National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 909b

Human Serum

This Standard Reference Material (SRM) is primarily intended for use in evaluating the accuracy of clinical procedures for the determination of specified constituents in human serum. It can also be used to validate working or secondary reference materials. A unit of SRM 909b consists of six bottles of lyophilized human serum (three bottles each of two different analyte concentration levels) and six bottles of deionized, autoclaved water. Before use, the serum in each bottle is to be reconstituted with 10.00 mL of the water provided. The volume of water in each bottle is 11.5 mL.

Certified Concentration Values: The certified concentrations of the serum analytes were determined by primary methods of measurement having the highest metrological qualities, i.e., definitive methods [1-13]. The concentrations and their uncertainties for the two analyte concentration levels (SRM 909b-I and SRM 909b-II) are listed in Tables 1, 1a, and 2. The certified concentrations apply only to reconstituted serum at room temperature (20 °C to 25 °C)-see Instructions for Use.

Information Values: Information values for the activity of selected enzymes, total bilirubin and pH are provided in Table 3. The analytical measurements were made by the supplier of the material.

NOTICE AND WARNINGS TO USERS

SRM 909b 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. The supplier of this serum has reported that each donor unit of serum or plasma used in the preparation of this product had been tested by an FDA approved method and found non-reactive for HbsAg and HIV-1 antibody. However, no known test method can offer complete assurance that hepatitis B virus, HIV, or other infectious agents are absent from this material. Accordingly, this human blood-based product should be handled at the Biosafety Level 2 or higher as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual [15].

Stability and Storage: The serum comprising SRM 909b is a lyophilized (freeze-dried) material and should be stored in a refrigerator at a temperature between 2 °C and 8 °C until ready for use. It should not be frozen or exposed to sunlight or ultraviolet radiation.

Reconstituted Material: After reconstitution, the contents should be used immediately or stored between 2 °C and 8 °C until ready for use, preferably within 4 h. Freezing of the reconstituted material is not recommended.

Expiration of Certification: The expiration date is stamped on the outer box label-DO NOT DISCARD. Note: Because glucose values are known to degrade with time, NIST will remeasure the glucose content of SRM 909b periodically. Should the glucose or any of the other certified values change before expiration of the certification, purchasers will be notified by NIST. Return of the attached registration form will facilitate notification.

The technical and support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the Standard Reference Materials Program by J.C. Colbert.

Gaithersburg, MD 20899
March 14, 1996
(Revision of certificate dated 2-29-96)

Thomas E. Gills, Chief
Standard Reference Materials Program

Analytical measurements were performed in the NIST Analytical Chemistry Division by R.G. Christensen, S.A. Margolis, K.E. Murphy, P.J. Paulsen, C.S. Phinney, K.W. Pratt, M.S. Rearick, L.T. Sniegoski, T.W. Vetter, and R.D. Vocke and by P. Ellerbe and S.E. Long, College of American Pathologists Research Associates at NIST. Technical advice was provided by R. Schaffer, consultant to the SRM Program.

Consultation on the statistical design of the experimental work and evaluation of the data were provided by S.B. Schiller of the NIST Statistical Engineering Division.

The overall direction and coordination of the analyses were under the chairmanship of M.J. Welch of the NIST Analytical Chemistry Division.

Instructions for Use: Remove a bottle from the refrigerator and equilibrate at room temperature before reconstitution. Tap the bottom of the bottle to dislodge any serum particles. Carefully remove the metal seal. Take extra care in removing the stopper, as the lyophilized serum may adhere to the stopper. To ensure complete recovery of the serum, carefully remove the stopper, maintaining its position over the bottle and using a Type I Class A calibrated volumetric transfer pipet or other dispenser of known accuracy, add 10.00 mL $\pm$ 0.02 mL of the diluent water (provided with the SRM). Replace the stopper, swirl the bottle two or three times, and let stand for 10 min. Mix the contents by gently swirling, let stand for approximately 30 min, swirl again, let stand for 10 min, and finally invert the bottle several times. Repeat this process as necessary until all material has gone into solution. **Do not shake**. Vigorous shaking or mechanical swirling should be avoided as it may cause the formation of foam which may lead to inhomogeneous distribution of the analytes within the bottle. Allow 2 h for reconstitution. After reconstitution, the contents should be used immediately or stored between 2 °C and 8 °C until ready for use, preferably within 4 h.

Fill Weight Correction: There is a small variability in the fill weights of dry serum. This variability contributes to the uncertainties reported in Tables 1 and 1a. To determine the concentration corrected for variations in fill weight (as was done for the results in Table 2), the mass of dry serum in the bottle must be determined. The following procedure is recommended: Prior to opening the bottle, completely remove the bottle label and adhesive by scraping and then wiping the bottle with a tissue moistened with a solvent, such as acetone or ethanol. Scratch an identification on the bottle, then remove the metal closure and lightly tap the bottom of the bottle to dislodge any serum particles adhering to the stopper. Carefully dislodge the stopper to equalize air pressure, then replace it. Wipe the surface of the bottle, and weigh to the nearest 0.1 mg. (Use a clean empty bottle of the same size as a tare.) Reconstitute the serum as described in the Instructions for Use. After the reconstituted serum has been removed, clean and dry the bottle and its stopper. Replace the stopper in the bottle and reweigh. Also, reweigh the tare at the same time to compensate for changes in temperature and humidity. The mass of dry serum is obtained by the difference between the original and final weighings. To compare results with those in Table 2, divide the values in Tables 1 or 1a by the grams of dry serum in the bottle. The mean mass densities at 22.0 °C were determined by pycnometer to be (1.0221 $\pm$ 0.0002) g/mL and (1.0370 $\pm$ 0.0003) g/mL for Levels I and II, respectively, and are provided to allow conversions between results in mass/mass and mass/volume units.

CERTIFICATION ANALYSIS

Source of Material: SRM 909b, lyophilized human serum was prepared by the Diagnostic Group, Bayer Corporation, Middletown, VA.

For each analyte, a stratified sampling plan was devised to test for homogeneity across the manufacturing process. Methods considered to be "definitive" [1] for clinical analytes by the National Committee for Clinical Laboratory Standards (NCCLS) were used for the determinations [2-13]. All of the organic analytes were determined using isotope dilution/gas chromatographic/mass spectrometric methods. The inorganic analytes were determined using only isotope dilution thermal ionization mass spectrometric methods (ID/TIMS) with the following exceptions: Sodium was determined using a gravimetric procedure and for chloride, a coulometric method was used in addition to ID/TIMS. References to the specific methods used for each analyte are given in Table 1.

Table 1. Certified Concentrations and Uncertainties for Analytes in Reconstituted SRM 909b, Level I (in mmol/L and mg/dL [14]).

Analyte	mmol/L			mg/dL		
Calcium [2]	2.218	±	0.016	8.890	±	0.063
Chloride [3,4,5]	89.11	±	0.57	315.9	±	2.0
Cholesterol [6]	3.787	±	0.047	146.4	±	1.8
Creatinine [7]	0.05618	±	0.00055	0.6355	±	0.0062
Glucose [8]	5.40	±	0.28	97.4	±	5.1
Lithium [3]	0.6145	±	0.0050	0.4265	±	0.0034
Magnesium [3]	0.7634	±	0.0050	1.855	±	0.012
Potassium [3,9]	3.424	±	0.025	13.387	±	0.096
Sodium [10]	120.76	±	0.92	277.6	±	2.1
Total Glycerides [11]	0.949	±	0.061	84.0	±	5.4[a]
Triglycerides [11]	0.804	±	0.011	71.22	±	0.96[a]
Urea [12]	5.51	±	0.15	33.11	±	0.91[b]
Uric Acid [13]	0.2809	±	0.0051	4.722	±	0.086

[a]Results in mg/dL are expressed as the equivalent concentration of triolein.
[b]To calculate urea-N, multiply the urea value in mg/dL by the factor 0.4667.

Table 1a. Certified Concentrations and Uncertainties for Analytes in Reconstituted SRM 909b, Level II (in mmol/L and mg/dL)

Analyte	mmol/L			mg/dL		
Calcium	3.532	±	0.028	14.16	±	0.11
Chloride	119.43	±	0.85	423.4	±	3.0
Cholesterol	6.084	±	0.077	235.3	±	3.0
Creatinine	0.4674	±	0.0053	5.287	±	0.060
Glucose	15.0	±	1.1	271	±	20
Lithium	2.600	±	0.023	1.804	±	0.016
Magnesium	1.918	±	0.021	4.661	±	0.051
Potassium	6.278	±	0.052	24.55	±	0.020
Sodium	141.0	±	1.3	324.3	±	2.9
Total Glycerides	1.529	±	0.035	135.4	±	3.1[a]
Triglycerides	1.271	±	0.014	112.6	±	1.3[a]
Urea	30.75	±	0.32	184.7	±	2.0[b]
Uric Acid	0.7579	±	0.0090	12.74	±	0.15

[a]Results in mg/dL are expressed as the equivalent concentration of triolein.
[b]To calculate urea-N, multiply the urea value in mg/dL by the factor 0.4667.

Each certified value in Tables 1 and 1a is the mean of measurements made using a definitive method, except for chloride for which two methods considered definitive were used. Each expanded uncertainty is approximately a 95 %/95 % statistical tolerance interval which reflects the combined effects of measurement imprecision and the variability of the mass of dry serum among bottles [17]. Each interval is constructed so that, at a confidence level of 95 %, it will include the concentration for 95 % of all of the bottles of SRM 909b, when reconstituted according to the procedure described in Instructions for Use on page 2 (without determining the dry mass of the serum).

Table 2. Certified Concentrations and Uncertainties for Analytes in Reconstituted SRM 909b (in mmol/L/g) [see Fill Weight Correction on page 2].

Analyte	Level I mmol/L/g			Level II mmol/L/g		
Calcium	2.5289	±	0.0080	2.3416	±	0.0067
Chloride	101.65	±	0.25	79.40	±	0.17
Cholesterol	4.315	±	0.031	4.030	±	0.026
Creatinine	0.06397	±	0.00036	0.3110	±	0.0019
Glucose	6.16	±	0.21	9.97	±	0.50
Lithium	0.6997	±	0.0027	1.7235	±	0.0061
Magnesium	0.8703	±	0.0022	1.2723	±	0.0062
Potassium	3.903	±	0.011	4.166	±	0.012
Sodium	137.15	±	0.45	93.30	±	0.31
Total Glycerides	1.080	±	0.046	1.014	±	0.013
Triglycerides	0.9153	±	0.0074	0.8428	±	0.0049
Urea	6.29	±	0.11	20.376	±	0.089
Uric Acid	0.3194	±	0.0036	0.5019	±	0.0029

Each of the certified values in Table 2 are the mean of measurements made using a definitive method, except for chloride for which two methods considered definitive were used. Each expanded uncertainty, computed according to the CIPM method as described in the ISO Guide [16], is at the 99 % level of confidence; that is, each certified value and expanded uncertainty define a range of values within which the true concentration is expected to lie with approximately 99 % confidence.

Table 3. Information Values

Constituent	Activity/Concentration Level I	Level II
ALP (orthophosphoric-monoester phosphohydrolase)	86 U/L	410 U/L
LDH (lactate dehydrogenase)	145 U/L	480 U/L
ALT (L-alanine:2-oxyglutarate aminotransferase)	49 U/L	150 U/L
AST (L-aspartate:2-oxyglutarate aminotransferase)	43 U/L	200 U/L
CK (creatine kinase)	92 U/L	300 U/L
Total Bilirubin	0.9 mg/dL	5.1 mg/dL
pH	7.9 @ 22.6 °C	7.8 @ 22.9 °C

The information values in Table 3 were measured using a conventional clinical analyzer (Technicon Chem-1 System[1]) and are the mean of three determinations. These are confirmation values determined in the supplier's quality assurance laboratory. The enzyme materials used to spike the SRM were as follows: ALP, chicken intestine; LDH, chicken heart; and ALT, AST, and CK, porcine heart. All enzyme activities were determined at 37 °C.

[1]The use of a tradename on this certificate is for identification only and does not imply endorsement of the product by the National Institute of Standards and Technology.

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