

NIST Analytical Chemistry Division (839)

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Chemical Science and Technology Laboratory

Analytical Chemistry Division

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I. Introduction

The Analytical Chemistry Division is one of five Divisions in the Chemical Science and Technology Laboratory, National Institute of Standards and Technology. The Division has approximately 100 scientists, technicians, and administrative/clerical support staff and an annual budget of about \$18M of which about \$8M supports programs for other Federal and State Government Agencies and/or American industry on a cost reimbursable basis.

The Division serves as the Nation's reference laboratory for chemical compositional measurements and standards to enhance U.S. industry's productivity and competitiveness, assure equity in trade, and provide quality assurance for chemical measurements used for assessing and improving public health, safety, and the environment. The Division maintains world-class metrologically based core competencies in:

- Analytical Mass Spectrometry
- Analytical Separation Science
- Atomic and Molecular Spectroscopy
- Classical and Electroanalytical Methods
- Gas Metrology
- Nuclear Analytical Methods
- Microanalytical Technologies

These core competencies reside in five Groups [Spectrochemical Methods; Organic Analytical Methods; Gas Metrology and Classical Methods; Molecular Spectrometry and Microfluidic Methods; Nuclear Analytical Methods] and provide the capability to carry out the Division's broad mission and the flexibility to respond to changing and evolving national priorities. The skills and knowledge derived from laboratory-based research concerning the phenomena that underpin the measurement of a wide variety of chemical species in a broad spectrum of matrices are applied to the development and critical evaluation of measurement methods of known accuracy and uncertainty. These internationally-recognized reference methods are benchmarked against those maintained by other National Metrology Institutes worldwide and are used to the deliver measurement services to the chemical measurements community through:

- Critically evaluated Reference Measurement Methods and Procedures;
- Standard Reference Materials (SRMs);
- NIST Traceable Reference Materials (NTRMs);

- Measurement Quality Assurance Programs.

II. Division Project Areas in Support of Chemical Science and Technology Laboratory Programs

Reference Methods and Standards for Clinical Diagnostics

The objective of this project is to develop and maintain the measurements and standards infrastructure to facilitate accurate decision-making regarding the diagnosis, treatment, and prevention of diseases. Measurements are responsible for 10% - 15% of the \$1.7 T annual costs of healthcare in the United States. A significant portion (25% - 30%) of health-related measurements is performed for non-diagnostic reasons (re-tests, error prevention and detection). Even modest improvements in measurement accuracy and quality assurance will result in multi-billion dollar savings in healthcare costs. Project drivers are therefore, measurement reliability as it impacts healthcare costs and medical decision-making, regulatory requirements, and international trade and competitiveness-related issues.

Project components include [1] Reference Methods for selected health status markers (electrolytes, small organic markers, toxic and/or speciated metals, protein-based markers) [2] Standard Reference Materials (pure primary chemical standards, optical filter standards for instrument calibration/assessment, body fluid-based materials), [3] interactive measurement quality assessment activities, [4] strategic international comparison exercises

NIST works with other government agencies (e.g., CDC, NCI, NIH), professional organizations (e.g., AACC, CAP, NCCLS), the private sector (e.g., AdvaMED, Mayo Clinic) and the international community through the recently formed Joint Committee on Traceability in Laboratory Medicine to prioritize measurement and standards needs.

Measurement Methods and Standards for Forensics and Homeland Security

Forensic chemical analyses have become important tools for solving crimes and assuring justice. Today, most forensic analysis techniques are qualitative and are used to identify or confirm the presence or absence of certain materials. However, in many cases, applying quantitative analytical techniques can provide important additional information about material sources or the significance of material identifications.

Project activities address measurement method and standards issues related to alcohol and drugs of abuse testing, crime scene investigations (gunshot and explosive residues, teleforensics), and chemical and biological weapons detection.

Project priorities are driven by input from the U.S. Departments of Justice and Homeland Security.

Measurement Methods and Standards for Nutrients, Contaminants, and Adulterants in Foods

The integrity of the nation's food supply is important for public health and safety. The development of reference methods and standards for nutrients, contaminants, and adulterants in foods is essential in this regard. Project components include measurement

and standards issues related to nutrients in food products, contaminants and adulterants in food products, chemical composition and contaminants in herbal supplements/nutraceuticals, and detection of genetic modifications in food products. Project priorities are determined in consultation with the AOAC, FDA, NIH, and the National Food Processors Association.

Environmental Measurements and Standards

Responsible stewardship of the environment is facilitated when measurements of known accuracy are used in monitoring and decision-making. Activities in this project involve the development of reference methods, the development of Standard Reference Materials, and the provision of other measurement quality assurance services to address measurement problems associated with Drinking Water Quality, wastewater Chlorination/Dechlorination Mechanisms, Atmospheric Monitoring and Global Climate Change Assessment, Automotive Exhaust Emissions, Atmospheric Particulate Characterization, Contaminants in Sediments and Soils, Contaminants in Biological Fluids and Tissues, and Specimen Banking Technology.

Activities in this area support directly and indirectly federal agencies such as, EPA, NOAA, DOE, DOD that have stewardship responsibility for the environment; state laboratories, and the large, environmental testing service sector that consists of private laboratories; secondary standards producers; and proficiency testing organizations. Our environmental projects also support industries and their consortia (e.g., AIGER, EPRI, etc.) that require high quality and/or traceable measurements to respond in a cost-effective manner to changing regulatory mandates concerning emissions and waste disposal. Other activities support basic studies and models that track the fate of pollutants over both time and space and their impact on quality of life.

Methods and Standards for Advanced Materials Characterization

Industrial and academic materials scientists often require accurate information regarding the chemical properties of advanced materials. These properties include chemical purity, trace element content, and the distribution of elements within the material. Such information not only allows optimization and control of production processes, but also facilitates a theoretic understanding of both chemical processes and material properties. As the Nation's reference laboratory for chemical measurements, CSTL must not only provide the fundamental basis of the nation's chemical measurement system, but also extend these capabilities to the real world. The Analytical Chemistry Division does this by providing direct measurements for customers and collaborators, by providing Standard Reference Materials for instrument calibration and for measurement quality assurance, and by making the latest measurement techniques and data available to the advanced materials community.

The Division has a broad array of analytical measurement tools including unique capabilities such as neutron activation analysis, prompt gamma activation analysis, and neutron depth profiling to provide analytical data and reference measurements needed to provide answers to important measurement problems.

Methods and Standards for Commodities Characterization

The provision of accurate, accepted chemical characterization determinations is critical for those materials whose commercial value, properties, or suitability for use depend on their chemical composition. These characterizations may require measurement of major component(s), identification and quantification of contaminants, and/or determination of the spatial distribution of components. This project requires ongoing identification of those commodity areas with critical needs that can be addressed by NIST and strategic selection of project tasks from among these.

Current project activities focus on sulfur in diesel fuels, composition of metals/metal alloys (e.g., low carbon silicon steel, phosphorized copper), a series of cement SRMs, and an electronic scrap artifact material – the first of a new class of SRMs addressing industrial “green product” goals in which key decisions, with significant economic and ecological consequences, depend on the quality of analytical characterizations of materials to be recycled.

Microanalytical Technologies – Lab on a Chip

This project focuses on facilitating the design and development of plastic microfluidic systems by developing techniques to achieve greater control of microflow and microchemistries performed in plastic microchannels. In both the analytical and biotech industries, there has been tremendous interest in recent years in the development of chip-based technologies incorporating microfluidics. It is predicted that miniaturization of chemical processes using these technologies will have a huge impact on rapid point-of-care screening as well as high throughput screening. For these devices to become commercially viable, the chemistries performed in microfluidic systems must be well-controlled to provide accurate and reliable results. In response to this need, the goal of our multi-year program is to develop novel methods to evaluate and control chemistries in microscale systems. First, we have developed methods to understand and manipulate microflow in a predictable manner, since a lack of flow control can lead to misinterpretation of analytical results. We will also develop methods to accurately evaluate and control temperature profiles in microchannels, since temperature is a critical parameter that has a profound effect on reaction kinetics. Finally, we will develop methods to fabricate and integrate components that allow for finer control of chemical reactions including passive micromixers and microarrays. Based on input from a young microfluidics industry, the fundamental control of chemical reactions performed in microchannels is the underpinning of a program designed to promote the commercial realization of plastic microfluidic measurement systems.

Further discussions regarding the Division’s core competencies, focused project areas, and products/services delivered to customers are provided in the Group Sections of this Overview and the Selected Technical Activity Reports.

III. SRMs: Tools for Providing Chemical Measurement Traceability

Increased requirements for quality systems documentation for trade and effective decision-making regarding the health and safety of the U.S. population have increased the need for demonstrating “traceability-to-NIST” and establishing a more formal means for documenting measurement comparability with standards laboratories of other nations and/or regions. Standard Reference Materials (SRMs) are certified reference materials (CRMs) issued under the National Institute of Standards and Technology trademark that are well-characterized using state-of-the-art measurement methods and/or technologies for chemical composition and/or physical properties. Traditionally, SRMs have been the primary tools that NIST provides to the user community for achieving chemical measurement quality assurance and traceability to national standards. Currently, NIST catalogs nearly 1400 SRMs; in FY04 NIST sold approximately 30,400 SRM units to more than 6,500 unique customers. Approximately 24,500 of the units sold were from the ~1000 different types of materials that are certified for or support measurements of chemical composition.

During the past year, measurements were made to support our value assignments for ~100 different SRMs. Thirty-eight new SRMs were completed in FY04 and another forty-eight SRMs were issued as renewals. Measurements were made to confirm stability and/or update certificates on an additional twenty SRMs. In addition, 40 gas mixture SRMs in cylinders were recertified for 11 different Specialty Gas Companies and approximately 220 sets of optical filter standards were re-value assigned for wavelength and/or absorbance in the UV and NIR spectral region for more than 130 different companies. Three new NIST Standard Reference Photometers were delivered (one to U.S. EPA and two to other NMIs) and four recertified during the past year. The following narrative descriptions of several new SRMs completed during the past year illustrate the diversity of the industries and societal segments impacted by the SRM services delivered by the Division:

- **SRMs for Dietary Supplements:** More than 50% of the U.S. population uses dietary supplements, accounting for roughly \$10 billion in sales every year. Standards are being developed to address needs expressed by the Food and Drug Administration (FDA) and the National Institutes of Health’s Office of Dietary Supplements (NIH-ODS) for reference methods and reference materials for dietary supplements to provide quality assurance of analytical measurements associated with the manufacturing process, verification of manufacturers’ label claims, and public health and safety concerns. Suites of SRMs have been completed for Ephedra (SRMs 3240 – 3245) and Ginkgo biloba (SRM 3246-3248). The Ephedra suite of SRMs consists of the authentic plant, plant extract and mimics of commercial finished products. They all have certified values for ephedrine alkaloids and several toxic metals. The Ginkgo biloba suite of three SRMs consists of Ginkgo biloba leaf, Ginkgo biloba extract, and Ginkgo biloba-containing tablets and are certified for prepared ginkgolides, flavnoids, and toxic metals. SRMs for multivitamin/multielement supplements, saw palmetto, bitterorange, and St. John’s wort will be completed in FY05-FY06.
- **SRMs for Clinical Diagnostics: Homocysteine** is an amino acid whose elevated levels in blood correspond with increased risk for heart disease and stroke, as well as other

conditions such as dementia. Folic acid is a vitamin that is known to reduce the risk of neural tube defects in newborns. In addition elevated levels of forms of folic acid (folates) in blood appear to reduce homocysteine levels. NIST worked closely with CDC to develop a three level, frozen human serum SRM (SRM 1955) for these two analytes. Value assignment involves use of higher order reference measurement procedures involving isotope dilution coupled with liquid chromatography-tandem mass spectrometry (LC/MS/MS) performed at both NIST and CDC. This is the first serum-based certified reference material for these two analytes

More than one million Americans will suffer a myocardial infarction, or heart attack, this year. The presence of **troponin I (cTnI)** in the blood is strong evidence that damage to heart tissue has occurred, and the level of this protein in the blood is indicative of the severity of the damage. Unfortunately, the different clinical assays used to measure troponin I may give widely differing results – currently by a factor of 30 -- complicating the diagnostic process. SRM 2921 is formulated as a dilute solution of human cardiac troponin complex of the troponin I, troponin C, and troponin T subunits, extracted from human heart tissue under non-denaturing conditions. This SRM is certified for troponin-I amount of substance content and has reference values for troponin-C and troponin-T. Preliminary data indicate the proper use of this SRM will decrease assay-to-assay measurement variability by a factor of 10.

- **SRMs for Support of Blood and Breath Alcohol Testing:** Two new **ethanol in water reference materials**, SRM 1828b and SRM 1847, with six and three concentration levels, respectively, have been issued to replace the current SRM 1828a, which had only four concentration levels. The concentration levels in SRM 1828b, Ethanol-Water Solutions (Blood-Alcohol Testing: Six Levels), have been tailored to correspond to legally relevant points, specifically, 0.02% and 0.04% for “zero tolerance” and occupational alcohol testing, 0.08% and 0.1% for state drunk driving laws, and 0.2% and 0.3% for an average and high level for blood alcohol measurements. In addition, SRM 1847 has been developed to support Breath-Alcohol Testing. This SRM has three concentration levels of ethanol in water (2%, 6%, and 25%) for calibrating breath-alcohol instruments. The development of this expanded suite of ethanol in water SRMs was supported in part by funding from Office of Law Enforcement Standards in FY03 and FY04.
- **New Sulfur in Fossil Fuel SRMs:** SRM 2693, **Sulfur** and Mercury **in Coal** is the first bituminous coal SRM to be issued with a certified value for sulfur of less than 1 %, and thus fills an important industry need as requested by ASTM. Newer coal-fired power plant are required to use coal containing less than 1% sulfur. These plants generally need to blend coals of varying S content, and need to be very confident that the blended compositions are below 1% S. This new SRM will be used to assure the accuracy of measurements made very near the regulatory limit..

The S concentration SRM 2770, Sulfur in Diesel Fuel, was designed to help the petroleum industry meet the 15 ppm sulfur limit imposed by the EPA for on-road diesel

fuel that will go into effect in 2006. The material was used as the test sample for the CCQM Key Comparison (K35) for Low Sulfur in Diesel Fuel.

- **SRMs for the Metals Industry: Silica fume** is a byproduct of producing silicon metal and ferrosilicon alloys, and its chemical and physical properties make it a very reactive pozzolan. No longer simply discarded, more and more silica fume is being recycled through use in concrete instead of being placed in landfills. High-Performance Concrete containing silica fume can have very high strength and durability. Increasingly, state highway and transportation administrations require high-performance concrete in bridges and roadways. Standard Reference Material 2696 Silica Fume, is the culmination of a five-year development project carried out by the Silica Fume Association and NIST with support from the Federal Highway Administration. SRM 2696 is primarily intended for use in evaluating chemical and instrumental methods of analysis of silica fume used in conjunction with product specifications. Certified values have been established for silicon expressed as SiO_2 and six other chemical constituents, plus reference values for five chemical constituents and the physical measurement parameter Specific Surface Area determined using nitrogen absorption. The SRM was issued in May 2004.
- **Galvalume®** is a hot-dip coating applied to sheet steel to protect it from the elements. As the molten alloy is used, it picks up Fe and Si from the sheet steel passing through the pot. At elevated levels, Fe and Si have a deleterious effect on the Galvalume® coating. Therefore, the pot must be analyzed frequently and recharged at the appropriate time. ASTM International Committee E01 requested that NIST develop **SRM 2426, Galvalume®** to support the US steel industry. The material was prepared and donated by industry laboratories. Homogeneity testing and quantitative measurements by XRF were completed in FY04. Collaborative testing by industry laboratories is expected to be completed in the fall of 2004 and we expect to issue the SRM in FY05.

Additional examples are provided in the Group Overviews and Technical Activity Reports that follow.

In order to address questions from the international community concerning the quality of data provided on SRM certificates, NIST 260-136 “Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements” was published in January 2000. NIST SP 1012: “An Approach to the Metrologically Sound Traceable Assessment of the Chemical Purity of Organic Reference Materials” was developed and published. In addition, the Division’s Quality Manual that summarizes and formalizes the Division’s policies and approaches to addressing quality-related issues concerning the services that we provide has been updated to assure appropriate compliance with ISO/EC 17025 and ISO Guide 34.

IV. NTRM Programs for Leveraging NIST Resources and Efforts

As it has the world's leading, most mature, and most comprehensive reference materials program, most of the world looks to NIST as the *de facto* source for high quality CRMs to support chemical measurements. NIST has met the reference materials needs of U.S. industry

and commerce for nearly 100 years. While our reference materials program has focused primarily on U.S. requirements, it is clear that these materials address international measurement needs as well. As demonstration of quality and “traceability” for chemical measurements have become increasingly global issues, the need for internationally recognized and accepted CRMs has increased correspondingly. Their use is now often mandated in measurement/quality protocols for analytical testing laboratories. Coupled with the fast pace of technological change and greater measurement needs, the demand for additional quantities and additional specific varieties of reference materials has mushroomed. NIST, by itself, does not have the resources to provide SRMs (exact sample types, unique compound combinations, concentrations, etc.) to meet all these needs. Without a significant shift in paradigm, we will not be able to address future needs for reference materials; neither nationally nor internationally.

The NIST Traceable Reference Materials (NTRM) program was created to partially address this problem of increasing needs for reference materials with a well-defined linkage to national standards. An NTRM is a commercially produced reference material with a well-defined traceability linkage to existing NIST standards for chemical measurements. This traceability linkage is established via criteria and protocols defined by NIST and tailored to meet the needs of the metrological community to be served. The NTRM concept was implemented initially in the gas standards area to allow NIST to respond to increasing demands for high quality reference materials needed to implement the “Emissions Trading” provisions of the Clean Air Act of 1990 (while facing the reality of constant human and financial resources at NIST). The program has been highly successful. Since its inception, 12 specialty gas companies (SGC) have worked with NIST to certify over 9000 NTRM cylinders of gas mixtures that have been used to produce more than 500,000 NIST-traceable gas standards. A recent study conducted by RTI International estimates that the “net benefits” of this NTRM program projected through 2007 will be between \$50M and \$63M with a social rate of return of about 225%.

Operationally, in the Gas NTRM Program, a specialty gas company prepares candidate gas mixtures in batches of ten or more cylinders, analyzes these, and submits the data to NIST. NIST selects 10% of the cylinders in the batch and analyzes them so that NIST can value-assign the batch. The cylinders are returned to the SGC with Certification documentation for the batch. The cylinders are under control of the SGC and can be sold to end users to provide a NIST traceable gas or the NTRM mixtures can be used by the SGC in the analysis of other mixtures that are then sold to provide traceability. During FY04, 14 new gas NTRMs were certified for three companies. These included two batches of very difficult 20 $\mu\text{mol/mol}$ nitric oxide in nitrogen NTRMs. An additional twenty nine NTRM batches were recertified this year for 4 companies to extend their certification period by 4 years.

During the past year, the NTRM program was expanded to include a newly defined “NTRM prime” designation to meet additional needs of the automobile industry. For NTRM prime batches NIST will individually certified each cylinder to provide the lowest uncertainty possible. Normal NTRM batches are batch certified using NIST and specialty gas company data. In effect the new NTRM prime cylinders will become industry sponsored SRM quality standards, which will better meet the need of the AIGER companies. The NTRM prime program is ready now; NIST is waiting on AIGER to initiate the first batch.

We had previously planned to develop NTRMs in the Elemental Standards area. Those plans are being re-evaluated. Our single element spectrometric solution standards program (SRM 3100 series) is now on its firmest footing since its inception. All elements are now in stock and the uncertainty model was updated to bring the certificates into full compliance with the ISO Guide to Uncertainty of Measurements (GUM). This uncertainty analysis includes incorporation of the new “Type B on Bias” method developed by the NIST Statistical Engineering Division, as well as a different treatment for transpiration correction. Improvements in packaging instituted several years ago are now paying off with longer shelf lives and larger lot sizes that have reduced the need for replacement of expired or sold out SRMs. Three years ago, over 20 SRMs in the series needed to be produced and certified over the course of a year. Over the past two years, only three SRMs needed to be replaced. Emphasis has shifted to stability testing of SRMs in stock, and working with commercial standards producers to develop strong and defensible claims of traceability between their CRM products and the SRM 3100 Series. Toward that end, we have developed a method for the rigorous comparison of single element solutions standards to the SRM 3100 Series that has been published in *Analytical Chemistry*. The paper includes, as supplemental information, a software tool to help laboratories outside NIST implement the method to establish their claim of traceability to the SRM

We recognize that the NTRM model is only applicable in selected areas where the science, metrology and economics are all favorable. In the remaining areas, such as complex matrix standards, we have begun to leverage our resources through increased strategic collaborations with other National Metrology Institutes and selected U.S. laboratories. Additional details are provided in Technical Activity Reports that follow concerning our collaborative activities with both private sector U.S. laboratories and providers of commercial reference materials and proficiency testing services as well as other National Metrology Institutes worldwide.

IV. Intrinsic Standards

Intrinsic standards “based on well characterized laws of physics, fundamental constants of nature, or invariant properties of materials” (ANSI/NCSL Z540) have many uses in physical and chemical metrology. Such standards can reduce the need for (1) labor-intensive artifact standard production by National Metrology Institutes (NMIs) and (2) repeated costly laboratory measurement comparisons associated with mutual recognition agreements.

For more than three decades the Division has provided a suite of Optical Filter Standards for validating the absorbance/transmittance scale and calibrating the wavelength scale of spectrophotometers from the near infrared through the ultraviolet spectral region. Current efforts are directed to developing a more cost-effective steady-state support structure for customers. The approach is to shift the burden of support for the mature technologies to the commercial sector and intrinsic standards in order to dedicate limited Division resources to emerging applications of near infrared and Raman technologies (for process chemistry) and fluorescence spectroscopy (for biotechnology). A paradigm is envisioned in which appropriate SRMs are not supported in perpetuity, but are produced through enough cycles to establish the material as an intrinsic standard or transfer the continued production to the private sector with an acceptable traceability strategy. A specific step in this direction resulted from our publication of band

positions of dilute acidic holmium oxide solution as an intrinsic wavelength standard. These values were determined from results of an international comparison involving fifteen institutions (fourteen National Metrology Institutes). In a related development, CSTL researchers have developed an algorithm to implement holmium oxide solution wavelength calibration and a proposed extended use of the material as a universal simultaneous standard for both wavelength and absorbance.

VI. International Standards Activities

International agreements and decisions concerning trade and our social well-being are increasingly calling upon mutual recognition of measurements and tests between nations. The absence of such mutual recognition is considered to be a technical barrier to trade and environmental and health-related decision-making. In recent years, mutual recognition agreements have been established related to testing and calibration services and in respect of the bodies accrediting such activities. All of these rest upon the assumption of equivalence of national measurement standards and reliability of the link between national measurement standards and the relevant testing services in each country. In October 1999, the Directors of National Metrology Institutes for the thirty-eight member states of the Meter Convention signed the mutual recognition arrangement on national measurement standards and calibration and measurement certificates issued by national metrology institutes (MRA); twenty-two additional signatories have joined in since. This MRA provides an open, transparent, and comprehensive framework for obtaining reliable quantitative information on the comparability of metrological services provided by the signatory NMIs. It also provides governments and other parties with a secure technical foundation for wider agreements related to international trade, commerce, and regulatory affairs. Signatories to this MRA have some very special responsibilities:

- declaring and documenting their calibration and measurement capabilities (CMCs in Appendix C of the MRA)
- participating in relevant international comparisons to benchmark the claims made in the CMCs (MRA Appendix B)
- documenting the existence of a system for assuring the quality of the measurement services provided.

NIST has taken a leadership role in the International Committee of Weights and Measures-Consultative Committee on the Quantity of Material (CCQM) and the Chemical Metrology Working Group of the Interamerican System for Metrology (SIM) in order to assure the effective, fair and metrologically sound implementation of this MRA. The CCQM has seven working groups: (1) Gas Analysis, (2) Organic Analysis, (3) Inorganic Analysis, (4) Electrochemistry (5) Biometrology, (6) Surface Analysis and, (7) Key Comparisons. These working groups are responsible for selecting and overseeing the operation of key comparisons that address chemical measurement-related issues important for international trade, environmental, health, and safety-related decision making. Analytical Chemistry Division staff are leading various activities within five of the seven working groups and Chairing the Organic Analysis Working Group.

The BIPM database includes approximately 3000 CMCs for chemical measurements. About 1000 will be from NIST. During the past six years, approximately 96 comparison studies have or are being conducted under the auspices of the CCQM to underpin CMC claims. The Analytical Chemistry Division has participated in ~80 of these, serving as Coordinating Laboratory in ~40.

Participation in CCQM Key Comparisons is available only to top-tier NMIs around the world. Within the Americas, only the U.S., Canada and Mexico have well-established programs in chemical metrology. In order to most effectively address the unique needs of all 32 countries within SIM, whose capabilities in chemical metrology span a very broad range, we have initially focused the SIM program on training and capability assessment rather than participation in MRA-driven Key and Supplemental Comparisons. During the past three years, eight intercomparison exercises were carried out to assess the proficiency of SIM NMIs and/or their designated laboratories. Five additional exercises are planned for 2005. While SIM is focusing entirely on training and capability assessment, Regional Chemical Metrology Working Groups in Europe and the Asian Pacific are forging ahead and conducting MRA-driven Key Comparison Studies. We have established agreements with the Chemical Metrology Working Group Leaders of both regions to allow non-CCQM member countries within SIM to participate in such studies as soon as they feel competent to do so. Once self-assessed capabilities of SIM member states are at an appropriate level, as determined based on performance in the SIM capability assessment studies, they can also request permission to participate in CCQM Comparisons.

In addition to these global and regional activities, we are also establishing a limited number of strategic bilateral collaborations and intercomparisons with NMIs around the world. For example, our collaboration with the Netherlands Measurement Institute (NMI) for determining the equivalence of primary gas standards has resulted in a formal "Declaration of Equivalence" that is recognized by the U.S. EPA and European environmental regulatory bodies as documenting the equivalence between seven NIST and NMI primary gas mixture suites. Our formal agreement with NRC-Canada (via the NAFTA Treaty) for cooperation in marine environmental studies has fostered collaborations between the U.S. and Canada in the certification of several certified reference materials important for trade and environmental decision-making. We have signed a Cooperative Arrangement with the National Metrology Institute of Japan for collaborative efforts in the area of pure volatile organic compound standards. These highly pure reference compounds will serve as primary references for many of our gas mixture SRMs. Several additional strategic bilateral arrangements with other National Metrology Institutes /Standards laboratories are being discussed.

VII. Collaborations with Other Government Agencies and Professional Organizations

Providing chemical measurement quality assurance services in support of other Federal and State government agency programs (on a cost reimbursable basis) continues to be an important part of our measurement service delivery portfolio. During the past year, we were involved with about 30 projects with 12 federal and state government agencies. The most prominent of these other agencies programs, based on level of funding, are described below:

- National Institutes of Health/Food and Drug Administration – In 2002 a collaborative effort was begun with the Office of Dietary Supplements (ODS) within the National Institutes of Health (NIH) and the Food and Drug Administration (FDA) to develop SRMs for use in validating analytical methods for chemical characterization of dietary supplements. This program is a multi-year effort (six years minimum) designed to provide SRMs for 8-10 selected dietary supplement materials such as ephedra, ginkgo biloba, St. Johns wort, saw palmetto, and green tea. NIH-ODS and FDA have identified high-priority dietary supplement materials for which SRMs will be developed, and NIST will prepare the reference materials and certify the concentrations of the active and/or marker compounds as well as contaminants in plant material, commercial extract, and finished product SRMs. This year, our on-going collaboration on development dietary supplement SRMs was expanded to include multivitamin/multielement dietary supplements. NIH-ODS is collaborating with the U.S. Department of Agriculture (USDA) to establish the Dietary Supplement Ingredient Database (DSID). The DSID project will report the results of a systematic survey of supplement composition, including chemical analyses of ingredients and indicators of data quality. The initial focus of the DSID project will be on vitamins and mineral supplements. SRM 3280 is a Multivitamin/Multielement Tablet formulation that will have NIST Certified and Reference Values assigned for the 18 elements and 15 vitamins/carotenoids to be listed on dietary supplement labels.
- National Oceanic and Atmospheric Administration (NOAA) – The Division has had a long history of involvement with NOAA related to environmental specimen banking and quality assurance activities. For example, we have coordinated quality assurance programs for organic and trace metal contaminants in the marine sediments and tissue for more than ten years
- Department of Justice through NIST Office of Law Enforcement Standards (OLES) – During the past year, the Division had several projects supported by OLES including the development of forensic SRMs (ethanol in water, arson test mixture, and drugs of abuse in serum), and the evaluation of drug spray reagent test kits. In late FY03 OLES requested assistance from the Division in the physical and chemical testing of pepper spray canisters and these efforts continued this year.
In a totally separate activity for the National Institute of Justice, the Division's Microfluidics Team is designing an automated miniaturized DNA analysis system based on microfluidic technology that will be capable of simultaneously analyzing 16 individual samples for forensic applications.
- Environmental Protection Agency (EPA) – The U.S. EPA has plans to publish a regulation that requires analysis of coal fired power plant emissions to determine adherence to a maximum emission rate. This regulation went into effect in December of

2003 The Division's Gas Metrology team has developed a suite of primary standards for mercury in air to underpin measurements of mercury measurements and to facilitate "fair trading" of these emissions in the future.

In 2000, a new program was initiated with EPA to provide quality assurance and reference materials to support measurements of organic components in air particulate matter less than 2.5 μm in diameter (PM_{2.5}). As part of this program, a third NIST QA program for contaminants was initiated, the NIST/EPA Intercomparison Program for Organic Contaminants in PM_{2.5} Air Particulate Matter. This program currently has 25 laboratories participating in two interlaboratory studies for organic constituents in air particulate matter. This program will continue through FY04. For development of an SRM for organic contaminants on PM_{2.5} we have started a collection of PM_{2.5} at a site in Baltimore MD. The goal is to collect approximately 200 g of material by late 2004 for the SRM. Because the collection and production of a PM_{2.5} SRM will require several years, an "interim reference material" has been prepared from 20g of PM_{2.5} collected at the same site in Baltimore. Characterization of the organic and toxic metal content of this material is currently in progress with measurements at NIST and as part of the intercomparison exercise described above.

- Defense Threat Reduction Agency (DTRA) – The Division and CSTL have had a long-term association (~10 years) with this agency to provide chemical consultation and peer-review of analytical methods and data to support chemical weapons treaty verification.
- Department of Homeland Security – The Department of Homeland Security has requested that the Division provide ongoing consultative services in the general area of standards for chemical measurements. Additionally, efforts have been initiated for developing a Particulate Reference Material for Quantitative Analyses of High Explosives and a Validated Instrument-Independent Raman Libraries for Forensic and Homeland Security Applications to provide both the physical standards and the validated Raman spectral libraries necessary to impart confidence in Raman measurements, provide measurement traceability to national standards, and ensure evidentiary acceptance of Raman measurements. Potential law enforcement and security customers include the FBI, DEA, ATF, TSA, Customs, state and local police departments and hazmat teams.
- Centers for Disease Control and Prevention (CDC) - A long-term program has been established with CDC to provide QA support for their NHANES and similar epidemiological and/or monitoring programs. Activities for the past year included:
 - o Frozen Urine SRM certified for arsenic speciation and trace elements;
 - o QC Materials for cyanide in frozen whole blood;
 - o QC Materials for fat-soluble vitamins, carotenoids and vitamin C in serum;
 - o SRM for homocysteine and folate in serum;
 - o two new human serum SRMs certified for organic contaminants;
 - o a Speciated Arsenic in Urine SRM.

We also had technical interactions that involve laboratory research and measurement activities with more than 20 professional organizations, trade groups, and societies including the American Industry/Government Emissions Research consortium (AIGER), American Association for Clinical Chemistry (AACC), American Society for Testing and Materials (ASTM), Certified Reference Materials Manufacturers Association (CRMMA), the International Federation of Clinical Chemistry (IFCC), National Food Processors Association (NFPA), National Council on Clinical Chemistry (NCCLS), and the National Environmental Laboratory Accreditation Council (NELAC). Specific details concerning many of these interactions are provided in the Group Sections of this Overview.

VIII. Future Measurement and Standards Activities

We will continue our strategy of maintaining core competencies in chemical measurement science to address measurement problems in areas of national priority. All Division research and service projects will continue to be reviewed on an annual basis for match to mission, progress, quality, and match to customer needs. Projects in several new areas are being initiated or expanded.

In the **Forensics Standards** area, we are working with CDC to provide quality assurance and measurement proficiency assessment for the “Chemical Counter-Terrorism Laboratory Network” which is comprised of several state public health laboratories and the CDC. In the event of a chemical terrorism attack, samples (urine or blood) would be shipped to CDC for analysis to determine what agents were used, who was exposed, and how much exposure occurred. CDC itself would most likely not be able to handle so many samples in a short time and would need the help of the state labs in the analyses. NIST’s responsibility is to provide Reference Materials and QA samples to assist CDC in assuring the quality of results from such tests. In addition, Division activities concerning detection of toxins in the water supply and the establishment of a mass spectral database to facilitate rapid and unambiguous identification/detection of pathogens are being expanded to address Homeland Security issues.

The primary focus of the Division’s component of CSTL’s **Food Safety and Nutrition Program** is being shifted from populating the AOAC Food Triangle with food-matrix reference materials (to address nutritional labeling issues) to reference methods and standards to address consumer safety and truth-in-labeling issues associated with Nutraceuticals/Herbal Supplements. A variety of botanical-containing products are marketed as dietary supplements; common examples include St. John’s wort, ephedra, and ginkgo. Taxonomically authentic botanical reference materials with assigned values for active and/or marker compounds are needed for quality assurance of analytical measurements associated with the manufacturing process and for the verification of manufacturers’ label claims for the dietary supplement industry. Assigned values for contaminants and adulterants are also necessary to address public health and safety concerns. Priorities for activities in this new project area are being set in consultation with the U.S. Food and Drug Administration, and the National Institutes of Health’s Office of Dietary Supplements.

Our research project on **Microfluidic Devices**, methods, and applications for chemical analysis including studies of materials and material properties affecting the flow of liquids in

microchannels will begin to focus more on the use of microchannel and other electrophoretic methods for forensic and toxicological applications and standards. Research in our new Competence area, **Single Molecule Measurements and Manipulations** will be expanded. Additional details concerning the latter two research activities are provided in the Group Sections of this Overview that follow.

Healthcare is a major focus area for NIST, the Chemical Science and Technology Laboratory and the Analytical Chemistry Division. The goal of obtaining comparability of laboratory diagnostic test results will be possible only when common reference systems can be established for worldwide use. A critical step in reaching this goal is achieving traceability of reference measurement procedures and reference materials to a universally recognized and accepted reference point such as the International System of Units (SI). Recently, traceability requirements for medical devices to be imported into the European Community have been codified. The European Community In Vitro Diagnostic Directive (EC IVDD) states that "The traceability of values assigned to calibrators and/or control materials must be assured through available reference measurement procedures and/or available reference materials of a higher order." (98/79/EC, Annex1 (A) (3) 2nd paragraph). The Joint Committee on Traceability in Laboratory Medicine (JCTLM) was created to meet the need for a worldwide platform to promote and give guidance on internationally recognized and accepted equivalence of measurements in Laboratory Medicine and traceability to appropriate measurement standards. At present, neither reference materials nor reference methods are available for the vast majority of the chemical or biochemical species that are measured in medical laboratories using IVDs on a routine basis. We are committed to work with the JCTLM in the development of new reference methods and blood/urine-based CRMs for several new health-status markers while maintaining previously developed reference systems for calcium, chloride, cholesterol, creatinine, glucose, lithium, magnesium, potassium, sodium, triglycerides, urea, uric acid, and vitamins A, C, E and beta carotene as well as several therapeutic drugs and drugs of abuse. While focusing increased attention on the health-care/clinical diagnostics area over the next 3-5 years, we will still provide renewals of existing SRMs that support measurements in areas critical to the global community and address critical measurements and standards needs in new high priority areas such as:

- **Detection of Chemical Agents in Body Fluids** *to support CDC's Chemical Counter Terrorism Network*
 - **Fluorescence Intensity Standards** *for calibration of microarray scanning instrumentation in collaboration with CSTL Biotechnology Division*
 - **Gunpowder Composition** *to support NIST Office of Law Enforcement Standards programs for validating forensic methods used to identify gunpowder residues*
 - **Near Infrared Transflectance/Reflectance** *for calibration of single- and double-pass transmission instruments finding increasing use in sensing applications for both wavenumber and wavelength*
 - **Botanical Dietary Supplements** *in collaboration with FDA and NIH to address consumer safety and truth-in-labeling issues*
 - **Raman Intensity Correction SRMs** *in response to priorities established by ASTM E13.08 Raman Spectroscopy Subcommittee for a suite of standards for providing relative Raman intensity corrections for Raman spectrometers at the commercially important*

excitation wavelengths. SRM 2241 at 785 nm has been completed. SRMs for excitation at 532 nm, 488 nm/514 nm, and 1064 nm remain

We expect that the NTRM approach will continue to expand as the basis for allowing the commercial sector to provide reference materials to end-users with a well-defined traceability linkage to NIST. High quality SRMs will continue to be developed to address both national and international measurement problems, but they will be much more expensive and targeted for purchase primarily by other national metrology institutes and commercial producers/distributors. Increasingly, end-user needs will be met via NIST-traceable commercially produced reference materials.

The complex nature of chemical measurements coupled with the increasingly global nature of trade, health, and environmental issues speaks to the need for having the world make measurements using a common “meterstick.” The comprehensive nature of the NIST program in chemical measurements puts us in position to make a very significant contribution to the international chemical measurements community in this regard. In order to do this in both a cost-effective and internationally congenial manner, we are investigating opportunities for partnering with other highly qualified NMIs to provide high quality Certified Reference Materials in specific standards areas. Potential advantages of these proposed partnerships include increased supply, breadth and quality of reference materials for the worldwide chemical measurements community as well as less duplication of effort for more efficient use of limited resources.

This Division overview plus the Group-specific sections and Selected Technical Activity Reports that follow provide additional details concerning our work and reflect the high quality of our staff. This information also shows how our efforts are impacting U.S. industry's productivity and competitiveness as well as providing the measurement and standards infrastructural support for environmental quality and human health assessments. The Overview and Reports also illustrate the critical synergistic relationships that exist among our research programs in chemical measurement science and the standards and quality assurance services that we provide to our customers.

IX. Group Overviews

Spectrochemical Methods

Research activities in spectrochemical methods are directed toward the development, critical evaluation, and application of techniques for the identification and measurement of inorganic species using x-ray, optical, and mass spectrometries. The focus of this research is measurement accuracy and precision, benchmarking industry's needs for traceability, advanced materials characterization, and commodity value assignment and specification.

A significant portion of the Group's research and measurement activities help maintain the Nation's healthcare measurement infrastructure by developing and maintaining reference methods and materials for electrolytes and toxic metals in clinical matrices. In FY03 NIST issued SRM 2670a, Toxic Elements in Freeze Dried Urine, a much improved replacement SRM developed in collaboration with the Centers for Disease Control and Prevention and the Mayo

Clinic. The new SRM is now being used as an important quality assurance tool for CDC population surveys of toxic element exposure. In FY04, we replaced NIST SRM 956a, Electrolytes in Frozen Human Serum, with SRM 956b. This SRM series is widely used for the calibration and validation of automated analyzers and electrolyte measuring systems based on ion selective electrodes. The potential impact of these SRMs is well illustrated by a recent report, commissioned by NIST, on laboratory testing of calcium in serum. This report concluded that the potential economic impact on the health-care system of even a modest analytical bias could lead to an annual cost of \$60M to \$199M resulting from unnecessary follow-up patient testing. The new serum SRM was certified using recently updated NIST methods based on ICP-MS rather than Thermal Ionization MS. Noteworthy is the new ICP-MS method for lithium, which was a particular challenge because of significant mass bias drift found for lighter elements and inter-sample memory effects. The updated methods for electrolyte determinations in clinical samples have been compiled as a NIST 260 Series Report, currently undergoing NIST editorial review.

In December of 2003 the European Union Directive on In-Vitro Diagnostic (IVD) went into effect mandating IVD traceability to “higher order” standards and methods. Sales of NIST electrolyte in serum SRMs have increased dramatically in response, as has the needs of U.S. IVD manufacturers for higher-order reference measurements. With the goal of building an efficient infrastructure for providing clinical reference measurements for IVD manufactures, NIST entered into a collaboration with Mayo Clinic and Dade-Behring, Inc. to test procedures that might be used to implement a NIST Traceable Clinical Reference Laboratory Network. Such a network could provide the IVD industry with reference measurements traceable to NIST standards in a timely and efficient manner. The specific test conducted involved the measurement of calcium at various levels in blood serum, plasma, and urine. A subset of real samples of serum, plasma, and urine were measured both at NIST and the Mayo Clinic, using NIST calibration and validation SRMs, in order to establish an equivalence between NIST isotope dilution mass spectrometry measurements and measurements performed at Mayo Clinic. Initial results have been encouraging, and could potentially lead to the development of a network of laboratories with an expanded analyte and matrix coverage.

In FY03, the Spectrochemical Methods Group, with much collaboration with the Organic Analytical Methods Group, began a major research activity in the area of metal speciation, with a variety of healthcare and environmental projects planned. Two new LC-ICP-MS systems to allow sensitive metal specific detection of separated species were installed—one each both our Gaithersburg and Charleston facilities. FY04 has seen much progress in this area. A new GC-ICP-MS was designed and built in Charleston, and has already been used to develop a new and very sensitive method for the determination of organo-mercury compounds. The new GC-ICP-MS method was used to value assign the methyl mercury concentration in SRM 966, Toxic Elements in Bovine Blood, and will result in an update of the Certificate of Analysis for this SRM. Such an update has been greatly sought by CDC. In Gaithersburg, the new LC-ICP-MS system has been used to investigate arsenic valence state variations in SRM 3103a, the arsenic spectrometric calibration solution SRM. The study confirmed that various lots of the SRM have different distributions of As III and As V, that these differences can cause biases in ICP-MS and ICP-OES measurements, and that these differences result from slower than expected kinetics in the acid digestion of arsenic metal used to prepare the SRM. A separate research project is developing a LC-ICP-MS measurement method for selenoproteins in clinical samples. Back in

Charleston, both GC-ICP-MS and LC-ICP-MS measurements of arsenic, tin, selenium, and mercury species in various existing SRMs and marine tissue samples are being made.

In the area of homeland security we have completed and implemented the development of an isotope dilution GC-MS method for the determination of cyanide in blood. This project was undertaken to enable the development of quality assurance and measurement proficiency standards for the CDC Laboratory Response Network for Chemical Terrorism. This laboratory network was developed by CDC to provide rapid measurement support in the event of a terrorist chemical attack. This new method has been adopted by CDC, and an interagency agreement between NIST and CDC has been put in place to value assign and test the stability of commercially prepared proficiency testing samples of frozen human blood spiked with cyanide at various concentrations.

Three years ago researchers within the Group achieved a breakthrough in the area of high-accuracy measurement of mercury at both trace and elevated levels with the development of an isotope dilution cold vapor ICPMS methodology. This work was recognized with the R&D Magazine award for being one of 100 notable innovations in 2001. After seeing the announcement of this award, NIST was contacted by the Department of Energy to help provide data that could affect impending regulation of mercury concentrations in crude oil at U.S. petroleum refineries. As it turns out, information regarding typical concentrations of mercury in crude oil is scant, and the levels are low enough that the accuracy of existing measurements can be considered questionable. As a means of assessing the typical concentrations of crude petroleum streams which find their way into U.S. refineries, we were asked to undertake a study of mercury concentrations in samples selected from the U.S. Strategic Petroleum Reserve. Thus far nearly 100 samples have been studied, and the results indicate that levels are an order-of-magnitude or more lower than estimates based on older measurements. This new information is likely to impact decisions regarding the need for regulation of mercury in crude oil. The Department of Energy continued and expanded this project in FY04.

The Group maintains strong ties within many U.S. industrial sectors through its active participation in various committees of ASTM and ISO. Contacts and interactions developed through these activities help guide decisions regarding the development of new SRMs and the renewal of existing SRMs. Listed below are brief summaries of several recent SRM certification projects to provide national standards to underpin important measurements in aerospace, automotive, agriculture, food packaging, steel, cement, construction, and mining industries.

- Suite of Six Aluminum Alloy SRMs. (SRM 853a, SRM 854a, SRM 1240c, SRM 1241c, SRM 1255b, SRM 1256b). A total of 15 elements were determined in these SRMs using Glow Discharge Optical Emission Spectrometry for the first time for NIST certification measurements. These SRMs are benchmark materials for several important aluminum alloys: 3004 (SRMs 853a and 1240c), 5182 (SRMs 854a and 1241c), 356 (SRM 1255b, and 380 (SRM 1256b). All of these alloys have varied infrastructural uses and are represented in automobile manufacture, for instance, from body parts to engine components. The aluminum industry has sophisticated in-house reference materials programs to supply their plants and customers with well-characterized materials for

product quality control and business transactions. These SRMs serve as the benchmarks to underpin industry reference materials.

- SRM 173c, Titanium Alloy. This alloy is one of a small list of high value, high production Ti alloys that are important products of the US and world titanium industry.
- SRM 1d, Limestone. Argillaceous limestone is a basic commodity that is used in the production of steel (roughly 50 kg is added per 1000 kg of steel). The chemistry of the added limestone is an important factor in the quality of the steel production. Limestone also finds important uses in agriculture and building materials, and the renewal of this SRM was strongly advocated by several industrial sectors via ASTM.
- SRM 2696, Silica Fume. Silica fume imparts resistance to corrosion and adds strength to concrete; it has an estimated billion-dollar impact on our national infrastructure. Once an expensive waste byproduct of silicon and ferrosilicon production, it also has an environmental impact. Every ton used in concrete reduces the greenhouse gas output of the cement industry, which releases CO₂ during cement manufacture. A number of national standard specifications for silica fume are being implemented in the United States, Canada, Europe, Japan and Australia. The silica fume SRM will be used by the construction industry, silica fume producers and over 60 state and federal agencies (mostly state highway administrations) to verify specifications of silica fume before it is admixed into concrete. Demand for this SRM is driven by its unique physical and chemical properties as well as its economic impact. Quantitative XRF analyses were completed at NIST using a matrix-independent methodology developed a few years ago.
- Crystalline Silica on Filter SRMs. A series of SRMs undertaken at the request and with funding provided by NIOSH and OSHA to provide a basis for the comparability of results from various laboratories to monitor mine safety conditions.

The group has a small but potentially important research effort to utilize our High Performance ICP-OES capabilities to determine the stoichiometry of crystalline materials with high accuracy and precision. Aluminum gallium arsenide (AlGaAs), used in optoelectronic applications, was studied in collaboration with EEEL. Another collaboration with EEEL, this time for the measurement of AlGaN, is planned for FY05. In collaboration with the Surface and Microanalytical Research Division we have also studied SiGe, a material that is being applied in wireless communication and computer applications. In the latter project, the major effort was the development of an accurate means of dissolving the sample for ICP-OES analysis.

Throughout the world spectrochemical instrumentation is calibrated using elemental solution standards that most laboratories purchase from commercial suppliers. In most, if not all, cases these commercial standards assert traceability to NIST, which translates to traceability to the SRM 3100 Series of 69 single-element solution standards certified for the mass fraction of the element in solution. This SRM series therefore plays a key role in the overall calibration infrastructure for elemental analysis, and these SRMs directly affect the results of millions of analyses performed around the world. The ability to maintain this large and critical suite of SRMs certified and in stock has been a challenge for many years, and a key element in making

this task feasible is the shelf life of the SRM. For the past three years the main activity in the maintenance of this SRM series has revolved around testing the stability of the existing SRM stock, looking to extend certification periods rather than discarding and replacing stock. New data on the transpiration of water through the walls of the plastic containers used for many of the SRMs collected over the past three years indicates that the rate of transpiration is negligible when the containers are sealed in aluminized mylar pouches, as they have been for several years. Since transpiration results in a change of the element mass fraction, this new evidence of the lack of transpiration has allowed us to extend the certification period.

The Group's activities at the Hollings Marine Laboratory in Charleston, SC have expanded with the addition of new staff. Activities are centered on providing a quality assurance infrastructure for marine environmental monitoring projects, often in collaboration with state and other federal government agencies. As part of our collaboration in the Seabird Tissue Archival Monitoring Project with the U.S. Fish and Wildlife Service, the Alaska Maritime National Wildlife Refuge, and USGS we have analyzed various sets of sea bird eggs collected in Alaska for mercury concentration using our high accuracy isotope dilution method. Another activity is the Dolphin Health Assessment Project in collaboration with the National Marine Fisheries Service, the Mote Marine Laboratory, and the Chicago Zoo, for which we have developed blood sampling protocols for trace element determinations and accurate high throughput methods for the determination of trace elements in marine animal blood. Further studies include a multi-agency program to develop and apply protocols to investigate the cause of the increase in debilitated sea turtle strandings and a project with the College of Charleston funded by SC Sea Grant on the use of diamondback terrapins as a sentinel species for monitoring mercury contamination in estuarine systems. The second round of the NIST National Marine Analytical Quality Assurance Program for trace metal analysis is nearly complete with the preparation, value assignment and distribution of new whale liver homogenate QC materials to 31 participating laboratories, data compilation and analysis, and the issuance of a draft report to participants.

Many of the above Group activities have been benchmarked against the measurement capabilities of other national metrology institutes through our participation in the Inorganic Analysis Working Group of CCQM. Our capabilities to perform measurements of metals and alloys were tested through a pilot study (P-34) on the determination of constituent elements in aluminum alloy, and a key comparison (K-33) on minor elements in steel. The aluminum alloy work will continue as a Key Comparison in FY05. We also served as the coordinating laboratory for the Key Comparison K-35 on the measurement of low sulfur in diesel fuel was run concurrently with a pilot study (P26.1) on the measurement of very low (~10 ppm) sulfur in kerosene. Both of these studies were designed to demonstrate NMI measurement capabilities needed to meet current and near-future regulatory limits on low sulfur concentrations in fuels. The regulatory push to lower sulfur concentrations is needed in order to enable extremely efficient and long-lived after-treatment emission technologies, primarily catalytic converters. The need for these after-treatment systems is being nationally and internationally mandated by regulatory actions seeking to reduce the level of nitrogen oxides and non-methane organic gases, which are the primary emission pollutants from internal combustion engines. In addition, we are coordinating a pilot study (P46) that compares the primary elemental solution standards of participating NMIs. These solutions are prepared by participants and sent to NIST where a relative comparison of element content relative to the prepared solution concentration is made

using our high-performance ICP-OES methodology. Measurements are ongoing, and include solutions of copper, magnesium, and rhodium.

Nuclear Analytical Methods

Research activities in this group are focused on the science that supports the identification and quantitation of chemical species by nuclear analytical techniques. Current laboratory research activities involve the full suite of nuclear analytical techniques based on reactor neutrons, including instrumental and radiochemical neutron activation analysis (INAA and RNAA), prompt gamma activation analysis (PGAA), and neutron depth profiling (NDP). In addition, we are developing analytical applications of neutron focusing technology. The measurement capabilities that reside within this group provide an excellent complement to those in the Spectrochemical Methods Group in that nuclear analytical methods depend upon characteristics of the nucleus of the element rather than the electron shells, and therefore are insensitive to the chemical state of the analyte. In addition, the nuclear methods are generally nondestructive and do not require sample dissolution, thus providing an independent assay. NDP and focused beam PGAA provide unique capabilities at NIST for location sensitive analysis and elemental mapping.

INAA and RNAA are powerful reference techniques that have been used at NIST for many years. New developments continue to provide improvements in detection sensitivities, elemental specificity, precision, and overall accuracy that allow nuclear methods to address new measurement needs. During the last several years, we have been involved in demonstrations that instrumental neutron activation analysis meets the CCQM definition of a primary ratio method of measurement. Our first step was to characterize all sources of uncertainty for INAA measurements and develop the tools to establish a complete uncertainty statement in terms of SI units. We next applied INAA as a primary method for certification of the arsenic content of SRM 2134, Arsenic Implant in Silicon. The INAA results used for this certification included the first complete, quantitative evaluation of all sources of uncertainty in an INAA measurement. The expanded relative uncertainty for the mean value of this SRM was 0.38 % and approximates the 95 % level of confidence.

In measurements for the NIST Surface and Microanalysis Science Division (837) similar low uncertainties were required for the determination of germanium in silicon. Silicon germanium can be epitaxially grown on silicon resulting in alteration of numerous physical and electronic properties and improved performance of transistors and circuits. It allows for increases in chip speeds of up to 35% while reducing their power consumption. IBM projects that SiGe technology will be dominant in the wireless semiconductor market, growing to a \$3 billion market in 2005. The need for compositional reference materials for secondary ion mass spectrometry (SIMS) analysis was indicated at a SIMS workshop held in 2001. This work was undertaken to assist in the development of such standards. Three different bulk alloy samples were acquired from Virginia Semiconductor. The samples have been characterized for homogeneity by extensive conventional electron microprobe work. Two samples, SiGe6.5 and SiGe14, were found to be suitable for use as microprobe standards. ICP and INAA work has been done on test samples from the 3.5%, 6.5% and 14% alloy. Preliminary measurements indicated that relative expanded uncertainties of several tenths of a percent should be achievable by INAA. However, a significant trend in the analytical data was discovered as a function of

decay time; this prompted a re-evaluation of the half-life for ^{77}Ge . A new value about 1% less than the literature value was found. When the new half life was this was introduced into the INAA calculations, excellent agreement was observed for all measured data. Considering that our half-life measurements were not yet independently validated, the uncertainty in the decay parameters remained a significant component in the expanded uncertainty of the final reported values: Ge3.5: (4.177 ± 0.025) mol Ge / mol Si (0.59%), Ge6.5: (6.455 ± 0.041) mol Ge / mol Si (0.63%), and Ge14: (14.360 ± 0.092) mol Ge / mol Si (0.64%).

Recently developed new INAA procedures for small sample analysis have been applied in the certification of SRM 2703 Inorganics in Marine Sediment for Solid Sampling Analysis. The innovative SRM has been developed to serve the many analytical techniques used in industry and academia, which rely on the analysis of very small samples (i.e., 1 mg), typically in the solid (undissolved) form. Taking advantage of the sensitivity and nondestructive properties of INAA, the use of this technique for homogeneity studies of small samples has been evaluated and implemented for the determination of sampling characteristics for a number of environmental SRMs. The small analytical uncertainty associated with the INAA measurements allows extraction of the variability due to material inhomogeneity from the observed total variability within a given set of measurements. Based on the evaluation of three sediment materials that previously have shown the potential for a high degree of homogeneity at very small sample sizes, we have processed a portion of sediment from the Baltimore Harbor, previously certified for “normal” sample sizes (SRM 2702) constitute SRM 2703. We have achieved a finely powdered sample with a median particle size of 3.5 μm with no particles exceeding 20 μm in diameter. The excellent homogeneity that had been expected from this processing has been confirmed with INAA determinations of minor and trace elements in 0.7 mg samples. We have observed relative standard deviations of 0.9 % for Al and 1.8 % for V, for example, in 22 samples of this material. Certified values have been assigned based on the NIST-INAA determinations of 22 elements in 0.7 mg samples of SRM 2703, and the results obtained from collaborators with similar INAA procedures as well as other solid sampling techniques including GFAAS, laser ablation or slurry sampling ICP-MS, and microbeam-XRF and -PIXE. Analytical evidence of equivalency in mass fraction values of SRM 2703 and the parent material SRM 2702 allowed us to broaden the analytical basis and consider the certification values of the latter for value assignment in SRM 2703.

Radiochemical neutron activation analysis has proven to be a powerful tool for measuring trace phosphorus in a variety of materials. An RNAA procedure has been developed to determine and value assign phosphorus in various SRMs. Phosphorus is quantified by beta counting of ^{32}P after radiochemical separation. Originally developed for the analysis of metals, the method was used to value assign phosphorus in SRMs 861 (Aircraft Superalloy) and 2175 (Refractory Alloy), both containing phosphorus at low mg/kg levels. Modifications to the procedure have allowed us to value assign phosphorus in two new SRMs: 1575a (Pine Needles) and 2702 (Inorganics in Marine Sediment). RNAA was also used for the first time as a primary method to certify the ion implanted phosphorus dose in SRM 2133, Phosphorus Implant in Silicon. This material has been a continuing high-priority need of the semiconductor industry for many years, and is intended for use as a calibrant for secondary ion mass spectrometry (SIMS). It has been produced and certified in collaboration with the Surface and Microanalysis Science Division.

RNAA methods are also being developed and evaluated for measurement of low-level sulfur and nitrogen in steels. Irradiation with thermal neutrons produces ^{35}S and ^{14}C (both pure beta emitters) from sulfur and nitrogen via the $^{34}\text{S}(n,\gamma)^{35}\text{S}$ and $^{14}\text{N}(n,p)^{14}\text{C}$ reactions. These nuclides are separated from the matrix and quantified by liquid scintillation counting. Preliminary measurements indicate detection limits of 5 ng/g and 30 ng/g for S and N respectively in 1 g of low alloy steel irradiated for 8 h at $3 \times 10^8 \text{ cm}^{-2}\text{s}^{-1}$. If successful, these procedures may be adapted for measurement of S and N in other materials. Development of methods for low-level measurement of these elements could impact numerous industries, including the U. S. aerospace industry, which requires primary standards for accurate measurement of these elements in alloys.

Delayed neutron activation analysis (DNAA) is being established at NIST for the measurement of small quantities of fissionable nuclides such as ^{235}U and ^{239}Pu . DNAA is rapid, specific, matrix independent, nondestructive, and sensitive. The system being built at NIST is calculated to have a detection limit for either of these species about 10 picograms, based on a straightforward extrapolation from published practice. After testing uranium standards in two shielding configurations, a final detection system has been designed and built. This consists of ten neutron detectors in a 30 x 30 cm cylindrical moderator of polyethylene, lined with 2 cm of lead to absorb gamma radiation. The design incorporates the existing pneumatic rabbit assembly for irradiation control and sample transport. The system is able to move the sample rapidly to the neutron detector through a polyethylene flight tube. The analysis time is less than 2 minutes per sample. On completion and verification, the Nation will have a readily accessible, rapid means of measuring traces of fissionable U and Pu in samples of forensic interest. In addition, the specificity and sensitivity of this method of analysis will be put to use in certifying trace uranium in Standard Reference Materials.

A method has been developed and apparatus built to produce titanium (and other metal) SRMs of known hydrogen concentration on the few-kilogram scale. The method is based on the controlled reaction of hydrogen with titanium in a closed system. After preparation, the hydrogen concentration is verified by cold-neutron prompt-gamma activation analysis and gravimetry. The first SRM, 2453 (Hydrogen in Titanium Alloy) has been prepared near the critical level of approximately 100 mg/kg. Cold neutron PGAA, neutron incoherent scattering, volumetry and gravimetry have been used together to certify a hydrogen mass fraction of $114 \pm 5 \text{ mg/kg}$. Two additional Hydrogen in Titanium SRMs, 2452 and 2454, have now been prepared and certified at bracketing levels of $62.5 \pm 1.6 \text{ mg/kg}$ and $211 \pm 4 \text{ mg/kg}$ respectively. This method has also been used to prepare standards for neutron-tomographic nondestructive analysis of turbine blades at McClellan Air Force Base.

Pioneering research is conducted by the Nuclear Methods Group on the use of cold neutron beams as analytical probes for both PGAA and NDP. PGAA measures the total amount of an analyte present throughout a sample by the analysis of the prompt gamma rays emitted immediately following neutron capture. NDP, on the other hand, determines the concentrations of several important elements (isotopes) as a function of depth within the first few micrometers of the surface by energy analysis of the prompt charged particles emitted during neutron bombardment. Both of these techniques continue to make important contributions in the characterization of advanced materials, particularly with the enhanced sensitivities now available

using cold neutrons from the NIST Reactor.

The neutron depth profiling facility continues to provide unique measurement capabilities directly to US industries. Current experiments of interest at the NDP instrument include the measurement of lithium concentration and distribution in thin films being studied for battery applications, studies of boron mobility in tungsten with the Army Research Laboratory, shallow-doped boron content in silicon in conjunction with Advanced Micro Devices, the study of lithium distribution in lithium niobate, and the measurement of nitrogen in layers such as TiN and GaN.

As recent examples, we have collaborated with Corning Laboratories on analyses of several high technology materials. One measurement was of nitrogen concentrations measured in GaN/GaAs bilayers. This material is a base material for construction of devices such as blue light emitting lasers. The N concentration, which we can determine quantitatively, is a crucial parameter for establishing the device characteristics. However, there are processing issues in the introduction of N at high levels without causing phase separation. An important need towards that goal is a reliable, accurate method for N concentration as a function of depth in GaAs-based alloys. Two other types of samples with Corning have been analyzed: 1) Boron profiles are measured in glasses to determine B loss due to its volatilization during manufacturing. Surface depletion of B is a key characteristic of borosilicate materials for both chemical vapor deposition and conventional melting processes; and 2) lithium niobate where a quantitative measure of Li concentration was able to differentiate congruent and stoichiometric compositions and any surface depletion in commercial wafers.

We have also measured the nitrogen concentration of MnN/ScN in conjunction with scientists from the NCNR and Ohio University. MnN is a metallic anti-ferromagnetic material that can be used with ferromagnetic semiconductors to make spintronic devices for data storage systems. The magnetic transition point of thin films made with this material is different from that of the bulk material. To measure this transition, which occurs at an elevated temperature, annealing is required. However, the annealing process is suspected of causing the nitrogen to escape, which in turn changes the transition temperature. Using NDP, we have determined that the original films were indeed nitrogen-deficient. Subsequent films were then made with a ScN layer and were again measured with NDP. These measurements show that this problem has been corrected. A joint publication on this work is in preparation. The total film thickness was determined (330 nm) and the total nitrogen concentration to a precision of better than 1% (1 σ).

Members of the Nuclear Methods Group have also worked on a number of high priority PGAA projects with more than 20 “outside clients” as part of our responsibility for supporting the NIST Center for Neutron Research “National Users’ Facility. Many of our current PGAA collaborations involve determining hydrogen in a wide variety of materials for different applications. PGAA has been used to measure hydrogen in semiconductor materials, in barium titanate, in mesoporous carbon, in glassy carbon plates developed for use in fuel cells, and in various deuterated polymers. PGAA has also been used to characterize elemental contents of Ag and Au doped vicor, zeolites, TiO₂ used in pigments, and Ag compounds used in ink jet printers. Native boron has been measured in silicon as part of a CCQM study. Boron mass fractions near 50 ng/g were measured. In collaboration with scientists at NASA-Goddard Space Flight Center,

we are helping to develop a method of obtaining spatially resolved elemental compositions using a focused neutron beam. The goal is to develop an instrument that could be used to evaluate a planetary body from a distance of 2 km.

In collaboration with scientists at Johns Hopkins University, PGAA was used to perform an efficiency calibration of a gamma-ray detector that will be used by the NASA Messenger spacecraft to map the composition of the crust of the planet Mercury. The detector will measure gamma rays emitted by elements in the planet's crust upon capture of cosmic ray induced neutrons. Efficiency was calibrated as a function of energy up to 9 MeV using prompt gamma rays emitted from NaCl and chromium targets, along with gamma rays emitted by calibrated radioisotope sources mounted in the same configuration. The results will allow the spacecraft to obtain an accurate compositional map. Messenger was launched this year and will begin mapping the surface of Mercury in 2011.

We have recently collaborated with Jefferson Laboratory to monitor the hydrogen content of niobium that is used in the construction of the accelerator for the Spallation Neutron Source at Oak Ridge National Laboratory. The presence of interstitial hydrogen in niobium is believed to have a detrimental effect on its mechanical and superconducting properties. It is suspected that chemical polishing (acid treatment) used to remove surface defects from the niobium introduces hydrogen, while vacuum heating may be used to remove it. We have used prompt gamma-ray activation analysis (PGAA) and neutron incoherent scattering to study the effects of these two processes on the hydrogen content of the niobium. The measurements indicated that both processes can significantly alter the metal's hydrogen content. The results of these and future studies should lead to significant improvement in the methods for manufacturing and processing the high purity niobium used in these cavities.

Plans are underway for reconstructing the cold neutron guide NG7. A segmentally curved section will be added to move the PGAA sample position from the present constricted location 3 cm below the SANS guide to the new end position. This reconfiguration will give greatly lowered background and improved detection limits, more space in the target area for the analysis of larger samples, and a much more flexible configuration: for the first time, the sample area will be accessible when the reactor is on, allowing use of a sample cryostat, adjustment of collimators and beam monitor, use of a chopped beam, and an adjustable position for the neutron lens. With the help of a summer student, a plan has been devised to make the PGAA and NDP instruments interchangeable at the new experimental station.

Organic Analytical Methods

Activities in organic analytical methods are directed toward the development, critical evaluation, and application of methods for the identification and measurement of organic and organometal species using analytical separation techniques and mass spectrometry. These separation techniques include gas chromatography (GC), liquid chromatography (LC), supercritical fluid chromatography (SFC) and extraction (SFE), and capillary electrophoresis (CE). These techniques are applied for measurement of organic compounds in support of development of SRMs, quality assurance programs, and other agency activities in clinical, food and nutritional, environmental, forensic, and homeland security areas.

Organic Mass Spectrometry: Recent research activities in organic mass spectrometry have focused on the development of techniques for characterization and quantitative determination of organic compounds, including proteins, in biological matrices. The acquisition of new MS instrumentation in 2003-2004, an LC with tandem mass spectrometry (LC/MS/MS) system, an LC/MS system, and a matrix assisted laser desorption time-of-flight mass spectrometer (MALDI-TOF), has significantly increased our capabilities for the determination of trace-level analytes of health, nutritional, forensic, and environmental importance, as well as protein characterization. The second MALDI-TOF was obtained with our increased support for activities in the Hollings Marine Laboratory in Charleston, SC and will expand our capabilities for characterizing biomolecules and natural products.

Much of our effort in mass spectrometry research has been applied to the measurement of health status markers. Levels of specific proteins and other biomolecules in blood are indicative of certain disease states. These so called “health status markers” often permit more rapid diagnosis of disease with greater certainty than is possible by other methods. Twelve health status markers have been identified as high priority for immediate studies. Recent efforts have been directed toward the development of reference methods for troponin I (a new marker of myocardial infarction), thyroxine and other thyroid hormones (markers for thyroid function), cortisol (a marker for endocrine function), speciated iron (how iron is associated with proteins is important for elevated or low iron levels), homocysteine (a risk factor for myocardial infarction), folic acid (an essential nutrient that reduces the risks of heart disease and neural tube defects), and prostate specific antigen (PSA) (a marker for prostate cancer). Because protein biomarkers occur naturally with considerable heterogeneity (i.e., with glycosylation, acetylation, phosphorylation), we have developed LC and MS techniques for chemical characterization of the target species, particularly the protein-based markers such as troponin I and PSA.

The Analytical Chemistry Division has a long-standing effort to promote accuracy in health-related measurements through development of reference methods and SRMs. With issuance of the in vitro diagnostic (IVD) device directive by the European Union, it has become even more critical for NIST to develop new reference methods and SRMs to provide traceability for the US IVD industry so that this industry can maintain its strong position in European markets. During the past year, work has focused on the development of reference methods for these health status markers based on isotope dilution LC/MS and LC/MS/MS and the completion of critical new SRMs to address the IVD Directive. SRM 2921, Cardiac Troponin Complex (see Technical Activity Report) and SRM 1955, Homocysteine and Folate in Human Serum (risk factor for heart disease and a substance that counteracts effects of homocysteine) have been completed. Methods development for selected thyroid hormones in serum have been completed and will be applied to the certification of an SRM 971 Hormones in Serum. In response to a need for a better reference material for detection of kidney disease, work has started on a new frozen serum SRM for creatinine. Three important clinical SRM renewals were completed this year: SRM 1951b Lipids in Frozen Human Serum is a two-level material certified for cholesterol and triglycerides; SRM 956b Electrolytes in Frozen Human Serum is a three-level material certified for five electrolytes with reference values for ionized calcium; and SRM 965a Glucose in Frozen Human Serum is a four-level material with a much wider concentration range than the previous

lot had. These new reference methods and SRMs will help the IVD industry meet new regulatory requirements for traceability.

Research is continuing on development of a reference method for another risk factor for heart disease, C-reactive protein (CRP). Modest increases in CRP have been linked to arteriosclerosis and the increased risk of heart attacks. NIST, working with scientists at the Laboratory of the Government Chemist (LGC) in the UK and the Physikalisch-Technische Bundesanstalt (PTB) in Germany, is using a proteomics approach to isolate characteristic peptides from CRP for measurement by LC/MS. An isotope labeled peptide will be used as an internal standard for this work. Research has also begun to investigate the quantitative potential of MALDI-TOF mass spectrometry for biomolecules. To explore this potential, studies are underway on promising approaches for quantification of transferrin, an important iron-transporting protein in blood. Research is also underway on measuring various selenium-protein (anti-cancer agents) and iron-protein (iron-transport) combinations in blood.

Separation Science: Research activities in separation science continue to focus on investigations of the physical and chemical processes that influence retention in LC, GC, SFC, CE, and CEC. Results from these fundamental studies are used to design stationary phases tailored to solve specific separation and analysis problems, and to assist in method development and optimization. Recently we have explored a novel approach to the synthesis of LC stationary phases based on polymer immobilization. Polyethylene acrylic acid copolymers were immobilized on silica as an alternative to conventional silane surface modification chemistry. The resulting columns were evaluated for the LC separation of carotenoid isomers, and results indicate exceptional selectivity for this class of compounds, which should result in improved measurement capability for carotenoids and related nutrients in food and dietary supplement samples.

Research in chiral separations is continuing in several areas using LC, CE, and GC. A CE method was developed for the determination of chiral ephedra alkaloids as part of our development of a suite of ephedra-related SRMs. The alkaloids of interest consist of pairs of diastereomers that can be difficult to separate on nonchiral stationary phases.

Our research in organometal speciation has continued with improvements in our method for methylmercury and alkyltin species. The GC-Atomic Emission Detection (AED) method used previously to certify several mussel and fish tissue SRMs has been replaced with an isotope dilution GC-MS that has been used to provide data for certification of methylmercury in SRM 1974b Organics in Mussel Tissue and SRM 1947 Lake Michigan Fish Tissue. Other GC-related separation science research has focused on improved the methodology for the determination of nitro-substituted polycyclic aromatic hydrocarbons (nitro-PAHs) and high molecular weight PAHs in environmental matrices. For the nitro-PAHs a normal-phase LC isolation of the nitro-PAHs was implemented prior to measurement by GC/MS with negative ion chemical ionization detection. An improved separation of the isomeric nitro-PAHs, particularly the nitropyrene and nitrofluoranthene isomers, was achieved using a 50% phenyl-substituted methylpolysiloxane. This improved methodology was used to assign values for concentrations of 25 mononitro-PAHs and 3 dinitrorene isomers in three diesel particulate-related SRMs (SRM 1650a, SRM 1975, and SRM 2975) and two air particulate SRMs (SRM 1649a and SRM 1648). Improved

separation of PAHs isomers of MW 302 has resulted from the use of a 50% phenyl methylpolysiloxane phase instead of the common 5% phenyl phase. GC/MS analysis of several environmental matrix SRMs (air and diesel particulate matter, two sediments, and coal tar) provided identification and reference values for 23 MW 302 isomers. Both the nitro-PAH and the MW 302 PAH studies resulted in the most extensive characterization of these groups of compounds in any environmental matrix and have provided reference values for these SRMs.

Methods development for new emerging environmental pollutants has focused on polybrominated diphenyl ethers (PBDEs), which are flame retardant compounds commonly added to many plastics, resins and textiles, which are then incorporated into products such as TVs, computers, furniture, and carpets. Ten natural matrix SRMs have been analyzed for determination of a suite of 26 PBDE congeners and the results will be used to assign certified concentration values (see Technical Activity Report).

Environmental Specimen Banking and Marine Analytical Quality Assurance: ACD has been involved in environmental specimen banking and marine analytical quality assurance programs for over two decades; these activities are collaborative efforts supported by both NIST resources and by funding for the other federal agency partners (see below for Other Agency Activities). ACD currently maintains the National Biomonitoring Specimen Bank (NBSB) at two locations, the NIST Gaithersburg campus and the Hollings Marine Laboratory (HML) in Charleston, SC. The NBSB is a cryogenic environmental specimen bank that resulted from the pilot Environmental Specimen Bank Program, which was established in 1979 with support from EPA, to develop collection and banking protocols for human liver specimens and to cryogenically store these specimens for retrospective contaminant analysis. Since 1982, the specimen bank has grown in number and types of specimens (e.g., sediments, fish tissues, mussels, oysters, human diet samples, marine mammal tissues, and seabird eggs and feathers) collected as part of several monitoring and research programs. The National Oceanic and Atmospheric Administration (NOAA), Minerals Management Service (MMS), and the U.S. Geological Survey (USGS) have been major consistent supporters since the mid-1980's. A major expansion in environmental specimen banking occurred in 1995 with an agreement between NOAA and NIST to establish a marine environmental specimen bank (ESB) in Charleston, SC as part of the Hollings Marine Laboratory (HML), a partnership with NOAA, two institutions of higher learning and research (College of Charleston and Medical University of South Carolina), and the South Carolina State Department of Natural Resources. ACD currently has seven permanent staff members, three postdoctoral research associates, and three graduate students in the HML working on specimen banking and marine quality assurance activities (see Technical Activity Report).

SRM Activities: During the past year, measurements have been completed on a number of SRMs of importance to the clinical, environmental, and food and nutritional communities; several important new SRMs are described below:

- Clinical Reference Materials
SRM 2921 Human Cardiac Troponin Complex (see Technical Activity Report). After evaluation of six different candidate reference materials using two round robin studies, the material was selected for SRM 2921. More than 20 different commercial cTnI

assays, made by 12 different manufacturers, from the US, Europe, and Japan, were used to evaluate the candidate materials. This choice was made after evaluation of the troponin material for purity, stability, ability to provide harmonization of assay results, and the commutability of the material among the 20 commercial cTnI assays used in the study. SRM 2921 was issued in May 2004 as the first protein health status marker SRM.

- Environmental Reference Materials

SRM 1947, Lake Michigan Fish Tissue. SRM 1947 is a cryogenically homogenized fish fillet prepared from adult lake trout (*Salvelinus namaycush*), which will complement SRM 1946 Lake Superior Fish Tissue issued in 2003. SRM 1947 will have certified values assigned for trace elements (8), methylmercury, PCB congeners (32), pesticides (15), and PBDE congeners (7) with reference values assigned for 17 additional PCBs/pesticides/PBDEs and fatty acids. This is the first tissue material with certified values for PBDE congeners.

SRM 2385 Contaminants in House Dust. House dust is a repository of pesticides and other chemicals used indoors or tracked in from outdoors. SRM 2585, Organic Contaminants in House Dust, will be issued in late 2004 with concentration values assigned for over 100 organic contaminants including pesticides, PAHs, PCB congeners, and PBDE congeners.

SRM 1650b Diesel Particulate Matter. SRM 1650b is a reissue of the original diesel particulate SRM issued in 1985. The recertified materials have values assigned for over 80 PAHs and nitro-substituted PAHs.

- Botanical Dietary Supplement SRMs

As part of the NIST/NIH/FDA collaboration to develop SRMs for use in validating analytical methods for dietary supplements, we have prepared a suite of ephedra-related SRMs (SRMs 3240-3244) including ground plant material, two extracts, and two finished products (powdered oral dosage form and protein powder). These materials have been value assigned for ephedra alkaloids using a combination of LC-UV, CE, LC/MS, and LC/MS/MS methods (see Technical Activity Report).

- Forensic SRMs

Three new SRMs were developed in response to feedback from the forensic community as to their most immediate needs for standards. The development of these new SRMs was initiated with support from the NIST Office of Law Enforcement Standards (OLES) and include: (1) an updated and redesigned SRM 1828b Ethanol in Water for blood- and breath-alcohol testing (see Technical Activity Report); (2) an SRM designed for qualitative arson analysis, SRM 2285 Arson Test Mixture; and (3) an SRM for drugs of abuse in human serum/blood is in progress to complement the current SRMs for drugs of abuse in urine because forensic laboratories often receive blood instead of urine for analysis.

The Organic Analytical Methods Group provides measurement quality support to n a number of Other Federal Agencies including but not limited to:

- The National Institutes of Health (NIH),
- The U.S. Department of Justice (DoJ),
- The National Oceanic and Atmospheric Administration (NOAA)
- The Environmental Protection Agency (EPA),
- The Defense Threat Reduction Agency (DTRA),
- The Department of Defense (DoD),
- The Centers for Disease Control and Prevention (CDC).

Support for these activities accounts for approximately 30% of the group budget.

Gas Metrology and Classical Methods Group Overview

Research and measurement service activities within the Gas Metrology and Classical Methods Group are focused on gas metrology, wet chemical methods (gravimetry, titrimetry), coulometry, and maintenance of the theoretical infrastructure for pH and conductivity measurements.

In the area of electrolytic conductivity we completed 5 SRMs; SRM 3190, 25 $\mu\text{S}/\text{cm}$, SRM 3191, 100 $\mu\text{S}/\text{cm}$; SRM 3192, 500 $\mu\text{S}/\text{cm}$; SRM 3198, 5 $\mu\text{S}/\text{cm}$; and SRM 3199, 15 $\mu\text{S}/\text{cm}$. We also continued our investigations of new standards packaging technology. The packaging used in the past was not suitable for storage of electrolytic conductivity solutions for more than one year. Each electrolytic conductivity solution standard has to be remade every year and uncertainties cannot be lowered below 1%. For the past three years all electrolytic conductivity solutions greater than 500 $\mu\text{S}/\text{cm}$ have been packaged in 50 ml glass ampoules. In sealed ampoules the transpiration problem, which has plagued us in the past, is solved and the solutions have multiyear self-lives. The leaching problem is minimal since the solutions have high ion concentrations already. This reduces the reissue rate for these high electrolytic conductivity standards and allows us to devote more time to the development of low electrolytic conductivity standards being requested by the pharmaceutical industry to address new water purity specifications. However there is no simple solution for the low electrolytic conductivity standards. Leaching from glass is the limiting problem and will require some exotic containers to solve. Until this issue is resolved, electrolytic conductivity standards will continue to increase in price, and perhaps outstrip the ability of our customers to pay. It is planned that the electrolytic conductivity SRMs above and including 500 $\mu\text{S}/\text{cm}$ will be discontinued in favor of a written procedure. This written procedure is in the form of a NIST SP-260 and implements an IUPAC standard, which describes the production of electrolytic primary standards.

CCQM Pilot Study CCQM-P47 evaluated the performance of NMIs in the measurement of electrolytic conductivity of two KCl solutions of nominal conductivity equal to 50 mS/m (500 $\mu\text{S}/\text{cm}$) and 5 mS/m (50 $\mu\text{S}/\text{cm}$). For the 5 mS/m solution, the pilot laboratory (NMI, Netherlands) discovered a time-instability in the solution as stored in the bottles used for distribution of the solution. Following discussion of the original results among the participants in April 2004, the pilot laboratory, NMI (Netherlands), decided to institute a time-dependent correction of the reference value. For the 50 mS/m solution, the NIST result was initially higher than the reference value. Following discussion at the CCQM Electrochemical Analysis Working Group, April, 2004, the cell calibration procedure used at NIST was modified to eliminate the non-IUPAC primary calibrants that previously had been used. With this modification, the NIST result for the 50 mS/m solution was in excellent agreement with the reference value.

In the area of pH and pure materials we completed 4 SRMs; SRM 187e Certification, Borate pH Buffer; SRM 999b, Potassium Chloride; SRM 915b, Calcium carbonate; and SRM 350b, Benzoic Acid. These renewals restock currently out-of-stock SRMs and were prepared with 5 years worth of inventory.

Significant progress was made in our comparability with other NMIs in pH. In the past a very small bias has been evident, along with a larger uncertainty, when compared with other NMIs. Research has yielded significant improvements in the Type A uncertainty (“scatter”), associated with measurement replication, of the NIST pH SRMs. These pH SRMs provide the primary benchmarks to establish the traceability of pH measurements. The decreased uncertainty results from the elimination of transient mixed potential phenomena within the Ag|AgCl electrodes, used in the primary measurement, after they are transferred from the storage solution (0.01 mol kg⁻¹ HCl) to the pH SRM buffer being certified. The mixed potential decays to a negligible level if the electrodes are stored in the given buffer solution for at least 12 h prior to performing the primary measurement. A corresponding pre-equilibration step was incorporated into the NIST procedure for primary pH measurements. This new protocol yielded a 3-fold improvement for the carbonate buffer (pH 10). Smaller improvements were noted for the phthalate buffer (pH 4). Typical Type A uncertainties with the new protocol are less than 0.0005 pH for each buffer with overnight equilibration. This uncertainty now equals or exceeds that of other NMIs that currently perform similar measurements. A model, based on modern mixed-potential theory, was developed and tested to explain the observed transient potentials at these Ag|AgCl electrodes.

There were two international comparisons completed in 2004. CCQM Key Comparison CCQM-K34 was designed to evaluate the agreement obtainable for the assay of potassium hydrogen phthalate (KHP) using high-accuracy assays. The seven participants each used coulometry. The NIST result and its estimate of uncertainty agreed well with those of the other “experienced” NMIs (who had participated in the corresponding pilot study, CCQM-P36). In addition, the NIST estimate of uncertainty was more complete than those submitted by all but one of the other participants (one included the same set of sources), in that more possible sources of uncertainty were included in the estimate.

SIM Pilot Study SIM.8.11P, piloted by CENAM, was a follow-up to the previous SIM.8.P4, which had been piloted by NIST in 2000. Five participants, including NIST, performed Harned cell pH methods (primary method). The NIST results were in excellent agreement (within ± 0.0017 pH) with other experienced NMIs (those that had participated in previous CCQM pH Key Comparisons CCQM-K9 or CCQM-K17). The NIST uncertainties, which included all known Type A and Type B sources, were equal to or smaller than those of the other participating NMIs in the primary measurement. The reduction in the NIST combined uncertainty resulted from a threefold reduction in the Type A uncertainty of the extrapolation of the acidity function to obtain $\text{p}a^\circ$, the acidity function in buffer without added chloride (from which the pH is directly obtained). This reduction was directly attributable to the implementation of pre-equilibration of the Ag|AgCl electrodes used in the Harned cells. The results of this Pilot Study correspond directly to the procedure for the certification of the phosphate pH SRM 186g and support the validity of its certification.

The concentration of ozone (O₃) in the atmosphere remains a significant issue from both scientific and political perspectives. At tropospheric levels, O₃ is a health concern and contributes to global climate change as a greenhouse gas, while stratospheric O₃ protects earth from harmful UV radiation. Since 1983, NIST has provided Standard Reference Photometers (SRPs) based on UV photometry to ten US Environmental Protection Agency (EPA) facilities to provide an infrastructure for the calibration and traceability of ozone measurements within the US. More recently, the international interest has prompted sixteen foreign laboratories to acquire SRPs. Current efforts focus on further anchoring the SRP through international comparisons, comparisons with NIST's primary nitric oxide (NO) gravimetric standards and a nitrogen dioxide (NO₂) permeation tube system, and the development of an advanced primary reference standard. Additionally, NIST and BIPM have been collaborating to transfer the responsibility of international O₃ measurement traceability to the BIPM. Collaboration with the Process Measurements Division will continue in the development of the next generation instrument for ozone determination.

The NIST SRP network continues to expand. Many more countries have requested NIST SRPs for establishing traceability for their atmospheric ozone measurements. New SRPs have been constructed and delivered to; Hong Kong Environment Protection Department, Hong Kong S.A.R., China (SRP34); National Institute for Environmental Studies, Tsukuba, Japan (SRP 35); and U.S. EPA Region 9 Laboratory, San Francisco, CA (SRP 36). In addition SRPs are regularly recertified against NIST SRP 2. Last year we recertified; New South Wales Environment Protection Authority, Sydney, Australia (SRP 21); Czech Hydrometeorological Institute, Prague, Czech Republic (SRP 17); National Physical Laboratory, London, United Kingdom (SRP 20); and U.S. EPA Radiation and Indoor Environments (SRP 7). In addition to the high level SRP work NIST does, NIST also offers a calibration service for commercial ozone monitoring instrumentation. This service is mostly offered to States and Federal Institutions, and commercial instrument makers.

In the gas metrology area, NIST certified and issued 10 SRMs during the past year; SRM 2629a, 20 µmol/mol NO in nitrogen; SRM 2737, 0.5 µmol/mol NO in nitrogen; SRM 2738, 1.00 µmol/mol NO in nitrogen; SRM 2629a, 20 µmol/mol NO in nitrogen; SRM 1683B, 50 µmol/mol NO in nitrogen; SRM 1684B, 100 µmol/mol NO in nitrogen; SRM 1693, 50 ppm Sulfur Dioxide; SRM 2731, 20 ppm Hydrogen Sulfide; SRM 1677, 10 ppm Carbon Monoxide in Nitrogen; and SRM 1663, 1500 ppm Sulfur Dioxide.

We continue to support U.S. industry through development and dissemination of high priority reference materials based on input from organizations such as the American Industry Government Emissions Research (AIGER) consortium and ASTM. Stakeholders in the AIGER group are working together to facilitate the automobile industry meeting more stringent 2003 Federal Tier II and California LEV II emission regulations. AIGER members include the U.S.EPA, California Air Resources Board (CARB), General Motors, Ford, and Daimler-Chrysler. In 1998, NIST worked with a Specialty Gas contractor to blend two cylinders each of low NO standards at concentrations of 0.5 µmol/mol, 0.75 µmol/mol, 0.95 µmol/mol, 1.05 µmol/mol and 1.25 µmol/mol. The ten new standards were prepared employing a newly developed cylinder pretreatment, a NIST SRM 2629a as the parent NO source and a diluent

balance gas of specially gettered nitrogen ($O_2 < 2 \text{ nmol/mol}$). The new standards have been analyzed periodically against NIST dynamic permeation standards and have exhibited excellent NO concentration stability for more than 4 years. In 2001, AIGER provided direct funding to NIST's contractor to expedite the development of two new NO SRMs at the lower concentrations of $0.5 \text{ } \mu\text{mol/mol}$ and $1.0 \text{ } \mu\text{mol/mol}$. NIST and its contractor used the same pretreatment etcetera to blend forty cylinders of $0.5 \text{ } \mu\text{mol/mol}$ and forty cylinders of $1.0 \text{ } \mu\text{mol/mol}$ NO in nitrogen candidate SRM mixtures. The ownership of 27 candidate cylinders of each new SRM (54 total) was transferred from AIGER to NIST; who certified twenty-five (25) stable cylinders of NIST SRM 2737 ($0.5 \text{ } \mu\text{mol/mol}$) and twenty-five (25) stable cylinders of NIST SRM 2738 ($1.0 \text{ } \mu\text{mol/mol}$). Additionally, thirteen cylinders of each concentration were recently analyzed by NIST and returned to AIGER members for their interim use – until the new SRMs become available in early 2005.

We participated in four international gas mixture comparison studies during FY04. In CCQM K-22, key comparison of volatile organic hydrocarbons, it was shown that the six NMI participants generally agreed to within 2 %. In CCQM K-15 International comparison on global warming gases, emission levels it was shown that the four participating NMIs agreed to within 0.3% on the concentration of sulfur hexafluoride and carbon tetrafluoride. In CCQM K-26a, ambient levels of nitric oxide in nitrogen, the agreement among the participant was well within 2 %. This comparison is still undergoing active review and official results should be available in April 2005. In the SIM region NIST piloted a study comparison on automobile emissions (carbon dioxide, carbon monoxide, nitric oxide, and propane). The results of this comparison, while improved over a previous study, was still not of the levels expected from NMIs. It was decided to repeat this comparison in FY2005. NIST is currently coordinating a key comparison on hydrogen sulfide (CCQM-K41). This comparison will run through FY2005 and a preliminary report will be available in Oct 2005.

NIST continued with the bilateral comparison program with the Netherlands Measurement Institute (NMI). Gases compared this last year included, sulfur dioxide, hydrogen sulfide, nitric oxide, and nitrogen dioxide. Generally, all comparisons with NMI have shown agreement to within 0.5% or better. The lone exception has been hydrogen sulfide, where a 2% agreement has been demonstrated. While the comparisons with NMI will continue, we will redirect our efforts into new gases needed by industry. Our first interaction with new gases will be with ammonia, which is needed by the automotive industry and the power industry. NIST expects to develop capabilities in ammonia to support gas standards at the 1 to $500 \text{ } \mu\text{mol/mol}$ level.

During the past two years, we have had discussions with EPA on traceability of mercury measurements from coal-fired power plants. EPA is publishing a regulation, which requires analysis of coal fired power plant emission for mercury emission, and adherence to a maximum emission rate. This regulation will go into effect over the coming years. EPA funded the Gas Metrology team to establish a traceability link for mercury gas standards. This work started in March 2003, with the deadline of December 2003 for traceable gas standards. Although NIST will not be able to establish the ultimate traceability protocols by December 2003, NIST delivered to EPA gas standards, which have been highly characterized by NIST, with a provisional certified value in FY2004. In FY2005, EPA again funded NIST to work on ways to better establish traceability, methods for traceability for $HgCl_2$, and to evaluate the stability of

the Hg gas standards. The ultimate traceable artifact for HgCL₂ will be based on generation HgCl₂ from a dynamic generation system, which will have traceable linkages to flow, mass, temperature, and pressure, or through catalytic conversion of Hg gas standard.

The certified cylinder mixtures delivered to EPA are to be used in their program to audit mercury monitoring sites to determine compliance with regulations. Up till this point they had no way by which to provide quality assurance for the audits. The program also provided data on the performance of a mercury generation device and this data showed that this device is a viable alternate option for calibration and audit of mercury monitors.

The gas mixture NIST Traceable Reference Material (NTRM) program is a procedure for producing and certifying traceable calibration gas standards. Specialty Gas Companies produce, with concurrence from NIST, groups of identical gas mixtures in cylinders, minimum of 10, analyze them and send the data to NIST which then selects 10% of the group to be sent to NIST for analysis. The group of cylinders is assigned one concentration value and uncertainty by using the data generated by NIST for concentration determination and the NIST and Producer data to determine the uncertainty. These NTRM mixtures are under the prevue of the Producer, or Specialty Gas Company, to be sold as traceable standards or to be used at the Producer facilities to analyze other mixtures that are then sold as traceable mixtures. In FY2004 three Specialty gas Companies produced 19 batches of NTRMs. The over 500 individual gas cylinders that comprised these 19 NTRM batches will be used by the specialty gas industry to produce approximately 50,000 NIST-traceable gas standards for end-users.

There have been requests, specifically from the automotive industry, for NIST to provide NIST analyzed mixtures with lower uncertainties than NTRMs. Lower uncertainties are needed to support the lower and tighter restrictions on automobile emissions. A method of accomplishing this is for NIST to analyze each NTRM mixture of a specific group and provide a concentration and uncertainty for each specific cylinder; these would be known as NTRM Prime (NTRM*). In this model the uncertainty is lowered because NIST provides all the analytical data, and the batch homogeneity is not considered. This new designation is available now and new NTRM* batches are expected to be produced in FY2005.

One way to meet the increasing needs of industry for standards at much lower concentrations is through dynamic dilution technology. Over the past two years we have collaborated with the United Kingdom's National Physical Laboratory (NPL) on the critical evaluation of a binary network dilution device that they patented in 2001. This dilutor consists of 6 mass flow controllers (MFC) arraigned in series. Each MFC, or bit, is adjusted to deliver precisely twice the flow of the preceding MFC. Thus calibration consists of matching flows precisely, and does not rely on absolute flow calibration. The binary network behaves like a binary number, and dilution is easily adjusted through switching the MFCs between the dilution gas and the standard gas. We have been working to further develop this concept into a "primary" dilutor. Work using Mol Bloc flow measurement technology and new state of the art mass flow controllers, shows great promise for reducing the uncertainty in the composition of gas mixtures delivered. This technology, and additional dynamic dilution approaches will be utilized in a new dynamic dilution facility to be developed at NIST in the gas metrology group over the next two years. This dilution facility will require new investments in instrumentation and research, but will allow

NIST to produce gases dynamically at low uncertainties. We hope to be able to produce primary gases at an uncertainty approaching or bettering 0.1 % relative. If we are successful we may be able to reduce our reliance on primary gas standards in cylinders and be able to respond faster to needs for new concentrations and new gases.

The Molecular Spectrometry and Microfluidic Methods Group conducts research on the metrology of molecular spectrometry and develops standards for calibration, validation, and performance of instruments for measuring molecular spectra; conducts research on microfluidic devices, methods, and applications for chemical analysis including studies of materials and material properties affecting the flow of liquids in microchannels and the use of microchannel and other electrophoretic methods for forensic and toxicological applications and standards; has responsibility for the development and certification of optical transmittance and wavelength standards in the ultraviolet, visible, and near-infrared spectral regions, of Raman intensity correction standards, and of fluorescence wavelength and intensity standards; and works with users and manufacturers of analytical instruments to assess and measure the performance of analytical methods and to determine and address existing and future needs for analytical instrument standards ranging from device calibration and instrument performance through specifications for remote device control and data interchange. Much of Group 839.04's FY 2004 activities are covered in our Technical Activity Reports, the goal of this report is to document other important activities carried out in FY 2004.

In FY 04, 46 sets of solid absorbance filter SRMs were certified (16 sets SRM 930, 20 sets SRM 2031a, and 10 sets SRM 2930), and 221 optical filter sets were recertified. Continuing measurements were made on a number of other filter sets. Five-hundred seventy-six units of SRM 931g Liquid Absorbance Filter were produced. Certificates were completed for SRM 2037 (Solvent Red 24 Diesel Dye) and SRM 2243 (Relative Intensity Standard for Raman Spectroscopy with 488 nm and 532 nm Excitation). Initial measurements were made on SRM 2244 (Relative Intensity Standard for Raman Spectroscopy with 1064 nm Excitation). Studies were carried out on a material for a Relative Intensity Standard for Raman Spectroscopy with 633 nm Excitation that may eventually become SRM 2245.

Certification was completed on SRM 293--an extended set of visible filters (0.1%, 0.3%, and 70% transmittance) that enlarges the range of transmittances covered by our visible optical filters and makes the range of our visible filter offerings more compatible with those of other NMIs (e.g., NPL) and meets requests by CORM, our NTRM vendors, and others for broadening the range of our standards.

The report on using holmium oxide solution as an intrinsic standard (one whose purity can be assessed inherently and whose wavelength "peak" values at given spectral slit widths can be certified independently and published as standard reference data) for UV/Visible wavelength spectrometer calibration was completed and published. The wavelength values were determined based on an holmium oxide wavelength intercomparison with several NMIs around the world. Accordingly, it should no longer be necessary for NIST to produce SRM 2034 (Holmium Oxide Solution Wavelength Standard). This year's batch of 247 units of SRM 2034 should be our last.

We continued our development of candidate materials for luminescence standards. We developed a uranium-based glass and a manganese-bearing glass that appear useable for fluorescence spectral emissivity standards in the green and yellow spectral regions respectively. After several difficulties in glass production, the glass bricks were sent off to Optiglass in England for cutting and polishing. Candidate materials for both blue- and violet-fluorescing emissivity standards are being evaluated. We are having some problems due to the absorbance of the base glass. We could go with the materials developed so far, but would have to make a very large correction for the self-absorbance or "inner filter" effects. Accordingly, we are looking for ways to fabricate glasses of different matrices to avoid this problem. This is made difficult because small amounts of platinum that dissolves from the crucibles used in the glass making process are a principal cause of this absorbance problem. We continue to search for a suitable candidate material can be used as an emissivity standard the red.

We have nearly fully qualified our SPEX-JY fluorometer as a reference instrument for making certified fluorescence measurements. We are now getting reasonable agreement between the standard-detector and the standard-light-source methods. We have developed a small light source for carrying out photobleaching studies under controlled, reproducible conditions. In collaboration with researchers at NIH, we have begun to construct a reference microarray scanner that can be utilized for the certification of artifact standards used for validating the performance of commercial microarray scanners. We have organized and begun a study on the comparison of fluorescence measurements involving five NMIs. The first steps compare relative emissivity measurements on SRM 936 (Quinine Sulfate Dihydrate) and a set of dyes covering the visible spectrum that were developed by BAM.

Three candidate materials for a new material supporting measurements of high explosives (TNT, RDX, HMX), RM 8105 Trace Particulate Explosives, were prepared, evaluated, and discussed at the major international meeting focusing on explosives detection (ISADE). Additional candidate materials are under development with the support of the Department of Homeland Security.

The National Institute of Justice estimates a backlog of a 542,700 cases for DNA analysis as of April 2004, and current forensic crime labs do not have the equipment capacity to address this backlog in a timely manner. Forensic DNA analysis or "fingerprinting" involves the measurement of the molecular size of several fragments of DNA produced in a specially designed molecular-biological reaction. NIST's development of a microfluidic device for forensic DNA analysis is addressing this backlog by allowing for analysis techniques that are both faster and more economical, while still ensuring the data created is of the highest quality. Current microfluidic device designs fabricated and tested for this purpose are approaching the desired performance for forensic analysis in salient figures-of-merit to current state-of-the-art equipment with the time required for analysis being reduced by nearly 90%.

The development of the initial Group Quality Manual QM-III-839.04 was a major accomplishment this year. The initial version covers the production, certification, and recertification of optical filter standards for absorbance and the production and certification of Raman intensity correction standards. Additions for the production and certification of the fluorescence standards, standard Raman libraries, and the explosives standards will be added as they are developed.

SELECTED TECHNICAL ACTIVITIES

Title: Marine Environmental Specimen Bank Activities

Authors: P.R. Becker, R.S. Pugh, M.B. Ellisor, J.R. Kucklick, S.J. Christopher, S.S. Vander Pol, R. Day, J.M. Keller, C. Bryan, H.M. Stapleton, and S.A. Wise

Abstract: NIST is expanding its environmental specimen banking activities through the recent development of the Marine Environmental Specimen Bank (Marine ESB) at the Hollings Marine Laboratory, Charleston, SC. Through the Marine ESB, the banking of marine mammal tissues has expanded to include the banking of blood and other kinds of samples collected as part of ongoing marine mammal health studies. In addition, the bank now includes bird specimens collected as part of two Department of Interior bird monitoring programs. Future banking will include tissue specimens from other kinds of marine biota and the development of a genetic cryo-bank for marine research. With the increased interest in newly emerging contaminants, the Marine ESB will be a valuable resource for establishing temporal trends for these compounds in marine ecosystems.

Purpose: Environmental specimen banking is the long-term preservation of representative environmental specimens for deferred analysis and evaluation. A systematic well-designed specimen bank program is not only a valuable component of real-time monitoring and basic research, but it also enables investigators to extend their research into the past and provides for future verification of analytical results. Formal environmental specimen banks are recognized internationally as integral parts of long-term environmental monitoring and research. Two national environmental banking systems in the U.S. are the CDC and ATSDR Specimen Packaging, Inventory and Repository (CASPIR™) which cryogenically archives specimens for national public health investigations and NIST's National Biomonitoring Specimen Bank (NBSB) which cryogenically archives specimens for environmental research. Both specimen banks include well developed banking protocols, computerized sample tracking (chain-of-custody) systems, maintenance of many forms of data associated with original specimens, and large investments in state-of-the-art facilities and equipment required to store specimens over relatively long periods of time. Both programs have emphasized cryogenic storage, using ultra-cold (-80 °C) electric freezers and liquid nitrogen vapor storage (-150 °C), security systems, and monitoring of storage conditions 24 hours a day, 365 days a year.

The NBSB was originally established in 1979 by NIST at its Center for Neutron Research in Gaithersburg, MD. In 2002, a NBSB satellite facility was established by NIST at the Hollings Marine Laboratory, Charleston, South Carolina. This facility, the Marine Environmental Specimen Bank (Marine ESB) is devoted to the cryogenic banking of environmental specimens collected as part of ongoing research and monitoring programs conducted by other agencies, such as the National Oceanic and Atmospheric Administration (NOAA) and the U.S. Department of the Interior (DOI), in the marine and coastal environment of the USA.

Major Accomplishments: Although the banking of marine specimens has always been a major part of the NBSB (banking of mussels and oysters for NOAA's Mussel Watch Program, sediments and fish tissues for NOAA's National Status and Trends Program, and marine

mammal tissues for NOAA and DOI), the establishment of the Marine ESB at the Hollings Marine Laboratory in association with NOAA, two institutions of higher learning and research, and a State marine research laboratory, has provided major resources and support for expansion of specimen banking nation-wide. The National Marine Mammal Tissue Bank, which was established by Federal legislation in 1992, is maintained by NIST for the National Marine Fisheries Service and the Fish and Wildlife Service as a component of the NBSB and Marine ESB, with the Marine ESB providing the lead. Presently, NIST maintains 2,504 tissue samples collected from 885 individuals representing 37 species of marine mammals from throughout the US coastal waters, including Alaska. These samples are collected from strandings, animals taken incidentally in fishing operations, and animals harvested by Alaska Natives for food. Since the establishment of the Marine ESB, NIST has used its cryogenic banking expertise to develop protocols and to collect and archive blood and blubber samples for NOAA's ongoing bottlenose dolphin health assessment studies, eggs collected as part of a DOI environmental monitoring program on Alaska seabird colonies, and eggs and feathers collected as part of a DOI peregrine falcon monitoring program. To date, blood and blubber have been collected from 215 bottlenose dolphins, 434 eggs have been archived from five species of arctic seabirds throughout Alaska as well as 9 eggs and 26 feathers from the peregrine falcon program.

The primary function of the Marine ESB is to provide samples for retrospective analysis. In 2004, aliquots of blubber specimens collected from California sea lions during 1993 to 2002 and banked in the NMMTB were analyzed by NIST for polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD), which are synthetic anthropogenic flame retardants that have now become ubiquitous contaminants and temporal trends have indicated concentrations are increasing rapidly in many matrices. The analysis of these banked samples was to determine if temporal trends for these compounds are also discernable in this species of marine mammal that is common on the west coast of the United States. The results indicate a significant temporal trend with HBCD with a doubling time of 2 years. The specimens archived in the Marine ESB will provide a valuable resource for investigating temporal environmental trends in these new compounds and for determining patterns of past exposure in marine biota.

Impact: The establishment of the Marine ESB in association with the Hollings Marine Laboratory has provided a renewed interest in specimen banking as part of marine environmental monitoring and research. Additional kinds of specimen have been added to the bank and additional sponsors have contributed to the expansion of the banking program. With the recognition that newly emerging compounds are appearing in the environment with potential environmental and health effects, the value of the specimens presently held by the bank for determining past exposure patterns and for evaluating temporal trends in concentrations of these compounds is becoming more apparent.

Future Plans: NIST will continue to work with the Hollings Marine Laboratory partners and other Federal agencies and partners to expand environmental specimen banking. NIST staff are presently working with these partners to add banking as part of a future sea turtle health assessment program, to reinstitute banking as part of the Mussel Watch Program, to establish a genetic cryo-bank as part of a National Genetics Archive initiative for marine biota, and to expand the banking of bird specimens to include other kinds of species and types of tissue samples on a national and international level. And finally, existing specimens being held by the bank are

being identified for future work on identifying and measuring new contaminants of interest as related to issues on ocean and human health.

Title: Studying the Chemistry of Pharmaceuticals in Wastewater

Authors: M. Bedner and W.A. MacCrehan

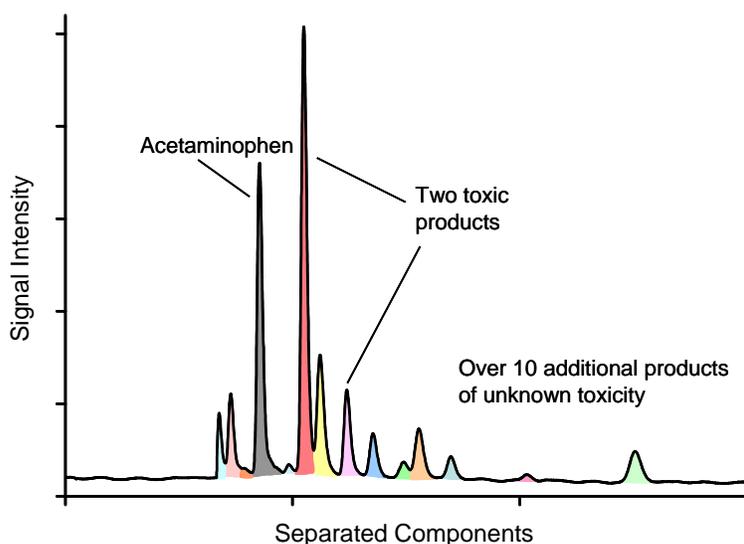
Vision: To establish the NIST role in the measurement of environmentally-important pharmaceutical compounds.

Purpose: Pharmaceuticals and personal care products (PPCPs) are increasingly being recognized as a threat to human health and the environment. PPCPS are continuously released into the environment from human and agricultural waste and are frequently subject to waste treatment processes. Chlorination is the most widely-used chemical process for disinfecting wastewater and drinking water. Chlorine is a strong, non-selective oxidant that is capable of rapidly transforming pharmaceutical compounds. Understanding the chemical and toxicological nature of the transformation products is an important first step to determining what compounds should be measured in the environment.

Context: This research represents a new focus area in CSTL and was awarded exploratory research funds in FY04.

Scientific and technical research and development: This project was made possible by the new LC/MS system in our laboratory. The identification of unknown transformation products using mass spectrometric detection is critical in investigating this measurement problem.

Major Accomplishments: Four pharmaceutical compounds that are frequently detected in the environment were evaluated and included acetaminophen, metoprolol, sulfamethoxazole, and diclofenac. We have begun investigations of the pharmaceutical transformations using calibrated doses of chlorine (added as hypochlorite). Liquid chromatography was used to separate reaction products, which were evaluated and identified using four detection modes. All compounds were significantly transformed by reaction with chlorine. Acetaminophen was shown to form two toxic products, benzoquinone and *N*-acetyl-*p*-benzoquinoneimine, and at least ten additional products (see chromatogram). Metoprolol reacted to form one major product, a chloramine. Sulfamethoxazole formed two major products, both of which have a chlorine atom in the molecule, but only one of which appears to be a chloramine. Diclofenac forms at least five products, one of which has an additional chlorine atom. For all compounds, the products of the reactions tended to be more hydrophobic than the parent



pharmaceutical, which might make them more bioaccumulative.

Impact: These preliminary investigations have revealed potential target compounds for measurement in the environment. This critical information will be used by environmental researchers determining the importance and fate of pharmaceutical compounds.

Future Plans: This project is still in its early stages, so there are many important aspects that need to be addressed. The transformation rates of the pharmaceutical compounds need to be evaluated at the ppb levels typically found in wastewater to verify that the reactions will be significant. Also, as products are identified, targets for measurement in the environment need to be determined based on the potential for toxicity and/or bioactivity. In addition, there are other chemical processes including chloramination, dechlorination, and ozone-disinfection that might transform pharmaceutical compounds. The effects of these water treatment processes on pharmaceuticals will be investigated.

Title: Candidate Material for an Explosive Residues on Soil Reference Material.

Authors: B.A. Benner, Jr. and W.A. MacCrehan

Abstract: A bulk soil sample was collected, processed, and characterized for a number of explosives and their degradation products. This material will be used as a candidate reference material to support the measurement of explosives in soil. Preliminary measurements to characterize this material have been completed.

Purpose: To provide a candidate material for development of a NIST Reference Material (RM) for environmental and forensic explosives analysis. This RM will support measurements required by the demilitarization of former munitions proving grounds and trace explosives detection.

Major Accomplishments: A total of approximately 45 kg of bulk soil was collected at a military munitions proving ground. The bulk material was dried and a portion was sieved to particle sizes between 90 μm and 212 μm , mixed, and irradiated by a standard ^{60}Co procedure to minimize microbial activity that might change the levels of analytes upon storage. This processing yielded about 1.5 kg of suitable candidate material. The concentrations of key explosive compounds were evaluated in this candidate material. One-gram sub-samples were solvent extracted and analyzed by gas chromatography with negative ion chemical ionization-mass spectrometric (GC/NICI-MS) detection for measurement of explosive compounds, including 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and 1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (HMX), and related degradation products. The concentrations of the nine analytes including the high explosives, smokeless powder additives, and their microbial degradation products were determined to be in the range of ng/g to $\mu\text{g/g}$.

Impact: Recently, concerns have been raised by the detection of explosive compounds in the ground water surrounding our military munitions facilities (1). There is insufficient understanding of the environmental fate of the components leaching of incompletely and unexploded ordinance. An explosives on soil material would support the explosive residues measurements in the on-going remediation of these munition proving grounds (2). In addition, this material would be appropriate for use in verifying measurements that are part of landmine detection, forensic post-blast investigations, and trace explosives residue detection.

Future Plans: We will continue to refine our GC/NICI-MS and liquid chromatography/mass spectrometric (LC/MS) methods for measurements of these explosive analytes in this natural matrix material. Evaluation of the long-term stability of this soil will be an important component of the development of a reference material for these unstable analytes. It will be interesting to see if the ^{60}Co irradiation was successful in eliminating any further microbial degradation of 2,4,6-trinitrotoluene (TNT) that was noted in this soil prior to irradiation.

References:

1. MacDonald, J. A. "Cleaning Up Unexploded Ordnance," *Environ. Sci. Technol.*, 35, 370A-374A (2001).
2. Environmental Protection Agency (EPA) Methods 8095 (Explosives by Gas Chromatography) and 8330 (Nitroaromatics and Nitramines by HPLC).

Title: Development of Standard Reference Material (SRM) 2921, Human Cardiac Troponin Complex: A Primary Calibrator for Assays Used to Diagnose Heart Attacks

Author: D.M. Bunk and M.J. Welch

Abstract: With the assistance of the American Association of Clinical Chemistry (AACC), the International Federation of Clinical Chemistry (IFCC), and the manufacturers of clinical cTnI assays, NIST has developed a reference material to address the need for standardization of clinical human cardiac troponin I (cTnI) assays. Based on the results of two interlaboratory comparison studies, NIST Standard Reference Material (SRM) 2921 was prepared from the purified human cardiac troponin complex of the troponin T, troponin I, and troponin C subunits.

Characterization of the structures of the troponin subunits in SRM 2921 was performed by mass spectrometry and a certified value for the concentration of the troponin I subunit was determined by a variety of analytical techniques, including amino acid analysis. Manufacturers of commercial cTnI assays can now use SRM 2921 for quality control purposes and to establish direct SI-traceability of assay measurements, as well as for the value assignment of secondary reference materials.

Purpose: The clinical measurement of serum cardiac troponin I has become an important tool in the diagnosis of acute myocardial infarction and myocardial damage. Unfortunately, considerable variability in clinical cTnI assay results has been reported. Standardization of clinical cTnI measurements is needed to provide more reliability in the use of cTnI assays for the diagnosis of myocardial infarction and damage.

Major Accomplishments: After evaluating several different forms of troponin as candidate reference materials through two round robin studies, a human cardiac troponin complex was chosen as the most suitable material for SRM 2921. This choice was made after evaluation of the troponin preparation for purity, stability, its ability to provide harmonization of assay results, and the commutability of the material among the twenty commercial cTnI assays used in the studies.

Certification of the concentration of cTnI in SRM 2921 was accomplished by two analytical methods, including amino acid analysis. Through the amino acid analysis, SI-traceability of the cTnI concentration in SRM 2921 was achieved. Because of the inherent structural complexity of human proteins and because this complexity can have a substantial impact on immunoassay response, a thorough investigation of the chemical heterogeneity of cTnI and the other two troponin subunits, cTnT and cTnC, in SRM 2921 was performed. SRM 2921 was issued in May 2004 and has been well received by the *in vitro* diagnostic industry.

Impact: Manufacturers of commercial cTnI assays can use SRM 2921 for quality control, calibration, and to establish direct SI-traceability for assay measurements.

Future Plans: A commutability study is underway, involving nineteen cTnI assays currently on the market worldwide. Once accomplished, this demonstration of the commutability will put SRM 2921 in compliance with ISO 15194 and eliminate the last hurdle for this new reference

material to be acknowledged by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) as a higher order reference material for use worldwide.

Title: Raman Libraries

Author: S.J. Choquette

Vision: To develop instrument independent Raman spectral libraries and the calibration protocols to enable their use with field portable instrumentation.

Purpose: Portable Raman spectrometers are currently in use by first responders—hazardous materials (hazmat) specialists, law enforcement officers, safety and security personnel—to identify unknown substances in real-time with minimal handling. Such materials include explosives, drugs of abuse, and bio/chemical agents. Our goal is to provide both the physical standards and the validated Raman spectral libraries necessary to impart confidence in Raman measurements, to provide measurement traceability to national standards, to improve the Raman determination efficacy, and to ensure evidentiary acceptance of Raman measurements.

Background: The issue with spectral libraries in general, is that the origin, purity, and physical state of the compound are typically never specified. It is also quite infrequent that the instrument state of the analyzing spectrometer is adequately known. Although parameters such as spectral resolution may be listed, the calibration state of either axis (wavelength and intensity) are rarely given. Quality systems, which might assure the veracity of the data, are infrequently, if ever, used for spectral acquisition. Raman spectral libraries have the additional challenge in that the spectra are instrument dependent, due to the variety of laser excitation wavelengths used and nature of the measurement. Because Raman is an emission process, the spectra are convolved with the instrument response. This is quite varied from vendor to vendor, even given the same laser excitation wavelength. Our goal is to develop a standardized protocol for measurement of these compounds by Raman spectroscopy and then develop the protocols for transferring this reference data to commercial instrumentation.

Major Accomplishments 2004: In 2004 a test set of compounds comprising 14 persistent organic pollutants (pop's) was chosen for assessment and to aid in the development of standardized protocols for inclusion of compounds into a spectral library. Our next goals are to study efficient methods for transferring this data to the commercial sector. These compounds were chosen based upon input from Raman vendors and NIST's prior experience in developing SRM's based upon these materials. GC-FID and differential scanning calorimetry were employed to verify the purity of the materials. Raman spectra were acquired on research grade instruments employing 514 nm, 785 nm and 1064 nm laser irradiation. Each instrument was calibrated in both x and y axes using NIST SRM's. Spectra were corrected for scattering and incorporated into a searchable library. Arrangements are in progress to measure these compounds upon commercial grade systems to assess the efficacy of software algorithms to identify these compounds utilizing this reference data.

Future Plans. A collaboration initiated with applied math (Anthony Kearsley) to develop algorithms for automated Raman spectral searches. This is an important and necessary extension of this work to compare instruments of widely disparate capabilities.

Title: New Series of Standard Reference Materials (SRMs) for Raman Intensity Correction

Authors: S. J. Choquette, W. Hurst, and E. Etz

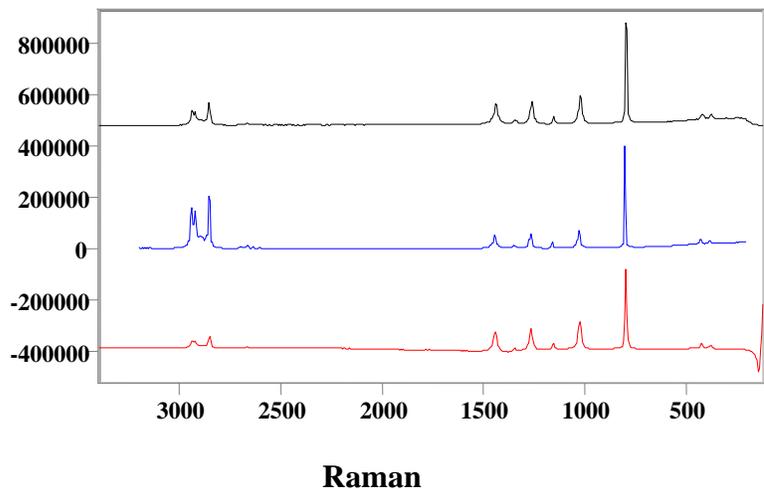
Purpose: Raman spectroscopy is a nondestructive analytical method that has many qualitative and quantitative analytical applications in the homeland security, forensic, pharmaceutical and biotechnology industries. It's major advantage over IR spectroscopy as an analytical tool is that it may be used with little or no sample preparation. Because Raman scattering is a single beam emission process, the appearance of a compound's Raman spectra is highly dependent on the wavelength of the excitation source and the construction of the spectrometer. As a result, Raman spectral libraries are only useful for the specific instrument configuration upon which the spectra were measured. The development of a simple to use emission standard would allow the user to correct the sample spectra for instrument response. Corrected spectra, much like absorbance spectra, would be transferable between instruments, would be amenable to searching in corrected libraries, and would allow verification of instrument performance.

Context: This work is part of a collaborative effort between division 839, 836 and 837 to develop a suite of inexpensive intensity correction standards for commercial Raman systems employing a number of laser excitation wavelengths. Currently three standards have been produced that are suitable for use with systems employing 785 nm lasers (SRM 2241), 532 nm lasers (SRM 2242) and 488/514.5nm lasers (SRM 2243). The development of the standards will allow a traceable calibration of the system response to NIST standards, which is a necessary step for qualification of chemical instrumentation in the pharmaceutical industry. In addition, the use of these standards will enable the creation of standardized Raman spectral libraries, an increasingly important task given the number of spectrometer vendors marketing this technique to 1st responders, forensic scientists and military personnel.

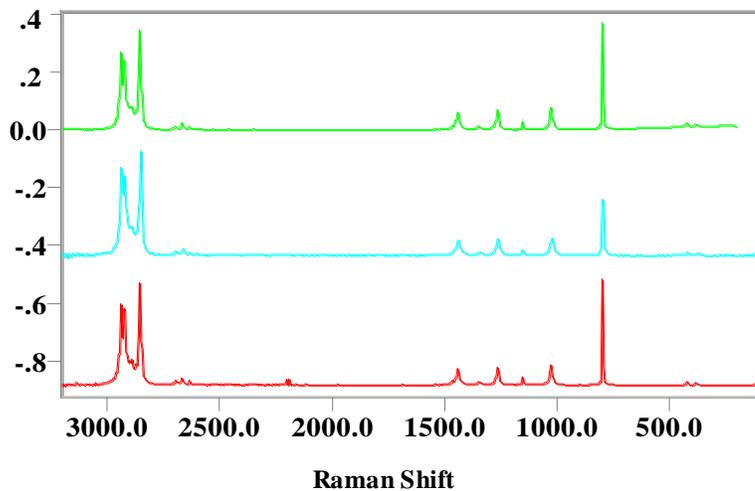
Major Accomplishments: This year we completed work on SRM 2243: a relative intensity correction standard for 488/514.5 nm systems and initiated work on SRM 2244: a relative intensity correction standard for 1064 nm lasers. The later is particularly important as our customer base for SRM 2244 is the pharmaceutical industry. FT Raman systems are widely used in this industry because of the large installed base of FT-IR systems (similar instruments/software) and also the Raman spectra of compounds excited with NIR excitation tend to exhibit less fluorescence interference. We have identified a suitable candidate glass for SRM 2244 and have initiated a round robin study with a number of instrument vendors to assess compatibility and utility of the glass. Based upon favorable feedback concerning this glass, a production melt was procured and is currently being processed by our glass shops. We anticipate delivery of this SRM in FY 2005.

The following graph depicts the uncorrected Raman spectrum of cyclohexane acquired on three different Raman systems. All three spectrometers used 785 nm laser excitation. As can be seen, the height of the bands is different with each spectrometer. The next graph shows that same spectra corrected with SRM 2241. The band heights of the spectrum are now similar.

Cyclohexane Raman Spectra on three Raman spectrometers utilizing 785 nm excitation.



SRM 2241 corrected Raman spectra of cyclohexane on the same spectrometers.



Title: Standard Reference Materials (SRMs) for Contaminants in Marine Tissue and Sediment

Authors: S.J. Christopher, R.D. Day, W.C. Davis, S.E. Long, R.S. Pugh, M.M. Schantz, J.R. Kucklick, D.L. Poster, J.M. Keller, H.M. Stapleton, K.S. Sharpless, C. Phinney, B. J. Porter, E.A. Mackey, R.O. Spatz, C.E. Bryan, J.R. Sieber, R. Zeisler, S.A. Wise, and G.C. Turk

Abstract: New classes of organic and organometallic contaminants have been measured in various marine tissue and sediment SRMs and in SRM 1947 Lake Michigan Fish Tissue. New analytical approaches have been developed to certify trace elements in SRM 1947 and SRM 2703 Marine Sediment for Microsampling.

Purpose: Targeted environmental monitoring efforts, regulatory pressures and compounds of emerging concern are expanding the list of potentially toxic contaminants that impact marine systems and challenging the analytical community to develop new measurement technologies for organic pollutants and trace metals. NIST is responding by providing marine matrix SRMs to the marine environmental research community for method validation and quality assurance purposes and is at the forefront of developing and disseminating new analytical technologies for measuring contaminants in marine reference materials.

Major Accomplishments: The value assignment of SRM 1947 Lake Michigan Fish Tissue highlights the collaborative effort undertaken to provide a marine reference material with a comprehensive suite of contaminant values, as well as information on nutritive properties. Several classes of compounds are represented in this material, ranging from selected trace elements, including total mercury and methylmercury, and organic contaminants, including polychlorinated biphenyl (PCB) congeners, chlorinated pesticides, and polybrominated diphenyl ether (PBDE) congeners to proximates and fatty acids. New methods were developed to measure compounds like PBDEs, which are flame retardant compounds added to consumer plastic products. These compounds are chemically similar to PCBs, with levels increasing in the U.S. population. PBDEs are currently being measured in two marine sediment SRMs (1941b Organics in Marine Sediment and 1944 NY/NJ Waterway Sediment) and three Mussel tissue SRMs (1974b, 2977 and 2978). All PBDE measurements required the preparation of custom primary standards from chemically pure solutions and solids, reduction of background levels in the analytical blank and development of gas chromatography mass spectrometry (GC/MS) methods to accommodate these thermally labile compounds.

Significant effort went into development of new complementary strategies for determination of organometallic species in marine tissue SRMs. For example, microwave assisted acid extraction was used in combination with solid phase microextraction gas chromatography-inductively coupled plasma mass spectrometry (SPME-GC-ICPMS) to measure organic and inorganic fractions of Hg and organotin compounds in SRMs 1947, 1946 (Lake Superior Fish Tissue), and bivalve tissues, SRM 1566b Oyster Tissue, SRM 2976 Mussel Tissue (Trace Elements and Methylmercury) and SRM 2977 Organics in Mussel Tissue. Some of these SRMs were also screened for As and Se content using liquid chromatography coupled to collision cell ICPMS,

with the goal being to eventually provide concentration values to SRM customers tasked with measuring speciated forms of metals in various environmental matrices.

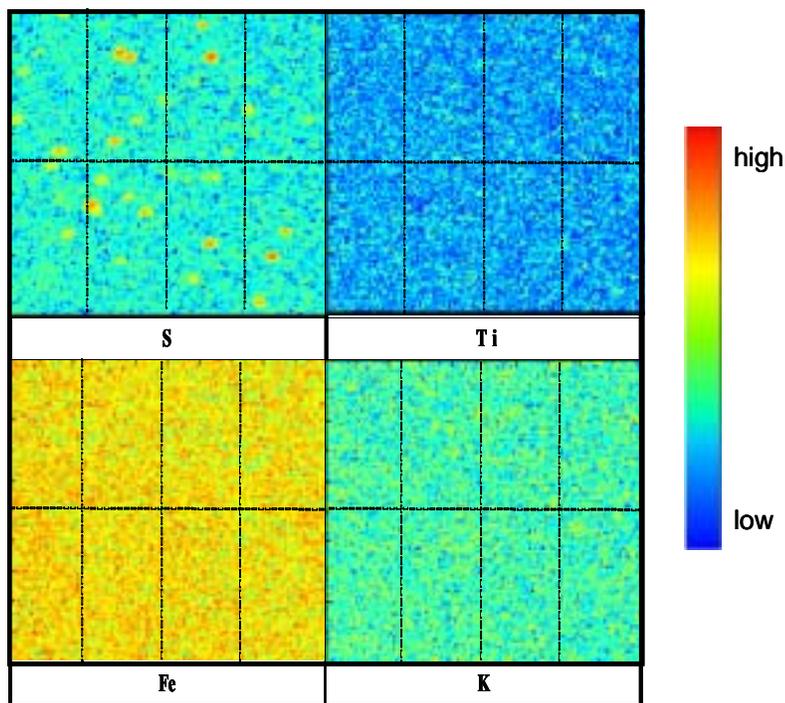
A new certification strategy was tested and implemented for certification of trace elements in SRM 1947 that is applicable to all homogeneous reference materials. Where multiple jars of a homogeneous NIST SRM need to be analyzed, a new standard additions/internal standard procedure was created to streamline certification measurement efforts. In the new procedure, multiple (typically 6) samples of the candidate CRM are accurately weighed into digestion vessels and gravimetrically spiked with an internal standard solution. Increasing amounts of a multi-element spike solution are then gravimetrically added to each vessel, ranging from no spike to a high spike, to effect a standard additions calibration curve. From this point on all measurements of the digested samples will be based on analyte/internal standard ratios, and all sample handling, including removal of the solutions from the digestion vessels and any subsequent dilutions, does not need to be done quantitatively. This is one of the main benefits of this approach. The other benefit is that we do not have to perform sample splits on each sample (SRM jar) to be measured. Values and uncertainties are calculated by linear regression of the measured analyte/internal standard ratios vs. the amount of standard added, and the slope of the standard additions curve can be used to predict concentration values in similar matrix controls run concurrently during experiments.

SRM 2703 is a marine sediment collected at the mouth of the Baltimore Harbor, primarily intended for use in evaluating analytical methods for the direct determination of selected elements in solid samples of marine or fresh water sediment and similar matrices. SRM 2703 was specifically developed for microsampling techniques. Direct and slurry sampling, as well as dissolution techniques using typically milligram size samples can employ this SRM to benchmark their procedures. A thorough homogeneity assessment of the material was conducted using instrumental neutron activation analysis (INAA), and laser ablation inductively coupled mass spectrometry (LA-ICPMS), micro-beam X-ray fluorescence (μ XRF) and micro proton beam induced X-ray emission (μ PIXE, see Fig. 1) techniques complemented the INAA assay with homogeneity results for significantly smaller sample sizes. Additional measurements with several solid sampling procedures and dissolution procedures were provided by collaborating laboratories with the following techniques: atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICPAES) and ICPMS, and X-ray fluorescence (XRF). All assays were designed to establish communicability of values between those conventionally certified in SRM 2702, representing the parent material, and the SRM 2703 measured at small sample sizes. These measurements confirmed that the composition of the material had not changed in processing and that the measured values in SRM 2702 can be utilized for value assignment of SRM 2703.

Impact: Study of complex marine environmental systems requires wide ranging chemical measurements performed in many laboratories over long periods of time. Meaningful knowledge can only be extracted from such studies if the quality of these chemical measurements is high. The program examples highlighted here show that CSTL's collaborative measurement capabilities lead to innovative reference materials and measurement technologies aimed at improving and assuring chemical measurement quality.

Future Plans: Work will be dedicated to developing analytical methods and measuring environmental compounds of emerging concern in current issue and future marine tissue and sediment SRMs, including, perfluorooctanesulfonate (PFOS), perfluorooctanoic acid (PFOA), other types of brominated flame retardants, classes of compounds like phthalate esters and their metabolites, which are prevalent in plasticizers, and representative compounds comprising the range of pharmaceutical and personal care products. We will also measure methylmercury and organotin compounds in marine sediment and tissue SRMs.

Figure 1. Micro-PIXE images of the SRM 2703 bottle #1291 sample by I. Bogdanović Radović, Ž. Pastuović, and M. Jakšić, Rudjer Boskovic Institute, Zagreb, Croatia. Samples were analyzed using a 3 MeV focused proton beam. Micro-PIXE images were collected for all elements found in the PIXE spectrum. The total scan size of $750\ \mu\text{m} \times 750\ \mu\text{m}$ was divided in 8 equal regions, each being quantitatively evaluated. Only sulfur showed heterogeneity directly observable from the 2D maps in all SRM 2703 samples. Detailed analysis of the spectra from each of the 8 smaller regions revealed homogeneity of SRM 2703 for Al, Si, Cl, K, Ti, Mn, and Fe at the probed $1\ \mu\text{g}$ to $7\ \mu\text{g}$ sample size



Title: Development of Reference Methods and SRM's for Toxic Species in Body Fluids

Authors: S.J. Christopher, W.C. Davis, C.E. Bryan, and R.D. Day

Abstract: We have applied new and accurate analytical methods for the determination of toxic trace elements in whole blood using collision cell inductively coupled plasma mass spectrometry. New analytical instrumentation and a new methodology for detection of organometallic species in blood was developed and applied to the certification of methyl mercury (Me-Hg) at ultra-trace levels in SRM 966 Toxic Elements in Bovine Blood.

Purpose: The role of metals in health and medicine is all encompassing, with current research focusing on toxicology, drug delivery, degenerative diseases, imaging diagnostics and development of various classes of metal-based drugs possessing chemotherapeutic, anti-inflammatory, anti-diabetic or anti-microbial properties. Genomics and proteomics research will eventually lead to a better understanding of the role of metalloproteins in disease-proteins that may eventually serve as disease treatment targets. Fundamentally, all of this research is underpinned by the quality of analytical measurements in clinical samples like blood and urine. To address this we are developing accurate and sensitive analytical methods that can measure interference prone elements in a timely manner and producing clinical reference materials, which are closely aligned with industry, needs.

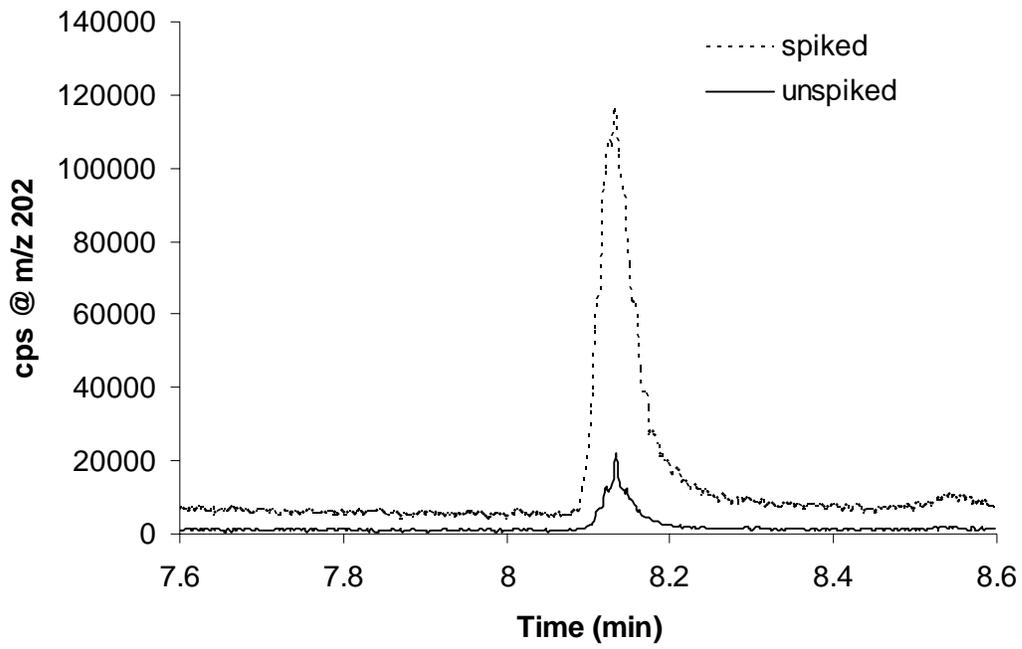
Major Accomplishments: One example involves the development of a new method for the determination of interference-prone trace elements (As, Cd, Co, Cr, Cu, Mo, Ni, Pb, Rb, Se, Sr, V, Zn) in dolphin whole blood using collision cell inductively coupled plasma mass spectrometry (ICPMS). The method is based on gravimetric solution handling, and an internal standard ratio-based approach to quantification using the method of standard additions. A mixing scheme that facilitated preparing and running the samples in a "dilute and shoot" manner was developed to avoid a time consuming, high temperature and pressure sample decomposition. All clinical samples were prepared in a diluent consisting of several high purity chemicals: 1% 1-butanol + 1% Ammonium Hydroxide + 0.01% EDTA + 1% Triton X-100 + 1% nitric acid in balance high purity water (all by mass). The combined effects of this chemical suite serve to reduce the viscosity of the nebulized samples and ameliorate the differential ionization effects induced by carbon in individual analytical samples: 1-Butanol acts to reduce matrix effects by elevating the carbon in all matrices so that variable, sample-to-sample ionization effects are minimized, especially for As and Se. Ammonium hydroxide lyses red blood cells, EDTA prevents the loss of metals by precipitation or absorption, and Triton X-100 prevents blockage of the ICPMS nebulizer and torch injector tube. The calibration scheme was streamlined to avoid the numerous sample splits typically encountered in a standard additions experiment by using the slope of a blood standard additions curve to predict the concentration in unknown blood samples successfully, with any subtle matrix effects being compensated by the internal standards. A collision cell gas was used effectively to open isotopic channels for numerous interference prone elements including As and Se, by breaking apart background species generated in the plasma.

Another example involves the certification of methyl-mercury at low levels in SRM 966 Toxic Elements in Bovine Blood. This project involved development of new instrumentation and highly sensitive methods to address a critical problem for the CDC, which needs to benchmark

Me-Hg measurements in clinical samples for various public health exposure efforts. A gas chromatograph (GC) was successfully coupled to an ICPMS via constructing a home built heated transfer interface, and solid phase microextraction (SPME) headspace sampling of microwave extracted samples was implemented to acquire the needed sensitivity to address this analytically challenging problem. Non-ideal extraction conditions can yield poor recovery or chemical transformation so several optimization studies were undertaken to gauge species liberation, degradation, and transformation during sample preparation and extraction, which are all circumstances presenting an obstacle for accurate quantification of any chemical species. Both methylmercury and inorganic mercury were monitored in SRM 966 and quantified during the method testing phase and the sum of the methyl and inorganic mercury species were in excellent agreement with certified values for total Hg. The method of standard additions provided the means for Me-Hg quantification. Detection limits for Me-Hg using the SPME-GC-ICPMS method were 4.2 pg/g, while identical headspace sampling and chromatographic conditions allowed for separation and detection of organo-tin species at the fg/g level.

Impact: The program examples highlighted here show that CSTL's measurement capabilities are well positioned to benchmark accurately the next generation of measurements for metals in clinical samples, regardless of chemical form or whether the samples are derived from human or non-human sources. The new calibration approaches developed for dolphin blood health assessments proved to be robust and applicable to similarly matched matrices of human and animal origin, suggesting that current-issue clinical SRMs can be used to benchmark whole blood measurements from any similar matrix. A similar approach could be developed for urine measurements.

Future Plans: Work will be dedicated to applying GC-ICPMS to solve ultra-trace speciation problems involving Hg and Sn chemical species and isotope dilution methods for species quantification will be explored. Liquid chromatography-collision cell-ICPMS methods will be used to certify organoarsenic compounds in a urine SRM. The method of double isotope dilution will be applied to a pharmacokinetics study (funded by S.C. Sea Grant through the College of Charleston). Staff from the FDA will assist with modeling the dynamics of orally administered MeHg in the blood of diamondback terrapins. Enhanced performance of the pharmacokinetics models will result from high accuracy measurements and the ability to quantify dose and background levels of blood Hg among replicates independently, eliminating model noise arising from individual variability.



Chromatogram for the spiked and unspiked extracted methylmercury in SRM 966 Toxic Elements in Bovine Blood.

Title: Fluorescence Standards Suite for Spectral Correction of Fluorometers

Authors: P.C. DeRose, D.H. Blackburn, and G.W. Kramer

Vision: To prepare, characterize and certify a set of fluorescent glass SRMs that will enable the shape and intensity of spectral emission to be calibrated on fluorometers in the near UV and visible regions of the spectrum.

Purpose: Luminescence measurements have become the detection methods of choice for new clinical and biochemical assays, and related high-throughput techniques, due to their extraordinary selectivity and sensitivity. These new analytical methods are becoming increasingly more quantitative, requiring standards to calibrate the luminescence measuring instruments that they utilize and aid in the validation of the methods. Ideally, users would like to employ the same organic dye probes used for analyte detection as standards for fluorescence intensity and spectral correction. Unfortunately, organic dyes photobleach quickly, do not have long shelf lives in solution, have environment dependent fluorescence and are expensive to produce at high purity.

Scientific Research: After studying the characteristics of the different types of fluorescent materials, we found metal oxide doped (MOD) glasses to be the best choice for use as fluorescence standards for spectral correction and intensity. These glasses are photostable, robust, relatively inexpensive and can be made to suit most detection formats. Our research-grade fluorometer is being used to characterize the spectral characteristics of a series of MOD glasses to determine which will have the correct spectral shape and intensity for appropriate standards. A UV light chamber and a laser are also being used to irradiate the candidate glasses over a timed period. The fluorescence intensity of each glass is measured before and after irradiation to determine its rate of photodegradation.

Major Accomplishments: Spectral emissivity standard candidates have been identified with UV/violet, blue, green and yellow/orange fluorescence, corresponding to CeO_2 , CuO , U_3O_8 and MnO_2 dopants, respectively. At this point, the U_3O_8 and MnO_2 glasses have been most thoroughly studied. They are both highly resistant to photodegradation and possess all of the desired characteristics for spectral emissivity standards. They are presently in the final stage of production and are expected to be certified within the next year. These standards are being made in the form of a standard sized cuvette with three polished long faces for 90-degree detection and one frosted long face for front-face detection. The CeO_2 and CuO glasses have both been shown to have desirable spectral characteristics and are presently in the prototype stage of development.

Context and Impact: Once certified, the U_3O_8 and MnO_2 glasses can be used in combination with SRM 936a quinine sulfate dihydrate; a blue spectral emissivity standard that is the only standard presently sold for spectral correction of fluorometers; to cover the visible region from 400 nm to 700 nm (see Figure). The CeO_2 glass will be used to cover the near UV/violet from 350 nm to 450 nm. We hope to replace SRM 936a with the CuO glass, as the present standard is an organic dye that has many of the stability problems associated with organic fluorophores. Since all of these glasses are highly resistant to photodegradation, they can also be used as day-to-day intensity standards for instrument qualification.

Future Plans: We have not yet found a suitable MOD glass for a red emission standard that would cover the region from approximately 680 nm to 800 nm, and beyond. We are presently working with a nanocrystal manufacturer to help us to identify such a nanocrystal-based material. We are also working with the same manufacturer and a glass manufacturer to develop fluorescent thin films to be used as intensity standards for microarray readers.

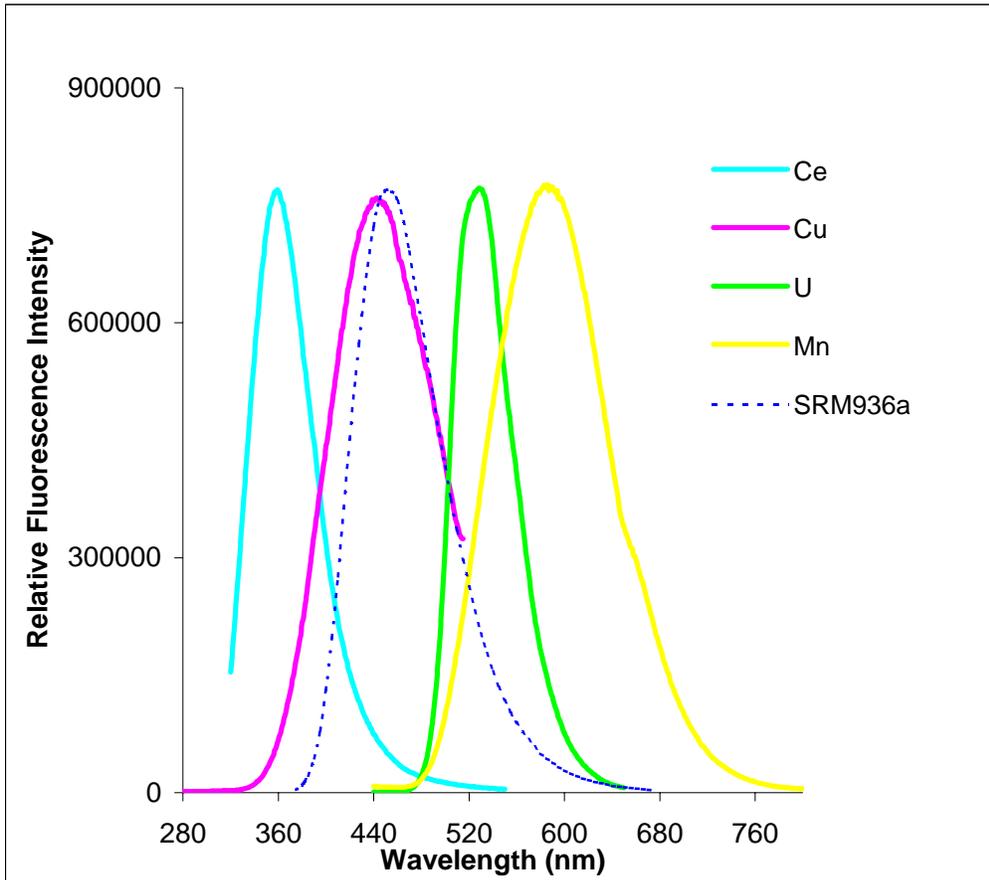


Figure: Spectral emission profiles of each of the four glasses in the suite of standards, compared with that of SRM 936a

Title: Protein Quantitation

Authors: N. Dodder and D.M. Bunk

Abstract: To meet the need for new reference methods and materials to support clinical protein measurement, new approaches to quantitative protein mass spectrometry have been investigated. Initial studies focused on the measurement of intact proteins by MALDI mass spectrometry. Limitations of the robustness of this approach required a shift to quantitation of peptides generated from the proteolytic digestion of the analyte protein.

Purpose: Proteins are measured in the clinical setting to assist in the diagnosis of a variety of diseases including heart attacks and cancer. Currently, the majority of these tests are performed using immunoassays. Although sensitive, fast, and relatively inexpensive, the specificity, accuracy, and precision of immunoassays can be a problem and necessitates a need for method validation through protein-based reference materials and methods.

The Analytical Chemistry Division is currently developing mass spectrometric (MS) methods for protein quantitation, direct methods without the potential for bias that can be observed in an indirect method, such as immunoassay. Two methods currently under development are based on liquid chromatography/tandem mass spectrometry (LC/MS/MS) and matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry.

Major Accomplishments: Initial studies explored the quantitative capabilities of MALDI-TOF mass spectrometry for the small protein human insulin. Investigations were made in sampling issues, such as the creation of homogenous sample spots, and in the automated use of the mass spectrometer, programmed to search sample spots to produce uniform sampling. Based on this research a quantitative MALDI-TOF method was developed for human insulin, using pig insulin as an internal standard. The linearity, dynamic range and error of this technique were acceptable. However, further studies, using larger proteins revealed limitations of this approach, particularly the limitations in finding internal standards that would be applicable for the measurement of large proteins.

An alternative approach is currently under investigation, using some of the methodologies developed in the growing field of proteomics. Instead of measuring intact proteins, the peptides produced from the proteolytic digests of intact proteins are measured. Using this proteomics-based approach, we are developing methods utilizing both MALDI-MS and LC/MS/MS for quantitative protein measurements.

Because proteolytic digests, particularly trypsin digests, are at the core of our proteomics-based approach, we have devoted a large effort to understanding the practical nature of trypsin through fundamental studies of how experimental factors affect trypsin digests of analyte proteins. Guest researchers from the national metrology institutes of the United Kingdom (LGC) and Germany (PTB) have collaborated on this research effort. Successful quantitative measurements have been made on serum C-reactive protein using this approach.

Impact: As the field of proteomics matures, more protein biomarkers will be discovered and used for clinical diagnoses. New immunoassays for protein biomarkers will require validation through more metrologically-sound methods, particularly methods based on mass spectrometry. Furthermore, accurate and precise protein quantification may help answer fundamental biological questions regarding protein expression and its relation to the genome and environment. Additionally, the techniques developed for protein quantification can also be used in the areas of drug discovery and biotechnology.

Future Plans: We are developing a quantitative method for serum transferrin using a combination of ion-exchange chromatography, tryptic digestion, and MALDI-MS. A quantitative method for serum transferrin will be an important component in the on-going research at NIST to develop methods for serum iron speciation because transferrin is one of the principle serum iron transport proteins.

We will also continue the development of a proteomics-based reference method for human C-reactive protein, a clinical biomarker for mild to severe inflammation as well as for cardiovascular disease. After further refinement of the C-reactive protein reference method developed thus far, a validation study utilizing patient serum samples is planned FY05.

Title: Gas Mixtures Standards for the Automotive Industry: The NTRM Prime Program

Author: W.D. Dorko

Abstract: The gas mixture NIST Traceable Reference Material (NTRM) program is a procedure for producing and certifying traceable calibration gas standards. Specialty Gas Companies produce, with concurrence from NIST, groups of identical gas mixtures in cylinders, minimum of 10, analyze them and send the data to NIST which then selects 10% of the group to be sent to NIST for analysis. The group of cylinders is assigned one concentration value and uncertainty by using the data generated by NIST for concentration determination and the NIST and Producer data to determine the uncertainty. These NTRM mixtures are under the purview of the Producer, or Specialty Gas Company, to be sold as traceable standards or to be used at the Producer facilities to analyze other mixtures that are then sold as traceable mixtures. There have been requests, specifically from the automotive industry, for NIST to provide NIST analyzed mixtures with lower uncertainties than NTRMs. A method of accomplishing this is for NIST to analyze each NTRM mixture of a specific group and provide a concentration and uncertainty for each specific cylinder; these would be known as NTRM Prime (NTRM).

Purpose: The Gas Mixture NTRM Program provides a mechanism to produce needed calibration gas standards on demand. Specialty Gas companies are in touch with end users who require traceable mixtures and inform the gas companies of their needs. If NIST has the infrastructure to certify the required standards then the Gas companies can produce the NTRM batch, have it certified by NIST and supply mixtures to meet the demand. Some end users require, one group being the auto industry, require pedigreed calibration standards with low uncertainty. The pedigree is required because of the need to comply with requirement in the Code of Federal Regulations (CFR), which specify that SRMs or NTRMs are to be used for instrument calibration for the measurement of vehicle exhaust emissions. Using an NTRM serves two purposes; (1) it is a calibration gas mixture specified in CFR and (2) it has a lower uncertainty than an NTRM since each mixture has individual concentration value and uncertainty, there are no batch statistics involved.

Major Accomplishments: NIST has met with representatives of the automotive industry requesting NTRM's and has defined a program to meet their needs. Members of the automotive industry will decide what gas mixtures are needed and then contact a NTRM producer to manufacture the total number of mixtures. NIST has already discussed this with the NTRM producers all of whom have stated that they will certainly cooperate with the needs of any industry or organization requiring NTRM's. After the NTRM mixtures are produced all of them will be sent to NIST for analysis after which a certificate will be issued for each individual cylinder and the cylinder sent to the company or organization requesting it.

Impact: A large number of gas measurements mandated by CFR require calibration standards traceable to NIST with low total uncertainty. Standards supplied by the SRM program are the most suitable but are not always available and are NIST resource intensive to provide. The normal NTRM program provides standards that by their nature are higher in uncertainty than the SRMs. The NTRM program provides a mechanism to provide required calibration gas standards in a timely fashion with NIST traceability and a low total uncertainty.

Future Plans: To support the need for NTRM material, NIST plans to increase the number and concentration ranges of gas species for which it maintains primary standards, which are the foundation of the program. A meeting is to be held at 2005 Pittsburgh Conference in Orlando, FL to which all interested parties are invited, NTRM Producers, regulating bodies and end users, to describe the program and to obtain and evaluate comments and suggestions.

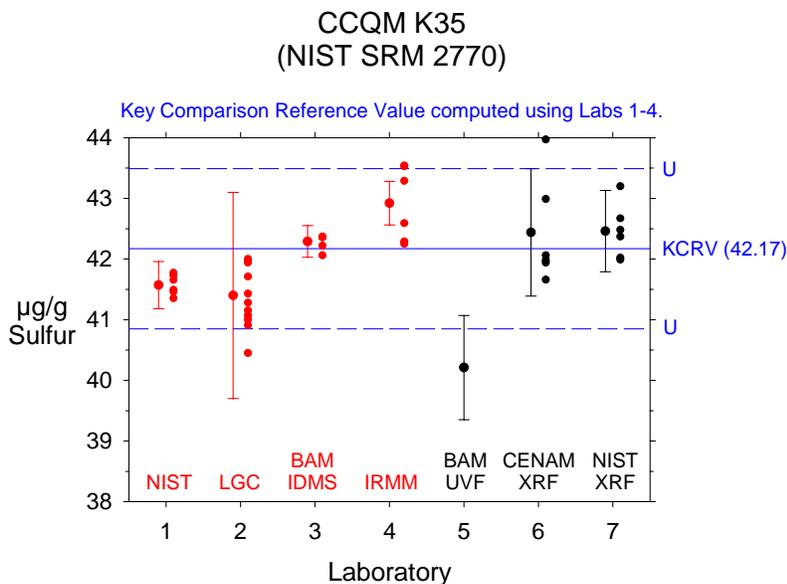
Title: Sulfur in Diesel Fuel by Isotope Dilution Mass Spectrometry: Results of the CCQM K35 Key Comparison

Authors: W.R. Kelly, R.D. Vocke, J.L. Mann, and G.C. Turk

Abstract: NIST planned and conducted a CCQM Key Comparison (K35) and a concurrent pilot study (P26.1) on sulfur in diesel fuel and kerosene. Participants in the studies measured sulfur concentrations in three candidate SRMs covering a wide range of sulfur concentrations. There was generally good agreement among the NMIs for both the Key Comparison nominal 40 µg/g diesel fuel and the nominal 8 µg/g kerosene used in the Pilot Study. Participants also measured a nominal 4000 µg/g check sample included in the studies to distinguish measurement issues associated with sulfur blanks at the lower concentrations from other potential measurement problems.

Purpose: There is a regulatory requirement in North America and Europe that the petroleum industry and regulatory environmental agencies be able to measure accurately sulfur in diesel fuel between 5 and 50 µg/g with a total combined uncertainty of less than 1 µg/g. Both Europe and the United States will be moving to 50 µg/g and below fuels in the next year. Lower sulfur in fuels will make possible extremely efficient and long-lived after-treatment technologies based on noble metal catalyst. This Key Comparison and associated Pilot Study was undertaken to assess and document the capability of National Metrology Institutes (NMIs) to perform accurate determinations of sulfur in diesel fuel at and below future regulatory limits.

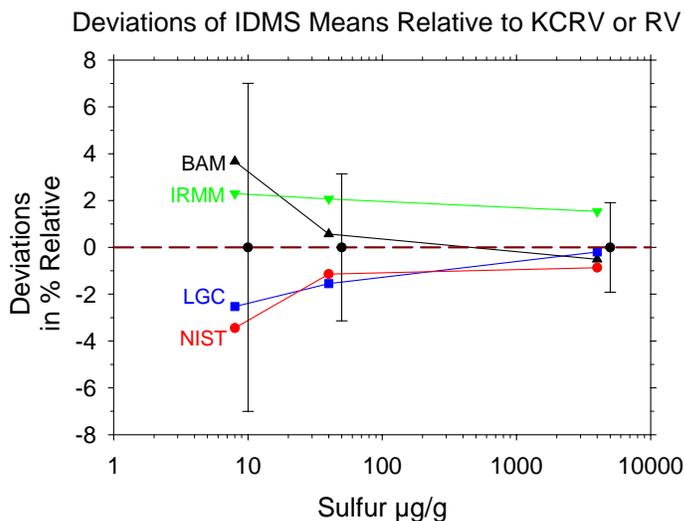
Major Accomplishments: Results of CCQM K35 and P26.1 were reported to the Inorganic Working Group of the CCQM at their meeting in April 2004. The Key Comparison data are shown graphically in figure below.



The four laboratories shown in red were registered Key Comparison participants using isotope dilution mass spectrometry either with thermal ionization instruments (NIST and BAM) or ICP-MS (LGC and IRMM). The other values were submitted as Pilot Study results. Shown in the figure are the means and total expanded uncertainties expressed as 95 % confidence intervals and the individual determinations (solid circles to the right of the estimates) submitted by the NMIs. The Key Comparison

Reference Value (KCRV) was computed using the Mixture Model Median method developed by D. Duewer of NIST. The agreement among the four NMIs is considered good since the computed standard deviation is less than 1 µg/g.

The results on all three samples are shown in the next figure as deviations from the reference values in relative percent. The reference values and total uncertainty are indicated by the points and error bars that are centered on the zero reference line. The reference values have been arbitrarily shifted to higher values for clarity of presentation. The greater spread in the data at the lower concentrations is a consequence of the greater influence of the blank.



Impact: The International Council on Clean Transportation states the following:

Worldwide, many jurisdictions have recognized the public health and environmental costs of allowing motor vehicle fuels to contain High levels of sulfur. The European Union, United States, and Japan have led the way in sulfur reduction, and will reach near-zero sulfur levels later in this decade.

CCQM K35 Key Comparison has demonstrated the capability of 4 NMIs to perform sulfur measurements in diesel fuel at a nominal 40 $\mu\text{g/g}$ concentration. Today the regulated upper sulfur limit in US on-road diesel fuel is 500 $\mu\text{g/g}$, but this limit will drop to 15 $\mu\text{g/g}$ in year 2006 and perhaps to near-zero ($< 5 \mu\text{g/g}$) in the next decade. Japan and Europe will adopt a 50 $\mu\text{g/g}$ limit in years 2004 and 2005. Europe will phase in a 10 $\mu\text{g/g}$ limit between 2005 and 2009 and Japan will adopt the same limit in year 2007. The incremental measurement challenges increase non-linearly as sulfur concentrations decrease from 500 to near zero levels. The regulatory agencies and the petroleum industry look to the national metrology laboratories to produce calibration standards with certified concentrations and uncertainties that will ensure a smooth and cost effective transition to low sulfur diesel fuel. CCQM K35 and P26.1 address present and near future measurement needs at the NMI level.

Future Plans: At the present time there are no plans to conduct a Key Comparison at the lower level, but this may change as sub-10 $\mu\text{g/g}$ capabilities will be needed by Europe and Japan starting in 2005 and that must be phased in completely by 2009.

Title: Standards for Fluorescence Microarray Analyses

Authors: G.W. Kramer, A.G. Gaigalas (831), and P.C. DeRose

Abstract: A working group representing all of the U.S. manufacturers of microarray scanners has asked NIST to develop standards for the quantitation of fluorescent microarray analyses. Specifications for a series of physical artifacts have been developed during several NIST workshops and monthly teleconferences. Work continues to find suitable fluorescent materials, to identify appropriate fabrication methods, and to create instrumentation for certifying the fluorescence of such standards.

Purpose: DNA microarrays, also known as DNA or gene chips, have become important tools for gene expression analyses and are poised to revolutionize clinical diagnostics and enable personalized medical care—where treatment can be individually tailored to a specific person through genetics-based diagnoses. The related protein microarrays show great promise for pharmaceutical drug discovery research as well as clinical diagnostic tools. For microarrays, single-stranded genetic or protein material (probes) is bound in an array to a surface the size of a standard microscope slide permitting tens of thousands of molecular reactions to be tracked in parallel when an analyte solution is washed over the array. To accommodate this many sites in such a small area, the individual spots of genetic material or protein must be very small, commonly on the order of 10 to 100 micrometers in diameter. Target DNA or protein in the analyte solution are labeled with a fluorescent dye and allowed to interact with the probes bound on the surface of the array. A favorable interaction leads to fluorescence signal from the appropriate spots. A device called an array scanner or reader detects which spots in the array fluoresce and how much.

Today, there is no consensus method for microarray assays; there are multiple technologies for fabricating microarrays ranging from nano-pipetting to pin-printing to photolithography, and there are several types of scanners. The method variations and uncertainties are so great currently that the biological information obtained from microarray assays is often a function of the method and equipment used, and comparisons of results generated by differing technologies is not possible. If artifact standards for microarray assays can be created and certified, assay quantitation can be improved, results will be intercomparable, and the measurements can be made traceable ultimately back to the SI. The purpose of this project is to develop such standards in collaboration with the manufacturers of microarray readers.

Major Accomplishments: This project began with a request from the scanner industry for NIST to develop standards for this field. A technical workshop on fluorescence standards for microarray assays co-sponsored by Agilent Technologies and NIST's Biotechnology and Analytical Chemistry Divisions was held in late 2002 and attended by representatives from the major array reader manufacturers. The purpose of the workshop was to develop technical specifications for fluorescence intensity, uniformity, and detection limit standards for the calibrating and validating microarray readers. Several parameters such as the excitation and emission wavelengths of the fluorescent tags seemed to be common across the industry; however, there were diverging views on other specifications such as spot size and the form factor of the array. Following the initial technical workshop, the participants worked out many issues concerning the types of standards, intensity levels, form factors, etc. in teleconferences. It was

agreed that unpatterned artifacts for each of two colors (similar to the dyes Cy3 and Cy5 that are commonly used now) would be developed. One set of artifacts with fluorescence intensities in the mid-to-high range would serve as uniformity/homogeneity standards and to measure signal-to-noise ratios for bright features, while a second set of materials would be developed with low fluorescence levels to serve as detection limit standards and to measure signal-to-noise ratios for dim features. A second technical workshop was held at NIST in the spring of 2003 to come to agreement on many of the physical factors and to begin the search for appropriate fluorescent materials and application processes to fabricate the standards.

Through quarterly teleconferences, the group continues to refine parameters and to search for suitable materials. Recently, NIST began collaborating with private sector firms in the search for candidate materials. We have begun to examine several glass technologies with the help of R&D personnel from Schott Glass, Inc. (Duryea, PA), and we have recently entered into a CRADA with Evident Technologies, Inc. (Troy, NY) to study the use of nanocrystal composites. The working group has also developed a set of procedures and specifications for testing the suitability of candidate materials. The primary difficulty is finding materials with acceptable spectral characteristics that can withstand the high laser light intensity without photodegrading.

Impact: The development of artifact standards for quantitative fluorescence assays on microarrays is a first step in multi-requirement process for standardizing microarray assays. However, it is essential that this process go forward to allow the use of microarrays in clinical settings instead of just research venues. Microarrays have a very promising future, not only in the clinical/biotech/pharmaceutical applications that are being developed now, but also for general chemical analyses. The impact of microarray technologies on the future of analytical chemistry could be huge.

Future Plans: We are currently searching for suitable materials to develop into the artifacts and are building the instrumentation necessary to make the certification and stability measurements. Once these issues are behind us, and we have viable materials, we can concentrate on developing standard protocols for their use. We have discussed these issues with the appropriate ASTM and NCCLS committees. It is likely that we will work jointly with both organizations to develop these documents.

Title: NIST Aids NOAA with Bottlenose Dolphin Health Assessments

Authors: J.R. Kucklick, S.J. Christopher, P.R. Becker, R.S. Pugh, M. Ellisor, J.M. Keller, C.E. Bryan, and J.E. Yordy

Abstract: NIST aids the National Oceanographic and Atmospheric Administration (NOAA) in their long-term bottlenose dolphin health assessments through developing and implementing a protocol for samples collected for persistent organic pollutant and trace element analysis. NIST also provides measurements of persistent organic pollutants and trace elements in dolphin samples, ensuring continuity for the project and high accuracy results. To date, the NIST protocol has been used in 11 dolphin health assessments and will be used in seven assessments planned for fiscal year 2005. The data and expertise provided by NIST are vital in interpreting toxicological and health-related information collected on the animals.

Purpose: Since 2000 NOAA has studied the health of bottlenose dolphins by collecting data and samples from animals captured and then released at locations along the US Atlantic and Gulf Coasts. Bottlenose dolphins accumulate persistent organic pollutants, such as polychlorinated biphenyls (PCBs) and brominated flame retardants to some of the highest observed levels in wildlife, hence they are at risk from the toxic effects of these compounds. For instance, in Sarasota Bay, where sampling occurs twice per year, there is nearly complete mortality of dolphin calves born to first-time mothers. It is hypothesized that persistent organic pollutants offloaded from the mother via milk play a major role in this reproductive failure. In 2002 NOAA asked NIST to provide technical assistance for the project by (1) designing a protocol for collecting, handling, and storing blood, blubber, and skin to be used for organic contaminant and/or trace element analyses (2) permanently archiving blood and blubber samples for future study, (3) analyzing persistent organic pollutants and trace elements in samples collected during dolphin health assessments, and (4) providing technical assistance in the field during sample collection. The collaboration between NIST and NOAA on this project emerged from the recognition by NOAA that NIST is a leader in the field of environmental specimen banking, quality assurance, and environmental analysis. Data supplied by NIST will provide basic understanding of contaminant burdens in bottlenose dolphins and provide exposure data for toxicological studies performed on the animals.

Major Accomplishments: NIST developed a detailed protocol for collecting samples from bottlenose dolphins during health assessments. The protocol covers the collection of skin, plasma, whole blood, and blubber samples to be analyzed for trace elements (including mercury), persistent organic pollutants, and perfluorinated compounds, such as perfluorooctane sulfonate (PFOS). Since 2002 NIST personnel have successfully applied the collection protocol during 11 health assessments from four locations resulting in samples from over 150 dolphins. Seven additional health assessments are scheduled for fiscal year 2005. Subsamples of plasma and blubber are being archived in the Marine Environmental Specimen Bank. Blubber and blood samples are being analyzed for over 100 individual persistent organic pollutants including brominated flame retardants. Blubber samples from 22 mother-calf pairs were analyzed revealing that the majority of the persistent organic pollutant load is passed to the calf. Milk samples collected from the mother dolphins and blood from mother and calf dolphins are currently being examined to better understand contaminant transfer from milk, especially for the

brominated flame retardant compounds. Trace elements including mercury were determined in blood samples from dolphins sampled in Sarasota Bay, Florida and coastal New Jersey. A technique has also been developed to measure mercury in skin biopsy samples. This may allow the use of skin collected from remote dart biopsies to be used for the assessment of mercury.

Impact: A number of different research groups are involved in bottlenose dolphin health assessments. The protocol established by NIST provides for a common procedure for all projects collecting samples for persistent organic pollutant or trace element analysis. Samples collected by NIST for NOAA are archived in the Marine Environmental Specimen Bank—a facility constructed specifically for the storage of environmental samples in a controlled, non-contaminating environment. Analytical work completed for NOAA ties measurements to a national metrology laboratory with a history of providing high-quality measurement data to NOAA. Since the dolphin health assessments are long-term projects, NIST’s involvement will provide continuity in the analytical data generated on the project.

Future Plans: NIST will continue to work with NOAA as they expand their dolphin health assessment project to other areas along the coastal US. Work is underway to make use of high throughput techniques for the analysis for persistent organic pollutants in blood and blubber. The target list will be expanded to include other types of compounds of emerging concern, such as additional flame retardants and perfluorinated compounds. A control material for dolphin blood will be developed and value assigned to provide a benchmark for blood analysis.

Title: Interlaboratory Comparison Exercises for Organic Contaminants and Trace Elements in Marine Mammal Tissues

Authors: J.R. Kucklick, S.J. Christopher, P.R. Becker, R.S. Pugh, M.B. Ellisor, S.S. Vander Pol, E.A. Mackey, R.O. Spatz, B.J. Porter, M.M. Schantz, G.C. Turk, and S.A. Wise

Abstract: The National Institute of Standards and Technology (NIST), in support of the National Oceanic and Atmospheric Administration's Marine Mammal Health and Stranding Response Program (NOAA/MMHSRP), conducts annual interlaboratory comparison exercises for the determination of chlorinated pesticides, polychlorinated biphenyl congeners, and trace elements in marine mammal tissues. The exercises provide one mechanism for laboratories to evaluate their measurement quality and comparability for these constituents in marine mammal tissues. In the 2003 exercise, a total of 49 laboratories participated in the organic contaminant and trace element exercises including laboratories from nine countries in addition to the U.S. Participants were requested to analyze a control material and an unknown sample for a variety of constituents. Cumulative results compiled from exercises conducted from 1999 to 2003 indicate that laboratory agreement with target exercise values improves with the number of years a laboratory participates in the exercise.

Purpose: Laboratories measuring contaminants in the marine environment must assess the accuracy and precision of their measurements. Quality control of measurements made on marine environmental samples is vital to the accurate assessment of marine pollution and its effects on wildlife and human health. Representative control materials needed to benchmark analytical methods and measurements are often limited or not available for many marine matrices of interest (*e.g.*, marine mammal tissues and marine fishes). Consequently, analysis may be undertaken on samples without control materials that are similar in nature to the sample. To help address this need, the National Institute of Standards and Technology's (NIST) Analytical Chemistry Division, with the help of other U.S. Government sponsors, initiated several programs to assess the data quality of laboratories performing chemical measurements on marine-related samples.

NIST helps benchmark and improve the quality of analytical data on the marine environment by conducting interlaboratory comparison exercise programs. The first such program for environmental measurements was initiated in 1987 and funded in part until 2000 by the National Oceanic and Atmospheric Administration's (NOAA) National Status and Trends Marine Monitoring Program (NOAA/NS&T). This program provides mechanisms for assessing the interlaboratory and temporal measurement comparability of polycyclic aromatic hydrocarbons, polychlorinated biphenyl (PCB) congeners, and chlorinated pesticides in bivalve mollusk, sediment, and fish samples. The program includes components for developing improved analytical methods, producing NIST Standard Reference Materials (SRMs) and other control materials, conducting annual interlaboratory comparison exercises, and coordinating workshops to discuss exercise results. This program continues as the NIST Intercomparison Program for Organic Contaminants in the Marine Environment with partial support from fees paid by the participants, and it served as the model for the Interlaboratory Comparison Exercises Program for Organic Contaminants and Trace Elements in Marine Mammal Tissues.

Major Accomplishments: NIST expanded the Interlaboratory Comparison Exercise for Organics in Marine Mammal Tissues in 1999; and the trace element complement to the exercise was formalized in 2000. Since this time the participation has increased dramatically. For example, the number of laboratories participating in the organic exercise increased from 10 in 1999 to 25 in 2003. The exercises are highly international with nine countries involved in the trace element exercise and six in the organic exercise in 2003. Results from the program led to the development of a suite of control materials for trace elements in marine mammal tissues and the production of one Standard Reference Material, SRM 1945 Organics in Whale Blubber. The organics exercise has been successful as a source of data on new constituents in SRM 1945. For example, fatty acids and brominated flame retardants were determined in the unknown and in SRM 1945 by five and six laboratories, respectively. The trace element exercise has led to the development of three trace element control materials from marine mammal livers, a pilot whale liver homogenate in 1991, and beluga whale and pygmy sperm whale liver homogenates in 1997 and 2003, respectively. Independent analytical methods at NIST (inductively coupled plasma mass spectrometry and instrumental neutron activation analysis) produced data to provide a reliable benchmark to assess the performance of participating laboratories. The trace element exercise also used an innovative approach to arrive at a consensus mean and uncertainty data using a maximum likelihood solution model developed by Rukhin and Vangel at NIST.

Impact: Comparability of measurements for organic contaminants has improved for those laboratories with a longer history of participation in the exercises, e.g., those that have participated since 1999 have improved their accuracy by 10% and their precision by 20%. The SRMs and control materials developed through this program and the interlaboratory comparison exercises are mandated for use by researchers in the U.S. who are funded by NOAA to perform measurements of trace elements and organic contaminants in marine mammal tissues.

Future Plans: NIST will continue to administer and coordinate interlaboratory comparison exercises for chemical analysis of marine mammal tissues. Future exercises will ask participants for compounds of emerging interest such as brominated and fluorinated compounds, toxaphene congeners, and organometal contaminants, such as butyl tins and methyl mercury. The next round of interlaboratory comparisons will occur in 2005. Also in 2005, the data compiled from the exercises using SRM 1945 as a control will be used to assist in updating the Certificate of Analysis with values for additional polychlorinated biphenyl congeners, brominated diphenyl ether congeners, toxaphene, and fatty acids.

Title: Determination of Traces of Fissionable Materials Using Delayed Neutron Activation Analysis

Authors: R.M. Lindstrom, E.A. Mackey, and G.P. Lamaze

Abstract: Detection and measurement of small traces of fissionable uranium and plutonium can be done by delayed neutron activation analysis. The method is intrinsically specific to nuclear fission, the sensitivity is excellent, and the procedure is simple, rapid, and readily automated for high throughput.

Purpose: As shown in the figure, an important tool in nuclear forensics is the collection and analysis of “swipe” samples at sites where materials of interest may be, or may have been at one time. Tiny traces of fissionable uranium or plutonium can be left behind whenever these materials are handled or transported. Using neutrons from the NIST research reactor, the delayed neutrons from fission in these traces can be used to detect and quantitate U and Pu in swipe samples with excellent speed, sensitivity, and specificity.



IAEA inspector collecting a swipe sample from process equipment in a nuclear fuel fabrication facility in 1994
(*Anal. Chem.* **74** (2002), 28A)

Major accomplishments:

Delayed Neutron Activation Analysis is being established at NIST for the measurement of small quantities of fissionable nuclides such as ^{235}U and ^{239}Pu . After a brief neutron irradiation, the sample is placed quickly into a neutron detector array and the neutron emission rate measured and compared with that of a standard. The method is well-tested, rapid, specific, matrix independent, nondestructive, and sensitive. The system being built at NIST is calculated to have a detection limit for either of these species about 10 picograms, based on a straightforward extrapolation from published practice. The analysis time is less than 2 minutes per sample.

Preliminary tests have been performed using uranium standards prepared by depositing solutions containing known amounts of uranium onto filter papers, irradiating, and counting on a detection system with one ^3He detector surrounded by hydrogenous moderator. Two shielding configurations were tested, one using water as the moderator and the other using polyethylene. Based on these results and a review of the literature, a final detection system was designed. This consists of ten neutron detectors in a 30 x 30 cm cylindrical moderator of polyethylene, lined with 2 cm of lead to absorb gamma radiation. The design of the manual system incorporates the existing pneumatic rabbit assembly for irradiation control. Incorporated in its design is the ability to unload a sample from the receiver after irradiation with compressed air. This feature will be used to move the sample rapidly to the neutron detector through a polyethylene flight tube.

Impact: On completion and verification, the Nation will have a readily accessible, rapid means of measuring traces of fissionable U and Pu in samples of forensic interest. In addition, the specificity and sensitivity of this method of analysis will be put to use in certifying trace uranium in Standard Reference Materials.

Future plans: To improve sample throughput, the transfer system can be readily automated with computer control in the coming year. It has been demonstrated elsewhere that ^{233}U , ^{235}U , and ^{239}Pu can be distinguished by the relative yields of delayed neutron precursors with different half-lives, and also of several fission products. We plan to add a gamma-ray detector into the neutron moderator to exploit this signature.

Title: Molecular Simulation of Alkylsilane Stationary Phases in Liquid Chromatography

Authors: K. Lipka and L.C. Sander

Abstract: Covalently modified surfaces represent a unique state of matter that is not well described by liquid or solid phase models. The chemical bond in tethered alkanes imparts order to the surface in the form of anisotropic properties that are evident in chromatographic and spectroscopic studies. An understanding of the structure, conformation, and organization of alkyl modified surfaces is requisite to the design of improved materials and the optimal utilization of existing materials. Computational simulations offer insights into the structure of covalently modified surfaces that may not be apparent through empirical observation.

Purpose: Simulations offer several advantages over physical experimentation. Models provide a means to visualize the important features of a surface at the molecular level in a way that is not possible by physical experimentation. Models can be precisely described in terms of bonding densities, chain placements, temperature, etc. The influence of changes in specific aspects of the model can be tested directly without altering other parameters. For example, alkyl chain length effects can be studied by extension of existing chains without changing their placement on the silica surface. Simulations can provide insights into the conformation and dynamic aspects of physical materials; however, the utility of the data depends on the suitability and accuracy of the computational approach employed.

Molecular dynamics (MD) is a time-dependent simulation of molecular motion. Initially, velocities are assigned to atoms (often based on Boltzmann distribution) such that the net momentum is zero (i.e., no flow). After a finite time interval, the energy of the system is calculated. Forces on atoms are calculated, new velocities are determined, and the process is repeated. Coordinates are saved periodically, and analysis of the coordinates over time provide information on molecular motion. Monte Carlo approaches utilize random sampling to choose positions, orientations, etc. of molecules. The energy of each sample is calculated, and the new configuration is accepted or rejected.

Molecular mechanics calculations are typically used to calculate the energy at intermediate steps. Molecular mechanics computations are based on algebraic relations that describe the energy of the molecule(s) in terms of stretching, bending, torsions, electrostatic and van der Waals forces, etc. These terms constitute the force field; any required constants are provided by experiment or ab initio calculation (parameterization).

Major Accomplishments: MD simulation of C₈, C₁₈, and C₃₀ modified silicas have been carried out to study changes in stationary phase architecture that may result from variations in chain length, bonding density, synthetic approach, and temperature. Surfaces were modeled using a quartz substrate with vicinal –OH groups (9.8 μmol/m²), and alkyldimethylsiloxane or alkyldisilanol siloxane groups were linked via siloxane bonds. Surface densities ranged from 1.6 μmol/m² to 5.9 μmol/m² for C₈, C₁₈, and C₃₀ ligands, and some models were constructed of 3 oligomer units to approximate bonding in polymeric phases. Simulations were carried out with the COMPASS force field, over time periods long enough so that the structural features were constant (> 800 ps). Several trends were apparent. The thickness of the phases increased with

increased bonding density as individual chains were more constrained by neighbors. Correspondingly, the *gauche* fraction also decreased with increased density. At the same bonding density, longer chain length phases were more ordered than shorter chain length phases. Finally, increased order was observed at reduced temperature. The snapshots shown in Figure 1 are representative of low energy conformational states for low density monomeric and high density polymeric C₁₈ stationary phases. A high degree of disorder is apparent with models representative of (commercial) monomeric C₁₈ columns. Chain extension and increased *trans* conformations are observed with models of polymeric C₁₈ phases. C₃₀ stationary phase models exhibit significantly increased *trans* conformations, even with lower surface densities (see Figure 2). With the exception of the end methyl group, the distal carbons are primarily *trans*, whereas the proximal carbons have more *gauche* states. It is believed that the *gauche* state is favored in proximal carbons to permit formation and association of *trans* segments at the distal end.

Impact: The models that have resulted from this study clearly indicate the extent of molecular order within alkyl modified surfaces and are consistent with spectroscopic studies (e.g., Raman, ¹³C NMR, and infrared spectroscopy). The results provide a compelling vision of alkyl modified surfaces that will guide future development of materials for chromatography and biotechnology.

Future Plans: An extension of the simulation effort is planned to probe the effect that various solvent environments in contact with the alkyl modified surface may have on alkyl chain conformation.

Title: Microanalytical Technologies to Support NIH Measurement Programs

Authors: L.E. Locascio (839), M. Gaitan (812), D. (812), N. Morgan (NIH/OD/ORS), P. Smith (NIH/OD/ORS), T. Pohida (NIH/CIT), T. Phillips (NIH/OD/ORS), E. Perruccio (NIH/NEI), P. Becerra (NIH/NEI)

Abstract: We recently established collaborations with NIH scientists for the purpose of enhancing medical research using microfluidics and microengineering. There are two distinct ongoing projects with different teams at NIH whose research involves the following:

- *Study of the immune response to Herpes Papilloma Virus (HPV)*
- *Study of vision-related diseases involving defective neuronal differentiation or cell survival*

For the first project, we are developing chip-based microfluidic devices to be used in multi-analyte immunoaffinity capture and detection of proteins related to HPV in cervical secretions. The second project involves patterned immobilization of mammalian retinal cells using microfluidic channels to guide their growth and to facilitate observation of their behavior under different conditions and treatments.

Purpose: The purpose of this collaborative effort is to fabricate microsystems based on MEMS and microfluidics to support and improve the measurement capabilities of medical researchers at the NIH laboratories.

Major Accomplishments:

Project 1. Human Papilloma Virus: This work involves an epidemiological study of the immune response to the Herpes Papilloma Virus (HPV), for which the simultaneous isolation of multiple proteins from microliter samples of cervical secretions is required. For this purpose, we are developing a microfluidic system capable of multi-analyte detection in a single small volume sample by performing multiple sequential heterogeneous immunoassays on chip. Microfluidic

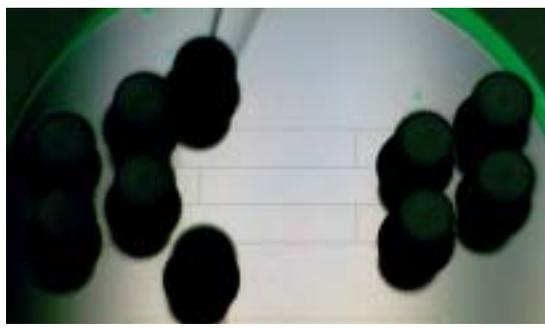


Figure 1a. Microfabricated silicon/glass device with 4 different arms for antibody

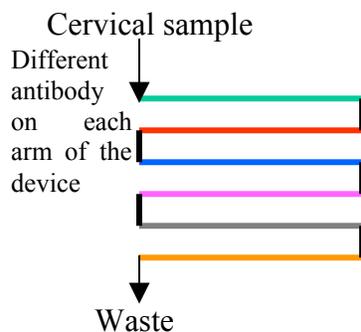


Figure 1b. Schematic diagram of device with 6 arms for measurement of 6 independent analytes.

channels designed in a serpentine pattern are fabricated at NIST using silicon and glass substrates as shown in Figure 1. Different antibodies are then covalently immobilized to each separate arm of the chip so that the system is capable of measuring a number of analytes equal to the number of arms. The channel device architecture has several advantages over existing array

technology including the fact that the device is reusable, and the captured proteins can be extracted after measurement with their biological activity intact. We are designing the system so that the basic methodology can be applied to many different applications relevant to clinical and biological research at both institutions.

Project 2. Vision-related disease: There exist a number of techniques that have been used for patterning cells on surfaces. The strategy often used to adhere single (mammalian) cells involves immobilization of extracellular proteins onto the surface for further adhesion of the cells by interaction with these proteins. In this work, immobilization of rat retinal epithelial cells

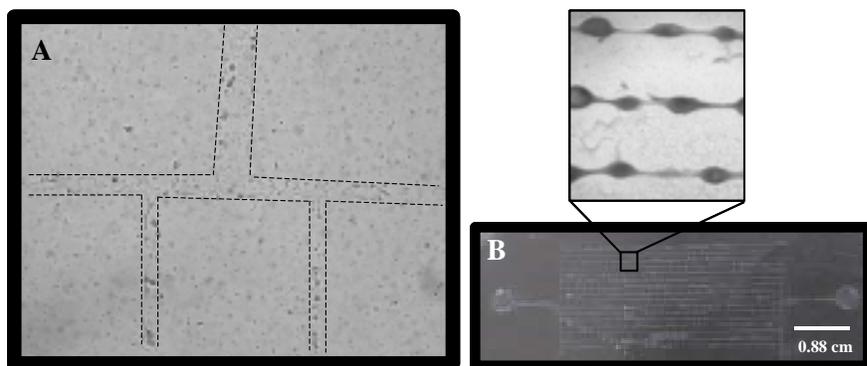


Figure 2. A) Overnight growth (~18hr) of retinal cells on poly(ethyleneimine). Cell growth is observed on the polycation surface areas (within the dotted black lines). B) Two weeks cells' growth on poly(ethyleneimine). Cells are covering all the polycationic area.

is accomplished using micropatterned polyelectrolyte multilayer (PEM) -coated surfaces. PEMs were deposited in discrete lines using a (poly)dimethyl-siloxane (PDMS) microfluidic network on top of a flat PDMS slab. The layers were formed by sequentially flowing the polyions throughout the microfluidic network. Retinal cells, seeded on

flat PEMs/PDMS surfaces adhered and grew on the PEM areas preferentially as shown in Figure 2. Cells were allowed to grow for up to two weeks showing the pattern delineated by the PEMs. We believe this approach could provide a useful and simple tool to pattern single cells or cell networks in specific locations to monitor neural activity of retinal cells as they are exposed to different stimuli.

Impact:

We have found these partnerships with NIH to be very beneficial to our research at NIST. In the last 8 years, we have developed strong microfabrication expertise and capabilities in-house, and have recently been working toward the application of microtechnologies to solving critical problems in environmental monitoring, forensics, nanotechnology, and now, healthcare. We believe that micro- and nanotechnology can be of great use to those involved in clinical research allowing for very elegant small volume sample manipulation and treatment as demonstrated by our successes in the first years of these projects.

Future Plans:

The goal of the first project in the next year is to demonstrate multianalyte capability and determine the sensitivity and detection limits of assays performed in this manner. In the next year we also plan to continue work with retinal cells to examine their viability and growth on patterned PEMs surfaces.

Title: Nanobiotechnology: Nanoscale Chemical Reactions and Separations

Authors: L.E. Locascio, W.N. Vreeland, A. Jahn (812), and M. Gaitan (812)

Abstract: This project focuses on the development of nanoscale structures to facilitate the performance of ultra-small volume chemical reactions and separations. The work is associated with the Single Molecule Manipulation and Measurement competence program whose purpose is to study the behavior of biomolecules one molecule at a time to elucidate the differences that make them uniquely beneficial or detrimental. The nanoscale structures that we are designing are composed most often of phospholipid molecules and are self-assembled spherical or tubular structures with diameters ranging from tens to hundreds of nanometers. We have demonstrated the use of these nanometer structures for controlled chemical reactions using picoliters of reagents.

Purpose: The purpose of this work is to develop nanometer-sized structures that can ultimately be incorporated into microsystems (microfluidics and MEMS) for use in studying the behavior of very small numbers of biological molecules with fine control.

Major Accomplishments: There were two major accomplishments associated with the project during the last fiscal year:

- *Fabrication of microfluidic systems to facilitate the rapid and reproducible formation of liposomes with encapsulated fluorescent molecules*
- *Demonstration of single molecule encapsulation inside liposomes*

Due to their amphiphilic nature, when phospholipid molecules are dispersed in water they self-assemble into bilayer membranes to form structures called liposomes that are often spherical and encapsulate an aqueous internal volume. Liposomes made using bulk techniques range in size from 50 nm to 10's of micrometers encapsulating volumes that are measured in attoliters to picoliters. Water-soluble molecules can be readily incorporated into the liposomes upon formation. The ultimate goal of our work is to use liposomes as discrete packages to sequester very small amounts of reagents in order to finely control their reaction. For this purpose, two characteristics of the liposome population are critical – the liposome size and the number of encapsulated molecules contained inside each liposome. Ideally, we would like all liposomes in a given population to be identical with a diameter of approximately 100 nm and containing one encapsulated molecule. However, liposomes prepared from bulk techniques generally exhibit a very large polydispersity with either uncontrolled or unpredictable encapsulation efficiency. For example, liposomes made in our laboratory using established techniques range in size from 70 nm to 200 nm in the same preparation.

Recently, we have demonstrated the automated and controlled formation of liposomes in microfluidic systems. In this work, we hydrodynamically focus a stream of lipid tincture at a microchannel cross-junction between two aqueous buffer streams. In a typical procedure, isopropyl alcohol (IPA) containing the dissolved lipids flows through the center inlet channel, and an aqueous phosphate buffered saline solution flows through the two side inlet channels as shown in Figure 1a. When the two liquid phases come into contact, the IPA rapidly diffuses into the aqueous phase and *vice versa*. A fluorescence image of the liposome formation process is

shown in Figure 1b where the IPA solution containing lipids also contains fluorescent dye that intercalates into the formed liposome membrane. Immediately downstream of the cross intersection (to the right in the image), the fluorescent intensity of the center stream increases indicating liposome formation (the quantum efficiency of the fluorescent dye in this experiment (DiIC₁₈) increases upon incorporation into a lipid membrane). The lipids self-assemble where the concentration of alcohol and buffer mixture is at a critical condition where lipids are no longer soluble. The flow rates of the alcohol and buffer channels are adjusted to control the degree of hydrodynamic focusing influencing both the dilution rate and the shear stress at the fluid/fluid interface. We have determined that changing this parameter allows us to maintain unprecedented control over the liposome size and homogeneity. After formation, the liposomes flow downstream for collection as a tightly focused stream owing to the low Reynold's number laminar flow typical of microfluidics and the low diffusion coefficient of liposomes.

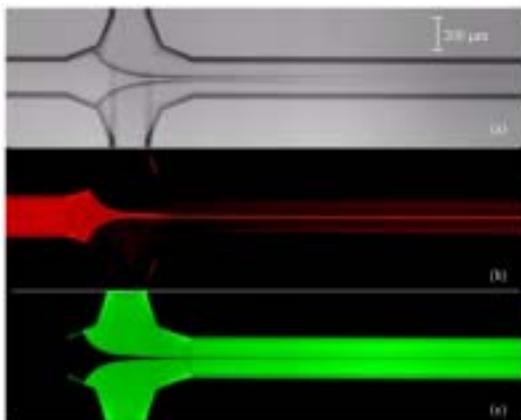


Figure 1. (a) White light image of hydrodynamic focusing of IPA by buffer streams. Fluorescence images of (b) DiIC₁₈ in IPA stream and (c) CF in buffer streams. Silicon/glass microchannels have trapezoidal cross sections with the following dimensions; depth = 40 μm , maximum width = 200 μm , minimum width = 147 μm .

We have also demonstrated encapsulation of fluorescent dye molecules into the aqueous interior of the liposomes. In these experiments, carboxyfluorescein (CF) is dissolved into the 2 buffer streams and encapsulation of the dye occurs upon liposome formation as shown in Figure 1c. The number of CF molecules encapsulated is controlled by changing the concentration of CF in the stream. Using this technique, we were able to finely tune the number of dye molecules inside the liposome population ultimately achieving single molecule encapsulation.

Impact: The characteristics of fluidic flow in a micrometer-scale channel can be used to precisely control the distribution of chemical conditions and mechanical forces so that they are constant on a length scale equivalent to that of a liposome. Hence, forming liposomes in micrometer-scale flow field results in more homogenous conditions during liposome self-assembly and resultant liposome populations that are more uniform in size, hence of low polydispersity.

Future Plans: The work is critical to our efforts in the development of new tools for observing and characterizing single molecule behavior. The characterization of single biomolecules, rather than the study of ensembles of biomolecules, is an important topic in the field of biology since it has been elucidated that the presence and behavior of the biological outlier or the mutant version of the biomolecule can facilitate amplification of that species resulting in catastrophic consequences as highlighted in recent reports on prions. This year, we have made considerable progress toward packaging single molecules, and performing controlled reactions with a few molecules. Future work will involve further characterization of encapsulated single DNA and RNA molecules so that we can study their behavior one at a time.

Title: Electrolytes in Human Serum

Authors: S.E. Long and K.E. Murphy

Abstract: We have developed new analytical methods utilizing inductively coupled plasma – mass spectrometry (ICP-MS) for the determination of electrolytes in blood serum and have employed these in the certification of a renewal material, SRM 956b, Electrolytes in Frozen Human Serum. SRM 956b is widely used for the calibration and standardization of automated analyzers and electrolyte measurement systems employing ion selective electrodes. The new methods are faster, and therefore more efficient than previous methods, which were based on thermal ionization mass spectrometry (TIMS). A new ICP-MS instrument fitted with both collision cell and shielded-plasma technology for reducing mass spectral interferences has recently been installed in our laboratory. The new system will complement the existing instrumentation and will provide enhanced capabilities for the determination of problematic clinical analytes such as calcium and potassium.

Purpose: Accurate and expedient clinical assessment of patient health status is often critically dependent on the quality of clinical testing measurements. For example, a recent report, commissioned by NIST, on the laboratory testing of calcium [1] concluded that the potential economic impact on the health-care system of even a modest analytical bias could be in the range \$60 to \$199M resulting from the need to provide (often unnecessary) follow-up patient testing. The availability of appropriate high-quality reference materials to provide a traceability basis for such clinical measurements is an important requisite. This was recognized by the European Union, which recently introduced the In-Vitro Diagnostics (IVD) Directive, which came into effect in December 2003. Electrolytes are undoubtedly the most commonly measured analytes in the clinical laboratory, and therefore the SRM 956 series, which provides certified values for electrolytes in blood serum, provides an extremely important resource to the clinical measurement community. At the time of the implementation of the IVD Directive, sales of SRM 956 increased dramatically. To support the continuing regeneration of this material, it is necessary to develop and maintain higher-order analytical methods, which utilize state-of-the-art measurement technology.

Major Accomplishments: New methods using isotope dilution ICP-MS have been developed for the determination of lithium, total calcium and potassium in human serum and have been used to certify SRM 956b, Electrolytes in Frozen Human Serum. Certification of five electrolytes, namely lithium, potassium, total calcium, sodium and magnesium in three levels comprising SRM 956b, has been completed. All of the certified values were obtained using ICP-MS, which has permitted a more efficient certification strategy. Certification measurements on the previous issue (956a) relied heavily on TIMS analytical methods. While TIMS can provide unsurpassed accuracy and repeatability, it is an extremely slow technique. The new methods for calcium and potassium are based on shielded (cool) plasma ICP-MS. Significant effort was made to develop a robust method for the determination of lithium by ICP-MS. Lithium has not been routinely measured using this technique because of severe problems with instrument mass bias drift and significant inter-sample memory effects. In the new method these effects have been almost eliminated by the careful optimization of ICP-MS instrument conditions.

The analytical methods currently used for electrolyte measurements have been compiled into a methods manual, which we intend to publish both as a NIST 260 Series report and as an on-line document available to external users of the NIST web site.

A quadrupole ICP-MS instrument, configured with both collision cell and shielded plasma technology to reduce or eliminate spectral interferences has been installed in the Analytical Chemistry Division. It is anticipated that this will provide new capabilities for the determination of clinical analytes, which have been difficult to measure accurately using traditional instrumentation.

Impact: The development of new analytical methods for electrolytes in clinical materials has provided significant enhancements to ACD measurement capabilities and has improved the efficiency of SRM certification activities. The new methods have been used to renew SRM 956b, which is an important clinical SRM and which should provide valuable traceability support for both domestic and European IVD markets.

Future Plans: Development work on new analytical methods using collision cell ICP-MS for the determination of electrolytes and other important clinical analytes in serum and urine will be actively investigated.

References:

1. Gallagher M.P, Mobley L.R., Klee G.G and Schryver P., “RTI Planning Report 04-1, The Impact of Calibration Error in Medical Decision Making,” April 2004.

Title: A NIST Reference Material to Support Explosive Device Measurements

Authors: W.A. MacCrehan and M. Bedner

Vision: To assure the best measurements to detect and investigate explosives crime.

Purpose: To deter explosives crime, national, state and local forensic laboratories measure the additives in smokeless powder (gunpowder). US military laboratories also measure the chemical composition of such propellant powders to determine the stability of munitions. CSTL is providing a reference material, RM 8107 Additives in Smokeless Powder to support the quality of these forensic and military smokeless powder measurements.



Pipe Bomb Ingredients and RM 8107

Major Accomplishments: This new reference material consists of 5 g of a rifle-type smokeless powder. Reference values were assigned for four commonly determined additives, nitroglycerin, diphenylamine, N-nitrosodiphenylamine, and ethyl centralite. An ultrasonic solvent extraction method was developed to quantitatively recover these analytes. Mean values determined by micellar capillary electrophoresis and liquid chromatography were in good agreement. Since the LC technique provided lower uncertainty in the measurements, it was used for the final value assignment. RM 8107 was released for sale August 20, 2004.

Impact: With the development of this smokeless powder RM, forensic laboratories will have a reliable material for the development and validation of measurement methods for improvised explosives devices. The RM may also be used as a proficiency challenge sample to test operator and laboratory performance in explosives measurements as part of laboratory accreditation activities under ASCLD/LAB. In addition, US military laboratories can use the additive reference values in the NIST RM to assure accurate measurement to assure the stability of munitions.

Future Plans: The smokeless powder RM 8107 was developed to help assure the quality of 'low explosives' type measurements. However, terrorist explosives' incidents often involve the use of 'high explosives' such as TNT and RDX (ingredients of military and plastic explosives). In conjunction with OLES and the Department of Homeland Security, we are developing a reference material for these high explosives. An inert solid matrix will be coated with commonly used military explosives. The goal is to develop a particulate explosives material that can be used to test explosives detection equipment used for airport security screening, evaluation of suspicious packages by first responders, and forensic laboratory post-blast investigations.

Title: Characterization of the New Thermal Neutron Prompt Gamma Ray Activation Analysis Instrument

Authors: E.A. Mackey, D.L. Anderson¹, P.J. Liposky², R.M. Lindstrom, H. Chen Mayer, and G.P. Lamaze

PGAA limits of detection have been greatly reduced compared with those of the original instrument due to reduced gamma-ray backgrounds and increased element sensitivities.

¹US Food and Drug Administration, Elemental Research Branch, College Park, MD 20740-3835

²NIST, Center for Neutron Research, Gaithersburg MD, 20899-8561

Abstract: A new thermal neutron PGAA instrument was built at the NIST Center for Neutron Research. It replaced the original PGAA instrument that was built in the late 1970's. This new instrument provides greater sensitivities, better detection limits, and much lower background radiation. The new instrument is also easier and safer to assemble for use.

Purpose: The new thermal neutron prompt γ -ray activation analysis instrument was designed to provide greater sensitivities, better detection limits, and much lower background radiation. (See Figure 1.) Experiments were performed to measure the improvements in element sensitivities and background count rates.

Major Accomplishments: A sapphire filter was placed in the neutron beam shutter assembly to reduce the fast neutron and low energy γ -ray components of the beam. The fast neutron component of the beam was reduced by a factor of five and the low energy background γ -radiation by factors of 5 to 10. A new external beam tube, sample chamber, beam stop, and support structure were built and a new detection system installed. The new beam tube is made of two cylindrical aluminum sections lined with a lithiated polymer. The sample chamber was also fabricated from aluminum and lined with lithiated polymer, and may be evacuated to minimize the number of neutrons scattered and absorbed by air. The beam tube and sample chamber assembly is suspended from the aluminum support structure. The detection system consists of a 40% efficient germanium detector (resolution 2.0 keV at 1332.5 keV) and bismuth germanate Compton suppressor. The detection system shield consists of lead surrounded by borated and lithiated polyethylene, placed on a table attached to the support structure. The new, more compact beam stop is welded to the support structure. Capture gamma-ray photopeaks from H, B, C, N, Na, Al, Fe, Ge, I, and Pb in the background spectrum were either of lower intensity or eliminated with the new PGAA instrument. The improved detection system, positioned closer to the sample, increased element sensitivity by 5% to 50%. During the past year work was completed on the determination of 0.1% N in oil materials which would not have been possible without the previous instrument. We have also completed a complete uncertainty assessment for this method and this instrument.

Impact: The new PGAA instrument is safer to assemble for use and provides greater sensitivities, better detection limits, and much lower background radiation. Analyses of SRMs and other materials will benefit from these improvements.

Future Plans: Additional work is in progress to prepare a suite of standards for the elements of interest for a broad range of neutron absorption and scattering matrices.



Title: Simultaneous Determination of Sulfur Isotopic Composition and Concentration in Environmental Samples Using a $^{33}\text{S}/^{36}\text{S}$ Double Spike Technique

Authors: J.L. Mann, W.R. Kelly, and R.D. Vocke

Abstract: A new analytical technique that is both highly accurate and precise has been developed for the simultaneous determination of sulfur isotope composition and concentration of low concentration samples. The technique combines multi-collector thermal ionization mass spectrometry (TIMS) with a $^{33}\text{S}/^{36}\text{S}$ internal standard and has been applied to three international sulfur standards (IAEA-S-1, IAEA-S-2, and IAEA-S-3) from the International Atomic Energy Agency (IAEA). The $\delta^{34}\text{S}$ values determined were $-0.32\text{‰} \pm 0.04 \text{‰}$ (1s), $22.65 \text{‰} \pm 0.04 \text{‰}$ (1s), and $-32.47 \text{‰} \pm 0.07 \text{‰}$ (1s) for IAEA-S-1, IAEA-S-2, and IAEA-S-3, respectively. The uncertainties reported for sulfur isotopic composition are comparable to or better than those obtained by isotope ratio mass spectrometry (IRMS). The precisions on the concentration measurements are typically 0.1 % or better for sample sizes of approximately 15 μg to 65 μg .

Background: Recently the National Resource Council (NRC) identified several “Grand Challenges” in environmental research for the next generation. Included in these is “understanding Earth’s major biogeochemical cycles and their interaction with the global climate”.

The in the atmosphere and the particles has important Sulfate aerosols interact outgoing terrestrial radiation and indirectly function as that influence concentration surface reflectivity, and the (IPCC, 1995). The majority derived from gas-to-particle emissions result in



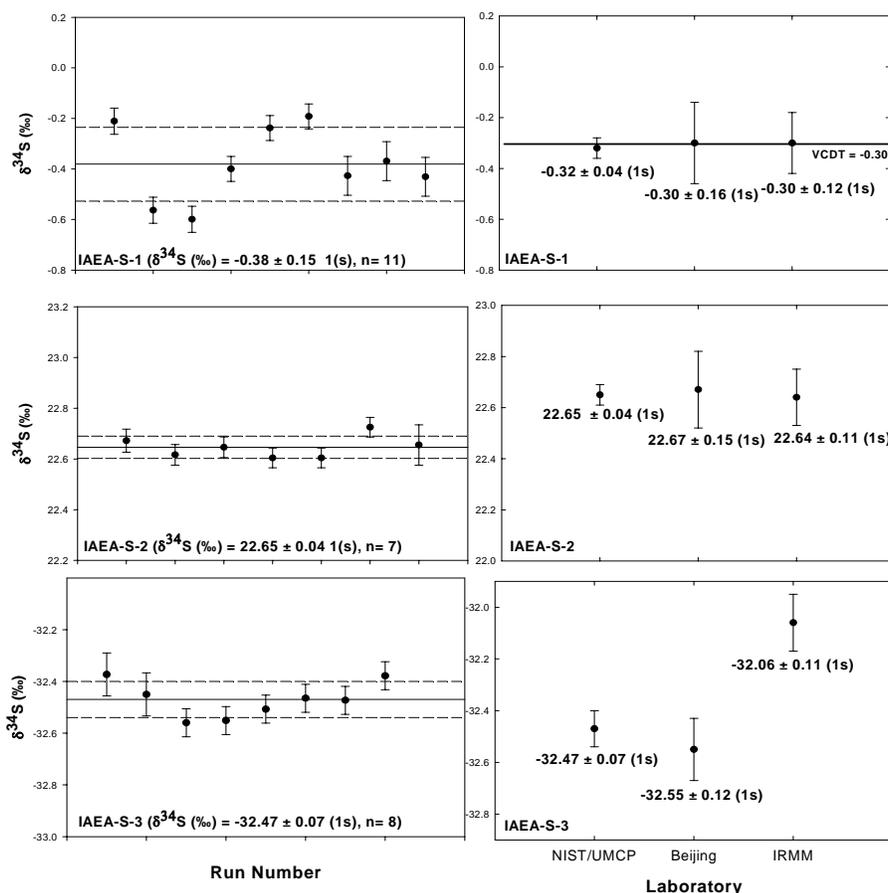
biogeochemical cycling of sulfur formation of sulfate aerosol consequences for global climate. directly with incoming solar and through scattering and absorption cloud condensation nuclei (CCN) and size of cloud droplets, droplet radiative properties of clouds of sulfate aerosol particles conversion of anthropogenic SO_2 submicron secondary aerosols.

These submicron aerosols, with a residence time in the atmosphere of a few days to a week, are able to influence atmospheric composition both downwind of industrialized areas and in remote areas of the world. In the 1990s researchers recognized the need to determine the origin of submicron sulfate aerosols in order to assess the impact of increasing anthropogenic sulfur emissions on the natural atmospheric sulfur cycle and global climate. Assessing the global influence of anthropogenic activity in the northern hemisphere on the climate is becoming increasingly more important with the growing population and commensurate use of fossil energy.

Purpose: The variability of sulfur isotope ratios in nature, caused by mass-dependent fractionation during biogeochemical processing, provides a chemical tool for tracing the various sources of sulfur aerosols and a useful tool for understanding the sulfur cycle. Snow and ice cores provide archives of the sources, sinks, and processing of sulfur that reflect changes in this cycle through time. These archives can be used to assess the current and historical changes in sulfur source contributions to remote regions of the Earth including polar, temperate, and tropical regions. Typical concentrations of sulfate in these regions are 25 to 100 ng/g (ppb); thus, 300 g

to 4000 g of sample is required to obtain enough sample (~ 33 $\mu\text{g S}$) for traditional isotope ratio mass spectrometric (IRMS) analysis. Global atmospheric sulfur cycling is a dynamic process that varies on short timescales and these large quantities of sample can mask seasonal changes in sulfur sources. Consequently, an analytical technique that allows the reduction of sample needed for analysis is required to minimize masking and increase the resolution.

Technical Background: In this study we have focused on the use of multi-collector TIMS combined with a $^{33}\text{S}/^{36}\text{S}$ internal standard for simultaneous determination of sulfur **isotope composition** $\delta^{34}\text{S}$ ($\delta^{34}\text{S} (\text{‰}) = [(^{34}\text{S}/^{32}\text{S})_{\text{sample}}/(^{34}\text{S}/^{32}\text{S})_{\text{standard}}-1]\times 1000$) and **concentration** of small (< 1 μg) sulfate (SO_4^{2-}) samples. The fundamental limitation to accurate and precise isotopic ratio measurements by thermal ionization is that the measured ratio differs from the true ratio in the source as a result of instrumental fractionation during vaporization of the sample from the filament. To address this changing ratio and to improve precision and accuracy in the $^{34}\text{S}/^{32}\text{S}$, a well-characterized $^{33}\text{S}/^{36}\text{S}$ internal standard was added to the samples and was used to calculate a fractionation factor (α) that corrects for this changing ratio (instrumental



fractionation) to give the true ratio in the source. The $^{32}\text{S}/^{33}\text{S}$ ratio that has also been corrected for instrumental fractionation was used to calculate the sulfur concentration. This technique has a significant advantage over the IRMS technique that is limited to only isotope composition measurements.

Major Accomplishments: The international standards were measured for isotopic composition (Figures 1a – 1c) and concentration. The $\delta^{34}\text{S}$ values (reported relative to VCDT ($\delta^{34}\text{S} = -0.3\text{‰}$)) determined were $-0.38\text{‰} \pm 0.15\text{‰}$ (1s), $22.65\text{‰} \pm 0.04\text{‰}$ (1s), and $-32.47\text{‰} \pm 0.07\text{‰}$ (1s) for IAEA-S-1, IAEA-S-2, and IAEA-S-3. Each standard showed less than 0.5‰ variability (IAEA-S-1 $\approx 0.4\text{‰}$, IAEA-S-2 $< 0.2\text{‰}$, and IAEA-S-3 $\approx 0.2\text{‰}$). The uncertainties are comparable to or better than those reported for IRMS the typical method used for isotope ratio analysis. Figures 1d – 1c show our (NIST/UMCP) results for the three standards relative to two other international labs, the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) and the Institute of Mineral Deposits (Beijing, China). These laboratories used a modified gas source (IRMS) mass spectrometer equipped with a molecular flow inlet system to determine the absolute isotope abundance ratios of the three standards. All labs obtained the same $\delta^{34}\text{S}$ value for IAEA-S-1, the benchmark standard, and IAEA-S-2 within the uncertainties. This does not hold true for IAEA-S-3 where our values are the same as that obtained for the Beijing laboratory but is significantly different than the value obtained by IRMM. The value obtained by IRMM is also significantly different than that obtained by Beijing. This may potentially be due to scale contraction that is related to the gas source technique and which is eliminated by our technique. In each case our uncertainties are comparable or better than those obtained by the other labs. It is important to note that these measurements were made on samples ranging from 13 μg to 65 μg , which is smaller than the amounts used by the other laboratories (84 μg to 125 μg). Our precisions on concentration measurements range from 0.07 % to 0.23 % (% rsd) which is comparable or an improvement over our present measurement capability (0.2 %) and includes a greater variability in the typical sample size taken for measurement.

Impact and Future Plans: The technique has shown considerable promise as a tool for the measurement of low concentration sulfate samples for both isotopic composition and concentration as demonstrated with standard measurements. The availability of TIMS instruments in laboratories around the world will make this technique immediately available to the scientific community and can be used for any applications requiring highly accurate and precise measurements of sulfur such as low-sulfur fossil fuels. We are presently in the process of applying this technique to low concentration snow and ice samples from Greenland and Krygstan. The data obtained will allow us to examine the degree of anthropogenic contribution to the sulfur cycle on a seasonal basis in these remote regions and to assess the spatial variability of global anthropogenic influence.

Title: Identification and Provision of “Higher Order” Certified Reference Materials and Reference Measurement Procedures Required for U.S. Industry Compliance with the EU IVD Directive

Authors: W.E. May and V.L. Vilker

Abstract: The goal of obtaining comparability of laboratory diagnostic test results will be possible only when common reference systems can be established for worldwide use. A critical step in reaching this goal is achieving traceability of reference measurement procedures and reference materials to a universally recognized and accepted reference point such as the International System of Units (SI). Recently, traceability requirements for medical devices to be imported into the European Community have been codified. The European Community In Vitro Diagnostic Directive (EC IVDD) states that “The traceability of values assigned to calibrators and/or control materials must be assured through available reference measurement procedures and/or available reference materials of a higher order.” (98/79/EC, Annex1 (A) (3) 2nd paragraph). The Joint Committee on Traceability in Laboratory Medicine (JCTLM) was created to meet the need for a worldwide platform to promote and give guidance on internationally recognized and accepted equivalence of measurements in Laboratory Medicine and traceability to appropriate measurement standards. At present, neither reference materials nor reference methods are available for the vast majority of the chemical or biochemical species that are measured in medical laboratories using IVDs on a routine basis.

Purpose: Excluding home diagnostics, the overall world-wide invitro diagnostic market is approximately \$20 billion. The total IVD market in Europe was about \$5.6 billion in 1998 and has shown growth of about 4% per year over the past five years (data from www.edma-ivd.be). Approximately 60% of the IVD products currently on the European market are imported from the US. The U.S. IVD industry (ADVAMED) has asked NIST to work with our counterparts in Europe and the Asia-Pacific to provide the reference materials and methods of “higher order” that are urgently needed to comply with the requirements of the EU IVD Directive. Without timely completion of these standards, the U S. IVD industry’s access into the European market will be seriously jeopardized.

Major Accomplishments: (A) NIST has provided the clinical measurements community with both neat chemical and human body fluid-based Standards Reference Materials for well-defined health status markers such as Electrolytes (sodium, potassium, lithium and magnesium), cholesterol, creatinine, glucose, triglycerides, urea, uric acid, vitamins A, C, E and beta carotene, and several therapeutic and drugs of abuse for more than 20 years.

Over the past few years, NIST has expanded its standards program in clinical diagnostics. A major portion of the expansion of the NIST program has involved the development of new standards for several protein, hormone, peptide, and other large biomolecule-based health status markers. Efforts have been initiated for development of reference methods and SRMs for: Cardiac Troponin I (heart attack occurrence and damage), C-Reactive Protein and Homocysteine (heart attack risk), Cortisol (endocrine function), Folates (neural tube defects), Glycated Hemoglobin (diabetes status), Prostate Specific Antigen (prostate cancer), and Triiodothyronine and Thyroxine (thyroid function). Many of these new markers show great promise from the

clinical diagnostic perspective, but offer new and more difficult challenges for standardization. They are presenting the blood at very low concentrations and many are thermally labile, very polar, and heterogeneous -- both in conformation and in what is attached to them. Because of the vast market for tests for these new markers, many different measurement approaches have been developed that often provide quite different results. These discrepancies can lead to erroneous diagnoses and/or the need for retesting -- both very costly.

During the past year, work was completed on three new SRMs and three high-priority SRM renewals. A calibration standard for Cardiac Troponin-I (SRM 2921) was completed along with materials for Toxic Metals in Urine (SRM) and Homocysteine and Folate in Human Serum serum (SRM 1955). Renewals were completed for 956b – Electrolytes in Human Serum, SRM 965a - Glucose 1851b - Lipids in Human Serum.

(B) During the past year, NIST led the efforts of the Joint Committee on Traceability in Laboratory Medicine (JCTLM) Working Group on Reference Materials and Reference Laboratory Procedures in establishing a process for identifying, reviewing against agreed upon criteria, and publishing a List of “higher order” Certified Reference Materials and Reference Measurement Procedures required for IVD industry compliance with the EC IVD Directive regarding in vitro diagnostic medical devices. In order to facilitate a fair and transparent review process, eight analyte categories were identified and Review Teams established for each. To the extent possible, each Review Team has representation from IVD manufacturers, National Metrology Institutes, accreditation organizations, and professional societies from the US, Europe, and the Asia Pacific Region.

On April 1, 2004, the JCTLM published its first List of Higher Order Reference Materials and Reference Measurement Procedures. This initial List (List I) comprises Certified Reference Materials and Reference Measurement Procedures for well-defined chemical entities or internationally recognized reference method-defined measurands. Reference Materials and Measurement Procedures included in List I are those that provide values that are traceable to the SI units; e.g., electrolytes, enzymes, drugs, metabolites and substrates, non-peptide hormones and some proteins. List II, to be published during the last quarter of 2004, will comprise International Conventional Reference Materials, i.e., those for which the measurand(s) is/are not SI-traceable and/or no internationally recognized reference measurement procedure is available; e.g., WHO reference materials for coagulation factors, nucleic acids, and some proteins.

The current List contains approximately 100 Reference Measurement Procedure entries for 58 different health status markers. Thirty of these “higher order” reference measurement procedures are from NIST. For Reference Materials, the List contains approximately 150 entries for 96 measurands; NIST SRMs provide traceability for 72 of these. A laboratory-based quality assurance audit program has been initiated to provide measurement results regarding the comparability of multiple “higher order” Reference Materials for the same measurand on the list as well as to verify the veracity of the review process. Nominations are currently being accepted for high purity substance reference materials and for human hair and body fluid-based reference materials and reference measurement procedures in the eight original plus five new analyte categories.

In a related activity, a NIST Traceable Reference Laboratory Network is being developed to provide higher order reference measurements to IVD industry. We are working with Mayo Clinic (a potential member of the reference laboratory network) and Dade Behring (an IVD manufacturer with a need for higher order reference measurements) to establish the guidelines and operating conditions. An exercise was completed this year to evaluate the comparability of reference measurements of calcium in serum, plasma, and urine made at Mayo Clinic with measurements made at NIST has been completed. This will serve as a model for establishment of the Network.

Reference Measurement Procedure					
Procedure Name and/or ID #	Analyte Name	Applicable Matrices	Measurement Principle	Reference Procedure Citation(s) or Document(s)	Reference Procedure Comparability Assessment Studies
NIST definitive method for serum cholesterol	cholesterol	lyophilized, fresh, or frozen serum	EDCAMS	Anal Chem 61, 1718-1723 (1988)	CCQM-K6, http://tsdtkb.nist.gov/external/ShippingInfo/ccqm-k6/ccqm-k6_final_report.pdf ; Clin Chem 36, 370-375 (1990)
U. of Ghent reference method for cholesterol	cholesterol	lyophilized, fresh, or frozen serum	EDCAMS	Clin Chem 39, 1001-5 (1993) [a part of Clin Chem 39, 803-1000 (1993)]; Eur J Clin Chem Clin Biochem 34, 653-60 (1996); Clin Chem 42, 521-5 (1996)	EUROMET 663
CGKC definitive Method for Serum Cholesterol	cholesterol	lyophilized, fresh, or frozen human serum or plasma	EDCAMS	Siekman et al., J. anal. Chem. 279, 145-146 (1976)	PTB - National Key Composites for Accreditation
CDCA/ASAP/ASAP method for cholesterol	cholesterol	lyophilized, fresh or frozen human serum	Spectrophotometry	Cooper, GR, et al. Clin Chem 32, 921-929, 1986	Clin Chem 36, 370-375 (1990)

Reference Materials							
Information about Material				Contact Information	References		Comments
Analyte	Matrix	Material Name and/or ID #	Estimated * Availability (months, as of Jan 2004)	- Producer - Country - Website - Email Address - Phone Number - Fax Number	Commutability Study Information and/or Citations	Other Relevant Publications	Hyperlink to Comparability Assessment Studies
cholesterol	cholesterol	20040026	30	CCQM-K6 NIST 100 Tel: 301-975-2719 Fax: 301-975-2709 Email: ccqm-k6@nist.gov	Primary calibration for higher order reference material		
cholesterol	cholesterol	20040176	21	NIST USA http://tsdtkb.nist.gov/external/ShippingInfo/ccqm-k6/ccqm-k6_final_report.pdf Email: ccqm-k6@nist.gov Tel: 301-975-2719 Fax: 301-975-2709	Primary calibration for higher order reference material		
cholesterol	serum (human)	20040121	30	NIST USA http://tsdtkb.nist.gov/external/ShippingInfo/ccqm-k6/ccqm-k6_final_report.pdf Email: ccqm-k6@nist.gov Tel: 301-975-2719 Fax: 301-975-2709	Primary calibration for higher order reference material		NIST only presented at 27th Meeting, June 21, 2004, SP4, Geneva, France
cholesterol	serum (human)	20041004	30	NIST USA http://tsdtkb.nist.gov/external/ShippingInfo/ccqm-k6/ccqm-k6_final_report.pdf Email: ccqm-k6@nist.gov Tel: 301-975-2719 Fax: 301-975-2709	Primary calibration for higher order reference material		
cholesterol	serum (human)	20041026	30	NIST USA http://tsdtkb.nist.gov/external/ShippingInfo/ccqm-k6/ccqm-k6_final_report.pdf Email: ccqm-k6@nist.gov Tel: 301-975-2719 Fax: 301-975-2709	Primary calibration for higher order reference material		NIST only presented at 27th Meeting, June 21, 2004, SP4, Geneva, France
cholesterol	serum (human)	20040026	30	NIST USA http://tsdtkb.nist.gov/external/ShippingInfo/ccqm-k6/ccqm-k6_final_report.pdf Email: ccqm-k6@nist.gov Tel: 301-975-2719 Fax: 301-975-2709	Primary calibration for higher order reference material		

Impact: Clinical measurement results that are reliable and comparable over both space and time are essential for optimal patient care, most efficient use of available healthcare funds, and full utilization of the potential of new information technology tools. Incorrect interpretation of

measurement results by the physician can lead to incorrect diagnosis and treatment, additional unnecessary tests and medical procedures, and increased pain and suffering for the patient. Measurements are responsible for 10%-15% of the \$1.7 T annual costs of healthcare in the United States. A significant portion (25% - 30%) of health-related measurements is performed for non-diagnostic reasons (re-tests, error prevention and detection). The “German Health Report of 1998” states explicitly that “the costs of repeat measurements amount to 1.5 billion US \$ per year in Germany.” If normalized to the U.S. GDP for that year, these costs would be \$7.4 B. Even modest improvements in measurement accuracy and quality assurance will result in multi-billion dollar savings in health-care costs. In addition to measurements reliability and related cost issues, timely completion of these standards will also assure U S. IVD industry’s continued access to the European market.

Future Plans: Work will continue on the high priority list presented above. Work will begin on standards for gene expression including RNA standards and a fluorescence standard for microarray scanning devices. The JCTLM List of “higher order standards” will be updated and expanded to include Reference Materials and Reference Laboratory Procedures for Blood Gases, Blood Groupings, Microbial Serology, Non-Electrolyte Metals and Vitamins.

Title: International Benchmarking of NIST Capabilities for Chemical Measurements

Authors: W.E. May, G.C. Turk, S.A. Wise, F.R. Guenther, G.W. Kramer, R.R. Greenberg, and R.M. Parris

Abstract: Traceability to stated references and global confidence in this realization are the basis for mutual recognition and confidence in data used to facilitate and underpin international trade and decisions regarding health, safety, commerce, and scientific studies. In October 1999, the Directors of National Metrology Institutes (NMIs) for the thirty-eight member states of the Meter Convention, and representatives of two international organizations signed a Mutual Recognition Arrangement on national measurement standards and calibration and measurement certificates issued by national metrology institutes (MRA). The NIST Analytical Chemistry Division has ongoing major activities to meet the MRA requirements for NMIs:

1. Declaring and documenting calibration and measurement capabilities
2. Evidence of successful participation in formal, relevant international comparisons
3. Demonstration of system for assuring quality of each NMI's measurement services

Purpose: Traceability to stated references and global confidence in this realization are the basis for mutual recognition and confidence in data used to facilitate and underpin international trade and decisions regarding health, safety, commerce, and scientific studies. In October 1999, the Directors of National Metrology Institutes for the thirty-eight member states of the Meter Convention, and representatives of two international organizations signed a Mutual Recognition Arrangement on national measurement standards and calibration and measurement certificates issued by national metrology institutes (MRA). This MRA provides an open, transparent, and comprehensive framework for obtaining reliable quantitative information on the comparability of metrological services needed for mutual recognition of national measurement standards and measurement certificates issued by national metrology institutes around the World. This Arrangement also provides governments and other parties with a secure technical foundation for wider agreements related to international trade, commerce, and regulatory affairs.

Major Accomplishments: For implementation of this MRA, the signatory NMIs agreed to:

1. declare and document their calibration and measurement capabilities (CMCs)[Appendix C]
 - CSTL's Analytical Chemistry Division has over 1000 CMCs for Chemical Measurements included in ~3000 CMCs for Chemical Measurements published in the CIPM MRA Appendix C (<http://kcdb.bipm.org/AppendixC/default.asp>)
2. participate in relevant international comparisons to support their CMCs [Appendix B] (<http://kcdb.bipm.fr/BIPM/KCDB/>, <http://icdb.nist.gov>)
 - CSTL's Analytical Chemistry Division has participated in >80 international comparisons to meet requirements of the CIPM MRA. Results of selected recent comparisons are shown below.
3. implement and document the existence of a system for assuring the quality of the measurement services provided.

- CSTL's Analytical Chemistry Division's Quality Manual that summarizes and formalizes policies and approaches for addressing quality-related issues concerning the services that it provides has been updated to assure appropriate compliance with ISO/EC 17025 and ISO Guide 34.

The implementation of the CIPM MRA is carried out by a Joint Committee of Regional Metrology Organizations (RMOs) and the BIPM (JCRB). The JCRB is made up of representatives from each RMO and the BIPM, and provides oversight for results included in the Key Comparisons Database (Appendix B of the MRA), as well as the determination of the degree(s) of equivalence of results from individual NMIs. RMOs have the responsibility for carrying out supplementary comparisons and other actions within their regions to support mutual confidence in the validity of calibration and measurement certificates through the Joint Committee of the RMOs and the BIPM (JCRB). They are also responsible for approval of calibration and measurement capabilities (CMCs) of their member NMIs.

Systema Interamericano de Metrologia (SIM) is the metrological regional organization (RMO) for the Americas. CSTL provides the Chair for the Chemical Metrology Working Group and SIM representative to the JCRB. In order to assure the effective, fair and metrologically sound implementation of the MRA. CSTL staff have led the critical review of both SIM and international chemistry CMC data for Appendix C of the BIPM.

CSTL staff also play a leadership role the International Committee of Weights and Measures-Consultative Committee on the Quantity of Material (CCQM). The CCQM has formed seven working groups: (1) Gas Analysis, (2) Organic Analysis, (3) Inorganic Analysis, (4) Electrochemistry (5) Biometrology, (6) Surface Analysis and (7) Key Comparisons and CMC Quality. These working groups are responsible for selecting and overseeing the operation of key comparisons that address chemical measurement-related issues important for international trade, environmental, health, and safety-related decision making. CSTL staff are active in all seven and has provided formal leadership for the Organic Analysis, Biometrology, and Key Comparisons Working Groups.

Impact: NIST and other National Metrology Institutes around the world have the responsibility for establishing, maintaining, and disseminating the highest level of metrological references for a given country or economy. The calibration and measurement services that these NMIs provide must be of high quality and delivered to our customers in a consistent and transparent manner.

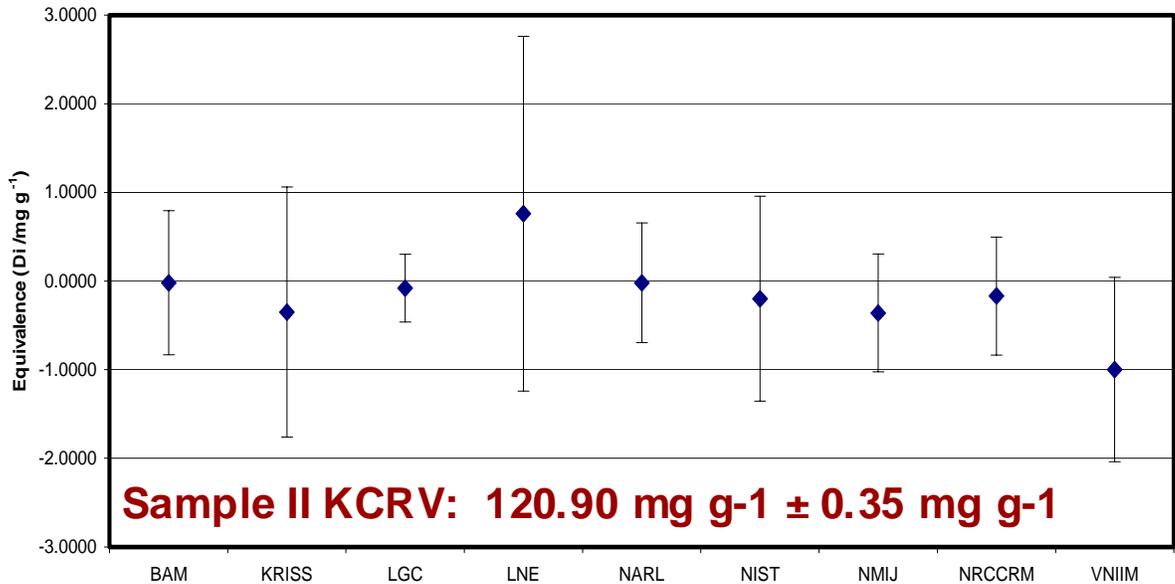
Future Plans:

Over 25 additional CCQM studies are planned to be conducted over the next two years and the Analytical Chemistry Division has already committed to coordinate at least 7 of these. In addition, the Division's Quality System for its measurement services will be presented to and assessed by the SIM Quality System Task Force. The results of this review will be reported to the CIPM JCRB the complete the requirements for maintaining our CMCs in the CIPM Database.

Examples of NIST participation in recently completed international comparisons

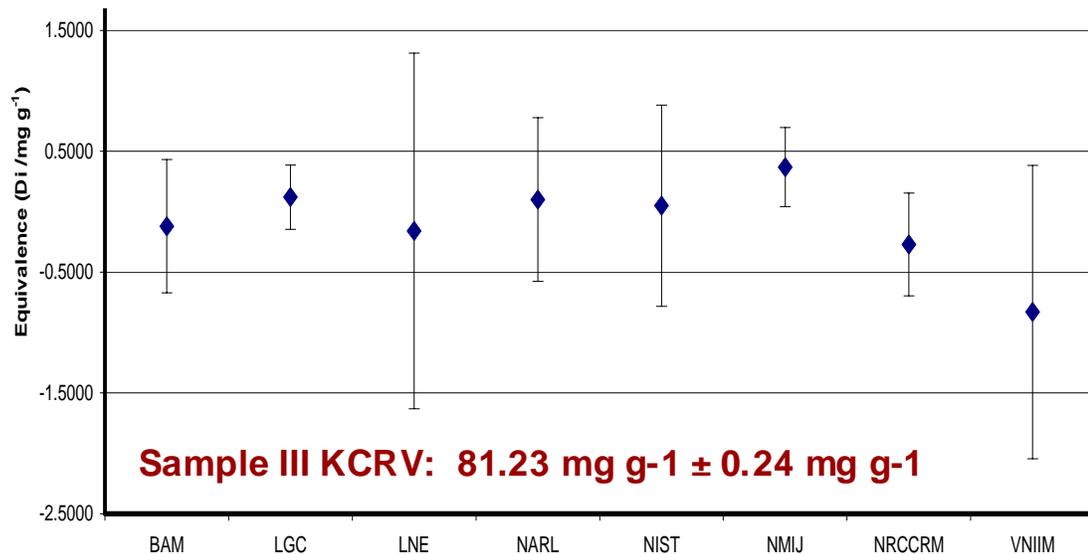
CCQM-K27a Forensic level

Samples II (aqueous solution spiked gravimetrically with ethanol)



CCQM-K27b Commodity level

Sample III (commercial red wine stabilized by irradiation)



Determination of Ethanol in Aqueous Matrix

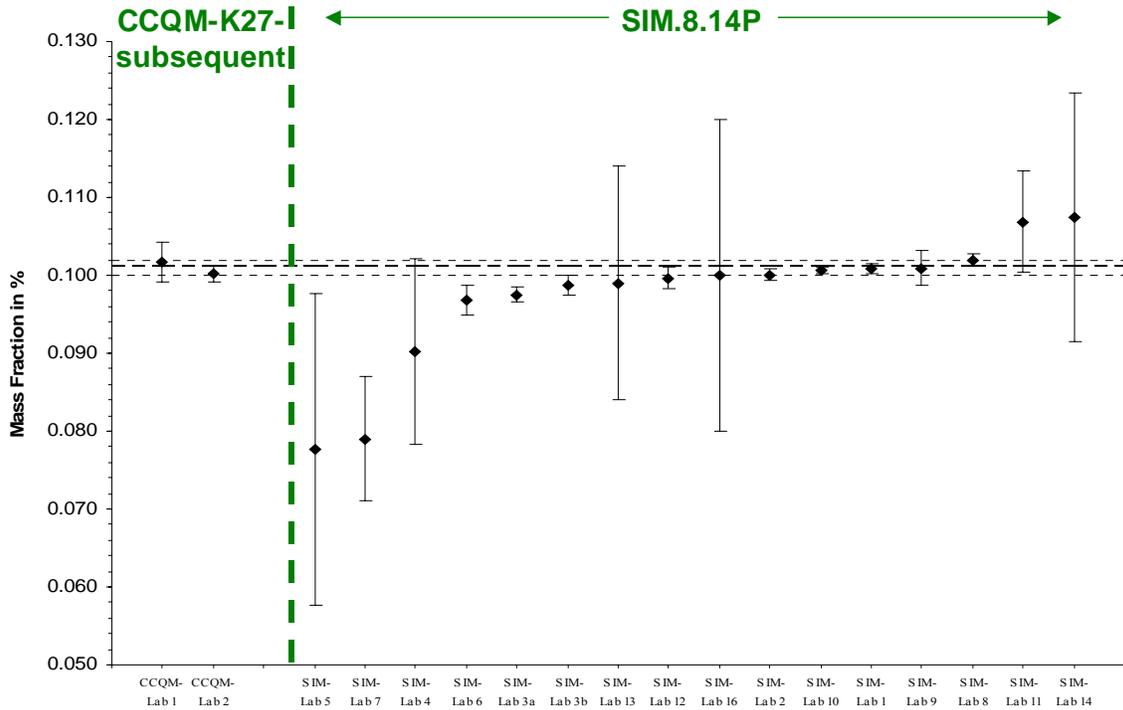
As SIM Pilot Comparison: SIM.8.14P (16 participants)

As Key Comparison: CCQM-K27-subsequent (4 participants)

Ethanol in Aqueous Matrix

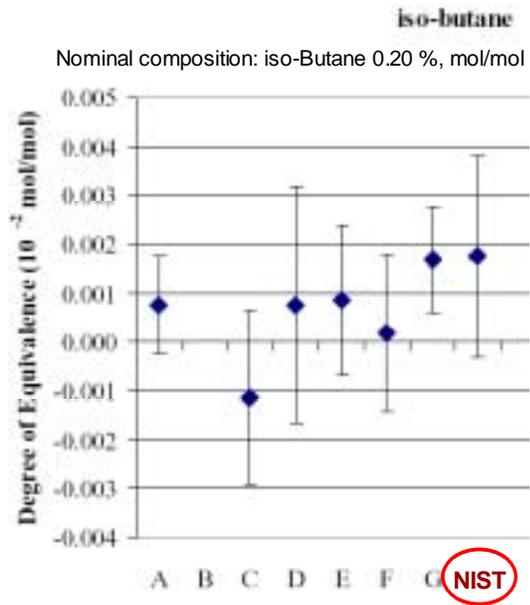
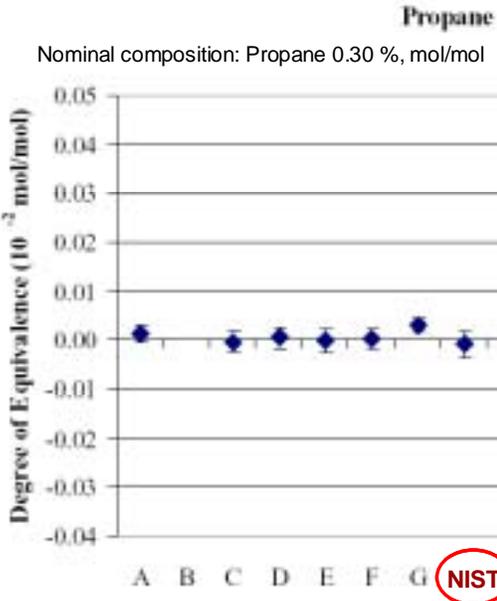
Sample SII: nominal concentration 0.1% ethanol in water

(showing gravimetric value and upper and lower limits of the 95% CI of the gravimetric value based on the CCQM-K27a study)

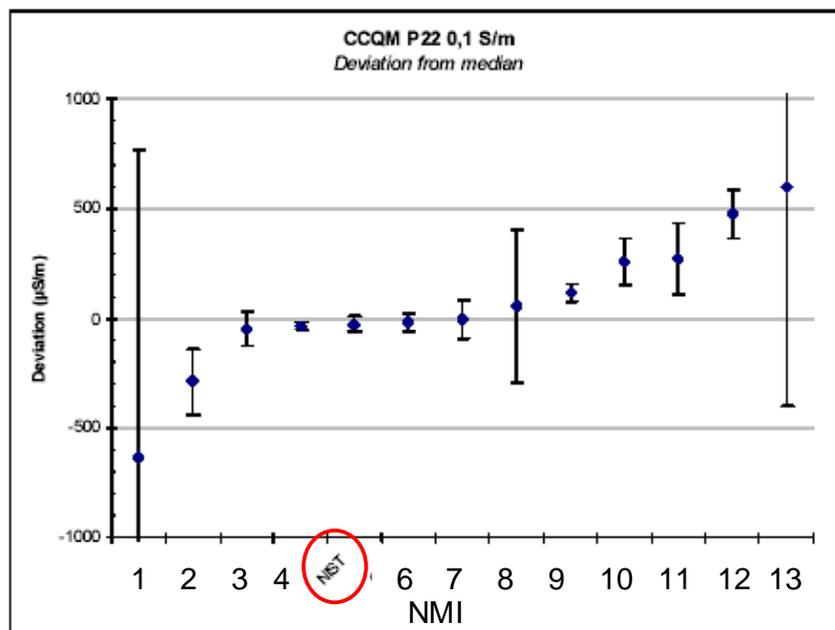


Determination of Components of Natural Gas

CCQM-K16a: Natural Gas



CCQM-P22: Electrolytic Conductivity (primary and secondary measurements)



Nominal 0.1 S/m (1000 $\mu\text{S/cm}$)

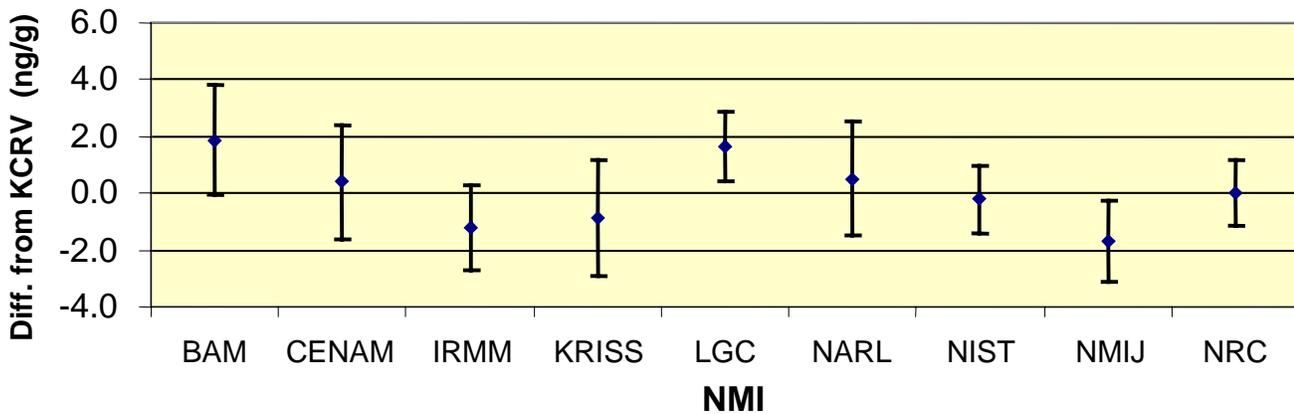
CCQM-K25: PCB Congeners in Sediment

The five PCB congeners measured in CCQM-K25 were selected to be representative of the approximately 150 congeners found in environmental samples. These five congeners also provided the typical analytical measurement challenges encountered including problematic GC separations and a wide volatility range and concentration range for the individual congeners. The results for PCT 153 are shown below.

- PCB 28 - volatile and potential coelution with PCB 31
- PCB 101 - potential coelution with minor congener, PCB 90
- PCB 105* - lower concentration and potential elution order changes with PCB 153 and/or PCB 132
- PCB 153 - potential coelution with PCB 132
- PCB 170 - lower concentration and potential coelution with PCB 190

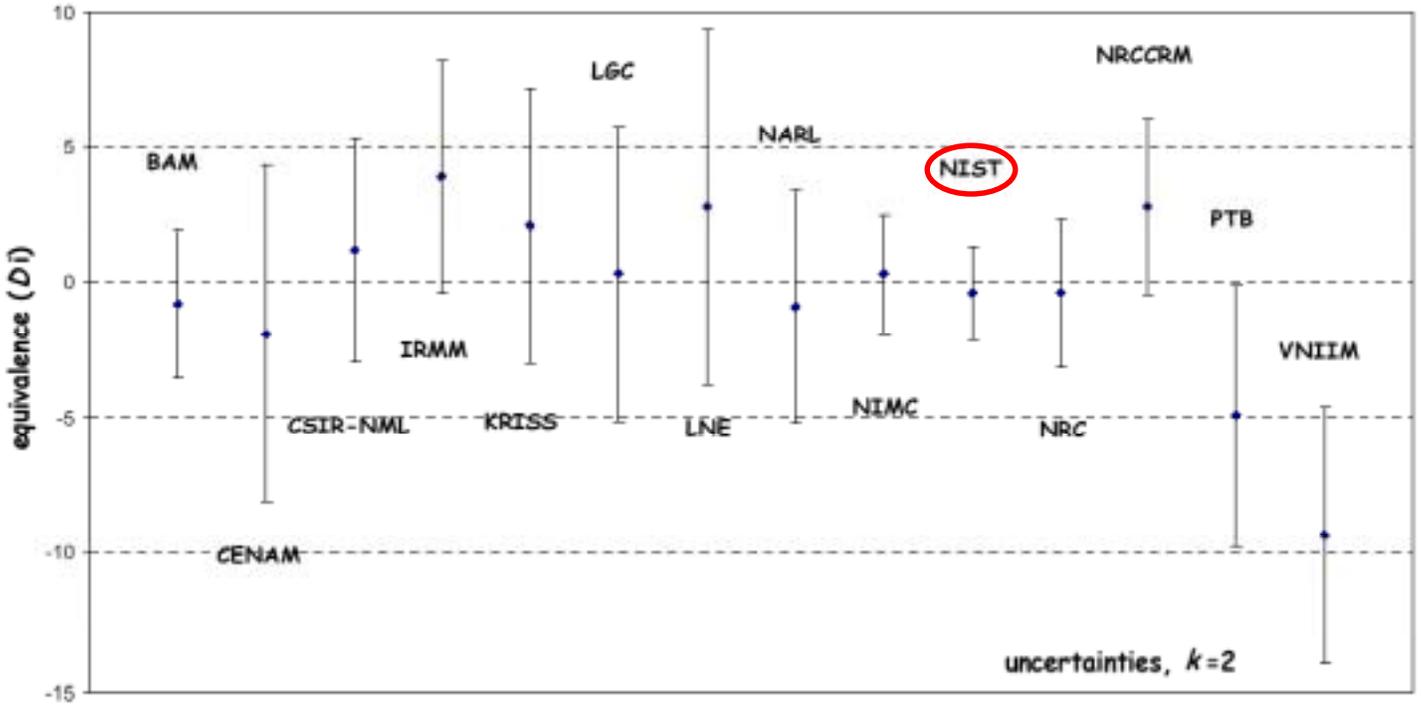
CCQM-K25 PCB 153 Equivalence

KCRV: 31.9 ng/g (dry basis) \pm 1.1 ng/g (dry basis)



Determination of Cadmium and Lead in Sediment

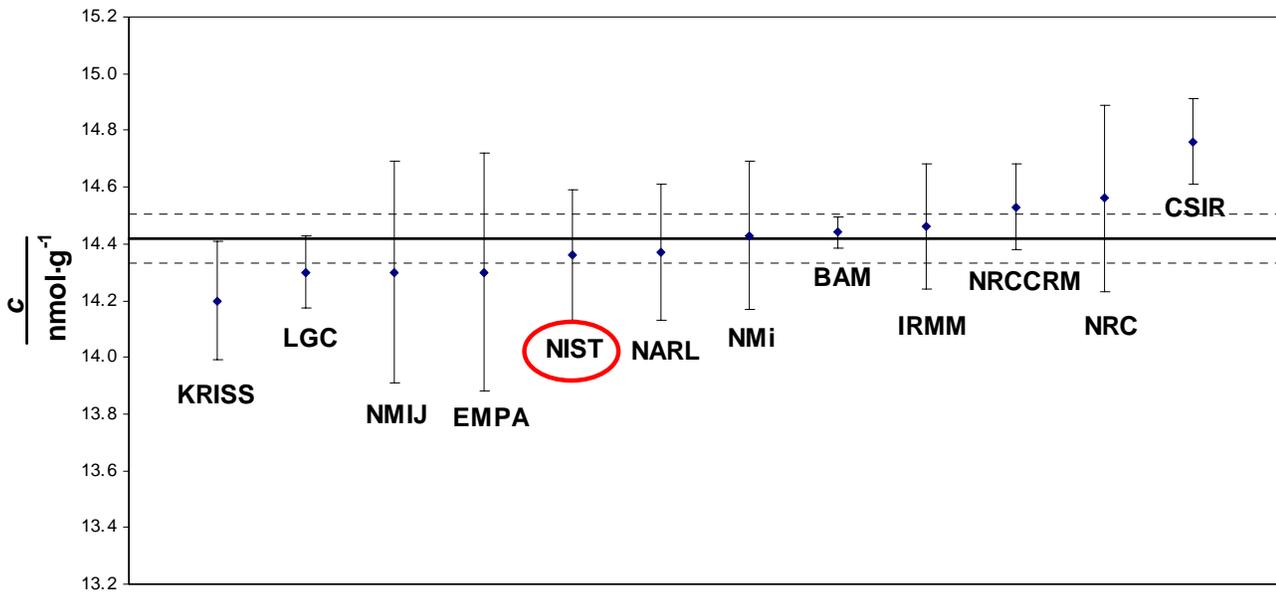
CCQM-K13 key comparison Pb in sediment



Determination of Cadmium in Rice

CCQM-K24: Cd in rice

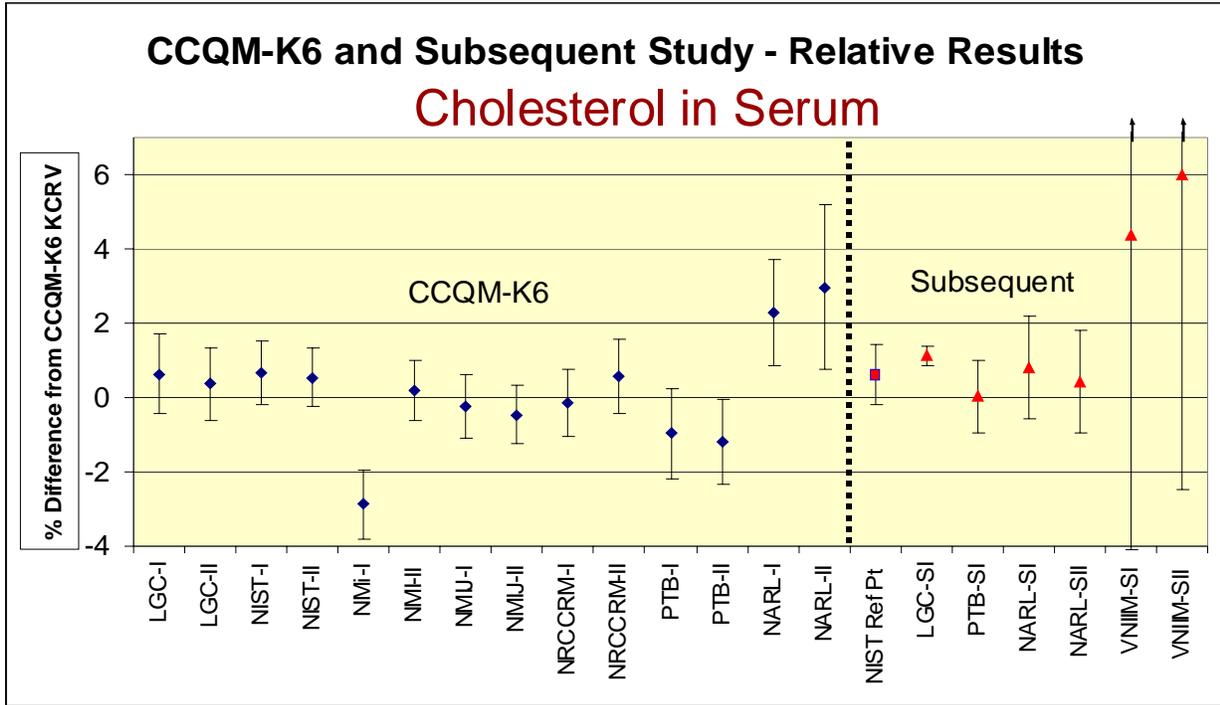
KCRV: $14.418 \pm 0.087 \text{ nmol}\cdot\text{g}^{-1}$; $U=ku_c, k=2$



Expanded uncertainties with coverage factor of $k=2$

Uncertainty of prescribed drying protocol was major component of NIST expanded uncertainty.

Determination of Cholesterol in Serum



K6 results are plotted as % differences from KCRVs

Subsequent results are plotted relative to NIST results in K6S and are offset by average (NIST-KCRV) result from K6 (NIST Ref Pt)

Title: Establishing Measurement Traceability for Gaseous Mercury Emissions Monitoring

Authors: G.D. Mitchell and W.D. Dorko

Abstract: NIST was tasked with providing traceability for measurements of gas phase elemental mercury (Hg^0) for EPA compliance purposes. The typical manner by which NIST provides traceability for the measurement of gas phase constituents is to certify gas mixtures containing those constituents contained in compressed gas cylinders. One of the Specialty Gas companies has developed a procedure for producing high-pressure mixtures of Hg^0 in nitrogen contained in aluminum cylinders. NIST was requested by EPA to work with this company and obtained mixtures with differing concentration levels of Hg^0 . NIST had SRMs of Fly Ash, Orchard Leaves and water that contained certified amounts of mercury, so the experimental plan was to use existing standards to calibrate a system to measure mercury in compressed gas mixtures. A commercial instrument was identified and purchased that could analyze samples containing mercury in any of the three matrices; solid, liquid or gas. The most suitable of the SRMs was the mercury in water, so this was quantitatively diluted to the proper concentration and used to calibrate the analytical instrument that was then used to analyze mixtures of Hg^0 in nitrogen at nominal concentration levels of $2 \mu\text{g}/\text{m}^3$, $5 \mu\text{g}/\text{m}^3$ and $20 \mu\text{g}/\text{m}^3$.

Another means of providing gas mixtures for instrument calibration purposes is to use a mercury vapor generator. One type of generator functions by passing a metered flow of nitrogen over a heated pool of mercury. The concentration of mercury in the gas stream is calculated by knowing the vapor pressure of Hg^0 at the thermostated temperature of the mercury pool, and the calibrated flow rate of the nitrogen. One system of this type was also evaluated by NIST by setting the parameters to generate mixtures at the same concentration levels as the cylinder gas mixtures.

Purpose: To reduce the health risk posed to people, the Environmental Protection Agency (EPA) has announced that it will regulate emissions of mercury from coal and oil fired power plants. The EPA is focusing its effort on both elemental (Hg^0) and ionic (Hg^{++}) mercury. Although coal-fired power plants are the largest source of mercury emissions to the air in the United States, mercury is also emitted from other sources such as municipal waste combustors, medical incinerators and hazardous waste combustors. Mercury emitted from power plants and other sources is carried by the wind and is eventually deposited onto the land and water. Once it enters the water (lakes, rivers and wet lands) it is converted to methylmercury and can enter the food chain. The EPA came to NIST for assistance in providing traceability in mercury gas standards, which are to be used to calibrate monitoring instruments to measure mercury to verify compliance with EPA emission allowances. . To certify a set of standard gas mixtures of mercury vapor in gas cylinders and to evaluate systems that generate mercury vapor mixtures.

Major Accomplishments: NIST worked with a commercial Specialty Gas Vendor to procure three sets of gas mixtures of Hg^0 in nitrogen. The nominal concentrations of the Hg^0 in the cylinders are $2 \mu\text{g}/\text{m}^3$, $5 \mu\text{g}/\text{m}^3$ and $20 \mu\text{g}/\text{m}^3$. NIST also purchased a Hg^0 mixture generator capable of producing mixtures at the same concentration levels as the cylinders. NIST delivered to the EPA three cylinders of mercury in nitrogen whose concentrations are certified and

traceable to the SI (International System of Units). Also, a report was submitted detailing the findings concerning the performance of the mercury gas vapor generator.

The primary analytical instrument used to quantify the amount of mercury is a Nippon Instrument Corporation Model MA-2000 Mercury Analyzer (MA-2), which is equipped with a cold vapor atomic absorption (CVAA) detector. The MA-2 is an analytical system that can measure mercury in discrete liquid or solid samples. A weighed amount of a solid or liquid is put into a ceramic boat that is then placed in a chamber of the analyzer. The way in which vapor samples are supplied to the MA-2 is to pass a measured volume of the gas containing the vapor through an external gold trap to collect the Hg. This gold trap is then placed in the MA-2 instrument boat and this is treated as described above for a solid sample. The traps that were used to trap the Hg⁰ from the gas samples, either from the cylinders or the generator, were tested to determine that there was complete collection. The analyzer was calibrated periodically with quantitatively diluted Mercury in Water SRM #1641d and the cylinders analyzed and delivered to EPA.

<u>Cylinder Number</u>	<u>Concentration, $\mu\text{g}/\text{m}^3$</u>
CC-162918	2.25 \pm 0.08
CC-162789	5.99 \pm 0.18
CC-162881	22.8 \pm 0.68

The generator was tested at output concentrations similar to those of the cylinders with the following results.

	<u>Concentration, $\mu\text{g}/\text{m}^3$</u>		
	<u>Hg-20</u>	<u>Hg-05</u>	<u>Hg-02</u>
Generator Setting	20.86	5.13	2.48
<u>Measured on MA-2</u>	21.3	4.97	2.50
Difference: Measured to Set	+ 2.1%	- 3.1%	+ 0.8%

The uncertainties given for the cylinders are the expanded Uncertainties and do not exceed 7% relative, which is within the desired range of the project which was to be less than 10%. The output of the generator was within 5% of set point.

Impact: The certified cylinder mixtures are to be used by EPA in their program to audit mercury monitoring sites to determine compliance with regulations. Up till this point they had no way by which to provide quality assurance for the audits. The program also provided data on the performance of a mercury generation device and this data showed that this device is a viable alternate option for calibration and audit of mercury monitors.

Future Plans: The cylinders containing the mercury mixtures are to be re-analyzed on a periodic basis to determine whether or not there is any degradation of the Hg⁰ concentration. Development work has already begun on a method for studying ionic mercury (Hg⁺⁺) to provide traceability for measurements being made in support of EPA's proposed Regulations.

Title: NIST Support of the CDC Laboratory Response Network for Chemical Terrorism

Authors: K.E. Murphy, M.M. Schantz, G.C. Turk, B.A. Benner, Jr., T.A. Butler, and L.J. Wood

Abstract: We have developed a new method for the determination of cyanide (CN) in human whole blood and used the methodology to evaluate the stability of CN in frozen blood-based proficiency standards. The proficiency standards are used by the Centers for Disease Control (CDC) to assess measurement capabilities of members of the Laboratory Response Network (LRN), a network of national laboratories that are equipped to rapidly test for human exposure to chemical and biological weapons. CN has a long history of use as a chemical weapon and the need for methods and standards for measurement of CN was identified by CDC as a top priority. CN exposure is indicated by measuring the CN content of whole blood, however CN is very reactive and blood CN concentrations can decrease or increase over time depending on the method of storage. As a result, there are no commercially available CN standards to underpin the accuracy of blood CN measurements. CDC contracted a commercial standards supplier to produce a suite of blood-based CN standards. The standards are composed of a blank level and three elevated levels containing nominally 0.075 mg/kg CN, 0.3 mg/kg CN, and 1.5 mg/kg CN. Standards are supplied as 0.5-mL aliquots of the CN-spiked blood packaged in 5-mL head space vials and stored frozen at $-50\text{ }^{\circ}\text{C}$. We have accurately measured the CN content of each level and have monitored the CN concentrations for over one year.

Purpose: CN is a likely chemical terrorism agent due to its toxicity, wide availability, and history of use as a chemical weapon. Fatalities from exposure to CN may be prevented by the administration of antidotes if exposure is diagnosed in a timely manner. Rapid response requires a well coordinated public health system. The anthrax incident in 2001 showed that laboratory capacities can be quickly overwhelmed if such coordination is lacking. Consequently there is a need for an organized network of laboratories with the capabilities to rapidly and accurately measure terrorism agents such as CN. New methods and standards are required to support and critically evaluate public health laboratories measurement capabilities.

Major Accomplishments: We have developed a new method for the measurement of CN based on headspace gas chromatography/mass spectrometry (GC/MS) using a labeled cyanide internal standard ($\text{K}^{13}\text{C}^{15}\text{N}$). The method has been adapted by CDC for use by laboratories in the LRN. In addition we have completed measurement of the CN content and stability of CDC supplied proficiency standards. Measurements of five samples from each level were performed on a bi-monthly basis for a period of 14 months. Results showing the stability of CN in the proficiency standards are depicted in Figure 1. The measured CN concentration is plotted versus analysis date. The solid black line shows the trend in CN concentration during the 14-month period for the blank level as well as the three elevated levels. The CN concentration remained stable for the nominal 0.3 mg/kg and 1.5 mg/kg levels, but increased slightly for the blank and nominal 0.075 mg/kg level. The average CN concentration for all levels was higher than the target nominal values. This may be partly due to endogenous CN in the blood stock, but may also be a result of the artifactual production of CN from freezing the blood. Overall, results for the three elevated levels show a variability of less than 6 % relative (1s, $n = 33$). These results demonstrate a

considerable improvement over data reported in the literature for the stability of CN-spiked blood standards and validate the viability of storage at $-50\text{ }^{\circ}\text{C}$.

Impact: An integrated public health system is vital in the event of a chemical terrorism attack. In the past two years Congress has appropriated \$95 million for chemical terrorism preparedness. There are currently 62 public health laboratories in the chemical component of the LRN, over 40 of which are Level 2 designate laboratories. Level 2 laboratories are tasked with the analysis of CN, toxic metals and lewisite in human samples. Inclusion in the network requires participation in a rigorous quality assurance program, which includes the analysis of proficiency standards. The data NIST has provided to characterize the CDC blood-based proficiency standards will help ensure that network laboratories provide accurate measurements in the event of a chemical terrorism incident involving CN.

Future Plans: NIST will continue to monitor and respond to future measurement and standards needs of the CDC Laboratory Response Network.

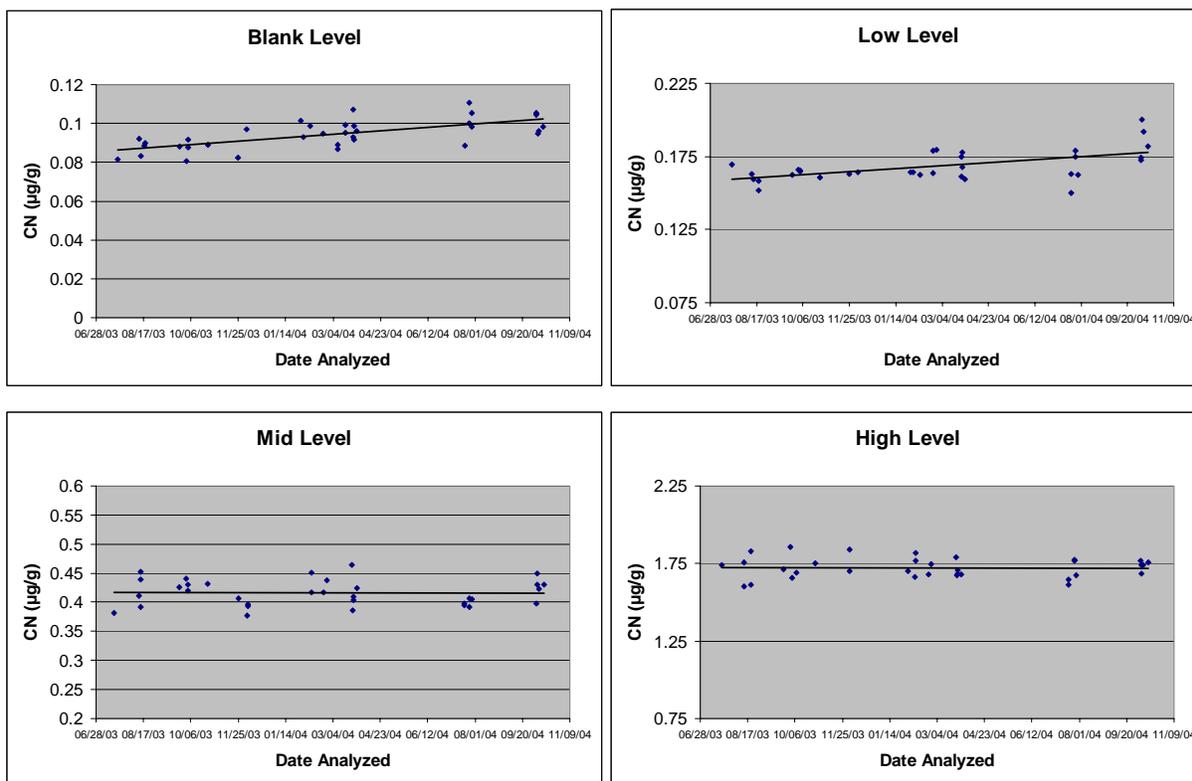


Figure 1. Plots showing the stability of CN in frozen blood-based proficiency standards over a 14-month period.

Title: International Comparisons in Electrochemical Analysis

Authors: K.W. Pratt

In FY 2004, NIST participated in three international comparisons in the area of electrochemical analysis: SIM.8.11P in pH measurement, CCQM-K34 in assay of potassium hydrogen phthalate (KHP), and CCQM-P47 in electrolytic conductivity.

SIM Pilot Study SIM.8.11P, piloted by CENAM, was a follow-up to the previous SIM.8.P4, which had been piloted by NIST in 2000. Five participants, including NIST, performed Harned cell pH methods (primary method). The NIST results were in excellent agreement (within ± 0.0017 pH) with other experienced NMIs (those that had participated in previous CCQM pH Key Comparisons CCQM-K9 or CCQM-K17). The NIST uncertainties, which included all known Type A and Type B sources, were equal to or smaller than those of the other participating NMIs in the primary measurement. The reduction in the NIST combined uncertainty resulted from a threefold reduction in the Type A uncertainty of the extrapolation of the acidity function to obtain p_a° , the acidity function in buffer without added chloride (from which the pH is directly obtained). This reduction was directly attributable to the implementation of pre-equilibration of the Ag|AgCl electrodes used in the Harned cells. The results of this Pilot Study correspond directly to the procedure for the certification of the phosphate pH SRM 186g and support the validity of its certification.

CCQM Key Comparison CCQM-K34 was designed to evaluate the agreement obtainable for the assay of potassium hydrogen phthalate (KHP) using high-accuracy assays. The seven participants each used coulometry. The NIST result and its estimate of uncertainty agreed well with those of the other “experienced” NMIs (who had participated in the corresponding pilot study, CCQM-P36). In addition, the NIST estimate of uncertainty was more complete than those submitted by all but one of the other participants (one included the same set of sources), in that more possible sources of uncertainty were included in the estimate.

CCQM Pilot Study CCQM-P47 evaluated the performance of NMIs in the measurement of electrolytic conductivity of two KCl solutions of nominal conductivity equal to 50 mS/m (500 $\mu\text{S}/\text{cm}$) and 5 mS/m (50 $\mu\text{S}/\text{cm}$). For the 5 mS/m solution, the pilot laboratory (NMI, Netherlands) discovered a time-instability in the solution as stored in the bottles used for distribution of the solution. Following discussion of the original results among the participants in April 2004, the pilot laboratory, NMI (Netherlands), decided to institute a time-dependent correction of the reference value. For the 50 mS/m solution, the NIST result was initially higher than the reference value. Following discussion at the CCQM Electrochemical Analysis Working Group, April, 2004, the cell calibration procedure used at NIST was modified to eliminate the non-IUPAC primary calibrants that previously had been used. With this modification, the NIST result for the 50 mS/m solution was in excellent agreement with the reference value.

In addition to the above three comparisons, NIST also completed and submitted to the CCQM the Final Report of Pilot Studies CCQM-P19/P19.1, Assay of 0.01 mol kg⁻¹ Hydrochloric Acid, in which NIST was the pilot laboratory. The analyses for the two phases of this Pilot Study had

been completed in preceding years, with 14 participants from NMIs worldwide. The submission of the Final Report formally completes this study and constitutes the official record of its results.

Title: Standards Development and Measurements to Support Global Climate Change

Author: G.C. Rhoderick

Abstract: Several species of gases found in the atmosphere that can contribute change to the radiative environment of the earth have been developed as gaseous primary standards (PSMs). These gases are considered greenhouse gases and have been monitored throughout the atmospheric environment community worldwide for many years. These gases have also gained increased importance since the Kyoto protocol was designed and implemented to promote reduced emissions of greenhouse gases. PSMs were developed previously for methane (CH₄), carbon dioxide (CO₂), tetrafluoromethane (CF₄), nitrous oxide (N₂O), and sulfur hexafluoride (SF₆).

This past year an additional compound, 1,1,1,2-tetrafluoroethane (Halocarbon 134a), was studied and standards developed for the State of California Air Resources Board (CARB). This compound is a replacement for halocarbons previously used as refrigerants but being phased out due to their high warming potential. Additionally, new PSMs were prepared at atmospheric levels and added to a suite of previously prepared standards. These compounds include carbon tetrachloride, chloroform, trichlorotrifluoroethane (CFC-113), 1,1,1-trichloroethane and trichloroethylene. NIST primary standards (PSMs) for global climate change measurements have served three purposes this past year. Firstly, CARB needed NIST traceable standards of 1,1,1,2-tetrafluoroethane for their ambient and automobile measurement programs. Secondly, our laboratory participated in three comparison studies for PSMs. One study, CCQM-P41 Greenhouse Gases, involves several national metrology institutes (NMIs) and laboratories from the World Meteorological Organization (WMO) in an effort to evaluate and improve the preparation and measurement capability of gravimetrically prepared methane and carbon dioxide mixtures. A second separate study in collaboration with NOAA/CMDL compared methane PSMs. The third was also an international comparison, CCQM-K15, for CF₄ and SF₆.

Purpose: PSMs were developed to support the in-house standards base for SRM certification, international intercomparison programs between national NMIs to determine equivalence, the NIST FTIR spectral database project and the International Halocarbon Experiment (IHALACE) program sponsored by the WMO, NOAA and NASA.

Major Accomplishments: A total of 22 gravimetrically prepared CH₄ in air primary standards, now exist and are used to define the NIST primary calibration methane scale for laboratory measurements and traceability. The entire suite of primary standards range in concentrations from 0.8 μmol/mol to 10 μmol/mol. Eight of these PSMs also contain nitrous oxide, dichlorodifluoromethane and trichlorofluoromethane. Two PSMs were submitted by NIST for evaluation in the CCQM-P41 Greenhouse Gas comparison study. One PSM contains methane in air and the second mixture contains carbon dioxide in air. Three PSMs containing methane in air were also compared to methane in air PSMs prepared at the Climate and Diagnostics Laboratory at NOAA. A suite of three standards containing CF₄ and SF₆ were prepared to value assign the CCQM-K15 sample. A suite of four standards containing carbon tetrachloride, 1,1,1-trichloroethane, chloroform, trichlorotrifluoroethane and trichloroethylene in air and five standards prepared in nitrogen containing the same compounds now exist at NIST. This suite of

primary standards range in concentrations from 5 pmol/mol (ppt) to 100 pmol/mol. The PSMs containing these halogenated species can now be used to analyze real air samples as part of the IHALACE program.

Impact: Development of these PSMs will underpin the SRM program and serve to establish equivalency between national NMIs and atmospheric research measurement laboratories.

Future Plans: In 2005, these new PSMs containing halogenated species will be used to analyze and determine concentrations in unknown real air samples from remote sensing sites as part of the IHALACE program; an international comparison between research laboratories that make real air measurements and/or prepare their own PSMs. Also, it is anticipated that PSMs will be developed for many species that contribute to global warming. These PSMs will lay the foundation for which atmospheric measurements can be underpinned and possible development of new SRMs to support national ambient monitoring measurements of those species.

Title: Development of Ephedrine Alkaloid-Based Dietary Supplement Standard Reference Materials (SRMs)

Authors: L.C. Sander, K.E. Sharpless, J. Brown Thomas, B.J. Porter, T.A. Butler, M. Satterfield, S.E. Long, L.A. Mackey, K.E. Murphy, L.J. Wood, R.D. Vocke, L.L. Yu, and S.A. Wise

Abstract: A suite of five ephedra-containing dietary supplement Standard Reference Materials has been issued with certified values for ephedrine alkaloids, synephrine, caffeine, and selected toxic trace elements. The materials represent a variety of natural, extracted, and processed sample matrices, which provide different analytical challenges. The constituents have been determined by multiple independent methods with measurements performed by NIST and by three collaborating laboratories. The methods utilized different sample extraction and cleanup steps in addition to different instrumental analytical techniques and approaches to quantification. In addition, food-matrix proximates were determined by National Food Processor Association (NFPA) laboratories, for one of the ephedra-containing SRMs. The SRMs are primarily intended for method validation and for use as control materials to support the analysis of dietary supplements and related botanical materials.

Purpose: The enactment of the Dietary Supplement Health and Education Act (DSHEA) in 1994 by the U. S. Congress has promoted growth in the nutritional supplement industry, due in part to the way in which dietary supplements are regulated. DSHEA provides a legal definition of dietary supplements, which classifies these materials separately from food additives and pharmaceutical drugs. Requirements for product labeling are less stringent than for drug substances, and the burden of proof for the safety of dietary supplements is placed on the Food and Drug Administration (FDA). In December 2003, the FDA issued a ruling that declared dietary supplements that contain ephedrine alkaloids to be adulterated. This ruling was based on mounting evidence of health risks associated with the use of ephedra, and in effect bans the use of ephedrine alkaloids (regardless of their botanical origin) in dietary supplements. Ephedra-containing dietary supplement SRMs are intended for use in method validation and as control materials for analytical methods used in the determination of ephedrine alkaloids, and should prove to be useful to support such methods and to demonstrate the absence of ephedrine alkaloids in ephedra-free products.

Major Accomplishments: NIST, working in collaboration with the National Institutes of Health Office of Dietary Supplements (NIH-ODS) and FDA, Center for Drug Evaluation and Research (CDER) and Center for Food Safety and Applied Nutrition (CFSAN), has recently issued a suite of Standard Reference Materials® (SRMs) that contain ephedra. Five SRMs are available: SRM 3240 *Ephedra sinica* Stapf Aerial Parts, SRM 3241 *Ephedra sinica* Stapf Native Extract, SRM 3242 *Ephedra sinica* Stapf Commercial Extract, SRM 3243 Ephedra-Containing Solid Oral Dosage Form, and SRM 3244 Ephedra-Containing Protein Powder. In addition, SRM 3245 Ephedra Suite is available and contains two bottles each of the five ephedra-containing materials. The SRMs are certified for levels of ephedrine alkaloids and selected toxic trace elements (As, Cd, Hg, and Pb). In addition, the level of synephrine (an ephedrine-like alkaloid present in many ephedra-free dietary supplements) is certified in SRM 3243, and levels of caffeine are certified in SRM 3243 and SRM 3244. Information on proximates (i.e., moisture, solids, ash, protein, carbohydrates, fat) and nutrient elements is also included with SRM 3244.

Impact: SRMs 3240 through 3244 represent the first in a series of dietary supplement SRMs to be offered by NIST with certified values for organic constituents and selected trace elements. These materials are provided primarily for use in method development and as control materials to support analytical methods for the determination of these constituents. In the absence of a formal regulatory environment, the SRM suites will assist manufacturers of dietary supplements to characterize raw materials voluntarily and to prevent the use of materials that are contaminated or adulterated. In addition, the SRMs will assist self-assessment of consistency and quality in finished products. The goal of this ongoing effort is to provide tools to the dietary supplement industry and measurement communities that will lead to improved quality of dietary supplements, and ultimately reduce public health risks that could potentially be associated with these products.

Future Plans: The development of dietary supplement SRMs is an ongoing effort. SRMs based on multivitamin/mineral tablets, *ginkgo biloba*, saw palmetto, bitter orange, green tea, St. John's wort, and carrots (carotenes) are in progress.



Figure 1. Photograph of voucher specimen representative of plants used in the preparation of SRM 3240 *Ephedra sinica* Stapf Aerial Parts

Title: AnIML—Analytical Information Markup Language

Authors: B.A. Schaefer, D. Poetz, A.D. Nguyen, and G.W. Kramer

Abstract: In collaboration with ASTM Subcommittee E13.15 on Analytical Data, we are creating an extensible markup language (XML) for analytical chemistry result data. Based in part on our previous SpectroML markup language for UV/vis result data, this ASTM effort is being called the Analytical Information Markup Language (AnIML). It is specifically designed for spectroscopy and chromatography data, but its design is generic and modular, making it suitable for use with many different analytical measurement techniques--including those not yet invented.

Purpose: The interchange and storage of analytical chemistry data has long been hampered by multiple, incompatible data formats. Over the past 15 years, several different interchange formats have been developed that allow data generated by different vendor systems of a given technique to be exchanged. For example, using JCAMP-DX, users of most commercial FT-infrared and NMR systems can interchange their respective data. However, in the last few years, the emergence of platform and application independence through the Internet has advanced the possibilities for data interchange beyond the capabilities of these protocols. Today's analytical chemists need not only to interchange data generated within a specific technique, they need to interchange, import, export, store, and combine all their data from multiple sources at multiple sites. Users are now no longer content with just having "pictures" of their data (i.e., data plots). They need real access to their data from within a report itself—they want to be able to expand plots, re-integrate peaks, overlay spectra, etc. without having to revert to the instrument or computer system that generated the original data. And, they don't want to have to purchase, learn, or even load proprietary programs to do this. In industrial settings, experimental data belong to and must be accessible to the entire organization, not just the laboratory personnel that generate it. Proprietary data formats and existing interchange programs simply cannot fill such corporate needs for data accessibility

Major Accomplishments: Over the past three years, we developed an XML-based mechanism for instrument-to-instrument, instrument-to-application, and application-to-application data interchange called SpectroML: an extensible markup language for molecular spectroscopy data. SpectroML was created initially to serve as a model implementation of a markup language for molecular spectroscopy and ultimately to provide a web-based mechanism for interchanging UV/visible spectral data generated on different spectrophotometers with our optical filters database. When our optical filters database was created as a replacement for paper records, we could import data only from the High Accuracy Spectrometer (HAS) used to certify the SRM optical filters. The complex and archaic data importation process badly needed to be modernized, and we wanted to be able to import data from our other spectrometers as well. Using SpectroML drivers/translators for the HAS-II, the PerkinElmer Lambda 900, and the Hewlett-Packard HP 8453 spectrometers, data from these instruments can now be converted into a single format SpectroML file and imported into our optical filters database. SpectroML is an integral part of the process for certifying and recertifying optical filter SRMs; plus, we can import research data taken on machines other than the HAS for comparison purposes.

Even before we developed SpectroML, we began to proselytize both instrument vendors and the ASTM E13 Molecular Spectrometry and Chromatography Committee about the merits of an XML-based approach to data interchange. In 2001, the ASTM E13 committee established a task group to develop a markup language for molecular spectrometry and chromatography, and in 2003 the task group was made into a full ASTM subcommittee—E13.15 on Analytical Data.

E13.15 has been working with instrument vendors and the IUPAC Committee responsible for JCAMP-DX to develop a unified XML-based approach for interchanging molecular spectrometry and chromatography data called the Analytical Information Markup Language (AnIML). AnIML is based on a hierarchical model that calls for the initial development of a core markup language containing the elements common to all applicable analytical techniques. The core schema defining the common notions of sample information, measurement data, system information, quality assurance, etc. will be handled by ASTM E13.15. Technique-specific base documents will build on the core and will be the responsibilities of the respective E13 subcommittees in conjunction with other interested expert groups such as those from the IUPAC, American Society for Mass Spectrometry, etc. Data/instrument vendors are responsible for implementations of the instrument/origin specific base document extensions that will build on both the core and the technique specific standards. Higher-level specifications could include organization-specified extensions that are company-specific and even end-user or application-specific extensions. The core is being built as much as possible to bridge previous data exchange standards work (e.g., ANDI and JCAMP-DX), and efforts are being made to collaborate with and include other organizations with similar interests and as much as possible to reuse terminology and concepts from existing standards.

Over the past year, the schemas for the core and technique definitions have been written and base documents for several techniques have been created. Since the metadata for individual techniques will be maintained in instance documents instead of schemas (to permit extension of technique metadata) the standard mechanism that applications use to validate XML documents syntactically cannot be used. Accordingly, we created a Technique Validator program that provides semantic and syntactical checking as well as some bounds/limits checking for AnIML files. We are now working with several groups of domain experts to develop AnIML. To solve the problem that most of the domain experts do not know XML, we have written a Technique Creator program that guides a user through the creation of a technique base document and then creates the XML code.

Impact: The development of AnIML as a universal molecular spectrometry and chromatography mark up language will revolutionize data interchange, eliminate long standing barriers to data exchange, enable new data manipulation software, facilitate new web-based notions for data usage, and permit the development of realistic schemes for long-term data archival. When coupled with parallel efforts in developing XML approaches to the manipulation of other chemical data, it will pave the way for the fully electronic chemistry data archive, where any known chemical fact or information is but a simple query away on your wireless Personal Digital Assistant from anywhere in the world.

Future Plans: Currently, work is underway in our Group to implement the AnIML core schema and to develop an example implementation of a technique-specific AnIML schema for liquid

chromatography photodiode array spectra. If these projects are successful, we can begin implementing AnIML for other techniques. The IUPAC committee has already converted their JCAMP-DX terminology to XML so progress should be rapid for those techniques supported by JCAMP-DX. The AnIML project also dovetails nicely with another IUPAC project to convert the IUPAC units and terminology documents (Green and Gold books) to XML.

Title: New Standard Reference Material (SRM) for Organic Contaminants in House Dust

Authors: M.M. Schantz, J.M. Keller, J.R. Kucklick, D.L. Poster, H.M. Stapleton, S. Vander Pol, and S.A. Wise

Abstract: House dust is a repository of pesticides and other chemicals used indoors or tracked in from outdoors. NIST has produced two Standard Reference Materials (SRM 2583 and SRM 2584) for lead and other trace elements in a house dust matrix. SRM 2585, Organic Contaminants in House Dust, will be issued with concentration values assigned for pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCB) congeners, and polybrominated diphenyl ether (PBDE) congeners.

Purpose: House dust was collected and analyzed in survey studies, such as the National Health Exposure Assessment Study (NHEXAS), as one indicator of indoor exposure. Pesticides associate with house dust primarily through interior use of pest control formulations, vapor intrusion from foundation and crawl space treatments, and tracking-in of lawn and garden chemicals. PAHs derive from indoor sources such as combustion, cooking, and smoking, and from track-in of contaminated yard soil or residues from garage floors. PBDEs are commonly used flame retardant compounds added to many plastics, resins and textiles which are then incorporated into products including TVs, computers, furniture and carpets. A house dust reference material with values assigned for a wide range of organic contaminants is needed for quality control in these measurements.

Major Accomplishments: Over 100 contaminants, including pesticides, PAHs, PCB congeners, and PBDE congeners, will be value assigned in SRM 2585 using an approach similar to that used for other natural matrix reference materials, such as mussel tissue¹ and sediment². Three methods were used to quantify a number of pesticides, PAHs, and PCB congeners in the house dust. Using one of the methods, the material was found to be homogenous at a 1 g sample size. *Cis*- and *trans*-chlordane are present in the material at concentrations between 150 µg/kg and 250 µg/kg. Fluoranthene and pyrene are present in the material at concentrations between 3000 µg/kg and 4500 µg/kg. The individual PCB congeners are lower in concentration (<50 µg/kg for each congener). Two methods were used to value assign the PBDE congeners in SRM 2585. PBDE 209 was the dominant PBDE congener in SRM 2585 at a concentration of 2600 µg/kg.

Impact: The house dust SRM 2585 will provide a control sample for laboratories routinely monitoring indoor dust. With the increased interest in the PBDE concentrations in all types of environmental samples, the assignment of concentrations for this group of compounds in many different matrices, particularly those associated with human exposure, will be important.

Future Plans: As methods are developed for additional groups of chemicals important in human exposure studies, the house dust SRM will be analyzed for these compounds to expand its utility.

1. Poster, D.L., Schantz, M.M., Kucklick, J.R., Lopez de Alda, M., Porter, B.J., Pugh, R., and Wise, S.A., "Three New Mussel Tissue Standard Reference Materials (SRMs) for the Determination of Organic Contaminants," *Anal. Bioanal. Chem.*, **378**, 1213-1231 (2004).

2. Wise, S.A., Poster, D.L., Schantz, M.M., Kucklick, J.R., Sander, L.C., Lopez de Alda, M., Schubert, P., Parris, R.M., and Porter, B.J. "Two New Marine Sediment Standard Reference Materials (SRMs) for the Determination of Organic Contaminants," *Anal. Bioanal. Chem.* 378, 1251-1264 (2004).

Title: Ethanol in Water

Author: M.M. Schantz

Abstract: Accurate calibration of instrumentation is critical in areas of forensic testing where quantitative analysis directly affects criminal prosecutions, as is the case with the determination of ethanol in blood and breath. Blood- and breath-alcohol testing can be imposed on individuals operating private vehicles such as cars, boats, or snowmobiles, or operators of commercial vehicles like trucks, planes, and ships. Two new ethanol in water SRMs, SRM 1828b and SRM 1847, with six and three concentration levels, respectively, have been issued to replace the previous SRM 1828a, which had only four concentrations levels.

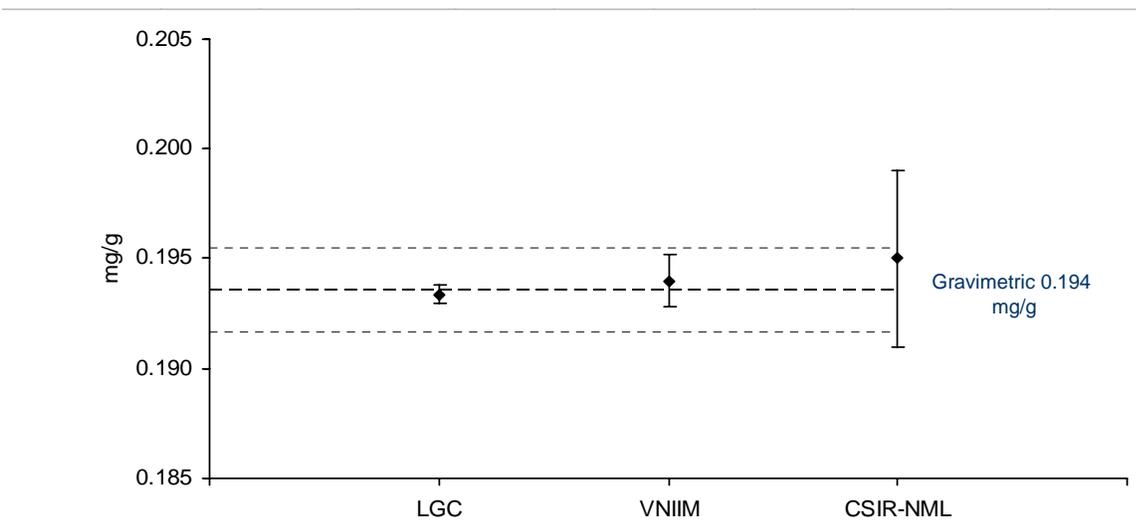
Purpose: The various levels of blood-alcohol that determine whether these operators are considered legally impaired vary depending on the circumstances, state, and even month in which the testing is occurring. As a result, practitioners in the field of alcohol testing have a need for reliable and stable standards at several concentrations. Most blood alcohol levels in driving under the influence (DUI) cases fall in the range of 0.1% to 0.3% (the average blood alcohol for a DUI traffic stop is 0.16 % to 0.17%). By providing SRMs with concentration levels set to legally relevant points, the accuracy of blood- and breath-alcohol testing will be improved.

Major Accomplishments: The concentration levels in SRM 1828b, Ethanol-Water Solutions (Blood-Alcohol Testing: Six Levels), have been tailored to legally relevant points, specifically 0.02% and 0.04% for “zero tolerance” and occupational alcohol testing, 0.08% and 0.1% for state drunk driving laws, and 0.2% and 0.3% for an average and high level for blood alcohol measurements. In addition, three concentration levels of ethanol in water (2%, 6%, and 25%) have been prepared as SRM 1847, Ethanol-Water Solutions (Breath-Alcohol Testing: Three Levels), for use as reference solutions for breath-alcohol instruments. The SRMs were prepared gravimetrically, and the concentrations of ethanol in water were confirmed at NIST by using gas chromatography with flame ionization detection, the analytical method of choice for blood- and breath-alcohol testing in the forensic laboratory. Because some laboratories, particularly in California, are required to verify their primary standards of ethanol in water by using titrimetry, the National Metrology Laboratory (CSIR-NML) in Pretoria, South Africa provided measurements for each of the nine concentrations of ethanol in water using a titrimetric method, shown to be very precise and accurate. The National Analytical Reference Laboratory (NARL) in Sydney, Australia (another national metrology laboratory) also provided measurements for four of the solutions (0.08%, 0.1%, 0.2%, and 6%) using an exact matching isotope dilution-gas chromatographic method, also demonstrated to be a very precise and accurate method. The certified concentrations of ethanol in water the SRMs are based on a combination of the gravimetry, NIST, CSIR-NML, and NARL measurements. The relative expanded uncertainties for the certified concentrations are less than 1.2% for each concentration level.

Impact: The redesigned concentration levels in SRM 1828b and SRM 1847 have made these SRMs more useful to the forensic community for use as reference solutions for breath-alcohol instruments.

Future Plans: An additional solution of the ethanol in water azeotrope will be made available in FY05 as SRM 2900. Feedback from customers indicated that the azeotropic mixture is needed in addition to those levels now available in SRMs 1828b and SRM 1847 for use as a starting point in the preparation of the customers' working calibration solutions.

Results for CCQM-K27-Subsequent study level 1 showing gravimetric value and upper and lower limits of the expanded uncertainty of the gravimetric value based on the CCQM-K27a study



Title: Critical Reference Materials for Mineral Commodities

Authors: J.R. Sieber, A.F. Marlow, S.A. Wilson (USGS), and J.T. Wolsiefer, Sr. (Silica Fume Assoc.)

Abstract: The Analytical Chemistry Division has long supported United States industries that rely on mineral resources. SRM 1d Argillaceous Limestone exemplifies this long-term commitment. Versions of SRM 1 have been provided by NBS/NIST since 1910. In contrast, new SRM 2696 Silica Fume exemplifies our responsiveness to new technology. SRM 2696 was developed to support manufacture and use of this high-grade, small particle size silica used to improve the strength and durability of concrete. Silica fume, a by-product of metals refining, metamorphosed from industrial waste to an important commodity in the past five to ten years.

Impact: Mineral wealth has always been a key element of a successful economy. As technology evolves, new mineral resources are exploited. However, older industries like construction and transportation still extract huge quantities of minerals. Over the years, the quality demands of these older industries have become more stringent, and their laboratories continue to find better ways to analyze raw materials and by-products. Consequently, they still need SRMs for the minerals they've been using for many years, but now, they need higher quality information. Plus, they need SRMs for byproducts, which are transformed into useful products ever more frequently as sustainability becomes a key element of economic planning.

SRM 1d is the fourth version of this important material. Argillaceous limestone – meaning ‘containing clay’ – is a critical natural resource. Besides its use as a building material, it is used to manufacture lime for agricultural and chemical processes, cement and concrete, and iron and steel. ASTM International Committee E01 on Analytical Chemistry of Metals, Ores, and Related Materials, Committee C01 on Cement, Committee C07 on Lime and Limestone, Committee C09 on Concrete and Concrete Aggregates, and the National Lime Association expressed support for renewal of SRM 1c. Like its predecessors, SRM 1d will be used extensively for validation of ASTM International and in-house test methods at hundreds of laboratories around the world.

Standard Reference Material 2696 Silica Fume is the culmination of collaboration by the Silica Fume Association and NIST with support from the Federal Highway Administration. SRM 2696 is intended primarily for evaluating chemical and instrumental methods of analysis of silica fume to satisfy product specifications. Certified values were established for silicon (as SiO₂) and six other chemical constituents, plus reference values for five constituents and Specific Surface Area by nitrogen absorption. All values are products of extensive testing by NIST and collaborating laboratories from manufacturers, distributors, state transportation departments, universities, and commercial laboratories. This allows us to leverage their expertise without the investment needed to develop it ourselves.

High-Performance Concrete containing silica fume can have very high compressive strength and durability due to low permeability. Reduced permeability helps prevent spalling due to the corrosion of steel reinforcing bars inside the structure following the infiltration of chlorine from salt. Silica fume is a byproduct of producing silicon metal and ferrosilicon alloys. No longer discarded in landfills, more and more silica fume is being used in concrete instead. Increasingly,

state highway and transportation administrations require high-performance concrete in bridges and roadways. In response to the demand and to protect against the use of contaminated material, standard-writing organizations around the world are implementing specifications for the chemical composition and physical properties of silica fume.

Accomplishments: Both SRM 1d and SRM 2696 were certified using X-ray fluorescence spectrometry (XRF) with borate fusion sample preparation in the matrix-independent approach developed during an Exploratory Research project several years ago [1,2]. Certified values were assigned after combining NIST XRF results with results from the private laboratories.

SRM 1d is certified for 12 constituents, with four reference values and 28 information values. In contrast, SRM 1c was certified for 11 chemical constituents. Table 1 provides a side-by-side comparison of SRM 1c and SRM 1d to illustrate the improvements in analytical uncertainty over a span of 25 years.

Future Plans: In the coming year, the level of activity in support of mineral commodities will remain at about the same level. Ongoing projects include the renewal of SRM 331 Copper Ore Mill Tails in support of the copper mining and refining industry and the renewal of SRM 57a, Silicon Metal in support of silicon refiners and their customers. In addition, plans are in place to begin renewals of three high-demand soil SRMs – SRMs 2709, 2710, and 2711.

References:

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Table 1. Comparison of Values and Uncertainty Estimates^a for Limestone SRMs.

Constituent	SRM 1c	SRM 1d
	December 1978	October 2004
SiO ₂	6.84 ± 0.16 (2.3 %) ^b	4.080 ± 0.071 (1.7 %) ^b
Fe ₂ O ₃	0.55 ± 0.06 (11 %)	0.3191 ± 0.0068 (2.1 %)
Al ₂ O ₃	1.30 ± 0.06 (4.6 %)	0.526 ± 0.013 (2.5 %)
TiO ₂ ^c	0.07 ± 0.02 (29 %)	0.0306 ± 0.0065 (2.1 %)
P ₂ O ₅	0.04 ± 0.02 (50 %)	0.0413 ± 0.0025 (6.1 %)
MnO ^d	0.025 ± 0.01 (40 %)	0.0105 ± 0.0003 (2.9 %)
CaO	50.3 ± 0.6 (1.2 %)	52.85 ± 0.16 (0.3 %)
SrO	0.030 ± 0.01 (33 %)	0.0303 ± 0.0010 (3.3 %)
MgO	0.42 ± 0.08 (19 %)	0.301 ± 0.010 (3.3 %)
Na ₂ O	0.02 ± 0.02 (100 %)	0.0109 ± 0.0016 (1.5 %)
K ₂ O	0.28 ± 0.02 (7.1 %)	0.1358 ± 0.0046 (3.4 %)
S	Not determined	0.1028 ± 0.0062 (6.0 %)
ZnO	Not determined	0.0022 ± 0.0003 (14 %)

^a Due to changes in definitions since 1978, the uncertainties for SRM 1c were multiplied by an expansion factor of 2 to make them comparable with SRM 1d.

^b The relative uncertainty is given in parentheses.

^c This constituent is a reference value for SRM 1d.

^d Mn is certified as the element in SRM 1d.

Title: Method Development and Measurements of Polybrominated Diphenyl Ethers (PBDEs) in Tissue, Serum, and Sediment Standard Reference Materials (SRMs)

Authors: H.M. Stapleton, J.M. Keller, M.M. Schantz, and S.A. Wise

Abstract: A gas chromatography/mass spectrometry (GC/MS) method has been developed to quantify the flame retardants Polybrominated Diphenyl Ethers (PBDEs) in a variety of existing natural matrix Standard Reference Materials (SRMs). The GC/MS method employs on-column cool injection to compensate for the thermal instability of some of the PBDE compounds. Using this method, the full range of PBDE congeners can be examined in tissue, human serum, and sediment matrices. Measurement of these compounds in these SRMs will be used to assign certified values for the concentrations of individual PBDE congeners.

Purpose: PBDEs are flame retardant compounds that are commonly added to many plastics, resins and textiles that are then incorporated into products such as TVs, computers, furniture, and carpets. PBDEs can volatilize or leach out of the products in which they are applied and be transported long distances in the environment, due to their physico-chemical properties. PBDEs are now considered ubiquitous environmental contaminants and much attention has been focused on their transport, uptake, and fate in both humans and the environment. Presently, there is an increased need for measurements of PBDEs in environmental matrices and human serum. SRMs with certified concentrations for individual PBDE congeners are currently unavailable, and they are needed to ensure quality control for these measurements.

Major Accomplishments: Ten existing natural matrix SRMs have been analyzed for determination of a suite of 26 PBDE congeners. These SRMs include marine mammal tissue, fish tissue, mussel tissue, human serum, marine sediment and house dust. The GC/MS method, which uses on-column cool injection, has allowed for the measurement of the fully brominated PBDE congener (2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether; BDE 209) using an isotope dilution quantification approach. This congener has often been difficult to measure due to its thermal instability. BDE 209 was observed to be the dominant PBDE congener in the sediment and house dust SRMs, and thus, these SRMs will be useful for laboratories interested in routine measurements for BDE 209.

Impact: The development of the GC/MS method and the certification of PBDEs in these SRMs have increased the usefulness of these existing NIST SRMs to laboratories that routinely measure environmental organic contaminants. Legislation in the U.S. is now being considered to reduce the use of PBDEs in consumer products. If this legislation is passed, numerous corporations that produce PBDE-laden products (i.e. Motorola, Dell, Apple) will be affected. These companies have expressed interest in having SRMs to test their products to ensure that they conform to these regulations.

Future Plans: Other flame retardants such as hexabromocyclododecane (HBCD) are now being used at higher rates as a replacement for PBDEs in some parts of the world. As such, there is interest in the measurements of HBCD in environmental samples. Additionally, evidence suggests that PBDEs may be metabolized by some organisms to hydroxylated and methoxylated derivatives. Preliminary evidence already suggests that methoxylated PBDEs (MeOBDEs) are

present in the marine mammal blubber and cod liver oil SRMs. Measurements are planned for HBCD and MeOBDEs in these environmental matrix SRMs to provide reference values for these new flame retardant related compounds.

Title: New Gas Standards for Calibrating Instrumentation used for Measuring Emissions from Next Generation Low Emission Vehicles

Author: W.J. Thorn III

Abstract: Stakeholders in the American Industry/Government Emissions Research (AIGER) group are working together to facilitate the automobile industry meeting more stringent 2003 Federal Tier II and California LEV II emission regulations. AIGER members include the U.S.EPA, California Air Resources Board (CARB), General Motors, Ford, and Daimler-Chrysler. In 1998, NIST worked with a Specialty Gas contractor to blend two cylinders each of low NO standards at concentrations of 0.5 $\mu\text{mol/mol}$, 0.75 $\mu\text{mol/mol}$, 0.95 $\mu\text{mol/mol}$, 1.05 $\mu\text{mol/mol}$ and 1.25 $\mu\text{mol/mol}$. The new standards have exhibited excellent NO concentration stability for more than 5 years and final concentration values with associated uncertainties have been recently assigned. In 2001, AIGER provided direct funding to a NIST contractor to work jointly with NIST to produce two new low NO SRMs at concentrations of 0.5 $\mu\text{mol/mol}$ and 1.0 $\mu\text{mol/mol}$. NIST and its contractor followed the “roadmap” used to prepare the ten low NO working Standards to blend forty cylinders of 0.5 $\mu\text{mol/mol}$ and forty cylinders of 1.0 $\mu\text{mol/mol}$ NO in nitrogen. Twenty-seven candidate cylinders of each new SRM (54 total) were given to NIST in January, 2002 to begin SRM certification analyses, which as of September, 2004 have been completed. The data analysis and completion memo should be issued by late December, 2004.

Purpose: NIST and the U.S. Motor Vehicles Manufacturers have worked together since 1975 to develop sixty (60) gaseous Standard Reference Materials, which are the Nation’s benchmarks against which all U.S. EPA mandated fuel economy and mobile source emission measurements must be traceable; by federal law. NIST currently supports AIGER members by maintaining reasonable inventories of required gas SRMs that consist of dilute mixtures of key pollutants such as hydrocarbons, carbon monoxide and nitric oxide. Newer vehicles produce lower levels of these pollutants because their engines employ fuel injection whose air to fuel ratio is optimized by an on-board computer, and their emissions are further reduced by more efficient catalytic converters. During testing the exhaust levels are diluted by the use of constant volume sampling bags or by new mini-diluter technology. AIGER stakeholders have identified the need for significantly lower NIST gas standards containing carbon monoxide, hydrocarbons and nitric oxide; with the completion of SRM 2737 (0.5 $\mu\text{mol/mol}$) and SRM 2738 (1.0 $\mu\text{mol/mol}$) nitric oxide in nitrogen being their highest priority. NIST is currently exploring working standards below 0.1 $\mu\text{mol/mol}$.

Major Accomplishment: NIST has assigned final values and uncertainties to ten low NO working standards after monitoring their stability for more than five years. These stable NIST working standards were then used to plot NO and NO_x calibration curves against which NO and NO_x concentrations were assigned to twenty-seven candidate SRM 2737 (0.5 $\mu\text{mol/mol}$ NO) cylinders and twenty-seven candidate SRM 2738 (1.0 $\mu\text{mol/mol}$ NO) cylinders with final values assigned in September, 2004; two years after blending. All candidate SRM cylinders were observed to be stable.

The new SRMs will be completed by December 31, 2004.

Impact: The project has developed a technical solution to NIST's long standing low NO SRM stability problems and provided AIGER members with interim standards to work with. Finally the project will provide too much needed lower concentration NIST certified NO SRMs in the next few months. These new SRMs will help facilitate vehicle manufacturers in meeting U.S.EPA and CARB's current and future lower emission regulations.

Future Plans: NIST will complete data analysis and report generation for SRM 2737 and SRM 2738 by December, 2004. NIST is continuing to move NO analytical standards and measurement capability lower to below 0.1 $\mu\text{mol/mol}$; defined as a high priority future requirement by AIGER.

Title: Leveraging Traceability for Chemical Spectrophotometry Through the Commercial Sector

Authors: J.C. Travis, M.V. Smith, M.D. Maley, and G.W. Kramer

Abstract: The controlled termination of a long-standing program for the individual certification of spectrophotometric absorbance standards will capitalize on the International Organization for Standardization (ISO)/NIST description of traceability in order to stimulate a more efficient and leveraged model for supplying certified reference materials (CRMs) for absorbance.

Purpose: The Analytical Chemistry Division (ACD), recognizing that the NIST model has been successfully emulated in commercially-produced traceable CRMs, is terminating the production of individually-certified neutral-density filter Standard Reference Materials[®] (SRMs[®]) for chemical spectrophotometry. Sales of SRMs 930e, 1930, and 2930 ($0.001 < T < 0.9$, neutral-density glass filters for the visible spectrum) will cease when existing stocks are depleted. Production of SRM 2031a ($0.1 < T < 0.9$, metal-on-fused-silica neutral-density filters for the UV and visible spectral regions) will be curtailed to end sales on schedule with the glass filters.

End users are encouraged to purchase CRMs from secondary suppliers who are appropriately accredited to Guide 17025 or Guide 34 of the ISO, or who possess documentation consistent with the NIST traceability policy. Such commercial suppliers may also recertify expired NIST SRMs, although NIST ACD will continue to offer recertification for the 3500+ sets of SRM filters in the field.

Major Accomplishments: In a related move, ACD transmittance and absorbance measurements, used in solid filter recertification and future batch SRM production, will soon feature traceability through the regular transmittance scale maintained by the NIST Optical Technology Division (OTD). This will result in a single U.S. national scale for regular spectral transmittance measurements conducted in support of both physical and chemical metrology. The OTD transmittance scale is routinely compared to other national transmittance scales through measurements coordinated by the Comité Consultatif de Photométrie et Radiométrie (CCPR) of the Bureau International des Poids et Mesures (BIPM).

Impact/Future Plans: In the long term, these changes are expected to benefit affected industries (e.g. pharmaceuticals, chemical products, and health technologies) by supporting a robust and responsive system of fit-for-purpose reference materials that are manufactured by competing commercial sources. The ACD is attempting to avoid near-term anxiety in the heavily regulated pharmaceutical sector by implementing extensive educational activities during the one-to-two year period required to sell out the stocks of these filters. Such publicity includes presentations at FACSS 2004 and EAS 2004 as well as on the NIST web site and anticipated announcements in trade magazines.

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Title: Technical Procedures for a NIST Traceable Clinical Reference Laboratory Network

Authors: G.C. Turk, S.E. Long, and D.L. Duewer

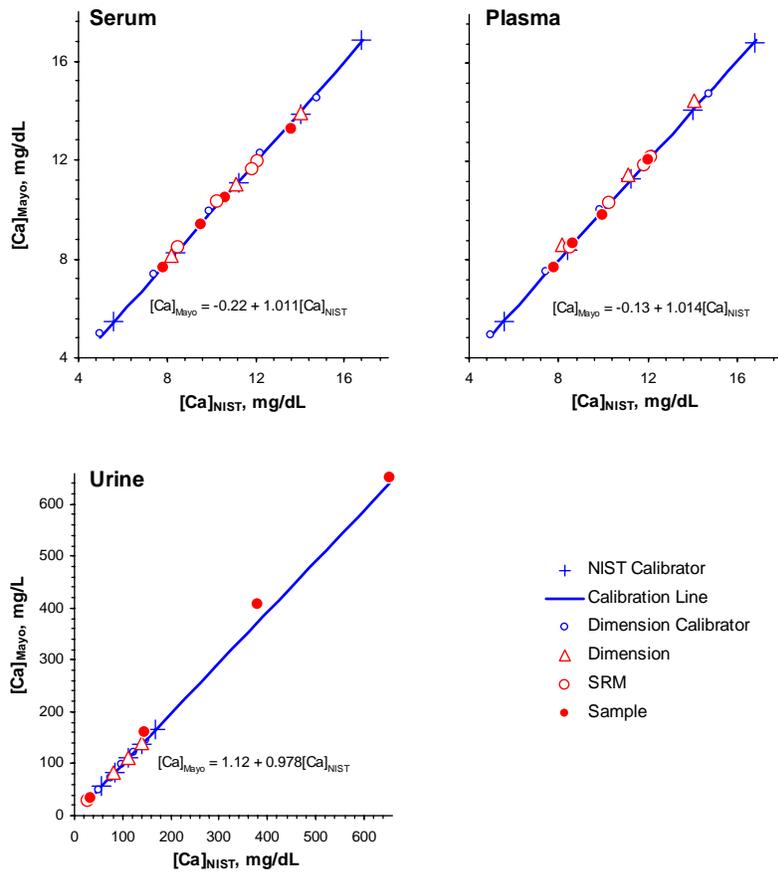
Abstract: In December of 2003 the European Union Directive on In-Vitro Diagnostics (IVD) went into effect mandating IVD traceability to “higher order” standards and methods. Sales of NIST electrolyte in serum SRMs have increased dramatically in response, as have the needs of U.S. IVD manufacturers for higher-order reference measurements. With the goal of building an efficient infrastructure for providing clinical reference measurements for IVD manufactures, NIST entered into a collaboration with Mayo Clinic and Dade-Behring, Inc. to test technical procedures that might be used to implement a NIST Traceable Clinical Reference Laboratory Network. Such a network could provide the IVD industry with reference measurements traceable to NIST standards in a timely and efficient manner. The specific test conducted involved the measurement of calcium at various levels in blood serum, plasma, and urine. A subset of real samples of serum, plasma, and urine were measured both at NIST and the Mayo Clinic, using NIST calibration and validation SRMs, in order to establish an equivalence between NIST isotope dilution mass spectrometry measurements and measurements performed at Mayo Clinic. Initial results have been encouraging, and could potentially lead to the development of a network of laboratories with an expanded analyte and matrix coverage.

Purpose: To design and test practical measurement procedures that could be used to establish traceability of non-NIST clinical reference measurements to NIST standards and measurements.

Major Accomplishments: This study was designed around the analysis of individual patient samples that were split and analyzed by both NIST and Mayo Clinic. The patient samples were pre-screened to cover the full range of calcium concentration for which the methods have been designed. All patient identification information was disassociated with the samples for this study. Further validation of the methods was provided by the analysis of NIST matrix SRMs by both NIST and Mayo. These SRMs include SRM 956a (Frozen Human Serum) and SRM 2670a (Freeze Dried Urine). In addition, both NIST and Mayo Clinic analyzed Candidate SRM 956b (Frozen Human Serum), which had not yet been certified, and for which the Ca concentration was unknown to Mayo Clinic at the time of the analysis.

The protocol called for Mayo Clinic to use their normal calibration procedures, based on their in-house supply of calibrator solutions. They also prepared a set of NIST Calibrators by dilution of SRM 3109a (Calcium Solution). Five NIST calibrators were prepared at concentrations similar to those of the Mayo Calibrators. Mayo used their normal setup and calibration procedures and then analyzed the samples included in the study using a run order supplied by NIST. Each sample, including the NIST calibrators, was treated as an “unknown”, and value assigned based on the normal Mayo calibration procedure with their in-house set of calibrators. The ratio of the determined values of the NIST calibrators relative to the prepared values using the NIST certified values was calculated. This value, and associated uncertainty, was then used to convert all of the Mayo determined values of the test samples (traceable to the Mayo calibrators) to values traceable to the NIST calibrators and SRM 3109a. The conversion shift was small, but

significant. The comparisons between NIST measurement results and those of the Mayo Clinic are illustrated graphically in the figure below for each sample matrix.



Impact: A NIST Traceable Clinical Reference Laboratory Network would become an important component within the clinical chemical measurement infrastructure, with a positive impact on the accuracy of such measurements and on the quality and cost effectiveness of our healthcare system.

Future Plans: Discussions are ongoing with our collaborators to evaluate the effectiveness and practicality of the approach used in this exercise. Possibilities for the future include formal development of the proposed laboratory network, and expansion to other analytes and matrices.

Title: Developing Trace Analytical Methods and Gas Standards for Ammonia in Nitrogen

Authors: T.W. Vetter and W.J. Thorn

Abstract: NIST has been asked to develop a primary method for assigning ammonia concentrations to gas standards in the 1.0 $\mu\text{mol/mol}$ to 100 $\mu\text{mol/mol}$ range. These standards are of interest to the U.S. EPA for mobile and stationary source monitoring; and of interest to specialty gas mixture vendors who service these applications. Preliminary measurements were made this year evaluating three primary methods on a series of ten ammonia working standards from 5 $\mu\text{mol/mol}$ to 640 $\mu\text{mol/mol}$. Ammonia concentrations for two of the working standards (40 $\mu\text{mol/mol}$ and 160 $\mu\text{mol/mol}$) were determined by a classical titrimetric method with relative expanded uncertainties of less than 1 %. All of the working standards were measured during an evaluation of a commercial ammonia instrument, which catalytically oxidizes ammonia to nitric oxide (>95 % efficiency) and then measures the nitric oxide by chemiluminescence. This method is of high interest because of the availability of a large quantity of nitric oxide primary standards in support of NIST SRMs. Additionally, all of the working standards were measured by FT-IR and a non-linear regression plot of the measured absorbances vs. concentration was developed. NIST has compared the ammonia values measured by the three analytical techniques and found that the 2 % to 4 % between the methods are difficult to resolve because the ten working standards of ammonia are decaying in concentration over time compared to their gravimetric preparation values.

Purpose: Natural gas turbines used to produce electric power generate significant NO_x and SO_x pollutants as emissions. Ammonia is added to the exhaust stream to neutralize the acid gases forming nitrate and sulfate salts, which are collected. The EPA regulations for continuous emission monitors (CEMs) require that ammonia not exceed 3 $\mu\text{mol/mol}$ excess in the stack emissions. Similarly, alternative fuels such as compressed natural gas (CNG) and hydrogen can generate ammonia in mobile source exhaust (cars and trucks), which must be monitored. Currently, there are no NIST SRMs or other reference materials available

Major Accomplishments:

1. NIST ammonia in nitrogen working standards: A contract was issued to BOC Gases to produce 14 ammonia in nitrogen calibration gas standards by gravimetric dilution. An FT-IR spectrum was run on each of the 14 mixtures analyzed. A regression plot of the measured ammonia absorbances as a function of the ammonia concentration resulted in a non-linear FT-IR response curve. Seven of the 14 mixtures were returned to BOC.

2. Titration primary method for determining ammonia: The ammonia concentration was determined in two working standards whose nominal levels were at 40 $\mu\text{mol/mol}$ and 160 ppm. The sample gas was metered into a solution of excess boric acid to form ammonium borate. The ammonium borate was neutralized by back titration with standardized hydrochloric acid to an

original pH endpoint of the of the boric acid solution. The titrimetrically-determined mole fraction of ammonia in the two mixtures is $(37.5 \pm 0.3) \mu\text{mol/mol}$ and $(151.5 \pm 0.8) \mu\text{mol/mol}$.

3. Oxidation of ammonia to nitric oxide and chemiluminescence analysis: NIST evaluated a commercial instrument, which oxidizes ammonia to nitric oxide and then measures the chemiluminescence response. The two highest working standards were diluted using NIST's Environics mass flow dilution system to generate ammonia reference mixtures similar to sample working standards being analyzed. Each of the working standards was assigned a concentration value, which was within 95 % of a similar NO response. The corresponding values for the two mixtures above are $(39.1 \pm 0.8) \mu\text{mol/mol}$ and $(155.7 \pm 0.8) \mu\text{mol/mol}$. All of the measured values are observed to be 4 % to 6 % below the gravimetric blend values; suggesting that these working standards are unstable.

Future Plans: NIST will invest in the ammonia chemiluminescence instrument and attempt to link ammonia concentration determination to our primary Nitric Oxide gas standards. Work will also continue on the titrimetric method to provide direct linkage to the SI. Permeation systems will also be investigated in order to provide the conversion efficiency parameter for the chemiluminescence instrument.

Title: Microfluidic Devices for Rapid DNA Analysis for Human Identification

Authors: W.N. Vreeland and L.E. Locascio (839); M.Gaitan, J. Geist, and J. Shah (812); N. Y. Morgan, P. Smith, T. Pohida, and J. Kakareka (NIH); and C.W. Kan, A. E. Barron (Northwestern University)

Abstract: We have developed a new microfluidic device with auxiliary optics, pneumatics, and software to allow for rapid analysis of DNA “fingerprints” for human identification. The microfluidic devices are fabricated from common low-cost commercial plastics facilitating their application as single-use devices and thereby eliminating concerns of sample-to-sample cross contamination. Current device design and configuration are approaching the performance in salient figures-of-merit to current state-of-the-art equipment with the time required for analysis being reduced by nearly 90%.

Purpose: The National Institute of Justice estimates a backlog of a 542,700 cases for DNA analysis as of April 2004 and current forensic crime labs do not have the equipment capacity to address this backlog in a timely manner. Forensic DNA analysis or “fingerprinting” involves the measurement of the molecular size of several fragments of DNA produced in a specially designed molecular-biological reaction. NIST’s development of a microfluidic device for forensic DNA analysis is addressing this backlog by allowing for analysis techniques that are both faster and more economical, while still ensuring the data created is of the highest quality.

Major Accomplishments: The plastics that are used to fabricate NIST’s microfluidic devices must fulfill a variety of chemical, mechanical, and optical properties. In particular they must have (1) low permeability to aqueous buffers and salts, (2) low electrical conductivity, (3) glass-transition temperature between 110 and 120 degrees Celsius, (4) low optical fluorescence, (5) high optical transparency, and (6) uniform thicknesses. Several commercial plastics were screened, and a medical-grade poly(methylmethacrylate) was determined to be the best match to these criteria (this is the same plastic that is used to fabricate disposable contact lenses for vision correction).

The microfluidic channel must also be of a particular architecture and design (see Figure 1 for a general device layout). Two physical parameters of the device design are important, including separation channel length and sample injector size. The separation channel length determines the time required for the analysis as well as its analytical selectivity, while the sample injector determines the sensitivity and efficiency. In both cases, the desirable aspect of one parameter is increased at the cost of the other. We have design, fabricated and tested a systematic assortment of microfluidic channel designs to determine the most appropriate physical parameters for the NIST forensic DNA analysis. The final device design had an injector size of 100 microns and a separation channel length of ~ 9 cm. Results from this device are presented in Figure 2.

Further we have designed, assembled and tested the necessary optical detection system for fluorescence detection of the DNA “fingerprinting” fragments. The current system consists of a ball lens that collects the microchannel fluorescence and guides that light down an optical fiber that is then imaged on a CCD camera with an optical grating. This system allows for detection of an arbitrary number of channels simultaneously. This approach allows for large scalability in

future device designs and has no moving parts (in contrast to all other optical detection strategies in current commercial use). The result is a more rugged device as is required for the ultimate deployment of this device in the field.

Figures

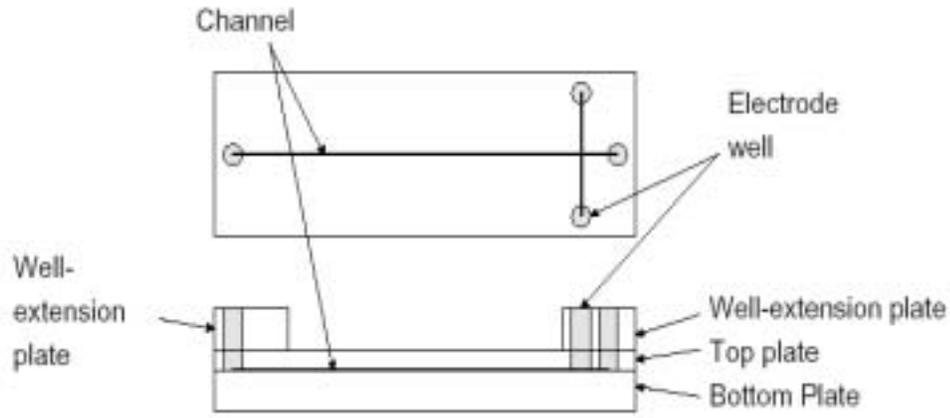


Figure 1. Top and side view of the single-channel microfluidic device currently being used in DNA separation experiments at NIST (not to scale). All lines are shown because the plastic substrates from which the device is made are transparent. The electrode wells, denoted as shaded circles, allow for the application of appropriate electrical fields. The DNA injector is formed from the intersection of horizontal and vertical channel (denoted as black lines) and the right-most portion of the horizontal line represents the separation channel

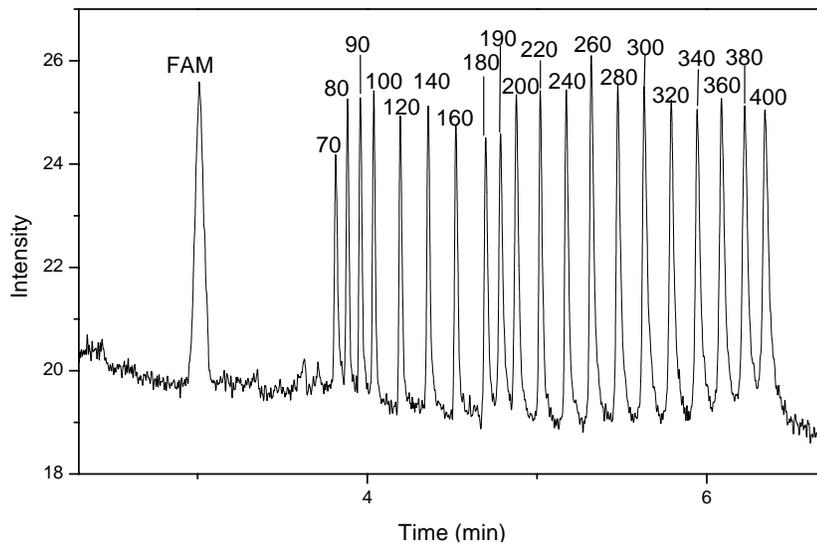


Figure 2. A demonstration of the NIST microfluidic DNA analyzer, showing the separation of a DNA “ladder” consisting of a variety of fragments of increasing size. Each peak represents a DNA fragment of particular size with larger fragments appearing later in time. The total analysis is performed in approximately 7 minutes.

Title: Development of Reference Methods and Reference Materials for Clinical Diagnostic Markers

Authors: M.J. Welch, D.M. Bunk, N. Dodder, B.C. Nelson, M.B. Satterfield, L.T. Sniegowski, and S. S-C. Tai

Abstract: NIST has a strong commitment to promoting accuracy in health-related measurements and providing measurement traceability to the U.S. *in vitro* diagnostic (IVD) industry through development of reference methods and SRMs. Work has been completed on SRM 2921 Cardiac Troponin Complex (heart attack marker) and measurements have been completed for SRM 1955 Homocysteine and Folate in Human Serum (risk factor for heart disease and a substance that counteracts effects of homocysteine). NIST has completed development of methods for certain thyroid hormones in serum, is developing methods for additional hormones, and will be applying these methods to certification of an SRM for hormones in serum. Collaborative work with other NMIs is continuing on development of a reference method for C-reactive protein, a risk factor for heart disease. In response to a need for a better reference material for detection of kidney disease, work has started on a new frozen serum SRM for creatinine. Three important clinical SRM renewals were completed this year: SRM 1951b Lipids in Frozen Human Serum is a two-level material certified for cholesterol and triglycerides; SRM 956b Electrolytes in Frozen Human Serum is a three-level material certified for five electrolytes with reference values for ionized calcium; and SRM 965a Glucose in Frozen Human Serum is a four-level material with a much wider concentration range than the previous lot had. These new reference methods and SRMs will help the IVD industry meet new regulatory requirements for traceability.

Purpose: NIST has a long-standing effort to promote accuracy in health-related measurements through development of reference methods and SRMs. With issuance of the *in vitro* diagnostic (IVD) device directive by the European Union, it has become even more critical for NIST to develop new reference methods and SRMs to provide traceability for the US IVD industry so that this industry can maintain its strong position in European markets.

Major Accomplishments: SRM 2921 Cardiac Troponin Complex is now available to the clinical laboratory community. This SRM is a buffer solution containing a complex of heart muscle proteins, troponins I, C, and T and certified for the concentration of troponin I, a widely used marker for detecting that heart attacks have occurred. This new material will help improve the accuracy and reduce the variability of the clinical assays in use for detection of heart attacks.

Reference method development is complete and papers published for thyroxine (T4) and triiodothyronine (T3), markers for thyroid function. This work along with ongoing work to develop reference methods and a new SRM for non-peptide hormones is described in technical report #. Measurements have been completed for a new SRM for homocysteine (HCY) and tetrahydrofolic acid (FOL) in serum. This new material has three levels: (high HCY, high FOL; normal HCY and FOL; and low HCY, low FOL). HCY is considered a risk factor for heart disease and other diseases associated with oxidative damage, while FOL is a strong antioxidant, which is known to reduce the risk of neural defects in fetuses and is believed to counteract the effects of homocysteine. This work is a collaborative effort between NIST and the Centers for Disease Control and Prevention (CDC), both of which provided measurements for certification.

The incidence of kidney disease is rising rapidly in the U.S. Early detection of kidney disease and treatment can prevent kidney failure, but early detection depends on better measurements of kidney function. Serum creatinine is the preferred measurement, but existing methods provide varying results, so NIST is developing a new creatinine in frozen human serum SRM to address this measurement problem. As part of this work, NIST has developed a new, rapid isotope dilution LC/MS method for serum creatinine to replace the tedious isotope dilution GC/MS method used previously at NIST.

The renewal of SRM 956b Electrolytes in Frozen Human Serum is described in detail in Tech report #. SRM 1951b Lipids in Frozen Human Serum was certified for total cholesterol and triglycerides (triglycerides only and total glycerides) this past year. This material is available, but additional information on HDL- and LDL-cholesterol, additional heart disease risk factors, will be added once measurements are completed at CDC using their proposed reference methods for these analytes. For the renewal of SRM 965a Glucose in Frozen Human Serum, a new low-level material was added to address measurements of patients with severe hypoglycemia. The high level material was raised so that it better addresses measurements of patients with severe hyperglycemia.

Research is continuing on development of a reference method for another risk factor for heart disease, C-reactive protein (CRP). Modest increases in CRP have been linked to arteriosclerosis and the increased risk of heart attacks. NIST, working with scientists at the Laboratory of the Government Chemist (LGC) in the UK and the Physikalisch-Technische Bundesanstalt (PTB) in Germany, is using a proteomics approach to isolate characteristic peptides from CRP for measurement by LC/MS. An isotope labeled peptide will be used as an internal standard for this work. Research has also begun to investigate the quantitative potential of MALDI-TOF mass spectrometry for biomolecules. To explore this potential, studies are underway on promising approaches for quantification of transferrin, an important iron-transporting protein in blood. Research is also underway on measuring various selenium-protein (anti-cancer agents) and iron-protein (iron-transport) combinations in blood.

Impact: These new reference methods and the SRMs that they will be used to certify will provide critical traceability to the IVD industry and will help improve the reliability of routine clinical measurements. Better clinical measurements leads to better diagnoses, enabling earlier and more cost-effective treatments.

Future Plans: Work will be completed in FY05 for the new human serum-based SRMs for homocysteine/folate, hormones, and creatinine. Work will continue on new approaches for quantification of biomolecules, including peptides, proteins, hormones, and species containing inorganic elements.

Title: Development of Reference Methods and Reference Materials for the Determination of Thyroid Markers and Other Non-Steroid Hormones

Authors: M.J. Welch and S.S-C Tai

Abstract: NIST is developing reference methods and SRMs to support accuracy and traceability for hormone assays. New methods, based on LC/MS, have been developed for thyroxine, triiodothyronine, and cortisol [1-3]. Method development is underway for estradiol, progesterone, and testosterone. These methods will be applied to the certification of a new hormones in human serum SRM.

Purpose: Many life functions are regulated by hormones. When hormone levels deviate from normal, serious health consequences may result. Timely and effective treatments require accurate diagnoses of hormone levels. New reference methods and reference materials are being developed to support accuracy and traceability of clinical laboratory measurements of non-steroid hormones related to thyroid function and other metabolic processes.

Major Accomplishments: Reference method development has been completed and the methods published for thyroxine (T4) and triiodothyronine (T3), important markers for evaluating thyroid function, and cortisol, an important hormone in metabolism. These methods are all based on isotope dilution, liquid chromatography/mass spectrometry (LC/MS). Tandem mass spectrometry (MS/MS) is used to provide greater specificity in the measurements. These methods will be applied to the certification of new hormones in human serum SRM. This new SRM consists of two pools, one from normal adult males and one from normal, premenopausal adult females. Research is underway to develop new LC/MS-based reference methods for estradiol, progesterone, and testosterone, important hormones in development and reproduction. These methods will be applied to this new SRM.

Impact: Improving the accuracy of clinical assays for hormones will improve diagnoses and result in earlier treatments. The new methods and the SRM will help improve accuracy of these assays and will also provide high order reference systems for traceability.

Future Plans: Method development will continue and new SRMs developed as needed. This work should lead to capabilities for reference methods for synthetic pseudo-testosterone substances reportedly used by athletes to enhance performance.

References:

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Title: Evaluation of Oleoresin Capsicum (Pepper Spray) Canisters for Chemical Content And Reliability

Authors: E. White V, R.J. Christensen (retired), K.W. Phinney, B.J. Porter, C. Presser (836), L.C. Sander, and R.Q. Thompson (guest researcher)

Abstract: A program to characterize the chemical compositions and the physical characteristics of pepper spray products has been established in the Analytical Chemistry Division at the request of the NIST Office of Law Enforcement Standards and with funding from the National Institute of Justice. A variety of products representing a cross section of those used by law enforcement agencies have been selected for study. The identities and concentrations of the active ingredients are determined by liquid chromatography/electrospray mass spectrometry and the identities of carriers and propellants by gas chromatography. Physical testing includes measurement of the number of 1-second bursts in a canister, a range test, a spray pattern test, drop tests, and, for the products that produce fogs, the droplet size. Improved analytical methods have been developed for the determination of the active ingredients.

Purpose: Pepper Spray is used by law enforcement officers to subdue non-cooperative individuals. It is effective 85-90% of the time and its use reduces the number of injuries to officers and suspects, and the number of use-of-force complaints. The causes of failures have not been established, but may include low concentrations of the active ingredients, poor delivery, and variable subject response. The goal of this study is the determination of the identities and concentrations of the ingredients and the mechanical performance of pepper spray units to establish potential modes of failure and hazards in handling.

Accomplishments: An improved liquid chromatographic (LC) separation for eight naturally occurring capsaicinoids, the active ingredients in pepper spray, and two internal standards, N-vanillyl octanamide and decanamide, has been developed. Satisfactory quantitative results have been demonstrated for the measurement of the analytes in mixtures with positive ion atmospheric pressure ionization electrospray mass spectrometry (API-ES-MS) for detection. Authentic samples of the compounds have been purchased or synthesized and their purity determined. The analytical method has been tested on several naturally occurring capsaicin mixtures and on pepper sprays.

New instrumentation has been developed to support this effort. A test chamber has been constructed for physical testing. Semi-automated devices have been fabricated and tested for determining canister spray capacity, for performing pattern and range tests, and for performing drop tests. Environmental chambers have been constructed for storage of canisters under controlled conditions. Phase Doppler interferometry instrumentation is presently being set up to measure particle sizes near the point of impact within the test chamber.

Tests have been performed on several canisters that have failed in actual field use. The composition of one such canister, reported to have caused injuries during a training exercise, was characterized by API-ES-MS. The spray was consistent with a synthetic (rather than natural) product, and the total level of active constituents was comparable to sprays formulated with

natural pepper extracts. A second canister reported to have failed during use was found to exhibit a weak spray pattern that was not centered in the direction the canister was aimed.

Impact: A reliable set of quantitative chemical and physical measurements on a representative set of canisters is expected to provide a basis for determining the most likely causes of failures in the field and to provide a benchmark against which manufacturers can assess the performance of their products.

Future Plans: Chemical and physical measurements are scheduled for approximately 1000 canisters. The resulting data is expected to provide indications of the presence/absence of design flaws or manufacturing defects that might lead to failures. The facility and capabilities will be made available to assist in determining the likely cause of future reported failures.

Title: Standard Reference Materials (SRMs) and Quality Assurance Activities to Support Measurements of Organics on Air Particulate Matter less than 2.5 μm ($\text{PM}_{2.5}$)

Authors: S.A. Wise, J.R. Kucklick, B.J. Porter, D.L. Poster, M.M Schantz, R.O. Spatz, and R. Zeisler

Abstract: The Analytical Chemistry Division is collaborating with the Environmental Protection Agency to develop SRMs to support measurements of organic species in fine particulate matter (PM). This collaboration focuses on three projects: (1) establishment of an interlaboratory comparison program to assess measurement comparability, (2) development of solution SRMs for compounds of interest for PM measurements, and (3) collection of bulk $\text{PM}_{2.5}$ for use as a future SRM. To date three interlaboratory comparison exercises have been conducted for the determination of organic contaminants and source markers in PM samples. A series of calibration solution SRMs are in progress for aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), nitro-substituted PAHs, hopanes/steranes, and ^{13}C -labeled and deuterium-labeled levoglucosan.

Purpose: In 1997 the Environmental Protection Agency (EPA) issued new standards for air particulate matter (PM) under the national ambient air quality standards including new regulations for PM less than 2.5 μm ($\text{PM}_{2.5}$), which is the respirable PM fraction, in addition to the existing PM_{10} standards. Research recommendations have been made by the National Research Council at the request of Congress and EPA to focus on evaluating what types of particles cause detrimental health effects. To support compositional analyses and other investigations on the fine PM, quality assurance materials are necessary; however, few appropriate fine particulate materials are currently available to support this research. NIST has entered an agreement with the EPA to develop SRMs to support analytical measurements of organic constituents in $\text{PM}_{2.5}$, to improve interlaboratory comparability of measurements, and to provide national measurement traceability.

Major Accomplishments: The NIST/EPA agreement has focused on three projects with the goal of providing quality assurance and SRMs to support measurements of organic compounds in fine PM including: (1) establishment of a interlaboratory comparison program to assess measurement comparability, (2) development of solution SRMs for compounds of interest for PM measurements, and (3) collection of bulk $\text{PM}_{2.5}$ for use as a future SRM. As part of the NIST/EPA collaboration, the Organic Speciation Working Group was formed in 2000 to assist in this effort by participating in interlaboratory comparison studies and by providing input for the development of SRMs to support these measurements. This group has participated in three interlaboratory comparison studies for the determination of polycyclic aromatic hydrocarbons (PAHs), nitrated PAHs, alkanes (including hopanes and cholestanes), sterols, carbonyl compounds (ketones and aldehydes), acids (alkanoic and resin), phenols, and sugars in PM-related samples. Based in part on the results of these studies and input from the Organic Speciation Working Group, priorities for the development of a number of calibration solution SRMs were identified including: aliphatic hydrocarbons, PAHs, nitro-substituted PAHs, hopanes/steranes, and ^{13}C -labeled and deuterium-labeled levoglucosan (for use as internal standards). SRM 1494 Aliphatic hydrocarbons in Iso-octane was issued in early 2004. SRMs

2260a PAHs in Toluene and 1491a Methyl-substituted PAHs in Toluene, which are redesigned solutions with an expanded list of 53 PAHs and alkyl-substituted PAHs, were completed in late 2004. The remaining solution SRMs are in progress and will be completed in 2005.

Collection of a 200-g bulk sample of ultra-fine particulate matter at a site in Baltimore, MD with a high-volume sampler has been only partially successful. Two collections of 20 g each have been used to prepare an interim reference material and for distribution in the NIST/EPA interlaboratory comparison exercises for the determination of organic compounds. The results from the second interlaboratory study using the interim reference material have been used in conjunction with NIST measurements to assign concentration values for the compounds of interest. This interim reference material is now available to laboratories involved in EPA PM_{2.5} research programs for use as a control or reference material. The second PM_{2.5} material collected in Baltimore has been distributed to over 20 laboratories as part of the third NIST/EPA intercomparison study, and results will be available in early 2005.

Impact: The development of SRMs to support measurements for organic species in fine PM will expand the quality assurance capabilities to important source markers and species critical to human health.

Future Plans: We are currently investigating alternative approaches to obtaining sufficient quantities of PM_{2.5} for preparation of an SRM including preparation of a fine fraction material from existing PM SRMs and/or other total suspended PM. Additional organic compound classes have been identified by EPA for development of additional solution SRMs including additional sugars, saturated and unsaturated acids, and quinones.

Title: Development of Standard Reference Materials (SRMs) for Dietary Supplements

Authors: S.A. Wise, L.C. Sander, and K.E. Sharpless

Abstract: The Analytical Chemistry Division is collaborating with the Food and Drug Administration (FDA), and the National Institutes of Health's Office of Dietary Supplements (NIH-ODS) in a multi-year program to develop Standard Reference Materials (SRMs) and analytical methods for a number of dietary supplement materials, including botanical and botanical-containing matrices as well as a multivitamin/multielement material. The first botanical dietary supplement SRMs, consisting of a suite of five ephedra-containing materials (ground dried plant, two extracts, diet pills, and protein drink powder), were completed in 2004. Measurements are currently in progress for the *Ginkgo biloba* SRM suite (plant, extract, and tablet). Acquisition of additional materials, including saw palmetto, bitter orange, carrot oil, and St. John's wort, was initiated in 2004. SRM 3280, Multivitamin/Multielement Tablets was prepared, and measurements were initiated for value assignment of 18 elements, 15 vitamins, and a carotenoid.

Purpose: The enactment of the Dietary Supplement Health and Education Act (DSHEA) in 1994 by the U.S. Congress has promoted growth in the nutritional supplement industry, due in part to the way in which dietary supplements are regulated. DSHEA provides a legal definition of dietary supplements that classifies these materials separately from food additives and pharmaceutical drugs. Requirements for product labeling are less stringent than for drugs, and the burden of proof for the safety of dietary supplements is placed on the Food and Drug Administration (FDA). More than 50% of the U.S. population uses dietary supplements, accounting for roughly \$10 billion in sales every year. A variety of botanical-containing products are marketed as dietary supplements, e.g., St. John's wort, ginkgo, saw palmetto, and green tea. Botanical reference materials with assigned values for active and/or marker compounds are needed to address two primary concerns in the botanical dietary supplements community: safety and efficacy. Potential health risks may result from contamination (e.g., pesticides, toxic elements), adulteration (presence of unlabeled foreign materials including pharmaceuticals), or variability in product composition (e.g., changes in levels of active constituents). Secondly, product quality and consistency must be maintained through verification of dietary supplement label claims. These needs can be addressed by the development of analytical methods and reference materials to support chemical composition measurements for dietary supplements.

In 2001 the Analytical Chemistry Division (ACD) of NIST, FDA, and NIH-ODS initiated a multi-year program to develop Standard Reference Materials (SRMs) and analytical methods for a number of botanical and botanical-containing dietary supplements. The goal of this collaborative program is to provide SRMs for eight to ten different botanical dietary supplements over a six-year period. NIH-ODS is also collaborating with the U.S. Department of Agriculture (USDA) to establish the Dietary Supplement Ingredient Database (DSID). The DSID project will report the results of a systematic survey of supplement composition, including chemical composition of ingredients with the primary focus on vitamin and mineral supplements. To support the DSID project, ACD and NIH-ODS have expanded their collaboration to develop dietary supplement matrix SRMs to include a multivitamin/multielement supplement. SRM

3280 Multivitamin/Multielement Tablets is a commercial multivitamin/multielement tablet which will be value assigned for concentrations of 18 elements and 15 vitamins and carotenoids for which label claims are made.

Major Accomplishments: Based on NIH-ODS priorities and needs, ACD is developing SRMs for the following botanical dietary supplement materials: ephedra, *Ginkgo biloba*, saw palmetto, bitter orange, green tea, St. John's wort, \exists -carotene and tocopherol mixtures, and multivitamin/multielement tablets. Each botanical material SRM will consist of a suite of matrices including plant, extract, and finished product. The first SRM suite for ephedra-containing dietary supplements (SRMs 3240-3244) has been completed (see Technical Report xxxx). The second SRM suite for *Ginkgo biloba* has been prepared and measurements of ginkgolides and flavonoids are nearing completion. Plant and extract materials for the next suites, saw palmetto and bitter orange, were obtained in 2004; a carrot oil, representing a natural carotene mixture, was also obtained. The candidate material for the multivitamin/multielement tablet SRM was received in mid-2004, and methods development and measurements are currently underway.

Impact: The availability of botanical dietary supplement matrix SRMs with certified concentrations of active/marker constituents and contaminants and a multivitamin/multielement tablet SRM will provide the measurement tools necessary to assess the quality of dietary supplements.

Future Plans: This program is a six-year effort to develop SRMs for eight to ten different botanical dietary supplements. After completion of the ephedra and ginkgo SRM suites, the next materials targeted for development of SRMs are: saw palmetto, bitter orange, \exists -carotene in carrot oil, St. John's wort, green tea, and tocopherol mixtures. Acquisition of the St. John's wort and green tea are in progress and will be completed in early 2005. The next priorities for SRM development include: β -carotene (e.g., a mixture of β -carotene isomers), vitamin E (e.g., d- α -tocopheryl acetate/d,l- α -tocopheryl acetate and a mixture of the tocopherol and tocotrienol isomers), black cohosh, and a number of berry materials (e.g., cranberries and blueberries). Completion of the multivitamin and multielement SRM is scheduled for early 2006.

Title: Investigation of Arsenic Oxidation State Speciation in Primary Calibration Solutions and Effects on ICP-OES Measurements

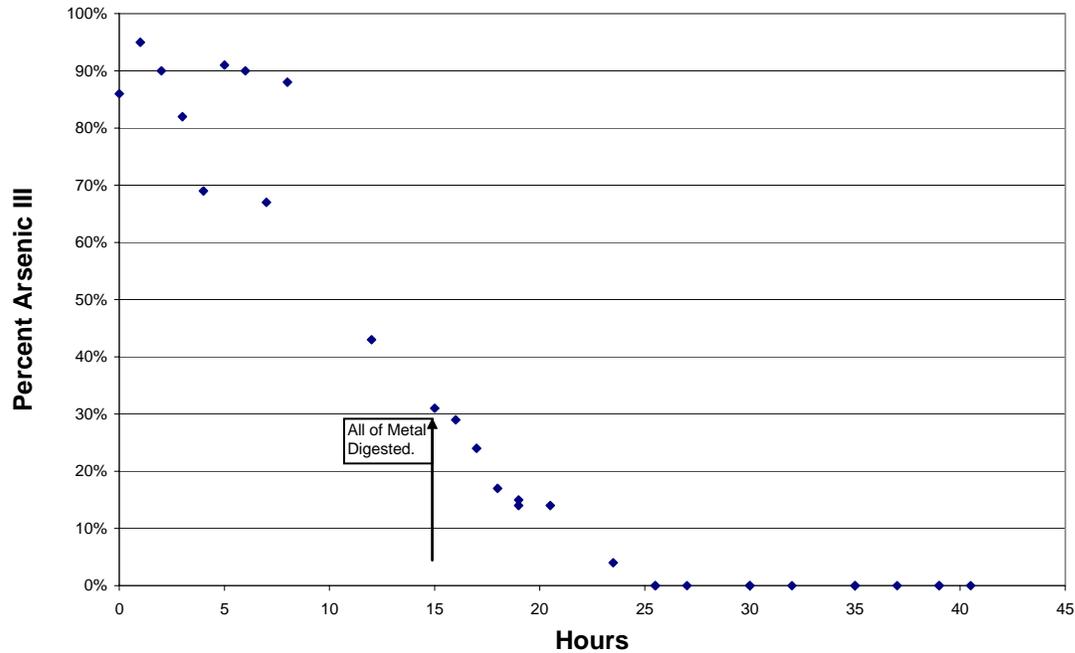
Authors: L.L. Yu, T.A. Butler, and G.C. Turk

Abstract: It is well known that the toxicity of arsenic is strongly tied to oxidation state speciation. Less understood is the effect that arsenic oxidation state has on measurement accuracy. Standards prepared from arsenic metal, which in principle should yield a more accurate and precise standard relative to those prepared from an arsenic compound, were frequently found to have inexplicable disagreements between batches when analyzed by using optical emission or mass spectrometry. The deviation, which can at times be as great as a few percent, frustrates the efforts of producing an accurate clinical standard for arsenic. Our studies of the cause of this disagreement between the arsenic standards point to differences in arsenic valence states between solutions. The results of an investigation into the kinetics of the reaction for the arsenic standard preparation elucidated why arsenic solutions comprised of varied arsenic species were produced. Guidelines were developed based on the investigation for producing a single species arsenic standard, thus eliminating the measurement bias in the certification process, ensuring the production of more accurate and precise arsenic standards for clinical and general use.

Purpose: To produce a highly precise and accurate arsenic standard by investigating and eliminating an unexplained bias between gravimetric preparation value and the inductively coupled plasma optical emission spectrometric value of arsenic standards.

Major Accomplishments: We analyzed sets of standard solutions prepared by dissolving arsenic metal with nitric acid and found both arsenic III and arsenic V to be present in these standards, including various lots of SRM 3103a, the arsenic spectrometric solution. Furthermore, we discovered that standards containing greater amounts of As III yielded somewhat lower signals in ICP Optical Emission Spectrometry than those in which the predominant form of arsenic was As V. Similar results have also been observed using ICP Mass Spectrometry. This difference in the instrumental response of these two forms of arsenic will result in a measurement bias if samples and calibration standards differ in the form of arsenic. The figure below shows the results of a study of the kinetics of the arsenic metal digestion process. In the experiment small aliquots of solution were periodically withdrawn over a period of 40 hours from a digestion vessel as a piece of arsenic metal was being dissolved in a 50 % (v/v) solution of concentrated nitric acid in water on a warm hotplate. The withdrawn aliquots were analyzed using liquid chromatography with ICPMS detection to determine the fraction of the total dissolved arsenic present as As III. The results show that at the beginning of the process most of the solution is present as As III, and all of the solution is present as As V after 25 hours. This discontinuities in the reaction curve occur when additional nitric acid / water is added. All of the metal appears to be dissolved after only 15 hours. If the process had been stopped after 15 hours, the resulting solution would contain a mixture of arsenic oxidation states. It is clear that this situation occasionally occurs in our production of arsenic solution standards, including SRM 3103a. We have now developed a procedure utilizing iodine as an oxidant to convert solution standards containing mixed oxidation states of arsenic to As V, thus eliminating the source of bias.

Kinetic Study of As Metal Digestion



Impact: The result of the research resolves a long-standing mystery regarding bias sometimes observed between the gravimetric preparation value of certain arsenic solutions and values determined spectrometrically using independent calibration solutions. A revised digestion procedure developed as a result of this research ensure the production of a more accurate and precise arsenic standard for clinical and general applications. The research also raises some questions regarding the accuracy of arsenic measurements by using plasma source based spectrometry techniques.

Future Plans: Further studies will determine if other elements are susceptible to similar systematic bias. The underlying cause of the bias in plasma source spectrometry remains unknown.

Title: Influence of Oxygen on the Shelf Life of Coal Standard Reference Materials (SRMs)

Authors: R. Zeisler and W.D. James (TAMU)

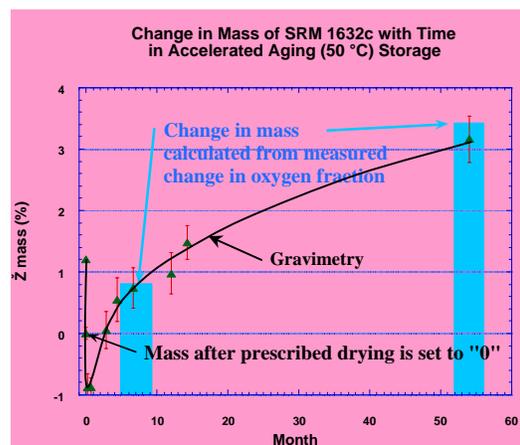
Abstract: Long-term stability of SRMs is essential to customers' use of the materials. CSTL scientists and collaborators validated the stability of coal SRMs under proper storage conditions. Oxidative environments in combination with elevated temperatures have been found to promote the oxidation of coal samples and lead to changes in composition.

Purpose: Based on reports by users of the previously (1985) issued SRM[®] 1632b Trace Elements in Coal of a change in the measured values of the carbon mass fraction, the new values being about 1.6% lower than previously measured, investigations to validate the long-term stability of coal samples were initiated. A definition of the chemistry of the changes was desired.

Major Accomplishments: Initial evidence of change was provided by trace element data in the SRM 1632b material, which indicated approximately a change in mass fraction values similar to the reported carbon change for a number of elements determined by INAA. While a change in carbon content through loss of CO₂ may have been a plausible explanation for lower than previously measured carbon values, the measured decrease of a larger number of elemental content values may be explained in a gain of matrix mass, such as by irreversible oxidation and therefore increased matrix mass.

The newly developed SRM 1632c Trace Elements in Coal (Bituminous) was selected for the study. Twenty-six samples each were stored at three conditions: in liquid nitrogen vapor and at room temperature in the original containers packaged under argon, and for accelerated aging at 50 °C open to air. Under proper storage, the samples showed stable mass during the more than 4-year observation; only the ones stored open at higher temperature gained weight. To directly measure the presumed oxidation, a fast (14-MeV) neutron activation analysis (NAA) procedure was employed to determine mass fractions of oxygen in the coal samples.

The 14-MeV NAA is an effective procedure for the direct determination of oxygen. It demonstrated sufficient precision and accuracy to determine a relative change of about 5 % or more, in the oxygen mass fraction of about 11 %. To evaluate the sample stability, test points were set at 6 and 54 months for the oxygen determination, more frequent gravimetric tests were carried out for the samples in the open 50 °C storage. The gain in mass and in oxygen for the latter samples is illustrated in the figure. All gain in mass can be explained by the gain in oxygen content. No change in oxygen content was measured in the other storage



conditions. As further validation of this gain in sample mass of coal under oxidative storage, an analytical comparison of trace element content in the three storage conditions showed a lowering in mass fraction for 22 trace elements corresponding to the gain in sample mass.

Impact: The measurements give assurance that properly packaged coal SRMs, protected from oxygen and heat, are stable during their shelf life. Gravimetric monitoring of coal SRMs during shelf storage should be employed by NIST and customers alike as a cost-effective quality measure.