Development of Reference Methods and Reference Materials for Clinical Diagnostic Markers

NIST has a strong commitment to promoting accuracy in health-related measurements and providing measurement traceability to the U.S. in vitro diagnostic (IVD) industry through development of reference methods and SRMs. With issuance of the in vitro diagnostic (IVD) device directive by the European Union, it has become even more critical for NIST to develop new reference methods and Standard Reference Materials (SRMs) to provide traceability for the US IVD industry so that this industry can maintain its strong position in European markets.

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NIST is adding several new SRMs to its available clinical standards suite. SRM 1955 Homocysteine and Folate in Human Serum is now available to the clinical laboratory community. Homocysteine is a risk factor for heart disease, and folate counteracts effects of homocysteine. This SRM is a three-level frozen serum material with certified concentrations for homocysteine, a risk factor for cardiovascular disease and other conditions associated with oxidative stress, and for 5-methyltetrahydrofolic acid, the principal form of folic acid in blood and an important antioxidant. Folic acid has been shown to reduce the risk of neural tube defects in fetuses and is believed to counter the effects of homocysteine. This work is a collaborative effort between NIST and the Centers for Disease Control and Prevention (CDC), both of which provided measurements for certification.

NIST has completed development of reference methods and Standard Reference Materials (SRMs) to support accuracy and traceability for hormone assays. New methods, based on liquid chromatography/mass spectrometry (LC/MS), have been developed for estradiol-17β [1], adding to the suite of reference methods previously developed for other hormones including cortisol [2], thyroxine (T4) [3], and triiodothyronine (T3) [4]. Method development is underway for progesterone and testosterone. These methods will be applied to the certification of a new hormones-in-human-serum reference material (SRM 971).

The incidence of kidney disease is rising rapidly in the U.S. Early detection of kidney disease and treatment can prevent kidney failure, but early detection depends on better measurements of kidney function than are currently available. Serum creatinine is the preferred measurement, but existing methods provide varying results, so NIST is developing a new reference material, SRM 967 Creatinine in Frozen Human Serum, to address this measurement problem. As part of this work, NIST has developed a new, rapid isotope dilution liquid chromatography/mass spectrometry (ID-LC/MS) method for serum creatinine to replace the tedious isotope dilution gas chromatography/mass spectrometry (ID-GC/MS) method used previously at NIST.

To address needs in healthcare and public safety, CDC has established a network of laboratories to monitor arsenic poisoning throughout the US by measuring the arsenic species in urine. To ensure accuracy of the measurements, CDC collaborated with NIST and sponsored the development of a series of reference materials of arsenic species in urine for use by CDC network laboratories throughout the US. The feasibility of producing and certifying such reference materials was assessed.

These new reference methods and the associated SRMs will provide critical traceability to the IVD industry and will help improve the reliability of routine clinical measurements. Better clinical measurements lead to better diagnoses, enabling earlier and more cost-effective treatments.

Recent state-of-the-art measurements of cadmium, mercury, and methylmercury in SRM 966, a bovine whole blood material certified for toxic metals, were used to upgrade the certification status of the material. This work considerably enhances the quality of the SRM and its usefulness to the clinical measurement community, especially for speciated mercury measurements. The updated Certificate of Analysis now has certified values for lead, cadmium, and total mercury in both Levels 1 and 2, and reference values for inorganic mercury and methylmercury in Level 2. Prior to this work, methylmercury was only listed on the Certificate as an information value.

Metabolomics is the study of products of metabolism and is a rapidly growing field. NIH asked NIST to develop a human plasma-based SRM to help assure the quality of data from NIH-sponsored laboratories that are performing research in metabolomics. More than 30 analytes will be measured in this new SRM, using reference methods developed at NIST.

Research is continuing on development of a reference method for another risk factor for heart disease, C-reactive protein (CRP). For quantitative measurements of proteins, one of the most promising approaches is to break the protein down into specific peptides and measure the concentration of the peptides. This proteomics-based approach relies on quantitative digestion of the proteins with enzymes such as trypsin. However, this digestion is often incomplete. Research at NIST is focusing on understanding this process and making it more quantitative. Research is continuing on the quantitative potential of matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry for biomolecules using this proteomics approach.

Future Plans: Work will be completed in FY06 for the new human serum-based SRMs for hormones and creatinine. A material will be acquired and measurements will begin for the metabolomics SRM. Work will continue on new approaches for quantification of biomolecules, including peptides, proteins, hormones, and species containing inorganic elements.