"Measurement Science and Measurement Standards to Support Innovation in Healthcare"

June, 2009

Contacts:
Michael D. Amos, Ph.D.
Biosciences Advisor to the Director, Chemical Science and Technology Laboratory
National Institute of Standards and Technology
U.S. Department of Commerce
Gaithersburg, MD, USA
e-mail contact: mamos@nist.gov
Phone: 301-975-8631

Or

Willie E. May
Director, Chemical Science and Technology Laboratory
National Institute of Standards and Technology
U.S. Department of Commerce
Gaithersburg, MD, 20899
e-mail contact: willie.may@nist.gov
Phone: 301-975-8300

The Purpose of this Document

This document presents a collection of authenticated unmet and/or under-met measurement needs that represent barriers to innovation and/or sound healthcare decision-making. These healthcare measurement needs have been systematically gathered from interactions with stakeholders from industry, non-profits, academia, other government agencies, and other global organizations. The measurement standards and technology needs identified in this document are intended to guide and inform research and measurement service delivery activities of the global measurement and standard community.

Introduction

Healthcare reform is a major issue in our world today. The rising cost of healthcare and increased prevalence of chronic diseases is having a devastating affect of economic security and quality of life in all parts of the world. Major efforts are underway to reform healthcare and reduce spending through increased efficiency and quality, focusing on prevention of disease and creating a healthier population. Improvement in the quality of healthcare measurements is a key to achieving these goals.

NIST, in cooperation with other U.S. government agencies, national measurement institutes (NMIs) from around the world and the Bureau International des Poids et Mesures (BIPM) in Paris has conducted extensive outreach efforts over the last 5 years to compile a list of measurement barriers impeding innovation in healthcare. The results of these efforts indicate that major improvements are needed in the measurement science and measurement technologies that support efforts to predict, diagnose and manage disease, as well as for those used to discover and develop safe and effective medical therapies. The lack of adequate standards to ensure accurate and comparable measurements for *in vitro* diagnostic and medical imaging biomarkers, predictive toxicology for drug safety, medical device materials biocompatibility, and genetic testing is having a staggering affect on healthcare innovation and contributing to the economic burden associated with the rising cost of healthcare. Stakeholders indicate that a lack of measurement standards and technologies are a large contributor to the dilemma that despite spending trillions of dollars on new drug development and biomarker discovery over the last 15 years, the number of new drugs approved by the U.S. Food and Drug Administration (FDA) has dropped dramatically and fewer than 20 new serum protein biomarkers have achieved FDA clearance/approval since 1995. All the while, the incidence and prevalence of the most expensive-to-treat chronic diseases (constituting over 80% of U.S. healthcare spending) continues to rise, along with their associated increased burden on the healthcare economy and quality of life.

Biomedical measurement technologies are heavily relied upon by physicians for making life and death decisions. In the U.S. healthcare system, 70% of medical decisions are based upon results from tests performed in a medical laboratory. Yet, the appropriate measurement infrastructure is sorely lacking. Only about 10% of the ~700 most-often-performed laboratory tests have internationally-recognized reference methods underpinning their performance. This situation is compounded by the fact that emerging biomedical measurement technologies are becoming increasingly complicated, and will require an evermore robust measurement infrastructure.

Potential Impact of Measurement Science and Measurement Standards on Efforts to Improve the Quality and Efficiency of Healthcare

In the U.S., it is a stated goal of the new Obama Administration to improve the quality of our healthcare while lowering its cost by computerizing all of Americans' medical records. In so doing, "this will cut waste, eliminate red tape, and reduce the need to repeat expensive medical tests …. it will save lives by reducing the deadly but preventable medical errors that pervade our health care system". While it is clear that modernizing information management will have a positive economic impact on healthcare, the full benefits of the Administration's planned efforts are not likely be fully-realized unless the patient's health-related data that is entered into the electronic records and used to make medical decisions are accurate and comparable over both space and time.

The figure below illustrates the conundrum that results when considering information that goes into the electronic health record.

Key Problems

- Patients and doctors expect test results to be accurate and comparable and interpreted in a reliable and consistent manner - ***but neither is true***
- Patients and doctors move around; results therefore need to be transferable between institutions - ***but measurement procedures give different results and reference ranges do not always take this into account***
- the electronic health record requires long term comparability of results over the lifetime of the patient - ***having to specify laboratory, instrument, reagent and calibrator for each investigation is unacceptable***
- knowledge of analytical validity, and metrological principles of traceability and calibration amongst laboratory professionals is inadequate - ***assay limitations are not appreciated***

Dr. Jonathan Middle
Deputy Director, Birmingham Quality (UK NEQAS)

The confidence levels required for sound healthcare decision-making can only be achieved if the appropriate measurement infrastructure is in place. Measurement infrastructure includes all of the underpinning reference materials, reference methods, measurement calibration, measurement validation, other measurement services, and appropriate technologies (physical, chemical and computational) required to ensure measurement accuracy and comparability over space and time. This infrastructure is necessary to lend confidence in the measurements that are performed within the global healthcare community which is comprised of industry, academia and government agencies

Measurement Science will Play an Increasingly Important Role in Future Personalized Medicine Initiatives

New efforts are underway to better understand the molecular basis of diseases that will result in diagnosis and treatment at earlier stages before the most deleterious effects are manifest. Although there have been many advances in recent years in medical technologies, it is not yet feasible to obtain reliable diagnosis of most diseases at the earliest stages before clinical symptoms appear. The dream of predictive, preventative and early interventional medicine will only be accomplished through development and deployment of new measurement technologies that enable a much deeper and broader understanding of the molecular processes responsible for health, disease and reactions to drugs and environmental insults. The advent of innovative new measurement

technologies will complement the new administration's healthcare priorities and contribute to economic security by creating biomedical technology companies and creating new jobs. There are, however, significant barriers that need to be overcome for these benefits to accrue.

Some general conclusions regarding the measurement barriers impeding healthcare innovation and the critical role measurement standards and measurement technologies play can be drawn from the NIST-led outreach efforts. Stakeholders agree that:

- Improvements in healthcare is a key to global economic security and quality of life in the future
- A much deeper understanding of complex human biological systems is needed
- Highly sophisticated measurements are needed in order to study the relevant changes that occur in complex biomolecular networks in health and disease
- Major measurement barriers exist that are stifling innovation
- Current technology is largely unreliable due to a very limited measurement infrastructure that, unfortunately, allows scientists and physicians to have confidence in only a very small percentage of the biomeasurements they conduct
- New multiplex, multiparametric measurement technologies will need to be invented and developed
- The new measurement systems will heavily rely upon the accuracy and comparability of the data obtained from current technologies as the basis upon which to build the new measurement systems
- Standardization of current measurement technologies is therefore needed to enable next-generation systems
- Standardization of next-generation biomeasurement systems that bridge historical and new data will be needed as new methods emerge.

To summarize, greater reliability of existing healthcare measurement systems is needed to support the effective utilization and innovation of new technologies based upon never-before-achieved multiparametric analytical capabilities. These new complex measurement methods will be needed to support personalized approaches to predicting and preventing chronic diseases and helping to stem the rising cost of healthcare. However, without the underpinning science and standards to improve existing and enable development of next-generation measurements, the rate of innovation will continue to slow. Physicians and medical scientists and engineers must have available the measurement technology and measurement standards infrastructure to provide the sound scientific basis for the confidence they need in the measurements performed on human systems to which they so-heavily rely upon to support sound decision-making.

The Critical Role of the Measurement Community in the Drive for Accelerated Innovation in Healthcare

The international metrology community is responsible for establishing and maintaining the infrastructure to provide confidence in the measurements. Patients and clinicians base critical medical and lifestyle decisions on diagnostic tests. Medical research

professionals need confidence in the data gathered from measurements made with the ever-increasing variety of tools used for discovery purposes as well as in clinical safety and efficacy determinations of new therapeutics. The inadequacy of current measurement infrastructure is a possible reason that, despite the apparent technological revolution that is occurring in medical research, there are decreasing numbers of new drugs and biomarker tests reaching market.

It is the role of the global metrology community to address those measurement barriers to innovation in healthcare that represent the highest risk to economic security and quality of life. *The NMIs and Designated Institutions serve as the ultimate reference point for measurements, standards, and technology research to support industry, science, health, safety, and defense.* At least for the U.S., ***it is congruent with the NIST mission to provide the measurement infrastructure that supports innovation.***

The global measurement community cooperates to leverage its collective expertise in the physical, bio/chemical and information sciences to maintain the references (methods, materials and data) and calibration capabilities for providing confidence in the results from biomedical measurements. Increased those efforts will enable and facilitate realization of optimal economic and societal benefits from new innovations in healthcare delivery.

Developing solutions to the measurement needs identified in this document will have a positive impact on more than just healthcare. Many of the same measurement issues in other critical areas of biological (such as the need to measure complex biological signals, the lack of consistency of data over space and time, etc) have been identified as being relevant to other areas of critical importance to economic security and quality of life, such as: energy, environment, manufacturing, national security and defense, and food and agriculture.

The following describes at a high level, the programs needed to address the barriers to innovation identified by NIST and others. It is not intended to be a comprehensive list but, instead is a reasonable starting point upon which to begin building international cooperation to address the identified measurement needs. The compilers of the roadmap would like additional feedback on its content and therefore, welcome suggestions for improvement.

The Roadmap to Addressing Measurement Barriers to Innovation in Healthcare

This document describes the measurement barriers in two sections: those relevant to current healthcare measurements and those critical to enable innovation of next-generation healthcare measurement capabilities. **NOTE: The following sections pertain to physical and chemical measurement (PCM) infrastructure only. The work described in both SECTION I and SECTION II require considerable computational biology support, much of it crosscuts the 2 sections and constitute approximately $50M of the $260M roadmap. The details of this critical component are found in Appendix A.**

Section I - Standards and Technology for Increased Quality in Current Generation Biomedical Measurements for Diagnostics And Therapeutics – (~$120M = $110M PCM + $10M IT) – Numerous *in vitro* diagnostic, medical imaging and therapeutic products are currently on the market for which little or no measurement infrastructure exists to help industry, regulators, health care providers and patients have confidence that the products will perform as intended. Discussions with stakeholders make it clear that the metrology and standards community should do more in this area. Measurement infrastructure is needed to enable better control over the quality and safety of healthcare products and services.

Section II – Standards to Support Next Generation Healthcare Measurements – *Tools to support discovery and utilization of "Disease Signatures"* (~$140M = $100M PCM + $40M IT) – To combat the rising cost of healthcare, new technologies will be developed to enable the detection of disease signature biomarkers for the earliest stages of the most costly-to-treat and devastating diseases (preventable chronic diseases constitute over 80% of our healthcare expenditures). Because of the highly complex nature of the disease signature biomarkers, these new technologies will require much greater accuracy, sensitivity and specificity than ever before. The metrology and standards community need to provide the measurement infrastructure to industry, regulators and standards development organizations to enable accelerated development of new revolutionary technologies for detection of the anatomical features and the multitude of proteins, nucleic acids and metabolites that make up disease signatures. By providing tools to ensure the accuracy of newly-developed biochemical and anatomical measurement systems, industry could have greater confidence in the measurements they use to develop new technologies. In turn, this effort would help accelerate the development of more sophisticated clinical assessment tools, thus helping the Nation to more quickly realize the benefits of personalized medicine.

Definition of Disease Signature - *For the purposes of this document – "Disease Signature" is defined as the biomolecular profiles and anatomical features indicative of the onset and progression of disease. These features are determined by analysis of integrated biochemical and anatomical measurements Disease signatures are specifically distinctive or characteristic of a disease or pathologic condition. It is a portion of the information that goes into describing a person's phenotype.*

Section I –
Standards and Technology for Increased Quality in Current Generation Biomedical Measurements for Diagnostics and Therapeutics

A. Diagnostics

Programmatic Areas

1. **Standards to Support Laboratory Medicine - C**urrent laboratory medicine *in vitro* diagnostic (IVD) technology suffers from a lack of attention to standards. Organizations such as the U. S. Food and Drug Administration (FDA), United States Pharmacopoeia (USP), the National Institutes of Health (NIH) – most notably the National Cancer Institute (NCI) and National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), U.S. Centers for Disease Control (CDC), College of American Pathologists (CAP), American Association for Clinical Chemistry (AACC) and numerous IVD manufacturers have articulated the need for additional measurement infrastructure to support laboratory medicine. NIST and other agencies around the world have provided support to this vital area, but considerably more effort is needed. Areas of need include:

   - **Higher Order Reference Methods and Certified Reference Materials for Clinical Analytes -** The IVD manufacturing, medical professional, and medical laboratory accreditation communities have requested that National Measurement Institutes (NMIs) around the world develop and conduct formal intercomparisons of additional Higher Order Reference Measurement Procedures and accompanying Certified Reference Materials. Priorities for this work are established in consultation with the American Association of Clinical Chemistry, the International Federation for Clinical Chemistry (IFCC) and the International Bureau of Weights and Measures (BIPM), and the International Laboratory Accreditation Cooperation (ILAC) Joint Committee on Traceability in Laboratory Medicine (JCTLM). Additional Measurement science, Reference Methods, Certified Reference Materials and technology and measurement services are needed in the following areas:

   - **Non-Peptide Hormones –** several clinically significant hormone comprise this category for which there are no standards – these include; serotonin, melatonin, dopamine and leukotrienes.

   - **Serum Proteins** – those single analytes with known clinical utility for medical decision-making – these include; C-reactive protein, HER-2-nu, protein hormones, infectious disease-associated antibodies, autoantibodies, metalloproteins. Important parameters include identity,

quantity, post-translational modification, sample integrity, standardized capture reagents, improving efficiency of probe generation, etc.

- **Nucleic acids** – important parameters include ribonucleic acids (RNA) and deoxyribonucleic acids (DNA) virus copy number, microRNA identity and quantity, fidelity of DNA amplification, low abundant genomes.

General measurement challenges and issues include; sample integrity and quality, information technology (IT)-based tools for data interpretation, annotation of results for the health record, security of genetic sequence data, and signal/noise.

Specific Projects

a. **Tools to Enable More Direct Measurement of Clinical Analytes using Alternative Technologies -** More laboratory protein analyte measurements are currently performed using immunoassays. There are numerous potential measurement biases associated with such. The introduction of more direct physical measurement methods could help alleviate many of the reliability problems with measuring proteins. Measurement infrastructure is needed to enable introduction of mass spectroscopy into clinical practice.

b. **Tools to Improve Pre-analytical Sample Quality** The integrity of the blood samples used for laboratory medicine needs to be assured. Factors affecting quality of samples include collection method, storage and utilization procedures. Measurement infrastructure is needed to insure the analyte in the sample being measured in an assay is present at the same concentration in the patient. These include standards such as integrity molecular markers for blood serum/plasma samples.

c. **Standardized Assay Data Formats And Statistics** - The statistical methods used for setting the point in an assay where a reading above the line is positive (called the "cut-off"), is set by the manufacturer. Different manufacturers use different methods to set the cut-off and some have established a quantitative data set point that calls a measurement "low-positive" and "high positive". Standards are needed for establishing the positive/negative/equivocal cutoff for any assay, standard operating procedures and standard reference materials for each assay according to the analyte measured. This would allow conversion between the readout scales in the different assay platforms and the ability to compare results across manufacturers. The availability of these procedures would allow evaluation of performance metrics for each assay platform.

d. **Standard Procedures For Assessing the Correlation Between Single Analyte Assays, Multiparameter Assays and Disease Prediction -** Development of robust statistical methods that allow the experimental

design of a clinical trial to assess assay performance - would allow evaluation of assay reproducibility and performance of the single parameter and multiparameter assay to predict disease outcome. Proper design of experiments would take into account the patient-to-patient variabilities and would provide metrics for determining how effective a particular assay is at predicting disease outcome. Research into the most appropriate statistical procedures required for such multiplex assays will be needed.

e. **Standard Assay Formats** The term "gold standard" is used in IVD circles to indicate the assay against which all others should be compared. To date, few "gold standard" assays have been established for assays in a manner involving rigorous scientific evaluation by standards authority. This must be done with due respect to the clinical relevance of the measurand being studied and the intrinsic patient-to-patient variability that may contribute inconsistencies in testing. The work being performed in collaboration with domestic and international collaborators such as JCTLM and CCQM needs to be expanded.

f. **Tools to Standardize Signal Transduction in Immunoassays** Immunoassays involve use of binding agents (monoclonal or polyclonal antibodies or autoantigens) that must be modified by coupling to another molecule that enable a signal to be generated. This can be an enzyme of a fluorophor. Such modification introduces measurement biases and must be controlled. Signal development and transduction standards are needed for the enzyme and fluorescent tags used in immunoassays. Sophisticated image analysis will be used to analyze the patterns of light generated in the assays resulting from the measurement of various levels of the selected proteins in cells and fluids.

g. **Develop Better Technologies to Minimize Assay Problems Caused by Interfering Substances** - Laboratory medicine assays are subject to erroneous results due sometimes to interfering substances in serum. These include rheumatoid factor, immune complexes, bilirubin, cholesterol, etc. and may or may not be related to the patients' diseases. Standards are needed to enable better measurements in the presence of interfering substances.

h. **Methods and Standards for Measuring Cell Volume and Size -** Companies performing analysis of human red blood cells using flow cytometry need the ability to obtain reliable measurement of red blood cell size and volume.

i. **Measurement Science for Autoantibody Determination -** Autoantibody testing for autoimmune disease is widely known for the variability of measurement that exists in commercial autoantibody assays. Even on standard sera developed by the Association of Medical Laboratory Immunologists (AMLI), results on samples tested in assays from different manufacturers don't always agree. These inconsistencies can be addressed through:

- Development of high-quality standard reference materials (reference samples and capture antigens)
- Development of measurement tools to study autoantibody fine specificity

Input for developing the items in this section was provided through:

- **Workshop with The U.S. Food and Drug Administration – Critical Path Committee** November 2007 – January, 2008
- **Joint Committee for Traceability in Laboratory Medicine (JCTLM) Industry Stakeholder's Workshop** - July 26, 2008, Washington, DC
- **Meeting with Mayo Clinic** - October 2007
- **Improved Antibody-Based Metrology in Flow Cytometry,** February 23, 2006, Gaithersburg, MD
- **Developing New Standards for Autoantibody Measurement; Bringing Metrology to Serology**, February 21-22, 2006, Gaithersburg, MD
- **Strategy for Health Care through Bio and Information Standards and Technologies,** September 24 – 25, 2007, Co-sponsored by NIST and Biotechnology Council of IEEE, ASME, BMES, HIMSS, SBE/AIChE
- **NIST conference "Accelerating Innovation in 21st Century Biosciences: Identifying the Measurement Standards and Technological Challenges"** - October 19-22, 2008
- **Abbott Diagnostics** - May – July, 2008
- **USMS Measurement Needs Documents**
  - Serum-Based Proteomics for Early Cancer Detection
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100320
  - Standards for Autoantibody Assays in Autoimmune Disease (AD) and Cancer
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100584
  - Measurement Standards Supporting *In Vitro* Diagnostic Device (IVDDs) Usage
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100596
  - Advanced Measurements for Auto-Immune Diseases and Cancer Detection
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100622
  - Measurements and Standards for Genetic Testing
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100319
  - Quality Control in Cytometry for Improved Clinical Diagnostics
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100491
  - Diagnostic Flow Cytometry
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100602

2. **Standards to Support Medical Imaging –** Considerable outreach has been conducted to determine the needs of the medical imaging industry.

Workshops conducted with FDA, NCI and the Radiological Society of North America (RSNA), Council on Ionizing Radiation Measurements and Standards (CIRMS). Preliminary work at NIST has set the stage (and raised community expectations) to establish a coordinated program aimed at 1) providing national standards for all the major diagnostic imaging methods being used clinically, and 2) supporting industrial and medical researchers in developing new and better medical imaging instruments and methods. The expanded program in quantitative medical imaging will address the following:

- Standards and measurement quality assurance processes for medical diagnostic imaging modalities
- Standard materials and methods for radiation therapy – methodologies for quantifying the uptake of radioactive tracers for diagnosing disease;
- New standards for quantitative nuclear medicine imaging
- Optical measurement standards to quantify appearance factors, in particular color and texture.
- Bone density measurement standards - well-defined x-ray phantom, traceable to standard measurements and the International System of Units
- Standards for molecular imaging (MI) – standards to support the further development and application of technologies for visualization of combined biochemical and anatomical features.

Specific Projects

a. **Quantitative Imaging Standards -** Standards and measurement quality assurance processes will be developed, in collaboration with the medical community, to enable quantitative medical imaging for the modalities most often used in medical diagnostics, including PET-CT, SPECT, Spiral CT, and MRI; in out-years, methods will be extended to include standards for new diagnostic instruments and techniques being developed by the U.S. health care instrumentation industry and medical researchers.

b. **Standards for Improved Dosing in Nuclear Medical Imaging** - Standardized methodologies for quantifying the uptake of radioactive tracers for diagnosing disease. Current ways of calculating so-called Standardized Uptake Values (SUV) are inadequate for making consistent clinical decisions because of the lack of calibration standards, inconsistent data collection and analysis protocols, and insufficient data to link observed uptake to clinical outcome.

c. **Medical Imaging Phantom Development -** Building on the successes of previous phantom development projects, a dual-modality PET-MRI phantom will be designed and developed to provide the necessary physical quantities to quantify medical images

in terms of spatial dimension, MRI contrast, and positron emission intensity.

d. **Standards for Quantitative Nuclear Medical Imaging -** New standards for quantitative nuclear medicine imaging need to be developed, including the development of 1) well-calibrated radioactive source standards that can be used to obtain accurate radioactivity distributions, providing a key benchmark for analysis of patient image data, and 2) standardized image databases for comparing internal dose models.

e. **Standards for *In vivo* and *In vitro* Optical Imaging -** Optical measurement standards need to be developed to quantify appearance factors, in particular color and texture. Optical imaging measurement science must be advanced to allow improved contrast, biopsy selection, and diagnosis in endoscopic and laparoscopic procedures in collaboration with medical researchers and industrial associations.

f. **Standards to Enable New Subsurface Imaging Techniques** - Near infrared tissue-equivalent artifact and electronic phantoms and radiation transport models need to be developed to help evaluate and advance new methods being developed to diagnose breast cancer and other diseases where tissue transparency allows for subsurface, non-invasive optical measurements.

g. **Standards to Improve Quantitative Analysis of Bone Loss -** For bone density measurements an absolute, well-defined x-ray phantom, traceable to standard measurements and units, need to be developed to provide a confident means of calibrating a variety of instruments and systems all back to a common point and reduce variability among instruments due to system-specific technical and engineering issues.

h. **Interoperability Standards for Medical Imaging Data Transmission -** Standards and methods for intercomparability of clinical imaging data need to be pursued to support improved response analysis, e.g., "change analysis" to determine treatment efficacy.

i. **Standards for Molecular Imaging** - Measurement infrastructure is needed to enable development and optimization of tools for discovery, characterization and measurement of biologic processes in preclinical models and clinical investigations at the molecular, cellular, molecular and organ spatial scales using both *in vitro* (laboratory) and *in vivo* imaging methods. Development of physical reference materials and validated computational software to enable researchers to optimize the performance of the imaging platforms (sensitivity and specificity) and to permit normalization of image data, thus allowing data sharing of both cellular and medical imaging, to meet the specific requirements for risk assessment, early detection, differential diagnosis, and image guided intervention of

disease. Support is needed for development of standardized methods to integrate imaging data with genomic and proteomic data and development of nanoscale carriers, such as semiconductor quantum dots and colloidal gold nanoparticles, that can transport molecular probes for detection, diagnosis and image and localized treatment will be developed and characterized for a range of imaging modalities (PET CT, MRI, US, and optical).

Input for developing the items in this section was provided through:

- **Workshop on Design and Performance Validation of Phantoms Used in Conjunction with Optical Measurements of Tissues** January 19-20, 2008, , San Diego, CA
- **Workshop with The National Cancer Institute (NCI) Center for Cancer Research (CCR)** 2007 – 2008
- **Workshop with The U.S. Food and Drug Administration – Critical Path Committee -** November 2007 – January, 2008
- **Standards for Bone Imaging and Bone Mineral Density Measurements** - February 4, 2006, International Society of Clinical Densitometry, San Diego, CA
- **Imaging as a Biomarker: Standards for Change Measurements in Therapy -** September 14-15, 2006, NIST, Gaithersburg, MD
- **USMS Measurement Needs Documents**
  - Remote image-based medical diagnostic tools
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100377
  - Minimally invasive *in vivo* optical tissue diagnostics
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100436
  - Next Generation High Resolution Magnetic Resonance Imaging (MRI) Systems
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100443
  - Nanomagnetic MRI Contrast Agents
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100462
  - Polarized 3He gas for advanced medical imaging
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100464
  - Biomedical Imaging as a biomarker for Change Measurement in Therapy
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100481
  - Room-Temperature Magneto-Cardiography
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100494
  - Quantitative Optical Medical Imaging
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100568
  - Medical imagery as a biomarker for disease extent and change measurement
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100576
  - Radio-diagnostic Molecular Imaging (Positron Emission Tomography - PET)
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100563

- Next Generation Dual Energy X-Ray Absorptiometry (DXA) Systems
  http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100441

3. **Standards to Support Molecular Pathology -** Clinical histopathology determinations from which therapeutic regimens are prescribed, are inherently subjective. This leads to increased chance of unnecessary or ineffective treatment and significant variability in clinical outcomes, including mortality. Organizations such as the CAP, Armed Forces Institute of Pathology and NCI have articulated that new enabling technologies are needed in order to help move pathology away from paraffin embedding to examination of fine needle aspirate biopsies and even examination of live cells from biopsies. Measurement challenges include: sample integrity, signal development, and signal interpretation.

Input for developing the items in this section was provided through:

- **Workshop with The Armed Forces Institute of Pathology -** February, 2008

B. Drug Therapeutics (Pharmaceuticals and Biopharmaceuticals)

Programmatic Areas

1. **Standards and Technology for Biopharmaceutical Manufacturing -** Biotechnology drugs, protein and cell-based medications, represent the fastest growing category of healthcare spending in the United States. Yet, there is very little measurement infrastructure in place to enable new innovations in this field. Uncontrollable and unpredictable variability in the manufacturing process have a profound effect on the quality and safety profile of final protein biopharmaceutical products. The biopharmaceutical industry wastes in excess of $15 billion per year due to inefficient manufacturing. Slight changes in the manufacturing process can cause unintentional biochemical and structural changes to the final product that affect safety and efficacy.

   - Minor changes in a manufacturing process that go undetected can have profound effects on product quality, causing it to fail QC. Even worse, the defective product could inappropriately pass QC tests that fail to detect the product flaw, and then be released into the market where it could harm patients and result in devastating regulatory sanctions and corporate financial liability. A primary reason for these problems is a lack of measurement tools to enable a rigorous

scientific understanding of both the biomanufacturing process and biological products.

Outreach activities have identified measurement needs for protein therapeutics: aggregation, post-translational modification, 3-dimensional structure, product contamination (host cell materials, viruses, endotoxin, etc.) and stability of the final product.

Specific Projects

a. **Tools to Measure Adulterants in Manufactured Proteins Tools for Host Protein Analysis -** Major problems associated with the intentional and unintentional introduction of substances in pharmaceuticals and biologic drugs. For example, the introduction of heavy metals into vaccines may have serious health ramifications. Measurement infrastructure is needed to enable stakeholders to identify and quantitate impurities including organic and inorganic chemicals using neutron scattering, mass spect and immunoassays.
b. **Tools to Measure Physical Properties of Manufactured Proteins -** Protein products are manufactured in cells and each cell system contributes endogenous protein impurities to final product – methods are needed to enable unbiased measurement of inappropriate proteins in the production run that come from the production cell line – current methods involve raising polyclonal antibodies against cell proteins, many of which are not immunogenic, thus: no antibodies are raised against them and the ELISAs will not detect them. Generic assays that apply to diverse products and are highly sensitive. Methods for measuring protein aggregation and determine its affect on the immunogenicity of the final manufactured protein product using electrospray differential mobility analysis (ES-DMA)are needed.
c. **Measurement Tools For Production Cells & Cell Processing** - A systems-biology-based understanding of the widely used cell lines for manufacture of biological therapeutics is needed in order to improve product quality. The development of these novel multiplex measurement tools for proteins, RNA and metabolites will lead to a more fundamental understanding of bioprocessing and enable the agile, low cost manufacturing of safe and effective protein- and cell-based products.
d. **Establishment of Quality by Design (QbD) Validation Services –** A center of excellence is needed to serve as a test-bed to develop and validate new measurement tools and to standardize biological manufacturing processes. Physical standards for analysis of data coming from enzymatic mapping studies of protein products to measure process consistency. Standards to assess the degree of conformity of the data to a standard procedure run on a standard reference material. Standard analytical methods for comparing complex chromatograms.

e. **Standards for Measurement of Three-dimensional Protein Structure -** Protein products must be folded into their final 3-D structure to become functional – this is performed both in the production cell or separately in a separate process following expression (if produced in bacteria) – all protein products will have a major 3-D structure with a potential distribution of 3-D structural variants that must be understood in order to enable thorough structural evaluation that does not depend on biological assays, which may lack precision, robustness, or other important features. Measurements needed include:
   - Identification of aberrant 3-D structures (misfolding)
   - Distribution of 3-D structures of whole proteins and domains within proteins
   - Confirmation of correct (major) 3-D structure
   - Impurities profile (if possible).

f. **Standards for Measurement of Post-translational Modification of Manufactured Proteins** - Protein products are polypeptides that often undergo a set of changes to their structure for them to function properly as drugs. Measurement tools and standards are needed to enable the understanding of these modifications. This is a critical problem that cuts across all protein manufacturing. In order to evaluate similarity between FOPP and innovator products, these modifications must be fully understood and characterized. Due to the complex and heterogeneous nature of the modifications, methods are currently lacking which (i) qualitatively and quantitatively assess the structure as it relates to the intact protein and (ii) provide an understanding of the relationship of the modifications to potency and thus, clinical performance:
   - Glycosylation
   - Protein and Carbohydrate phosphorylation
   - Adduct addition.

g. **Validation of Viral Clearance Methods -** Protein products purified from human sources or manufactured using bioprocessing can contain viruses which are in part removed by a filtration step in downstream processing. Manufacturers of filters use different virus preparations to evaluate filter performance and strategies to assess the efficacy of that process need to be standardized. For example, virus preparations obtained from contract testing labs used to challenge filters need to be of better dimensionally defined and more consistent.

h. **Improved Functional Assays for Protein Therapeutics** - New tools and standards need to be developed to assess the physical and chemical mechanisms of the recognition and binding of protein biologic (PBs) to their cellular or plasma protein targets. All PBs operate by interacting with another biomolecule either in the blood plasma or on the surface of cells. In almost every case, that target is a glycoprotein. Particular attention is needed on measuring the specific interactions between PBs and integral membrane proteins, the most common target of these drugs. New tools and standards need to be developed to understand how small

molecule drugs affect protein targets and how these activities can be used to better understand protein function.

i. **Measurements to Ensure the Stability and Purity of Manufactured Proteins -** Most proteins, especially those greater that 30,000mw, are unstable when stored improperly. For example, 30% to 60% of new protein pharmaceuticals will be freeze-dried. No systematic way to stabilize these currently exists. Measurement tools and underlying theory need to be developed to provide this industry with systematic and deterministic approach to stabilizing any protein, given knowledge of its salient physical properties.

Input for developing the items in this section was provided through:

- **Development of Biologic Drugs: Scientific Issues in Assessing the Similarity of Follow-on Protein Products -** Conference cosponsored by FDA, NIST, and the New York Academy of Science - December 12-14, 2005, Brooklyn, NY.
- **Workshop with The U.S. Food and Drug Administration – Critical Path Committee** November 2007 – January, 2008
- **Industry Workshop on Biopharmaceutical Manufacturing Measurement and Standards Needs** – June 12-14, 2006, World Antibody Summit, San Mateo, CA
- **USMS Measurement Needs Documents**
  - Real Time Measurements for Pharmaceuticals and Biologics Manufacturing
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100431
  - Nuclear Magnetic Resonance Spectroscopy Tools for Drug Design
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100413

2. Standards and Methods to Support Measurements of Nanodrug Safety- Heavy investments in medical nanoscience and nanoengineering is resulting in breakthrough drugs for targeted cancer therapeutics as well as new agents to enhance medical imaging. Nanomaterials are used as "carriers" to convey drugs to their site of action (tumors, etc). Along with any new treatment, there certain risks that must be well understood. Introduction of nanomaterial to medical practice requires assessment of their quality and safety. This represents a major measurement challenge according to participant of several workshops on the subject including the NIST/FDA 2008 workshop. Measurement science, standards, technology, and measurement services are needed for determination of nanomaterial parameters such as; raw material and final product chemical composition, size, final product quality, location in biological materials, adulteration.

Specific Projects

A. Tools and Standards for Assessing Biodistribution of Nano-Particles *In vivo* There are currently no tools for real-time tracking nanoparticles once injected into a living organism. FDA

stakeholders articulated this as a need to help them understand *In vivo* toxic affects of nanoparticle drug delivery systems.

B. Model Development - *In vitro* experimental models that are good mimics of humans and foods are needed in order to predict the health affects of nanomaterials. *In vitro* cell-based models need to be developed to enable analysis of as few as one cell. Advanced cell culture platforms (multi-organ mimics) and bioinformatics tools are needed.

C. Coordination of International Studies – Studies to compare and validate existing nanotoxicology screening methods to promote adoption and acceptance of 'nano' products in the international community. This work would also support better acceptance of *in vitro* cellular measurements that can hopefully someday minimize testing on living systems as there is momentum to reduce animal testing globally.

Input for developing the items in this section was provided through:

- **Workshop on Engineered Nanoscale Materials** - September 12-13, 2007, NIST, Gaithersburg, MD
- **Workshop with The U.S. Food and Drug Administration – Critical Path Committee -** November 2007 – January, 2008
- **USMS Measurement Needs Documents**
  - Toxicology of Nano-particles in Biological Systems. http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100430
  - Health Care/Nanotechnology - Cancer Diagnosis and Treatment http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100629

3. Standards to Support Small Molecule Drug Manufacture – Updated standards and practices for drug manufacture to improve quality control and increase safety are needed. Measurement science, standards, technology, and measurement services are needed to support the work with stakeholder organizations such as the U.S. Pharmacopeia (USP) to develop the measurement infrastructure to enable safer name brand and generic pharmaceuticals.

Input for developing the items in this section was provided through:

- **USMS Measurement Needs Documents**
  - Nano-Scale Drug Delivery http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100411
  - Nuclear Magnetic Resonance Spectroscopy Tools for Drug Design http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100413
  - Advancing the Fundamental Science of Nanobiotechnological Systems http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100608

B. Non-Drug Therapeutics

Programmatic Areas

1. **Standards for Advanced Radiation Therapy -** New modalities of radiation therapy have enabled the availability of better information for use in delivery of radiation with increasingly higher conformity to tumor volumes. These new modalities require measurement standards for radiation dosimetry of narrow beams, which do not yet exist but are necessary to ensure efficacy and safety. Measurement science, standards, technology, and measurement services are needed for determination of parameters to address useful quantity, absorbed dose and calibrations of dosimeters

   Specific projects

   a. National measurement standards for HDR Ir-192 dosimetry - Iridium is the primary radiation source used in brachytherapy Standards are needed for traditional quantity air kerma and for the more directly useful quantity absorbed dose.
   b. **X-ray and proton beam standards -** National-standards-based direct calibrations of dosimeters in accelerator-produced high-energy x-ray beams and in proton beams.
   c. **Field reduction calibration standards** - Measurement and calibration methods to transfer traditional instrument calibrations in large fields to the small, narrow fields increasingly used in therapy.
   d. Electron paramagnetic resonance standards - New methods of alanine-EPR measurements and analysis, now the gold-standard in high-dose radiation-processing dosimetry, for accurate dosimetry at therapy levels, taking advantage of the small size and tissue equivalence of the passive dosimeter for the most challenging applications of small-beam modalities.
   e. Electron beam standards - New national standards for electron-beam dosimetry, used for superficial and shallow-seated tumors.
   f. **Standards for low-dose brachytherapy -** Improved national standards and measurement methods are needed for the dosimetry of radioisotope-based low-dose-rate brachytherapy sources (e.g., prostate seeds) and of electronic brachytherapy (micro x-ray tubes).

   Input for developing the items in this section was provided through:

   - **Council on Ionizing Radiation Measurements and Standards (CIRMS) meeting,** October 22 – 24, 2007, Brachytherapy Subcommittee of the AAPM meeting, July 27, 2008

   USMS Measurement Needs Documents

- Brachytherapy dosimetry
  http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100487

2. **Standards to Support Cell-Based Therapeutics** - Accurate, non-invasive assessment of cell population heterogeneity and quality will be critical to the development and clinical application of any cell- based therapeutic regimen. Lead regulators from the FDA have articulated the need for new measurement science, standards, technology, and measurement services to assess the health (viability) and function of cells used for therapeutic purposes. Needs include: cell quantitation, identity, stability, adulteration, and elucidation of metabolic processes to determine health status and location in patients following injection.

   Specific Projects

   a. **Methods to Assure Accurate Quantification of Cells –** The measurement community needs to work with the FDA and the cell therapy community to identify best practices and reliable assays that will permit unambiguous quantification of cell characteristics and markers. These enabling activities technologies will include standards for benchmarking optical imaging and flow cytometry, and validated models for interpreting data such as cell volume measurements.
   b. **Methods to Identify Terminal Differentiation Markers** - Cell therapeutics will involve the manufacture, storage and delivery of human cells to patients for therapeutic purposes. The desired therapeutic cell will express a set of characteristic proteins, functional activities, and other phenotypic parameters that indicates its fitness for therapeutic use. Measurement tools are needed to ensure the integrity of the cells intended for therapeutic implantation. Standards are needed to enable manufacturers to measure the features of therapeutic cells.
   c. **Methods to Determine Status of Key Signaling Pathways That Control the Differentiation of Therapeutic Cells or Tissues** - Future use of stem cells for therapeutic purposes depends upon the ability of the stem cell to differentiate (change) into the desired body cell type required for therapeutic purposes. It may also be critical for the stem cells to lose their "stemness" in order to avoid oncogenesis. These changes occur via complex interactions in cells known as signaling pathways or networks. Methods are needed to interrogate these networks to ensure the stem cells acquire and lose the desired attributes when injected into humans. The measurement community will work with stakeholders (FDA) to determine the appropriate standards needed to ensure accurate measurements.
   d. **Methods to Track the Fate of Injected Cells in the Body, in Terms of both Pharmacologic Disposition and Differentiation -** Once cells are injected into humans or animal models, there needs to be reliable ways to monitor where they go and what they do in real time. The

measurement community will work with stakeholders to develop methods to reliably track injected cells.

Input for developing the items in this section was provided through:

- **Workshop with The U.S. Food and Drug Administration – Critical Path Committee** November 2007 – January, 2008

3. **Standards for Regenerative Medicine (RM)** -Although the field of RM has been in existence since the 1980s, very few RM products have been commercialized. This is largely due to the inability to solve engineering and manufacturing problems. Measurement science, standards, technology, and measurement services are needed for determination of RM parameters, including: biocompatibility of tissue scaffolds, chemical and environmental influences on tissue manufacturing processes that have effects of product quality and safety.

   Specific Projects

   a. **Measurements and Standards for Tissue Scaffolds -** Successful RM products involve processed natural materials (e.g., collagen) and synthetic materials as scaffolds for cell and tissue growth. The chemical and morphological properties of these scaffolds strongly impact cell behavior, and these properties can vary significantly, depending on processing conditions. Needed are characterization methods and reference materials for tissue scaffold properties that impact cell behavior.
   b. **Standards and Measurements for Improved Tissue Production Process and System Design -** RM products such as replacement tendons, are typically manufactured in a culture media-containing reactor vessel containing a scaffold into which cells enter and begin to grow. The design of such systems is empirical. New approaches are needed to explore systematically the parameters that influence cell response to environment (various reactor conditions and scaffolding materials, etc) in order to develop predictive models for optimal production system design.
   c. **Standards for Process Monitoring -** Methods are needed to monitor both the synthesis of scaffolding materials and the health and phenotype of cultured cells during the production process. Scaffold synthesis involves use of either synthetic materials (often biodegradable polymers such as polylactic and glycolic acid co-polymers) or processed natural materials (collagen). Cells can easily change their phenotypic if cultured in the improper environment. Although cells of a particular type may be placed in scaffolds during the manufacturing culture process, there are currently no nondestructive methods for ensuring that cells have achieved or maintained the desired phenotype in the final product. The

ability to adhere to the scaffolding is a critical parameter and requires measurements and standards. Needed are methods that will allow manufacturers to apply in-line monitoring of tissue culture constructs to ensure proper growth and differentiation states of cells in the constructs.

d. **Measurements and Standards for Assessing Safety and Efficacy of Cell-based Products -** Methods are needed that provide discrete and reliable metrics for the safety and potency of cell-containing therapeutic products. The lack of such assurance has stymied advances in these therapies. Criteria such as cell viability, state of differentiation, growth rate, division rate, metabolic status and other phenotypic characteristics need to be assessed. Needed are quantitative validated assays that can be used to assess these products.

e. **Measurements and Standards for Preservation of Final Products and Product Release** - RM products have a very short shelf-life and must be used within a few hours of production. In addition, better measurements are needed to ensure the quality of the product at the completion of the manufacturing process. Non-destructive characterization of finished RM products are needed and interpretation of images requires standardization.

Input for developing the items in this section was provided through:

- **USMS Measurement Needs Documents**
  - Tissue-Engineered Medical Products (TEMPS) *In Vivo* Monitoring
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100423
  - Materials-Guided Tissue Regeneration
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100519
  - Biomimetic Regenerative Materials in Dental Applications
    http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100606

4. Standards for Gene Therapy – Gene therapy has reemerged from its low point in 2002 when safety issues halted all clinical trials. New forms of nucleic acid-based therapeutics are emerging that are safe and effective. According to representatives from the diagnostic industry who attended a 2008 workshop hosted by the JCTLM, certain measurement issues are interfering with the speed of innovation. E.g., one of these new therapeutic modalities is small interfering RNA (siRNA), a drug that, once injected into cells, inhibits gene expression. siRNA represent a unique measurement challenge because of its size and difficulty to sequence. Measurement science, standards, technology, and measurement services are needed for determination of siRNA parameters such as; size, sequence, distribution once injected and concentration inside targeted cells.

Input for developing the items in this section was provided through:

- **Joint Committee for Traceability in Laboratory Medicine (JCTLM) Industry Stakeholder's Workshop -** July 26, 2008, Washington, DC

C. Toxicology

1. **Standards to Support Nanotoxicology Measurements -** New nanomaterials with unique properties are being developed in the electronics, chemical, and materials industries that are anticipated to revolutionize some existing products in core market areas. According the participants in national nanotoxicology workshops that included representatives from the Environmental Protection Agency (EPA), National Institute for Occupational Safety and Health (NIOSH) and numerous corporations and academic institutions and the report from the National Nanotechnology Initiative, to promote the use of nanomaterials for these various applications, and to ensure a continued U.S. position of leadership in this promising field, the U.S. must also take the lead in the assessment of nanomaterial toxicity to alleviate public concerns regarding this contentious issue. Measurement science, standards, technology, and measurement services are needed for nanobiometrology, chemical composition and physical properties analysis, health effects model development, *in vitro* toxicology measurements, and development of nanotoxicity standards.

   Specific projects

   a. **Nanobiometrology: Measurement Science for Nanomaterials in Biological Systems** – Reference materials and measurement methods are needed to assess the presence of nanomaterials in biological systems (nanometrology) – Technologies will be advanced imaging techniques for nanomaterial analysis in biological systems (cells, tissues, organs); nanomaterial physical and chemical characterization methods.
   b. **Standards to support nanotoxicology Studies** – Advanced technologies for robust high throughput nanotoxicology measurements will be developed to support establishment of reference materials, methods and data needed to support the determination of biological affects of synthetic nanomaterials on biological systems.
   c. **Nanotoxicology Standards -** Nanomaterial physical standards are needed to support the development of the measurement science needed for nanotoxicology determination. Needed are reference methods required to develop the reference materials and data needed to support nanotoxicology measurements.

Input for developing the items in this section was provided through:

- **USMS Measurement Needs Documents**
  - Toxicology of Nano-particles in Biological Systems. http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100430

Section II –
Standards to Support Next Generation Healthcare Measurements –
Tools to support multiplex methods for discovery and utilization of "Disease Signatures" ($140 M - $100M PCM + $40M IT)

NIST, with the cooperation of international colleagues, has conducted considerable outreach to solicit the opinions of leaders and visionaries in industry, academia, other government agencies, and non-profit organizations. These efforts identified the lack of advanced diagnostic tools capable of determining "disease signatures" as a key barrier to advances in medicine (i.e., personalized medicine). Disease signature analysis represents a completely new and comprehensive way of understanding the molecular events associated with disease.

Disease signatures are the computer-integrated collection of quantitative disease-associated changes in 1) concentrations in blood and cells of thousands of biomolecules involved in the complex biomolecular networks that maintain life processes, as detected by chemical, physical and electronic measurement tools and 2) anatomical abnormalities (lesions) detected by medical imaging. Disease signatures reflect the biochemical processes that occur during the course of disease and can be detected sometimes years before symptoms appear. Current technologies allow for elucidation of only a small fraction of the highly complex disease signature.

Reports issued articulating the concerns of over 300 industry, academic, FDA, and NIH participants in the 2008 NIST/UMBI "Accelerating Innovation in 21st Century Biosciences: Identifying the Measurement, Standards and Technological Challenges" and the 2007, NIST/IEEE conference, " Strategy for Health Care through Bio and Information Standards and Technologies Conference, indicate that a new research approach driven by innovation of new measurement tools is needed to define disease signatures for their optimal utilization in personalized medicine. Particular emphasis must be placed on tools to enable the elucidation, at their earliest stages, the complex "disease signatures" associated with chronic diseases. Over 80% of U.S. healthcare dollars are spent on treatment of debilitating chronic diseases such as, diabetes, obesity-related diseases, cancer, cardiovascular and rheumatic diseases.

Development of new technologies for disease signature-based diagnostics will require significant advances in biomolecular and bioimaging measurement techniques, standards, and computational tools. Standard methods, reference data, and reference materials are needed to enable developers of such tools to know that their systems are working properly and measuring the right thing. This measurement infrastructure to enable industry, academia, and other government organizations to develop the quantitative measurement tools needed to understand the well-being and disease signatures of individuals. In addition, a new understanding of systems biology will emerge that will be able to link individual and groups of biochemical changes to anatomical lesions such as tumors and allow for better diagnostic and prognostic capabilities.

A multiphase program is needed to establish the measurement infrastructure (biochemical and physical analytical and imaging tools and measurement science) necessary to develop the requisite reference methods, Standard Reference Materials, and data to enable industry and academia to innovate products and services for 1) discovery of the molecular basis of health and disease, 2) discovery of new drugs and therapeutics, and 3) discovery and routine clinical use of the complex biological signatures that distinguish health from disease (disease signatures).

Many measurement methods in the biosciences are currently semi-quantitative at best. Benchmark methods, data and physical standards will be established to enable the fundamental measurements that underpin medical research and discovery. The measurements of highest priority will include RNA, proteins, and metabolites in cells and blood, intermolecular interactions between biomolecules (e.g. protein-protein binding measurements) to enable discovery of critical biomolecular networks, modifications of DNA in the genome, and next-generation DNA sequencing technologies for sequencing individuals' personal genomes. IT tools are needed for interoperability and database reliability, standards for systems medicine, sequence analysis, genotype and phenotype annotation (static and dynamic), analysis of gene regulation, disease mutations and protein expression, prediction of protein structure, comparative genomics and intermolecular interactions. Standards for computational tools for capturing experimental metadata, elucidating human disease signatures are also needed.

Additional measurement science, standards and technology are needed for:

A. DNA measurements - Whole human genomic sequencing technology is undergoing a revolutionary transition. It is likely that it will be possible to sequence a person's genome in just a day or two at a cost of under $1000. For this to become a reality, though, standards are needed to assure the reliability of multiplex, high-throughput DNA sequencing. Measurement science, standards, technology, and measurement services are needed for ensuring the integrity of DNA samples, integrity and security of DNA sequencing data, and for robust genomic analysis including measurement of epigenetic changes:

1. **Standards for Next Generation DNA Sequencing -** Whole human genomic sequencing technology is undergoing a revolutionary transition. It is likely that it will be possible to sequence a person's genome in just a day or 2 at a cost of under $1000. For this to become a reality though, standards are needed to assure the reliability of the sequence data. The measurement community needs to work with industry to validate emerging sequencing methods by providing standard reference data and materials to help ensure confidence in genomic sequencing.
2. **Measurement of Epigenetic Genomic Modifications -** The silencing and activation of genes by the body's own ability for direct chemical modification of the DNA sequences plays a major role in many life processes such as fetal development and cancer development. This effect is termed epigenetics. Experimental reprogramming of adult cells to become stem-like cells also

involves this process. To date, there are no reliable methods for measuring the global or local epigenetic status of the genes. The measurement community needs to develop tools to enable validation of current and development of new technologies to measure the degree of modifications to DNA segment (e.g. – methylation) and, in addition, should help develop technologies to monitor and measure the multiple molecular events driving epigenetic regulation of the genome.

3. **Standards for Fidelity of DNA Amplification -** Many clinical DNA diagnostic procedures require the DNA in the patient sample be amplified to produce enough copies with which to analyze. Various methods are used for this, the most common being polymerase chain reaction (PCR). To amplify DNA, PCR uses replication enzymes that can have variable efficiencies, especially when being determined using multiplex measurement technologies or in the presence of different sample matrices and may introduce errors in the DNA analysis. The measurement community needs to develop standards for DNA amplification to help confidence in these procedures to researchers and clinical laboratorians.
4. **Standards for Multiplex Amplification of DNA -** Numerous methods are emerging for rapid, highly paralleled amplification and analysis of DNA. No standards currently exist to ensure the fidelity of these methods. Platform standards for multiplex DNA amplification are needed.
5. **Standards for DNA Sampling and Storage** There are currently no standards available to calibrate the processes used for DNA sampling and storage. The measurement community should undertake a systematic approach to determining appropriate analytical methods to ensure sample integrity.
6. **Technologies for Measurement of Low-Abundance and Under-Represented Genomes** - DNA is frequently analyzed as a heterogeneous mixture of DNA genotypes. DNA sequencing cannot detect a genotype when present at 20 % or less abundance. One very important application in this area is the elucidation of microbiomes – the full complement of microbes existing in and on various body parts and structures. A major effort is underway at NIH in this area. The measurement community needs to work with developers to help validate new methods for measuring the heterogeneity of DNA.

B. RNA Measurements – Current technologies capable of simultaneously measuring thousands of messenger RNA (mRNA) molecules are still only able to interrogate cells at static time points. Perturbations in complex biological networks occur dynamically and may involve hundreds of changes over the course of minutes to hours. New measurement and IT technologies are needed to visualize those important changes in real time. Measurement science, standards, technology, and measurement services are needed for determination of parameters such as sample integrity, messenger RNA quantity and sequence in cells, and microRNA quantity and sequence in blood and for data annotation into health records.

1. **RNA Copy Number Quantitation -** Quantitation of the number of individual copies of specific RNA sequences is critical for the understanding of the signatures of chronic infectious diseases (e.g., Viral load testing – for example, estimating the number of copies of HIV in infected humans). Technologies and standards are needed to enable reliable RNA sequence quantitation and direct molecular detection.
2. **Identification and Quantitation of MicroRNA -** MicroRNAs are small (17-23 base long) sequences involved in gene expression regulation and disease development. Their small size results in major measurement challenges. New methods and standards are needed to enable more robust determination of the base sequence and amount of microRNAs in tissue, cells and blood.
3. **siRNA Quantitation -** Small interfering RNA (siRNA) has become a very important tool for analysis of complex biological networks. These molecules are introduced into cells to perturb networks in order to understand affects of individual genes. The utility of this tool depends upon the ability to accurately measure the quantity of siRNA molecules that enter the cells being studied. No such measurement capabilities exist. The measurement community needs to develop new methods and standards required to measure intracellular siRNA.
4. **Standards for RNA Sampling and Storage -** There are currently no standards available to calibrate the processes used for RNA sampling and storage. The measurement community needs to undertake a systematic approach to determining appropriate analytical methods to ensure sample integrity.
5. **New Technology for Real-Time Monitoring of Dynamically Changing Levels of mRNA in Cells** - Current technologies capable of simultaneously measuring thousands of mRNA molecules are still only able to interrogate cells at static time points. Perturbations in complex biological networks occur dynamically and may involve hundreds of changes over the course of minutes to hours. There is no technology available to visualize those important changes in real time. The measurement community will work with industry to develop new technologies and appropriate standards to enable development of such technology.

C. Protein Measurements – Proteins are an important component of disease signatures because they show the most direct correlation with health status. It is estimated that, in order to obtain a meaningful disease signature, it will be necessary to measure up to 50 different proteins for each of the 50 or so important cell types in the body. For practical purposes, all 2500 measurements will need to be performed simultaneously. Measurement of single protein represents a significant challenge in laboratory medicine. Therefore, multiplex protein analysis will require considerable new innovation in the areas of binding reagent technology, sample quality, engineering for process miniaturization and IT based visualization of the disease signature. Measurement science, standards, technology, and measurement services are needed for determining protein identity structure and function. Critical measurement issues include sample integrity and quality (presence of interfering

substances), heterogeneity of proteins (phosphorylation, metal partners of metalloproteins), computer integration of multiplex signals, computer modeling for differentiation of normal protein patterns versus those associated with disease, small volume manipulation and analysis, multimodal data integration, capture reagent generation (antibody and non-antibody) quality, and annotation of information into the health record.

1. **Technologies and Standards for Quantitative Measurements of Proteins** - Technologies, tools and standards will be developed to identify and quantitatively measure particular proteins, and understand the physicochemical mechanisms of the recognition and binding of affinity probes to protein analytes. These efforts will impact all areas touched by bioscience research.
    a. **Standardization of Affinity-based Measurements** - State-of-the-art measurement methods need to be applied to gain a more complete understanding of this measurement technique and apply this knowledge to development of multiplex protein measurements. A goal is to understand sources of error in affinity measurements that are platform specific and are not specific to analysis platform, in order to satisfy the needs for reproducible and credible clinical analysis. Measurement platforms studied could include immunoassay (signal generation systems will include optical, spectral, plasmonic, mass and electronic), flow cytometry and imaging systems (light and other spectra).
    b. **Standardize Capture Agents for Autoantibody Measurement** - Autoantibodies are recognized diagnostic markers for chronic autoimmune diseases such as rheumatoid arthritis, lupus and stroke. They are also a potentially important part of the disease signature for cancer. The measurement community needs to apply state of the art measurement science to understand the chemical and physical interactions involved in capture, signal transduction and statistical analysis of autoantibodies. Standard autoantigens materials are needed.
    c. **Methods and Procedures to Test Alternative Affinity Technologies** - Aptamers, recombinant antibodies, click chemistry and other engineered recognition/binding reagents are alternative capture agents that can be tailored to have properties that facilitate quantitative measurements. But suffer from a lack of methods to assess the performance capabilities as replacements for antibodies.
    d. **Validation of Multiplex Protein Measurement Platforms** - The measurement community needs to develop tools and methods to better understand the precision and reliability of existing and aid in the development of emerging multiplex protein measurement platforms. Capabilities in biochemistry, biomaterials, biophysics, bioelectronics and statistical analysis and biocomputing should be brought to bear on this problem.

2. **Technologies and Standards to Enable Elucidation of the Human Disease Proteome -** New multiplex systems will be developed to study critical measurement issues associated with multiplex measurement of proteins in human serum. This effort is critical to the future of personalized medicine. Lessons learned from such analysis will be applicable to all large scale protein analysis technologies (agricultural, environmental and marine biological)
   a. **Standards for Ensuring the Integrity of Blood and Tissue Samples**
   b. **Measurement Platforms for Multiplex Serum Protein Analysis**

3. **Measurements and Standards for Determination of Protein Structure** - Protein structure capabilities are critical to the understanding of protein biopharmaceuticals, small molecule pharmaceuticals and industrial enzymes (important for biofuels). Structural analysis of proteins involves structure stability measurements and estimates, analysis of glycan residues, and methods to accurately quantitate glycoproteins and metalloproteins.
   a. **Structural Determination in Isolated Proteins -** Technologies employed could include; x-ray crystallography, NMR, biophotonics, neutron scattering, neutron reflectometry, single molecule detection systems. Research should focus on protein biopharmaceuticals and diagnostically-relevant proteins.
   b. **Structural Determination of Proteins *In Situ* –** Standards to support the study of proteins as they exist in biological systems, either as integrated in membranes, in body fluids or in intracellular networks are needed.
   c. **Reconciliation of Isolated vs. *In Situ* Protein Structures –** Standards and measurement technologies to support studies to understand discrepancies between structural measurements performed on isolated proteins as compared to when they are functioning in their natural crowded environment are needed.
   d. **Standards for Metalloprotein Measurements -** Tools are needed to determine at the atomic level, the relationship between their correct metal partner and the perturbations caused by the binding of the wrong metal instead of its proper cofactor. The measurement community needs to develop and optimize methods for metalloprotein isolation, chromatographic separation, and characterization/quantification in blood, cells and tissues.

4. **Standards for Protein Function Measurements** – Measurement science and protocols, models and theories are needed to elucidate the fundamental biophysical and biochemical properties of proteins as they function. This effort will impact the drug discovery, biofuels and biomanufacturing industries. Although functional assays exist for proteins with a well-understood mechanism of action, new methods are needed to the performance of more meaningful functional testing.
   a. Protein Function Determination by Intermolecular Associations Protein networks operate through a series of dynamic functional

interactions that regulate a biological function (growth, division, blood clotting etc.). The measurement community needs to develop the measurement infrastructure to support new technologies to measure the physical interactions between proteins to enable functional discernment.

b. Protein Function Determination by Expression Analysis - The measurement community needs to develop standards to support the establishment of tools better correlate mRNA measurements with protein measurements in dynamic biological regulatory networks.

c. Protein Function Determination by Cellular Metrology - Measurement infrastructure is needed to validate cell-based protein functional determination - tracking temporal/spatial relationships between active and structural cellular proteins and tools to understand perturbations in cellular processes secondary to alterations in protein and metalloprotein structure/function.

d. Functional Alteration of Cellular Proteins by Small Molecule Pharmaceuticals - The measurement community needs to develop the measurement infrastructure to support the studies to understand the behavior and interaction of the structure/function changes that occur when a cellular protein is perturbed by a small molecule drug.

5. **Standards for Protein Sampling and Storage** -- Practical issues of simultaneous testing and storage of large numbers of samples will be studies.

a. Microfluidics and Nanofluidic Tools – tools and services are needed to support testing the robustness of multiplex protein measurements and to understand sample handling issues associated with very large scale studies that will be required for personalized medicine.

b. Sample Storage Standards - Approaches for making multiplexed stability measurements on an entire proteome in blood and cells have not been developed. Significant efforts are required to develop tools and procedures to assess tissue preservation methods as well as optimize the preservation methods for protein-containing samples.

c. Sample Handling Systems - Large-scale specimen handling systems will need to be established and optimized – including robotic based systems for sample manipulation.

D. Metabolite Measurements - Metabolomics is the simultaneous measurement of metabolites in blood. This emerging area shows great promise for diagnosing and monitoring disease, development of personalized therapeutic regimens, and identification of potential drug and environmental toxicity. Integrating knowledge of an individual's metabolome with information about his or her genome and proteome would be a significant step forward in understanding the complex pathways at work in the human body and their interrelationships. Measurement science, standards, technology, and measurement services are needed for assessing metabolomic sample integrity, multimodal data integration and interpretation, multiplex determination of lipids, peptides, saccharides, etc.

1. **Advanced Multimodal Multiplex Measurement Technologies -** Multiplexed analytical techniques that integrate disparate measurement technologies for measuring the diversity (peptides, saccharides, lipids, etc) of metabolites are needed enable reliable identification and quantitation of groups of metabolites in various biological matrices (cells, plasma, etc.).
2. **Imaging Techniques for Metabolomics -** Imaging systems are needed to identify and quantify metabolites in cells and tissues, and to monitor changes in these concentrations over time *in vivo*. This will allow metabolite fluxes in various systems to be monitored.

E. Cell and Tissue Measurements – The ability to identify perturbations in networks of proteins, RNAs and metabolites in diseased cells is a key to discovery of the individual components of the disease signature in the blood. It is these very biomolecules produced and secreted into circulation that are the most likely individual components of the disease signature measured in blood. Cells represent several measurement challenges because they are small and their biochemical networks are massively complex and highly dynamic. Measurement science, standards, technology, and measurement services are needed to support development of tools to analyze the complex features of cells at the molecular level (light microscopic and ultrastructural). Critical measurement issues include sample integrity, cell population dynamics, isolation of activity of single cells, and mimicking *in vitro* the *in vivo* environment to reduce measurement bias, multiplex identification and quantitation of cellular RNA, proteins and metabolites, visualization of minor changes in patterns of analytes due to environmental or pharmacological influences, visualization and discernment of the functional biochemical network nodes (i.e., respiration, cell division, growth, etc.), establishment of models for comparison of normal versus diseased cells (measurement and interpretation of intermolecular interactions), and integration of multimodal imaging data. Computational tools will need to be developed for image feature extraction and selection, segmentation, registration, clustering, classification and annotation, and reconstruction for visualization.

Specific projects

1. **Standards for Cell and Tissue Sample Integrity -** The integrity of the blood samples will need to be assured – collection, storage and utilization procedures will need to be established and standardized – this would involve the development of an integrity molecular marker for blood serum/plasma samples.
2. **Standards for Stem Cell Characterization –** Measurement infrastructure is needed to support the characterization of stem cells - approximately 216 different stem cell types known to exist in the human. Each of those cells could represent a potential therapeutic used for regenerative medicine.
3. **Standards for Single Cell Measurements -** Single cell cultures and manipulation technologies represent an exciting and important opportunity to explore the development of disease signatures from normal complex

biomolecular networks. Measurement infrastructure to support development of tools to perform measurements of RNA, proteins and characteristics of intermolecular interactions are needed.

4. **Models for Quantifying and Understanding Diversity of Cell Populations -** Appropriate measurements and statistical analysis are required to correctly distinguish normal from abnormal and to accurately characterize cell response.
5. **Standards and Methods for Quantitative Histopathology -** Enabling technologies are needed to move pathology away from paraffin embedding to examination of fine needle aspirate biopsies and even examination of live cells from biopsies. The measurement community needs to address this by providing objective, quantitative metrics for pathology-based diagnosis and stratification of disease states.
6. **Standards for Measurement of Environmental Affects on Cells –** Measurement infrastructure is needed to support developing tools to assess the various environmental toxins and chemicals are known to affect cellular function.
7. **Standards for Measuring Secreted Proteins from Cells -** Technologies to measure rates and amounts of proteins actively being secreted from cells will be required. Measurement infrastructure to support their development are needed
8. **Cell Imaging Reagent Standardization -** Standards are needed for optimization of signal development and transduction using fluorescent tags in multiplex assays. Sophisticated image analysis are needed to analyze the patterns of light generated in the assays resulting from the measurement of various levels of the selected proteins, DNA, RNA and metabolites in cells and fluids.
9. **Intermolecular Interaction Imaging Methods** Standards for measuring the "importance" (functional interactions vs. casual sticking together) of protein pairs are needed.
10. **Standards for New Imaging Modalities –** Measurement infrastructure is needed to support the development of new imaging methods such as Raman spectroscopy and surface plasmon resonance (SPR) for cells and tissues. These methods have the potential to enable specific molecular information about cells that may be used to characterize cells as to their type and function.
11. **Standards for Subcellular Imaging -** Standards for several of the new EM methods on biological materials are needed to ensure precision in identification and quantitation of molecules and anatomical features. Subdiffraction imaging methods to achieve higher spatial resolution need advancing.
12. **Standards for Multimodal Imaging** - Measurement infrastructure to support correlative and comparative analyses of images obtained from multiple techniques are needed. Proper sampling and image interpretation methods are not well defined.

13. **Improved Reagents for Following Intracellular Molecular Events in Real Time –** Evaluation of a growing trend in reagent development for cell studies involving molecular beacon technologies is needed.
14. **Single Molecule Imaging of Cells –** Technologies such as super-resolution light microscopes are in need of further development. Such methods hold the promise of 10 nm resolution of fluorescently tagged specimens, surpassing classical electron microscopy by combining the specificity of fluorescent labeling with the ability to image intact, living specimens. In the limit, single molecules can be detected and their behavior observed over time in the living cell.

Appendix A

Advanced IT and Biocomputing Tools – *Health Care Information Technology (HIT) and Systems Level Integration Of Data And Models of Health and Disease* (Section I - $10M, Section II - $40M)

Biocomputing Tools

Advances in personalized medicine will require validated computational tools for improved qualitative and quantitative biochemical and imaging measurements, advanced informatics infrastructure that allows for the seamless capture, search, retrieval and exchange of large-scale biological data, and systems level tools that allow scientists and physicians to visualize human disease and enable them to make educated, science-based decisions for research and patient care.

1. Computational Tools for BioMeasurements for Discovery Purposes and in Support of Diagnostics and Therapeutics

Medical research scientists and clinicians need confidence in their measurements. Each measurement method produces data with associated analytical uncertainty, data file formats, experimental details, conventions, and nomenclature that are unique compared to data from other measurement methods. Many models are developed from such data for limited subsets of biological reactions. Efforts will focus on computational and informatics standards that will allow data and models from different sources to be efficiently combined and compared. By developing reference datasets and algorithm test methods, and new ways of naming measurement-related information, NIST will help assure interoperability of data and models from the many scientific contributors, and help derive knowledge from biomolecular data. In particular, for each biomeasurement, will improve will be:

A. **Standards for Capturing Experimental Data/Metadata** Advances in high-throughput experimental techniques have allowed the acquisition of data on a large scale. To manage and use this data within and between laboratories, and in particular, to achieve a level of integration that is necessary to support personalized medicine, research is needed to move life science information standards beyond the current *ad hoc* and community-based methods. Working with industry and academia, the measurement community needs to:

- Harmonize information standards for capturing experimental metadata, and defining and exchanging RNA, DNA, Protein, and Metabolite data, including experimental protocols, instrument settings, and analysis techniques. Such metadata will allow for downstream normalization and integration of experimental data.

- Establish formal models to allow for exchange of experimental data between laboratories.
- Develop platform-specific implementations for life science data, to allow for automatic integration of experimental data.

B. **Mathematical and Statistical Methods for Improving Signal/Noise** Research laboratories must deal with instrument or protocol-dependent data inconsistencies. Data if often inconsistent due to systematic bias, which often includes heterogeneous calibration methods or protocols, environmental conditions, instrument background effects, or noise dependent on expression levels of the sample. A number of statistical and mathematically techniques have been proposed – deconvolution, blind deconvolution, guassian filtering, nonlinear filters, anisotrophic filters, and spline and polynomial fitting -- with no clear indication of which technique to use under what circumstances. In addition, new techniques will need to be developed to address real-time monitoring of dynamically changing measurements

C. **Validated Computational Methods for BioImage Analysis** Biological science is rich in images. Most familiar are images taken through microsopes or medical images taken by X-ray, CT-scans or MRI's. The deluge of complicated biological and biomedical images has led to increased focus on developing novel image processing, data mining, database, and visualization techniques to extract, compare, search and manage the biologically relevant information. Biological images are diverse and complex, spanning from the whole organism level down to the single molecule level. They may involve two-dimensional (2D) or 3D spatial information, multiple colors which may correspond to various molecular reporters, 4D spatio-temporal information for developing tissues or moving cells, various co-localized biological signals such as mRNA expression levels of different genes (Peng et al, 2007), or other screening experiments related to RNA interference, chemical compounds, etc. Analyzing theses images is critical to biological insight, such as differentiating cancer cell phenotypes. Dramatic variations in morphology and intensity can occur from image to image, and stacks of images only compound the analytical issues. Automatic analysis of these images will be necessary to support current and emerging high-throughput techniques. Using reference data sets and principles of experimental design, NIST will conduct comparative evaluations of existing image processing and analysis algorithms to support automated analysis in the following critical areas

   i. **Image Feature Extraction and Selection** In recent years, a number of feature extraction techniques originally developed for aerial images, have been successfully applied to biological images to extract visual features, such as texture and shape. Using a defined distance metric, such as nearest neighbors, allows similarity comparisons to be conducted across a set of images. Low-level visual features are useful in similarity-based retrieval tasks, but

cannot answer questions such as the presence of certain proteins. Recent research in this area is focused on using higher order feature sets to enable automatic annotation of gene expression patterns, or to characterize the 3D protein location patterns associated with major subcellular organelles and structures. Successful techniques are based on learning algorithms where a priori knowledge of existing patterns are needed to train an algorithm. Future challenges include applying these techniques to more complex images of multiple cells or tissues to recognize previously unknown patterns formed by proteins

ii. **Image Segmentation** Segmentation provides a mechanism for identifying biological objects of interest, such as cells or medical tumors, within an image. There are no common methods or class of methods applicable to even the majority of images. Segmentation is easiest when the objects of interest have intensity or edge characteristics that allow them to be easily separated from the background and noise, as well as from each other. Many algorithms are used without sufficient evaluation of their accuracy and can introduce as much as a 40% measurement error in biological images. A novel approach to image segmentation is the use of multi-modal data

iii. **Image Registration -** To achieve maximum benefit from different modes of imaging instrumentation, such as combining structural and protein distribution information derived from confocal, multiphoton, and electron microscopy, or anatomical information from both X-ray and CT-scans, multiple images must be aligned. Techniques are categorized based on the features that are being matched. For example, such features may be external markers that are fixed, internal anatomic markers that are identifiable on all images, the center of gravity for one or more objects in the images, crestlines of objects in the images, or gradients of a predefined object. Image registration is well defined for rigid objects, but is more complicated for deformable objects

iv. **Image Clustering, Classification, and Annotation -** Since in many cases, the number of distinct patterns expected may not be known, an important pattern recognition area that needs to be addressed is that of high-dimensional clustering. Typical image descriptors are high-dimensional feature vectors with dimensionality ranging from a few tens to a few hundreds. Research in clustering has four major thrusts: (1) raw data clustering; (2) discriminative classification, regression, and detection methods; (3) interactive analysis; and (4) hierarchical techniques for the analysis of the large, high-dimensional datasets that arise from high-dimensional visual features

v. **Image Reconstruction/Visualization -** Determination of 3D structure and configuration plays a central role in biological inquiry. Insight into the spatial and geometric properties of components

within a biological entity can be achieved through the reconstruction of a set of 2D images into a 3D interactive visual representation that can be viewed from different angles and perspectives. Current techniques can miss or misrepresent subtle features, and lead to erroneous conclusions, such as relative changes in tumor size. New algorithms that take advantage of better feature extraction and enhancement techniques will be needed to keep pace with advances in high-resolution image acquisition and the development

2. IT Standards and Methods to Support Annotation of the Human Phenotype

As new technologies are developed and disease signatures are discovered, it is certain that the way in which disease is classified will change. A person's molecular phenotype will need to be correlated with his or her health status (physical) phenotype.

Now that both the public and private sectors have reached major milestones in the quest for the human genome sequence, the focus is shifting to the genetic variability of our species. Lying buried in human genetic variability is the source not only of all genetic disease, but the entire range of normal phenotypic variation, including susceptibilities to pathogens and environmental factors, and individual differences in response to drug treatment. Emerging high-throughput technologies like the DNA microarray are enabling for the first time large-scale genotyping and gene expression profiling of human populations. Emerging technologies combined with standard methods for accessing, analyzing and exchanging biological information form the basis for developing complex models of human phenotypes. These network models can define disease as through the modular collection of genomic, proteomic, metabolomic, and environmental networks that interact to yield the pathophenotype. Disease network analysis ultimately provides a mechanistic basis for defining phenotypic differences among individuals with the same disease through consideration of unique genetic and environmental factors that govern intermediate phenotypes contributing to disease expression. These disease networks ultimately provide a unique method for identifying therapeutic targets or combinations of targets that can alter disease expression.

A. **IT Standards for Systems Biology -** High-throughput technologies are generating large amounts of complex data that have to be stored in databases, communicated to various data analysis tools and interpreted by scientists. Data representation and communication standards are needed to implement these steps efficiently. Standards are needed to encapsulate biological knowledge as well as evidence-based data. On-going efforts are aimed at defining genomics, functional genomics, pathways, proteomics, and metabolomics/metabonomics. These efforts

are at differing levels of maturity and have often been developed via ad hoc or community based methods. To achieve full exchange and integration of biological information necessary to support annotation of the human phenotype, it will be necessary to harmonize and extend these efforts on a global scale

B. **Interoperable and Reliable Databases -** At the core of modern genomic research is the generation of enormous amounts of raw sequence data. As the volume of genomic data grows, sophisticated computational technologies are needed to manage the data deluge. Storing and handling the staggering amounts of data is often accomplished through the use of biological databases. Based on their content, biological databases are roughly divided into three categories: primary databases, secondary databases, and specialized databases. Primary databases contain original biological data – archives of raw sequence or structural data generated by the scientific community. GenBank and the Protein Data Bank are examples of primary databases. Secondary databases contain computationally processed or manually curated information, based on original information from primary databases. Translated protein sequence databases containing functional annotation fall into this category, as do SWISS-Prot and Protein Information Resources. Specialized databases are those that cater to a particular research interest. For example, Flybase and the HIV sequence database specialize in a particular organism or a particular type of data. Biological research is hampered by the lack of standard access mechanisms and formats as well as inaccurate or conflicting information within these databases. Standard methods for defining, storing, and accessing this information are needed. Tools to uncover and improve data accuracy are also needed

Integrative Bioinformatics - Bioinformatics involves the information technology tools that are used for storage, retrieval, manipulation, and distribution of information related to biological macromolecules such as DNA, RNA, and proteins. Tools are used to automate the tasks in genomic data analysis that are highly repetitive or mathematically complex, and are indispensable in mining genomes for information gathering and knowledge building. High-speed genomic sequencing coupled with sophisticated informatics technology will allow a doctor in a clinic to quickly sequence a patient's genome and easily detect potential harmful mutations and to engage in early diagnosis and effective treatment of diseases. In the last couple of decades, many advances have been made in bioinformatics tools that are used to identify and integrate molecular sequence, structure, and function analysis information. For example, knowledge of the three-dimensional structures of proteins allows molecules to be designed that are capable of binding to the receptor of a target protein with great affinity and specificity. For all of its advances in recent years, bioinformatics tools are still quite limited. The raw data that is produced for analysis is fraught with errors resulting from experimental

techniques. These errors are propagated through downstream analysis and produce misleading results. Most algorithms lack the capability and sophistication to truly reflect reality. They often make incorrect predictions that make no sense when placed in a biological context. Errors in sequence alignment, for example, can affect the outcome of structural or phylogenetic analysis. Many accurate, but exhaustive algorithms cannot be used because of the slow rate of computation. Instead, less accurate, but faster algorithms serve as substitutes.

Despite the pitfalls, there is great promise that bioinformatics can serve a pivotal role in revolutionizing biological research in the coming decades. Reliable and more rigorous computational tools for sequence, structural, and functional analysis will be coupled with advances in biomeasurement technologies. Additional major challenges include the development of tools for elucidation of the functions and interactions of all gene products in a cell. Advances will be necessary in the following areas

i. Sequence Analysis Computational Tools
ii. Genome Annotation Computational Tools
iii. Computational Tools for Analysis of Regulation
iv. Computational Tools Analysis of Protein Expression
v. Computational Tools Analysis of Disease Mutations
vi. Computational Tools for Prediction of Protein Structure
vii. Computational Tools for Comparative Genomics
viii. Computational Tools for Protein-Protein Docking
ix. Tools for Computational Evolutionary Biology

3. Networks Models for Elucidating Human Disease

With the complete sequence of the human genome a reality, and with a growing body of transcriptomic, proteomic, and metabolomic data sets in health and disease, we are now in a unique position in the history of medicine to define human disease precisely, uniquely, and unequivocally, with optimal sensitivity and specificity. Theoretically, this precise molecular characterization of human disease will allow us to understand the basis for disease susceptibility and environmental influence; offer an explanation for the different phenotypic manifestations of the same disease; define disease prognosis with greater accuracy; and refine and, ideally, individualize disease treatment for optimal therapeutic efficacy. Research in this area will be to build network-based analysis of the associations among the individual genes, proteins, metabolites, intermediate phenotypes, and environmental factors that conspire to yield the pathophenotype. Defining the network interactions among these modular elements (and their probabilistic relationships, where appropriate) not only will account for the ultimate pathophenotype but also can lead to the identification of potential regulatory nodes within the network that can modify phenotype (i.e., potential therapeutic target).

Appendix B

NMI Roles

NMIs have a clear role in supporting measurements performed by healthcare scientists and practitioners. Standards and the metrology that enables them form the scientific basis for establishing confidence in the data from which critical decisions are made. NMIs support:

Diagnostics

Laboratory medicine – Fundamental methods, materials, data and calibrations activities supporting medical/ clinical testing to facilitate recognition of a disease and/or the underlying physiological/biochemical cause(s) of a disease or condition. Suppot measurements on biosystems relevant to medical tests; support for discovery, development, manufacturing, and use of medical measurement activities (reagents, instruments, sensors, probes, software) used to discover, qualitatively detect, quantitate or identify biomolecular structures, dynamics, and function, and biochemicals and biochemical processes for diagnostic, predictive or prognostic purposes (blood chemistry, urinalysis, immunodiagnostics, biological activity assays, cell counting/identification) – includes also activities that support measurements for identification, epidemiology and control of microbes and infections, and laboratory based measurements in support of clinical trials.

Imaging diagnostics – Fundamental methods, materials, data and calibrations supporting measurements made for discovery and clinical analysis of gross (non-microscopic) anatomical structures and tissue. Also, the development or manufacturing of medical imaging systems – includes optical, CT, MRI, PET, x-ray, ultrasound, nuclear medicine, mammography, etc) – includes discovery, development and use of contrast or image enhancement reagents (metals, nanomaterials and biochemical probes or metabolites).

Molecular pathology (diagnostics)– Fundamental methods, materials, data and calibrations supporting discovery, development and utilization of microscopic analysis of fluids, tissues and cells by pathologists for disease identification, staging, prognostication. Technologies required for light and particle based imaging (e.g. electron microscopy).

Therapeutics

Drugs (Pharmaceuticals and Biopharmaceuticals) – Fundamental methods, materials, data and calibrations activities supporting biochemical and cellular measurements used in basic research for discovery, and applied research for development, manufacturing and delivery of small molecule, nucleic acid and protein biochemical drugs (enzymes, antibodies, clotting factors, etc) – includes activities that support measurements made as part of the manufacturing and QC of

medicines (process analytical technologies, pressure/temp/pH/metabolite monitoring, filtration, chromatography, etc) – includes IT in support of these activities: Does NOT include healthcare products that are not under the regulatory purview of the US FDA (excludes dietary supplements, homeopathic remedies and nutraceuticals).

Non-Drug Therapeutics

Tissue engineering - Fundamental methods, materials, data and calibrations activities supporting measurement technologies used to characterize cells, biomaterials, and engineered or native tissues for use as tissue engineering/regenerative medicine therapeutics. Activities include development of instrumentation and methods for characterizing cell response to materials and to mechanical stimuli, as well as characterization of biomolecules at interfaces.

Dental - Fundamental methods, materials, data and calibrations activities supporting measurement technologies used to characterize, develop or manufacture materials used for dentistry.

Cell-based therapeutics – Fundamental methods, materials, data and calibrations activities supporting measurement technologies used to characterize cells for use as therapeutics – includes phenotyping (qualitative and quantitative assessment of appearance, activational status, viability, pluripotency, etc.) and genotyping cells.

Gene therapies – Fundamental methods, materials, data and calibrations activities supporting measurements used to determine the safety and effectiveness of genes and genetic materials for therapeutic purposes.

Radiation therapies -- Fundamental methods, materials, data and calibrations activities in support of measurements used in ionizing-radiation therapy, including standards used to support the use of radiation-emitting implantable or attachable materials for therapeutic purposes (brachytherapy).

Environmental

Toxicology - Fundamental methods, materials, data and calibrations activities supporting measurements made on living organisms (plants, microbes, humans and other animals) to discover toxicological mechanisms or perform health assessments on potential environmental toxins - including Nano environmental health and safety (EH&S) - measuring biological damage caused by environmental agents.

Appendix C

Standards for Annotation of Human Phenotype

This section is included as an appendix because it falls out of the range of responsibility of the measurement community. It is, however, critical to the establishment of standards because the establishment of phenotypic standardized nomenclature will be directly affected by the results molecular measurements performed on patients.

Standards are needed to tie together measurements and clinical assessments. The association of data obtained on patients through quantitative measurements of their genotype, gene expression profiles, proteome profiles, immune status, etc. with the information reflecting their contemporaneous clinical status is necessary to define the signatures of various clinical states of individuals such as health, at risk for specific conditions, diseased, recovering or responding to treatment, active or not, etc. This information includes some very objective data as well as some very subjective data. While the genotype (genetic sequence) of an individual provides the baseline information about the DNA, it is the ability to measure the results of the expression of the genome (the phenotype –at the molecular level, at the level of the organ, as well as at the visible clinical level) and how it interacts with environmental influences that will provide the information needed to enable the biggest gains in personalized medicine.

Establishing and implementing phenotype standards in description, annotation, and dynamic change in status are critically needed to facilitate transferring useful information, for making clinical assessments, for establishing reliable diagnoses, to ensure accuracy and reliability in clinical research and clinical trials, and to enhance understanding of disease states and processes. Such standards will, therefore provide clinical benefit to patients and physicians, scientific benefit to investigators, and provide and improved basis for regulatory decision-making. In the short term, this can improve the conduct of clinical trials and enhance the ability of pharmaceutical, biotechnology, device and diagnostics manufacturers to move the fruits of scientific discovery to improvements in public health.

The discovery and establishment of disease signatures MUST be coordinated with the language used by the clinical community to describe what they observe in patients. Only through establishment of consensually agreed-upon and clearly defined descriptors for annotating the clinical assessments made by physicians, can disease signatures become clinically useful. The clinician and measurements community MUST speak the same language. Clinical assessments will help drive disease signature discovery as well as incorporation into clinical practice. Likewise, the discovery of new disease signatures will likely drive the way clinicians describe and assess diseases. The international measurement community will work with stakeholders (NIH, FDA, physicians groups, patient advocacy groups and the electronic records community) to:

A. **Develop an Established Procedure for Defining Clinical Phenotype** – Standards are need for deciding on which defining features (height, weight, mobility, size of lesion) need to be measured, as well as the procedures by which the measurements are conducted. An example already being implemented is molecular pathology based grading of tumors. Development of an accepted "standard phenotypic" description of disease and normal populations is neededas are interoperable data features for complete phenotype descriptions (history, physical, lab, imaging). These are needed to move towards a standardized clinical and research practice and and to establish best practices. Health status descriptions will be developed that incorporate the most useful current descriptors (-itis, -osis, -oma, etc) with newly established ones based on the disease signature – as based on measured molecular, cellular, organ-based and organism-based events, as well as environmental interactions

B. **Standards for Annotating Clinical Assessments** - signs and symptoms, history, disease subtype, heterogeneity of disease, severity, therapeutic outcomes, iatrogenic events, clinical outcomes, trial outcomes, histology/ pathology will be developed. Requirements - robust, reproducible, platform independent standards, implementable with minimal training

C. **Standards to Enable Traceability of Biological Measurement** - More confidence is needed in clinical measurements, tying results to an absolute or relative reference - functionality, dynamics, definition of environmental information. The establishment of predictive disease signatures will result in the establishment of a set of pre-disease phenotypic observations for "transitional" phenotypes in patients

D. **Sample Resource Standards** - The availability of biological specimens will be a requirement for standardizing molecular phenotype. Needed are established standards for management of sample banks to facilitate the study and understanding of long-term outcomes of defined phenotypes. This will be required for validation of disease signature biomarkers

E. **Standards for Annotation of Temporal Changes in Patients** – establishment and annotation of clinically-valid observations that occur over time in a patient's lifetime – will enable measurement of dynamic disease symptoms/ characteristics and tie-back to disease signature

F. **Standards to Assure Consistency of Descriptions of Physical Attributes** Physical attributes such the color, texture, shape and size of anatomical lesions observed in patients are important descriptors of phenotype. Physical artifact standards are needed for calibration of instruments and telemedicine devices to improve the quality and objectivity of observational measurements used in diagnosis and to improve the clarity of communication in clinical medicine

Establishing and implementing such standards in description, annotation, and dynamic change in status are critically needed to facilitate transferring useful information, for making clinical assessments, for establishing reliable diagnoses, to ensure accuracy and

reliability in clinical research and clinical trials, and to enhance understanding of disease states and processes. Such standards will, therefore provide clinical benefit to patients and physicians, scientific benefit to investigators, and provide and improved basis for regulatory decision-making. In the short term, this can improve the conduct of clinical trials and enhance the ability of pharmaceutical, biotechnology, device and diagnostics manufacturers to move the fruits of scientific discovery to improvements in public health.

Input for developing the items in this section was provided through:

- **Workshops with The Institute for Systems Biology -** May 2006 – March 2008
- **Joint Committee for Traceability in Laboratory Medicine (JCTLM) Industry Stakeholder's Workshop -** July 26, 2008, Washington, DC
- **NIST conference – "Accelerating Innovation in 21st Century Biosciences: Identifying the Measurement Standards and Technological Challenges"** October 19-22, 2008
- **Workshop with National Cancer Institute (NCI) Center for Cancer Research (CCR)** 2007 - 2008
- **Strategy for Health Care through Bio and Information Standards and Technologies,** September 24 – 25, 2007, Co-sponsored by NIST and Biotechnology Council of IEEE, ASME, BMES, HIMSS, SBE/AIChE
- **Measurement Challenges in Proteomics Workshop -** March 12, 2006, Human Proteome Organization, Boston, MA
- **USMS Measurement Needs Documents**
  - Advanced DNA Analysis Using Lab-on-a-Chip Technology - http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100427
  - Rapid and Low-Cost DNA Sequencing http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100579
  - Nanoscale biological imaging http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100414
  - Cell-Based Measurements http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100418
  - Diagnostic Tissue Imaging http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100424
  - Microarray Gene Expression Profiling http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100321
  - Genomics: Array-Based Comparative Genomic Hybridization http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100590
  - Proteomes Mass Spectrometry http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100468
  - Rare Gene Microarray Detector http://usms.nist.gov/index.cfm?event=search.mn&IDMeasurementNeed=100592