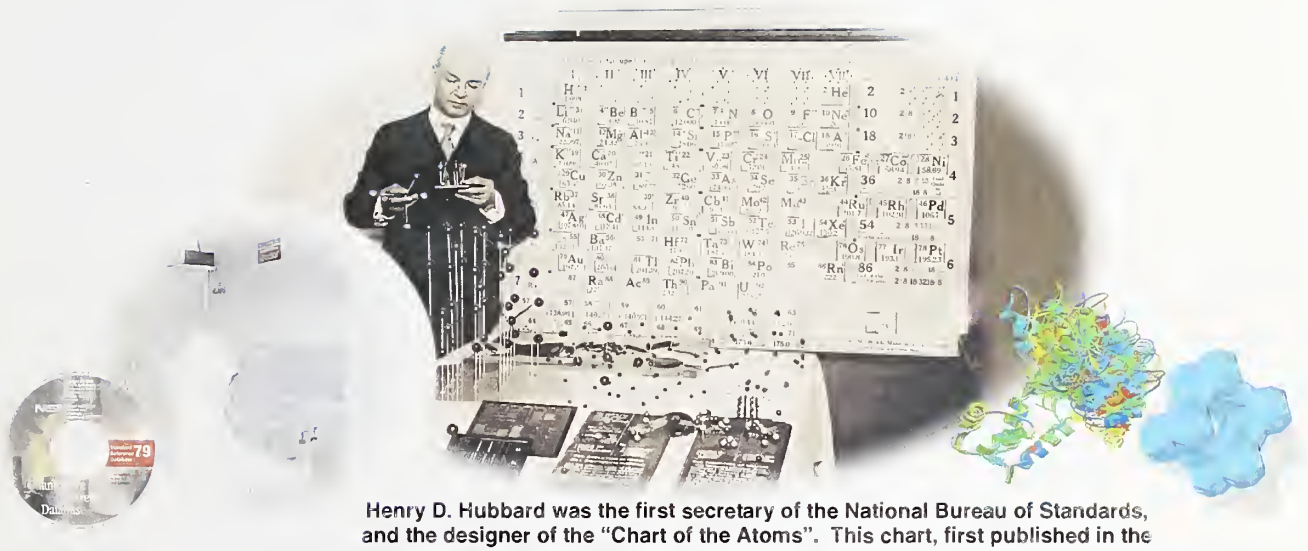
Celebrating One Hundred Years of Chemistry at the National Institute of Standards and Technology



Henry D. Hubbard was the first secretary of the National Bureau of Standards, and the designer of the "Chart of the Atoms". This chart, first published in the 1920's, has been updated over the years, and is still used by chemists today.

Chemical Science and Technology Laboratory

NIST
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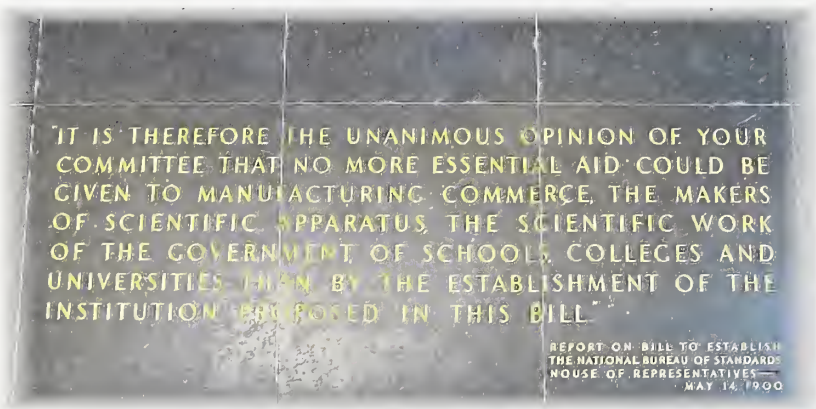
logy Administration
epartment of Commerce

NISTIR 6388 2001 ED
Pittcon Version, March 2001

Welcome to the
National Institute of Standards and Technology

"Upon entering the Administration Building of the NIST Gaithersburg site, the feeling of spacious aesthetic design is not lost. The large reception area is flooded in black terrazzo, and its walls are white or black marble. When entering one sees on the far wall an inscription taken from a House Committee report on the bill to establish the Bureau, dated May 14, 1900. In gold letters, incised into black marble, the quotation states:



It forms an impressive greeting."

Excerpt from: *A Unique Institution, The National Bureau of Standards, 1950 – 1969*
Elio Passagalia, Editor
U.S. Government Printing Office, Washington DC

NISTIR 6388 2001 ED

Celebrating One Hundred Years of Chemistry at the National Institute of Standards and Technology

Chemical Science and Technology Laboratory

March 2001

U. S. DEPARTMENT OF COMMERCE
Donald L. Evans, Secretary

TECHNOLOGY ADMINISTRATION
Karen H. Brown, Acting Under Secretary of Commerce for Technology

NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
Karen H. Brown, Acting Director

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www.cstl.nist.gov

Version of March, 2001

National Institute of Standards and Technology

The Centennial Celebration

from 1901 ...

to 2001 ...

Since its creation in 1901, the National Institute of Standards and Technology has been the source of measurements and standards on which U.S. manufacturing, commerce, scientific institutions and all branches and levels of government depend. NIST will celebrate its centennial on March 3, 2001.

THE EVENING STAR, MONDAY, MARCH 11, 1901

CORRECT MEASURES

Function of the New Bureau of Standards.

LABORATORY TO BE ERECTED

Prof. Stratton, the Director, Details Need of Establishment.

A HANDICAP REMOVED

A new bureau of the government, authorized by the last Congress, will be established in this city in the near future and will give employment to a number of persons. It is to be known as the national bureau of standards and is to be under the control of the Treasury Department. A separate building for a laboratory, to cost not to exceed $250,000, is to be erected on a site to be purchased at a cost of $75,000.

Mr. Samuel W. Stratton of Chicago has been appointed by the President to be chief of the bureau at an annual salary of $6,000. Prof. Stratton is to have the following as-

Director Stratton.

Chartered by Congress, NIST (originally known as the National Bureau of Standards) was the first physical science research laboratory of the federal government. It was established at about the same time as the nation's first commercial laboratory. At that time, the United States had few, if any, absolute national standards Instead, there was a patchwork of regional standards, often arbitrary, which proved to be a burden as much as an aid to commerce. Yet, at that time, the United States was becoming a world power, with an industrial economy driven by the steam engine, the railroad and the expanding reach of electricity. Other industrialized nations already had established standards laboratories.

After strong advocacy by leading scientists and industrialists, Congress established the National Bureau of Standards. Because Lyman Gage, Secretary of the Treasury under President McKinley, initiated and led the campaign for a national standardizing laboratory in the federal government, the Bureau of Standards was originally in the Treasury Department. In 1903, the new Department of Commerce and Labor was established, and the House Committee on Interstate and Foreign Commerce recommended that *"The newly created National Bureau of Standards is a bureau which necessarily goes into a department primary devoted to manufacturing and commercial interests. This Bureau is destined to exercise great influence on the development of business and commerce of our country"*. When the Commerce and Labor Department was divided, NBS went with the Commerce Department where it remains.

Industrial Central Research Laboratories established:

1900 General Electric
1903 DuPont
1904 Westinghouse
1908 Corning Glass
1912 Eastman Kodak
1925 Bell Telephone
1928 U.S. Steel

The Early Decades: Both World Wars found NBS deeply involved in mobilizing science to solve pressing weapons and war materials problems. After WWII, basic programs in nuclear and atomic physics, electronics, mathematics, computer research, and polymers as well as instrumentation, standards, and measurement research were instituted.

In the 1950s: NBS research helped usher in the computer age and was employed in the space race after the stunning launch of Sputnik. A second NBS campus was dedicated in Boulder CO in 1954. This new facility permitted an explosion in the number of calibrations of radio-related equipment.

In the 1960s: The Bureau's technical expertise led to assignments in the social concerns of the Sixties; including the environment, health and safety. This decade saw the move of NBS laboratories from the Van Ness site in Washington DC, to the then rural Gaithersburg MD, where is it located today. By the Seventies, energy conservation and fire research had also taken a place at NBS.

The mid-to-late 1970s and 1980s: NBS returned with renewed vigor to its original mission focus in support of industry and commerce. In particular, increased emphasis was placed on addressing measurement problems in the emerging technologies.

The Omnibus Trade and Competitiveness Act of 1988: This act augmented the Institute's uniquely orchestrated customer-driven, laboratory-based research program aimed at enhancing the competitiveness of American industry by creating new program elements designed to help industry speed the commercialization of new technology. To reflect the agency's broader mission, the name was changed to the National Institute of Standards and Technology (NIST).

Technology Competitiveness Act The Omnibus Trade Bill of August 23, 1988

"To modernize and restructure (NBS) to augment its unique ability to enhance the competitiveness of American industry while maintaining its traditional function as the lead national laboratory for providing measurements, calibrations and quality assurance techniques which underpin U.S. commerce, technological progress, improved product reliability and manufacturing processes and public safety."

http://www.nist.gov

NIST's four major programs are designed to help U.S. companies achieve their own success, each one providing appropriate assistance or incentives to overcoming obstacles that can undermine industrial competitiveness. Each of the four programs addresses different components of the technology pipeline.

Measurement and Standards Laboratories

The Measurement and Standards Laboratories provide technical leadership for vital components of the nation's technology infrastructure needed by U.S. industry to continually improve its products and services. NIST's seven discipline-based Measurement and Standards Laboratories work at all stages of the pipeline from advancing basic science and pioneering new measurement methods to the development of standard test methods, materials, and data to ensure the quality of commercial products. The seven NIST Laboratories are: Electronics and Electrical Engineering Laboratory (EEEL), Manufacturing Engineering Laboratory (MEL), Chemical Science and Technology Laboratory (CSTL), Physics Laboratory (PL), Materials Science and Engineering Laboratory (MSEL), Building and Fire Research Laboratory (BFRL) and, Information Technology Laboratory (ITL).

Advanced Technology Program

The Advanced Technology Program (ATP) bridges the gap between the research lab and the market place, stimulating prosperity through innovation. Through partnerships with the private sector, ATP's early stage investment is accelerating the development of innovative technologies that promise significant commercial payoffs and widespread benefits for the nation.

Manufacturing Extension Partnership

In 1989, NIST established the first federally funded extension centers to help small manufacturers improve their capabilities and performance—a necessity for survival in the global marketplace. Today, the Manufacturing Extension Partnership is a nationwide network of more than 400 not-for-profit centers and field offices that, in 1999, provided technical assistance to nearly 27,000 smaller manufacturers. MEP makes it possible for even the smallest firms to tap into the expertise of knowledgeable manufacturing and business specialists all over the U.S.

Baldrige Quality Program

The Baldrige Quality program is an outreach program associated with the Malcolm Baldrige National Quality Award that recognizes business performance excellence and quality achievement by U.S. manufacturers, service companies, educational organizations, and health care providers. The Malcolm Baldrige National Quality Award, created in 1987, is widely credited with making quality a national priority.

U.S. Dept. of Commerce
Technology Administration

Chemical Science and Technology
… leading into the 21st century …

Introduction:

In 2001, the world marks the beginning of a new millennium, and the National Institute of Standards and Technology marks the beginning of its second century. As we begin our next hundred years, it seems fitting not only to recall achievements, but also - and more importantly - to consider what lies ahead.

Advances in science and technology fuel the growth of the modern economy. In fact, the technology sector is said to account for fully one half of the United State's economic growth. Each year the Federal Government invests about $300M in the NIST Measurement and Standards Laboratories in order to meet the standards and other measurement needs of this growing economy. The knowledge and capabilities generated by these Laboratories support a $10B-per-year, private-sector measurement services enterprise; in turn, about half of the United States' GDP ($7.6T) depends on these measurement capabilities to develop their products and services. New technologies require entirely new standards, measurement capabilities, and new types of reference data and materials, and established industries demand improvement to and expansion of existing standards and data collections. Other economic trends, especially globalization, will put further demands on the international system of measurements and standards. Because of these growing demands for standards and reliable data, it seems inevitable that NIST will continue to play a vital role in the economic growth of the U.S.

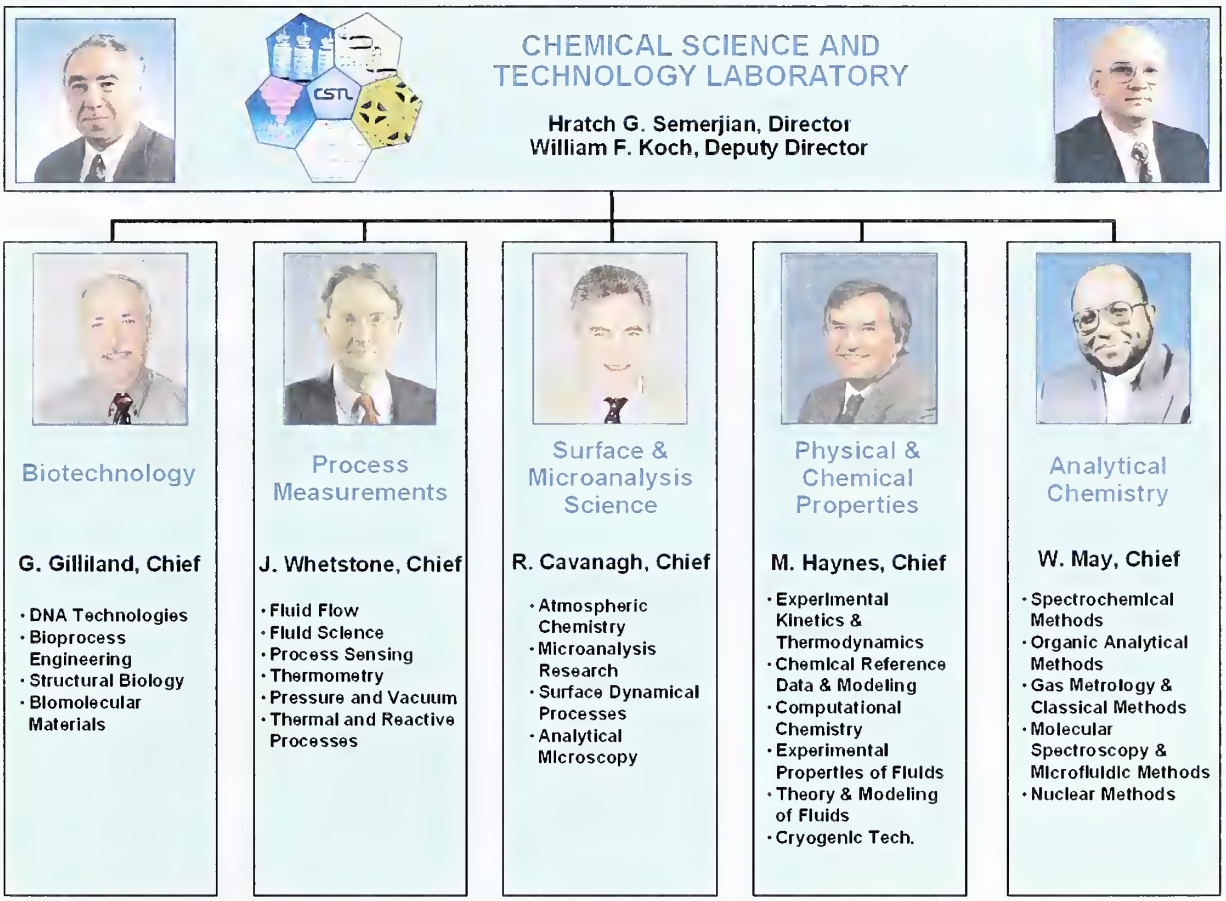
Measurement and Standards Laboratories

The Chemical Science and Technology Laboratory is one of NIST's seven Measurements and Standards Laboratories. Reflecting the importance of chemical and chemical engineering measurements and standards to the rapid industrialization of the U.S., the Chemistry Division and the Heat and Thermometry Division were among the first established by the founders of NBS/NIST in 1901. Building on a one hundred year history of technical excellence, today's Chemical Science and Technology Laboratory has the most comprehensive array of chemical, physical, and engineering measurement capabilities of any group working in chemical science and technology. We are well positioned to achieve our Vision to be a world-class research laboratory that is recognized by the nation as the primary source for the chemical, biochemical, and chemical engineering measurements, data, models, and reference standards that are required to enhance U.S. competitiveness in the world market.

Hratch G. Semerjian, Director
Chemical Science and Technology Laboratory

As the nation's chemical reference laboratory, CSTL's ***mission*** is to provide the chemical measurement infrastructure to enhance U.S. industry's productivity and competitiveness, assure equity in trade, and improve public health, safety and environmental quality. An important part of CSTL's efforts is to access future measurement needs through interaction with a broad base of customers and through extensive ties to industry, academia and other government agencies.

CSTL is organized to reflect the technical expertise that is the foundation of our technical program and allows us to accomplish our mission. The Laboratory consists of five Divisions: Biotechnology Division, Process Measurements Division, Surface and Microanalysis Science Division, Physical and Chemical Properties Division, and Analytical Chemistry Division. Each Division employs a group structure organized to achieve synergy and critical mass in its technical program areas.



Chemistry at NBS/NIST is steeped with rich traditions and established partnerships with industry and academia. While the nature of the partnerships may change with societal expectations of the time, the dedication to addressing industry needs remains a hallmark of the institution. Certainly chemical science has evolved over the past century and enabled many new technologies. This book describes our present activities with a historical perspective, in hopes of providing insights into the years to come.

Our Customers ... You

... responsive and responsible service
to industry, academia, and other agency partners
for the American people and the broader global community ...

CSTL is a multifaceted, synergistic organization with a broad customer base. The Laboratory has technical capabilities in analytical chemistry, surface chemistry and microanalysis, chemical and physical properties, process measurements and modeling, and biotechnology. Its core competencies in physical and chemical measurement standards, data, and science are its strength and are the source of its unique contribution to technological development. These core competencies are not stagnant, but rather evolve and change and have become an integral part of advancing technology. These competencies both influence advances and are influenced by them.

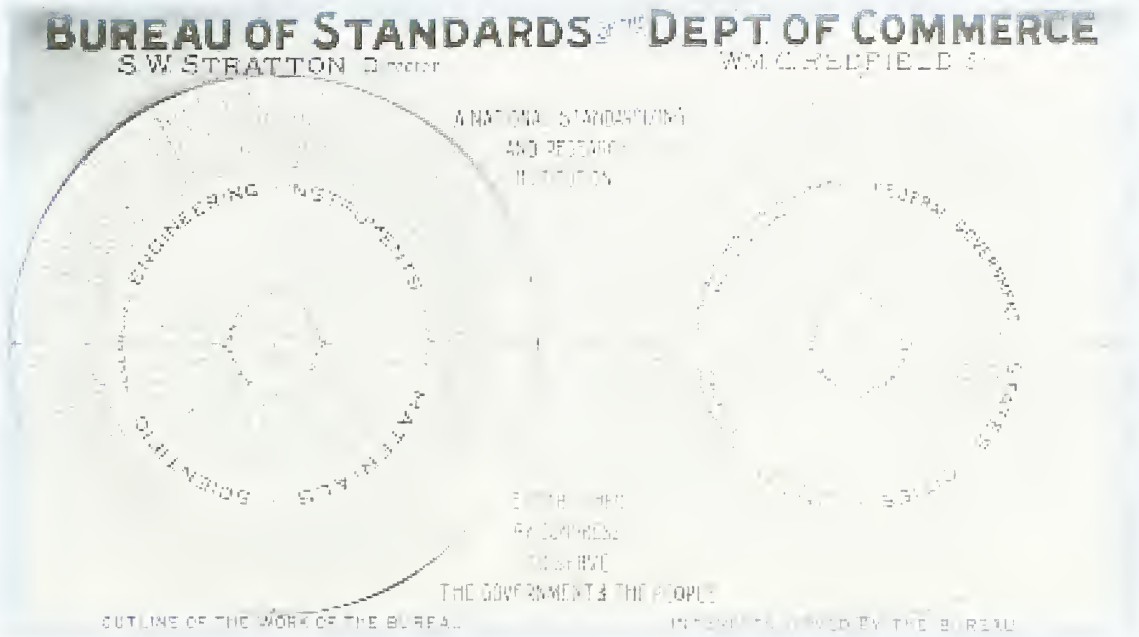
CSTL's core competencies support its programs. These programs are a reflection of our customer needs. This basic philosophy is depicted in the figure below.

CSTL Programs Support a Diverse Customer Base

The **primary customers** we serve are: industry (chemical, electronics/microelectronics, automotive and transportation, energy, petrochemical, instrumentation, metals and materials, biotechnology, environmental technologies, food, health care and pharmaceutical); federal, state, and local government agencies. We also serve standards and industrial trade organizations, and the academic and scientific communities.

To meet these customer needs CSTL currently has the following twelve **programs**: Chemical Characterization of Materials; Process Metrology; Chemical and Biochemical Sensing; Nanotechnology; Healthcare Measurements; Environmental Measurements; Microelectronics; Physical Property Data; Chemical and Biochemical Data; Bio-molecules and Materials; DNA Technologies; and International Measurement Standards.

Identifying and defining our customer base, and articulating our services and programs while reaffirmed in the present day, are not new to NIST. This is evidenced by the "Activity Wheel" (below) constructed during the early days.



"The proliferation of Bureau interests, abetted by special congressional appropriations for investigations not covered in the Organic Act, inspired the wheeled chart of NBS activities. It was probably prepared for an appropriations hearing before congress about 1915." ***Measures for Progress – A History of NBS, published in 1966 by NBS and DoC.***

Budgetary Issues:

Proposal Bill for Staffing the newly founded NBS – Lyman Gage, Secretary of the Treasury – 1900

Position	Annual Salary
Director	$6000
One Physicist	$3500
One Chemist	$3500
Two Assistants (Physicists or Chemists)	$2200 each
Two Laboratory Assistants	$1400 each
Two Others	$1200 each
One Secretary	$2000
Two Clerks	$1400, and $1000
One Messenger	$750
One Engineer	$1500
One Fireman	$720
Three Mechanicians	$1400, $1000, and $840
One Watchman	$720
Two Laborers	$600 each

Historical note:

Final appropriations cut back salary schedule by reducing director's salary to $5000 and eliminating 8 of the 21 proposed positions, including the two laboratory assistants, the secretary, a clerk, the fireman, 2 mechanicians, and a laborer.

The sum for equipping the main laboratory was reduced from $25,000 to $10,000

… And at the turn of the millennium …

NIST's Budget: ***$800 million (operating budget from all sources)***

Staff: ***3,300 scientists, engineers, technicians, business specialists, and administrative personnel. In addition, about 1,500 visiting researchers complement the staff. NIST also partners with 2,000 manufacturing specialists and staff at affiliated centers around the country.***

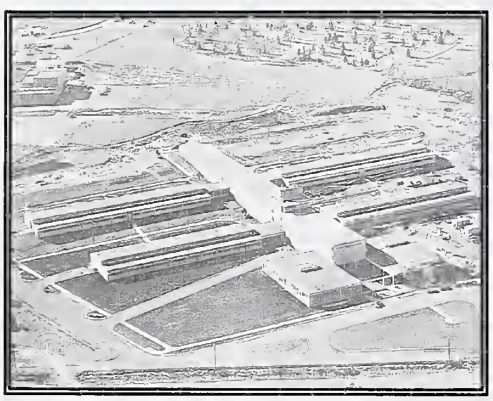
Chemistry Laboratories at NBS/ NIST: then and now

Facilities and Locations: By 1903, construction of the new laboratory facilities were underway. The site selected was a then sparsely populated and remote residential section on Connecticut Avenue to the north of the business center of Washington DC.

The first World War prompted the expansion of NBS. Chemicals and steels, used in weapon production, were the primary concerns of the Bureau throughout the war. Under the stimulus of war, industry turned out scores of new alloy steels – containing nickel,

chromium, tungsten, zirconium, molybdenum, vanadium, manganese, and cobalt – and sent them to the Bureau for chemical composition analysis and properties determinations. Groundbreaking for a new chemistry building was in early fall 1915, with full occupation of the then state-of-the-art laboratory in 1917.

The Boulder Site:



The Boulder Laboratories were formed by the National Bureau of Standards in the early 1950's when radio research was moved out of the Washington, D.C. area. President Eisenhower dedicated them on September 14, 1954. Even before that date, however, work had begun at the Boulder Labs. Working for the old Atomic Energy Commission (AEC), NBS had established a hydrogen production facility on site in conjunction with the hydrogen bomb project at Los Alamos, NM. Within a short time, the AEC decided to go in a different direction and did not need liquid hydrogen from NBS. However, this capability led to the formation of the Cryogenic Engineering Division. So, for many years the Boulder Labs specialized in radio propagation physics, radio propagation engineering, radio standards, and cryogenics.

Current areas of research include time standards, optical fibers, antenna measurements, superconducting electronics, wireless communication, magnetics, advanced materials, semiconductors, optoelectronics, physical and chemical properties, alternative refrigerants, information technology, and quantum physics.

The Gaithersburg Site: The NBS facilities on the Van Ness site served the country well for more than fifty years. However, by 1955 it was clear that the physical plant suffered greatly … and plans were initiated to relocate to a site near the then "small, sleepy town of Gaithersburg". Preservation of the NBS culture was articulated in letter to the mayor of Gaithersburg in 1956:

"The Bureau wishes to develop on its new site a university campus-type atmosphere similar to the one which had been achieved on the present site. It has been found that such surroudings are an asset in attracting and maintaining scientists and in producing the environment which stimulates scientific productivity".

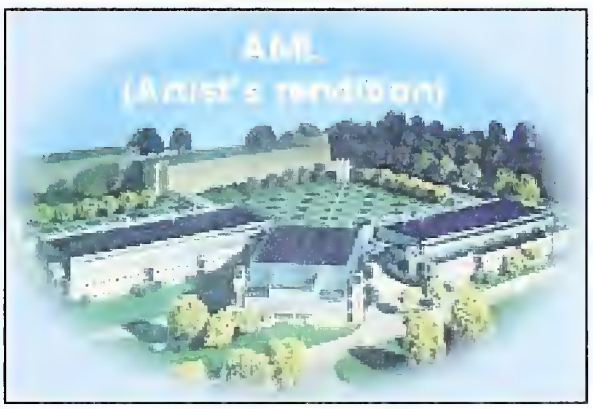
NIST Gaithersburg Campus, Spring 2000

New facilities mark the new millennium:

Fully occupied in May 1999 NIST's *Advanced Chemical Science Laboratory* is a state-of-the-art research facility that provides the means to meet 21st century needs in pharmaceutical manufacturing, medical diagnostics, pollution monitoring and clean-up, nutritional analysis, and advanced materials research. The building features high-efficiency air handling, precise temperature and humidity control, an uninterruptible power supply, an emergency back-up generator, and a wiring backbone that will satisfy data transmission needs well into the future.

The ground-breaking for the new *Advanced Measurement Laboratory* (AML) took place in June 2000. Expected to be ready for occupancy in 2004, the AML will enable CSTL and NIST to keep pace with the rapid developments in emerging technologies requiring molecular and atomic-level precision.
AML will provide improved power quality and stringent controls on:

- Air quality - Class 10,000 Building, Class 100 Clean Rooms
- Temperature - controlled to ± 0.25 °C in Instrument Labs, ± 0.1 °C and ± 0.01 °C in Metrology Labs
- Vibration - <3μm/sec
- Humidity - 40% ± 1%



Measurements and Standards: from the early 1900's to the new millennium

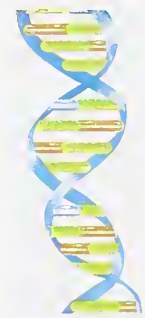
Chemical Standards: addressing needs of mature and emerging industries

The Bureau's First Standard Samples (1905)

"Standard Samples" (with chemical properties certified) were first sold by the Bureau in **1905** when it undertook to certify and distribute the composition of various types of iron provided by the **American Foundrymen's Association.** In **1906**, at the request of the **Association of American Steel Manufacturers,** the Bureau began the preparation and certification of samples of seventeen types of steel, and thus the Standard Samples Program was born.

By **1951**, the Bureau had a whole catalogue of 502 standard samples, with 98 of these samples of steel that were certified for the concentration of up to ten elements. Another 224 samples were hydrocarbons and organic sulfur compounds produced with the help of the **American Petroleum Industry** for the analysis of process streams in petroleum refineries.

New Standards for New Technologies: Human Identity Testing has captured the interest of molecular biologists, as well as the public, since the late 1980's. NIST/CSTL's involvement in this developing technology for the nation's crime laboratories has resulted in a robust and widely accepted discipline today.



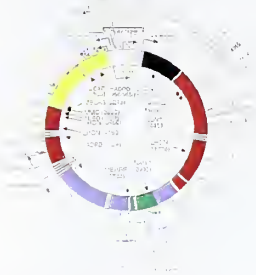
> "The general quality of DNA fingerprinting evidence currently being introduced into U.S. courts appears to be quite mixed," Eric S. Lander, a Harvard University research professor, told a House subcommittee in March 1992, "largely due to an absence of rigorous accepted standards."

To address the principle areas of need for measurement standards in DNA testing, the **first NIST DNA Profiling Standard** was completed and released in the fall of 1992. This Standard Reference Material (SRM 2390) was a DNA profiling standard developed to include existing *de facto* standards such as the female cell line, K562. The SRM was recognized as one of that year's top 100 technological advances, with a prestigious R&D 100 Award in 1993.

The first Polymerase Chain Reaction (PCR)-based DNA profiling Standard Reference Material, SRM 2391, was released in June 1995. This SRM was developed to support the rapid advances in PCR-based technologies in the early 1990's. SRM 2391 was the first standard available that supplied DNA material with multiple loci and genetic typing information. Within a short time, SRM 2391 was enhanced to support the newly developed Short Tandem Repeat (STR) genetic systems for DNA profiling. An extensive set of STR data for the cell lines and genomic DNAs were added to include new forensic markers available from commercial laboratories. This was done to comply with requests from the FBI and other

forensic laboratories to have the SRM 2391 certificate updated to include all genomic DNA components for these tests. NIST has also developed a website (STRBase) to provide a single site where STR information can be obtained by users.

STRBase
http://www.cstl.nist.gov/div831/



The latest release in the suite of DNA Standards:
Human Mitochondrial DNA Standard Reference Material (SRM 2392). This SRM is used in quality control, in sequencing, forensic identification, medical diagnostics and mutation detection. The SRM includes extracted DNA and all information for performing PCR amplification process, cycle sequencing steps, gel preparation, data analyses to determine DNA sequence, and materials to assess the accuracy of the results. In addition, sequences of 58 sets of unique primers are also included to allow any area of all mtDNA to be amplified and sequenced.

Today, CSTL partners with the NIST Technology Service's **Standard Reference Materials Program** to provide scientists and engineers in industrial and academic research with internationally accepted SRMs for critical technical decision-making. NIST pioneered, and continues to lead, in the development of certified reference materials used for quality assurance. NIST provides more than 1,200 different SRMs certified for specific chemical composition or physical properties. CSTL provides technical leadership for most of the chemical and compositional standards produced by NIST.

SRMs are used for three main purposes: to help develop accurate methods of analysis (reference methods); to calibrate measurement systems; and to assure the long-term adequacy and integrity of measurement quality assurance programs. NIST SRMs also legally constitute part of the National Measurement System infrastructure of the United States and, as such, are essential transfer mechanisms for national as well as international measurement traceability. SRMs are available for many applications, including: engine wear materials, environmental materials, ferrous and non-ferrous metals, food and agriculture, fossil fuels, gas mixtures, geological materials, high-purity materials, health/clinical/industrial hygiene, microanalysis, optical and thermophysical properties. The complete list of available SRMs can be found on the following web site.

http://ts.nist.gov/ts/htdocs/230/232/232.htm

Physical Standards: thermometry over the years

NBS 1901 - Division I
Heat and Thermometry
"As primary standards, this section had acquired a number of specially constructed ... thermometers in Europe, and was prepared to certify almost any precision thermometer used in scientific work, as well as industrial and commercial thermometers."

... and the rest is history...
The Bureau's first director was reluctant to hire women since "the sight of his scientists in shirtsleeves might offend them." However, almost 100 women came to the Bureau during the war, making significant contributions in many technical areas. ... Among them was Miss Johanna Busse, a researcher in *thermometry*, who in 1929 became the chief of the section until her retirement in 1949."
Measures for Progress – A History of NBS

International temperature scale had its inception in 1911 when the national laboratories of Great Britain, Germany, and the United States proposed to adjust the differing "practical" temperature scale to absolute. This proved more difficult than anticipated, and in 1927, the three laboratories proposed adoption of an "international temperature scale" (ITS) that might be more readily realized. This practical temperature scale ranged from the temperature of liquid oxygen to that of luminescent incandescent bodies – serving the immediate needs of industry. Agreement on the basic fixed point was reached a year later. For the first time it became possible to certify temperature measurements for a wide variety of industrial purposes. The international temperature scale has been updated and amended in several times during the past century.

History of Temperature Scales:
1. The normal hydrogen scale (adopted in 1889)
2. The International Temperature Scale of 1927 (ITS-27)
3. The International Temperature Scale of 1948 (ITS-48)
4. The International Practical Temperature Scale of 1948, amended edition of 1960 IPTS-48(60)
5. The International Practical Temperature Scale of 1968 (IPTS-68)
6. The International Practical Temperature Scale of 1968, amended edition of 1975 IPTS-68(75)
7. The 1976 Provisional 0.5 K to 30 K Temperature Scale (EPT-76), adopted in 1979
8. The International Temperature Scale of 1990 (ITS-90)

NIST First to Realize International Temperature Scale (ITS-90) from 0.65 K to 1234.94 K with uncertainties (<0.1 to 1.1 mK)

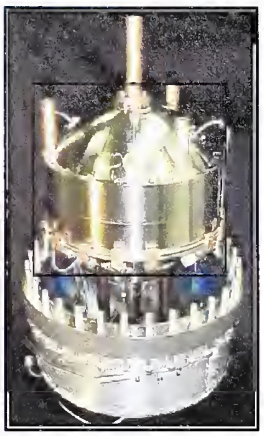
Dissemination of ITS-90

- **Calibration of precision resistance thermometers**
- **Extensive use of SRMs**
  - **Fixed-point cells**
  - **High purity metals**
  - **Reference thermometers**

Customers

- **Aerospace**
- **Cryogenic fuel industries**
- **Academia**
- **Other agency sponsors**

Cutting-edge research in thermometry for the next generation temperature scale



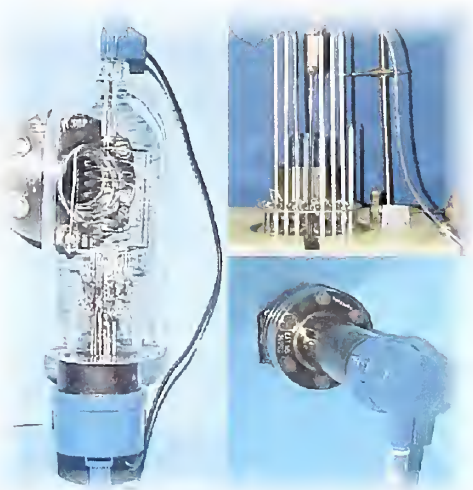
Acoustic Thermometry takes advantage of the fact that the speed of sound of a monatomic gas is simply related to the thermodynamic temperature. The speed of sound is measured via acoustic techniques in a basketball-sized spherical shell. The acoustic resonator shown is resting inside half of a spherical 3 L pressure vessel and furnace cell.

Acoustic thermometry promises to improve the accuracy of thermodynamic temperature measurements above 500 K by a factor of five. These measurements will provide the basis for the next international temperature scale, and an improvement in the consistency of thermometer calibrations.

NIST's calibration services link the makers and users of precision instruments to basic and derived units of the International System (SI) of units. As one of the cornerstones for ensuring the consistency of measurements in the United States and internationally, this measurement transfer system is a critical factor in controlling manufacturing, assembly processes, and marketing, as well as assuring the quality of manufactured goods. Users of these services send transfer standards to NIST where they are calibrated according to a measurement process that is stable, predictable, and statistically analyzed. Currently NIST provides more than 500 different calibrations, tests, and measurement quality assurance programs in seven major measurement areas.

CSTL partners with NIST's Technology Services and provides calibration services for the following:

- **Thermometers (including thermocouples)**
- **Humidity Measuring Instruments**
- **Air Speed Instruments**
- **Volumetric Test Measures**
- **Pressure and Vacuum Gauges**
- **Fluid Flow Meters**
- **Optical Filters**



http://ts.nist.gov/ts/htdocs/230/233/calibration/index1.html

Standard Reference Data

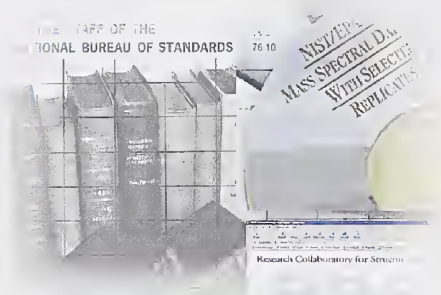
"In 1909 the American Society of Refrigeration Engineers, in search of physical data for more efficient refrigeration, asked the Bureau to determine the specific heats of several calcium chloride brines." And so it began … ***(Measures for Progress – The History of NBS)***

NISTDATA The NIST Standard Reference Database (SRD) series has grown to over 50 electronic databases in chemistry, physics, materials, building and fire research, software recognition, and electronics. Versatile interactive databases provide easy access to high quality NIST data. Traditionally, CSTL has been the key source for evaluated data and Standard Reference Data. Through this program CSTL provides SRDs for Analytical Chemistry, Atomic and Molecular Physics, Biotechnology, Chemical and Crystal Structure, Chemical Kinetics, Industrial Fluids and Chemical Engineering, Materials Properties, Surface Data, and Thermodynamics and Thermochemistry. Methods of data dissemination have changed dramatically over the last century – from tables in voluminous books to computer disks and the World Wide Web.

http://www.nist.gov/srd/

IR Spectral Data – *what goes around comes around*

In 1905, the NBS IR Specta program was established by William Coblentz, and the first book of spectra was published in 1910. By mid-century the IR Spectra resided with the Coblentz society. However, in the late 90's the data was returned to NIST and is being prepared for web-based dissemination.



CSTL led the efforts to provide the mass spectral community with a fully evaluated mass spectral database. **NIST/EPA/NIH Mass Spectral Library** was first published in 1976 in three volumes with three supplements. It is currently disseminated via CD and the Web and is installed on more than 3000 mass spectrometers annually. It contains evaluated spectra for more than 100,000 compounds and it is continually updated with the addition of high quality data.

The NIST Chemistry WebBook, now in its 6th edition, was first released in 1996. In addition to the abundance of thermochemical, thermophysical, and ion energetics data, the new release of February 2000 contains Henry's Law data, UV/Visible Spectral data, and new data on critical constants such as Vapor Pressure and Ion Energetics. In addition, there has been data expansion in areas that support chemical design, including; thermochemical and thermophysical properties of pure fluids and aqueous solutions, vapor-liquid equilibrium data, and equation of state information.

User Statistics:

- **Between 6000 and 12000 users per week**
- **45-55% are return users**

New tool **for sub-structure searching for chemical structures drawn by user**

http://webbook.nist.gov

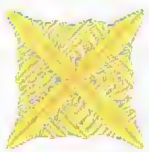
Multi-discipline, synergistic teams are required to address the needs of modern technology. To this end CSTL partners with all other NIST Measurements and Standards Laboratories.

Building and Fire Research (BFRL)
http://www.bfrl.nist.gov/

Electronics and Electrical Engineering Laboratory (EEEL)
http://www.eeel.nist.gov/

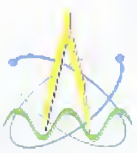
Information Technology Laboratory (ITL)
http://www.itl.nist.gov/

Manufacturing Engineering Laboratory (MEL)
http://www.mel.nist.gov/



Materials Science and Engineering Laboratory (MSEL)
http://www.msel.nist.gov/

Physics Laboratory
http://www.pl.nist.gov/



CSTL supports NIST's ATP to accelerate the development of innovative technologies for broad *national benefit* through *partnerships* with the *private sector*

CSTL Programs

The following pages describe a variety of activities in selected CSTL Programs. These are:

- *Chemical Characterization of Materials*
- *Chemical and Biochemical Sensing*
- *Healthcare Measurements*
- *Environmental Measurements*
- *Chemical and Biochemical Data*
- *DNA Technologies*

CSTL's twelve programs

1. Chemical Characterization of Materials
2. Process Metrology
3. Chemical and Biochemical Sensing
4. Nanotechnology
5. Healthcare Measurements
6. Environmental Measurements
7. Microelectronics
8. Physical Property Data
9. Chemical and Biochemical Data
10. Bio-Molecules and Materials
11. DNA Technologies
12. International Measurement Standards

Chemical Characterization of Materials

The role of NIST and in particular its chemistry laboratories in the chemical characterization of materials dates back to the earliest history of NBS, when the first metal and mineral standards were produced. However, it was not until the early 1970s that the program blossomed into the substantial activity that exists today. Now more than 65% of the nearly 1300 different types of Standard Reference Materials (SRMs) are certified for chemical composition by the staff of the Chemical Science and Technology Laboratory. The Analytical Chemistry Division and the Surface and Microanalysis Science Division of CSTL perform the major part of the work involving the determination of organic and inorganic constituents in macro-, micro-, and nano-domains for reference materials as well as for many other applications. The methods and materials resulting from our work are relevant to a wide range of uses, from environmental and industrial processes to forensic and clinical studies.

Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements

W.E. May, R.M. Parris, C.M. Beck II, J.D. Fassett, R.R. Greenberg, F.R. Guenther, G.W. Kramer, S.A. Wise (839), T. E. Gills, J.C. Colbert, R. Getting, and B. MacDonald (TS)

The Analytical Chemistry Division of CSTL, in collaboration with the Standard Reference Materials Program of Technology Services, has developed a document that provides definitions of terms and descriptions of current practices used at NIST for value-assigning SRMs for chemical composition and related properties. NIST Special Publication 260-136, *"Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements,"* provides a complete description of the seven modes used at NIST to acquire analytical data for the value assignment of SRMs and RMs for chemical measurements and links these modes to three data quality descriptors: NIST Certified Values, NIST Reference Values, and NIST Information Values.

Exploiting Traceability Between Reference Materials: A New Mode for the Value Assignment of Reference Materials

M.R. Winchester, W.R. Kelly, J.L. Mann, and G.C. Turk (839)

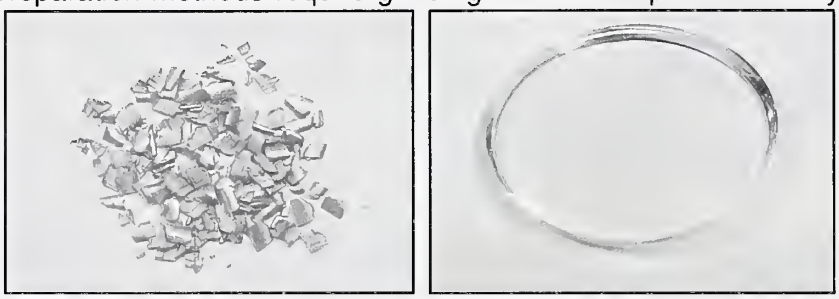
A new approach for the value assignment of reference materials that establishes a direct, robust, traceability link between a candidate material and previous NIST measurements is being explored.

Traditional certification of chemical composition in SRMs at NIST involves the use of a definitive method, or two or more independent analytical methods. In this new approach, a candidate reference material is directly and rigorously compared to a single existing NIST SRM of an appropriate matrix using a single high-precision analytical method. The measurand for the candidate reference material is then computed from the certified value for the calibrant and any observed differences between the two materials. This approach has been applied successfully to the determination of sulfur mass fraction in coal SRMs 2682b, 2684b, and 2685b using high temperature combustion/IR absorption detection.

A Matrix-Independent XRF Method of Analysis for Metals

J.R. Sieber and A.F. Marlow (839), J. Blanchette and F. Claisse (Corporation Scientifique Claisse)

A matrix-independent method of x-ray fluorescence (XRF) analysis is being developed for a wide variety of metals and alloys in disk or chip forms and with calibration using primary reference materials. Conventional sample preparation methods require grinding of metal chips before they can efficiently react with fusion flux materials. The method under development combines sample pretreatment of the sample with acid and hydrogen peroxide followed by a borate fusion to produce a homogeneous solid glass disk from a heterogeneous solid without grinding.

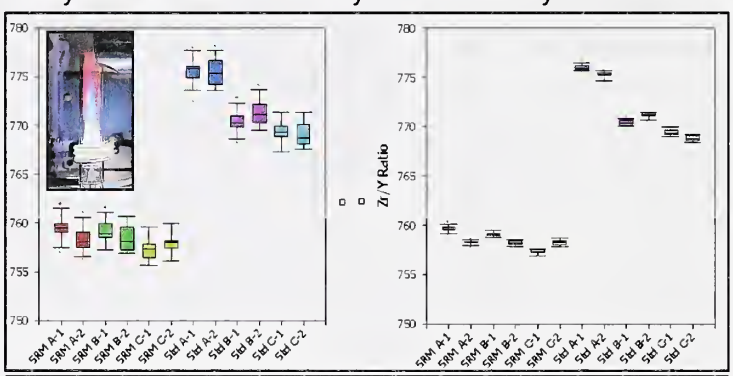


From metal chips to a homogeneous glass disk

A High Performance Primary Method for Elemental Composition

M. Salit and G.C. Turk (839)

A commercially-viable primary method is being developed for elemental analysis that permits traceable measurements with small uncertainty in the chemical analysis community. The method involves inductively coupled plasma (ICP) emission spectrochemical analysis (photo at the left is the high-temperature ICP emission source) coupled with a ratio-based measurement and quantitation scheme, a novel drift correction procedure, gravimetric sample handling, and an experiment design that permits complete evaluation of uncertainty. The result is a method of the highest metrologic order that uses unmodified, widely deployed (≈2000 instruments), commercially available equipment. It has been applied in the certification of major constituents in a high-temperature alloy, the characterization of a $LiAlO_2$ ceramic material, and in the certification of 64 single element SRMs.



Concentration comparisons of a candidate SRM (Zr spectrometric solution standard) with independently-prepared standards with (right) and without (left) the ratio-based measurement scheme illustrating the vast improvement in precision and information content provided by the high performance method.

On the international level, this method was used in the CCQM K8 Key Comparison of standard solutions (Al, Mg, Cu, Fe), completed in December 1999. The NIST results compared favorably in terms of both precision and accuracy with classical methods applied by other National Measurement Institutes (NMIs) that used titrimetry, coulometry, gravimetry, and isotope-dilution mass spectrometry (IDMS). These results support our claim that the improved ICP method can be placed in the context of "replacing classical analysis." It matches the precision and approaches the understanding of sources of error of classical analysis, especially for cases where the sample can be reduced to a simple dilute solution. This claim was submitted to the CIPM/CCQM during its Primary Methods Symposium.

CCQM Primary Methods Symposium

W. E. May, T.W. Vetter, R.R. Greenberg, M. Salit, M. Welch, and R.M. Lindstrom (839)

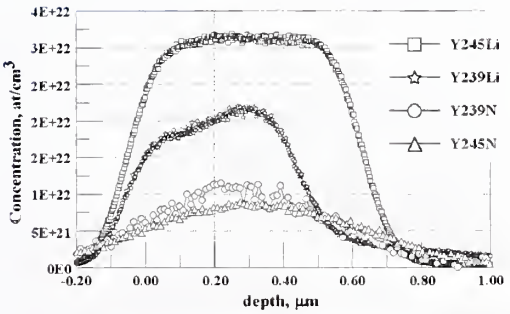
The CCQM has described a primary method as "...a method having the highest metrological properties, whose operation can be completely described and understood, for which a complete uncertainty statement can be written down in terms of SI units."

A symposium titled "CCQM Primary Methods Symposium: How Far Does the Light Shine?" was organized on behalf of the BIPM Consultative Committee for Amount of Substance (CCQM) and held 4-5 April 2000 at the BIPM in Sèvres, France. The purpose of the symposium was to discuss analytical measurement methods that are, or have the potential to be primary direct methods or primary ratio methods. Methods discussed by CSTL staff included (1) gravimetric methods such as the carefully controlled gravimetric determination of sodium in serum using precipitation as sodium sulfate; (2) instrumental neutron activation analysis (INAA) for the measurement of arsenic in silicon, in which all uncertainty sources and the magnitude of the actual measurement uncertainty are identified; (3) ICP optical emission spectroscopy for the determination of major elements in a high temperature alloy, and (4) isotope-dilution gas chromatography-mass spectrometry for various organic analytes.

Measuring Lithium Depth Profiles for Thin Film Battery Applications

G.P. Lamaze and H. Chen-Mayer (839)

Neutron depth profiling (NDP) is being used to measure depth profiles of several light elements up to a few micrometers in thin films. A great advantage of the NDP technique is that it is non-destructive, which allows repeated observations of the concentrations under different conditions. NDP is being applied to measurements of lithium in battery materials that will aid in designing the thermo-mechanical properties of amorphous lithium phosphorous oxynitride (lipon), a solid ionic conductor, particularly the thermal expansion coefficient. The resulting thermal stress is related to starting composition and temperature of evaporant, and of the composition and pressure of the background gas during deposition.

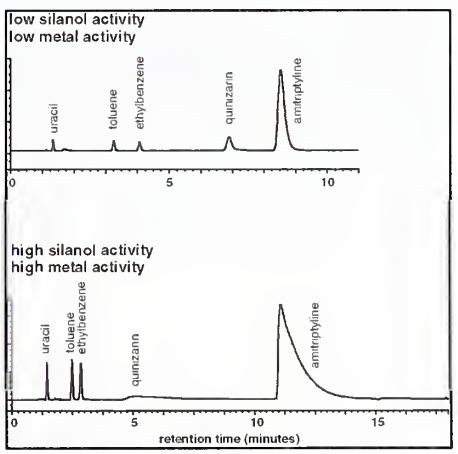


Depth profiles for two lipon samples manufactured under different conditions.

Characterization of Liquid Chromatography Column Performance

L.C. Sander, K.W. Phinney, and S.A. Wise (839)

Uniform approaches are being developed for characterizing the chromatographic performance of columns used in liquid chromatography. Based on fundamental studies, chromatographic tests have been developed that serve as broad indicators of column performance and expected retention behavior. These tests are the basis for two new SRMs intended for the characterization and classification of liquid chromatography columns. SRM 870 (Column Performance Test Mixture for Liquid Chromatography) is an indicator of silanol activity, trace metal activity, retentiveness, methylene selectivity, and



Comparison of SRM 870 separated on dissimilar C_{18} columns

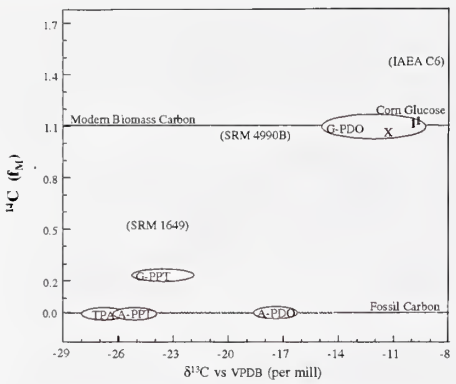
column efficiency. SRM 877 (Chiral Selectivity Test Mixture) is intended for the evaluation of column selectivity toward chiral species. Both materials are useful for column evaluation and classification, as an objective indicator of changes in column performance over time with use, and for control of column manufacturing processes.

Applying Isotope Metrology to the Authentication of Polymers Produced Through Biotechnology

L.A. Currie (NIST Scientist Emeritus), D.B. Klinedinst, and R.M. Verkouteren (837)

Industry watchers anticipate that PPT will supplant PET (Polyester) in importance. PET is *a multi-billion dollar global industry*.

Polypropylene terephthalate (PPT) is a superior alternative to polyethylene terephthalate (PET, a.k.a. "polyester"). The DuPont Company has recently succeeded in developing a new route to its manufacture involving the biosynthesis of an essential intermediate, 1,3-propanediol (PDO), metabolized through a bioengineered yeast feeding on glucose in corn syrup. This new route is more economical (and "greener") than using the alternative synthesis of PDO from petroleum-sourced acrolein. However, since the PDO products from either source were chemically indistinguishable, no "composition of matter" patent was possible and future high investments in PET-to-PPT plant conversion were jeopardized. Measurements with NIST traceability were needed to distinguish PDO and PPT made by the unique bio-assisted process for legal authentication and protection of fair commerce. Since carbon from bio-sourced feedstocks is isotopically distinct from that derived from petroleum, measurements of carbon-13 and carbon-14 enabled the differentiation needed to authenticate the new PPT and PDO. As part of the CSTL Bio-Molecules and Materials Program, a CRADA was created to design the relevant isotopic measurements having the highest possible traceability. The method was tested through blind analysis of a G-PDO sample provided by DuPont. This isotopic method may benefit other sectors of the chemical manufacturing industry when similar barriers to commercialization are encountered.



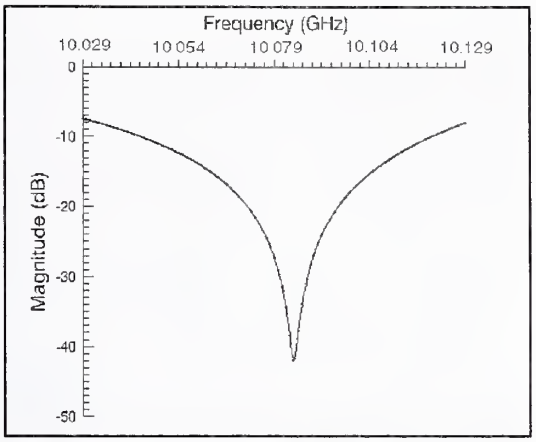
Verification and authentication of PDO and PPT derived from glucose (versus acrolein) by dual isotope (^{13}C + ^{14}C) stoichiometry:
G-PPT = 3/11(G-PDO) + 8/11(TPA)

CSTL helps industry to protect its technological R&D investments.

Microwave Evanescent Probe Microscopy for Materials Analysis

S.J. Stranick, C.A. Michaels, and S.W. Robey (837)

The Surface and Microanalysis Science Division is developing robust microwave-frequency microscopy methodologies and data reduction strategies for dielectric imaging and high-throughput screening of chemically textured surfaces of thin films and semiconductors, with emphasis on accuracy in the measurement of small sample volumes and high-throughput methodologies. We are extending near-field microscopy to the microwave region using radiation up to 20 GHz that is coupled evanescently to the sample surface using a sharp proximal probe that is part of a resonant cavity or a transmission line structure. Our current focus is on the evaluation of probe designs and on the fabrication of geometry-controlled calibration artifacts.



Shown above is a mode of the microwave resonator that along with a sharpened metal tip forms the evanescent probe used in the microscope design.

Phase Mapping of Plasma-Sprayed Yttria-Stabilized Zirconia

J.R. Verkouteren, R.B. Marinenko, and D.S. Bright (837)

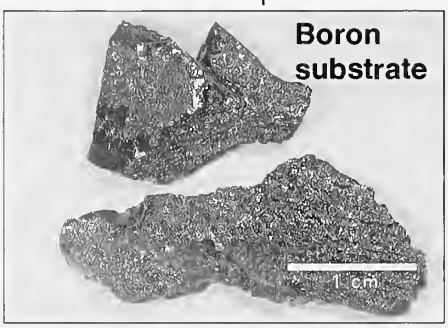
Elemental wavelength dispersive spectrometry (WDS) x-ray compositional mapping and microbeam x-ray diffraction (μXRD) are being used to determine compositional and crystalline phase changes upon heating of plasma-sprayed $(Zr,Y)O_x$ ceramic coatings. These coatings, used as thermal barriers on aircraft and land-based turbines, and on diesel engines, fail after a number of thermal cyclings for an unknown reason. Redistribution of yttria in the coating from the tetragonal (t) phase to a low-yttria monoclinic (m) phase and a high-yttria cubic (c) phase is thought to contribute to the failure. Bulk diffraction methods (neutron and x-ray) are used primarily for phase identification in these materials, but the analysis is challenging due to the similarity in structure between the t and c phases. The lattice parameters of each phase are used to track the redistribution of yttria, but the challenging nature of the analysis raises doubts as to the accuracy of measurements using these methods. The results of our measurements on coatings obtained from a collaboration with the Materials Science and Engineering Laboratory clearly showed that the distribution of yttria and the resulting phase compositions are dependent upon the choice of feedstock powders used in the preparation of the coatings, and that individual coatings display different responses to heating, in some cases following routes not predicted by phase equilibria studies.

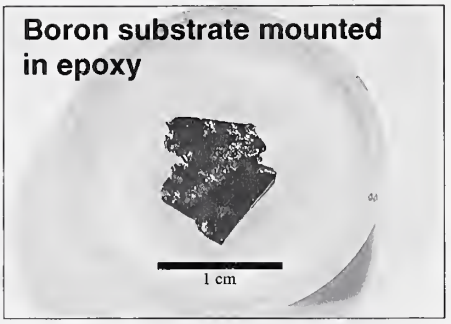
WDS compositional x-ray map with low-yttria areas in red. μXRD pattern of t-ZrO_2 from 10 μm wide area shown at the right.

New Boron Substrates for Particle Microanalysis

E.S. Windsor, D.E. Newbury, and J.D. Kessler (837)

A new substrate is being developed for the chemical analysis of carbonaceous particles with the electron probe microanalyzer. Since carbon planchets interfere with carbonaceous particle analysis, and beryllium planchets are undesirable because of that element's toxicity, we have been studying boron substrates. These substrates should easily be adoptable by laboratories performing air pollution and atmospheric particle analyses and will provide a safe and instrumentally ideal (low background) way to measure carbonaceous particles.





Use of Single Particle Radioactivity Measurements to Expand Microanalysis Capabilities

C.J. Zeissler (837)

Using a photostimulated luminescence phosphor system, particulate samples of up to several grams are autoradiographed and sample subdivision methods are incorporated for preconcentration and for particle isolation.

Electron and ion radionuclide microanalysis capabilities are being expanded by: (1) developing pre-screening method for samples 10^6 times more massive than can be analyzed by electron and ion beam methods, (2) preconcentrating radionuclides in samples prior to ion and electron microscopy, and (3) providing a means to associate chemical and morphological information with radionuclides at concentration levels significantly below the detection limits of conventional beam and spectrometry methods. This new method lowers detection limits to the 0.02 mBq to 2 mBq range, depending on the radioactivity decay mode, corresponding to levels that are far below the detection limits of electron and ion probe methods.

Standards to Determine Moisture in Crude Oil for the Petroleum Industry.

S.A. Margolis (839)

The accurate measurement of water in crude oil is of significant economic importance in the crude oil industry because crude oils contain appreciable amounts of both sulfur compounds and water. Refineries do not wish to pay for water and the crude oil producers do not want the water content overestimated. A variety of compounds (including mercaptans and other sulfur containing compounds) reduce iodine to iodide, and are present in crude oils and refined oils, and can bias the measurement of water as determined with the primary assay technique, the Karl Fischer method. Researchers in the Analytical Chemistry Division have developed SRMs 2721 and 2722 for the calibration and standardization of instruments and refined oil reference materials for the measurement of water. A new coulometric method has been developed that allows the manipulation of measurement parameters and has facilitated our development of methodology to estimate non-water interferences. Preliminary results with a representative group of refined and crude oils including SRMs and RMs indicate that refined oils may contain 5 mg/kg to 10 mg/kg of interfering substances and that crude oils may contain from ≈5 mg/kg (low sulfur crude) to ≈800 mg/kg (high

sulfur crude) of interfering substances. The total amount of material reacting with the Karl Fischer reagent in the high sulfur crude oil was 956 mg/kg indicating that 84% of the measured material was not water. Future plans include (1) the validation of this method, (2) its application to the measurement of the interfering materials in current RMs and the crude oil SRMs, (3) the measurement of water in oils such as motor and lubricating oils which also contain interfering substances, (4) the patenting of this method for which we already hold a preliminary patent and (5) the submission of this method to ASTM as a standard method to be used in conjunction with the Karl Fischer method for the measurement of water in a wide variety of oils.

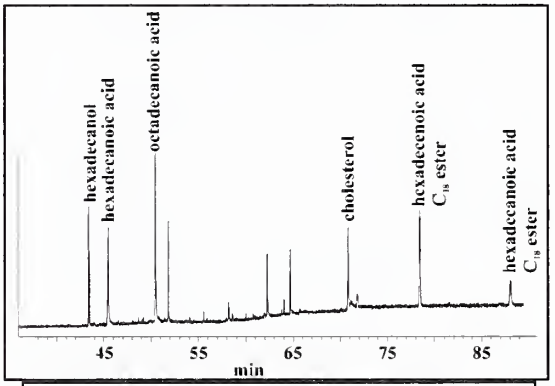
Providing Measurement Tools and Standards for Forensic Chemistry

G.W. Kramer, L.C. Sander, B.A. Benner, D.L. Duewer, W.A. MacCrehan, K. Phinney, J.D. Secl, M.J. Welch (839), and M.C. Kline (831)

CSTL addresses the needs of the forensic chemical measurements community by
1. Developing and evaluating new measurement technology that will have applicability in criminal investigations; and
2. Providing *reference materials and interlaboratory comparison studies* to help forensic laboratories validate their methods and to provide compelling evidence of competent measurement performance.

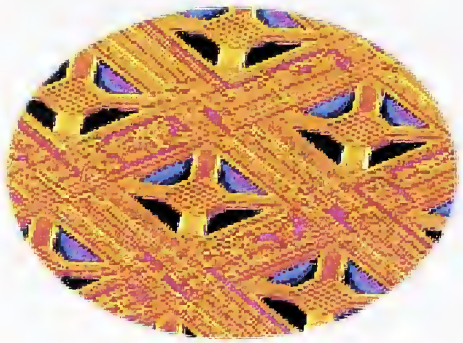
The admission of scientific evidence is coming under increased scrutiny in the courtroom, and forensic investigators and crime laboratories are heavily burdened with casework, providing little or no opportunity to develop new, more effective chemical measurement tools. CSTL has responded to these measurement needs in a number of key areas: human identification through the use of DNA and hydrocarbon signatures, the determination of handgun and pipe bomb use through organic gunpowder analysis, and accurate evaluation of drugs of abuse in hair.

Hair is routinely collected at crime scenes for subsequent forensic analysis by microscopic examination. However, a new method for determining chemical characteristics of small samples of hair could aid law enforcement officials in narrowing the pool of suspects of a crime. ACD is investigating a method for chemically characterizing very small samples (100 µg to 1 mg) of human hair using on-line supercritical fluid extraction, gas chromatography/mass spectrometry (SFE-GC/MS). The method offers a number of benefits including greater sensitivity compared with liquid extraction-based techniques because the entire extractable mass is transferred to the analytical system, providing higher recoveries of volatile species. The mass spectra of species detected by this method can confirm their identities. We have employed SFE-GC/MS to measure components associated with human hair from 20 volunteers to investigate if the method yields consistent yet unique chemical profiles from peoples' hair. Components detected in hair after SFE-GC/MS include fatty acids, alcohols, fatty acid methyl esters, squalene, cholesterol, and a number of other species from secretions of the sebaceous and sweat glands in the scalp, shampoos, and hair treatments, and from environmental exposure (see figure). This preliminary study suggests that analysis of the surface components of small hair samples by SFE-GC/MS may help match hair samples taken at a crime scene with those of specific individuals. Further research is planned including utilizing a larger and more statistically developed data base of hair samples, investigating the effects of archiving on the chemical profiles of an individuals hair, and employing other analytical techniques in addition to GC/MS (e.g., liquid chromatography/mass spectrometry) for measurement of components in hair.



SFE-GC/MS analysis of 149 µg of hair from an 8 year old female

Chemical and Biochemical Sensing



CSTL is a leader in the development of small surface-mounted detection technologies with the capabilities of detecting single molecules. The evolution in engineering of surface-mounted nanodevices has resulted in the development of gas sensing with micro-hotplate sensor arrays by measuring variations in conductivity of temperature-cycled, treated surfaces due to adsorbed molecules, which provide identifiable "fingerprints" for the specific gases. These sensors may lead to a single chip device that can monitor changes in mixed gases, vapor composition and the introduction of environmental contaminants, which are critical for many chemical and industrial processes.

There are few detectors that are as sensitive and specific as those developed by nature. Efforts to incorporate natural, cellular detectors in integrated and, ultimately, arrayed devices are in progress. The critical factor in the design of these devices is the attachment of the sensor molecules to a device in a manner that permits the molecules to function properly so that species can be detected electronically, thermally or optically. These novel sensing technologies are expected to have broad impact, with spillover into areas ranging from pharmaceuticals, chemical production, environmental monitoring, health diagnostics, workplace safety, and treaty monitoring.

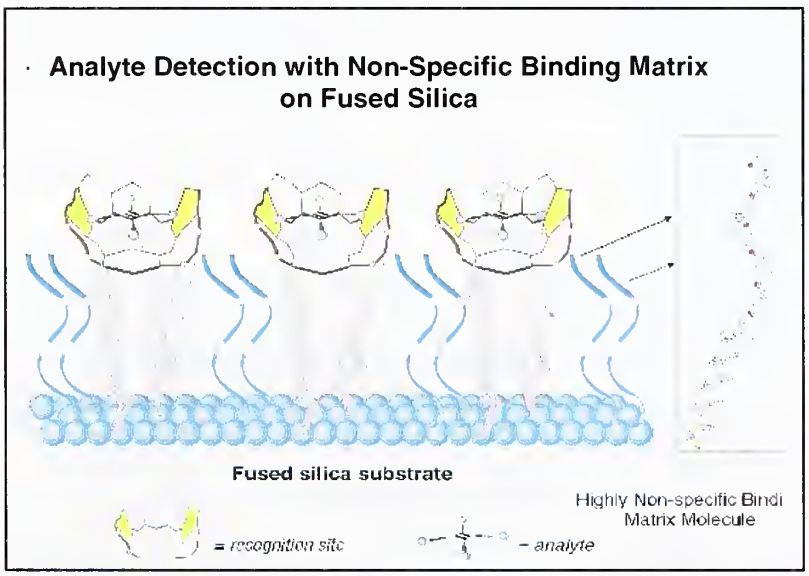
CSTL researchers exploit the efficiency of "natural sensors"

In cells, most of the biological sensing molecules are membrane bound molecules, usually receptors, and there are a number of projects that are exploiting the cellular mechanisms and machinery to create molecular detectors.

Optical Methods for Chemical Sensing

A.C.R. Pipino, J. T. Hodges (836), D.J. Vanderah, and C.W. Muese (831)

Ultra-sensitive optical techniques for probing the surface chemistry of SiO_2, which plays a critical role in many applications areas including microelectronics, are under development. Cavity ring-down spectroscopy (CRDS) measures the optical absorption of gases with extremely high sensitivity by utilizing the photon decay time in a high-finesse optical cavity as the absorption-sensitive observable. CSTL researchers are developing a novel implementation of CRDS, termed evanescent wave cavity ring-down spectroscopy (EW-CRDS). This approach extends the CRDS technique to surfaces and condensed media. EW-CRDS utilizes novel optical cavity designs that employ total internal reflection (TIR) at ultra-smooth surfaces, and provides a new tool



Molecular recognition scheme for chemical selective sensing at the surface of an EW-CRDS resonator.

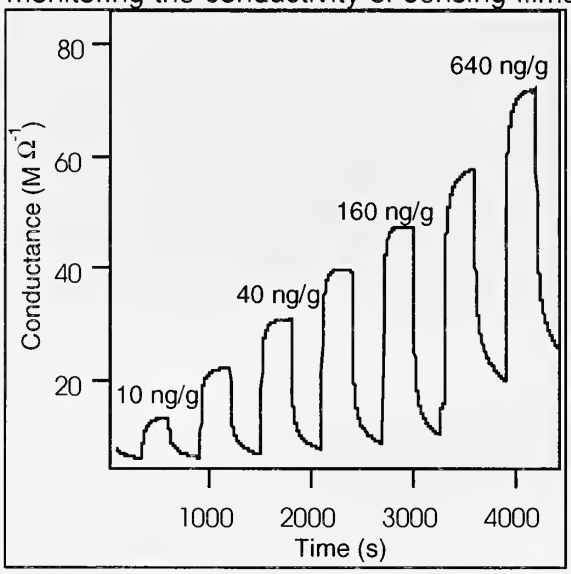
for fundamental studies of surface chemistry and physics, while forming the basis for a new chemical sensing technology. The goal is to combine the sensitivity of EW-CRDS with the selectivity of molecular recognition chemistry by functionalizing the ultra-smooth TIR surfaces of EW-CRDS resonators.

Gas Sensing with Micro-Hotplate Sensor Arrays.

R. Cavicchi, S. Semancik, C. Wheeler, J. Allen, J. Tiffany, M. Carrier, J. Melvin (836), J. Suehle (EEEL), D. DeVoe, and B. Panchapakesan (University of Maryland)

Accurate gas-phase measurements are required for applications ranging from environmental monitoring at hazardous waste sites to chemical agent detection.

Increasing global competition has placed new demands on the chemical process industry for more efficient use of materials, better process reproducibility, and environmental safety. Meeting these demands requires a low-cost technology for the measurement of gas species, which can provide immediate, onsite analysis for the detection of reaction products, exhaust gases, leaks, etc. The micro-hotplate sensor array used in these studies has three functional layers: a heater, a thermometer/heat distribution plate, and electrical contacts for monitoring the conductivity of sensing films. There are three key components to the microsensor research program: advancing sensor materials, understanding transducing mechanisms, and developing new methods for sensor operation and signal analysis. The sensor generates repetitive response signatures that are characteristic of adsorbed species/sensing material combinations. Neural network and chemometric-based approaches to this problem are being used to optimize the generation of patterns and to analyze signals during sensing.

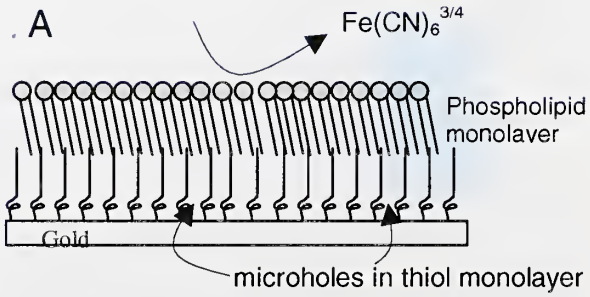


The response of a tin oxide nanoparticle sensing film to methanol in air (concentration increases twofold at each step as shown in the figure).

Fabrication of an Electrochemical Biosensor Sensitive to Structural Defects in Hybrid Bilayer Membranes.

J. Elliott and A. Plant (831)

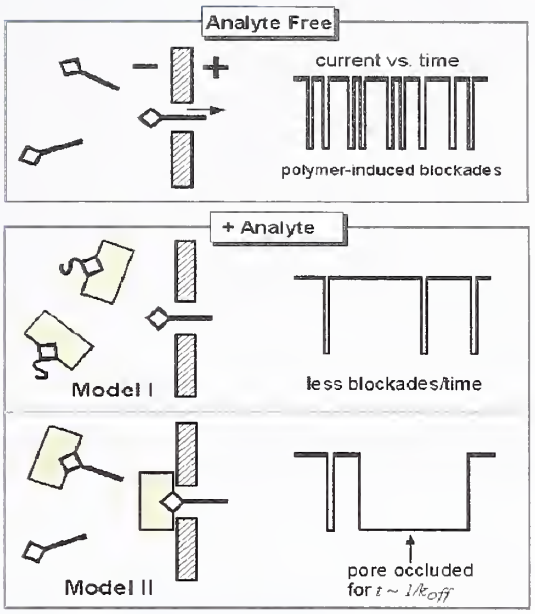
This project involves the fabrication of a phospholipid hybrid bilayer membrane that permits monitoring of membrane integrity using impedance spectroscopy and electrochemistry. This technique might prove useful as a biosensor for detecting peptide toxins and lipid enzymes that act on cell membranes. Our goal was to produce Hybrid Bilayer Membranes that could be used to electrically monitor defect formation in the supported phospholipid monolayer. We therefore measured the electrochemical properties of a variety of mixed and partially packed alkylthiolate monolayers that decrease the insulating effect of the self-assembled monolayer (SAM).



Simultaneous and Rapid Multianalyte Quantitation Using a Nanopore.

J.J. Kasianowicz, S.E. Henrickson, B. Robertson (831), and H.H. Weetall (ATP)

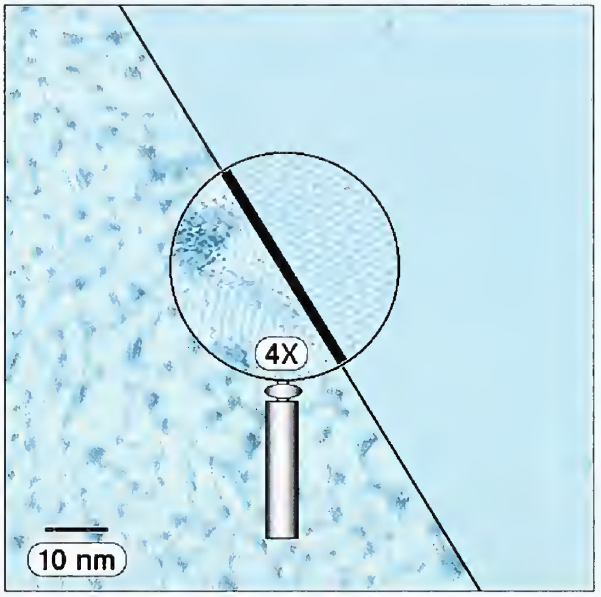
The ability to rapidly and simultaneously quantitate many analytes represents the next frontier in sensing. A wide variety and large number of analytes could be detected simultaneously by affixing recognition elements to polymers that thread completely through a nanometer-scale pore. We used the ion channel formed by *Staphylococcus aureus* α-hemolysin (αHL) as a model nanopore, biotinylated single-stranded DNA (bT-poly[dA]) as the pore-permeant polymer, and Avidin as the analyte. Electrophysiological methods were used to measure the rates at which polynucleotides thread into the pore in the presence and absence of analyte.



Self-Assembled Monolayers for Diagnostics and Sensing.

M. Tarlov, G. Poirier, T. Huang, G. Saupe (836), and K.G. Olsen (Loyola College)

Self-assembled monolayers (SAMs) as model systems are being studied for fundamental, quantitative knowledge of generic molecular recognition and sensing reactions of ultrathin films. Development and application of ultrathin film measurement methods may help correlate molecular-scale structure of films with their diagnostic and sensing performance. Biosensors and diagnostics are being developed to perform rapid, accurate, and low-cost multi-analyte measurements in devices such as DNA and protein chips. These two-dimensional diagnostic arrays are revolutionizing health care, biomedical research, and drug discovery. While the feasibility of this technology has been demonstrated, there is still little known about factors such as molecular conformation and structure that influence hybridization of surface-bound probes. To better understand these systems it will be necessary to develop novel measurement methods for correlating biomolecular layer structure with molecular recognition properties.



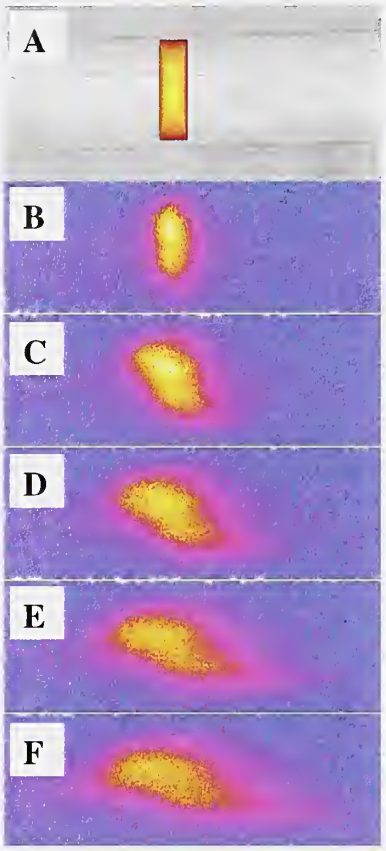
STM images of decanethiol SAMs formed on Au (111) prepared by conventional self-assembly methods (left) and a new method (right) derived from the understanding of phase behavior. The new method yields nearly defect-free monolayers.

Flow Imaging Techniques for Characterization of Microfluidic Systems

L. Locascio, E. Waddell, T. Johnson, G. Kramer (839), M. Gaitan (EEEL), D. Ross, R.E. Cavicchi, S. Barker, and M. Tarlov (836)

Microfluidic, or so-called "lab-on-a-chip," devices are generating much excitement because of their potential for high-speed and high-throughput chemical analysis. However, expansion of the "lab of the future" to other applications is held back by numerous technical barriers, such as poorly characterized and irreproducible microfluidic behavior, the high cost of silicon-based devices, and the lack of integrated and chemically selective detection elements. We have been improving methods for forming microchannels in polymer substrates as well as developing an optical method to measure the electroosmotic flow (EOF) that moves fluids through the system. Polyelectrolyte multilayers (PEM) are being used to modify microchannel surfaces so as to generate reproducible surfaces and allow tailoring of channels for specific chemical analysis problems. PEM coatings on channel walls can be used to control the direction of flow in the microchannels as shown in the figure, where there is a positively charged polyelectrolyte on the walls of one half of the channel and a negatively charged polyelectrolyte on the other half, producing EOF in opposite directions in a single channel. The figure shows a plug of fluorescent dye in such a channel that separates due to the opposite flows. Flow profiles have been measured in a number of different plastic microchannels in order to examine the effects of different materials and fabrication methods on EOF in plastics. Such fundamental data relating flow to surface properties will enable developers of this technology to tailor plastic microfluid channels for specific applications.

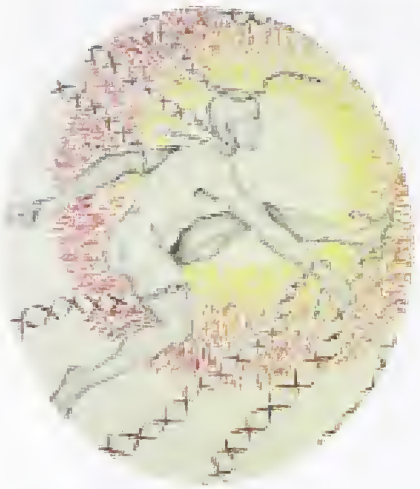
Time-step images an uncaged dye plug moving in a PEMs derivatized channel. (A) shows a photomicrograph of the microchannel with schematic depiction of dye at the instant of uncaging. Sequential fluorescence images of the plug movement, acquired every 67 ms, are shown in (B) – (F).



Using methods to probe the surface charge and surface charge density in plastic devices using fluorescent labeling and microscopy to identify the presence of active charged moieties, we have determined that the process used to fabricate plastic microfluid devices, as well as the plastic material itself, can have a profound influence on the surface charge on the microchannel walls. Using flow-imaging techniques, we evaluated the effect of charge distribution on sample dispersion. By understanding the influence of different fabrication techniques on the surface of the plastic microchannels, we are beginning to develop microfluidic systems designed for specific applications. The idea of tailoring the microchannel surface by varying the fabrication method is pioneered by our group and is unique to plastic microfluidic devices, indicating that there are advantages other than cost to utilizing plastics as the preferred material.

Fluorescence images showing localization of channel charge (bright spots on images). Channels approximately 50 μm wide. (A) room temperature imprinted; (B) hot imprinted; (C) laser ablated channel.

Healthcare Measurements



Chemical metrology is at the heart of accurate medical diagnosis and the development of measures to improve our health and ensure long life. In the U.S. about 1.5 trillion dollars are spent each year on health care, which is over 14 % of our GDP. More than 25% of these expenditures are for measurements. It is estimated that over one third of these measurements are performed for non-diagnostic purposes, such as QA/QC measurements and retests at a cost of about $40B annually. Clearly, improvements in the reliability of chemical measurements in this area would have a significant economic impact for our country.

NIST/CSTL works closely with the American Association for Clinical Chemistry (AACC), the Centers for Disease Control and Prevention (CDC), NCCLS, and other organizations interested in health-related standards to help prioritize our standards activities and the development of SRMs.

CSTL's Healthcare Measurements Program addresses measurements and standards needs of the medical industry – in the areas of prevention, diagnostics, and treatment.

NCCLS

CDC Centers for Disease Control and Prevention

AACC Advancing Clinical Laboratory Science Worldwide

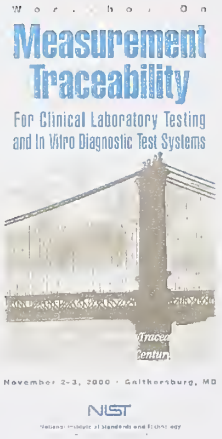
For more than 20 years CSTL has developed, maintained, and refined "Definitive Methods" for 12 health status markers to support the national reference system for clinical measurements: calcium, chlorine, cholesterol, creatinine, glucose, lithium, magnesium, potassium, sodium, triglycerides, urea, and uric acid. NIST definitive methods for these health status indicators have been used to value-assign SRMs and high-priority serum pools used to serve as the anchor point for CDC developed reference methods and by the College of American Pathologist (CAP) for proficiency testing of more than 20,000 U.S. clinical laboratories. Improved accuracy facilitated by this program has led to better diagnosis, treatment, and reduced healthcare costs. Maintaining these anchor points for the clinical measurements reference system also facilitates the development and use of new technologies that are better, faster and cheaper.

A new generation of health status markers, emerging now, shows great promise from the clinical diagnostic perspective, but offers new and more difficult challenges for standardization. Many of the new markers are proteins, peptides, or other large biomolecules, usually present at very low concentrations. Because of the large market for tests for these new markers, many different approaches have been developed commercially resulting in vast disagreements among manufacturer test kits. NIST/ CSTL has focused on basic research to establish reference systems for several new biomarkers, shown in the box to the right.

• Troponin	*heart attack marker*
• Homocysteine	*risk of heart disease*
• Glycated Hemoglobin	*diabetes status*
• Cortisol	*endocrine function*
• Thyroxine	*thyroid function*
• Cadmium	*heavy metal toxicity*
• Folic Acid	*neural tube defects*
• Mercury	*heavy metal toxicity*
• Speciated Iron	*hemochromatosis, anemia*
• Human Serum Albumin	*renal failure*
• Prostate Specific Antigen	*prostate cancer*
• P53 DNA	*breast cancer*
• Thyroid Stimulating Hormone	*thyroid function*

Another driving force for more clinical reference standards is the new European Community (EC) *In Vitro* Diagnostic Devices (IVDD) directive that requires traceability of IVD devices to recognized national standards. By December 2003, all IVD products sold in Europe must have the "EC Mark" verifying compliance with the directive. U.S. manufacturers are major exporters of IVD products and thus are directly impacted by this directive.

In November 2000, NIST hosted a workshop on Measurement Traceability for Clinical Laboratory Testing and *In Vitro* Diagnostic Devices. CSTL worked closely with the workshop cosponsors, NCCLS, AACC, AdvaMed (formerly HIMA), CDC, and College of American Pathologists (CAP) to ensure that the needs of the IVD industry were presented and met. The workshop goals were to develop recommendations regarding the needs for measurement traceability for health status markers to (1) address IVD industry needs for compliance with international standards (e.g., EU IVD Directive) and (2) improve comparability of clinical measurement data to facilitate better decision making by medical professionals.



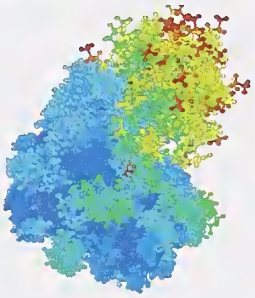
The status of current health status marker work, grouped by disease state, is described below.

Markers to Improve Disease Diagnosis and Management

Cardiac Troponin-I (cTnI): Marker to Diagnose Heart Disease

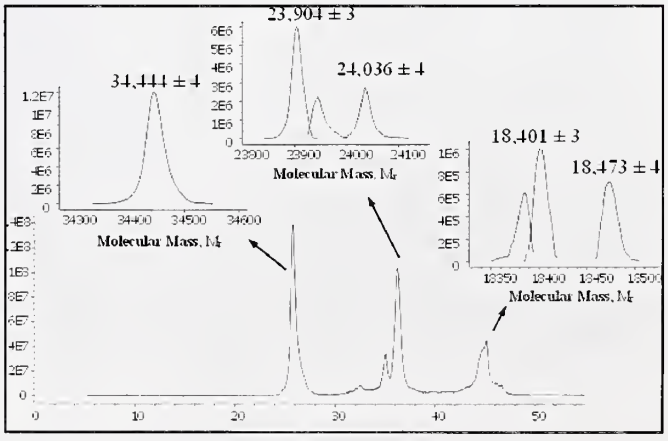
D. Bunk, J. Dalluge, M. Welch (839), R. Christenson, and S.H. Duh (University of Maryland)

The measurement of cTnI in serum provides a highly selective and sensitive means for diagnosing myocardial infarction. For clinical cTnI measurement, there are numerous cTnI assays that have been developed. Unfortunately, cTnI measurements using different methods on identical samples may differ by 100-fold, creating a serious problem for the clinical community. The Troponin-I subcommittee chose ten candidate reference materials, which were analyzed at NIST for purity and structural heterogeneity using liquid chromatography coupled with mass spectrometry (LC/MS). The candidate reference materials were sent to all manufacturers of commercial cTnI assays used in the United States and Europe (13 manufacturers in total) for analysis using their cTnI assays.



NIST, the American Association for Clinical Chemistry (AACC), and the International Federation of Clinical Chemistry (IFCC) have formed a subcommittee to address inter-method variability problems in clinical cTnI measurement, through development of a cTnI reference material to harmonize results.

Results from these analyses were collected and subjected to statistical analysis that found that two candidate reference materials, both complexes of troponin I with other troponin isoforms, provided the most linear response for all thirteen cTnI assays. The



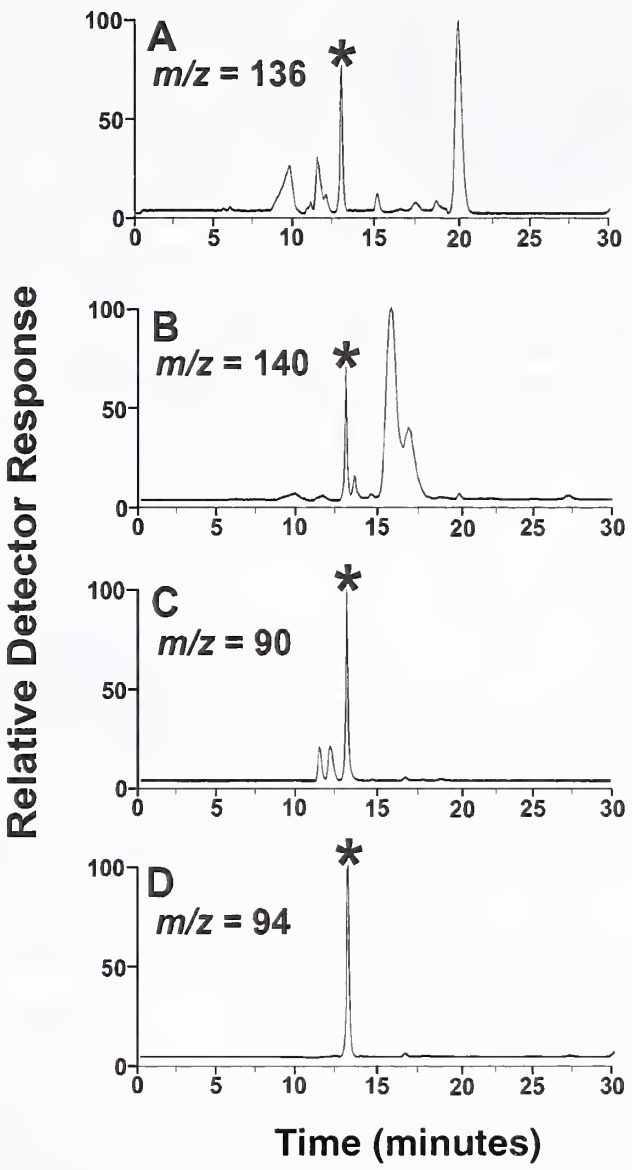
Total ion chromatogram from the LC/MS analysis of the cardiac troponin CIT complex from the University of Miami. The inserts show the molecular mass distributions from the troponin T, troponin I, and troponin C peaks, respectively.

next comparison study will involve these two materials and blood samples from patients with myocardial infarction. The candidate reference materials will be used to calibrate the commercial cTnI assays prior to measuring the patient samples. This study should determine the material that will best harmonize the commercial cTnI assays.

Homocysteine: Marker to Diagnose Heart Disease

J. Dalluge, B. Nelson, L.T. Sniegoski, and S. Margolis (839)

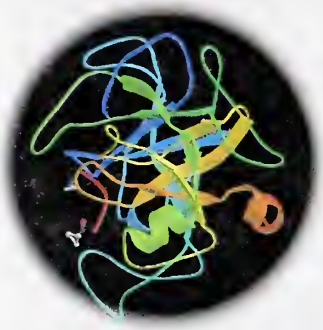
Total homocysteine (tHcy) has emerged as an important independent risk factor for cardiovascular disease, as well as other serious health conditions. tHcy is measured clinically using a variety of methods including immunoassays and LC-based methods requiring extensive derivatization procedures and frequent analysis of quality control samples. Because clinical laboratories use a variety of different methods for its measurement, interlaboratory comparisons of tHcy measurements are poor. Work at NIST has focused on development of LC/MS and GC/MS methods. For the LC/MS method, chromatographic columns and elution conditions were investigated to achieve retention of homocysteine. This led to development of an elution system that allowed unambiguous detection of two homocysteine-specific ions and two isotopically labeled homocysteine-specific ions (internal standard) in human plasma. The quantitative capabilities of this approach will be tested in conjunction with the GC/MS method under development and in collaboration with scientists at the Mayo Clinic. Use of GC/MS requires additional separations and derivatization prior to analysis. An anion exchange method was found that provided the necessary analyte isolation. Thus, the GC/MS method should be ready for further testing in the near future. Once the methods have been validated, they will be applied to the determination of homocysteine in plasma-based reference materials.



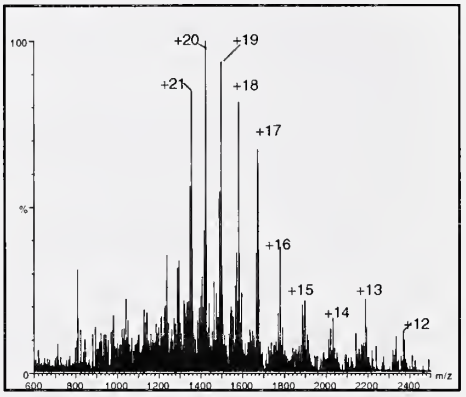
Identification of Hcy and d_4–Hcy in human plasma using LC/MS. The peaks corresponding to Hcy and d_4–Hcy in the mixture are labeled with an asterisk. Concentration of Hcy and d_4–Hcy in the plasma sample was 15 µmol.

Prostate-Specific Antigen (PSA): Marker to Diagnose Cancer

C. Nelson, J. Dalluge, D.M. Bunk, and M.J. Welch (839)



PSA is a single chain glycoprotein (~ 7% - 8% carbohydrate) present in the prostate and seminal fluid. The presence of PSA in the serum is measured as a diagnostic indicator of prostatic cancer. Clinical laboratories currently measure PSA by immunoassays, which are confounded by lack of common standards, and the heterogeneity of the antisera and antigen being measured. Measurement of PSA is a significant challenge due to the structural heterogeneity of PSA in serum, its low concentration in this complex matrix (<10 ng/mL), and lack of knowledge regarding what form is actually being measured in clinical PSA tests. Current research efforts are focused on characterization of a variety of PSA standards at the molecular level by LC/MS in order to understand further the heterogeneity of the analyte, arising predominately from differential lycosylation of the protein. Reproducible full scan (m/z 600 to 2500) positive charged mass spectra were obtained with a commercial source of PSA. Deconvolution of the mass spectrum showed a predominant molecular species at M_r 28,447; the predicted M_r for the glycosylated mature PSA is 28,430. Selected ion monitoring of specific positive charge states was utilized to detect PSA standards at levels as low as 50 ng. Further efforts will focus on: 1) production of a well-characterized SRM for improvement of between-method variations in calibration of immunoassay measurements, 2) determination of specific forms of PSA in patient samples to understand better the clinically relevant forms of this compound, 3) isolation of PSA from serum using non-immunological methodology including combinatorial production of aptamers with high binding affinities for PSA, 4) development of LC/MS and LC/MS/MS methods for the measurement of PSA in serum.

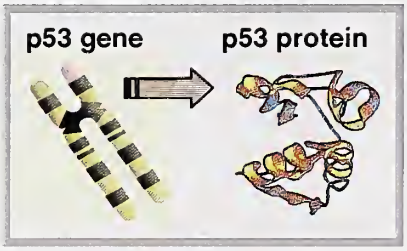


Full scan mass spectrum of prostate-specific antigen obtained by liquid chromatography/electrospray ionization mass spectrometry

Evaluation of p53 Standard Reference Materials Using Mutational Scanning Technologies: Marker to Diagnose Cancer

C.D. O'Connell, B.S. Akbasak, L. Tully, D.H. Atha (831), J. Delaney, and M. Marino (Transgenomic, Inc.)

We have created a panel of p53 mutation standards for use in mutation detection technologies as part of our molecular diagnostic measurements program. These materials were used as positive controls to assess the mutational status of clinical specimens. Although DNA sequencing remains the "Gold Standard" for the detection of mutations, a number of mutational scanning technologies are being developed to reduce the region of the gene that needs to be sequenced, thus saving both time and cost. Measurement standards are required to accurately compare these mutation detection technologies.

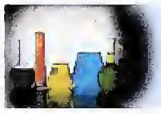


Measurements on clinical specimens were performed using proposed NIST SRM materials. Archival clinical samples were evaluated for p53 mutations within exons 5 through 9, the most commonly mutated region of this gene. The NIST materials, used for both positive and negative mutational controls, consisted of 11 mutant and 1 wild-type clone.

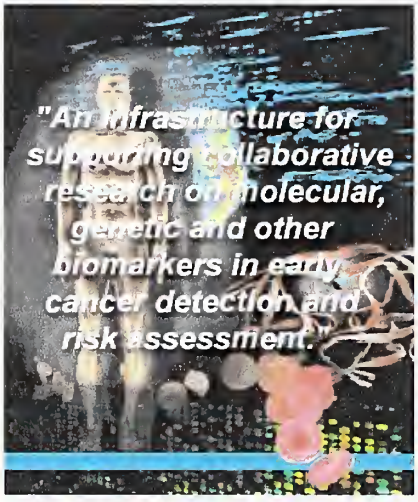
Four different mutation detection technologies were used in this study: 1) DNA sequencing, 2) single strand conformation polymorphism analysis (SSCP), 3) denaturing gradient gel electrophoresis (DGGE), and 4) denaturing high performance liquid chromatography (DHPLC).

The two heteroduplex-based methods (DGGE and DHPLC) yielded identical results: 6 mutations were detected in the 33 samples analyzed. These were confirmed by DNA sequencing. Difficulties in gel-based separation (DGGE) of exon 5 heteroduplexes were not observed in the DHPLC measurements. All but one mutation detected by heteroduplex analysis were also observed by slab gel and capillary SSCP measurements. Multiple conformers were detected by SSCP in both slab- and capillary-based measurements, consistent with previous reports using this technology. The presence of multiple conformers representing a single species of DNA (wild-type or mutant) is a complication of the SSCP assay system. The wild-type standard was important in distinguishing wild-type conformers from mutations. Both slab gel SSCP and capillary SSCP detected additional conformers with respect to wild type in samples not reported as mutant by heteroduplex analysis. Automated fluorescent DNA sequencing did not confirm these putative mutations. These "mutations" may be present at too low a percentage in the mixed population of normal and tumor tissue to be detected by sequencing or heteroduplex analysis. Alternatively, additional conformers representing wild-type DNA are formed in the SSCP analysis of clinically derived genomic DNA samples. Future studies are aimed at determining the detection limits of these mutational scanning technologies for the identification of mutations in heterogeneous clinical materials.

NIST Biomarker Validation Laboratory
P.E. Barker and C.D. O'Connell (831)



Early Detection Research Network



As a collaboration with the National Cancer Institute's new consortium The *Early Detection Research Network* (EDRN), CSTL's Biotechnology Division was selected to serve as the ***Biomarker Validation Laboratory*** for new cancer detection biomarkers discovered in this network. This 5-year, $2.2 million Interagency Agreement will focus on biomarkers with a strong nucleic acid component that are well within the expertise and interests of the DNA Technologies Group. Projects approved by the EDRN for validation include a project "Validation of the mutagen sensitivity and chromosomal hotspot assays" to be performed in collaboration with M.D. Anderson Cancer Center. Other biomarkers still in approval stage include assays for mtDNA sequence changes in head and neck tumors and serum levels of the enzyme telomerase.

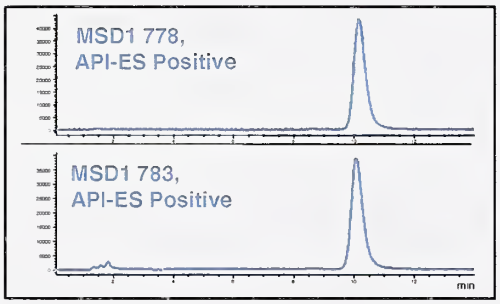
Thyroid-Stimulating Hormone and Thyroxine: Marker to Diagnose Thyroid Disease

S.S. Tai, L.T. Sniegowksi, and M.J. Welch (839)

Human thyroid-stimulating hormone (TSH) is a hormone secreted by the pituitary gland. Its role is in the stimulation of the thyroid gland to produce the thyroid hormones triiodothyroxine (T3) and thyroxine (T4). Serum TSH levels are used as a diagnostic tool for assessing thyroid function. Increased TSH levels can be indicative of hypothyroidism, a condition in which the thyroid gland fails to recognize TSH and decreases its production of T3 and T4. TSH is structurally heterogeneous and present at very low levels in human serum, and therefore presents a significant measurement challenge. Work to date has focused on developing approaches for characterizing TSH preparations. We found that treating TSH with sialidase to remove sialic acid residues from the carbohydrate moieties on these proteins significantly improved their mass spectrometric characterization. Full characterization of the TSH preparations will require peptide mapping to verify identity and primary structure and characterization of carbohydrate heterogeneity.

Image of a Normal Thyroid

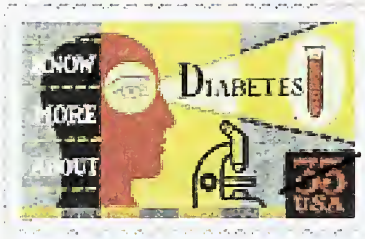
Thyroxine is a hormone secreted by the thyroid gland that is a stimulator for a number of functions and is important in growth, development, and sexual maturation. The concentration of total thyroxine in serum is about 50 ng/mL - 110 ng/mL, and is used as a measure of thyroid function. A new method based on isotope dilution liquid chromatography/mass spectrometry using electrospray for ionization (LC/MS-ESI) has been developed for the determination of total thyroxine in serum. Samples of CAP survey sera were prepared and measured on three separate sets. Excellent precision was obtained for all three levels of serum samples with within-set CVs ranging from 0.2% to 1.0%. Excellent linearity was obtained with the correlation coefficients of all linear regression lines ranging from 0.999 to 1.000. Positive and negative ion measurements agree within 0.8%. The detection limit at a signal to noise ratio of approximately 3 to 5 for thyroxine with this method is estimated to be 30 pg and 20 pg for positive and negative ion, respectively. The LC/MS-ESI method was tested against field methods (about 1900 laboratories from CAP surveys). The results of the LC/MS-ESI method and field methods compared well with an average difference of 5% for all three levels. This method will be used to measure the thyroxine level in some existing serum-based SRMs. Future plans are to develop a new SRM with both low and elevated levels of thyroxine and cortisol. More than 99.9% of thyroxine in blood is bound to protein. There is considerable interest in measuring free thyroxine, but clinical methods give widely varying results. The isolation of the free thyroxine and its measurement by LC/MS will be investigated.



Single Ion Chromatograms by LC/MS-ESI for Thyroxine and Thyroxine-d_5 from a Serum Sample

Glycated Hemoglobin: Marker for Improved Management of Diabetes

D. Bunk and J. Dalluge (839)



Approximately 16 million people in the United States have diabetes, with nearly 2,200 new cases diagnosed each day. About 95% of the people with diabetes have type II disease.

Glucose accumulates in the blood of those with type II produce insulin since the cells in their bodies are "insulin resistant" and do not respond properly to the hormone. Left untreated, diabetes can result in cardiovascular disease, kidney disease, eye diseases, and nervous-system maladies. In addition to the daily monitoring of blood glucose levels, the measurement of glycated hemoglobin (HbA_{1c}) plays an important role in the diagnosis and treatment of diabetes. When blood glucose levels rise above normal, the glucose can react with hemoglobin in the blood, forming HbA_{1c}. Since the lifetime of hemoglobin in the bloodstream is approximately 3 to 4 months, the measurement of HbA_{1c} provides a record of the levels of blood glucose over the course of 3 to 4 months. Glycated hemoglobin measurement provides doctors with information on the efficacy of diabetes treatment over a longer period than daily blood glucose measurements. While the medical utility of HbA_{1c} measurement has been clearly demonstrated, the clinical application of HbA_{1c} measurement has demonstrated considerable problems with method-to-method variability. There is a strong need for HbA_{1c} measurement standardization. The International Federation for Clinical Chemistry (IFCC) has developed two reference methods for the determination of HbA_{1c} in blood hemolysates. One method uses liquid chromatography coupled with mass spectrometry (LC/MS) and the other used capillary electrophoresis (CE) for HbA_{1c} determination. Both methods have demonstrated high precision (average inter-laboratory coefficient of variance ≤ 2%) and excellent agreement with each other. These reference methods have been implemented in a network of laboratories worldwide. Currently the IFCC reference methods are being used to value-assign an international HbA_{1c} reference material

NIST has participated in inlaboratory comparison studies of IFCC reference materials and has provided critiques for improvements.

Iron-Containing Proteins: Marker for Diseases Related to Abnormal Serum Iron Levels

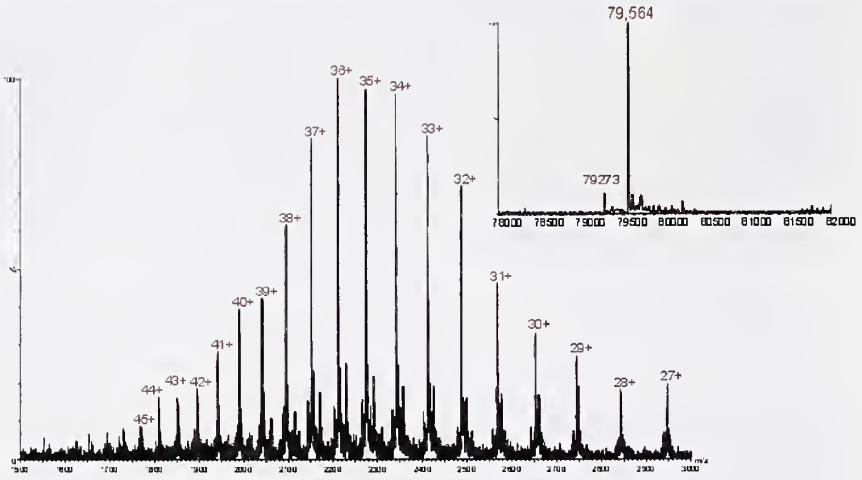
D. Bunk and S.E. Long (839)

Serum iron determinations are an important clinical measurement. According to the CDC hereditary hemochromatosis is the most common genetic disorder in the United States, with approximately one in one hundred people at risk for developing the disease. Typically, for the purpose of medical diagnosis, the total serum iron concentration is measured. However, in many iron-related diseases, the distribution of iron bound to serum proteins is altered. For improved medical diagnosis, it would be beneficial to determine the distribution of iron among serum proteins as well as the total serum iron concentration. Work has begun on development of methodologies that will lead to a reference material for speciated iron in serum, a material that could be used for the more routine clinical measurements of total serum

***Low serum iron levels* are indicative of diseases such as anemia, rheumatoid arthritis, and certain infections.**

Hemochromatosis, the most common form of *iron overload disease*, is an inherited disorder that causes the body to absorb and store too much iron, often resulting in failure of the liver, heart, or pancreas.

Transferrin
Molecular Mass = 75,143



Human Transferrin (Tf)
Electrospray-ionization mass spectra

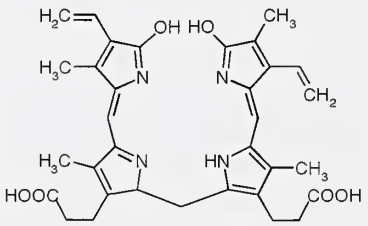
iron, but also used for the more advanced diagnosis of iron-related diseases. The two important iron-containing proteins in human serum are transferrin and ferritin. The concentration of ferritin in serum is approximately one thousand times lower than that of transferrin. However, medical research is indicating that serum ferritin determination can often be a more selective diagnosis tool than determination using transferrin. Development of a quantitative method for serum transferrin using liquid chromatography coupled with mass spectrometry (LC/MS) and affinity chromatography is underway. The concentration of iron associated with the transferrin will be measured using high-resolution inductively coupled plasma mass spectrometry (ICP/MS). Together, the LC/MS and the ICP/MS measurements provide highly selective and high-precision values for both serum transferrin and serum iron levels.

Bilirubin: Marker for Elevated Liver Function

Y.Y. Davidson, L.T. Sniegoski, and M.J. Welch (839)

Elevated levels of bilirubin in blood are indicative of impaired liver function.

Bilirubin, the orange-yellow bile pigment, is produced from protoporphyrin IX by microsomal heme oxygenase. There are three principal isomers of bilirubin, which may be free or complexed with other blood constituents. Clinical laboratory measurements of bilirubin exhibit considerable variability because of method differences and calibration errors. Our goal is to develop an LC/MS reference method to measure bilirubin. Efforts have focused on first establishing the spectrophotometric reference method based upon a direct diazo reaction. Plans are to use this method as a tool for validation of the LC/MS method, and then to use both methods for certification of bilirubin in serum-based SRMs.



Folic Acid: Marker for Neural Tube Defects

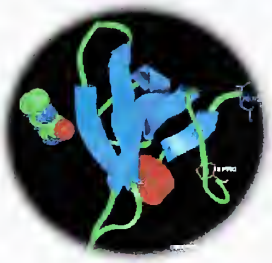
B.C. Nelson, J.J. Dalluge, S.A. Margolis, D.Z. Bezebeh, and L.C. Sander (839)

Folic acid is a water-soluble B vitamin that plays a significant role in human health:

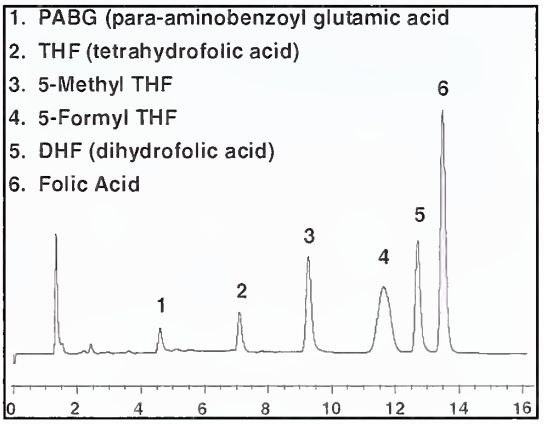
1) A deficiency of folic acid during gestation causes neural tube malformations that result in spina bifida.

2) A deficiency of folic acid causes the accumulation of high levels of amino acid homocysteine that has been linked to the increased risk of cardiovascular disease and other diseases.

The ability to determine folic acid status accurately is a significant challenge due to the existence of up to eight metabolites, many of which are unstable, and their low levels in plasma and serum (total folate concentration ≤ 20 ng/ml). A gradient elution liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) method for the separation and detection of the five most biologically relevant folates has been developed and applied to the quantitation of folates in human plasma/serum. The method involves the *in vitro* stabilization of plasma samples via a dual antioxidant system (*L*-ascorbic acid/*L*-cysteine), protein precipitation and sample concentration. The major circulating folate (5-methyl tetrahydrofolic acid) in human plasma/serum is detectable and quantifiable based on a standard additions procedure using an endogenous plasma component as an internal standard. The other biologically relevant folates are not detectable in normal human plasma. This group of researchers is also developing a second method based on the use of liquid chromatography with coulometric detection for the determination of folates in plasma. Current efforts are focused on developing a folate quantitation procedure based on the use of external standard calibrants so that the LC/MS method might be more adaptable to routine clinical use.



Folic Acid Binding Protein



Diagnostic and Therapeutic Standards

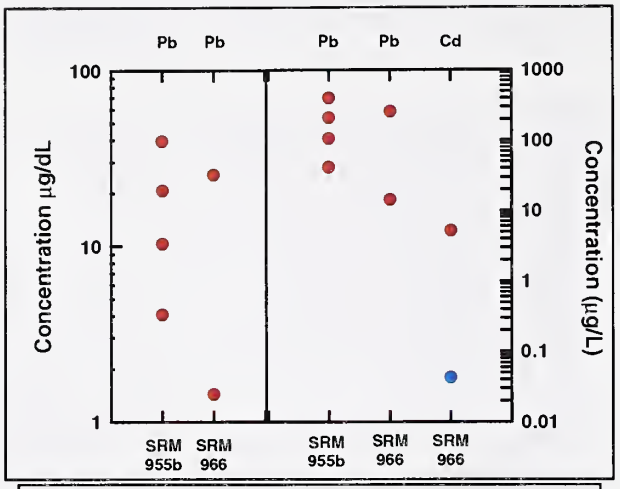
Lead, Cadmium, Total Mercury, and Methyl Mercury

S.E. Long, M.S. Rearick, R.D. Vocke, and E.A. Mackey (839)

A whole blood reference material, SRM 966, for evaluating the accuracy of lead (Pb), cadmium (Cd), total mercury (Hg) and methyl mercury (MeHg) measurements in whole blood at natural and slightly elevated levels has been released this year. Analysis of whole blood is one of the most common ways exposure to these toxic trace elements is monitored in the human population. Also, a large number of organizations in the human health monitoring area (e.g. CDC, NIEHS, EPA, and WHO) have indicated their interest in biological SRMs (especially blood) certified for MeHg. A unit of this SRM consists of two vials from each of two levels (natural and elevated), containing approximately 2 mL of whole blood each. The bovine blood base material was prepared for NIST at a USDA licensed facility from cows bled after dosing with lead nitrate.

New OSHA workplace monitoring standards now require measurements of blood cadmium; prior to this SRM, only proficiency samples with consensus cadmium values existed as "standards".

Endogenous Pb concentrations were certified by isotope dilution (ID) ICP-MS, a primary method for this analyte in clinical materials. Cd concentrations in the elevated level were certified by combining data results from two independent methods, instrumental neutron activation analysis (INAA) and ID ICP-MS. Total Hg was certified in the elevated material by combining data from ID ICP-MS and cold vapor atomic absorption spectrometry (CVAAS). This material will support workplace and general public health monitoring for lead, cadmium, total mercury and MeHg in blood.



SRM 966 released in FY 2000 is a new addition to the NIST SRMs for Pb and Cd in Whole Blood

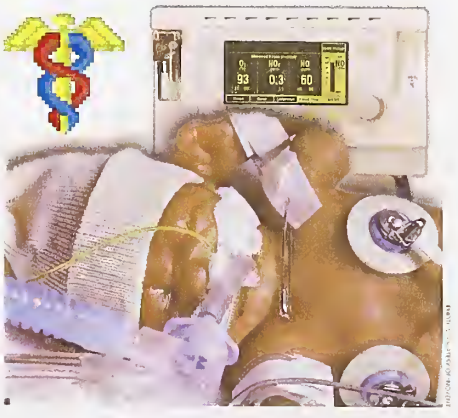
Inhaled Nitric Oxide Therapy

W.J. Thorn (839)

CSTL's work facilitates the implementation of Inhaled Nitric Oxide (INO) Therapy that could help save the lives of 2,000 U.S. newborns annually.

For more than five years, researchers in CSTL's Analytical Chemistry Division have been working with the National Insitute for Child Health and Development (NICHD) to facilitate the implementation of Inhaled Nitric Oxide (INO) Therapy. Early work involved the investigation of the kinetics of NO_2 formation from the NO used in INO. Dilute concentrations (<100 µmol/mol) of nitric oxide have shown dramatic results as a pulmonary vasodilator in some newborn patients when inhaled in oxygen via a ventilator. However once the nitric oxide is mixed with oxygen, the clock starts for the spontaneous formation of nitrogen dioxide. Accurate measurement of nitrogen dioxide is important because nitrogen dioxide (>5 µmol/mol) is considered detrimental - possibly leading to pulmonary edema and other negative consequences. Based on the NIST data it was concluded that at anticipated dwell times of <0.5 s, no significant levels of the harmful NO_2 should reach the patient during treatment.

At a workshop held at NIST last year, several of both types of instruments used for NO/NO_2 monitoring (chemiluminescence-based and electrochemical-based devices) were found to measure NO and NO_2 inaccurately under high oxygen conditions. NIST developed the primary standards that provide the accuracy base for these measurements and support the production of the necessary standards by a commercial specialty gas company of NTRM gas mixtures that are used to calibrate these monitoring devices. At the request of the NICHD NIST developed a NIST Traceable Reference Material (NTRM) at 85 µmol/mol NO in nitrogen.



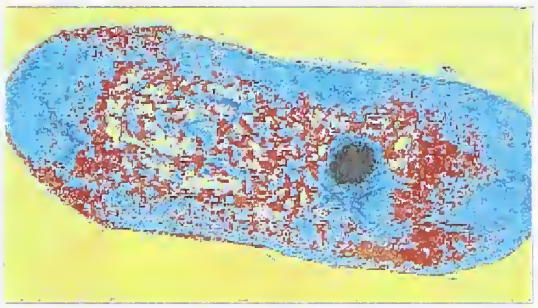
The methodology developed at NIST to simulate and deliver INO ventilator circuit mixtures of known concentrations was also used to evaluate the accuracy of redesigned monitoring devices in measuring NO and NO_2 and also was used to investigate possible biases due to oxygen quenching.

Based on the NIST research, nitric oxide, which helps babies breathe easier, was approved as a drug by the FDA in December 1999. Currently, NIST and the FDA are working with doctors and instrument manufacturers to develop written protocols and standards so that new INO devices can gain FDA approval. Once the devices are approved it is expected that this therapy will be commonplace in hospital neonatal care units.

NIST's role now is to serve on ASTM subcommittee F29.1.11 working with FDA anesthesiologists, medical doctors, and INO medical device manufacturers on a device acceptance "standard" which when followed will facilitate FDA device approval. NIST provides input to NO and NO_2 performance tests for gauging accuracy and the negative effects of interfering anesthesia gases.

Cloning, Expression, and Characterization of Chorismate Mutase from Mycobacterium Tuberculosis

P. Reddy (831)



Mycobacterium tuberculosis genome sequence revealed a gene for chorismate mutase. Chorismate mutase is present only in bacterial and lower eukaryotic systems but is absent in higher eukaryotes. Hence, this enzyme is a popular target in metabolic engineering and drug development. It is intriguing to determine the biochemical properties and three-dimensional structure of chorismate mutase in *M. tuberculosis*.

Chorismate mutase I gene was amplified by polymerase chain reaction (PCR). The primers for PCR amplification included NdeI and BamHI restriction endonuclease recognition sequences at the 5' and 3' ends, respectively. Amplified product was digested with the restriction enzymes, and the gene was cloned into a similarly digested pRE1 protein expression vector. A recombinant plasmid was introduced into *E. coli* strain MZI for protein expression. Expressed chorismate mutase was purified to homogeneity, and the enzymatic properties were studied.

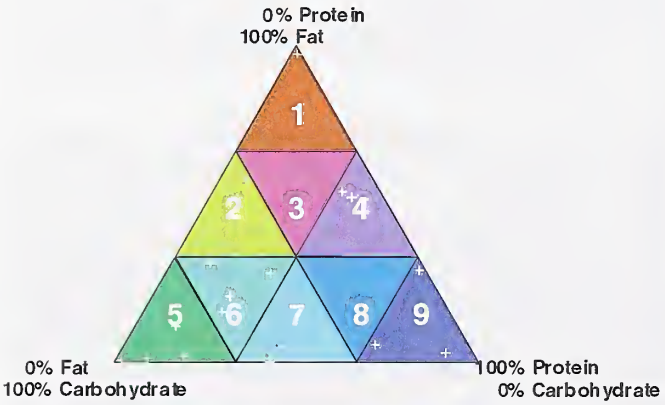
Chorismate mutase catalyzes the pericyclic rearrangement of chorismate to prephanate, which can be converted to either tyrosine or phenylalanine. We investigated the functional nature of chorismate mutase from *M. tuberculosis*. Chorismate mutase is a 199 amino acid protein with an amino terminal signal sequence that is cleaved from the mature protein. The role of the signal sequence remains to be investigated. The enzyme has no associated activity for either prephanate dehydratase or prephanate dehydrogenase. Therefore, chorismate mutase from *M. tuberculosis* belongs to monofunctional class mutases. Preliminary structural characterization revealed that this enzyme belongs to monofunctional chorismate mutases but differs in structural diversity having only alpha helices in contrast to *B. subtilis* chorismate mutase which is also monofunctional containing alpha helices and beta strands.

We will determine the three dimensional structure of chorismate mutase from *M. tuberculosis,* and design small molecule inhibitors, and test for inhibition of chorismate mutase activity. This research will revolutionize drug discovery for *M. tuberculosis*.

Methods and Standards to Support Nutritional Labeling of Food Products

K.E. Sharpless, J. Brown Thomas, S.A. Margolis, B.C. Nelson, C.S. Phinney, and L.J. Wood (839)

The Nutrition Labeling and Education Act requires that specific nutritional information be provided on all processed foods sold in the U.S. In a 1996 study by the U.S. Food and Drug Administration, the accuracy of label information ranged from 98% to 54% for nutrients for which labeling is required. As more food-matrix SRMs become available, label accuracy should improve when the food and nutrition communities employ these SRMs in their analyses.



Association of Official Analytical Chemists (AOAC) International has developed a nine-sectored triangle in which foods are positioned based on their fat, protein, and carbohydrate content. AOAC's belief is that one or two reference materials within each sector should be representative of other foods within that sector and could be used for quality assurance and method validation when analyzing those other foods.

New Food SRMs in the Certification Process for FY 2000

SRM 2384 Baking Chocolate – Sector 2 – highest priority
The first reference material available from NIST with values assigned for caffeine, theobromine, and catechins.

SRM 2385 Spinach – Sector 7
To replace existing freeze-dried, finely ground SRMs with more natural material.

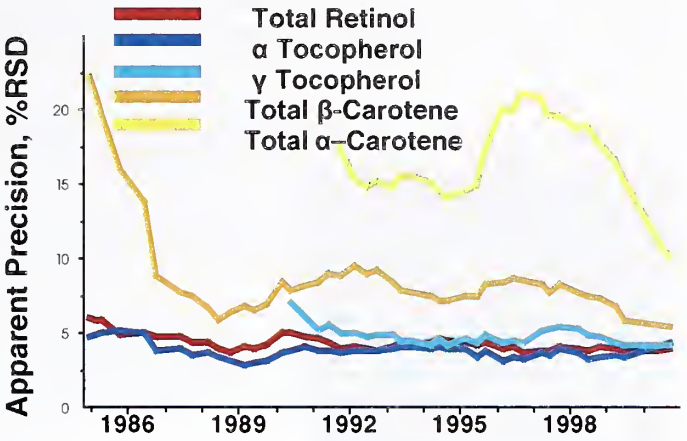
SRM 1946, Lake Superior Fish Tissue – Junction of sectors 4,8, and 9
In addition to the analytes of nutritional interest, this material will be certified for toxic trace metals, polychlorinated biphenyls, pesticides, and methylmercury.

High priority needs that have been identified as a result of a workshop included SRMs for aflatoxins and allergens such as peanut protein. (Foods must be labeled if they contain – or may contain – unexpected allergens. Permissible levels of aflatoxins are also regulated.) A peanut butter SRM would address the needs for aflatoxin and allergenic peanut protein reference materials as well as providing a reference material in sector 3 of the AOAC triangle, which is not occupied by any other SRMs.

The Micronutrients Measurement Quality Assurance Program

J. Brown Thomas, D.L. Duewer, S.A. Margolis, K.E. Sharpless (839), and M.C. Kline (831)

The Micronutrients Measurement Quality Assurance (QA) Program was organized to support measurement technology for selected fat- and water-soluble vitamins and carotenoids in human serum and plasma. It was initiated in 1984 as part of investigations supported by the National Cancer Institute (NCI) Division of Cancer Prevention and Control to study the possible role of these analytes in reducing the risk of developing certain types of cancers and diseases. Today it is the "the only QA program available for the fat-soluble vitamins and carotenoids" and currently includes more than 60 laboratories worldwide.



Micronutrients Measurement QA Program Comparison Exercise (from 1986 – 2000)

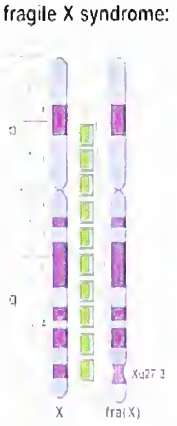
NIST provides laboratories with the tools for comparability assessment through use of interlaboratory comparison studies, Standard Reference Materials (SRMs) and control materials, and methods development and validation. Serum-based samples with assigned values for the target analytes and performance-evaluation standards are distributed by NIST to laboratories for analysis. NIST staff provide the laboratories with technical feedback concerning their performance as well as suggestions for methods development and refinement. The results from the comparison studies are used to establish a laboratory performance database, which is used to help laboratories to improve their measurement comparability and to obtain reliable data needed to make accurate clinical and health-care decisions

As a result of the QA program, the accuracy of laboratory measurements resulting in increased interlaboratory comparability for retinol, α-tocopherol, and β-carotene has improved substantially over time (see figure). The average estimated coefficient of variation for retinol and α-tocopherol has been approximately 5% for the past five years and about ≤10% for β-carotene for that same period of time.

DNA-Based Measurements

Standardization of Trinucleotide Repeat Measure

K.L. Richie, D.H. Atha, and C.D. O'Connell (831)

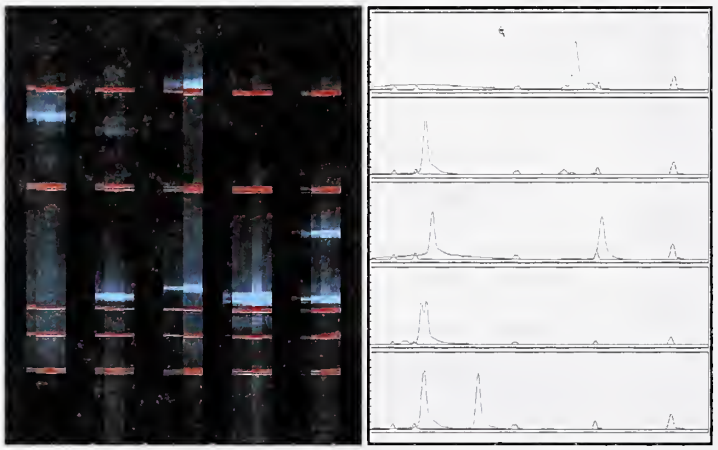


CSTL provides the clinical diagnostics community with accurate protocols and measurements for the detection of genetic disease. The Fragile X disease system used in this study requires the accurate quantitation of triplet repeats that confer important diagnostic information.

In this study, we focused on the triplet repeat causing Fragile X Syndrome for the following reasons. First, it is one of an increasing number of triplet repeat expansions associated with disease. Expansion of these repeat elements results in the interruption of gene expression and disease. Interruption of the FMR-1 gene occurs when more than 200 copies of the triplet repeat are present. Mental retardation is associated with this interruption in virtually all males with over 200 copies of the repeat, and females are affected to a lesser degree. Moreover, a "premutation"

state can be detected when 50 to 200 copies of the repeat are present. Secondly, Fragile X is the leading heritable cause of mental retardation; because it is inherited, accurate quantitation of the number of repeat sequences can be used for carrier screening in family planning in addition to diagnosis of disease and pre-natal screening. As such, we feel that our research will have the largest impact on diagnostic testing by focusing on the accurate quantitation and standardization of Fragile X measurements.

PCR-based testing methods were examined and optimized to determine which protocols proved to be more robust for amplification of Fragile X repeat elements. We purchased a panel of Fragile X cell lines from the Coriell repository and have validated 5 of these cell lines for Fragile X testing using slab gel and capillary separation systems. We have applied statistical analysis to these measurements determining inter-gel variation as well as inter-lane variation for repeat elements of varying sizes. The effect of PCR amplification was also measured by conducting the amplification experiments in triplicate. We concluded from these data that reproducibility is independent of size but may depend on other factors such as DNA purity, concentration, and interruption of the repeat units. Recently, a PCR-based Fragile X assay was released in the marketplace. It is expected that some of the clinical laboratories will shift from their current assay systems (primarily home-brew) to this assay system. Future plans call for the evaluation of measurement sensitivity and variability of this new system in comparison to our current validated assay system. We will also maintain our collaborations with the clinical diagnostics community, providing them with accurate measurements and standards to aid in the development of guidelines to ensure accurate measurement for diseases associated with triplet repeats. The results of this research will be presented at the "Annual Clinical Genetics Meeting" in March 2001.



Left: Gel image of fluorescently labeled fragile X gene products from 5 individuals with normal and pre-mutation alleles. Right: Electropherogram of the size separations between these alleles, where 29 and 31 triplet repeats are clearly resolved by GeneScan™ analysis.

Environmental Measurements

CSTL activities that support stewardship of the environment extend from the enhancement of industrial process efficiency for pollution prevention to the assessment and remediation of environmental problems through the development of measurements, data, models, and reference standards. CSTL has served as a partner to both industry and measurement laboratories for many years in dealing with environmental concerns by providing the tools needed for sustainable development and the shared goals of environmental protection and socio-economic growth. Environmental activities within CSTL fall under the broad categories of *Assessment* (identification and measurement of pollutants and monitoring of environmental health) and *Green Chemistry* (pollution prevention through the improvement of industrial processes).

Environmental Assessment

CSTL projects related to the assessment of environmental health cover a wide range, including the development of methods to measure pollutants, the monitoring of atmospheric and marine environments, the compilation of reference data and certification of reference materials for use in environmental measurements, and the accreditation of commercial water testing laboratories.

NIST scientists collect sediment from the Buffalo River in the development of SRM 2704

Commerical Laboratory Proficiency Testing (PT) Studies

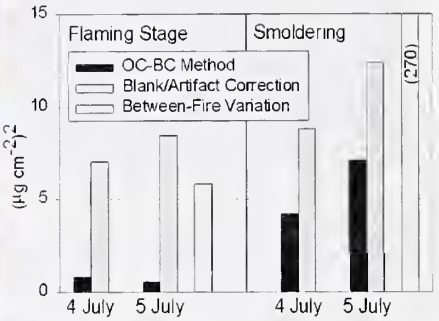
R.M. Parris, W.E. May (839), and C.D. Faison (TS)

The Analytical Chemistry Division and National Voluntary Laboratory Accreditation Program have completed the first year of this program that was designed to provide a system under which private sector companies and state laboratories are accredited by NIST to provide proficiency testing that meets the needs of the Environmental Protection Agency and states for regulated chemical, microbial, and radiochemical parameters. In our role of developing and managing the program for chemical and microbial species, Standard Reference Materials have been established and maintained to support the program, and we have conducted blind sample audits of PT samples on an ongoing basis for the 12 currently accredited providers covering all 48 chemistry and microbiology PT program fields.

Sampling and Measurement of Atmospheric Aerosol Carbon from Forest Fires

J.M. Conny, G.A. Klouda (837), and J.F. Slater (University of New Hampshire)

Agricultural burning and wildfire activity are major sources of atmospheric aerosols that adversely affect global climate, health, and visibility. The chemical characterization of particles from burning is essential for assessing its contribution in ambient air and, in turn, for predicting the health and environmental effects of unabated burning. Due to emission variation, sampling artifacts, and measurement method differences, fundamental challenges exist in determining the carbon composition of aerosols that accurately represent large-scale vegetative burning. The Surface and Microanalysis Science Division has been developing a procedure that uses the thermo-optical method, which involves the absorption of laser radiation by filter-collected aerosol particles at varying temperatures, to improve the accuracy and uncertainty in the measurement of the black carbon to total carbon ratio. This ratio is of great significance in parameterizing climate change models that relate to global warming.

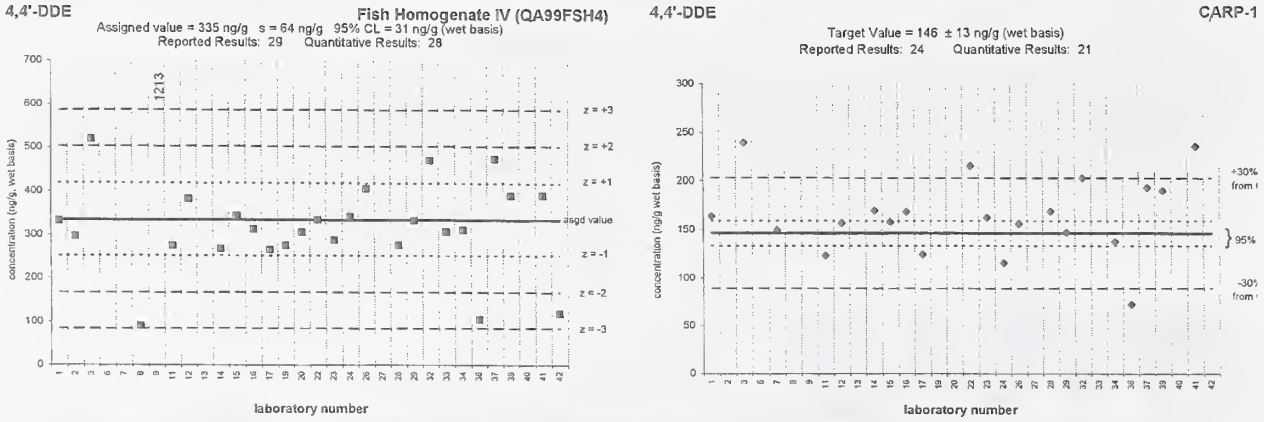


At left, aerosol sampling during flaming (arrow points to sampler) At right, differences in collected aerosol particles from flaming and smoldering fire conditions

Measurement Quality Assurance for Contaminants in the Marine Environment

M.M. Schantz, R.M. Parris, and S.A. Wise (839)

For over a decade, in collaboration with the National Oceanic and Atmospheric Administration's National Status and Trends Program, the Analytical Chemistry Division has coordinated annual intercomparison exercises for the measurement of polycylic aromatic hydrocarbons, polychlorinated biphenyl congeners, and chlorinated pesticides in marine tissue and sediment to assess the comparability of analytical measurements among members of the marine environmental measurement community. Current participants include 42 federal, state/municipal, university/college, private sector, and international laboratories. The data received are combined to provide an assigned value for the unknown used in the exercise. Participation in the program is written into a number of federal government solicitations, including at least one Mineral Management Service and eight U.S. Environmental Protection Agency solicitations in the last year.

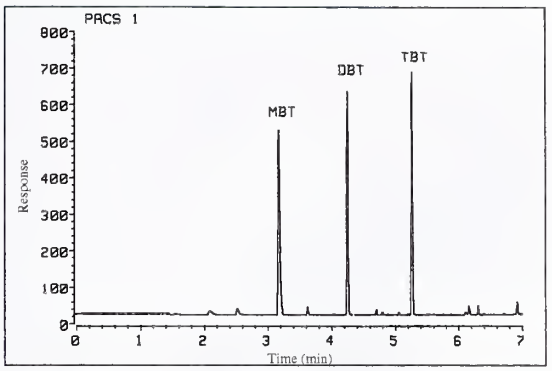


Shown above are the results for 4,4'-DDE from 42 laboratories in the 1999 exercise in which Fish Homogenate IV (candidate NIST SRM 1946) and Marine Sediment IX (candidate NIST SRM 1941b) were distributed

New Analytical Methodologies for Environmentally Significant Organic Species

M.M. Schantz, D. Bezabeh, W.W. Brubaker, Jr., J. R. Kucklick, S. Tutschku, and S.A. Wise (839)

Gas and liquid chromatographic methodologies are being developed for the quantification of organotins, dibenzo-*p*-dioxin (PCDD) and dibenzofuran (PCDF) congeners, polychlorinated naphthalenes (PCN), and nitro-substituted polycyclic aromatic hydrocarbons (nitro-PAHs) in natural matrices. These highly toxic substances have been measured in a number of SRMs with some verification through interlaboratory studies coordinated jointly by NIST and Environment Canada.



A typical chromatogram for the measurement of organotin species in SRMs using solid-phase microextraction – gas chromatography with atomic emission detection

NIST Traceable Reference Materials (NTRM™) from Commercial Sources

W.E. May, F. Guenther, W. Dorko, G.W. Kramer, J.C. Travis, G. Turk, J. Fassett, and R. Greenberg (839)

NTRMs are reference materials that have a well-defined traceability linkage to NIST that has been established using criteria and protocols defined by NIST and tailored to meet the needs of the metrological community to be served. Formal programs have been established to facilitate the commercial production and distribution of these materials in the areas of gas mixtures and optical filter standards, and programs for elemental solution and metal alloy standards are in the planning stages. Many of these materials, particularly pollutant gases and toxic element solutions, are critical for quality assurance of environmental measurements, and the increased availability of reference materials with a well-defined traceability linkage resulting from NTRM programs in these areas will significantly benefit our customers.

Aqueous-Phase Atmospheric Chemistry of the Nitrate Radical

P. Neta, G.A. Poskrebyshev, and R.E. Huie (838)

The behavior of the nitrate radical, NO_3, is being studied to establish its role in the chemistry of the aqueous phase of the atmosphere. NO_3, which is formed through the interaction of NO_2 and O_3, is the most important free radical in the atmosphere at night, and is a critical component to atmospheric chemistry models. Since there is great difficulty in generating NO_3 in aqueous solution, laboratory results of reactivity studies are often discordant. This study has provided reliable values for rate constants of reactions critical to atmospheric chemistry.

NO_3 is the most important free radical in the atmosphere at night, and is a critical component to atmospheric chemistry models.

Reference Methods and Standards for Environmentally-Significant Inorganic Species

R.R. Greenberg, R. Demiralp, W.R. Kelly, R.M. Lindstrom, S.E. Long, and R. Zeisler (839)

Environmental monitoring and research programs are being supported through the development of analytical methods and SRMs. A primary example is the development of ICP-MS and RNAA methods for mercury that has led to the certification of the mercury concentration in SRM 1632c (Bituminous Coal). The improved accuracy and precision of the NIST methods have resulted in SRMs that provide traceability for power industry measurements of mercury. The Environmental Protection Agency (EPA) has stated that mercury is the top priority elemental pollutant in the environment, but in recent years no SRMs have been available to provide the traceability for power industry measurements due to the difficulty in making accurate and precise low-level determinations of mercury in a coal matrix. The Electric Power Research Institute (EPRI) has been conducting a round-robin study to assess the status of the most commonly used industry methods for mercury in coal, but the lack of a reliably certified coal SRM has initially limited the assessment of data accuracy. The improved accuracy and precision (method agreement was within ~1%) of the NIST methods will alleviate these problems as well as facilitate reliable long-term stability testing of SRMs certified for mercury.

The improved accuracy and precision of the NIST methods have resulted in SRMs that provide traceability for power industry measurements of mercury.

Structure, Adsorptive Separations, and Characterization of Surfactant/Clay Complexes

C.D. Muzny, T.J. Bruno, and H.J.M. Hanley (838)

Clay is a key inorganic substance in a broad array of applications ranging from pollution prevention and remediation, enhanced oil recovery, the treatment of petroleum liquids, the manufacture of cosmetics and pharmaceuticals, and the synthesis of polymer nanocomposite materials. An understanding of clay-organic chemical interactions and the effects these interactions have on the structure of clay complexes is a critical issue for future developments in all of these applications. Characterization of structural changes induced in polymer-clay nanocomposite materials by variation of the organic clay surface modification is being examined by small-angle neutron scattering (SANS), wide-angle x-ray scattering (WAXS), and dynamic light scattering (DLS), to permit correlation of small-scale structural changes with macroscopic property changes. In addition, advances were made in physicochemical gas chromatography by devising stable clay- and organoclay-coated capillary columns to study the chemical kinetics of the clay-organic interactions. The capillary column approach is more efficient, requires lower column temperatures, and produces values of the enthalpy of adsorption (H_{ads}) with a much lower uncertainty than conventional techniques. The scattering results have provided evidence that complete exfoliation of clay particles in a polymer matrix is very difficult to achieve. When nanocomposite materials were produced from well-dispersed organoclay solutions, an apparent flocculation transition was observed during polymerization preventing the complete dispersion of the clay in the polymer matrix. Future plans are to combine computer simulation techniques with the results of the scattering investigations in order to accurately model the local structures present in clay solutions and nanocomposite materials, and to coordinate structure surface studies with thermodynamic information obtained from the coated capillary column. In addition, we have begun extending the chromatographic technique to the measurement of the diffusion of pollutants into the clay and organoclay system.

Clay is a key inorganic substance in a broad array of applications ranging from pollution prevention and remediation, enhanced oil recovery, the treatment of petroleum liquids, the manufacture of cosmetics and pharmaceuticals, and the synthesis of polymer nanocomposite materials.

A Primary Reference Photometer for Ozone Measurements

P.M. Chu, J.E. Norris, and F.R. Guenther (839)

In partnership with researchers at the Bureau International des Poids et Mesures (BIPM), the Analytical Chemistry Division is developing an advanced primary ozone photometer with improved SI-traceability to assure the comparability and SI-traceability of global ozone measurements. The new instrument will allow for optical probes at multiple wavelengths rather than at a single Hg line, so that ozone, nitrogen dioxide, nitric oxide concentrations and other contaminants can be measured simultaneously.

Viewing the Absorption Cells of the Standard Reference Ozone Photometer

Gas Sensing with Microhotplate Sensor Arrays

R. Cavicchi, S. Semancik, C. Wheeler, J. Allen, J. Tiffany, M. Carrier, J. Melvin (836) J. Suehle (EEEL) D. DeVoe, and B. Panchapakesan (University of Maryland)

Details provided in the **Chemical and Biochemical Sensing** section.

Development of Optical Methods for Chemical Sensing

A.C.R. Pipino, J.T. Hodges (836) D.J. Vanderah, and C.W. Muese (831)

Details provided in the **Chemical and Biochemical Sensing** section.

National Particulate Matter Research Program

R.M. Verkouteren, G.A. Klouda, R.A. Fletcher, J.A. Small (837), J.R. Sieber, R.L. Zeisler, S.A. Wise (839), and J.M. Ondov (University of Maryland)

New Standard Reference Material for Air-borne Particulate Concentrations (SRM 2784 Urban Dust On Quartz Filters)

As a consequence of increased interest in atmospheric particulate matter (PM) research that resulted from the 1997 revisions to the National Ambient Air Quality Standards designed by the EPA, the Analytical Chemistry Division and the Surface and Microanalysis Science Division of NIST along with the University of Maryland have generated proposals to address measurement issues. This has resulted in an interagency agreement between EPA and NIST to 1) develop an urban PM-on-filter reference material for carbonaceous species; 2) develop methods and metrology needed to provide PM calibration materials for organic and elemental carbon analysis; 3) develop a special thin-film glass standard for X-ray fluorescence analysis; 4) develop technology for large scale collection of air particulate matter less than 2.5 micrometers in diameter (for subsequent SRM development); and 5) develop a modern bulk urban dust material (and soluble extracts) with reference values for selected organic constituents.

Quantitative Infrared Database Developed to Support Remote Sensing Applications

P.M. Chu, F.R. Guenther, G.C. Rhoderick, and P.A. Johnson (839)

NIST Standard Reference Database 79 (SRD 79) "Quantitative Infrared Database" is being developed to support optical-based measurements of chemical emissions and hazardous air pollutants (HAP). Currently, absorption coefficient data for 30 HAPs on the U.S. EPA priority list is available in the standard JCAMP-DX format to enable universal access to the data. Future direction for the project will involve continued data acquisition for compounds listed in the 1990 US EPA Clean Air Act Amendment, as well as for those compounds that are of concern in global warming and emissions trading. Additionally, intercomparisons of NIST primary standards and molar absorptivity data with other National Metrology Institute's data will be expanded to facilitate the use of this database in issues of global interest and impact.

Green Chemistry

CSTL projects related to Green Chemistry result from our mission as the Nation's Reference Laboratory to provide the chemical measurement infrastructure to both enhance U.S. industry's productivity and competitiveness, and to improve environmental quality. Towards that shared goal, the projects described below involve the development of innovative procedures and reference materials and the compilation of critical data that will significantly improve efficiency and reduce waste in industrial processes.

Standards to Assist Perfluorocarbon Reductions in Aluminum Production

G.C. Rhoderick, P.M. Chu, and F.R. Guenther (839)

The Analytical Chemistry Division is helping the Voluntary Aluminum Industrial Partnership (VAIP) to reduce emissions of perfluorinated carbon compounds (PFCs) and greenhouse gases and improve the efficiency of aluminum production through the development of a suite of eight primary gas standards for CF_4 and C_2F_6. The development of these transfer PFC gas standards has assisted the aluminum industry in establishing traceability and mutual confidence in data generated for PFC emissions. The improved accuracy of measurements resulting from these standards has permitted better understanding and control of the "anode effects" that cause the production of PFCs, and in 1999 the VAIP reported reductions in PFC emissions by 41 %, equivalent to a reduction of nearly 8 million metric tons of CO_2, from 1990 levels.

The development of these transfer PFC gas standards has assisted the aluminum industry in establishing traceability and mutual confidence in data generated for PFC emissions

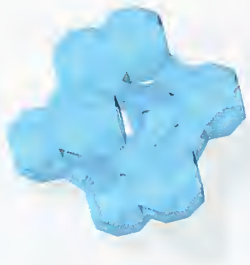
Atmospheric Lifetimes, OH Kinetics, and UV Spectra of Bromine-Substituted Fluoroalkenes

V.L. Orkin, F. Louis, M.J. Kurylo, and R.E. Huie (838)

Details provided in the **Chemical and Biochemical Data** section.

Reference Standards for Zeolites

J.R. Sieber, T. W. Vetter, T.F. Marlow, R.L. Zeisler (839), and S. Turner (837)



Zeolites are environmentally friendly: non-hazardous, regenerable, and non-corrosive. Zeolite catalysts are widely used in industry, but the characterization of zeolite composition is critical since variations in silica-to-alumina ratios, pore sizes, surface area, cations, and incorporated metals produce a wide range of adsorbent and catalytic properties. The need for zeolite reference materials was identified at a National Science Foundation sponsored workshop with participants from the research community, users, and suppliers. The lack of common materials for comparative measurements was identified as an important barrier to the development of new materials. These SRMs will provide a basic set of zeolite types to benchmark physical and chemical measurements. Chemical composition measurements for these standards were made in the Analytical Chemistry Division using X-ray Fluorescence Spectrometry, and nuclear and classical methods. The physical parameters (particle size and lattice parameters) were measured in the Surface and Microanalysis Science Division, which championed this project, and the Materials Science and Engineering Laboratory.

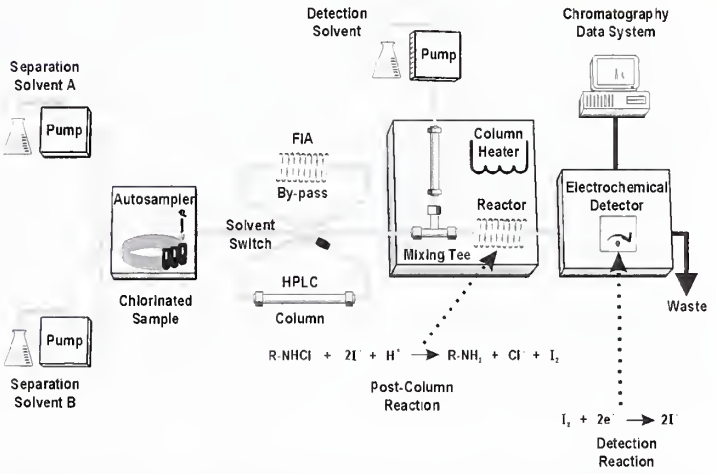
Zeolite Standard Reference Materials (SRMs) have been developed for use in the minimization of pollution and the treatment of effluents in industrial processes.

MSEL

Wastewater Chorination/Dechlorination Mechanisms

W.A. MacCrehan, M. Bedner (839), and G. Helz (University of Maryland)

To support national initiatives in developing environmentally-friendly, "green" chemical processing, the Analytical Chemistry Division is collaborating with the Water Resources Center of the University of Maryland to investigate new wastewater processing chemistries. The work is directed towards understanding and controlling chlorination residuals by developing a reliable measurement tool, liquid chromatography with post-column electrochemical detection, which can speciate the various forms of active residual chlorine. By understanding how the various chlorine species behave in their reaction with dechlorination agents, we hope to help sewage process engineers minimize the amount of wastewater chlorination by-products discharged into the environment.



A schematic diagram of the separation system designed to toggle between a flow injection analysis (FIA) (with no separation column) and the liquid chromatographic (LC) separation, permitting the direct evaluation of the factors that influence on-column losses such as solvent composition, pH, and separation column material

Detoxification of Polychlorinated Compounds in Water, Oil, and Soil by Electron Beam Irradiation

D.L. Poster (839), R.E. Huie, P. Neta (838), M. Chaychian, C. Jones, J. Silverman, and M. Al-Sheikhly (University of Maryland)

The radiolytic degradation of PCBs present in contaminated matrices is being examined to evaluate techniques that increase the efficiency of PCB dechlorination. Methods include using non-toxic additives, surfactants, and sonication during the electron beam treatment, using additives (alcohol, alkali) and applying an external high voltage electric field during electron beam irradiation in transformer oil, as well as evaluating the cost and performance of radiolytic treatment methodologies for the remediation of the most prevalent types of PCB contamination at polluted sites. One hundred percent dechlorination of PCBs in BG&G transformer oil has been achieved using electron irradiation and added triethylamine.

One hundred percent dechlorination of PCBs in BG&G transformer oil has been achieved using electron irradiation and added triethylamine.

Chemical and Biochemical Data

Data acquisition, manipulation and handling are mandates for NIST activities. Thus, CSTL activities in the area of chemical and biochemical data are numerous and broad. These activities can be subdivided beyond chemical or biochemical activities, into areas of experimental data and derived data, which are obtained from modeling processes or statistical analysis of other data. Some of the data included in the databases are rigorously measured and evaluated, and thus can be called NIST Standard Reference Databases (SRD). Other databases, due to the nature and source of their data, are less rigorously evaluated, and therefore not termed a reference database. Both classes of NIST databases have comparable utility and significance, because they contain the most accurate data in the country, if not the world.

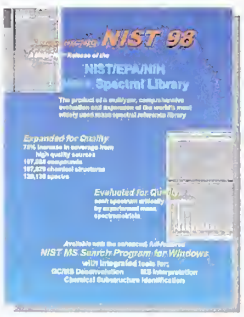
CSTL addresses the needs of data handling in industry by focusing on the areas of highest impact across those industries. Tackling these problems requires both NIST's expertise in data handling as well as the extensive chemical and biochemical expertise of CSTL brought to bear on them.

The NIST WebBook – NIST Chemical Reference Data for Industry

W.G. Mallard, P.J. Linstrom, P.J. Christian (838), and J.F. Liebman (University of Maryland, Baltimore County)

During FY00 the sixth edition of the NIST Chemistry WebBook was released providing data for more than 35,900 compounds and between 8000 and 17000 users per week.

The major goal of the WebBook (http://WebBook.nist.gov/) is to supply data from NIST critical evaluations, though the WebBook is also a broad resource of chemical data from many sources. In parallel with the efforts to gather and evaluate data, another major part of this project is aimed at providing the mechanisms needed to make these and other NIST chemical reference data available on the Internet. These efforts are part of NIST's program on Systems Integration for Manufacturing Applications (SIMA). There is a substantial amount of organic thermochemical data (heats of formation, entropies, heat capacities, heats of reaction) as well as thermophysical property data (vapor pressure, viscosity, boiling point, melting point, etc.) that have not appeared in widely available compilations and, hence, they are largely unknown to the technical community. One part of this project is to find, organize, and evaluate those data. In addition, there is a need to make ancillary thermochemical data, such as phase-change enthalpies, available. Data on infrared (IR), ultraviolet (UV) and mass spectra, and other analytical techniques are also important resources that are often difficult to find.



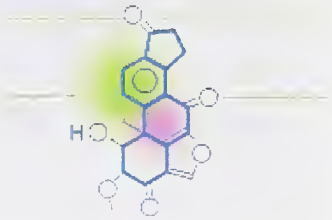
The NIST Mass Spectral Database: Extending the Evaluation

S.E. Stein, A. Mikaya, J. Klassen, D. Tchekhovskoi, C.L. Clifton, W.G. Mallard (838), and D. Zhu (Guest Researcher)

The determination of the identity of a compound is a central problem in chemistry. For volatile substances, the most widely used, sensitive, and definitive "fingerprint" for making such identifications is the electron-ionization mass spectrum. In practice, identifications begin with automated mass spectral "library searching" against a comprehensive library of reference spectra. The reliability of such identifications depends directly on both the quality of the reference library and the algorithms for matching mass spectra. Three parallel activities are involved in this program: addition of new data; quality control; and algorithm development. **Addition of New Data**: High quality mass spectra are acquired to both fill gaps in coverage and confirm the accuracy of spectra for important compounds. Related information such

as retention indices and chemical identification information are also sought. **Quality Control**: Expert evaluators use a variety of software tools along with a traditional structure-spectrum analysis to confirm the accuracy of spectra. Starting with the 1998 version of the library, all 129,136 distributed spectra for 107,886 compounds have been subject to evaluation. **Algorithm Development**: This includes the optimization and testing of automated computer methods for both identifying compounds from their mass spectra and for finding possible defects in library spectra.

Over the past year, evaluation has been completed for nearly all spectra received since the previous release.



Automated Gas Chromatography/Mass Spectral Decomposition and Analysis: Tools for Automating and Improving the Use of GC/MS Instruments

S.E. Stein, J.J. Reed, J. Klassen , W.G. Mallard (838), and O. Toropov (Guest Researcher)

This program has been supported by the Defense Threat Reduction Agency (DTRA) to provide a method for determining whether chemical weapons banned under the Chemical Weapons Convention are present in samples analyzed by GC/MS. The software implementing the algorithms must function without the need for the operator to examine the data. This ensures that any proprietary information that may be contained in the underlying data of treaty participants is protected. Moreover, the conventional method involving the manual analysis of GC/MS data files can be time consuming, operator dependent and error prone. So, in this project, GC/MS data is examined and a detailed noise analysis is performed, followed by a deconvolution of each of the chromatographic peaks in the GC/MS analysis. The extracted component spectra are then compared to reference spectra using a series of algorithms that reflect the degree of confidence that the reference and reference spectra originated from the same compound. The process of extracting the individual components in a complex data file proceeds in a series of four steps: noise analysis, component perception, signal extraction, and compound identification. Ongoing testing involves a number of laboratories both in the United States and abroad where specific chemical agent samples are examined.

Quantitative Infrared Database Developed to Support Remote Sensing Applications

P.M. Chu, F.R. Guenther, G.C. Rhoderick, and P.A. Johnson (839)

Details provided in the **Environmental Measurements** section.

Computational Chemistry Comparison and Benchmark Database

R.D. Johnson III (838)

As computer power increases there is more reliance on modeling and computational chemistry in the chemical industry, due to the increased safety, speed, and the low cost of computational modeling, as compared to laboratory measurements. *Ab initio* computational chemistry methods can provide accurate values for structures, entropies, and heats of formation. However, the calculation time, and therefore cost, increases greatly as the accuracy sought increases. The errors in the computational methods are systematic and dependent on the methods used and functional groups that compose the molecule. In order to take advantage of computational methods, accuracy and cost need to be evaluated. A set of benchmark molecules and reactions with reliable thermochemical and spectral data, including evaluated values and uncertainties, were selected. The measured data also included gas-phase enthalpies of formation, entropies, vibrational frequencies, and structures. Data are currently being generated from *ab initio* calculations for comparison with the experimental data. The calculations cover eighteen *ab initio* methods and more than fifteen basis sets.

The results of over 32000 calculations are available on the web, including data from over 600 species with well-known enthalpies of formation, and calculations employing 20 methods and 10 basis sets.

Chemical Kinetics Database on the Web

T.C. Allison, J.A. Manion, R.D. Levin, R.E. Huie (838), and C.Y. Lin (Guest Researcher)

A web-based interface to the NIST Chemical Kinetics Database has been developed and is available at http://kinetics.nist.gov/. The interface is implemented in HTML to ensure portability. More sophisticated features make use of common web languages such as CGI, JavaScript, and Java, which are readily available via most browsers. The database and supporting code have been completely rewritten, permitting development of a number of enhancements. In addition to the previous features, such as links between bibliographic and kinetic data and a rate constant plotting function, a number of enhancements have been made, including the ability to query on several fields using logical operators. A new data-entry system was developed to allow the database to be updated by a number of people working simultaneously on either data entry or quality control. Updates to the database will occur much more frequently than in the past and errors can be corrected very rapidly. Hypertext links direct the user to the page in the NIST WebBook containing information on the selected compound. A "thermodynamics calculator" was developed to read information on chemical structure and energetics from the output of several popular quantum chemistry codes and compute thermodynamic quantities from this information. Enhancements to the searching capabilities of the database include the recognition of chemical names and formulas associated with compounds in the database.

CSTL has made many advances to the Chemical Kinetics Database including more frequent updates and rapid error correction.

Targeted Evaluations of Kinetic and Thermodynamic Data

J.A. Manion, T.C. Allison, J.W. Hudgens, D.R. Burgess, W. Tsang, R.E. Huie, R.D. Levin, A. Fahr (838), and C.Y. Lin (Guest Researcher)

The development of models can have tremendous economic and environmental impact by permitting the rapid exploration of methods to improve yields of products and avoid unwanted or toxic byproducts. These tools are often not being utilized, primarily due to the absence of reliable, evaluated kinetic and thermodynamic data and the inability to extrapolate from available data. Production, disposal, and environmental fates of chlorinated hydrocarbons are extremely important, since they are utilized in many industries and products, including plastics, pharmaceuticals, computers, pesticides, and medical devices. Many of the processes involving chlorinated hydrocarbons are amenable to modeling if the necessary data are available; thus, chlorinated hydrocarbons were targeted for study. The available experimental thermodynamic data of chlorinated compounds were found to be internally inconsistent and the methodologies for predicting their enthalpies of formation, badly flawed. Development of methods to extend these data to larger chlorine systems is ongoing. Also under development are tools that take the molecular property output from, e.g., GAUSSIAN calculations, and immediately calculate JANAF-like thermodynamic tables as well as place the information in formats useful to common modeling packages. Parallel to these efforts was the implementation of reviews of experimental data on the kinetics of key reaction classes important in chlorination systems. In the course of these reviews, various problem areas were identified. These were resolved using both computational and experimental approaches and have extended our fundamental understanding of these reactions.

Currently, the evaluated heats of formation of over 70 commercially important chlorinated species have been evaluated and posted, along with their molecular and thermodynamic properties derived from quantum mechanics calculations. In addition, the kinetics of approximately 100 reactions have been reviewed and evaluated.

Fundamental Properties of Trace Components of Fuel Gas

T.J. Bruno, A.F. Lagalante, G.M. Bachmeyer (838), and K.I. Henning (Institute Louis Pasteur, France)

The United States consumes approximately 22 trillion cubic feet of fuel gas each year, with fuel gas being defined broadly as natural gas and liquefied petroleum gas.

Natural gas and liquefied petroleum gas (LPG) consist primarily of methane or petroleum, respectively, but between 300 and 400 additional compounds may be present at varying levels, with most of these naturally occurring and others intentionally added during processing. Most fuel gas is used industrially and for electric power generation, whereas only 20 % is used residentially. Efficient and safe design of plant equipment operated with fuel gases depends upon sufficient knowledge of the properties of the individual components of natural gas and LPG. Moreover, components present at low or trace levels can have a significant impact on the overall properties of the fuel gas mixture. Our most recent work with fuel gas has included (1) development of chromatographic databases for trace compound identification, (2) measurement of odorant diffusion in natural gas, and (3) measurement of the hydrolysis reaction of carbonyl sulfide (COS) in propane. The chromatographic databases permit trace components to be identified on the basis of retention indices. These databases are used in performing ASTM methods requiring the determination of gas composition. Work on the diffusion of sulfur odorants in natural gas resulted from the problem of odorant fading. We determined that the actual diffusion was significantly lower than what is predicted by theory, an observation that has caused the industry to evaluate odorization procedures. In LPG, we measured the kinetics of COS hydrolysis, and discovered that the hydrolysis only occurs in a separate aqueous phase, not in water dissolved in LPG, impacting how LPG producers test for COS. Moreover, we have developed a separation method to remove COS from LPG based upon molecular recognition technology.

Structure, Adsorptive Separations, and Characterization of Surfactant/Clay Complexes

C.D. Muzny, T.J. Bruno, and H.J.M. Hanley (838)

Details provided in the **Environmental Measurements** section.

Atmospheric Lifetimes, OH Kinetics, and UV Spectra of Bromine-Substituted Fluoroalkenes

V.L. Orkin, R.E. Huie, M.J. Kurylo (838), and F. Louis (Guest Researcher)

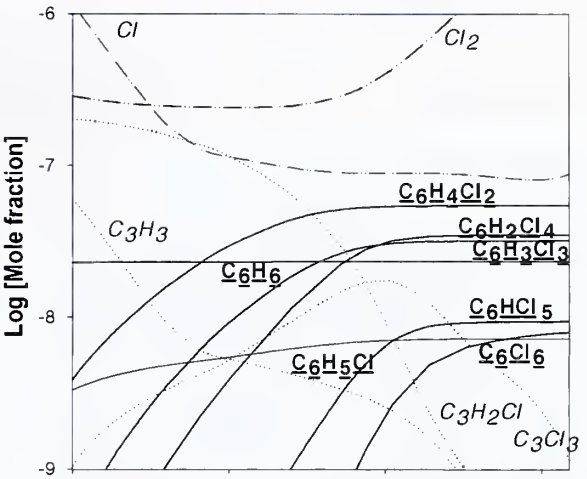
Although a number of bromine-free substances have been proposed and tested as fire suppressants, bromine-containing compounds continue to attract interest as very efficient, chemically-active flame suppressants. Both bromofluoroalkenes and hydrobromofluoroethers are under consideration as Br-containing halon replacements. The presence of either a carbon-carbon double bond or a carbon-hydrogen moiety is expected to render these substances reactive towards the tropospheric hydroxyl radicals, resulting in a short atmospheric lifetime. In order to quantify these atmospherically important properties, the reactivity of bromofluoroethers and brominated fluoroalkenes to hydroxyl radicals (OH) was investigated. Rate constants were measured over the temperature range 250 K to 370 K using a flash photolysis-resonance fluorescence technique. In addition, ultraviolet absorption spectra of all these compounds between 160 nm and 280 nm and infrared absorption spectra between 400 cm^{-1} and 2000 cm^{-1} were measured, and the atmospheric lifetimes of the compounds calculated. Preliminary results of *ab initio* calculations were obtained for reactions of fluoroalkanes and fluoroethers with OH. Present experimental activity is focused on OH reactions with other classes of industrial compounds. The results are being utilized together with the *ab initio* calculations in order to create a screening tool for the reactivity estimations of industrial compounds.

Fully substituted chlorofluorobromocarbons are excellent chemicals for use in various industrial applications and for fire suppression; however, their production is being phased out due to the considerable danger they pose to the Earth's ozone layer.

Mechanisms for the Formation of Polychlorinated Organics During Combustion

W. Tsang (838) and V. Babushok (Guest Researcher)

The formation of polychlorinated aromatics in an incineration system represents an interesting mechanistic problem since the overall chlorine concentration as well as the initial chlorine content of any particular organic compound is low. Thus, the concentration of chlorine in particular molecules is a reflection of the special mechanism that must be operative. Such effects have particular implications on the formation of dioxins or dibenzofurans. The failure to find a reasonable gas phase mechanism has led to the general assumption that these compounds must be formed by surface mediated processes. The incineration environment was simulated on the basis of chemical kinetic modeling using published results pertaining to the combustion of chlorinated hydrocarbons with the addition of reactions involving propargyl-type radicals. Particular emphasis was placed on the mixing and quenching of rich and lean mixtures. This model is a more faithful reflection of actual incineration operation than that of premixed fuel-air combustion. Initial results confirmed that it is impossible to obtain any degree of chlorine enrichment with a premixed sample, and in the absence of propargyl, enrichment could not be obtained even with mixing and quenching. The simulations showed that the temporal behavior of a number of species formed varies as a result of adding a very small amount of propargyl radicals into a quenched mixture of products from propane combustion with small amounts of typical chlorinated aromatic molecules. Distribution of chlorinated benzenes peaks at the two-to-four chlorine level. Combinations of these compounds result in the chlorines in dioxin or dibenzofurans peaking at the four chlorine level, in surprisingly good agreement with what is usually observed. These results demonstrate that even in the presence of trace chlorine, there is a very rich chemistry in the exhaust duct of incinerators. Furthermore, it is clear that for truly meaningful modeling of combustion systems, both fluid dynamics and chemical considerations must be combined.



Chlorination products from the addition of mixture from opposed propane air diffusion flame at the maximum propargyl concentration (≈ 1ppm) into quenched position of combustion products of ethylene/dichloroethylene (5% enriched in oxygen [Cl]/[C] = 0.005 at 1000 K)

Improved Methodologies for the Proper Treatment of Tunneling in Computational Kinetics

C.A. Gonzalez , T.C. Allison, and F. Louis (838)

This new methodology has already allowed scientists to properly compute the kinetics of a series of reactions relevant in the area of atmospheric chemistry.

Ever since the realization by Hund 63 years ago that tunneling might be important in the kinetics of chemical reactions, the chemical literature has been "flooded" with fundamental work focusing on theoretical predictions as well as experimental confirmation of the role tunneling plays in chemistry. Tunneling is the result of quantum effects that tend to couple the reaction path coordinate to the remaining degrees of freedom of the reacting system due to curvature along the reaction path. The methodology developed in this project was tested on a series of H-atom abstraction reactions. The results show a significant improvement over the conventional approach currently adopted by researchers, even in the case when simple tunneling models are used. In addition, research groups in industry and academia have recognized the potential impact of such a method in computational kinetics and have requested tools containing this method. We are extending the theory in order to devise robust diagnostic tools that will help

scientists choose the most appropriate one-dimensional tunneling correction method in an automated fashion.

Development of Efficient Tools for Computational Kinetics

C.A. Gonzalez, F. Louis, T.C. Allison, R.E. Huie, and M.J. Kurylo (838)

Despite progress in computational chemistry, the area of computational kinetics still remains more of an art than a science, and therefore is used mostly by experts in the field. The work by CSTL in this area will help make computational kinetics more of a science than an art and thus open the field to more researchers.

Application of quantum chemistry calculations in the area of computational kinetics will have a significant impact if the state-of-the-art methodologies are made widely available to the scientific community. Accordingly, the generation of computational tools that will allow scientists to perform reliable studies of the kinetics governing a large variety of chemical reactions is critical. In this project, a series of modules that compute rate constants as a function of the temperature have been implemented into generic software. Given its general acceptance, the Canonical Transition State Theory (CTST) has been chosen as the standard method to compute rate constants. Tunneling corrections are calculated using the one-dimensional Wigner, symmetrical Eckart, or asymmetrical Eckart approaches. Ability to treat normal modes as hindered rotors was implemented. Additionally, properties such as vibrational frequencies, heats of reaction, entropies, and partition functions are also computed. The program was written in standard FORTRAN 77, and has the capability of reading output files from one of the most popular quantum chemistry programs. The computational kinetics tools utilized in this project have been applied to study the kinetics and reactivity of hydroxyl radicals towards a series of halogenated organic compounds, part of an ongoing project developing environmental impact "screening tools" for these compounds. Theoretical studies on more than twenty molecules have been performed using the program. Currently, variational transition state theory is being applied to reactions characterized by low barriers and for which CTST gives poor results. The methodologies used for tunneling correction calculations are being improved, and efficient algorithms are under development to enable rapid characterization of reactant rotational conformers that may contribute to the kinetics of the reaction. Additional calculations are being performed for halocarbons and ethers with one and two carbons.

Fundamentals of van der Waals Interactions in Aromatic Clusters

C.A. Gonzalez (838)

The results obtained in this research indicate that the combination of molecular dynamics simulations followed by full geometry optimizations seem to provide a reliable tool for the study of vdW aromatic clusters.

Aromatic-aromatic interactions play important roles in many chemical and biological systems, including base-base interactions of the double helix of DNA, the function of photosynthetic reaction centers, the packing of aromatic crystals, the formation of aggregates, and the conformational preferences of polyaromatic macrocycles and chain molecules. Proper description of the interactions between monomers forming clusters of aromatic molecules is critical for the fundamental understanding of these phenomena. The molecular systems ideally suited for a detailed study of the intermolecular potentials are van der Waals (vdW) dimers and higher clusters of aromatic hydrocarbons that are experimentally generated by free jet expansion techniques. Since these species form as a direct consequence of intermolecular interactions, the geometrical structures of the vdW molecules not only reveal the nature of the forces between the molecules but also provide an understanding of the cluster's other properties, including dynamics. Although significant efforts have been dedicated to experimental characterization of these clusters, the results are usually inconclusive. Highly correlated *ab initio* electronic structure methodologies could be a valuable tool to complement the experimental efforts. However, given the large size of these systems and the fact that the number of possible structures dramatically increases with the size of the cluster,

reliable theoretical calculations have been limited to small clusters of benzene (dimer and trimer). It was therefore necessary to assess the validity of these methodologies in the case of larger clusters of different aromatic molecules and possibly determine if alternative methodologies that incur lower computational expenses can be applied to larger systems. We are currently extending the study to assess the basis set dependence of the theoretical treatment and, at the same time, to find more efficient alternatives to the methodologies so far used.

Thermodynamic Interpretation of Gas-Phase Ion Chemistry

K.K. Irikura (838)

CSTL researchers have recommended corrected procedures for extracting quantitative information from ion-molecule reactions. This type of measurement is practiced internationally by dozens of research groups in academia and government. The resulting data are an important part of compilations, such as the NIST Chemistry WebBook, that are used by scientists and engineers throughout the chemical sciences.

There is a large amount of thermochemical data on gas-phase reactions between ions and neutral molecules. Neutral thermochemistry is often derived from thermodynamic cycles of such experiments. The usual interpretation of the experiments is that if the reaction is observed to occur, it is presumed to be exothermic ($\Delta_r H° \leq 0$). An alternative interpretation assumes exoergicity ($\Delta_r G° \leq 0$). Often, either interpretation is assumed without a clear reason for choosing one over the other, though the two interpretations can lead to different results. The discrepancies can be large (tens of kJ mol^{-1}) for reactions with large entropy changes ($\Delta_r S° >> 0$). This confusion is a serious problem in extracting thermochemical data from ion-molecule reactions. Practitioners must update their methods, and data compilations must be examined for major errors. Recent controversy over the adiabatic ionization energy of the CF_3 radical arose with two nearly concurrent reviews recommending values differing by more than the uncertainties in any of the many experiments cited. Upon analysis, the conflict could be traced to the interpretation of ion-molecule reactions that have large entropy changes (two reactants leading to three products). The disagreement was reconciled using the exoergicity interpretation ($\Delta_r G° \leq 0$) with the final result in excellent agreement with very high-level *ab initio* calculations from the two different research groups. We concluded that the exoergicity interpretation is more accurate and should be used by all investigators, thus resolving the decades-old confusion.

Monte Carlo Methods for Optimizing the Quantitative Analysis of Thin Layers, Microparticles and Irregular Surfaces

J.T. Armstrong (837)

Electron microbeam analysis is very well suited for *qualitative* characterization of layered materials, particles and rough surfaces; however accurate *quantitative* analysis of specimens with irregular boundaries has remained elusive. Many industrial applications require accurate analyses of such samples and a variety of methods have been proposed.

One of the most promising approaches is the use of Monte Carlo simulations to model the electron and X-ray path lengths in complex samples. However, the various models and physical parameters commonly used in these calculations give significantly different analytical results. The Monte Carlo algorithms of Joy and Armstrong were modified to develop a versatile simulation program that calculates X-ray emission from thin films, particles and rough surfaces of given boundary conditions. The program allows defocusing, or rastering, of the electron beam to any given size, and bombardment of any portion of the particle or rough surface. It calculates the electron trajectories at a rate of approximately one million electrons per minute and is modularized to allow for easy substitution for the various physical expressions that make up the model. The program was used to determine analytical, geometrical and compositional conditions that are particularly sensitive to the choice of physical parameters used in the Monte Carlo models and the results were in good agreement with previously developed geometric corrections and experimental analyses,

particularly in showing the magnitude of electron sidescattering and its effect on X-ray production. This scattering is sensitive to the parameters of the program, resulting in large variations in the relative X-ray intensities with closeness to a sample side, with beam to sample angle, and with beam voltage. The Monte Carlo calculations showed variation in emitted X-ray intensities of particles of AlNi by greater than a factor of 100 with model-dependent variations of over a factor of two. The calculations are being used to identify the best measurement experiments and sample compositions to test the physical parameters. Appropriate analyses will then be made on such specimens to determine the best equations to use for quantitative analysis.

The Protein Data Bank (PDB)

T.N. Bhat, H. Cheng, D. Hancock, V. Ravichandran, N. Thanki, M. Tung, G.L. Gilliland (831), and P. Fagan (TS)

Research Collaboratory for Structural Bioinformatics

PROTEIN DATA BANK

Protein Data Bank

The PDB is the international depository for three-dimensional biological macromolecular structural data, including X-ray, NMR, cryoelectron microscopy and theoretical modeling data. Users include a diverse group of researchers in biology, chemistry, as well as scientists, educators, and students at all levels. A key component of creating the public archive of information is the efficient capture and curation of the data, termed data processing. Data processing consists of data deposition, annotation, validation and uniformity. Historical inconsistencies in the way data are reported within PDB files may lead to inaccurate and incomplete query results. The introduction of the advanced querying capabilities of the PDB makes it critical to accelerate the data uniformity process for these data. Tables of consistent data are being developed to improve query results. Numerous data fields have been re-examined across the archive, made uniform and added to the database resources to make search results reliable. Cross links were created for files of related NMR determined structures, providing minimized average structures linked with their ensemble structures on the query results pages. Paper files of the Master Archive were bar-coded and transferred to an automated filing system, and a scanning station is used to convert the paper archive to an electronic archive. Missing data are being sought from old depositor tapes that were read and transferred to CD-ROM. The Master Archive also produces quarterly snapshots of the PDB resource as a set of CD-ROMs that are distributed to the user community. Work to archive snap shots of the PDB Website and reproduce them for historical reference has begun. The completion of the human genome has made the role of structure in nucleic acid function of particular interest. The PDB users show an increasing demand for more and better data and access. It is estimated that the PDB, which currently contains more than 13,600 structures, could triple or quadruple in size over the next 5 years. The approach of using modern data management practices should permit scaling to accommodate a large data influx.

CSTL is meeting the needs of the emerging and rapidly growing biotechnology industry through database development, measurement development, data acquisition for modeling, and modeling and tool development for taking advantage of the enormous amounts of biological data being obtained.

The PDB processes and incorporates about 50 structures per week into the database. Usage is currently about 90,000 web hits per day and 70,000 PDB files downloaded per day.

Lispix Image Processing System Available for PC and Macintosh

D.S. Bright (837)

Over the last decade, MacLispix, a public domain image processing system for the Macintosh, has been applied to a variety of image processing problems. Due to interest from the PC community as well as the migration of the NIST Microanalysis Group to Windows machines, the software was ported to Windows, renamed 'Lispix', and distributed for both platforms, along with

example images, source and documentation. MacLispix was written in an enhancement of the Common Lisp programming language, providing graphical user interface (GUI) functions using special calls to the Macintosh Tool Box. Common Lisp (CL) for other operating systems do not have such calls, but instead have special routines for graphics and for mouse input. Our approach was to split the MacLispix code into a larger CL portion, and two smaller libraries. The development of image processing tools for our research is done entirely in the CL portion, so that development can be done using either the Mac or PC platform, and the tool will work similarly on both platforms without any additional or special code. The libraries, on the other hand, have the special graphics and GUI calls for their respective systems, but they look alike as far as the CL portion is concerned. Sufficient MacLispix tools have been ported to Windows to make the system useful both as a research tool for the Microanalysis Group and as an image processing system for outside users. We now are planning to port Lispix to Linux, and add more image processing tools to all platforms.

A Prototype Database for Evolutionary Analysis of Gene Families

A. Stoltzfus (831), W.-G. Qiu (CARB/UMBI)

The integration of evolutionary methods of character analysis into bioinformatics is a logical development that has yet to take place. Strategies for genome annotation and functional inference, a kind of comparative evolutionary inference (rather than an *a priori* prediction), are usually based on ranking of sequence alignments (BLAST scores), rather than on phylogenetic analysis. Phylogenetic methods that take into account the hierarchical structure of variance in biological data are superior for recovering functional relationships and identifying conserved functional features. However, the application of such methods to the analysis of genes on a broad scale faces several obstacles, including technical obstacles, like heterogenous tools and file formats, and conceptual difficulties, such as i) developing a system for querying relationships among gene families with different trees, and ii) accessing the uncertainty of phylogenetic trees and reconstructed ancestral character states. As a test case for automated analysis of character evolution in gene families, issues concerning the evolution of introns in eukaryotic genes were addressed using a prototype database system. Introns were designated as presence/absence characters, and the focus for detecting patterns and testing hypotheses, relate to their loss and gain rather than changes in intron length or sequence. The hypothesis that introns are gained at a non-random "target site" is of special interest. Data for three gene families, triosephosphate isomerase, Cu,Zn superoxide dismutase, and Mn superoxide dismutase, were collected from GenBank. The resulting gene family data sets were augmented with reconstructed histories of the loss and gain of introns. Database tools were developed to query the phylogenetic reconstructions to identify segments of genes corresponding to sites at which an intron was gained in a sister taxon, representing an estimate of the intron "target site". Results confirmed that introns are gained at a target site which is non-random at positions upstream and downstream of the intron, with the consensus sequence being AAG^GT. Current work includes (1) continued development of software tools for automated phylogenetic analysis, (2) designing more rigorous statistical tests and (3) expanding the database to include more gene families

Biothermodynamics

R.N. Goldberg, Y.B.Tewari (831), N. Kishore (Indian Institute of Technology, Bombay)

Knowledge of chemical equilibrium is essential for predicting the feasibility of chemical reactions, particularly when optimizing industrial processes.

Reaction conditions such as temperature, pH, ionic strength, and co-factor concentrations can substantially affect the equilibrium of many biochemical reactions. Chromatography, microcalorimetry, thermodynamic modeling, and literature data are used to measure thermodynamic quantities and assemble databases for enzyme-catalyzed reactions. The microcalorimetric capability has been particularly important for the extrapolation of data to higher temperatures. This effort has also resulted in the development of estimation schemes based upon limited and carefully chosen sets of data. Recent research

focused on reactions in the chorismate metabolic pathway that has been a focal point of interest because of its potential industrial importance. CSTL researchers have now characterized the thermodynamics of a major portion of this pathway by studying the reactions catalyzed by tryptophan synthase, prephenate dehydrogenase, prephenate dehydratase, chorismate lyase, chorismate mutase, glutaminase, and tyrosine aminotransferase. This past year, microcalorimetry and high performance liquid chromatography were used to conduct a thermodynamic investigation of reactions catalyzed by DAHP synthase, the first enzyme in the metabolic pathway leading to chorismate. Currently, the thermochemistry of adenosine(s) is being studied by combustion calorimetry, adiabatic calorimetry over the temperature range 11 K to 328 K, and HPLC for determining the saturation molality of adenosine in water. These measurements will be useful for calculating standard molar formation properties for the adenosine 5'-monophosphate (AMP), adenosine 5'-diphosphate (ADP), and adenosine 5'-triphosphate (ATP) series of aqueous species.

Collation of Experimental Data on Amino Acid Exchangeability

A. Stoltzfus (831) and L.Y. Yampolsky (CARB/UMBI)

Bioinformatics methods that rely on a matrix of amino acid similarity scores typically use Dayhoff-type matrices based on evolutionary divergence. From the perspective of understanding the structure and evolution of proteins, such matrices are problematic in that they are influenced in an unknown way by mutation rates, so that one does not know, for instance, whether the Cys-to-Met value is low for reasons of protein structure, or for reasons of mutation, since converting a Cys codon (TGY) to a Met codon (ATG) requires mutational change at all three nucleotide positions. To develop a measure of amino acid exchangeability that is uninfluenced by mutation requires a different approach. Results of experimental amino acid exchanges were collated from published studies. The resulting exchangeability matrix was evaluated by testing for expected *a priori* correlations, such as a positive correlation with widely used amino acid scoring matrices. Data on over 8000 individual amino acid exchanges were collated. The resulting exchangeability values correlated significantly with chemical indices of amino acid similarity, and with divergence-based matrices used for database searching. In spite of a variety of sources of heterogeneity in experimental conditions, the exchangeability matrix contains a significant and potentially useful signal. Work is underway to incorporate the exchangeability matrix into a general codon-based model of protein sequence evolution that includes separate parameters for mutational effects and protein effects. This model may then serve as the basis for generating tunable, task-specific amino acid score matrices, whose suitability will be evaluated for alignment tasks (database searching, fold-recognition), functional inference, and phylogenetic reconstruction.

Individual-Based Models of the Effect of Mutation Bias on Evolution

A. Stoltzfus (831) and L.Y. Yampolsky (CARB/UMBI)

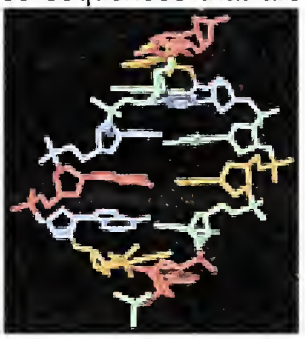
The general objective is to develop the theoretical basis for understanding the role of mutation in evolution. More specifically, this work aims to provide theoretical models for the influence of biases in mutation on the direction of evolutionary change. According to contemporary evolutionary theory, mutation biases influence the direction of evolution only in the special case of neutral evolution. Nevertheless, modern sequence divergence data suggest that mutation biases have figured importantly in the evolution of genes and proteins. Rather than implicating neutral evolution, such results may instead suggest the need to re-evaluate the manner in which mutation is treated in evolutionary models. In classical evolutionary theory, the existence of allelic variation is taken for granted, so that the introduction of novel alleles by individual mutation events is seen to play no appreciable role. The key to understanding the role of mutation bias in evolution may lie in exploring this novelty-introducing role of mutation. Our theoretical study of this role is based on a simple 2-locus, 2-allele case of the classic Bateson-Dobzhansky-Muller fitness scheme. An initial population of ab individuals may evolve toward one of two genotypes of higher fitness, Ab or aB, each of which is more fit than AB. Since the initial population is composed solely of ab individuals, mutation (rather than an assumption of initial variation) is

required. To explore the influence of mutation bias, mutation to aB occurs at a higher rate than mutation to Ab. The stronger the mutation bias, the more likely is evolution to proceed in the direction favored by mutation, an effect that does not rely on a high overall mutation rate (u). When mutations are rare (uN << 1), the biasing effect of mutation is approximately proportional to the mutation bias and to the bias in selection coefficients, and is not strongly affected by the magnitude of mutation or of selection coefficients. As anticipated, this effect requires that mutation act as a novelty-introducing process: mutation bias is ineffectual if variant alleles are simply assumed to exist initially, even at low frequencies. Future plans include further theoretical work with the goal of exploring many-locus (not just 2-locus) models, and empirical studies to gauge the extent to which mutation biases are reflected in evolutionary divergence of genes and proteins.

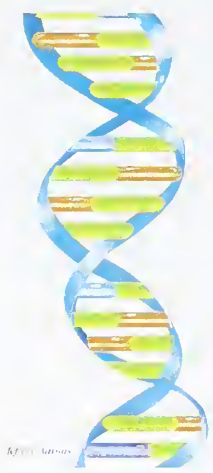
Predicted versus Experimental PNA/DNA and DNA/DNA Duplex Stabilities

F.P. Schwarz, M. Chakrabarti (831), and S. Krueger (MSEL)

Peptide nucleic acids (PNAs) are analogues of DNA, where the four nucleosides, A, G, C, and T are linked by a N-(2-aminoethyl) glycine backbone instead of the phosphate backbone in DNA. PNAs are able to form Watson-Crick pairs with complementary DNA base sequences that are more stable than their analogue DNA/DNA duplexes. Unlike DNAs, PNAs are electrostatically neutral and biologically inert, which have lead to their use in many areas of the health sciences, including single nucleotide polymorphism genotyping, PCR clamping, and inhibition of enzymatic activity in cells. All of these applications are critically dependent on ΔG° for binding of PNA, as well as DNA, to their complementary DNA sequences over a wide range of temperatures and thus, would be facilitated by a model predicting ΔG° from the sequence of the PNA. Predictive models have been developed for predicting the stabilities of DNA duplexes but have only been validated from 310-350 K. The thermodynamic parameters were determined from isothermal titration calorimetry (ITC) measurements on the binding of PNA and of DNA to their complementary DNA sequences to form, respectively, the PNA/DNA hybrid and DNA duplexes at ambient temperatures, and from UV absorption melting and differential scanning calorimetry measurements on the dissociation of the duplexes at high temperatures. Contributions to the thermodynamic parameters from conformational changes in the single PNA and DNA strands were determined from small angle neutron scattering (SANS) measurements on the PNA and DNA strands over this temperature range. The high temperature thermodynamic parameters were extrapolated to ambient temperatures and compared to their values determined from ITC measurements. Implications of these studies are that the conformational contributions may be predictable from the strand sequence. The ΔG° values for the DNA duplexes were in good agreement with predicted values from the literature. A comparison of the 8 and 10 base pair duplexes show significant contributions to the ΔG° values from changes in the single 10 base pair strand conformations. Any predictive model applicable over a wide temperature range must take this into account. The ΔG° values for the shorter 8 base pair DNA duplexes, however, are predictable from the nearest-neighbor Watson-Crick model using the literature assignments. Thermodynamic binding parameters are being determined for longer 12 base pair PNA/DNA and DNA duplexes to determine if single strand conformational changes also contribute to these binding parameters. These values will also be used along with the 10 and 8 base pair PNA/DNA duplex ΔG° values to determine the 16 assignments needed to predict the hybrid duplex stabilities. In addition, the dependence of the single strand conformational contributions on sequence will also be determined and used to refine the nearest-neighbor Watson-Crick model to correctly predict ΔG° values for all the duplexes over a wide range of temperatures.



DNA Technologies



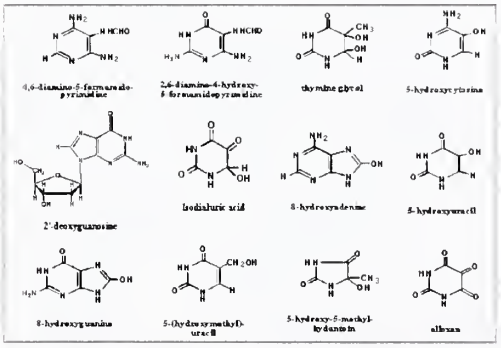
CSTL's role in the area of DNA technology primarily focuses on the needs of the diagnostic and forensic communities. CSTL efforts are concentrated in three areas, standards development, nucleic acid characterization, and measurement development. The CSTL work has become an essential touchstone for crime laboratories by providing a solid accuracy base. In addition, we are developing standards for the measurement of oxidatively modified DNA bases that will provide diagnostic tools for monitoring and establishing early treatment for the elderly. These standards will play a critical role in quality control and monitoring of tissue and tissue engineered products. Currently, there are no government or industry accepted quality control standards and biomarkers.

CSTL has responded to a vital industry need for high throughput analysis of genetic samples by developing innovative mass spectrometric methodology in the newly established DNA mass spectrometry laboratory that houses a state-of-the-art high-speed MALDI time-of-flight spectrometer with automated sample preparation. CSTL has lead the effort in the development of electrochromatographic preparative bioseparations, to respond to urgent industry needs for separation methods for large quantities of multiple physical forms of DNA

Development of Standards for the Measurement of Oxidatively Modified DNA Bases

H. Rodriguez, P. Jaruga, and M. Dizdar (831)

The fastest-growing segment of the United States population is age 65 years and older. Longevity Medicine will then become one of the most profound social and economic forces of the 21st Century, restructuring and redirecting the annual trillion-dollar-plus economics of the health care establishment in radical new ways. Scientists now know that oxidant by-products from normal metabolism and exogenous sources cause extensive damage to DNA, protein and lipid (fat). Scientists now argue that this damage, known as oxidative stress is a major contributor to aging and the degenerative diseases associated with aging. In 1998, a European Standards Committee on Oxidative DNA Damage was organized to implement the use of measurement standards for the various technologies used in monitoring oxidative stress. It was concluded that measurement standards were needed for this purpose. Furthermore, clinical reference laboratories in the United States have also expressed a need for such standards. Cambridge Isotope Laboratories synthesized five stable isotope-labeled compounds to be used as reference standards. Cambridge Isotope Laboratories provided GC/MS and NMR data with each of the five compounds purchased and certified the purity of the compounds and the isotopic content to be better than 98%, which was confirmed at NIST. NMR measurements of the five compounds were performed. The data was used to verify the purity of the compounds. Each of the compounds was analyzed by GC/MS according to the procedures previously developed at NIST. The isotopic content of the compounds were verified by mass spectrometry. A methodology was developed for analysis of the compounds by LC/MS using a reversed-phase column. All compounds had molecular ions and other characteristic ions expected from this class of compounds. Mass spectra confirmed the isotopic content of the compounds in agreement with GC/MS measurements. Their purity was found to be better than 99%. UV spectra of the compounds were



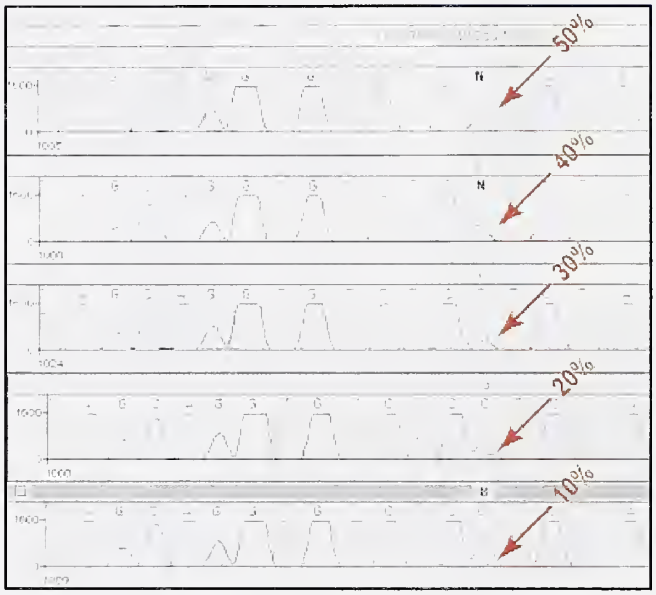
SRM 2396 - Oxidatively Modified DNA Base Biomarkers

recorded. The spectra were in agreement with those previously published data. The concentration of the solutions was confirmed by this technique as well. Future plans are to continue synthesizing and characterizing the six remaining compounds needed.

Development of a Heteroplasmic Mitochondrial DNA Standard Reference Material for Detection of Heteroplasmy and Low-Frequency Mutations

B.C. Levin, F.P. Schwarz (831), and L.A. Tully (National Institute of Justice)

A heteroplasmic human mitochondrial DNA (mtDNA) standard reference material (SRM) is being developed to provide quality control to forensic, medical, and toxicological scientists who wish to determine their detection limits when examining low frequency mutations or heteroplasmic sites in DNA. While the detection of a mutation present in every mtDNA molecule is routine, it is extremely difficult to detect mutations present in only a small proportion of the molecules. Human mtDNA mixtures containing a single polymorphic/wild-type site in different percentages have been constructed from Polymerase Chain Reaction (PCR) products from two different cell culture lines that differed by one base pair in the amplified region. Various mutation detection techniques, including automated sequencing, denaturant gradient gel electrophoresis (DGGE), peptide-nucleic acid (PNA)-directed PCR clamping, were used to determine the lowest detectable level of the heteroplasmy in our mixtures. The mixtures were sent to twelve laboratories in an Interlaboratory Evaluation of the SRM. Using automated DNA sequencing chemistries (ABI Dye-Terminator, dRhodamine Terminator, Big Dye Terminator), unambiguous detection of the polymorphism present at the 30% level was achieved. Although visible at the 10% and 20% concentrations, it was difficult to distinguish the polymorphism from the background. With DGGE, resolution at the 5% level was achieved. Addition of PNA complementary to the wild-type sequence, decreased PCR amplification of the wild-type DNA, but selectively amplified the polymorphism, thus detection by sequencing became possible at the 5% level. The results of the Interlaboratory Evaluation of this SRM are currently under evaluation. This SRM can be employed to test and perfect more sensitive mutation detection techniques.



Pre-symptomatic mtDNA diseases may become treatable, and perhaps even preventable. These more sensitive techniques may permit toxicologists to detect the effects of chemical and physical mutagens before they cause adverse health and environmental problems.
In addition, forensic scientists will be able to detect low frequency heteroplasmic sites thereby increasing their ability to make correct human identifications.

Determination of Commercial STR Kit Primer Sequences

J. Butler and P. Vallone (831)

Experts in CSTL developed a novel strategy for determining the previously unreported primer sequences contained in commercial multiplex STR kits by integrating four techniques, HPLC, mass spectrometry, informatics, and molecular biology.

Multiplex PCR amplification of short tandem repeat (STR) markers is commonly performed in forensic DNA laboratories with commercially available kits. These kits, which target between 3 and 16 sites in the human genome, contain a forward and reverse PCR primer for each STR marker amplified. One primer in each primer pair is labeled with a fluorescent dye to enable multi-color fluorescence detection of the PCR products. While a number of STR kits are in widespread use around the world, the sequences of the primers contained within these kits have not been completely reported by their manufacturers. HPLC is used to separate the primer mixture and isolate the individual components. Dye-labeled versus unlabeled primers can be identified base on their retention times on the HPLC column. The hydrophobic dyes cause the dye-labeled primers to be retained longer on the column. Fractions corresponding to each peak in the HPLC chromatogram are collected and concentrated for further characterization via mass spectrometry. Each fraction is subjected to exonuclease digestion and analyzed by mass spectrometry in order to elucidate partial sequence information. Sequence information obtained is input into a computer program that assists in determining the full sequence identity of the primer. A report on this work is being prepared for the National Institute of Justice, who funded our efforts. A detailed manuscript describing this integrated methodology for solving primer sequences will be submitted to a peer review journal.

Quality Control Methods for Primers in Commercial STR Kits

J. Butler and P. Vallone (831)

Forensic DNA laboratories utilize commercial kits to perform DNA tests involving short tandem repeat (STR) markers. These kits contain primers or short oligonucleotides that are utilized for targeting unique sites in the human genome to provide DNA amplification with the polymerase chain reaction (PCR). Results from STR tests are being placed in DNA databases around the world in order to link serial crimes and solve crimes committed by repeat offenders. Good quality control of the PCR primers included in STR multiplex kits will help maintain consistent and reliable results over time as these kits are used to amplify DNA samples from convicted offenders and crime scene samples. Altering even a single PCR primer within a kit has the potential to impact the accuracy of STR results. Therefore development of methods for an independent verification of the PCR primers contained in multiplex STR kits was undertaken. Using high performance liquid chromatography and time-of-flight mass spectrometry, new methods for rapid quality control testing of primers contained within

Comparison of mass spectral analysis of 4 different STR kits demonstrating that primers present within these kits have the same mass and therefore are unchanged from kit to kit. Not all primers are present in all kits. Only one primer, D3-F, is present in all four kits shown here.

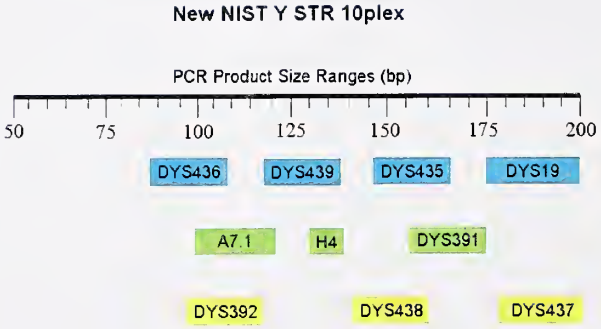
CSTL's efforts resulted in the development of a unique profile for each kit that can be used to verify that primer sequences in the kit remain constant over time.

commercial STR kits were developed. Our quality control methods utilize only 5 µL of a primer mix, which is a small fraction of the material supplied within a kit. HPLC retention time versus mass is plotted for each primer sequence.

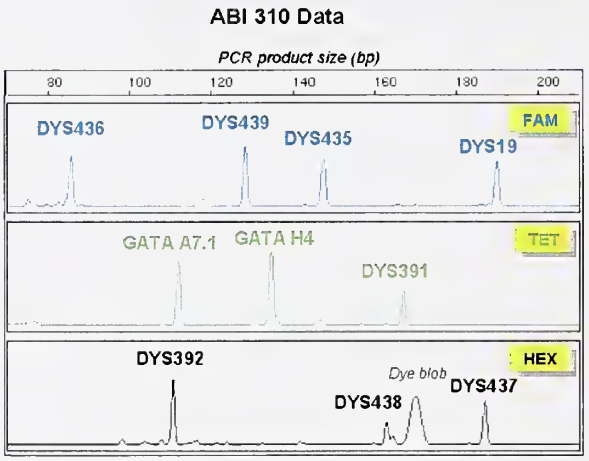
Development of New Y Chromosome Short Tandem Repeat Multiplex Assays

J. Butler, C. Ruitberg, and M. Kline (831)

The Y chromosome is in many ways the final frontier of forensic DNA analysis. Most forensic tests now focus on short tandem repeat (STR) markers present on non-sex chromosomes. In forensic cases where a mixture of body fluids might be present, such as in a sexual assault, the ability to target the male portion of a DNA sample has the potential to lead to more rapid identification of the perpetrator of such crimes. However, a standard set of DNA markers on the Y chromosome have not yet been agreed upon and no kits are commercially available to enable widespread use of a set of Y chromosome markers. Therefore production of improved Y chromosome assays was undertaken in order to aid the forensic DNA typing community. New primer pairs were designed to simultaneously amplify 10 separate regions of the Y chromosome in a multiplex PCR reaction. The resulting PCR products are labeled with three different colored fluorescent dyes, FAM (blue), TET (green), and HEX (yellow). Previously, no group had successfully amplified more than 6 regions of the Y chromosome simultaneously. Our new multiplex amplifies 10 Y STRs, permitting a greater power of discrimination in each test of the Y chromosome. The markers in our multiplex include DYS436, DYS439, DYS435, DYS19, Y GATA A7.1, Y GATA H4, DYS391, DYS392, DYS438, and DYS437. Development of SRM 2395 is ongoing, and will contain male DNA samples and certified results using multiple Y chromosome markers. The new Y STR 10plex will be examined to distinguish unrelated male individuals from one another by examining the variation in STR alleles in a population of 100 or more individuals and to publish the results of this population study. We also hope to expand our multiplex to perhaps as many as 20 Y STRs in order to further increase the power of discrimination in this DNA assay.



Primers were redesigned for most of these loci in order to keep the PCR products under 200 bp so that degraded DNA could be more successfully typed.



Data from a single PCR amplification showing that 10 different Y chromosome STR markers can be measured simultaneously

Examination of DNA Stability on Different Storage Media

M. Kline, J. Redman (831), and D. Duewer (839)

The recovery of genetically typeable DNA is important to the Human Identity Communities; Federal and state forensic DNA repositories for convicted offenders, the Department of Defense's DNA Registry for military personnel reference samples, and individuals storing samples from their children against future need. CSTL initiated this study in 1994 with S&S 903™ paper (Schleicher & Schuell, Inc.Keene, NH). Bloodstains on this medium were stored at ambient temperature, -20° C, -80° C, and (-150° C, the liquid nitrogen vapor temperature. Two additional complementary studies included bloodstains stored on S&S IsoCode® paper (stored ambient) with S&S 903 paper control stains stored at -20° C (5Y), and FTA® paper (FITZCO, Inc. Maple Plain,MN) and S&S 903 paper with bloodstains vacuum sealed in mylar pouches and stored at +37° C, ambient temperature, and –20° C (3Y). On a regular basis we removed samples for analysis from all studies. The data obtained from these studies was examined quantitatively using the average peak heights obtained from the Short Tandem Repeat typing data. Throughout the six year study typeable DNA from all samples at all storage temperatures was obtainable. The samples stored at –150° C appeared to be the same as fresh bloodstains, while the ambient temperature samples appear brown in color. Results for the six year time point samples show a slight decrease in the amount of PCR product from the ambient temperature stored material as compared to the bloodstains stored frozen. From 12 to 24 months the ambient samples amplified better, while from 39 to 72 months, the –20° C samples amplified better. In 1995, fresh bloodstains were prepared on both media as controls. Analysis showed no real differences in amplification between five year S&S 903 samples and the Time Zero (TZ) S&S 903 samples. The TZ IsoCode samples amplified better than the TZ S&S 903 samples. The TZ IsoCode samples amplified better than the five year IsoCode samples. Finally the five year S&S 903 samples amplified better than the five year IsoCode samples. Analysis of three year samples of FTA/S&S 903 bloodstains resulted in typeable DNA from all samples regardless of the media or the storage temperature. Continuation of the storage studies will be pursued.

CSTL efforts addressed a critical need to document the long-term stability of DNA extracted from bloodstains that have been stored on different paper based storage media.

Effect of Single Mutations on the Specificities of DNA Repair Enzymes

M. Dizdar (831), J. and P. Radicella (Guest Researchers)

Oxidative DNA damage has been implicated in mutagenesis, carcinogenesis and aging. In cells, there are repair systems that oppose DNA damage. If not repaired, DNA damage may lead to detrimental biological consequences. Mutations in repair genes cause significant modifications in substrate specificities of DNA repair enzymes coded by these genes and may play an important role in carcinogenesis. The effect of single mutations on the substrate specificity of *E. coli* Fpg protein and its homolog human Ogg1 protein has not been reported. Oxidative damage generates a multitude of modified bases in DNA. An accurate measurement of these modified bases is achieved by the use of gas chromatography/isotope-dilution mass spectrometry. Because of its ability to simultaneously measure a multitude of modified bases in DNA, this technique permits to study the substrate specificities of DNA repair enzymes and their excision kinetics. In this study, we used this unique approach to determine the effect of single mutations on the substrate specifities of two major DNA repair enzymes that are involved in the DNA repair in *E. coli* and in humans. A variety of mutated proteins were obtained using site-directed mutagenesis and PCR-based techniques. Fpg proteins were overexpressed in *fpg*⁻ *E. coli* and purified to apparent homogeneity. The results indicated Lys-57 had an important role in the activity of the Fpg protein.

Micrograph of *Eschrichia coli*

Mutations involving Lys-155 and Pro-2 had a dramatic effect with Pro-2→Glu leading to complete loss of activity, indicating a significant role of these residues. Two different mutant forms of the wild type human Ogg1 protein have been identified in human kidney tumors and a gastric cancer cell line. The mutant proteins were expressed in *E. coli* and purified to homogeneity. All three enzymes excised two purine-derived lesions from γ-irradiated DNA containing a multiplicity of base lesions. Michaelis-Menten kinetics of excision was measured, and significant differences between excision kinetics of these three enzymes were observed. These results show that point mutations significantly change the specificity of *E. coli* Fpg protein and human Ogg1 protein and suggest that point mutations are can be expected to change the specificities of other DNA repair enzymes. This work will be extended to other DNA repair enzymes and their mutant proteins that are found in *E. coli* and in human tumors.

Development of Measurement Technologies for the Measurement of Oxidative DNA Damage by Liquid Chromatography/Mass Spectrometry (LC/MS)

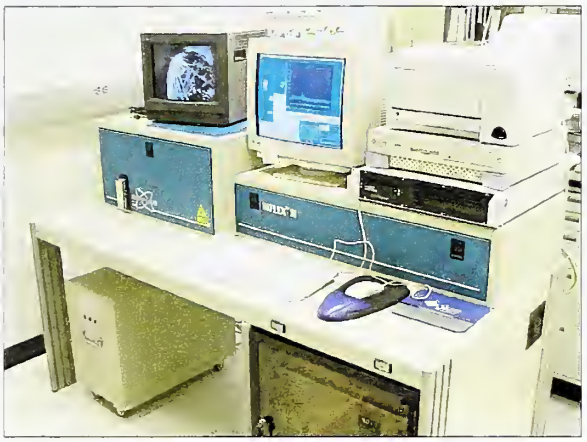
M. Dizdar, P. Jaruga, and H. Rodriguez (831)

Tissue-engineered products require precise measurement oxidative DNA damage for quality control, but there are no generally accepted standards to do this at this time. LC/MS technology has not been applied for this purpose before. So, this project was undertaken to determine if tissue engineered skin products have oxidative DNA damage due to the manufacturing processes, with results obtained used to complement the data obtained using gas chromatography/mass spectrometry (GC/MS). A number of LC columns and solvents were examined to explore optimum measurement conditions of intact nucleosides and bases, and an excellent LC separation of intact DNA nucleosides was achieved. Their mass spectra were recorded and were in accord with previously published mass spectra of DNA nucleosides. Next, aqueous solutions of individual nucleosides were exposed to ionizing radiation to generate typical, oxidatively induced products of DNA. Irradiated samples were then analyzed by LC/MS. Mass spectra of the products were recorded and interpreted on the basis of products of DNA previously known from GC/MS studies done at NIST. In particular, the efforts were concentrated on one particular product, 8-hydroxy-2'-deoxyguanosine (8-OH-dGuo), since this compound is mostly measured by different techniques around the world as a biomarker of oxidative DNA damage and thus oxidative stress. No studies on this compound using LC/MS technology exist. Preliminary results indicated that 8-OH-dGuo can be well measured in DNA by LC/MS. A stable isotope-labeled analogue of this compound, was used as an internal standard for quantitative measurement. Mass spectra of both authentic 8-OH-dGuo and 8-OH-dGuo-^{18}O standard were recorded and interpreted, and showed intense molecular ions and typical fragment ions suitable for positive identification. Calibration plots for quantitative measurement were obtained using these two compounds. Analysis by LC/MS of DNA damaged by ionizing radiation showed that 8-OH-dGuo could be measured precisely by LC/MS under our experimental conditions. Irradiation of DNA at several different radiation doses provided linear dose-yield plots, indicating the accuracy of this technology. Future plans include the application of this measurement technology to measure the level of oxidative DNA damage in tissue-engineered skin cells.

Establishing a Novel DNA Mass Spectrometry Laboratory

J. Butler and P. Vallone (831)

The current hot topic for the biotechnology, genomics, and pharmaceutical industries is the examination of single nucleotide polymorphisms (SNPs) for understanding human variation at the DNA level and development of new drug targets. More than 2 million SNPs are known to exist within the human genome. The ability to perform testing on a portion of these SNP markers in a high-throughput fashion can help identify the SNPs that are most relevant to a particular disease state. Likewise, a battery of SNP markers can have useful applications in human identity testing



Bruker BIFLEX III TOF Mass Spectrometer

MWG RoboAmp Robotic Workstation

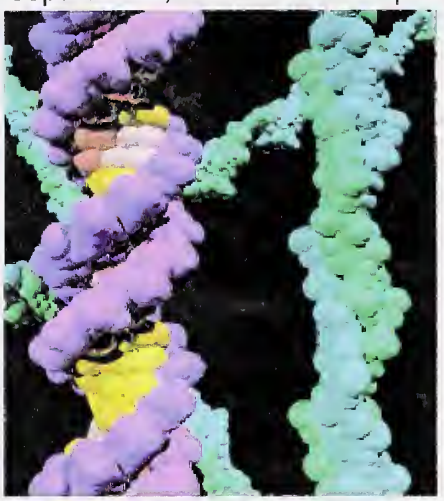
to link two biological samples originating from the same source. Time-of-flight mass spectrometry coupled with robotic sample preparation has the potential to meet the needs of high-throughput SNP genotyping because of rapid data collection with high accuracy. Through funding from the National Institute of Justice, we recently purchased a state-of-the-art time-of-flight mass spectrometer and robotic sample preparation workstation for performing DNA research projects. The Bruker BIFLEX III is capable of automated data collection using a fuzzy logic search algorithm. Multiple DNA samples can be processed in parallel using a liquid handling robot with integrated thermal cycler. We have developed methods for high-resolution DNA measurements important for quality control testing of PCR primers. We have also begun testing several approaches to typing SNP markers using the mass difference between the primer and extension product(s) in single base extension reactions. We intend to improve the capability of automated data collection through more homogeneous DNA-matrix crystal formation and smaller spots. We also want to develop robust protocols for testing SNP markers from the Y chromosome and mitochondrial DNA.

Preparative Bioseparations

K.D. Cole (831) and C.M. Tellez (University of Illinois)

Large-scale purification of nucleic acids is necessary to meet the needs of research and development laboratories and for uses as new classes of therapeutic agents. Production of large amounts of DNA for these industrial needs is a new challenge in Bioprocessing. Electrophoresis has proven to be a highly successful method for the separation of nucleic acids on an analytical scale. Electrophoresis in gels and capillaries is a high-resolution analytical method, but scale-up is difficult. Electrochromatography (EC) is proving to be a promising method to scale up the electrophoresis of nucleic acids. EC is the application of an axial electrical field to a chromatography column. This research has been done in collaboration with the company Life Technologies, Inc., which has provided a crude cellular extract that contained supercoiled circular DNA, nicked circular plasmid DNA (relaxed), linear genomic DNA (from host bacteria), RNA, and other cellular constituents. We have shown that EC can effectively separate supercoiled and open circular forms of plasmid DNA. The best separations were obtained using porous chromatography media made with the highest agarose concentration. Selective elution of plasmid

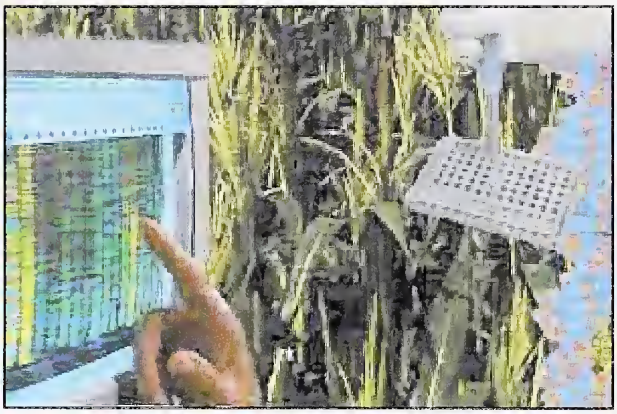
DNA with different forms was obtained by either increasing the flow rates or decreasing the electric field strength (by steps or a gradient). In all the separations, the more compact supercoiled form of the plasmid was retained less strongly than either the open circular form (nicked) or the linear form, and high molecular weight host genomic DNA was more strongly retained. Increasing the ionic strength of the buffer improved resolution and capacity. The capacity of the separation was determined by injecting increasing amounts of plasmid DNA. The retention of DNA depends upon a counter current flow of electrophoresis and convective flow and could be regarded as a type of field flow fractionation. Another approach is gel electrophoresis with reversible media. Gel electrophoresis is another high-resolution technique that is not widely used as a preparative tool because of the difficulty of getting DNA or proteins out of the gel when the separation is achieved. Investigations of gel-forming polymers that change to solutions when the chemical environment is changed are also underway. Current work is focused on investigating and controlling the structure of gels to influence the trapping of circular DNA.



Model of DNA helix with gellan molecules

Biocatalytic Systems

W.M. Byrnes, D.T. Gallagher, M.J. Holden, M.P. Mayhew, V.L. Vilker,(831) A.E. Roitberg, (GeoCenters, Inc.), and B. Coxon (NICHHD/NIH)



A new effort has been undertaken to work with the Grain Inspection, Packers and Stockyards Administration (GIPSA) of the U.S. Department of Agriculture, and the Institute for Reference Materials and Measurements (IRMM) of the European Commission Joint Research Center to develop measurement methods and reference materials for identifying biotech grains. Researchers in CSTL's Biotechnology Division are working with IRMM scientists to identify and inhibit or eliminate factors affecting DNA degradation, to select non-biotech housekeeping genes for quantitative comparison with event DNA, and to establish the purity of lines used for reference materials.

A Computer Program to Determine the Effects of Single Nucleotide Polymorphisms (SNPs) and Mutations in Human Mitochondrial DNA

B.C. Levin and M.S. Lee (831)

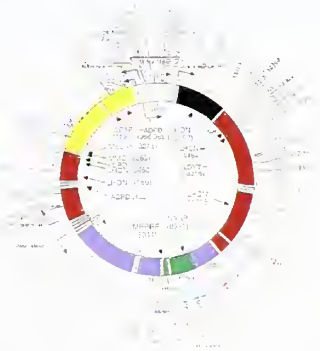
The program, MitoAnalyzer, compares any changes found during sequencing human mtDNA with the sequence and numbering system published by Anderson et al. in 1981. The user inserts the number of the nucleotide change and the new nucleotide (i.e., A, C, G, or T). The program was written in C++ and Java. MitoAnalyzer provides the researcher useful information about the particular polymorphism or mutation. The program identifies where the change occurs, such as in rRNA, tRNA, or one of the 13 proteins coded by human mtDNA. The program specifies whether the area of interest is in the coding region, and if so, whether it affects the first, second or third base pair of a codon. If the change is in a protein, the amino acid change, the nature of the amino acid change, and the position of the change in the resultant protein is provided, along with the new amino acid sequence of the entire protein. Finally, association of the mutation with a disease in the scientific literature is provided. The program is intended to facilitate rapid analysis and evaluation of polymorphisms and mutations in human mtDNA and their possible pathogenicity.

Intergenerational Transmission of a Human Mitochondrial DNA Heteroplasmy Among Thirteen Maternally-Related Individuals

K. Sekiguchi, K. Kasai (National Research Institute of Police Science, Japan) and B.C. Levin (831)

The observation that the transmission of a mtDNA heteroplasmy from one individual to her offspring is likely to differ among the first generation offspring and between that generation and subsequent generations lends further credence to the bottleneck theory of inheritance of mtDNA.

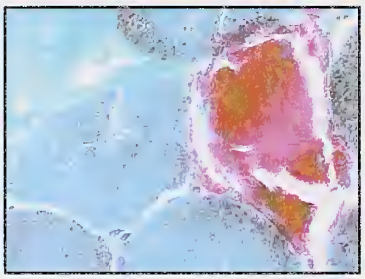
Mitochondria are maternally inherited, though it is not known how individual mitochondria are passed between generations. The presence of one of the heteroplasmic base pairs (different base pairs at the same mtDNA site) in one tissue sample and the other heteroplasmic base pair in the second sample can result in a forensic scientist calling it an exclusion rather than a match. At this time, it is not clear whether these heteroplasmies exist in the mtDNA in individual mitochondria, in different mitochondria in the same cell, or in mitochondria from different cells within the same tissue. The transmission of a cytosine:thymine (C:T) heteroplasmy found at position 16291 in the HV1 region of the human mitochondrial DNA (mtDNA) control region in thirteen maternally–related individuals across three generations and among the tissues in two of those individuals was studied. One disadvantage of using mtDNA for human identification is the possibility that a heteroplasmy may exist. This heteroplasmy was examined in buccal cells from thirteen maternally related family members across three generations and in additional tissues from two members, a mother and son, of this family. Sequencing was performed with the ABI PRISM™ 310 Genetic Analyzer using the BigDyeTerminator and BigDyePrimer sequencing kits. The ratio of C to T at position 16291 showed wide intra- and intergenerational variation. The variation was also observed within tissues from single individuals. These studies indicate that the proportion of the two components of a heteroplasmy can vary widely among maternally-related family members and among tissues within a single individual. Thus, a single base pair difference should not be used as the sole determinant that two samples are from different individuals or families. Such a discrepancy may be resolved by the detection of the heteroplasmy through the examination of additional tissues from the individual or other maternally-related family members.



A Patient with Chronic Progressive External Opthalmopegia Reexamined Thirty Years Later

B.C. Levin, L.A. Tully, J.T. Chen (831), K. Sekiguchi (National Research Institute of Police Science, Japan), and A. Gropman (National Human Genome Research Institute)

In 1972, seven cases of Idiopathic Progressive External Ophthalmoplegia with ragged-red fiber myopathy were described [Olson, W. et al., Arch. Neurol. 26:193-211(1972)]. At 14 years of age, Case 5 developed right-sided ptosis and paresis of eye movements (ophthalmoplegia). When examined at NIH at age 17, he showed moderate bilateral ptosis and severe restriction of all eye movements. Muscle strength as well as general health and height were said to be normal. A muscle biopsy revealed ragged-red fibers with gomori trichrome staining and abnormal mitochondria in clusters. Since other members of his family were normal, the conclusion in 1972 was that the disease must be acquired or, if genetic, must be inherited as an autosomal recessive trait. In 1999, following the development of human mtDNA Standard Reference Material 2392 for sequencing, mitochondrial disease diagnosis, and mutation detection at NIST, we were given the opportunity to examine the mtDNA from Case 5, who had been diagnosed with Chronic Progressive External Ophthalmoplegia (CPEO) but whose mtDNA had never been analyzed. We also sequenced the entire mtDNA of his mother and brother. In addition, he was re-examined clinically at NIH and found to be cachectic with severe muscle wasting and proximal weakness. He had moderate dysphagia, complete ophthalmoplegia and ptosis, which are symptoms consistent with a progressive mitochondrial myopathy. The sequence of the entire mtDNA from the blood of Case 5 (now age 50), as well as that of his unaffected brother (age 53) and mother (age 79) were found to be identical. Although differing at several positions from the sequence published by Anderson et al. in 1981, these differences did not correlate with any published mutations associated with mitochondrial disorders. The DNA samples were then analyzed for the "common deletion", associated with CPEO, Kearns Sayre Syndrome, and Pearson's Marrow Syndrome, and also been found to accumulate during aging in normal (i.e., unaffected) individuals. Initial analysis using primer pair F8164/R13611 in a two round PCR assay revealed the presence of the 4977 bp deletion in Case 5, but not in his mother or brother. Sequence analysis confirmed the presence of this deletion in Case 5. Subsequent analyses using deletion-specific PCR primers (provided by Dr. Steven Zullo, NIH) corroborated these findings, and again demonstrated that the 4977 deletion was present at detectable levels exclusively in Case 5. The presence of the 4977 deletion at low levels in the blood of Case 5, and not in the blood of his mother and brother, indicates that CPEO is the most likely explanation for his symptoms.

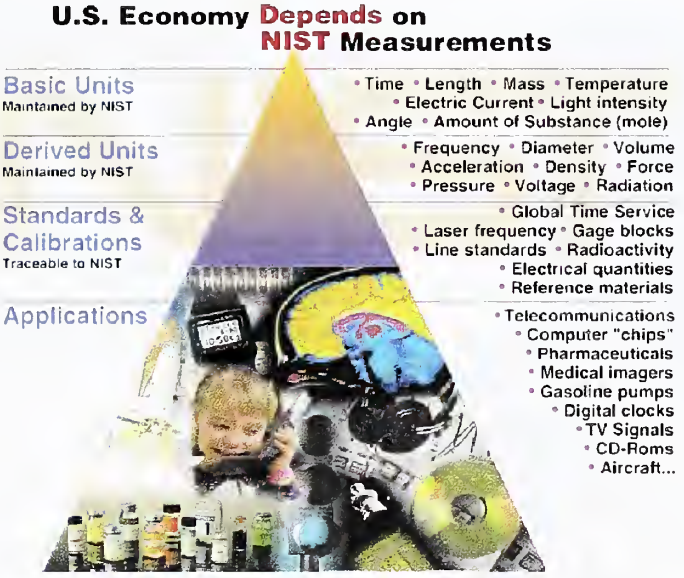


Example of gomori trichrome stain of muscle tissue that shows marked mitochondrial proliferation

Advances in the field of mitochondrial genetics provided the tools that made this molecular confirmation of the CPEO diagnosis possible.

Impact of CSTL Programs

U.S. industry depends on NIST to assure the quality of measurements, their traceability to national standards, and recognition of measurements made by U.S. manufacturers in the global market place. This places a considerable responsibility on NIST to establish and maintain the U.S. system of measurements. The hierarchy of units, standards, and calibrations is depicted in the following figure. NIST maintains the basic International System (SI) of units, such as time, mass, electric current, and the amount of substance (mole) through measurements of the physical phenomena that define these units, and maintenance of the U.S. mass artifact standards. Standards for derived units are similarly maintained. In order to transfer the accuracy base provided by the SI, NIST provides standards, calibrations, and reference materials to U.S. industry, supporting a wide range of applications, services, and products. NIST's investment is approximately $500 million per year, and undergirds an approximately $10 billion private sector investment in measurements and standards. More than half of the $7.6 trillion per year U.S. GDP is supported by this measurement infrastructure.



In the U.S., more than fifty percent of the economic growth is attributed to technological advances; such advances require measurements of higher resolution, sensitivity and selectivity, and measurement standards that are globally recognized. To meet the increasing demand from industry for infrastructure support, NIST needs to expand its resources. To justify such expansion, NIST must demonstrate the impact of metrology programs on economic growth and ensure that investments are highly leveraged.

NIST's extensive ties to industry, other government agencies, and academia provide a mechanism to identify and assess measurement, standards, and data needs of U.S. industry. Indeed, industry input helps to set the direction and emphasis of NIST programs, and ensure a significant impact. However, it is not always easy to assess the impact of metrology programs because of the infrastructural nature of measurement science. Therefore, both anecdotal information and more formal impact studies are needed to document their impact. The results of studies conducted to date have consistently shown high rates of return from NIST research, relative to both private investments in technology and other public technology investments. These results are not surprising, given that NIST targets its research at specific infrastructure problems that are typically faced by a large number of firms and/or industries, and that have been identified through cooperative strategic planning with industry. Methodologies and approaches developed for assessment of the economic impact of metrology programs may be used as models for articulating the critical importance of metrology for economic growth.

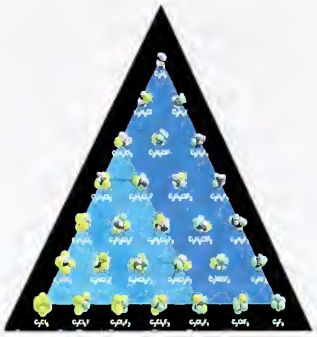
Formal Impact Studies

Evaluations of the economic impact of NIST's metrology programs in specific technical areas are carried out through NIST-commissioned studies, mostly performed by external contractors. These studies provide both qualitative assessments and quantitative estimates of the economic impacts resulting from the several categories of technology infrastructure that NIST provides to U.S. industry. Quantitative estimates are provided either as benefit-to-cost ratios or as rates of return to the nation (social rate of return). The results of these impact assessments not only respond to the need to measure and analyze current and past performance but also contribute to future strategic planning. Practically, only a few of these in-depth analyses are possible each year. Some of the results of these formalized impact studies are described below.

Thermocouples: The NIST thermometry program includes both calibration services and research on thermocouples. Thermocouples are among the most commonly used sensors for monitoring and control of manufacturing processes. The annual sales of thermocouple products sold by the U.S. thermocouple industry (suppliers of wire and thermocouple assemblies) into the U.S. market are approximately $280 million. The incorporation of these devices into higher levels of product structures across a broad base of domestic industries affects a much larger portion of the manufacturing sector, estimated to be on the order of $80 billion.

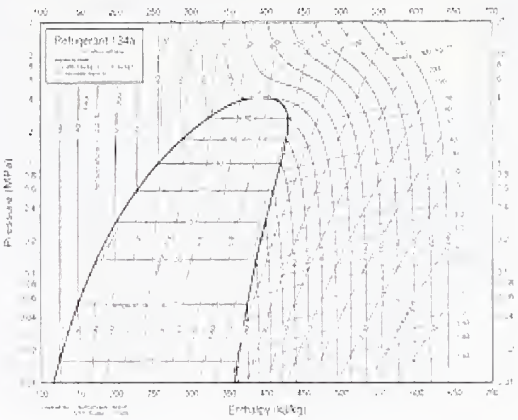
Benefits were estimated based on surveys and interviews of the thermocouple industry. Participants were asked to estimate the additional expenses that would have been incurred if NIST were to cease to provide primary calibration services. NIST's expenditures in the thermocouples program from 1990 to 1993 included support for research on the basic physical properties that underlie the measurement science to incorporate the change from IPTS-68 to ITS-90. For this effort, NIST led the development of the ITS-90 update and shouldered 60 % of the costs with eight other national standards laboratories. Costs over the whole period of the study (1990 – 1996) also include support for R&D on test methods as well as the calibration services themselves. Therefore, there was a significant time during which NIST expenditures on the fundamental and infrastructural aspects of thermocouple principles, measurement, and test methods did not result in immediate benefits to industry. But once benefits are realized, they are substantial, and these estimates do not include the much larger, though diffuse, community of device users. This study, greatly affected by both the short time-line and limited scope, conservatively estimated the social rate of return to be 32 %, and a benefit-to-cost ratio of 2.95.

Alternative Refrigerants: Occasionally, an accelerated R&D program must be undertaken to respond to industry needs that are constrained by set deadlines. Such was the case for NIST's program on the chemical and physical properties of alternative refrigerants used to replace chlorofluorocarbon (CFC)-based refrigerants. Until the past decade, most refrigerants used throughout the world were made up of CFCs. But as a result of research findings on the deleterious effects of CFCs on the earth's ozone layer, a global agreement to phase out the production and consumption of CFCs and replace them with alternative refrigerants was signed in 1987 (the Montreal Protocol).



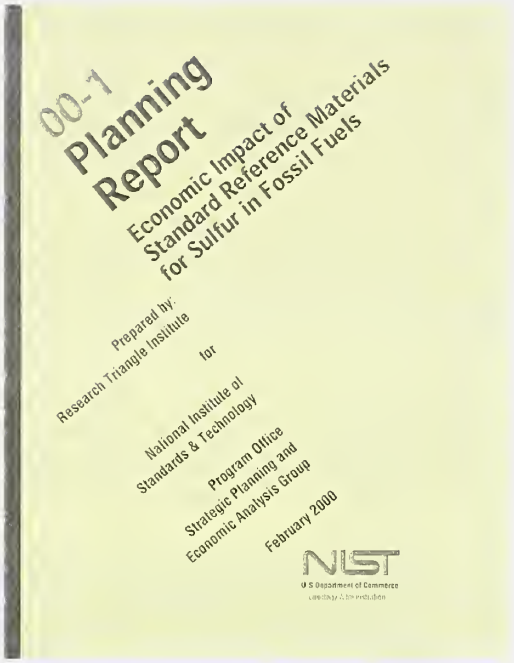
NISTDATA

With the timetable imposed by the Protocol as an incentive to develop new alternatives to CFCs, NIST engaged in research that would allow industry to make the switch to alternative refrigerants in a timely and economical fashion. NIST began by identifying the basic requirements for new refrigerants according to the new rules, and then started research on determining the physical properties of such candidate alternatives. NIST's most effective form of information dissemination has been the REFPROP program, a computer software package that is available through NIST's Standard Reference Data Program. The REFPROP program enables manufacturers and users of alternative refrigerants to model the behavior of refrigerant mixtures in their respective manufacturing processes, a key method in developing CFC replacements. A comparison of industry benefits with the funding stream of NIST's research program estimated a social rate of return of at least 433 %, and a benefit-to-cost ratio of 4 to 1.



SRM®

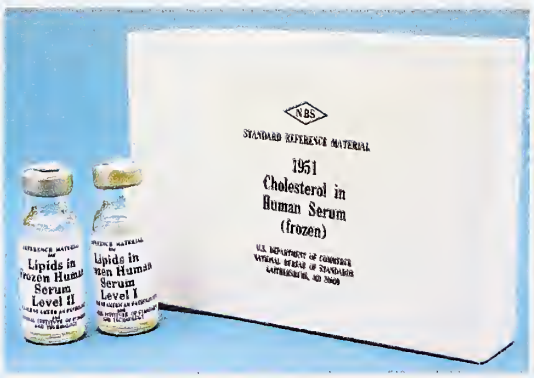
Sulfur in Fossil Fuels: The economic impact of Standard Reference Materials for *Sulfur in Fossil Fuels* was completed in February 2000. The sulfur content of fossil fuels is one of the most important intrinsic factors that determine fuel prices. The accurate determination of the sulfur concentration in fossil fuels is required as a result of environmental regulation that places increasingly lower limits on their sulfur content and the imposition of large fines for non-compliance. At every stage in the process (mining, transportation, buying and selling, and combustion) the sulfur content of both oil and coal must be determined in order to meet buyer and seller specifications that are dictated in large part by government environmental regulations. The efficient and cost effective movement of coal and oil from the mine and well to power plants and refineries requires precise and accurate determination of sulfur content in two or more laboratories. For equity in trade and the efficient production of energy, it is mandatory that instrumentation in these laboratories be calibrated using accurate standards.



NIST has developed a primary method based on isotope dilution mass spectrometry (IDMS) to determine sulfur in fossil fuels to an accuracy of better than ± 0.1 % relative. Using the IDMS method, NIST has certified the sulfur content in about 30 coal and fuel oil SRMs. These SRMs provide industry with the primary calibration materials needed for instrumentation used in routine measurements. SRMs also provide industry with a strong traceability link to NIST for such measurements, whether they be for setting the price of fuel or for demonstrating compliance with environmental regulations.

The recent study quantifies a portion of the economic benefits associated with these SRMs beginning in 1984, and projected through 2003. Surveyed industry representatives indicated that NIST SRMs have decreased the level of uncertainty associated with their measurements of sulfur content. This reduction has led to economic benefits throughout the supply chain. Included in the measures of economic benefits are improvements in product quality, production efficiency, and reductions in transaction costs and sulfur emissions to the environment. This study estimates a benefit-to-cost ratio of 113, and a social rate of return of 1,056 %. The Net Present Value was calculated to be more than $400 billion.

Cholesterol Standards: Chemical metrology is at the heart of accurate medical diagnosis and the development of measures to improve our health and ensure long life. In the U.S. about 1.5 trillion dollars are spent each year on health care, which is over 14 % of the U.S. GDP. More than 25 % of these expenditures are for measurements. It is estimated that over one third of these measurements are performed for non-diagnostic purposes, such as retesting, error prevention, and detection limitations.



In the area of cholesterol measurements alone, it has been estimated that measurement uncertainty was on the order of ± 18 % relative in 1969, before any reference materials were available. Over the last three decades, NIST, in cooperation with the College of American Pathologists (CAP), has developed a series of highly accurate and precise methods for a number of clinically important serum constituents, including cholesterol. These methods are recognized by the international clinical laboratory community as "definitive" and have been used to certify a series of

cholesterol SRMs. The first pure crystalline cholesterol (SRM 911) was introduced in 1967. Using the definitive method, serum cholesterol SRMs were developed in 1981 (SRM 909) and again in 1988 (SRMs 1951 and 1952). These SRMs have led to a steady decrease in the number of false positives and negatives resulting from clinical laboratory results for cholesterol in blood to between ± 5.5 % to 7.2 %, relative. These improvements represent potential savings of almost $100 million per year in treatment costs for misdiagnosed patients, in addition to the lives saved through timely and accurate diagnosis.

The latest impact study quantifies a portion of the economic benefits associated with these SRMs beginning in 1986. The economic consequences of NIST's Cholesterol Standards Program are experienced at several levels of the supply chain from manufacturers, to network laboratories, to clinical laboratories that ultimately deliver medical services to the consumer. The benefits to industry resulting from the NIST investment have changed over the more than three decades of NIST involvement. However, this analysis timeframe was limited to 1986-1999 covering only part of the program's life cycle, thus biasing the measured impacts downward. The results indicate that NIST has played an important economic role in support of a national effort to monitor, measure, and control cholesterol levels, thereby contributing to the reduced level of heart disease. This economic impact study estimates a benefit-to-cost ratio of 4.5, and a social rate of return of 154 %. The Net Present Value was calculated to be more than $3.6 million.

A Centennial Scrapbook
remembering the past ... a key to the future

Dignitaries gather for the groundbreaking of state-of-the-art laboratories:

Left: The new chemistry building on the Connecticut and Van Ness site in Washington DC Fall 1915

Right: Federal and local officials break ground for NIST's new Advanced Measurement Laboratory, June 2000

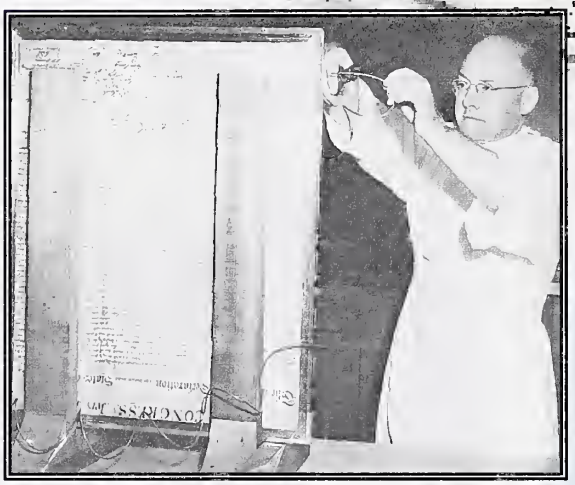
This institute has a rich history of membership and leadership in international standards organizations and committees to help ensure that our standards, and U.S. manufactured products, are globally accepted.

Left : **International Technical Committee Members of 1910**

Below: **CCQM Members of 1999**

Charters of Freedom:

The Declaration of Independence, the Constitution and the Bill of Rights (on permanent display at the National Archives in Washington, DC) have guaranteed the rights and freedoms of Americans for more than 200 years. Since 1951, these great documents--known collectively as the Charters of Freedom--have been preserved in helium-filled cases created by the Commerce Department's National Bureau of Standards, predecessor to the National Institute of Standards and Technology. Now, NIST, the National Archives and Records Administration, NASA and Heery International have teamed to design new state-of-the-art enclosures for the Charters of Freedom. ***CSTL researchers are NIST team members and supply expertise regarding the measurement of low-level impurities in gases.***



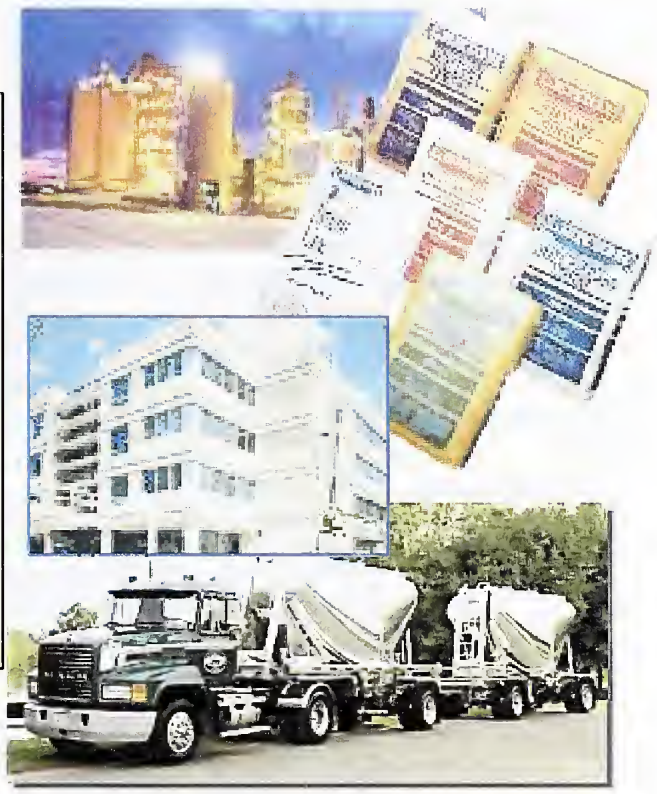
Deterioration of the glass in the current encasements appears as small surface cracks, crystals and droplets. This deterioration will eventually cause the glass to become opaque. Additionally, contact between the parchment and the glass may cause abrasions. Correcting these problems in the current cases is not possible: these cases are soldered shut and cannot be opened without compromising the seal.

Conservators will be able to be open and reseal the new cases—if it's ever necessary—to examine the documents or modify the interior components. The documents will be mounted so that glass never touches parchment. Ultra-smooth surfaces and the use of atomically larger argon gas rather than helium will prevent leakage. The new design will afford the flexibility to incorporate future conservation techniques as they are developed.

Working with Industry:

Standards for the Construction Industry:

In 1911 the cement laboratories of NBS tested over 23,900 samples, representing almost 2.5 million barrels of cement purchased for Government construction projects. The sampling required 512,000 physical tests, for fineness, specific gravity, tensile strength, and time of setting. ... In many cases the Government specifications were far from clear. ... In early 1912, the Bureau called manufacturers and Federal engineers to the first Portland Cement Conference, in order to consider preparation of a single standard specification. As a result, an Executive Order was issued in April of 1912, declaring that all portland cement purchases by the Government was to conform to the agreed upon specifications." (*Measures for Progress – A History of NBS*)

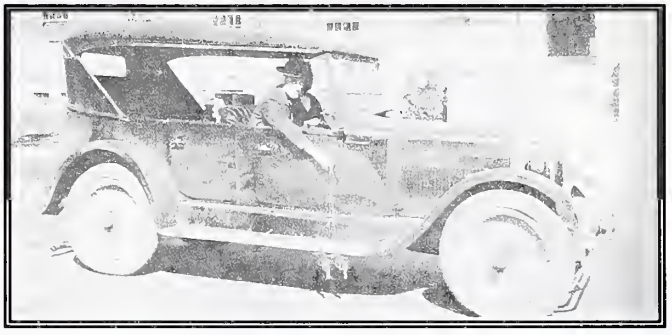


In 2000, ten new cement SRMs, certified for chemical composition, were produced by NIST in response to a continuing and increasing demand for cement standards. In the U.S., 45 companies and 118 plants in 37 states produce more than 80 million tons of cement yearly. This suite of SRMs is comprised of 6 different Portland cements, one white cement, one blended cement, and two different calcium aluminate cements. The SRMs are used by the industry primarily for production quality control in order to meet manufacturing specifications. Industry laboratories must demonstrate their competence to perform American Society for Testing and Materials (ASTM) Standard Test Methods, which require high levels of accuracy and repeatability, better than 1% relative for major components. Standards are also used for research and development related to strength, stability and durability of new products, and associated environmental concerns. As the variety of cement products grows, standards must cover a wide range of composition. One of the new SRMs contain slag and fly ash and addresses some of the environmental issues where cement production is viewed as an avenue for productive use of waste materials.

Standards for the Automotive Industry

By 1922 work in progress at NBS included research on automobile engines to find ways to increase their operating efficiency, ... of power losses in automobile tires, *and reclamation of used lubrication oil.*

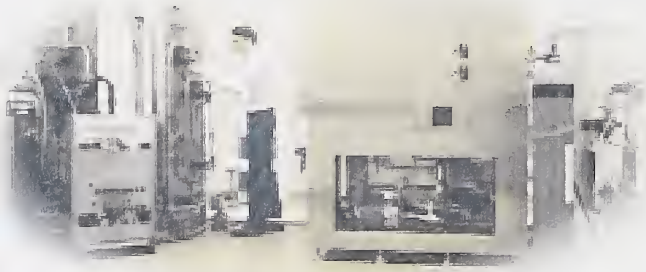
Automobile pictured to the right was used in the Bureau's famous study of the reaction times of drivers. (*History of NBS*)



Today, the **U.S. lubricants industry**, estimated to be worth $5 billion with a projected growth of about 5 % per year, has identified a standard certified for additive elements in passenger car motor oil as a top priority. The

new NIST *Lubricant Additive Package* SRM provides traceable standards for testing engine wear, and is needed for linking measurements made on finished products to national or international standards for compliance with ISO 9000 and QS 9000 requirements.

The U.S. automotive industry's long-term goal is to develop affordable mid-sized cars that are environmentally friendly and will travel the equivalent of 80 miles per gallon of gasoline. A NIST/Industry

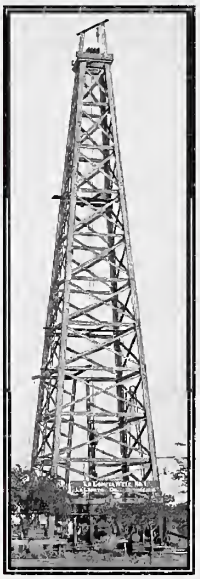


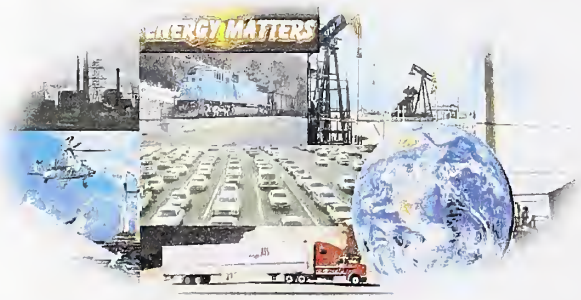
collaboration resulted in the production of NIST's low concentration gas standards that allow verification of next generation vehicle emission. In addition, the NIST High Temperature Gas Flow Calibration Facility (pictured above) provides U.S. industry with a unique capability to test and evaluate flow meters used in emissions testing under realistic usage conditions of variable gas composition, temperature, and flow.

Between 1920 and 1930, the number of cars on the road rose from 9 to 26.5 million. Even at that time, it was believed that "the nation's supply of gasoline and oil must be conserved." Depletion of this country's known resources were said to be as little as 10 years away. The need for conservation, as well as improvement of the quality of available gasoline was unquestioned.

During the 1920's, in cooperation with the American Petroleum Institute, NBS issued a series of papers establishing the characteristics of efficient motor engines, fuels, and oils.
(History of NBS)

Fuel Standards: An economic impact of **Standard Reference Materials for *Sulfur in Fossil Fuels*** was completed in February 2000. The sulfur content of fossil fuels is one of the most important intrinsic factors that determine fuel prices. The accurate determination of the sulfur concentration in fossil fuels is required as a result of environmental regulation that places increasingly lower limits on their sulfur content and the imposition of large fines for non-compliance.



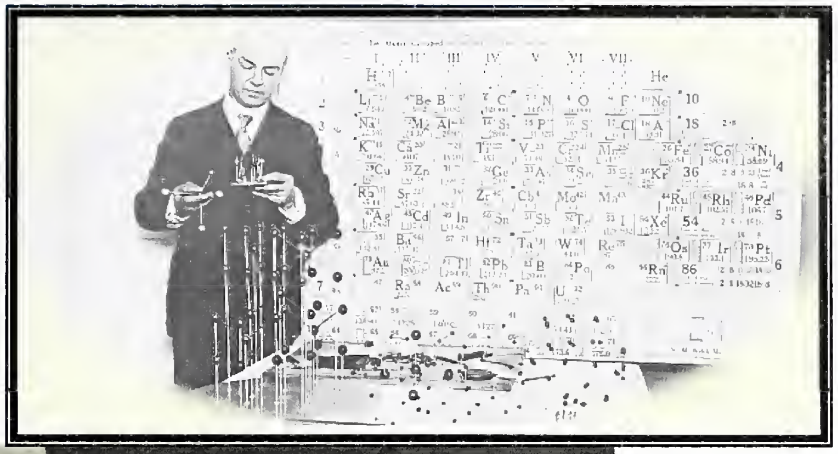


This impact study quantifies a portion of the economic benefits associated with these SRMs beginning in 1984, and projected through 2003. Surveyed industry representatives indicated that NIST SRMs have decreased the level of uncertainty associated with their measurements of sulfur content. This reduction has led to economic benefits throughout the supply chain. Included in the measures of economic benefits are improvements in product quality, production efficiency, and reductions in transaction costs and sulfur emissions to the environment. This study estimates a benefit-to-cost ratio of 113, and a social rate of return of 1,056 %. The Net Present Value was calculated to be more than $400 billion.

Technical Excellence:
The staff is the institute's greatest asset both past and present

Right: Henry D. Hubbard, the designer of the "Chart of the Atoms," was the first secretary of the National Bureau of Standards.

Pictured below – seminar on the Gaithersburg Site in the late 1960's



Pictured in the collage to the right are some of CSTL's new hires, Post-Doctoral and Graduate Fellows

... the hope and promise for the future ...

In conclusion ... thanks to you
our customers and colleagues
collaborators, associates, and friends
for 100 years of stimulating technical interactions
and looking forward to continued partnerships in the 21st century and beyond...

THE CSTL DIVISIONS

CSTL's laboratory activities are primarily located at the NIST headquarters site in Gaithersburg, MD. We also have research activities in NIST laboratories in Boulder, CO, and at the Center for Advanced Research in Biotechnology (CARB) in Rockville, MD. CARB is a collaborative effort of NIST, the University of Maryland, and Montgomery County, MD.

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- *Protein structure determination, properties, and modeling*
- *Biomaterials, biosensors, and bioelectronics*
- *Biocatalysis, bioprocessing measurements, and separations technologies*

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  - *Micro-machined gas sensor arrays*
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  - *Thin-film thermometry devices*
  - *Pressure, vacuum, and flow sensors*
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- *Engineering models and measurements for control of thermal systems and industrial processes*
- *Multiphase chemical reaction engineering*

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- *Particle characterization and standards*
- *Electronic and advanced materials characterization*
- *Chemical measurement process*
- *Surface and interface chemistry*
- *Advanced isotope metrology*
- *Molecular scale chemical characterization of organic and biomolecules*

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- *Properties of energy-related fluids*
- *Environmental fates of industrial chemicals*
- *Tools for chemical analysis*
- *Fundamental studies of fluids*
- *Cryogenic technologies*
- *Computational chemistry*
- *Basic measurements and standards*

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  - *Classical analytical methods*
  - *Gas metrology*
  - *Laboratory automation technology*
  - *Nuclear analytical methods*
  - *Organic analytical methods*
  - *Spectrochemical measurement methods*
- *Tools for achieving international comparability of chemical measurements*
  - *NIST Primary Reference Materials (SRMs)*
  - *NIST Traceable Reference Materials (NTRMs)*
  - *Interlaboratory proficiency testing programs*
  - *International intercomparisons of measurement methods and standards*
- *Critical analytical data and specialty analyses*
  - *Quantitative FTIR database for open path sensing applications*
  - *Aqueous solubility and Henry's Law constants database for environmentally relevant compounds*
  - *Analysis/Chemical characterization of high priority samples*

Visit the CSTL Website at:
http://www.cstl.nist.gov/

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