Reference Material Institute for Clinical Chemistry Standards (ReCCS)

Certified Reference Material for Measurement of C-Reactive Protein (CRP) in Human Serum

JCCRM 612-1

Certificate of Analysis

■ Intended use

This Certified Reference Material (CRM) is intended for use in calibrating and evaluating the accuracy of routine methods and validating working reference materials for measurement of C-Reactive Protein (CRP) in human serum, especially in low-concentration range of CRP (below 1.0 mg/dL). In order to ensure the reliability of evaluation of routine methods, this CRM has five concentration, levels 0 - 4.

■ Certified concentration values and uncertainty

The certified concentration values and uncertainty at 25 ℃ are listed in Table 1. Metrological traceability is to the International System of Units (SI) derived unit for mass concentration (expressed as milligrams per deciliter) and amount-of-substance concentration (expressed as micromoles per liter).

Table 1a. Certified Mass Concentration Values and Uncertainty

Unit: mg/dL

Level	0※2	1	2	3	4
Certified value	0.000※3	0.093	0.278	0.921	4.625
Uncertainty※1	—	0.005	0.015	0.049	0.243

Table 1b. Certified Amount of Substance Concentration Values and Uncertainty

Unit: μmol/L

Level	0※2	1	2	3	4
Certified value	0.000※3	0.040	0.121	0.400	2.009
Uncertainty※1	—	0.002	0.007	0.021	0.106

※1. The expanded uncertainty U (95% confidence interval) shown in the tables is obtained by combining standard uncertainty calculated according to the ISO GUM [1]. The coverage factor (k) is 2.

※2. Level 0 is a human CRP free serum.

※3. Based on the data of measurement for CRP concentration (see reference).

■ Preparation

This CRM was prepared by gravimetric dilution method using purified recombinant human CRP (Oriental Yeast Co., Ltd.) and human CRP free serum (Oriental Yeast Co., Ltd.) [2,3].

■ Measurement of certified values

The certified values for each level were calculated by mass of purified recombinant human CRP and human CRP free serum in gravimetric dilution method. The density of each prepared solutions was determined by using pycnometer.
The concentration of purified human recombinant CRP was determined by the weighted mean of the results of two amino acid analyses, in which following independent hydrolysis and isotope dilution mass spectrometry (ID/MS) [4].

■ Traceability

The certified value is traceable to the International System of Units (SI). The traceability is as indicated below:

The measurement for concentration of purified human recombinant CRP:
Amino acid analysis which was combined in part with primary method ID/MS and calibrated with NMIJ CRMs (L-arginine (NMIJ CRM 6017-b),L-glutamic acid (NMIJ CRM 6026-a), L-histidine (NMIJ CRM 6024-a), L-Leucine (NMIJ CRM 6012-a), L-Lysine monohydrate (NMIJ CRM 6018-a) , L-methionine (NMIJ CRM 6023-a), L-phenylalanine(NMIJ CRM 6014-a) , L-proline (NMIJ CRM 6016-a) , L-valine (NMIJ CRM 6015-a)).

The gravimetric dilution:
The balance which was used in the gravimetric dilution and pycnometer were both calibrated by Japan Calibration Service System.

■ Instructions for use

(1) Take out vial tubes of this CRM and thaw the frozen serum by allowing the tube to stand with the cap-side up at room temperature for approximately one hour.
(2) While holding the tube vertically with the cap-side up, hold the cap with fingers, and confirm that the cap is tightly screwed on. If the cap is loose, tighten it securely. Then mix the serum by gently rotating the tube approximately 20 times. Next, turn the tube upside-down slowly at least 40 times to secure homogeneity.
(3) Conduct sampling of the mixed serum for measurements. Unless used immediately, tighten the vial cap and refrigerate it for use in the same day.
Note: Once thawed, the serum should not be frozen again for future use.

■ Precautions for use: In vitro use only

This CRM is prepared from human serum and shown to be negative to the HBs antigens, HCV antibodies and HIV antibodies. However, since other infectious agents are not completely ruled out, handle this CRM as a biohazardous material capable of transmitting infectious diseases.

■ Storage and expiration

This CRM is must be stored in a in a deep freezer (below－70 ℃) upon receipt.
Under this condition, its shelf life is 6 months from the receipt date (see the label of the outer case).
The shelf life for storage at－20 ℃ is one month.

■ Product specifications

A single set of this CRM consists of 5 vials, each vial contains 0.5 mL of human serum. There are 5 different concentration levels 0,1,2,3,4.

Reference

■ Concentration of CRP in human CRP free serum used in preparation

Method	Result
Nephelometry method (Siemens Healthcare Diagnostics)	< 0.0008 mg/dL (Not detected)

■ Characteristics

The data in the table is level 0 of this CRM.

Item	JCCRM 612-1 (Level 0)	Unit	Method
Density (25 ℃)	1.011	g/cm^3	Pycnometry
pH (37 ℃)	7.3	−	Glass electrode
Total protein	6.2	g/dL	Biuret
Albumin	3.9	g/dL	BCG
Total cholesterol	3	mg/dL	Enzymatic
Triglycerides	7	mg/dL	Enzymatic

■ References

[1] Guide to the expression of uncertainty in measurement, ISBN 92-67-10188-9,1st Ed .,ISO, Geneva, Switzerland(corrected and reprinted,1995).
[2] Tanaka T, Horio T, Matuo Y. Secretory production of recombinant human C-reactive protein in *Escherichia coli*, capable of binding with phosphorylchoine, and its characterization. Biochem Biophys Res Com 295:163-166. 2002.
[3] Rinsyobyouri 50: 13-19, 2002.
[4] National Institute of Advanced Industrial Science and Technology - Certificate of NMIJ CRM 6201-c.
[5] Bunseki 3:119-125, 2010.

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Provider of JCCRM 612-1

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