4.2 Estándar de referencia L039

4.2.1 Preparación y uso del estándar de referencia

La referencia L039 se elaboró el 20 de mayo de 2003 en la planta de Caaguazú siguiendo el proceso de elaboración de los lotes de la primera serie de producción (vea la sección 3.2.S.2.6 Desarrollo del proceso de elaboración). Esta referencia se caracterizó utilizando métodos analíticos distintos.

La referencia se almacenó a +5°C ± 3°C y se monitoreó utilizando gráficos de control.

Este estándar de referencia se utilizó en el análisis ELISA Monolisa Plus para lo siguiente:

- Estudio de estabilidad de los lotes de la primera serie de producción L006, L008 y L009 a partir del momento de medición de 30 meses;
- Estudio de estabilidad de los lotes de la segunda serie de producción AA001, AA002 y AA003 hasta los 23 meses;
- Liberación de los lotes de la primera serie de producción L049 a L055;
- Liberación de los lotes de la segunda serie de producción AA001 a AA010.

Este estándar de referencia se utilizó en el análisis ELISA Rhein Biotech para lo siguiente:

- Estudio de estabilidad de los lotes de la segunda serie de producción AA001, AA002 y AA003 a partir del momento de medición de 24 meses.

4.2.2 Cualificación del estándar de referencia

El estándar de referencia se liberó de acuerdo con las especificaciones vigentes en ese momento.

Esta referencia se calibró con respecto a un lote de la vacuna GenHevac; el título obtenido con 14 determinaciones mediante el análisis ELISA Monolisa Plus fue 6,11 mg/mL.

El contenido proteico (método de Lowry) fue de 1,38 mg/mL.

4.3 Estándar de referencia AA004

4.3.1 Preparación y uso del estándar de referencia

La referencia AA004 se elaboró el 08 de junio de 2005 en la planta de Pilar siguiendo el proceso de elaboración de los lotes de la segunda serie de producción (vea la sección 3.2.S.2.6 Desarrollo del proceso de elaboración).

Este lote estaba incluido en un lote de Hexaxim utilizado en un estudio clínico de fase III.

La referencia se almacenó a +5°C ± 3°C y se monitoreó utilizando gráficos de control.

Este estándar de referencia se utilizó en el análisis ELISA Rhein Biotech para lo siguiente:

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- Estudio de estabilidad de los lotes de la tercera serie de producción AC004 y AC005 hasta el momento de medición de 18 meses y del lote de la tercera serie de producción AC007 hasta el momento de medición de 12 meses;
- Liberación de los lotes de la tercera serie de producción AC001 a AC046.

4.3.2 Cualificación del estándar de referencia

4.3.2.1 Calibración

El estándar de referencia se liberó de acuerdo con las especificaciones vigentes en ese momento.

El título de la referencia se obtuvo considerando los datos de proteínas totales (método de Lowry; promedio de 18 resultados = 1,398 mg/mL) y pureza (promedio de 10 resultados = 97,17 %):

Título = Lowry x pureza/100 = 1,36 mg/mL

4.3.2.2 Caracterización

Se desarrollaron diversos métodos bioquímicos, inmunoquímicos y biofísicos para caracterizar y documentar la partícula de HBsAg, como se describe en la sección 3.2.S.3.1 Elucidación de la estructura y otras características. Con base en el conocimiento adquirido a partir de todos los datos de caracterización, seleccionamos los parámetros clave para que se documenten de todos los estándares de referencia a fin de caracterizar todos los aspectos del antígeno de HBs relacionados con la estructura primaria (aminoácidos y secuencia N-terminal), estructura secundaria (contenido de hélice α) y estructura terciaria (fluorescencia intrínseca, enlaces disulfuro) de la proteína S y la estructura de la partícula (composición de lípidos, tamaño y antigenicidad).

En la Tabla 1 se presenta un resumen de los resultados obtenidos con el estándar de referencia AA004.

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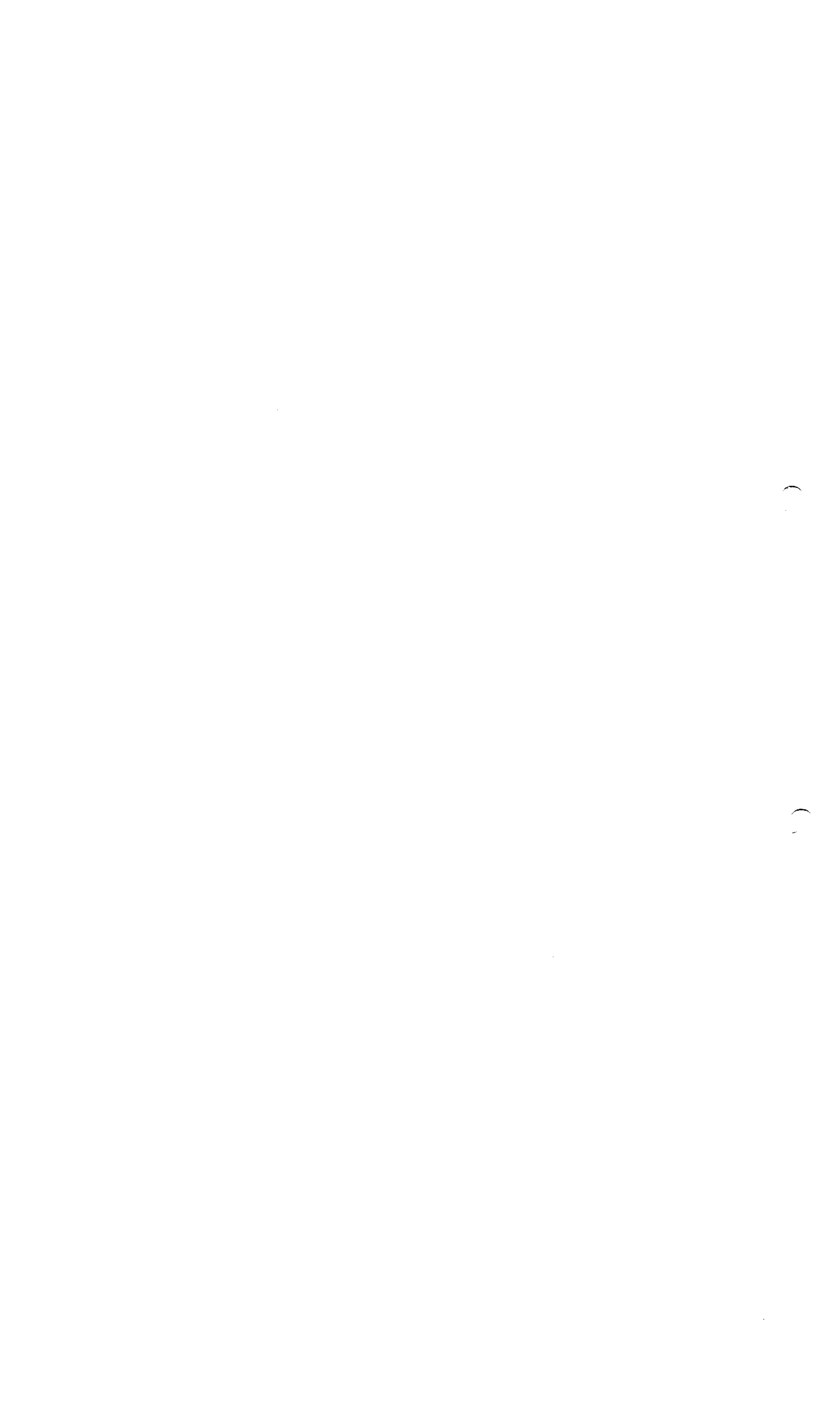

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Tabla 1: Caracterización del lote AA004

Característica	Método	Resultado
Tamaño de la partícula	Microscopía electrónica	21-25 nm
Composición de lípidos de la partícula	Gravimetría	0,82 µg de lípidos totales/µg de proteínas totales
	Análisis de fósforo	0,54 µg de fosfolípidos/µg de proteínas totales
	GLC	Ácidos grasos totales: 0,74 µg de ácidos grasos totales/µg de proteínas totales Ácidos grasos libres: 0,04 µg de ácidos grasos libres/µg de proteínas totales Esteroles totales: 0,002 µg de esteroles totales/µg de proteínas totales Triglicéridos: 0,01 µg de triglicéridos/µg de proteínas totales Composición de ácidos grasos: mayoría de ácidos grasos no saturados C18:1 (34,0 %) y C18:2 (25,7 %)
Antigenicidad de la partícula	Resonancia de plasmones superficiales	RF-1 Mab: ka 7,3 $10^3 M^{-1} s^{-1}$ kd 2,9 $10^{-4} s^{-1}$ KD 4,1 10^{-8}M
Estructura primaria de la proteína S (integridad de la secuencia N-terminal)	Secuenciación N-terminal	Integridad de N-terminal confirmada
Estructura primaria de la proteína S (secuencia AA)	MS/MS	Secuencia AA teórica confirmada
Estructura secundaria de la proteína S (contenido de la hélice α)	Espectroscopía infrarroja de transformada de Fourier	59,8 % de hélice α
Estructura terciaria de la proteína S	Fluorescencia intrínseca	Emisión máxima: 332 nm
Estructura terciaria de enlaces disulfuro de la proteína S (cuantificación de tioles libres)*	Método de Ellman	Tioles libres/proteína S (sin SDS): 0,19 Tioles libres/proteína S (con SDS): 0,26

* El análisis se realizó 22 meses después de la producción del lote, dado que el método no estaba disponible en este momento.

4.4 Estándar de referencia AC012

4.4.1 Preparación y uso del estándar de referencia

La referencia AC012 se elaboró el 03 de mayo de 2008 en la planta de Pilar siguiendo el proceso de elaboración de los lotes de la tercera serie de producción (vea la sección 3.2.S.2.6 Desarrollo del proceso de elaboración).

Este lote estaba incluido en un lote de Hexaxim utilizado en un estudio clínico de fase III.

La referencia se almacena a +5°C ± 3°C y se monitorea utilizando gráficos de control.

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Este estándar de referencia se utilizó en el análisis ELISA Rhein Biotech para lo siguiente:

- Estudio de estabilidad a +5°C de los lotes de la tercera serie de producción AC004 y AC005 a partir del momento de medición de 24 meses y del lote de la tercera serie de producción AC007 a partir del momento de medición de 18 meses;
- Liberación de los lotes de la tercera serie de producción AC047 a AC106.

4.4.2 Cualificación del estándar de referencia

4.4.2.1 Calibración

El estándar de referencia se liberó de acuerdo con las especificaciones vigentes en ese momento.

El título se obtuvo considerando los datos de proteínas totales (método de Lowry; promedio de 28 resultados = 1,47 mg/mL) y pureza (promedio de 18 resultados = 97,7 %).

$$\text{Título} = \text{Lowry} \times \text{pureza}/100 = 1{,}44 \text{ mg/mL}$$

4.4.2.2 Caracterización

Se aplicó el mismo paquete de caracterización definido en el párrafo 4.3.2.2 para la caracterización del lote AC0012.

En la Tabla 2 se presenta un resumen de los resultados obtenidos con el estándar de referencia AC012.

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Tabla 2: Caracterización del lote AC012

Característica	Método	Resultado
Tamaño de la partícula	Microscopía electrónica	21-25 nm
Composición de lípidos de la partícula	Gravimetría	0,94 µg de lípidos totales/µg de proteínas totales
	Análisis de fósforo	0,49 µg de fosfolípidos/µg de proteínas totales
	GLC	Ácidos grasos totales: 0,65 µg de ácidos grasos totales/µg de proteínas totales Ácidos grasos libres: 0,06 µg de ácidos grasos libres/µg de proteínas totales Esteroles totales: 0,003 µg de esteroles totales/µg de proteínas totales Triglicéridos: 0,02 µg de esteroles totales/µg de proteínas totales Composición de ácidos grasos: mayoría de ácidos grasos no saturados C18:1 (32,4 %) y C18:2 (29,3 %)
Antigenicidad de la partícula	Resonancia de plasmones superficiales	RF-1 Mab: ka 1,7 $10^4 M^{-1} s^{-1}$ kd 2,4 10^{-4} s^{-1} KD 1,4 10^{-8}M
Estructura primaria de la proteína S (integridad de la secuencia N-terminal)	Secuenciación N-terminal	Integridad de N-terminal confirmada
Estructura primaria de la proteína S (secuencia AA)	MS/MS	Secuencia AA teórica confirmada
Estructura secundaria de la proteína S (contenido de la hélice α)	Espectroscopía infrarroja de transformada de Fourier	59,6 % de hélice α
Estructura terciaria de la proteína S	Fluorescencia intrínseca	Emisión máxima: 334 nm
Estructura terciaria de la proteína S: enlaces disulfuro (cuantificación de los tioles libres)	Método de Ellman	Tioles libres/proteína S (sin SDS): 0,39 Tioles libres/proteína S (con SDS): 0,53

4.5 Estándar de referencia AC079

4.5.1 Preparación y uso del estándar de referencia

La referencia AC079 se elaboró el 02 de junio de 2010 en la planta de Pilar siguiendo el proceso de elaboración de los lotes de la tercera serie de producción.

La referencia se almacena a +5°C ± 3°C y se monitorea utilizando gráficos de control.

Este estándar de referencia comienza a utilizarse para controlar los lotes a partir de la temporada de elaboración 2011:

- Liberación del lote AC107 y posteriores.

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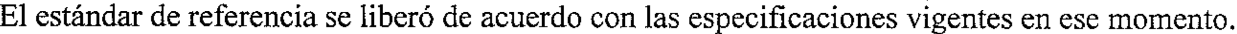
4.5.2 Cualificación del estándar de referencia

4.5.2.1 Calibración



El estándar de referencia se liberó de acuerdo con las especificaciones vigentes en ese momento.

El título se obtuvo considerando los datos de proteínas totales (método de Lowry; promedio de 30 resultados = 1,53 mg/mL) y pureza (promedio de 18 resultados = 97,4 %).

Título = Lowry x pureza/100 = 1,49 mg/mL

4.5.2.2 Caracterización

Se aplicó el mismo paquete de caracterización definido en el párrafo 4.3.2.2 para la caracterización del lote AC0079.

En la Tabla 3 se presenta un resumen de los resultados obtenidos con el estándar de referencia AC079.

Tabla 3: Caracterización del lote AC0079

Característica	Método	Resultado
Tamaño de la partícula	Microscopía electrónica	21-25 nm
Composición de lípidos de la partícula	Gravimetría	1,03 µg de lípidos totales/µg de proteínas totales
	Análisis de del fósforo	0,61 µg de fosfolípidos/µg de proteínas totales
	GLC	Ácidos grasos totales: 0,58 µg de ácidos grasos totales/µg de proteínas totales Ácidos grasos libres: 0,04 µg de ácidos grasos libres/µg de proteínas totales Esteroles totales: 0,003 µg de esteroles totales/µg de proteínas totales Triglicéridos: 0,02 µg de esteroles totales/µg de proteínas totales Composición de ácidos grasos: mayoría de ácidos grasos no saturados C18:1 (33,6 %) y C18:2 (31,7 %)
Antigenicidad de la partícula	Resonancia de plasmones superficiales	RF-1 Mab: ka 9,6 $10^3 M^{-1} s^{-1}$ kd 1,2 $10^{-4} s^{-1}$ KD 1,2 10^{-8}M
Estructura primaria de la proteína S (integridad de la secuencia N-terminal)	Secuenciación N-terminal	Integridad de N-terminal confirmada
Estructura primaria de la proteína S (secuencia AA)	MS/MS	Secuencia AA teórica confirmada
Estructura secundaria de la proteína S (contenido de la hélice α)	Espectroscopía infrarroja de transformada de Fourier	61,4 % de hélice α
Estructura terciaria de la proteína S	Fluorescencia intrínseca	Emisión máxima: 333 nm
Estructura terciaria de enlaces disulfuro de la proteína S (cuantificación de tioles libres)	Método de Ellman	Tioles libres/proteína S (sin SDS): 0,29 Tioles libres/proteína S (con SDS): 0,50

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4.6 Procedimiento para cualificar nuevos estándares

Un nuevo lote que se utilice como estándar de referencia debe ser un lote que cumpla con todas las pruebas de liberación y se cualifique de la siguiente manera.

4.6.1 Calibración de un estándar de referencia

La calibración de un nuevo estándar de referencia se lleva a cabo mediante un método absoluto. Considerando un promedio del contenido de proteínas totales (método de Lowry) y el promedio correspondiente de pureza, el título de una nueva referencia se determina de la siguiente manera:

Título del estándar de referencia = Contenido de proteínas totales (Lowry) x pureza/100

4.6.2 Caracterización de un estándar de referencia

En base al conocimiento adquirido a partir de todos los datos de caracterización (vea la sección 3.2.S.3.1 Elucidación de la estructura y otras características), se seleccionaron los parámetros clave para que se documenten de todos los estándares de referencia a fin de caracterizar todos los aspectos del antígeno de HBs relacionados con la estructura primaria (aminoácidos y secuencia N-terminal), estructura secundaria (contenido de hélice α) y estructura terciaria (fluorescencia intrínseca, enlaces disulfuro) de la proteína S y la estructura de la partícula (composición de lípidos, tamaño y antigenicidad).

Por consiguiente, cada lote que se utilice como estándar de referencia debe caracterizarse de la siguiente manera:

Tabla 4: Características de un estándar de referencia

Característica	Método
Tamaño de la partícula	Microscopía electrónica
Composición de lípidos de la partícula	Gravimetría
	Análisis de fósforo
	GLC
Antigenicidad de la partícula	Resonancia de plasmones superficiales
Estructura primaria de la proteína S (integridad de la secuencia N-terminal)	Secuenciación N-terminal
Estructura primaria de la proteína S (secuencia AA)	MS/MS
Estructura secundaria de la proteína S (contenido de la hélice α)	Espectroscopía infrarroja de transformada de Fourier
Estructura terciaria de la proteína S	Fluorescencia intrínseca
Estructura terciaria de la proteína S: enlaces disulfuro (cuantificación de los tioles libres)	Método de Ellman

Los métodos se enumeran a modo de ejemplo. Pueden utilizarse métodos equivalentes.

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4.6.3 Condiciones de almacenamiento

Los estándares de referencia se deben almacenar a +5°C ± 3°C y utilizarse durante no más de 30 meses (la misma vida útil que el producto).

5 Material de referencia marcador de bajo peso molecular

Se utiliza un marcador de bajo peso molecular (LMW), proporcionado por un proveedor externo, en el estudio SDS-PAGE, en condiciones reductoras y no reductoras, con tinción con azul de Coomassie, a fin de determinar el peso molecular de las distintas bandas de HBsAg (monómero, dímero y trímero). La temperatura de almacenamiento es de entre +2°C y +8°C.

Un ejemplo del certificado de análisis se presenta al final de esta sección.

6 Material de referencia para las endotoxinas

El material de referencia usado para determinar el contenido de endotoxinas bacterianas es un polvo liofilizado de endotoxinas de *E. coli* que se adquiere con un proveedor externo estadounidense. La potencia de esta referencia se determina respecto de un estándar de referencia internacional siguiendo el método descrito en la guía sobre la validación de la prueba de lisado de amebocitos de Limulus publicada por la FDA de EE.UU.

La solución se almacena a entre +2°C y +8°C una vez reconstituida.

Al final de esta sección se presenta un ejemplo del certificado de análisis

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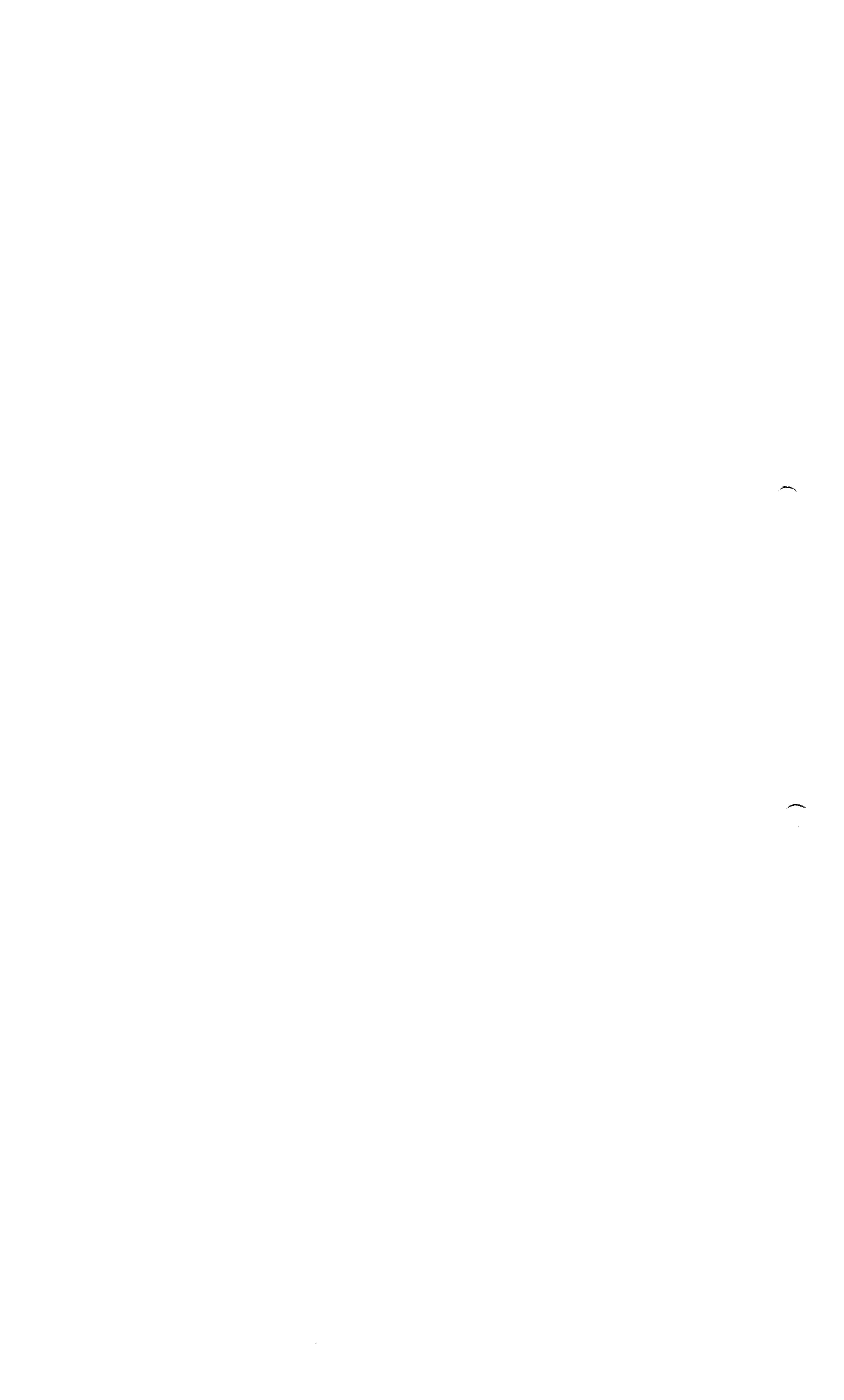

3.2.S.5

Certificate of Analysis - Bovine Serum Albumin Reference Standard - HBsAg

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National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 927d

Bovine Serum Albumin (7 % Solution)

(Total Protein Standard)

This Standard Reference Material (SRM) is intended primarily for use in the standardization of procedures employed in clinical analyses for total serum protein, for critical evaluation of daily working standards used in these procedures, and as a reference standard for assays of total protein by colorimetric methods. This SRM is a solution (mass fraction 7 %) of known protein concentration and purity. It conforms to the specification for standardized protein solution approved by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) [1]. The protein content of this SRM was determined using the biuret reference method [2] that is recommended for use in standardizing laboratory-prepared protein solutions and "normal" serum pools. Such standardized "normal" sera could then be used to calibrate refractometers or other instruments for serum protein estimations. SRM 927d may also be used for other procedures, such as gel diffusion, amino acid analysis, electrophoresis, nitrogen assays, or other tests that require well-characterized protein for calibration or evaluation. A unit of 927d consists of 10 ampoules, each containing 2.2 mL of solution.

In addition to the measurements using the biuret method, NIST made measurements of the bovine serum albumin (BSA) concentration using amino acid analysis. There is a discrepancy between the results from the two approaches. As the two different approaches are not determining the same measurand, it was decided to separately the report results from the two approaches as follows: 1) certified BSA concentration by amino acid analysis and 2) reference total protein concentration by the biuret method.

Certified Bovine Serum Albumin (BSA) Concentration and Uncertainty from Amino Acid Analysis: Two different methods of amino acid analysis were used to measure the concentration of bovine serum albumin (see "Analytical Methods" section). The certified results are shown in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or accounted for by NIST.

Reference Total Protein Concentration and Uncertainty as Determined Using the Biuret Method: The biuret reference method [2] was employed to determine protein concentration in SRM 927d using SRM 927c as an external standard. The reference protein concentration and uncertainty are shown in Table 2. Reference values are noncertified values that are the best estimates of the true values; however, the values 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 analytical methods.

Additional Reference Values and Uncertainties: Reference values are provided in Table 3 for additional properties including fill volume, pH, density, absorbances at various wavelengths, and relative molecular mass as determined using electrospray ionization mass spectrometry.

Expiration of Certification: This certification is valid until **30 September 2010**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification will be nullified if the SRM is contaminated or modified.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 02 February 2006

Robert L. Watters, Jr., Chief
Measurement Services Division

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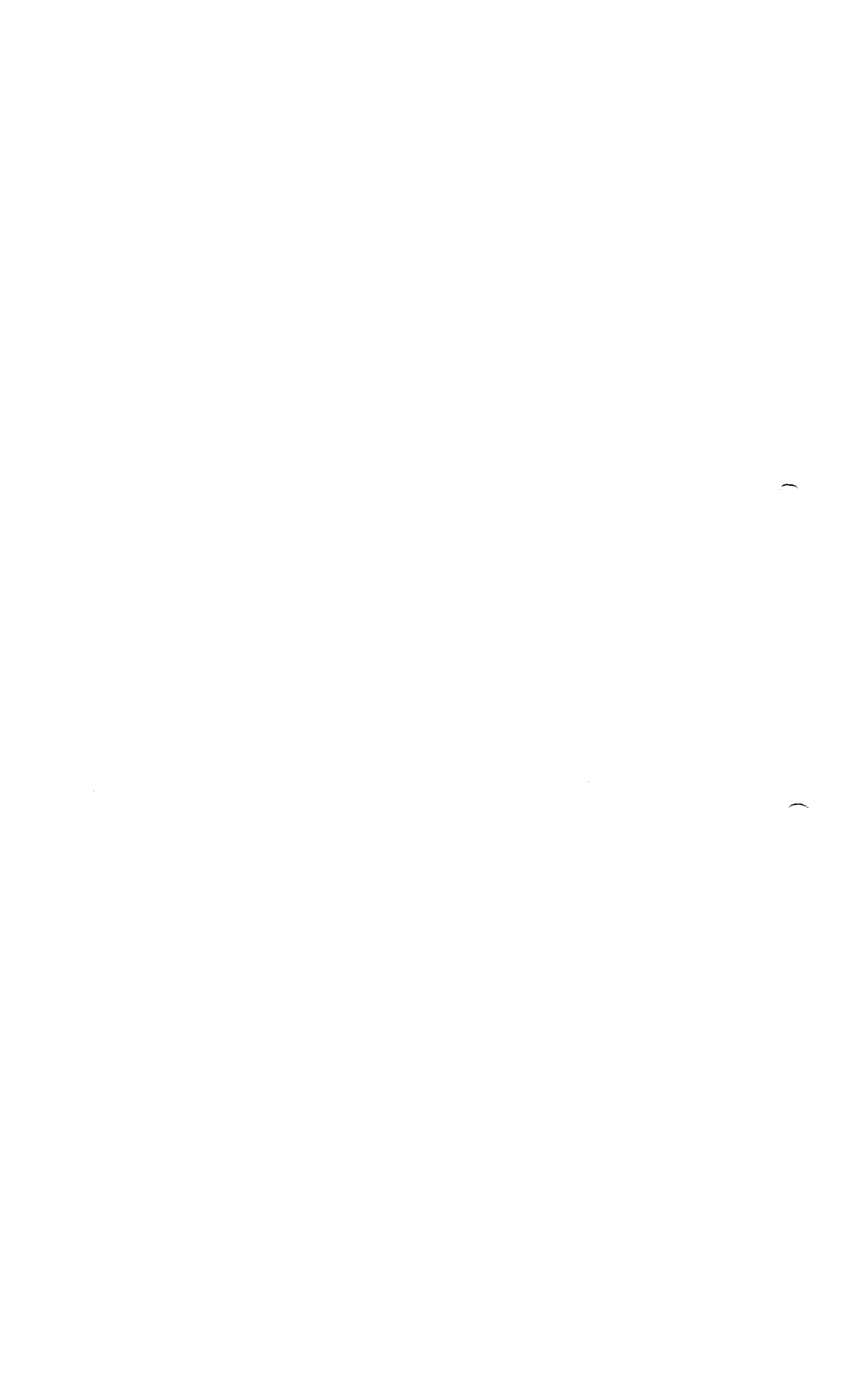

Information Value: A literature value for the optical absorbance of bovine serum albumin is given in Table 4. This is a noncertified value with no reported uncertainty as there is insufficient information to assess uncertainty. This information value is given to provide additional characterization of the material.

The overall direction and coordination of technical measurements leading to the certification were performed by M.J. Welch and D.M. Bunk of the NIST Analytical Chemistry Division.

Analyses were performed by D.M. Bunk, L.T. Sniegoski, and M. Vergne of the NIST Analytical Chemistry Division.

The statistical analysis of the data used for certification was performed by N.F. Zhang of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its value assignment. If substantive technical changes occur that affect the value assignment before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

NOTICE AND WARNING TO USERS

SRM 927d IS INTENDED FOR IN-VITRO DIAGNOSTIC USE ONLY. The blood used in the preparation of SRM 927d Bovine Serum Albumin (7 % Solution) was collected from cattle sourced in the United States. Only blood from cattle/carcasses that have passed ante-mortem and post-mortem USDA-Food Safety Inspection Service (FSIS) inspection was used. This material was collected prior to any known case of Bovine Spongiform Encephalopathy (BSE) in the United States. There were no additives to the pooled serum prior to protein purification.

INSTRUCTIONS FOR USE

Storage: This SRM is supplied to the user in sealed ampoules. The SRM should be stored in a refrigerator at a temperature between 2 °C and 8 °C. The ampoules should not be frozen because of possible breakage of ampoules during the thawing process. Once an ampoule is opened, the solution should be used promptly. Any unused solution in opened ampoules should be discarded.

Inappropriate Uses: This SRM is not intended to be used as a standard for dye-binding tests, for checking precalibrated refractometers, for immunochemical methods, or as an additive for bilirubin standardization.

SOURCE, PREPARATION, AND ANALYSIS[1]

Source of Material: The bovine serum albumin solution was prepared by Bionostics Inc., (Acton, MA, USA) under contract with Bioreclamation, Inc., (Hicksville, NY, USA). The bovine serum was produced for manufacture into products for pharmaceutical use by West Laboratories, Inc. at USDA EST. #245-J, Iowa Beef Packers, Inc., (Joslin, IL, USA).

The BSA for this SRM was dissolved in 0.02 mol/L sodium chloride and the pH adjusted to 6.5 – 6.8 with 1.0 mol/L sodium hydroxide. The material was sterilized by membrane filtration and tested for sterility by approved methods [3].

Preparation of Dilutions: Protein solutions of lower concentration may be prepared by transferring the appropriate aliquot to a volumetric flask and diluting to volume. Diluents are not furnished with the SRM; however, an aqueous sodium chloride diluent, such as a solution having a concentration of 0.15 mol/L, may be used.

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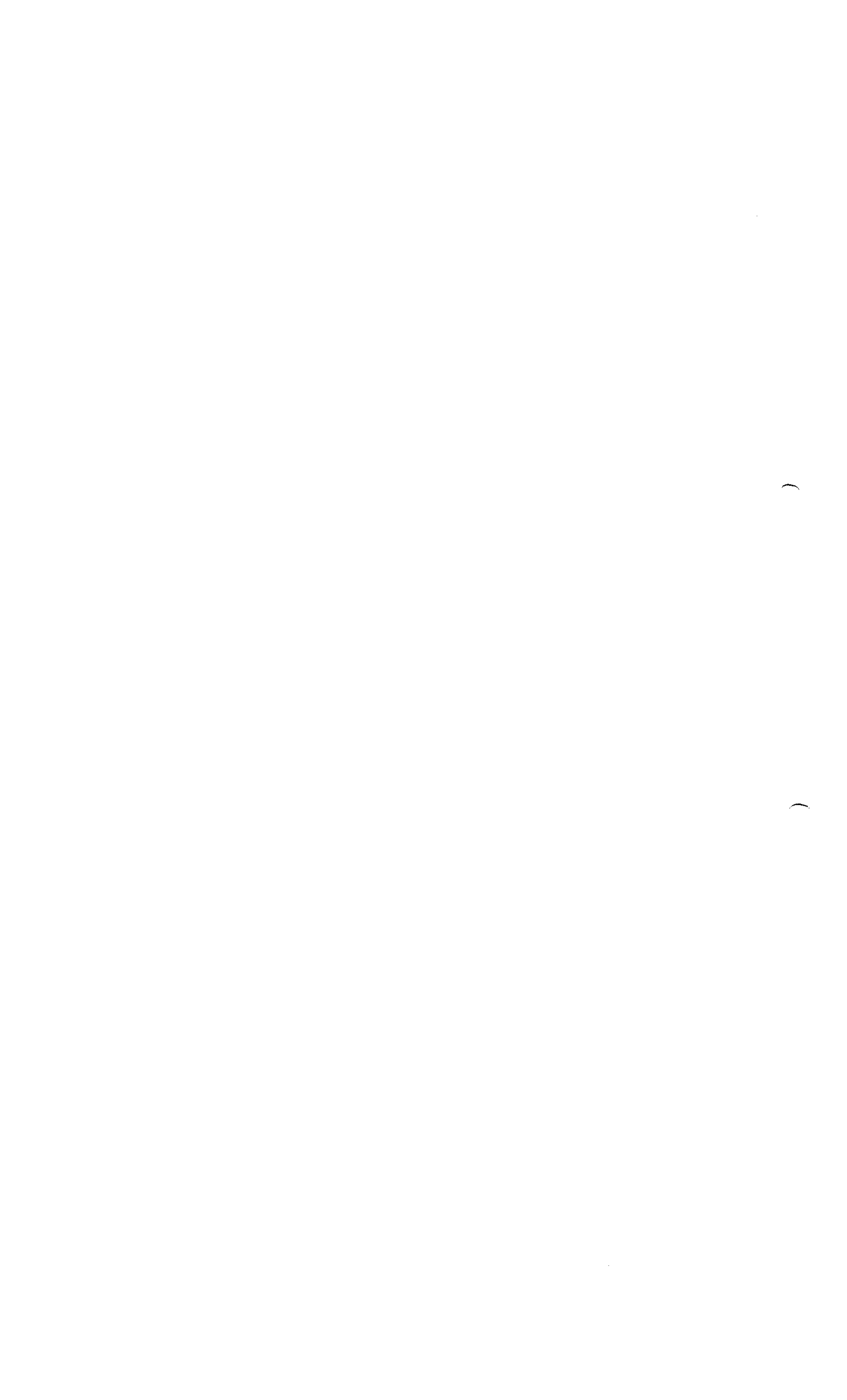
[1]Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.



Analytical Methods: All analyses for the certified and reference values were performed at NIST. For the determination of BSA, two different methods for determining amino acids were used. The first method involved isotope dilution liquid chromatography/tandem mass spectrometry (ID-LC/MS/MS). Samples of SRM 927d and 927c (as a control) were hydrolyzed with vapor phase hydrochloric acid (HCl) for 2 h at 150° C in sealed vials. After hydrolysis, the samples were lyophilized and then reconstituted with 0.2 % formic acid in acetonitrile containing isotope labeled analogs of phenylalanine, proline, and valine. Amino acids were separated using hydrophilic interaction chromatography (HILIC) on a polyhydroxyethyl aspartate column with gradient elution. Measurements were performed on a triple quadrupole mass spectrometer, monitoring specific transitions for each amino acid. The measurements were calibrated with different dilutions of SRM 2389 *Amino Acids in 0.1 mol/L HCl*. Data were collected for phenylalanine, proline, and valine. Based upon the known amino acid sequence for BSA, the concentration of BSA was calculated from the concentrations determined for each of the amino acids. For the second method, a commercial amino acid analyzer was used following hydrolysis as described above. This analyzer uses an ion exchange resin column to separate the amino acids, followed by derivatization with ninhydrin, and detection by spectrophotometry at 570 nm and 440 nm. These measurements were calibrated with dilutions of SRM 2389. The amino acids used for quantitation were alanine, leucine, lysine, and aspartic acid/asparagine.

The reference total protein concentration was measured using the biuret reference method for total serum protein [2]. The measurements involve a direct comparison between the current SRM lot (927d) and the previous lot (927c) and were performed on a Varian Cary 219 spectrophotometer.

The reference values for various properties determined for SRM 927d are given in Table 3. Absorbances were measured in accordance with requirements specified for a standard BSA solution [1]. Measurement of the pH was performed using an Orion Model 501 pH meter with a glass body combination pH electrode calibrated with pH 4 and pH 7 buffers. Density was measured using the Lang-Levey pipet method [4]. Fill masses were determined gravimetrically and fill volumes were calculated from the fill masses and mean density.

Relative molecular mass was determined using liquid chromatography/mass spectrometry (LC/MS). Measurements were performed on a single quadrupole mass spectrometer operated in the positive ion mode and coupled to a capillary LC with a commercial C-4 column, 0.5 mm × 50 mm, held at 40 °C. Gradient elution using 0.05 % trifluoroacetic acid in water and in acetonitrile was used. Horse apomyoglobin was used for mass calibration of the mass spectrometer. The molecular masses of the seven major forms of BSA found in SRM 927d are shown in Table 3 in decreasing order of abundance. The previous lot (SRM 927c) had a similar range of molecular masses.

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Table 1. Certified Bovine Serum Albumin Concentration by Amino Acid Analysis[a]

BSA Concentration: 65.41 g/L ± 0.82 g/L

[a] The certified value is the equally weighted mean of results obtained from two chemically independent methods (see "Analytical Methods"). The expanded uncertainty in the certified concentration is calculated as $U = ku_c$. The quantity u_c is the combined standard uncertainty calculated based on a Bayesian approach in [5] and ISO and NIST Guides [6]. The coverage factor, $k = 2$, represents an approximate 95 % level of confidence.

Table 2. Reference Total Protein Concentration by the Biuret Method[a]

Protein Concentration: 70.10 g/L ± 0.74 g/L

[a] Because only a single method was used, the results do not meet the NIST criteria for a certified value [7]. Therefore this method specific result is considered a reference value. The expanded uncertainty in the reference concentration is calculated as $U = ku_c$. The uncertainty u_c is based upon quadratically combining the measurement uncertainty with the uncertainty in SRM 927c, which served as the external standard for the measurements. The major component in the uncertainty for SRM 927d is the uncertainty in the concentration of SRM 927c. The coverage factor, $k = 2$, represents an approximate 95 % confidence interval.

Table 3. Reference Values for Various Properties of SRM 927d

Mean Fill Volume:	2.245 mL	±	0.006 mL
pH:	6.70	±	0.01
Density:	1.0178 g/mL	±	0.0003 g/mL
Spectral Properties			**Absorbance**
Ultraviolet (A_{252}/A_{279} ratio @ 1.0 g/L)	0.467	±	0.002
Soret Band (Visible) A_{405}	0.140	±	0.002
A_{500}	0.0320	±	0.0021
A_{600}	0.0187	±	0.0024

Major molecular forms of BSA in decreasing order of abundance

Relative Molecular Mass		
66 432	±	7
66 549	±	12
66 473	±	14
66 593	±	11
66 344	±	13
66 222	±	14
66 389	±	14

Theoretical BSA relative molecular mass from Amino Acid Sequence [8]: 66 399

The uncertainties in the reference values are calculated as $U = ku_c$. The quantity u_c is the combined standard uncertainty calculated according to the ISO and NIST Guides [6], where u_c is intended to represent the measurement error at the level of one standard deviation. The coverage factor, $k = 2$, represents an approximate 95 % confidence interval.

Table 4. Information Value for Optical Absorbance

Optical Absorbance @ 279 nm for 1 g/L [9] 0.660

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[1] NCCLS Approved Standard ACS-1; *Specification for Standardized Protein Solution (Bovine Serum Albumin)*, 2nd ed.; National Committee for Clinical Laboratory Standards: Villanova, PA (1979).

[2] Doumas, B.T.; Bayse, D.D.; Carter, R.J.; Peters, T., Jr.; Schaffer, R.; *A Reference Method for the Determination of Total Protein in Serum*; Clin. Chem. 27, pp. 1642–1650 (1981).

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[5] Liu, H.K.; Zhang, N.F.; *Bayesian Approach to Combining Results from Multiple Methods*; In Proceedings of the Section of Bayesian Statistical Science of American Statistical Society (2001).

[6] ISO; *Guide to the Expression of Uncertainty in Measurement*; ISBN 92-67-10188-9, 1st ed.; International Organization for Standardization: Geneva, Switzerland (1993); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at http://physics.nist.gov/Pubs.

[7] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-135; U.S. Government Printing Office: Washington, DC (2000); available at http://www.cstl.nist.gov/nist839/NIST_special_publications.htm.

[8] Swiss-Prot database, Swiss Institute for Bioinformatics (http://us.expasy.org/sprot/sprot-top.html).

[9] *All About Albumin: Biochemistry, Genetics, and Medical Applications*; Peters, T., Jr.; Academic Press Inc.: San Diego, CA, p. 25 (1995).

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 at http://www.nist.gov/srm

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CERTIFICATE OF ANALYSIS

PRODUCT NAME: Protein Standard, 2 mg/vial (Bovine Serum Albumin)

CATALOG NUMBER: P7656

LOT NUMBER: 060K6073

This product meets all of Sigma Diagnostics quality specification, and performs as outlined in the product insert.

Approved by: [signature]

Date: JAN 15 2000

p7656a.coa

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3.2.S.5

Certificate of Analysis - Sucrose Reference Standard - HBsAg

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Council of Europe
EUROPEAN DIRECTORATE FOR
THE QUALITY OF MEDICINES
EUROPEAN PHARMACOPOEIA COMMISSION

SAFETY DATA SHEET

according to ISO 11014-1 – 93/112/EC.

Warning! Reference Substance - important notice

The chemical substance to which this Safety Data Sheet relates is supplied exclusively as a reference substance for chemical test and assay purposes and on the basis of the Conditions of Supply and notes on Use of Reference Materials set out in the European Pharmacopoeia catalogue. It is to be used for no other purpose and is not for human consumption. The information set out in this sheet is applicable solely to the substance when used as a European Pharmacopoeia Chemical Reference Substance (Ph. Eur. CRS) and is not intended to apply to any other use or preparation of the substance (e.g. at different concentrations, in drug dosage form or in bulk quantities).

The information which follows has been assembled by Ph. Eur. staff from sources considered reliable, in particular from material provided in the ordinary way by the manufacturer or supplier. It has not been independently verified by the Ph. Eur. The accuracy of the information cannot therefore be guaranteed, nor does it constitute any expression of opinion by the Ph. Eur. concerning the substance. This information is accordingly not to be regarded as a representation or statement concerning the quality or safety of the substance, the presence of any defect in it, or its fitness for any particular purpose except that of use as a Ph. Eur. CRS by professional persons having technical skill and at their own discretion and risk.

Any recipient of this Safety Data Sheet with responsibility for other persons in a workplace should determine the risks associated with the substance according to the conditions of use and should take appropriate measures, including provision of appropriate information to persons working with the substance.

Information given in sections 2, 3, 4, 5, 6, 9, 10, 11, 12 and 13 relates to the bulk substance and is not necessarily relevant to the CRS regarding the small quantities contained in vials. Information in sections 1, 7, 8 and 14 applies only to the CRS.

1. IDENTIFICATION OF THE SUBSTANCE AND OF THE COMPANY

SUCROSE CRS

Council of Europe
European Directorate for the Quality of Medicines
European Pharmacopoeia
B.P. 907 - F 67029 Strasbourg Cedex 1
Tel. +33(0)3 88 41 20 35

2. COMPOSITION/INFORMATION ON INGREDIENTS

$C_{12}H_{22}O_{11}$ CAS: 57-50-1

3. HAZARDS IDENTIFICATION

May be harmful by inhalation, ingestion or skin absorption. May cause irritation.

4. FIRST AID MEASURES

Inhalation : Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.
Skin contact : Immediately wash skin with soap and copious amounts of water. Wash contaminated clothing before reuse.
Eyes contact : Immediately flush eyes with copious amounts of water for at least 15 minutes.
Ingestion : Call a physician. Wash out mouth with water provided person is conscious.
In any case seek medical advice and call the nearest poison center.

5. FIRE FIGHTING MEASURES

Extinguishing media : Water spray. Carbon dioxide, dry chemical powder or appropriate foam.
Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. Emits toxic fumes under fire conditions.

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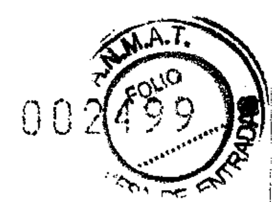

6. Accidental release measures

Sweep up, place in a bag and hold for waste disposal. Avoid raising dust. Ventilate area and wash spill site after material pick up is complete. Use protective clothing, gloves and mask.

7. Handling and storage

Handling :	Compatible chemical-resistant gloves. Chemical safety goggles. Approved respirator in non ventilated areas and/or for exposure above the ACGIH TLV. Rubber gloves. Safety shower and eye-bath. Mechanical exhaust required. Do not breathe dust. Do not get in eyes, on skin, on clothing. Wash thoroughly after handling.
Storage :	Keep unopened in the original container at about 2-8°C.

8. Exposure controls/personal protection

Refer to section 7.

9. Physical and chemicals properties

Appearance : White crystalline powder or lustrous, dry, colourless or white crystals.

mp: 189-191°C, α_{20}^{D} : +66.3° to +67.0°, very soluble in water, slightly soluble in alcohol, practically insoluble in ethanol.

10. Stability and reactivity

Incompatibilities :	Strong oxidising agents.
Hazardous combustion or decomposition products :	COx. Hazardous polymerisation will not occur.

11. Toxicological information

RTECS No : WN6500000

LD50 (oral-rat): about 29 700 mg/kg.

See actual entry in RTECS for complete information.

12. Ecological information

Not available.

13. Disposal considerations

Dissolve in water and dilute to a 5% solution. Check the pH and adjust it to 7 if necessary. Pour the solution down the drain with running water and continue to flush the drain system for 10 minutes, provided that rules at your place of employment or local, state and federal guidelines allow you to do so.

Observe all federal state and local environmental regulations.

14. Transport information

Not applicable.

15. Regulatory information

EINECS No : 200-334-9

16. Other information

SDS No. S1600000-02 15/12/99

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National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 17f

Sucrose
Optical Rotation

This Standard Reference Material (SRM) is intended primarily for use as a saccharimetry standard in calibrating polarimetric systems. The certified chemical purity and reference values for the optical rotation of a "normal solution" (described below) of SRM 17f Sucrose at 20.00 °C ± 0.01 °C in a 100.00 mm cell and a 200.00 mm cell are provided. A unit of this SRM consists of one bottle containing 60 g of crystalline sucrose.

Certified Purity and Uncertainty: A NIST Certified Value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified chemical purity of sucrose was determined by measuring the mass fractions of impurities including water, other saccharides, and residue from ashing, summing the impurities, and subtracting this sum from 100 %.

Certified Purity of Sucrose as a Mass Fraction: 99.303 % ± 0.004 %

The uncertainty in the certified value is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO Guide and NIST Guidelines [2]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the uncertainty in the measurement of the impurities. The coverage factor, $k = 2$, is determined from the Student's t-distribution corresponding to the appropriate degrees of freedom and approximately 95 % confidence.

Reference Values and Uncertainties: Reference values are noncertified values that represent a best estimate of the true value; however, the values do not meet the 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 [1]. Reference values for the optical rotation of a "normal sugar solution" of SRM 17f at four wavelengths are provided in Table 2a in both milliradians and degrees (See "Preparation of a Normal Sugar Solution"). Table 2b includes the reference value for the °Z value for a "normal sugar solution" of SRM 17f. The unit, °Z, is the unit of the "International Sugar Scale" defined by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA). The reference value for the specific rotation is provided in Table 2c.

Expiration of SRM Certification: The certification of SRM 17f is valid, within the measurement uncertainties specified, until **1 July 2013**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Notice and Warnings to Users"). However, the certification is invalid if the SRM is damaged, contaminated, or otherwise 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 the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The overall direction and coordination of the technical activities were under the leadership of K.W. Phinney of the NIST Analytical Chemistry Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

Robert L. Watters, Jr., Chief
Measurement Services Division

Gaithersburg, MD 20899
Certificate Issue Date: August 08, 2008

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