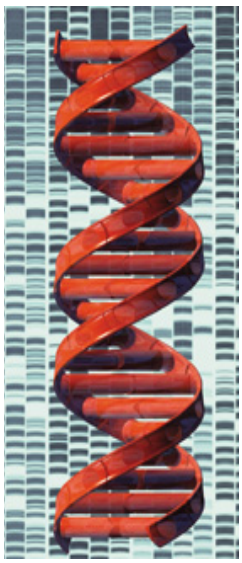
SRM SPOTLIGHT

February 2005 Editor: Regina R. Montgomery

Inside:



New NIST RMs/SRMs

NIST SRM 2394 Heteroplasmic Mitochondrial DNA Mutation Detection Standard

Human mitochondrial DNA (mtDNA) mutations are important for forensic identifications and mitochondrial disease diagnostics. If a mutation is present in every mtDNA molecule, detection is routine; however, low-frequency mutations, heteroplasmies (the existence of two nucleotides at the same site), or single nucleotide polymorphisms (SNPs), scattered throughout the DNA in the presence of a majority of mtDNA with the Cambridge Reference Sequence are almost impossible to detect. Therefore, NIST has developed heteroplasmic human mtDNA Standard Reference Material (SRM) 2394 to provide quality control to forensic, medical, and DNA scientists who wish to determine their sensitivity in detecting low-frequency mutations, single nucleotide polymorphisms (SNPs) in either mtDNA or in pooled nuclear DNA samples, or in heteroplasmic sites in mitochondrial DNA (mtDNA).

SRM 2394 is composed of mixtures of two 285 base pair (bp) PCR products from two cell lines (CHR and GM09947A) that differ at one nucleotide position. The CHR cell line designated polymorphic has a T at np 6371 and the GM09947A cell line containing the Cambridge Reference Sequence has a C at that site. SRM 2394 is composed of 10 tubes, one tube containing only the DNA with the polymorphism, one tube containing the DNA whose sequence agrees with the Cambridge Reference Sequence and 8 tubes containing different percentages of the polymorphic/CRS mtDNA mixtures (in which the mass % polymorphic levels are 1%, 2.5%, 5%, 10%, 20%, 30%, 40% and 50%). Before the final SRM was prepared, twelve laboratories including NIST, participated in an Interlaboratory Evaluation (ILE) of a prototype of SRM 2394. This ILE was a blind study in which the investigators could use any mutation detection method of their choice. The methods included automated DNA sequencing with three different chemistries and different sequencers; denaturing gradient gel electrophoresis (DGGE)**;** the use of a designer peptide nucleic acid (PNA); the Luminex 100 system: the LigAmp procedure; and denaturing high performance liquid chromatography. Most of these procedures were unable to detect the heteroplasmy if present below 20%; an indication that, in many real life cases, low-frequency mutations remain undetected and that more sensitive mutation detection techniques are urgently needed.

Technical Contacts: Barbara C. Levin, Diane K. Hancock, and Kristy L. Richie
Emails:barbara.levin@nist.gov, diane.hancock@nist.gov, kristy.richie@nist.gov

NIST SRM 2399 Fragile X Human DNA Triplet Repeat Standard

Fragile X syndrome is the most common form of inherited mental retardation and affects approximately 1/4000 to 1/6000 males. Symptoms range from mild to severe mental retardation, hyperactivity, autism-like characteristics, and distinctive physical attributes. This genetic disease has been associated with the expansion of an unstable CGG repeat in the FMR1 gene on the X chromosome.

Fragile X Human DNA Triplet Repeat SRM 2399 is intended to provide clinical diagnostic laboratories with the quality control and quality assurance that they are correctly and accurately determining the number of triplet repeats in fragile X patient families (those individuals with normal and pre-mutation numbers of repeats). This SRM provides the fragile X positive control required by the American College of Medical Genetics Guidelines for any genetic testing. Late-onset of neurological symptoms has recently been shown in male carriers of pre-mutation alleles, a range covered by this SRM.

SRM 2399 consists of 9 vials of polymerase chain reaction (PCR) products generated from DNA obtained from fragile X cell lines or patient samples. Each vial of PCR product contains a different number of CGG repeats. An ILE with nine laboratories was completed.

Technical Contacts: Barbara C. Levin, Diane K. Hancock, and Kristy L. Richie
Emails: barbara.levin@nist.gov, diane.hancock@nist.gov, kristy.richie@nist.gov

NIST SRM 1494 Aliphatic Hydrocarbons

This SRM is composed of a mixture of Aliphatic Hydrocarbons including Pristane and Phytane.

SRM 1494 Aliphatic Hydrocarbons in 2,2,4-Trimethylpentane is a solution of 20 compounds, including even and odd carbon number aliphatic hydrocarbons from *n*-decane (C_{10}) to *n*-eicosane (C_{20}), even carbon number aliphatic hydrocarbons from *n*-eicosane (C_{20}) to *n*-tetratriacontane (C_{34}), and pristine and phytane in 2,2,4-trimethylpentane (*iso*-octane). This SRM is intended primarily for use in the calibration of chromatographic instrumentation used for the determination of aliphatic hydrocarbons. The distribution of normal chain aliphatic hydrocarbons from C_{10} to C_{40} is used as a chemical fingerprinting tool for the identification of petroleum. The ratios between or among *n*-alkanes and pristine and/or phytane are important additional diagnostic tools for oil source identification. Pristane and phytane are primarily derived from the chlorophyll molecule and can be used as biomarkers for phytoplankton. Pristane and phytane are highly lipophilic and resistant to metabolic degradation.

Because aliphatic hydrocarbons are ubiquitous, the quantification in environmental samples may be complicated by laboratory background levels. In an interlaboratory study quantifying selected aliphatic hydrocarbons in air particulate samples, between 30% and 130% differences were observed among the laboratories reporting data for some of the aliphatic hydrocarbons. A calibration solution, such as SRM 1494, will aid in the identification and quantification of aliphatic hydrocarbons.

Technical Contact; Michele Schantz
Email; michele.schantz@nist.gov

NIST SRM 2930 Extends Transmittance Coverage for Visible Molecular Absorption Spectrophotometry

A new set of neutral density glass Standard Reference Material® (SRM) optical filters extends the present coverage of transmittance standards to accommodate the useful dynamic range of modern research grade visible spectrophotometers.

With nominal transmittances of 0.1 %, 0.3 %, and 90 %, SRM 2930 complements pre-existing SRMs 930 (10 %, 20 %, and 30 %) and 1930 (1 %, 3 %, and 50 %). The extension of the range to low transmittance permits the qualification of instruments to determine higher chemical concentrations without the bias-prone step of dilution required otherwise. At the other end of the extended range, the new SRM supports the evaluation of uncertainties in concentration and/or absorption cross-section near the limit of detection for low concentrations or nearly transparent samples.

Although SRM 2930 is available to all interested customers, this last production lot is particularly appropriate as a means for certified reference material (CRM) producers to establish traceability for the measurement results of their products as described in the NIST traceability policy and supplementary materials. For more information, see

(**http://www.nist.gov/traceability**).

The production of NIST neutral density SRM filters is ending with over 3000 sets in the field. The NIST Analytical Chemistry Division (ACD) will continue to offer biennial recertification of these filters to support existing customers.

Technical Contact: Melody Smith
Email: melody.smith@nist.gov

NIST SRMs 3240 through 3245 First Botanical Dietary Supplement SRMs to be Issued for Ephedra

Ephedra-containing products once represented a large share of the U.S. market for dietary supplements until concerns about their safety were raised and they were ruled as adulterated by the Food and Drug Administration (FDA) in February of 2004. In late 2001, FDA began working with NIST and the National Institutes of Health's Office of Dietary Supplements (NIH/ODS) to produce a suite of five ephedra-containing Standard Reference Materials (SRMs) against which analytical methods could be validated and the accuracy of analytical results could be judged. This suite consists of: SRMs 3240 *Ephedra sinica* Stapf Aerial Parts, 3241 *Ephedra sinica* Stapf Native Extract, 3242 *Ephedra sinica* Stapf Commercial Extract, 3243 Ephedra-Containing Solid Oral Dosage Form, and 3244 Ephedra-Containing Protein Powder.

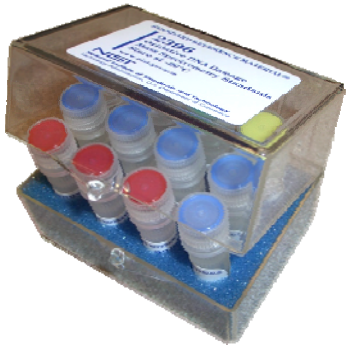
SRM 3245 Ephedra Dietary Supplement Suite contains two bottles of each of the five materials. Values are assigned for the ephedrine alkaloids and elements (including the potentially toxic arsenic, cadmium, lead, and mercury) in these materials. In addition, values for synephrine (a compound in some of the "ephedra-free" weight loss products that are currently being marketed), caffeine, and chromium are provided in SRM 3243. Values for caffeine, theobromine, and theophylline are provided in SRM 3244, as well as for nutrients including fat, protein, carbohydrate, individual fatty acids and amino acids, vitamins, and nutritive elements. (SRM 3244 lies in sector 9 of the triangle that NIST uses to categorize food-matrix reference materials; this triangle was developed by AOAC International.) Materials in the ephedra suite of SRMs are intended for use as primary control materials when assigning values to in-house (secondary) control materials and for validation of analytical methods. The ephedra SRMs are the first in a series of botanical dietary supplement SRMs under development as part of a multi-year collaboration with NIH/ODS and FDA. Future dietary supplement SRMs will include: *Ginkgo biloba*, saw palmetto, bitter orange, carrot oil, green tea, and St. John's wort.

Technical Contact: Katherine E. Sharpless
Email: katherine.sharpless@nist.gov

NIST SRM 2396 Oxidative DNA Damage
Measuring DNA Damage Related to Disease and Aging

Lifestyle, diet, and environment influence our physical and mental abilities. These factors influence our longevity and incidence of various diseases including cancer. In many of these diseases, oxidative DNA damage and its repair is believed to play a major role. Thus, it becomes exceedingly important to understand, at the fundamental level, the mechanisms of oxidative DNA damage, and its processing by DNA repair enzymes as well as how unrepaired DNA lesions may lead to cytotoxicity, mutagenesis and eventually to diseases and aging. Accurate measurement of these modifications is essential for understanding of mechanisms of oxidative DNA damage and its biological effects.

A new measurement standard from the National Institute of Standards and Technology (NIST) will help scientists to better measure oxidative DNA damage implicated in the progression of cancer and other diseases, as well as in the aging process. The new standard can be used to calibrate methods for measuring oxidative damage in a DNA sample by mass spectrometric techniques such as gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry. Oxidative DNA damage is caused by free radicals, highly reactive molecules generated by both normal metabolism and external sources such as ionizing radiation and carcinogenic compounds. These free radicals produce more than twenty different types of lesions in the nitrogen-containing compounds or "bases" of DNA. Oxidative DNA damage can be repaired by enzymes in normal cells, but for a variety of reasons the cellular repair process may fail or slow down, resulting in elevated levels of DNA damage implicated in disease and aging.



Standard Reference Material (SRM) 2396 is the first standard provided by NIST to be used in the field of oxidative DNA damage and repair. This SRM is a set of the twelve stable isotope-labeled compounds (ten analogues of oxidatively modified DNA bases, one analog of an oxidatively modified nucleoside and one analog of a normal DNA nucleoside for DNA quantification) contained in a protective plastic box. Each vial of SRM 2396 contains of 0.2 mL of a designated component at a specified concentration.

Users can calibrate their methods and equipment by using the components of the new SRM, and comparing their results to the NIST-certified values. The SRM is expected to help establish measurement accuracy and consistency among different laboratories as well as traceability to the NIST standard.

NIST scientists pioneered the development of methods for detecting and measuring oxidative damage at levels less than one modified base per million DNA bases. NIST's analysis techniques together positively identify and accurately quantify numerous DNA base lesions caused by free radicals. Other techniques generally measure only one modification and present no structural evidence for verification; such results might be misleading and might not reflect the overall rate of DNA damage.

Technical Contact: Miral Dizdar and Henry Rodriguez
Email: miral.dizdar@nist.gov, henry.rodriguez@nist.gov

New Calibration Standards for Differential Scanning Calorimetry; NIST SRMs 2234 and 2235

Differential scanning calorimetry is an instrumental method that follows the enthalpy change of a sample while an independent variable, usually temperature, is varied with time. The method often makes use of small samples and can employ very large rates of change of the controlled variable. Differential scanning calorimetry has many uses including the measurement of enthalpies of reactions, heat capacities, and temperatures characteristic of material change properties, such as glass transitions.

Differential scanning calorimeters require calibration of their temperature scale and their enthalpy flux response. There exist several protocols regarding the calibration of these instruments; most notable are the standard methods from ASTM International. Also important in practice is the validation of instrumental responses.

To accomplish calibration and/or validation of differential scanning calorimeters, reference materials are required that have well-determined temperatures and enthalpies that accompany an obvious transition from one state to another.

NIST is adding two new SRMs that have enthalpies of transition that were determined with adiabatic calorimetry and temperatures of transition that were determined in partial-melting studies using thermometry calibrated directly on the International Temperature Scale of 1990.

The two new SRMs are SRM 2234 Gallium for Thermal Analysis and SRM 2235 Bismuth for Thermal Analysis. The gallium SRM has a transition (fusion) temperature of 302.915 K and the bismuth standard has a fusion temperature of 544.556 K. Because the two new standards have been certified with adiabatic calorimetry, their enthalpies of fusion are better determined (lower uncertainties) than is the enthalpy of fusion of NIST's SRM 2232 Indium DSC Calibration Standard - Temperature and Enthalpy of Fusion
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Technical Contact: Donald Archer
Email: donald.archer@nist.gov

Renewals

SRM 2260a Aromatic Hydrocarbons in Toluene, and SRM 1491a, Methyl-substituted Polycyclic Aromatic Hydrocarbons in Toluene

SRMs 1491 and SRM 2260 were first issued in 1989 and 1991, respectively, with values assigned for 24 polycyclic aromatic hydrocarbons (PAHs). Both SRM solutions contained the same 24 PAHs at concentrations differing by a factor of 10, and all of the components in each solution were present at the same nominal concentration. SRMs 1491a and 2260a have been redesigned. SRM 2260a contains 36 parent PAHs ranging in molecular weight (MW) from naphthalene (MW 128) to three dibenzofluoranthene/pyrene isomers (MW 302). The concentrations of the individual PAHs range from approximately 2 µg/g to 11 µg/g and are varied so that the ratios of the PAH concentrations relative to each other are similar to those ratios found in many environmental matrix SRMs. SRM 1491a contains 18 methyl-substituted PAHs including methyl- and dimethylnaphthalenes (MW 142 and 156), methyl- and dimethylphenanthrene/anthracenes (MW 192 and 206), retene (MW 234), methylpyrene/fluoranthenes (MW 216), and methylchrysenes (MW 242) at concentrations ranging from approximately 1 µg/g to 2.5 µg/g.

These two solutions will be useful in the calibration of gas chromatographic methods for the quantification of a wide range of PAHs in environmental samples. The additional parent PAHs will expand the range of isomers quantified in many laboratories to include potentially coeluting isomers, such as chrysene/triphenylene, benzo[*b*] and benzo[*j*]fluoranthene, and dibenz[*a,c*] and dibenz[*a,h*]anthracene, as well as some of the potentially more mutagenic higher molecular weight PAHs. The additional solution of methyl-substituted PAHs will be useful particularly for those laboratories performing measurements to support combustion source apportionment research.

Other SRM Renewals…

SRM 1633b Constituent Elements in Coal Fly Ash

SRM 931g Liquid Absorbance Standard for Ultraviolet and Visible Spectrophotometry

SRM 2034 Holmium Oxide Solution Wavelength Standard

SRM 956b Electrolytes in Frozen Human Serum

SRM 1800b Eighteen Non-Methane Hydrocarbon Compounds in Nitrogen

Revisions

Certificate Revisions—Are you Using These Materials?

This is a list of our most recent certificate revisions. Users of NIST SRMs should ensure that they have the most recent certificates. NIST updates certificates for a variety of reasons, such as to extend the expiration date or to include additional information gained from stability testing. If you do not have the most recent certificate for your material, you can print or view a copy from the website at: http://www.nist.gov/srm, or contact SRM at:
Phone: (301) 975-6776
Fax: (301) 926-4751
Email: srminfo@nist.gov.

SRM 983 Radioagenic Lead Isotopic Standard
Editorial Changes

SRM 39j Benzoic Acid Calorimetric
Editorial Changes

SRM 1507b THC-COOH
SRM 1507 THC-COOH In Freeze-Dried Urine
New Expiration Date:
31 December 2008

1932 Fluorescein Solution
Updated reference values
New Expiration Date:
31 December 2006

SRM 997 Isotopic Standard for Thallium
Editorial Changes

SRM 2724b Sulfur in Diesel Fuel Oil
Addition of a certified mercury value

SRM 1619b Sulfur in Residual Fuel Oil
Addition of a certified mercury value

SRM 3158 Thallium Standard
Lot # 993012
New Expiration Date:
11 May 2008

SRM 1001 X-ray Film Step Tablet
Editorial Changes

SRM 1548a Typical Diet
New Expiration Date
30 April 2009

SRM 3151 Silver Standard Solution
Lot # 992212
New Expiration Date:
25 May 2008

SRM 3124a Indium Standard Solution
Lot # 991219
New Expiration Date:
11 July 2008

SRM 3117a Europium Standard Solution
Lot # 991307
New Expiration Date:
13 September 2008

SRM 3106 Bisumuth Standard Solution
Lot # 991212
New Expiration Date:
11 May 2008

Revisions continued…

SRM 970 Ascorbic Acid in Frozen Human Serum
Recertified values for total ascorbic acid.

SRM 3133 Mercury Standard Solution Lot # 991304
Uncertainty updated.
New Expiration Date:
13 September 2008

SRM 2384 Baking Chocolate
Additional reference values

SRM 2387 Peanut Butter
Additional reference values

SRM 909b Human Serum
Updated certified values for uric acid;
Uncertainties updated;
Editorial changes

SRM 2297 Reformulated Gasoline
New Expiration Date:
31 December 2012

SRM 2294 Reformulated Gasoline
New Expiration Date:
31 December 2012

SRM 2295 Reformulated Gasoline
New Expiration Date:
31 December 2012

NIST SRM News

Dr. Robert L. Watters, Jr. became the new Chief of the Measurement Services Division (MSD) at the National Institute of Standards and Technology (NIST) in March 2004. Bob has over 28 years of experience at NIST in SRM development and international metrology comparisons.

Bob received his B. S. in Chemistry from the University of Notre Dame in 1970 and his Ph.D. in Analytical Chemistry from the University of Wisconsin in 1976. He joined the National Bureau of Standards in 1976, and became Group Leader for the Atomic and Molecular Spectrometry Group in the Analytical Chemistry Division of NBS in 1987. He has participated in the analysis and certification of over 150 Standard Reference Materials. He was a member of the NIST *Ad Hoc* Committee on Uncertainty Statements, which developed the NIST policy on implementing the ISO Guide to Uncertainty in Measurement and has presented numerous workshops on applying the ISO principles to measurements in analytical chemistry. He also served as Senior Program Analyst for the NIST Director and as Deputy Chief in the Analytical Chemistry Division. Bob was a founding member of the *Comité International des Poids et Mesures* (CIPM) Consultative Committee on Amount of Substance. He led a team that developed an international database system for comparison measurements performed by the world's National Metrology Institutes. He is also responsible for maintaining the NIST Traceability web site, wherein the NIST policy on traceability is articulated, and through which many of NIST's customers obtain answers to their traceability questions.

Bob is excited about leading MSD, which performs all the business and customer service functions related to the delivery of NIST calibration services, Standard Reference Materials, and Standard Reference Data.

Now Order NIST SRMs Online

You can now order NIST SRMs through our new online ordering system. This system is efficient, user-friendly and secure. Our improved search picks up keywords on the detail page along with the words in the title of each SRM.

In addition, we are in the midst of a project to add numerous certificate references for each SRM online. Please also note we are also adding numerous historical archive certificates online for your convenience.

https://srmors.nist.gov

SRM MARKETING CATALOG 2005 NOW ON CD

If you would like a copy of our January 2005 SRM Marketing Catalog on CD please call, fax, or email us at: Ph: 301-975-6776 Fax: 301-948-3730
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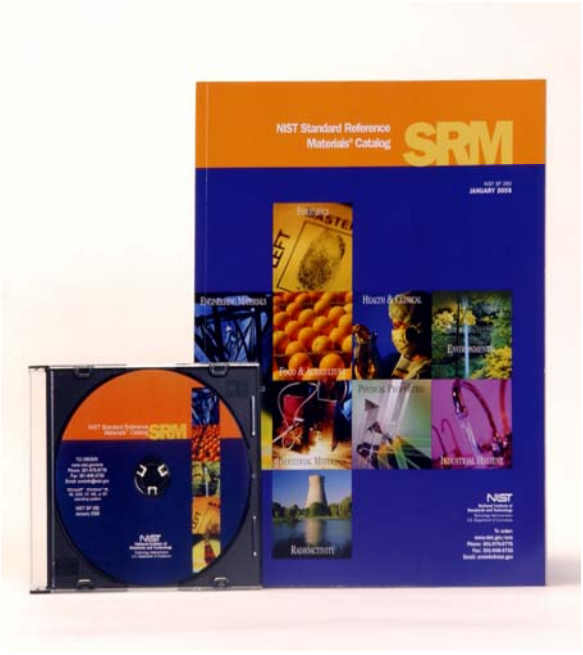
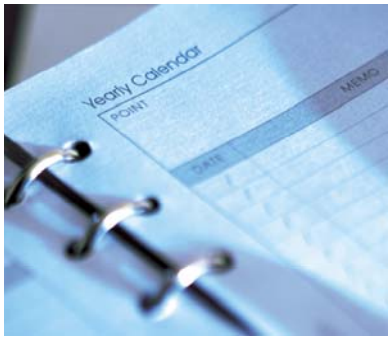


NIST SRM 2005 Exhibit Schedule



American Academy for Forensic Science (AAFS)
February 21 - 26, 2005
New Orleans, LA

Pittsburgh Conference (PITTCON)
February 27 - March 4, 2005
Orlando, FL

American Chemical Society (ACS)
March 14 - 16, 2005
San Diego, CA

NOBCChE
March 20 - 26, 2005
Orlando, FL

Chesapeake Bay Division – Intl. Assoc. for Identification Spring Meeting (CBD-IAI)
April 8 – 9, 2005
Morgantown, WV

BIO2005 PPCA (BIO2005)
June 19 - 22, 2005
Philadelphia, PA

Institute of Food Technologist (IFT)
July 17 - 19, 2005
New Orleans, LA

American Association of Clinical Chemists (AACC)
July 26 –28, 2005
Orlando, FL

NCSL Symposium (NCSL)
August 7 - 10, 2005
Washington, DC

American Chemical Society (ACS)
August 29 - 31, 2005
Washington, DC

Association of Official Analytical Chemist (AOAC)
September 11-15, 2005
Orlando, FL

Materials Science & Technology (MS&T 05)
September 25 - 28, 2005
Pittsburgh, PA

CHEM Show (CHEM)
November 1 - 3, 2005
New York, NY

Eastern Analytical Symposium (EAS)
November 14 - 17, 2005
Somerset, NJ