Frozen liquid  
 Levels and contents: Three concentration levels: M, H, HH

A single set of this CRM consists of three vials: one vial for each concentration level, and each vial contains 0.5 ml of human serum.

**Use: IMPORTANT MATTERS**

A vial is taken out from its case and is thawed at room temperature while placing it cap-side up. Next, the vial is left standing for about 30 minutes to bring the temperature of the serum to room temperature. Once this procedure is completed, hold the cap of the vial; gently rotate the vial in complete circles; several times and tighten the cap; and then mix the content of the vial by turning the vial upside down at least 20 times (the reference material in this manner must be used within two hours. Once thawed, the serum cannot be frozen for reuse.



**Storage and expiration after purchasing**

This CRM must be stored in a deep freezer (below  $-70^{\circ}\text{C}$ ). Under this condition, its shelf life is 1 year from the date of your (end user's) receipt.

**Precautions for use**

This CRM is a human source material, and handle it as a biohazardous material capable of transmitting infectious disease. This product has been shown to be non-reactive for HBs antigens, HCV and HIV antibodies by our test methods. However, no known test method can give complete assurance of absence of HIV, HCV antibodies, HBs antigens, and any other infectious agents. Thus assume that this CRM would be infectious, and exercise the same caution as for handling any other clinical specimens with the risk of infectious disease.

**In Vitro Use Only**

## Certificate of Analysis

**Certified Reference Material for Measurement of  
Glycated Albumin (GA) in Human Serum****JCCRM 611-1****Certified GA(mmol/mol)concentrations**

Levels	Glycated Albumin concentrations and uncertainties	units
JCCRM 611-1M	230 ± 7	mmol/mol
JCCRM 611-1H	365 ± 10	"
JCCRM 611-1HH	564 ± 18	"

The above glycated albumin (GA) concentrations were determined by the JSCC reference method for GA on the basis of isotope dilution-mass spectrometry [2, 6, 7].

The analytical measurements were performed at the Laboratory of ReCCS by E. Shimizu and W. Tani.

The expanded uncertainty U (95% confidence interval) shown in the above table for each certified value is obtained from the equation  $U=ku$ , where u is the combined standard uncertainty calculated according to the ISO Guide 35 [3] and k is a coverage factor. GA concentration was measured five times in one day on 15 separate days. Then the coverage factor, the Student's t distribution with 14 degrees of freedom, was  $k=2.145$ .

<b>Outline of measurement method for GA</b>
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Albumin fraction including glycated albumin in serum was chromatographed and separated by ion-exchange HPLC, which is published elsewhere. [2]

DOF-Lys :N<sub>ε</sub> - (1-deoxy-D-fructos-1-yl)-L-lysine [4, 5] and Lysine generated from the albumin fraction hydrolyzed by HCl were assayed by ID-MS using <sup>13</sup>C<sub>6</sub>- DOF-Lysine and D<sub>4</sub> -Lysine as isotopes, respectively.

All the analytical procedures were performed at ReCCS.

<b>Traceability</b>
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A Lysine standard solution prepared with the primary reference material (NMIJ CRM 6018-a, purity 99.8%) and a DOF-Lysine standard solution prepared with high-purity DOF-Lysine (ReCCS, purity 99%) were used for the calibration of the ID/MS.

The concentration of the DOF-Lysine standard solution was assayed against α-Benzyloxy carbonyl-DOF-Lysine (purity 98%).

**GA (%) with Lucica GA-L Kit**

Mean relative ratios (%) of glycated albumin(GA) in this CRM using Lucica GA-L Kits from Asahi Kasei Pharma were as below:

Level	Glycated Albumin (%)*
JCCRM 611-1M	12.8
JCCRM 611-1H	20.2
JCCRM 611-1HH	31.1

\*The above definition GA (%) is originally relative area ratio of glycated albumin to total albumin in the chromatogram of affinity HPLC

**Characteristics**

The characteristics of this CRM are as follows:

		JCCRM 611-1M	JCCRM 611-1H	JCCRM 611-1HH	
Density	(g/cm <sup>3</sup> , 25°C)	1.02~1.03	〃	〃	Pycnometer method
Total protein	(g/dl)	7.0	7.1	6.8	Biuret method
Albumin	(g/dl)	4.3	4.1	3.9	BCG method
Triglycerides	(mg/dl)	64	66	62	Enzymatic method
Glucose	(mg/dl)	75	179	260	Enzymatic method
Total Cholesterol	(mg/dl)	173	159	144	Enzymatic method
Turbidity	Abs	0.2	0.2	0.2	710nm, 1cm
Hemoglobin	(mg/dl)		< 2		
Viscosity	(mPa.sec 20°C)	1.6	1.6	1.6	

**Certification**

July 1, 2013

*Hirohito Umemoto Ph.D.*

Hirohito Umemoto, Ph. D. (President)

ReCCS (Reference Material Institute for Clinical Chemistry Standards)

## REFERENCES

- [1] "Preparation and Validation of Commutable Frozen Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures; Approved Guideline," NCCLS Publication C37-A, National Committee for Clinical Laboratory Standard, Wayne, PA, (1999).
- [2] Rinsyo Kagaku 37:178-19, 2008
- [3] NCCLS Publication C37-A: Preparation and validation of commutable frozen serum pools as secondary reference materials for cholesterol measurement procedures, CLSI, Wayne, PA, 1999.  
Guide to the Expression of Uncertainty in Measurement, ISBN 92-67-10188-9, 1st Ed, ISO, Geneva, Switzerland (corrected and reprinted, 1995)
- [4] Paul J. Thornalley, Annika Langborg and Harjit S. Minhas: Formation of glyoxal, methylglyoxal and 3 - deoxyglucosone in the glycation of proteins by glucose, *Biochem. J.*, 344: 109–116, 1999.
- [5] John W. Baynes, Suzanne R. Thorpe, Martha H., Murtiashaw Nonenzymatic glycosylation of lysine residues in albumin, *Methods in Enzymology*, 106: 88–98, 1984.
- [6] Rinsyo Kagaku 37:23-24(2008)
- [7] Rinsyo Kagaku 42:68-7, 2013

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