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 [1-12]. 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".

Reference Concentration Values: Reference concentration values for total bilirubin are provided in Table 3. The reference concentrations were derived from results reported by two collaborating laboratories and NIST. The reference values are noncertified values that **DO NOT** meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple methods.

Information Values: Information values for the activity of selected enzymes and pH are provided in Table 4. 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 *Biosafety in Microbiological and Biomedical Laboratories* [13].

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

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Certificate Issue Date: 19 November 2003
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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. Technical advice and coordination for the total bilirubin measurements were provided by B. Doumas of the Medical College of Wisconsin, Milwaukee, WI. Analytical measurements for the determination of total bilirubin were performed in the NIST Analytical Chemistry Division by Y.Y. Davidson and L.T. Sniegoski and by scientists at the Wisconsin State Laboratory of Hygiene (Madison, WI) and the Children's Hospital of Wisconsin (Milwaukee, WI).

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. Consultation on the statistical design of the experimental work and evaluation of the data for total bilirubin were provided by N-F. Zhang of the NIST Statistical Engineering Division.

The support aspects involved in the revision and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert and B.S. MacDonald of the NIST Measurement Services Division.

Stability and Storage: The serum comprising SRM 909b is 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.

Expiration of Certification: The certification of this SRM lot is valid, within the measurement uncertainties, until the date stamped on the outer box label **(DO NOT DISCARD)**, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification is invalid if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

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.

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. Using a Type I Class A calibrated volumetric transfer pipet or other dispenser of known accuracy, add 10.00 mL ± 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 that 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 Table 1 and Table 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" section. 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 Table 1 or Table 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 ± 0.0002) g/mL and (1.0370 ± 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.[1]

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 [2-12]. All of the organic analytes were determined using isotope dilution gas chromatographicmass spectrometric (ID-GC/MS) methods. The inorganic analytes were determined using only isotope dilution thermal ionization mass spectrometric (ID-TIMS) methods 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
Lithium [3]	0.6145	±	0.0050	0.4265	±	0.0034
Magnesium [3]	0.7634	±	0.0050	1.855	±	0.012
Potassium [3,8]	3.424	±	0.025	13.387	±	0.096
Sodium [9]	120.76	±	0.92	277.6	±	2.1
Total Glycerides [10]	0.949	±	0.061	84.0	±	5.4[a]
Triglycerides [10]	0.804	±	0.011	71.22	±	0.96[a]
Urea [11]	5.51	±	0.15	33.11	±	0.91[b]
Uric Acid [12]	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
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	±	0.20
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 Table 1 and Table 1a is the mean of measurements made using a definitive method, except for chloride, which two methods considered definitive were used. Each expanded uncertainty [15] 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 [16]. 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 of 5 (without determining the dry mass of the serum).

[1] Certain commercial equipment, instrumentation, or materials are identified in this certificate in order to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 2. Certified Concentrations and Uncertainties for Analytes in Reconstituted SRM 909b (in mmol/L/g) [See "Fill Weight Correction" on page 2 of 5]

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
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 [15], 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. Reference Concentrations and Uncertainties for Total Bilirubin in Reconstituted SRM 909b

Total Bilirubin	mmol/L	mg/dL
Level I	0.0139 ± 0.0021	0.81 ± 0.12
Level II	0.0754 ± 0.0024	4.41 ± 0.14

The reference values are the overall means of the measurements made by NIST and two collaborating laboratories all using the same reference method [17]. The expanded uncertainty, U, is a 95 % prediction uncertainty for a future measurement. The expanded uncertainty is calculated as $U = ku_c$, where u_c is at the level of one standard deviation and represents the combined effects of between-laboratory components of uncertainty and measurement errors. The coverage factor k is determined from the Student's t-distribution corresponding to the appropriate degrees of freedom at the 95 % confidence level.

Table 4. Information Values

Constituent	Activity/Concentration Level I (pH 7.9 @ 22.6 °C)	Level II (pH 7.8 @ 22.9 °C)
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
ALP (orthophosphoric-monoester phosphohydrolase)	86 U/L	410 U/L
CK (creatine kinase)	92 U/L	300 U/L

The information values in Table 4 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.

[2]Certain commercial equipment, instrumentation, or materials are identified in this certificate in order to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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Certificate Revision History: 19 November 2003 (This technical revision reports the correction of the uncertainties for bilirubin); 13 December 2002 (This technical revision reports the addition of reference values for total bilirubin); 31 October 1997 (This technical revision reports the de-certification of the analyte glucose.); 07 May 1996 (Editorial changes); 14 May 1996 (Original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet http://www.nist.gov/srm.