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

**Certified Reference Material for Measurement of Serum Iron**
**JCCRM 322-7**

# Certificate of Analysis



## ■ Intended use

This certified reference material （CRM）is intended primarily for use in evaluating the accuracy of procedures and calibrating procedures for determination of serum iron.

## ■ Instructions for use

(1) Take out a serum vial tube of this CRM and thaw the frozen serum by allowing the tube to stand with the cap-side up at room temperature for approximately 30 minutes.
(2) While holding the serum 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.
Note: Since the concentration of iron in the serum is very low, exercise caution to avoid external iron contamination, for instance, by using clean sample cups and pipettes.

## ■ 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 after purchasing

Since this CRM is frozen, it must be stored frozen till use.
Expiration from the date of shipping (listed on the product label).

9 months at －70 ℃
2 months at －20 ℃

## ■ Specifications

2 levels (Low & Medium ): 2 vials for each concentration: 1 ml per vial ,Total 4 vials

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## ■ Certified values and uncertainties

The certified values and expanded uncertainties of this CRM are as follows (25 ℃)

| Item | Low concentration JCCRM 322-7L | Medium concentration JCCRM 322-7M | Unit |
|---|---|---|---|
| Serum iron | **36.6 ± 1.1** | **133.6 ± 3.6** | μg/dL |
| | **6.6 ± 0.2** | **23.9 ± 0.6** | μmol/L |

The expanded uncertainty U (95% confidence interval) shown in the above table is obtained by combining standard uncertainty calculated according to the ISO GUM. Uncertainty of SRM and uncertainty of gravimetric method are also included and coverage factor (*k*) are 2.0.

## ■ Preparation of this CRM

This CRM used different human serum pools for each levels. JCCRM 322-7M was added iron chloride (III) (purity more than 99.9 %) to adjust Iron concentrations.
All the plastic vials were washed by hydrochloric acid which was excluding pollution of iron.

## ■ Measurement methods for certified values

The certified values were determined at the laboratory of ReCCS according to the international reference measurement procedure for serum iron (ICSH 1978) (hereinafter referred to as the international reference method) in combination with the standard addition method. By the use of the standard addition method, volume displacement error caused by the deproteinization associated with the international standard method was corrected (see Reference documents). In addition, Copper interference was eliminated.

## ■ Serum characteristics

This CRM is human serum material, and its characteristics are listed in the table below. It contains neither preservative nor stabilizer.

| Item | JCCRM 322-7L | JCCRM 322-7M | Unit | Measurement method |
|---|---|---|---|---|
| Density (25 ℃) | 1.024 | 1.024 | g/cm$^3$ | Pycnometer |
| Total protein | 7.1 | 7.1 | g/dL | Burette method |
| Albumin | 3.8 | 4.0 | g/dL | BCG method |
| TIBC | 415 | 377 | μg/dL | CPBA method |
| Ferritin | 7 | 11 | ng/mL | Immune method |
| transferrin | 339 | 313 | mg/dL | nephelometry method |
| Hemoglobin | Less than 1 | Less than 1 | mg/dL | Colorimetric method |
| Copper | 138 | 129 | μg/dL | Colorimetric method |
| Zinc | 61 | 97 | μg/dL | AAS |
| Total cholesterol | 156 | 166 | mg/dL | Enzyme method |
| Triglyceride | 74 | 85 | mg/dL | Enzyme method |

## ■ Reference documents

(1) Comparison between the international reference method using the standard addition technique and the direct methods.

Concentrations of serum iron in fresh serum samples were compared among the international reference method (ICSH1978) with the calibration curve technique, direct methods using different chelate-color reagents by employing each calibrator and the international reference method with the standard addition technique. Table1 shows the results. With the calibration curve technique, the measurement obtained by the international reference method was apparently higher than that obtained by the standard addition technique. The reason for this is that the standard addition technique corrects the errors associated with the international reference method (volume displace errors attributable to serum deproteinization). Blank errors, which cannot be corrected by the standard addition technique, were also corrected: Copper interference was corrected and the background was eliminated by a two wavelength method.

The concentration of serum iron in this CRM were compared between the international standard method and direct methods using Nitroso-PASP and Bathophenanthroline, respectively.

Comparison of serum iron measurements between the international reference method and routine direct methods

Unit: μg/dL

| | Certified value | Nitroso-PSAP method | Bathophenanthroline method |
|---|---|---|---|
| **JCCRM 322-7L** | 36.6 | 36.7 | 35.7 |
| **JCCRM 322-7M** | 133.6 | 131.6 | 132.7 |

(2) Interference from Hb iron in frozen and lyophilized serum samples.

Because the iron targeted by serum iron measurements is transferrin bound iron, Our Serum Iron Standard Method Investigation Committee investigated interference from hemoglobin iron (Hb iron), which

coexists with transferring—bound iron in serum, with regard to frozen and lyophilized samples. The results showed no interference from Hb iron in frozen serum samples when measured according to the international standard method as well as the direct methods. However, if these serum samples have been lyophilized, except for the direct method C (Ferren method), a slight interference from Hb iron of about 1± 3 % was observed (Hb concentrations in the serum sample ranged from 5 to 15 mg/dL). Since lyophilized serum samples are not suitable as reference materials or external accuracy controls as above, a serum reference material for serum iron should be prepared as a frozen material.

## ■ References

1) Evaluation of measurement data - Guide to the expression of uncertainty in measurement. ISO/IEC Guide 98-3 (JCGM 100:2008).
2) International Committee for Standardization in Haematology Recommendations for measurement of serum iron in human blood. Brit J Haematol 1978; 38 :291-294.
3) Iron Panel of the International Committee for Standardization in Haematology Revised recommendations for the measurements of the serum iron in human blood. Brit J Haematol 1990;75: 615-616.
4) Derman D.P, et al. A systematic evaluation of bathophenanthroline, ferrozine, and ferene in an ICSH-based method for the measurement of serum iron. Ann Clin Biochem 1989; 6: 144-147.
5) National Institute of Advanced Industrial Science and Technology (AIST) : study of Clinical Chemistry Standard Material 2008. p37-43.

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**Provider and Certification of JCCRM 322-7**

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