



# National Institute of Standards & Technology

## Certificate of Analysis

### Standard Reference Material<sup>®</sup> 1959

#### Drugs of Abuse in Frozen Human Serum

This Standard Reference Material (SRM) is intended primarily for use in evaluating the accuracy of procedures for the determination of drugs of abuse in human serum and blood. It is also intended for use in validating working or secondary reference materials. A unit of SRM 1959 consists of two vials of frozen human serum that has been fortified with seven drugs of abuse. Each vial contains approximately 5 mL serum.

**Certified Concentration Values:** The certified concentration values for benzoylecgonine, methadone, methamphetamine, morphine, phencyclidine, and nordiazepam are provided 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 taken into account [1]. The certified concentration values for these analytes are based on the agreement of results from isotope dilution gas chromatography with mass spectrometric detection (ID-GC-MS) and isotope dilution liquid chromatography with mass spectrometric detection (ID-LC-MS). The certified concentrations apply only to serum thawed to room temperature, 20 °C to 25 °C (see “Instructions for Storage and Use”).

**Reference Concentration Values:** The reference concentration value for 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-9-COOH) is provided in Table 2. A reference value is a noncertified value that is the best estimate of the true value based on available data; however, the value does not meet the NIST criteria for certification and is provided with an associated uncertainty 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 [1]. The reference value for THC-9-COOH is based on measurements by ID-GC-MS, ID-LC-MS, and isotope dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS). The reference concentrations apply only to serum thawed to room temperature, 20 °C to 25 °C (see “Instructions for Storage and Use”).

**Expiration of Certification:** The certification of SRM 1959 is valid, within the measurement uncertainties specified, until **01 October 2015**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for Storage and Use”). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

**Maintenance of SRM Certificate:** 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 preparation and analytical measurements leading to the certification of this SRM were performed by K.W. Phinney and M.J. Welch of the NIST Analytical Chemistry Division.

Analytical measurements were performed by L.T. Sniegowski, S.S.-C. Tai, and J.L. Prendergast of the NIST Analytical Chemistry Division.

Design of the sampling protocol and statistical analysis of the data were 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.

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Certificate Issue Date: 21 March 2011

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## NOTICE AND WARNINGS TO USERS

**Warning:** SRM 1959 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 has been tested by an FDA-approved method and found non-reactive/negative for hepatitis B surface antigen (HbsAg), human immunodeficiency virus (HIV) 1 and 2 antibodies, hepatitis C virus (HCV), and syphilis. However, no known test method can offer complete assurance that hepatitis B virus, HCV, 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 [2].

## INSTRUCTIONS FOR STORAGE AND USE

**Storage:** The serum is shipped frozen (on dry ice) and, upon receipt, should be stored frozen until ready for use. A freezer temperature of  $-20^{\circ}\text{C}$  is acceptable for storage up to one week. If a longer storage time is anticipated, the material should be stored at or below  $-50^{\circ}\text{C}$ . The SRM should not be exposed to sunlight or ultraviolet radiation. Storage of thawed material at room or refrigerator temperatures may result in changes in analyte concentrations.

**Use:** Vials of the SRM to be analyzed should be removed from the freezer and allowed to stand at room temperature ( $20^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ ) until thawed. After the material is thawed, it should be used immediately. The material should be swirled gently to mix before aliquots are withdrawn.

## PREPARATION AND ANALYSIS<sup>(1)</sup>

**Preparation of SRM Serum Pool:** SRM 1959 was prepared by Bioreclamation, Inc., Hicksville, NY. It was prepared by pooling drug-free serum collected from healthy adult donors, passing it through  $0.2\text{ }\mu\text{m}$  filters, and spiking it with appropriate quantities of the analytes of interest. The SRM was dispensed into amber cryovials suitable for storage at  $-80^{\circ}\text{C}$ .

**Analytical Methods:** Samples for analysis were selected by a stratified random process applied across the manufacturer's filling run. For each analyte, two independent methods were used, one based upon ID-GC-MS and one based upon ID-LC-MS. For THC-9-COOH, a third method based upon ID-LC-MS/MS was also used. Isotopically labeled internal standards were employed for all measurements.

*Analysis by ID-GC-MS.* Measurement of benzoylecgonine, methadone, morphine, nordiazepam, and phencyclidine was performed in a single analysis. For these analytes, 1 mL aliquots of serum were weighed into centrifuge tubes and adjusted to pH 6 with 0.1 mol/L phosphate buffer. Labeled internal standards were added to give approximately 1:1 ratios between labeled and unlabeled analytes. Solid phase extraction (SPE) cartridges (Bond Elut Certify, Varian, Inc., Lake Forest, CA) were used to isolate the drugs of abuse from the serum matrix, and the analytes were then derivatized with N,O-bis(trimethylsilyl)acetamide. Analysis of methamphetamine was performed in a similar manner, but the methamphetamine was derivatized with heptafluorobutyric acid anhydride. For analysis of THC-9-COOH, 1 mL serum aliquots were weighed into centrifuge tubes, and acetate buffer (0.1 mol/L, pH 4) and the labeled internal standard were added. The analyte was isolated by SPE (Bond Elut Certify) and derivatized with N,O-bis(trimethylsilyl)acetamide. All samples were analyzed by GC-MS using electron impact ionization and a 30 m fused silica capillary column with a 5 % phenyl/95 % dimethyl arylene siloxane phase (DB-5ms, J & W Scientific, Rancho Cordova, CA).

*Analysis by ID-LC-MS and ID-LC-MS/MS.* Measurement of methadone, methamphetamine, morphine, and phencyclidine was performed in a single analysis. Aliquots of serum (1 g) were weighed into centrifuge tubes and adjusted to pH 6 with 0.1 mol/L phosphate buffer. Labeled internal standards were added to give approximately 1:1 ratios between labeled and unlabeled analytes. The drugs of abuse were isolated from the serum matrix by SPE with Clean Screen DAU (United Chemical Technologies, Bristol, PA) cartridges. Measurement of nordiazepam was performed in a similar manner. For analysis of THC-9-COOH, 1 g serum aliquots were weighed into centrifuge tubes and adjusted to pH 3 to 4 with 0.1 mol/L acetate buffer (pH 4). Labeled internal standard was added to give approximately 1:1 ratios between the labeled and unlabeled analyte. The THC-9-COOH was isolated from the serum matrix by SPE with a  $\text{C}_{18}$  cartridge (Sep-Pak, Waters Corporation, Milford, MA). Analysis by LC-MS was

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<sup>(1)</sup>Certain commercial equipment, instruments, or materials are identified in this certificate to adequately specify 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.

performed with electrospray ionization in the positive ion mode. Chromatographic separations for determination of methadone, methamphetamine, morphine, nordiazepam, and phencyclidine were performed on a C<sub>18</sub> stationary phase (Luna C18(2), Phenomenex, Torrance, CA) with a water:methanol mobile phase containing an acetic acid volume fraction of 1 mL/L. For THC-9-COOH, a Zorbax Eclipse XDB-C18 stationary phase (Agilent Technologies, Santa Clara, CA) was used with a 0.2 mol/L ammonium acetate:methanol (40:60, volume fractions) mobile phase. Analysis of THC-9-COOH by LC-MS/MS was performed in a similar manner as the LC-MS analysis.

**Homogeneity Assessment:** The homogeneity assessment was made at the time the certification analyses were performed. A stratified sampling plan was devised to test for homogeneity across the manufacturing process. The results indicated that there was no apparent trend in the data when plotted against the sequence in which the vials were prepared.

**Value Assignment:** The certified or reference value assigned to each measurand is a weighted average of all the measured values obtained for it. The weights were determined by a Gaussian linear mixed effects model fitted to the data by the method of restricted maximum likelihood [3]. The Type A and Type B assessments of uncertainty components were combined in accordance with the ISO Guide [4]. The former reflect contributions from differences between analytical methods, samples, and replicates. The latter reflect contributions from purity, volumetric determinations, and possible interferences. The combined expanded uncertainty listed alongside the estimate of each measurand should cover the measurand's true value with approximately 95 % probability; the corresponding coverage factor was  $k = 2.1$ . Mass concentration levels were calculated from mass fractions using measured serum density: 1.025 g/mL.

Table 1. Certified Values for Drugs of Abuse in SRM 1959

Analyte	Mass Fraction (ng/g)	Mass Concentration (ng/mL)
Benzoylecgonine	1007 ± 94	1033 ± 97
Methadone	985 ± 89	1010 ± 92
Methamphetamine	989 ± 62	1014 ± 63
Morphine	987 ± 31	1012 ± 31
Phencyclidine	979 ± 31	1004 ± 32
Nordiazepam	1026 ± 42	1051 ± 43

Table 2. Reference Values for THC-9-COOH in SRM 1959

	Mass Fraction (ng/g)	Mass Concentration (ng/mL)
THC-9-COOH	983 ± 100	1008 ± 103

## REFERENCES

- [1] 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 260-136; U.S. Government Printing Office: Gaithersburg, MD (2000); available at [http://www.cstl.nist.gov/nist839/NIST\\_special\\_publications.htm](http://www.cstl.nist.gov/nist839/NIST_special_publications.htm); (accessed Mar 2011)
- [2] *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed.; HHS publication No. (CDC) 21-1112; Chosewood, LC; Wilson, DE, Eds.; US Government Printing Office: Washington, D.C. (2009); available at [http://www.cdc.gov/OD/OHS/biosfty/bmbl5/BMBL\\_5th\\_Edition.pdf](http://www.cdc.gov/OD/OHS/biosfty/bmbl5/BMBL_5th_Edition.pdf); (accessed Mar 2011).
- [3] Searle, S.R.; Casella, G.; McCulloch, C.E.; *Variance Components*; John Wiley & Sons; Hoboken, NJ (2006).
- [4] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (ISO GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (JCGM) (2008); available at [http://www.bipm.org/utls/common/documents/jcgm/JCGM\\_100\\_2008\\_E.pdf](http://www.bipm.org/utls/common/documents/jcgm/JCGM_100_2008_E.pdf) (accessed Mar 2011); 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/> (accessed Mar 2011).

*Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 926-4751; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or via the Internet at <http://www.nist.gov/srm>.*