U. S. Department of Commerce
Malcolm Baldrige
Secretary

National Bureau of Standards
Ernest Ambler, Director

National Bureau of Standards

Certificate of Analysis

Standard Reference Material 909

Human Serum

This Standard Reference Material (SRM) is intended for use in evaluating the accuracy of clinical procedures for the determination of specified constituents in serum, in calibrating instruments and equipment used in these procedures, and in validating working or secondary reference materials.

CERTIFIED VALUES OF ANALYTES: The concentrations of the analytes were determined by methods having the highest accuracy, i.e., definitive methods. The certified values are given in two ways, corresponding to whether reconstitution is done with or without weighing the freeze-dried serum contents of a vial. Concentration values having smaller uncertainties may be obtained by weighing the freeze-dried serum and multiplying this mass by the certified concentration values of the analytes per unit mass of dried serum. These certified values appear in Table 1. When the contents of a vial are not weighed, the values for the certified concentrations and their uncertainties in Table 2 should be used. These values apply to all vials of reconstituted SRM 909. Certified trace element concentrations for the two reconstitution procedures appear in Tables 1′ and 2′.

INFORMATION VALUES OF TOTAL PROTEIN AND ENZYMES: The concentration of total protein determined by a candidate reference method appears on page 5. The catalytic concentrations of seven enzymes are shown in Table 3. Table 3′ compares estimated enzyme catalytic concentrations at 37.0 °C with those at 29.77 °C in Table 3.

NOTICE AND WARNINGS TO USERS

USE: HANDLE AS IF CAPABLE OF TRANSMITTING HEPATITIS! Although this product was tested with licensed third generation reagents and found nonreactive for the presence of hepatitis B surface antigen (HB_SAG), no known test method can offer assurances that products derived from human blood will not transmit hepatitis.

SRM 909 IS INTENDED FOR "IN VITRO" DIAGNOSTIC USE ONLY.

STORAGE: The freeze-dried serum should be stored in a refrigerator at a temperature between 2 and 8 °C. It should not be frozen nor exposed to sunlight or ultraviolet radiation. Under the recommended storage conditions, this SRM is expected to be stable for at least one year; should evidence indicate a more rapid degradation of the certified properties, purchasers will be notified by NBS. The material is not certified for use after one year from date of purchase.

The statistical analysis of the data was performed by K.R. Eberhardt of the Statistical Engineering Division.

The overall direction and technical measurements leading to the certification were under the chairmenship of E. Garner, H.S. Hertz, T.J. Murphy, D.J. Reeder, R. Schaffer and E. White V.

The technical and support aspects concerning the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by R. Alvarez.

Gaithersburg, MD 20899
September 15, 1980
Certificate revised in 1981, 1982, 1983, and 1984 (2)
March 1985 (revision)*

Stanley D. Rasberry, Chief
Office of Standard Reference Materials

*This revision reports trace element concentrations and estimated enzyme catalytic concentrations at 37.0 °C.

(over)

INSTRUCTIONS FOR USE

HANDLE AS IF CAPABLE OF TRANSMITTING HEPATITIS! SRM 909 is supplied as a set of six vials of freeze-dried human serum and six vials of high-purity, diluent water for use in reconstituting the serum.

Two procedures for reconstituting SRM 909 are described. Selection of a procedure depends on the uncertainties required for the concentrations of the analytes. If lower uncertainties than those shown in Table 2 are required, the freeze-dried serum contents of a vial must be weighed as described in Procedure A.

Procedure A. Reconstitution with weighing of the freeze-dried serum: Completely remove label and adhesive by scraping the vial and then wiping it with a tissue moistened with a solvent, such as acetone or ethanol. Scratch an identification on vial. Remove metal closure and lightly tap bottom of vial to dislodge any serum particles adhering to the stopper. Dislodge stopper to equalize air pressure, then replace, wipe surface of vial, and weigh to the nearest 0.1 mg. (Use a clean empty vial of the same size as a tare.) Carefully remove stopper to avoid possible loss of serum particles. Use a Type 1 Class A volumetric transfer pipet or other dispenser of known accuracy to slowly add 10.00 ± 0.02 mL of the diluent water at 20 - 25 °C to the sides of the vial while continually turning the vial. Replace stopper, swirl vial two or three times, and let stand for 10 minutes. Mix contents by gently swirling, let stand for approximately 30 minutes, swirl again, let stand 10 minutes, and finally invert the vial several times. *Do NOT shake vigorously* because this will cause frothing. Total time for reconstitution is approximately 1 hour. After reconstitution, use contents as soon as possible. If not used immediately, store between 2 and 8 °C until ready for use, preferably within 8 hours. After the reconstituted serum has been used, clean and dry the vial and its stopper. Reweigh after replacing stopper. The tare is reweighed at the same time to compensate for changes in temperature and humidity. The mass of dry serum is given by the difference between the original and final weighings.

The concentration of an analyte, after the contents of a vial is weighed and reconstituted with 10.00 mL of diluent water, is calculated by multiplying the mass of freeze-dried serum, in grams, by the certified concentration of the analyte per gram of freeze-dried serum given in Table 1. For example, if the mass of freeze-dried serum in a vial were 0.8703 g, the certified concentration of lithium in this vial would be:

$$1.945 \text{ mmol} \cdot L^{-1} \cdot g^{-1} \times 0.8703 \text{ g} = 1.693 \text{ mmol/L}.$$

The uncertainty is calculated similarly and for this example becomes:

$$\pm 0.010 \text{ mmol} \cdot L^{-1} \cdot g^{-1} \times 0.8703 \text{ g} = \pm 0.0087 \text{ mmol/L}.$$

Table 1. Certified Analyte Concentrations and Uncertainties per Gram of Freeze-Dried Serum after Reconstitution of SRM 909 according to Procedure A.

Analyte[1]	Concentration,[2] per gram, $mmol \cdot L^{-1} \cdot g^{-1}$	Uncertainty,[3] per gram, $mmol \cdot L^{-1} \cdot g^{-1}$
Calcium[a]	3.560	± 0.004
Chloride[a]	128.0	± 0.4
Cholesterol[a]	4.359	± 0.017
Creatinine[a]	0.17963	± 0.00072
Glucose[a]	7.56	± 0.28
Lithium[a]	1.945	± 0.010
Magnesium[a]	1.425	± 0.015
Potassium[a]	4.155	± 0.011
Sodium[b]	158.4	± 1.0
Urea[a]	11.387	± 0.049
Uric Acid[a]	0.570	± 0.003

1. Analytical Methods
   a. Isotope dilution mass spectrometry.
   b. Ion-exchange separation, gravimetric method (concentration confirmed by atomic absorption spectrometry).
2. The certified concentrations apply to reconstituted serum at room temperature (20-25 °C).

The user should note that the certified values listed in the October 8, 1982 Certificate for cholesterol, 4.346 ± 0.030, and for uric acid, 0.5681 ± 0.0050, have changed slightly as a result of reanalysis of the original data. The new values are well-within the original uncertainty limits.

The concentration of glucose is known to decrease with time and had changed from 7.811 ± 0.095 $mmol \cdot L^{-1} \cdot g^{-1}$ originally listed in the September 15, 1980 Certificate to 7.74 ± 0.11 $mmol \cdot L^{-1} \cdot g^{-1}$ listed in the October 8, 1982 revision.

The stability of SRM 909 will continue to be monitored and users will be advised of any changes.

3. The uncertainties for all analytes except glucose are given as 99% confidence intervals, and include allowances for possible systematic error.

For glucose, the uncertainty is given as a 95%/99% statistical tolerance interval so that at a confidence level of 95% the interval includes 99% of all vials. It also includes an allowance for the decrease in glucose concentration with time (about 0.05 $mmol \cdot L^{-1} \cdot g^{-1}$ per year.)

Table 1' Certified Trace Element Concentrations and Uncertainties Per Gram of Freeze-Dried Serum after Reconstitution of SRM 909 According to Procedure A

Analyte[1]	Concentration[2], per gram	
	$\mu g \cdot mL^{-1} \cdot g^{-1}$	$\mu mol \cdot L^{-1} \cdot g^{-1}$
Copper	1.29 ± 0.08	20.3 ± 1.2
Iron	2.34 ± 0.24	41.9 ± 4.3
	$ng \cdot mL^{-1} \cdot g^{-1}$	$nmol \cdot L^{-1} \cdot g^{-1}$
Cadmium	1.46 ± 0.08	13.0 ± 0.7
Chromium	108 ± 4	2080 ± 80
Lead	23.7 ± 2.1	114 ± 10
Vanadium	3.19 ± 0.55	63 ± 11

[1]Analytical Method - Isotope dilution mass spectrometry. Analyses were performed at the NBS Center for Analytical Chemistry by: K.A. Brletic, J.D. Fassett, H.M. Kingston, J.R. Moody, L.J. Powell, and E. Michiels (NBS Guest Worker).

[2]The certified concentrations apply to reconstituted serum at room temperature (20-25 °C). The uncertainties are 99% confidence intervals and include allowances for possible systematic error.

Procedure B. Reconstitution of SRM 909 without weighing freeze-dried serum: Remove metal closure and lightly tap bottom of vial to dislodge any serum particles on stopper. Carefully remove stopper to avoid possible loss of serum particles. As described in Procedure A, reconstitute with 10.00 ± 0.02 mL of the diluent water, and use immediately or store between 2 and 8 °C until ready for use, preferably within 8 hours. Table 2 gives the certified concentrations of the constitutents and the tolerance limits for use with this procedure.

Table 2. Certified Concentrations and Uncertainties for Analytes in Reconstituted SRM 909 for Use with Procedure B.

Analyte[1]	Concentration[2], $mmol \cdot L^{-1}$	Uncertainty[3], $mmol \cdot L^{-1}$
Calcium[a]	3.013	+0.088 −0.048
Chloride[a]	108.4	+3.4 −2.0
Cholesterol[a]	3.69	+0.12 −0.07
Creatinine[a]	0.1521	+0.0049 −0.0029
Glucose[a]	6.41	+0.41 −0.37
Lithium[a]	1.65	+0.05 −0.04
Magnesium[a]	1.21	+0.05 −0.03
Potassium[a]	3.52	+0.11 −0.06
Sodium[b]	134.1	+4.6 −2.8
Urea[a]	9.64	+0.31 −0.19
Uric Acid[a]	0.483	+0.016 −0.010

1. Analytical Methods:
   a. Isotope dilution mass spectrometry.
   b. Ion-exchange separation, gravimetric method (concentration confirmed by atomic absorption spectrometry).
2. The certified concentrations apply to reconstituted serum at room temperature (20-25 °C).

The user should note that the certified values listed in the October 8, 1982 Certificate for cholesterol, 3.68 (+0.22, −0.08), and for uric acid, 0.481 (+0.031, − 0.012), have changed slightly as a result of reanalysis of the original data. The new values are well within the original uncertainty limits.

The concentration of glucose is known to decrease with time and had changed from 6.62 (+0.44, −0.20) originally listed in the September 15, 1980 Certificate to 6.55 (+0.46, −0.21) listed in the October 8, 1982 revision.

3. The uncertainties for all analytes except glucose are 95%/97% statistical tolerance intervals and reflect the combined effects of measurement imprecision and the variability of the mass of dry serum among vials. The intervals are constructed so that, at a confidence level of 95%, they will include the concentrations for 97% of all vials of SRM 909, when reconstituted according to Procedure B. For glucose, the statistical tolerance interval is 95%/95%.

The stability of SRM 909 will continue to be monitored and users will be advised of further changes.

The major source of uncertainty in the certified concentrations shown in Table 2 is vial-to-vial variability in the mass of freeze-dried serum. Information on the relation between mass of dry serum and concentration was obtained directly during the course of experimentation by weighing the contents of each vial as an intermediate step in determining the concentration of an analyte. Dry serum masses were obtained for 129 vials in this way. Supplementary information on the variability in mass was obtained from differential refractometry experiments in which 184 vials were analyzed. The data obtained from these two sources indicate that the distribution of masses is non-Gaussian, containing outliers in both directions. Except for three vials, all masses were contained in an interval from 1.5% below to 1.8% above the median mass, 0.8465 g. The three outliers were located at 14.8% below, and at 2.7% and 5.2% above the median mass.

Table 2' Certified Trace Element Concentrations and Uncertainties in Reconstituted SRM 909 for Use with Procedure B

Analyte[1]	Concentration[2]	
	$\mu g \cdot mL^{-1}$	$\mu mol \cdot L^{-1}$
Copper	$1.10^{+0.10}_{-0.083}$	$17.2^{+1.5}_{-1.3}$
Iron	$1.98^{+0.27}_{-0.23}$	$35.4^{+4.8}_{-4.1}$
	$ng \cdot mL^{-1}$	$nmol \cdot L^{-1}$
Cadmium	$1.24^{+0.10}_{-0.09}$	$11.0^{+0.9}_{-0.8}$
Chromium	$91.3^{+6.1}_{-5.0}$	1760^{+120}_{-100}
Lead	$20.0^{+2.5}_{-2.1}$	97^{+12}_{-10}
Vanadium	$2.70^{+0.56}_{-0.51}$	53^{+11}_{-10}

[1]Analytical Method - Isotope dilution mass spectrometry. Analyses were performed at the NBS Center for Analytical Chemistry by: K.A. Brletic, J.D. Fassett, H.M. Kingston, J.R. Moody, L.J. Powell, and E. Michiels (NBS Guest Worker).

[2]The certified concentrations apply to reconstituted serum at room temperature (20-25 °C). The uncertainties are statistical tolerance intervals to cover 97% of all vials of SRM 909 with a confidence level of 95%.

CONCENTRATION OF TOTAL PROTEIN

The following value is based on the results of a candidate reference method. It is a method-specific value.

Protein Concentration in SRM 909.

Reconstituted using "Procedure A"	$74.72 \pm 0.46\ g \cdot L^{-1} \cdot g^{-1}$
Reconstituted using "Procedure B"	$63.3^{+2.2}_{-1.3}\ g \cdot L^{-1}$

The protein measurements were made in accordance with the procedure described in "A Candidate Reference Method for Determination of Total Protein in Serum I. Development and Validation", Doumas, B.T., Bayse, D.D., Carter, R.J., Peters, T., and Schaffer, R. Clinical Chemistry 27 (10):1642-1654 (1981). This method currently (June, 1983) is under review for acceptance into the National Reference System for Clinical Chemistry (NRSCC). The method was previously approved by the Standards Committee of the American Association for Clinical Chemistry for publication in Clinical Chemistry.

The analysis was designed to assure appropriate controls for instrumental drift, random errors of measurement, and vial-to-vial variability. Ten vials of SRM 909 were reconstituted and used within one day. The protein concentration was calculated by comparison with absorbance measurements made on a standard protein solution, SRM 927. Individual as well as pooled samples were used for both SRM 909 and SRM 927.

Source of Material: The human serum for SRM 909 was processed, vialed, and packaged by Hyland Division, Travenol Laboratories Inc., Round Lake, Illinois.

Analyses were performed in the NBS Center for Analytical Chemistry by I.L. Barnes, K.A. Brletic, R.G. Christensen, A. Cohen, J.W. Gramlich, D.K. Hancock, W.R. Kelly, L.R. Machlan, J.R. Moody, L.J. Powell, T.C. Rains, D.J. Reeder, L.T. Sniegoski, and M.J. Welch.

This Standard Reference Material has been measured and certified at the Laboratories of the National Bureau of Standards, Gaithersburg, Maryland. All inquiries should be addressed to:

Office of Standards Reference Materials
Room B311, Chemistry Building
National Bureau of Standards
Gaithersburg, MD 20899

The date of issuance and certification of this Standard Reference Material is September 15, 1980.

INFORMATION ON ENZYMES

Enzymes: The catalytic concentrations in terms of U/L* of seven enzymes were determined in an interlaboratory study, and are given in Table 3. The results are in a form that follows ANSI/ASTM E691-79 "Standard Practice for Conducting an Interlaboratory Test Program to Determine the Precision of Test Methods".

Because of the state-of-the-art for the determination of enzymes, their concentrations cannot be stated on an accuracy basis. The enzyme values shown below are material and method-dependent. Thus, any laboratory using this SRM and the catalytic concentrations of these enzymes must follow the methods as described. The values given are consensus values, *not certified by NBS*, and were obtained through the interlaboratory study. They are provided for information only.

*Catalytic concentration is expressed in terms of International enzyme units per liter (U/L) where U is given as micromoles of substrate converted per minute. (1.0 U equals 16.67 nkat.)

Table 3

Catalytic Concentrations of Enzymes in Reconstituted SRM 909

Enzyme	Number of Laboratories	Overall Mean, U/L	Repeatability[1] Std. Dev.	Reproducibility[2] Std. Dev.
Acid Phosphatase[3]	3	0.23	0.02	0.04
Alkaline Phosphatase	6	75.4	1.9	2.2
Alanine Aminotransferase	5	24.2	0.8	1.8
Aspartate Aminotransferase	5	30.7	0.4	0.9
Creatine Kinase	4	123.0	9.7	10.2
Lactate Dehydrogenase	4	229.2	5.0	9.6
γ-Glutamyltransferase	5	16.4	0.3	0.4

1. Repeatability (within-laboratory standard deviation) for each enzyme was obtained by calculating the standard deviation of the four daily means (5 replicates per mean) obtained in each laboratory, and pooling the results across laboratories.

2. Reproducibility standard deviation combines the within-laboratory and between-laboratory standard deviations. It represents the typical variability among results obtained by different laboratories, each analyzing one vial under the present protocol (which involves averaging five replicate values).

3. Native catalytic concentrations. Prostatic acid phosphatase was not added to the serum base.

Reconsitution Procedure for Enzyme Determinations: The enzyme concentrations listed in Table 3 were measured after the lyophilized serum had been reconstituted with ice-cold water for about one hour as described below. This procedure is different from those given previously in the Certificate.

1. Remove the vials of SRM 909 lyophilized serum and diluent water from the refrigerator.
2. Immerse the vial of water in finely divided ice-water (slush) for 5 minutes. Water-ice should contact the vial, not air-ice.
3. Tap the SRM 909 vial lightly on a solid surface to dislodge any serum particles adhering to the stopper. Remove metal cap and stopper, being careful to avoid loss of serum particles.
4. Transfer 10.0 mL of the cold diluent water** to the vial of serum as follows: Using a class A, 10-mL pipette at room temperature, dispense the water slowly to the side of the vial while continually turning the vial, to wet all serum particles and enhance dissolution.
5. Replace the stopper, swirl to wet beads of serum, gently invert vial 10 times, and immerse the vial in ice water. Repeat the gentle swirling and inverting operations at 10 minute intervals for one hour, always returning the vial to the ice-water bath.
6. Inspect the reconstituted SRM 909 visually. Undissolved serum particles should not be evident. Invert the reconstituted SRM gently several times before use for specific enzyme procedures.

**A slight excess over 10.0 mL is actually delivered in this procedure. For enzyme concentration measurements, the error is not considered significant.

Enzyme Methods: The methods used in this study are outlined below. Details of the methods and the experience with their use during the interlaboratory study have been published in the NBS 260 Series. (1) (This series is used to disseminate information on the preparation, measurement, certification, and use of NBS SRM's.) All methods use a reaction temperature of 29.77 °C, which can be verified by using a Gallium Melting Point Cell, SRM 1968 (2).

1. Acid Phosphatase [Orthophosphoric monoester phosphohydrolase (acid optimum), EC 3.1.3.2].

The catalytic concentration of acid phosphatase in U/L is determined using thymolphthalein monophosphate as substrate. Enzymatic hydrolysis at pH 5.4 yields a colorless product, thymolphthalein, which becomes a self-indicating chromogen (λ= 595 nm) when the enzymatic reaction is terminated by the addition of alkali. The method of Ewen and Spitzer (3) is employed with the following modifications: a) the reaction temperature is 29.77 °C and b) multiple tests are performed at 15, 30, 45, and 60 min. The final reaction conditions are:

Reaction temperature	29.77 °C
pH	5.4
Acetate buffer	0.15 mol/L
Thymolphthalein monophosphate	1.0 mmol/L
Brij-35	1.5 g/L
Volume fraction (sample/total)	0.083 (1:12)

2. Alkaline Phosphatase [Orthophosphoric monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1].

The catalytic concentration of alkaline phosphatase in U/L is measured by following the liberation of 4-nitrophenol from the substrate 4-nitrophenyl phosphate in the transphosphorylating buffer, 2-amino-2-methyl-1-propanol. It utilizes the method of Bowers and McComb (4) with the single modification of changing the reaction temperature from 30 °C to 29.77 °C. The final reaction conditions are:

Reaction temperature	29.77 °C
pH	10.5
4-Nitrophenylphosphate	16.0 mmol/L
2-Amino-2-methyl-1-propanol	1.0 mol/L
Magnesium acetate	1.0 mmol/L
Volume fraction (sample/total)	0.0164 (1:61)

3. Alanine Aminotransferase (L-Alanine: 2 oxoglutarate aminotransferase, EC 2.6.1.2).

The catalytic concentration of alanine aminotransferase (AlaAt) in U/L is measured by coupling pyruvate production with reduced nicotinamide adenine dinucleotide (NADH) and lactate dehydrogenase (LDH). It utilizes the IFCC/EPE and AACC/AlaAt Study Group reference method conditions (5,6) at a reaction temperature of 29.77 °C instead of 30 °C. The volume fraction of serum allows convenient use of standard volumetric glassware. The starting reagent is 2-oxoglutarate, added after 15 minutes preincubation of specimen with an otherwise complete reaction mixture at 29.77 °C and after a stable absorbance is reached at 339 nm. The coupled catalytic reaction is followed for 300 seconds. A reagent blank reaction rate (water as specimen) is deducted from the overall rate. The final reaction conditions are:

Reaction temperature	29.77 °C
pH	7.3
L-Alanine	500 mmol/L
2-Oxoglutarate	15 mmol/L
Pyridoxal-5'-phosphate	0.11 mmol/L
Tris buffer	89 mmol/L
NADH (assuming NADH $Na_2 \cdot 2H_2O$)	0.16 mmol/L
LDH (EC 1.1.1.27)	2.2 U/mL (25 °C)
Volume fraction (sample/total)	0.083 (1:12)

4. Aspartate Aminotransferase (L-Aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1).

The catalytic concentration of aspartate aminotransferase in U/L is measured by coupling oxalacetate production with reduced nicotinamide adenine dinucleotide (NADH) and malate dehydrogenase (MDH). It utilizes the IFCC/EPE reference method conditions (7) at a reaction temperature of 29.77 °C instead of 30 °C and with minor modifications suggested by the AACC Study Group (8). In addition, lactate dehydrogenase is omitted from the reagent system. The starting reagent is 2-oxoglutarate, added after 15 minutes preincubation at 29.77 °C and after a stable absorbance is reached at 339 nm. The coupled catalytic reaction is followed for 300 seconds. The final reaction conditions are:

Reaction temperature	29.77 °C	
pH	7.8	
L-Aspartate	175	mmol/L
2-Oxoglutarate	15	mmol/L
Pyridoxal-5'-phosphate	0.11	mmol/L
Tris buffer	89	mmol/L
NADH (assuming NADH-Na_2·$2H_2O$)	0.16	mmol/L
MDH (EC 1.1.1.37)	0.95	U/mL (25 °C)
Volume fraction (sample/total)	0.083 (1:12)	

5. Creatine Kinase (ATP: creatine N-phosphotransferase, EC 2.7.3.2).

The catalytic concentration of creatine kinase (CK) is measured by coupling adenosine triphosphate production with nicotinamide adenine dinucleotide (NAD^+) through the use of intermediate reactions catalyzed by hexokinase and glucose-6-phosphate dehydrogenase. It follows the method recommended by the IFCC/EPE (9) and the AACC/CK Study Group (10) with the following modifications: a) adenylate kinase inhibitors are removed; b) a blank reaction is run; c) disodium ethylenediamine tetraacetate (Na_2EDTA) is added to the system; and d) the reaction temperature is 29.77 °C. The final reaction conditions are:

Reaction temperature	29.77 °C	
pH	6.6	
Imidazole acetate	100	mmol/L
Creatine phosphate	30	mmol/L
Adenosine-5'-diphosphate	2	mmol/L
D-Glucose	20	mmol/L
NAD^+	2	mmol/L
Hexokinase (EC 2.7.1.1)	2500	U/L
D-Glucose-6-phosphate dehydrogenase (EC 1.1.49) (from Leuconostoc mesenteroides)	1500	U/L
Magnesium acetate	10	mmol/L
N-Acetyl cysteine	20	mmol/L
Na_2EDTA	2	mmol/L
Volume fraction (sample/total)	0.043 (1:23)	

6. Lactate Dehydrogenase (L-Lactate: NAD^+ oxidoreductase, EC 1.1.1.27).

The catalytic concentration of lactate dehydrogenase in U/L is measured by following the oxidation of reduced nicotinamide adenine dinucleotide (NADH) at 339 nm. It utilizes the method of Bowers (11) with minor modifications at a reaction temperature of 29.77 °C. The final reaction conditions are:

Reaction temperature	29.77 °C	
pH	7.2	
Sodium pyruvate	1.2	mmol/L
NADH	0.15	mmol/L
Tris buffer	96.8	mmol/L
Volume fraction (sample/total)	0.016 (1:61)	

7. γ-Glutamyltransferase [(γ-Glutamyl)-peptidase: amino acid γ-glutamyltransferase, EC 2.3.2.2.].

The catalytic concentration of γ-glutamyltransferase in U/L is measured by following the liberation of the 4-nitroaniline at 410 nm. The acceptor substrate glycylglycine also serves as the buffer. This assay utilizes the IFCC/EPE proposed reference method (12) with a modification in reaction temperature from 30 °C to 29.77 °C. This assay is initiated by the addition of serum to the combined substrate/buffer mixture equilibrated to 29.77 °C. It is followed at 410 nm for at least 300 seconds. The final assay reaction conditions are:

Reaction temperature	29.77 °C	
pH	7.90	
L-γ-Glutamyl-3-carboxy-4-nitroanilide	6	mmol/L
Glycylglycine	150	mmol/L
Volume fraction (sample/total)	0.091 (1:11)	

References:

(1) Bowers, G.N., Jr., Alvarez, R., Cali, J.P., Eberhardt, K.R., Elser, R., Ewen, L.M., McComb, R.B., Reeder, D.J., Rej, R., Schaffer, R., Shaw, L.M., and Uriano, G.A., Standard Reference Materials: The Measurement of the Catalytic (Activity) Concentration of Seven Enzymes in NBS Human Serum SRM 909, NBS Spec. Publ. 260-83 (June 1983).

(2) Mangum, B. W. and Thornton, D. D., Eds., Standard Reference Materials: "The Gallium Melting Point Standard", NBS Spec. Publ. 481 (June 1977); also Clin. Chem. 23, 711-724 (1977).

(3) Ewen, L. M. and Spitzer, R. W., Improved determination of prostatic acid phosphatase (sodium thymolphthalein monophosphate substrate), Clin. Chem. 22, 627-632 (1976).

(4) McComb, R. B., Bowers, Jr., G. N., and Posen, S., Alkaline Phosphatase. Plenum Press, New York, N.Y. (1979), pages 903-907, or also Clin. Chem. 21, 1988-1995 (1975).

(5) IFCC Methods for the measurement of catalytic concentrations of enzymes, Part 3. IFCC Method for alanine aminotransferase (L-alanine 2-oxoglutarate aminotransferase, EC 2.6.1.2) (Stage 2, Draft 1, 1979-11), Clin. Chim. Acta 105, 147F-172F (1980).

(6) Recommendations of the Alanine Aminotransferase Study Group, Subcommittee on Enzymes, Committee on Standards, American Association for Clinical Chemistry - Rej, R., Clin. Chem. 26, 1023 (1980) and personal communication from the author.

(7) IFCC Methods for the measurement of catalytic concentrations of enzymes, Part 2. IFCC Method for aspartate aminotransferase (L-Aspartate 2-oxoglutarate aminotransferase, EC 2.6.1.1) (Stage 2, Draft, 1977), Clin. Chim. Acta 80, F21, F22, following page 394 (1977) Redefinitions of conditions previously published as part 2 in Clin. Chim. Acta 70, F19-F42, following page 470 (1976).

(8) Recommendations of the Aspartate Aminotransferase Study Group, Subcommittee on Enzymes, Committee on Standards, American Association for Clinical Chemistry - Clin. Chem. 26, 1023-1024 (1980).

(9) IFCC Methods for the measurement of catalytic concentrations of enzymes, Part 7. Method for creatine kinase (ATP : creatine N-phosphotransferase, EC 2.7.3.2) (Stage 1, Draft 1981) obtained from IFCC Expert Panel on Enzymes, M. Horder (Chairman), Department of Clinical Chemistry, University of Odense, Odense, Denmark.

(10) Recommendation of the Study Group on Creatine Kinase, Subcommittee on Enzymes, Committee on Standards, American Association for Clinical Chemistry, 1979 - personal communication with R. Elser.

(11) Bowers, G. N., Jr., Lactic Dehydrogenase. In Standard Methods of Clinical Chemistry (Vol. 4), Seligson, D., Ed., Academic press, New York 1963, p. 163.

(12) IFCC Methods for the measurement of catalytic concentrations of enzymes, Part 4. IFCC Method for γ-glutamyltransferase [(γ-glutamyl)-peptide: amino acid γ-glutamyltransferase, EC 2.3.2.2] (Stage 1, Draft 1981-3) - personal communication with L. M. Shaw.

Team 1 - Acid Phosphatase (ACP)

L. M. Ewen, Royal Columbian Hospital, New Westminister, B. C., Canada (Team Leader)
R. Bondar, Worthington Diagnostics, Freehold, N.J.
W. Miller, Dupont - ACA Division, Wilmington, Del.
G. N. Bowers, Jr., and M. Onoroski, Clinical Chemistry Laboratory, Hartford Hospital, Hartford, Conn.
L. M. Shaw, HUP/Pepper Laboratory, Philadelphia, Pa.

Team 2 - Alkaline Phosphatase (ALP)

G. N. Bowers, Jr., Hartford Hospital, Hartford, Conn. (Team Leader)
R. Miller, Technicon Instrument Company, Tarrytown, N.Y.
W. D. Fellows, Health Safety & Human Factors Laboratory, Kodak Park, Rochester, N.Y.
J. P. Bretaudiere, N.Y. State Department of Health, Albany, N.Y.
D. C. Hohnadel, Christ Hospital, Cincinnati, Ohio
W. Sowers, Hyland Division of Travenol, Round Lake, Ill.

Team 3 - Alanine Aminotransferase (AlaAt)

R. Rej, N.Y. State Department of Health, Albany, N.Y. (Team Leader)
R. Miller, Technicon Instrument Company, Tarrytown, N.Y.
W. Ryan, Beckman Instruments, Inc., Brea, Calif.
L. M. Shaw, HUP/Pepper Laboratory, Philadelphia, Pa.
G. Sims, Hycel Inc., Houston, Texas

Team 4 - Aspartate Aminotransferase (AspAt)

R. Rej, N.Y. State Department of Health, Albany, N.Y. (Team Leader)
L. M. Shaw, HUP/Pepper Laboratory, Philadelphia, Pa.
W. D. Fellows, Health Safety & Human Factors Lab., Kodak Park, Rochester, N.Y.
N. W. Tietz, University of Kentucky Medical Center, Lexington, Ky.
A. Bacharach, Strong Memorial Hospital, Rochester, N.Y.

Team 5 - Creatine Kinase (CK)

R. Elser, York Hospital, York, Pa. (Team Leader)
R. Davis, Dupont - ACA Division, Wilmington, Del.
W. Ryan, Beckman Instruments, Inc., Brea, Calif.
J. H. Strømme, Ulleval Hospital, Oslo, Norway
J. F. O'Brien, Mayo Clinic, Rochester, Minn.

Team 6 - Lactate Dehydrogenase (LDH)

R. B. McComb, Hartford Hospital, Hartford, Conn. (Team Leader)
K. Y. Jackson, N. Y. State Department of Health, Albany, N.Y.
B. Howell, National Bureau of Standards, Washington, D.C.
S. Buhl, Technicon Instrument Company, Tarrytown, N.Y.
D. S. Young and J. F. O'Brien, Mayo Clinic, Rochester, Minn.

Team 7 - γ-Glutamyltransferase (γ-GT)

L. M. Shaw, HUP/Pepper Laboratory, Philadelphia, Pa. (Team Leader)
R. Miller, Technicon Instrument Company, Tarrytown, N.Y.
S. Osaki, Hycel Inc., Houston, Texas
J. H. Strømme, Ulleval Hospital, Oslo, Norway
A. Wahlefeld, Boehringer Mannheim GmbH, Tützing, West Germany.

Table 3' Estimated Catalytic Concentrations at 37.0 °C

Based on a brief study he made, G.N. Bowers, Jr., MD, Hartford Hospital, Hartford, Conn. has estimated the catalytic concentrations of six enzymes at 37.0 °C. In this study, the enzymes were determined at 29.77 °C and 37.0 °C by IFCC methods using a commercial instrument.

The following table compares the reported catalytic concentrations at 29.77 °C to Bowers' estimates at 37.0 °C, the ratios of the concentrations at 37.0 °C to those at 29.77 °C, and the literature values of the concentration ratios 37.0 °C/29.77 °C. The catalytic concentrations are expressed in terms of International enzyme units per liter (U/L) where U is given as micromoles of substrate converted per minute.

	Concentration, U/L				
Enzyme	29.77 °C*	37.0 °C**	Ratio Found	Ratio Liter.	(Ref)
Alkaline Phosphatase	75 ± 2	102 ± 2	1.36	1.35	(1)
Aspartate Aminotransferase	31 ± 1	46 ± 2	1.48	1.59	(2)
Alanine Aminotransferase	25 ± 1	34 ± 2	1.36	1.34	(3)
Creatine Kinase-AK(AACC)	123 ±10	200 ± 10	1.63	1.63	(4)
Creatine Kinase + AK Inhibitors (IFCC)	117 ±10	190 ± 10	1.63	1.63	(4)
Lactic Dehydrogenase	229 ± 5	350 ± 10	1.53	1.71	(5)
Gamma Glutamyl Transferase	16.4 ± 1	22 ± 1	1.34	1.36	(6)

*From NBS Special Publication 260-83.

**Estimates from analyses made in November 1983 at Hartford Hospital by use of a Cobas-Bio instrument set at 37 °C.

1. McComb, et. al., Alkaline Phosphatase, Plenum, NY 1979, pages 334-337.
2. Rej, et. at., Clin. Chem. 18, 374-383 (1972).
3. Heerspink, et. al., Enzyme 25, 333-341 (1980).
4. Bowers and McComb, Clin. Chem. (to be published).
5. Buhl, et. al., Clin. Chem. 24, 261-266 (1978).
6. Szasz, Clin. Chem. 15, 124-126 (1969).