Rare Disease Workshop

Standardization for Data and Reference Sets

Fall 2010

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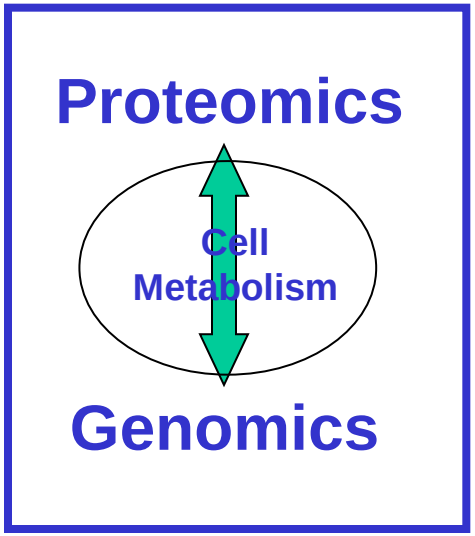
Outline

- Biomarkers
- Reference Measurement Systems
  - Measurand
  - Measurement procedures
  - Reference Materials
- Matrix Effects
- Commutability
- Laboratory Standardization
- Measurement Services

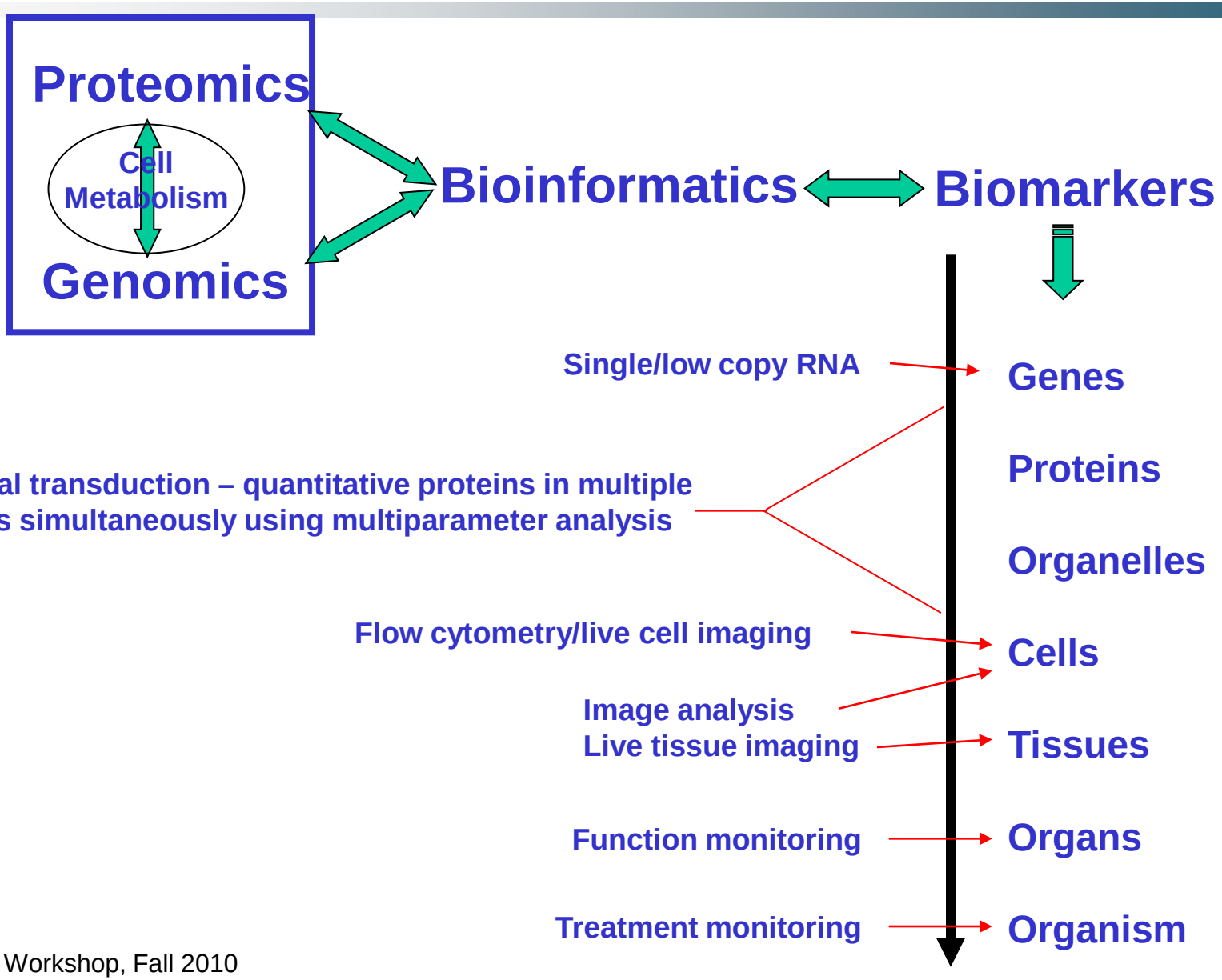


Biomarkers

A biomarker is a measurable entity used to help in the

- Diagnosis of disease/normal physiological state
- Monitoring of clinical responses to a therapeutic intervention
- Prognosis/monitoring of disease progression
- Delivery of personalized treatments

Biomarkers


Proteomics
Cell Metabolism
Genomics
Bioinformatics
Biomarkers
Single/low copy RNA
Genes
Proteins
Signal transduction – quantitative proteins in multiple paths simultaneously using multiparameter analysis
Organelles
Flow cytometry/live cell imaging
Cells
Image analysis
Live tissue imaging
Tissues
Function monitoring
Organs
Treatment monitoring
Organism

Biomarkers in the IVD Industry

Proteins

Immunoglobulins

Tumor Markers

Peptides

Enzymes

Cell Surface Receptors

Cytokines

Metabolites

Amino Acids

Biomarkers are the analytes detected by IVD assays and devices!

Carbohydrates

RNA

Lipids

DNA

Vitamins

Neurotransmitters

Lipoproteins

Trace Elements

Blood Gas

Electrolytes

Therapeutic Drugs

Drugs of Abuse

Toxic Compounds

Definitions:

Biomarkers Definitions Working Group, NIH:

- **Biological marker (biomarker):** a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention;
- **Clinical endpoint:** a characteristic or variable that reflects how a patient feels, functions or survives;
- **Surrogate endpoint:** a biomarker that is intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit (or harm or lack of benefit or harm) on the basis of epidemiological, therapeutic, pathophysiological or other scientific evidence. Surrogate endpoints are thus a subset of biomarkers.

Definitions:

Food and Drug Administration:

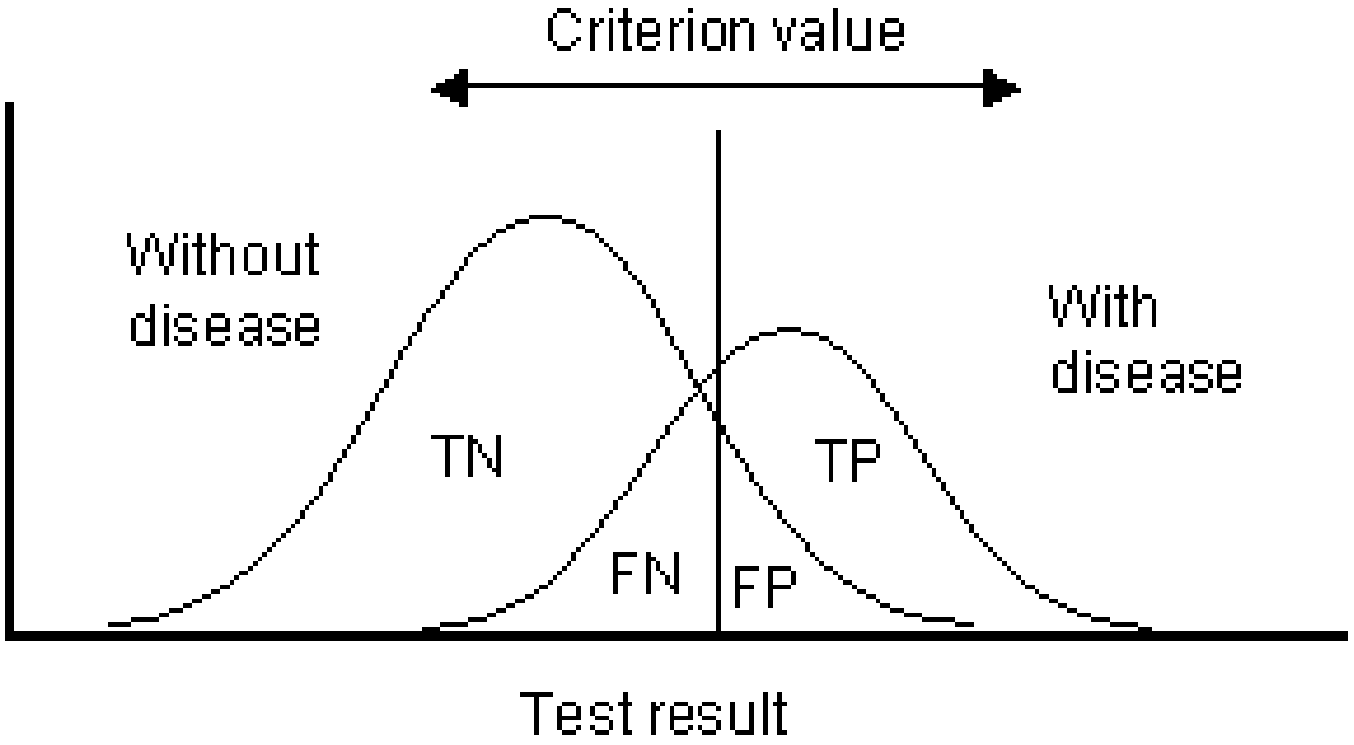
- ‘may grant marketing approval for a new drug product on the basis of adequate and well-controlled clinical trials establishing that the drug product has an effect on a surrogate endpoint that is reasonably likely, based on epidemiological, therapeutic, pathophysiological or other evidence, to predict clinical benefit or on the basis of an effect on a clinical endpoint other than survival or irreversible morbidity’.

Future Biomarkers

- Analytical performance-how reliably and correctly test measures analyte
- Clinical performance
  - How reliably test measures clinical condition
  - What action will clinician/patient take
- Labeling-directions for use, warnings, limitations, reporting recommendations

FDA 2006

Discriminating disease and normal cases



$$\text{Sensitivity} = \frac{TP}{TP+FN}$$

$$\text{Specificity} = \frac{TN}{TN+FP}$$

Reference Measurement Systems

- Diagnosis and treatment of patients requires accurate and precise measurements of clinical analytes
  - Independent of hospital, reference laboratory, Country and time
- Establish a reference measurement system
  - Laboratory result linked to a higher-order standard
- National Metrology Institutes
  - National Institute of Standards and Technology (NIST, USA)
  - Institute of Reference Materials and Measurements (IRMM, EU)
  - National physical Laboratory (NPL, UK)
  - National Metrology Institute Japan (NMIJ)

Reference Measurement Systems

- Essential Components
  - Definition of the Measurand
  - Measurement Procedures
  - Reference Materials and Calibrators

Reference Measurement Systems

- Definition of the Measurand
  - What is being measured
    - Single molecular structure with a known molecular formula – Cholesterol
    - Complex molecular structure – Proteins
      - Post transcriptional modification
        - » Addition of small chemical groups- Phosphate
        - » Addition of large chemical groups- Carbohydrates
      - Covalent complexes
      - Non-covalent complexes

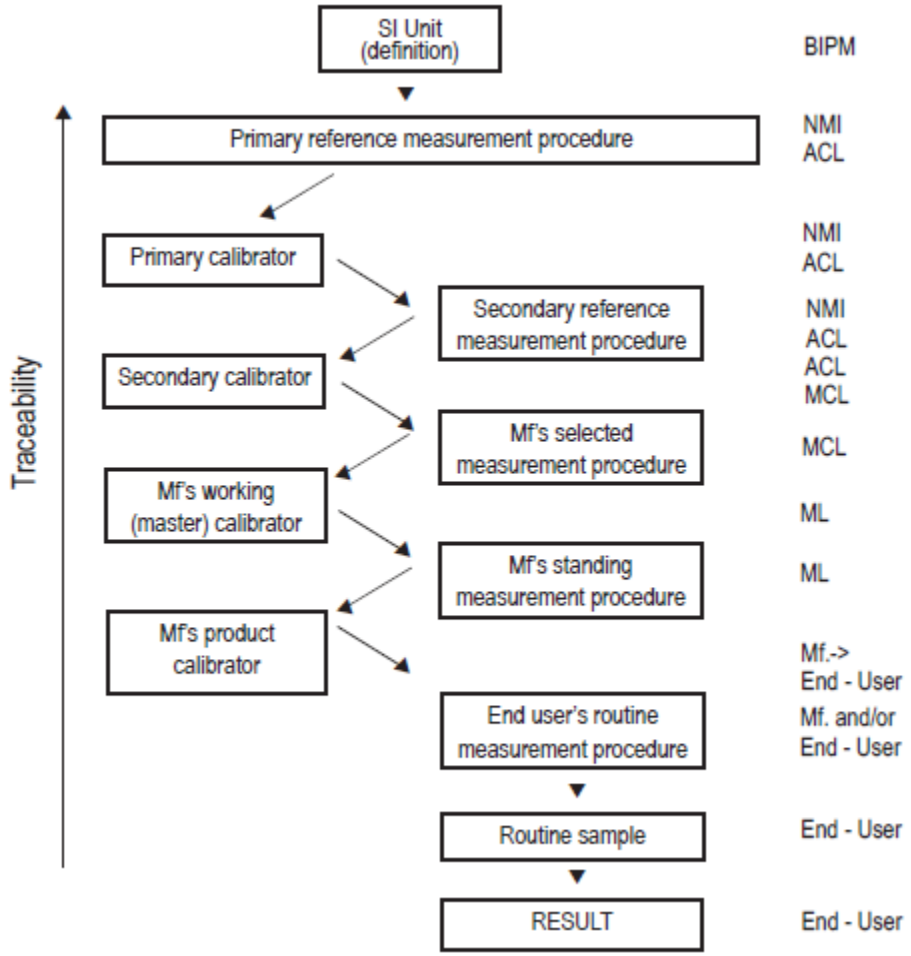
International System of Units (SI)

- Base Quantity

	Name	Symbol
	SI base unit	
length	meter	m
mass	kilogram	kg
time	second	s
electric current	ampere	A
thermodynamic temperature	kelvin	K
amount of substance	mole	mol
luminous intensity	candela	cd

NIST Reference

Reference Measurement Systems



BIPM: International Institute of Weights and Measures,NMI: National Metrological Institute,ACL: Accredited Calibration Laboratory
MCL: Manufacturer 's Calibration Laboratory,ML: Manufacturer 's Laboratory,Mf: Manufacturer

Siekmann L. Clin Biochem Rev 28, 149-154, 2007

Reference Measurement Systems

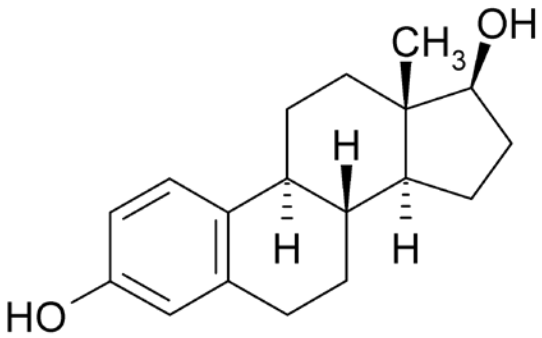
- Reference Materials
  - Homogeneous stable materials for Reference Measurement Procedures
    - Calibration
    - Assigning values to other Reference Materials
    - Quality control
    - Assessment of other Reference Measurement Procedures

Reference Measurement Systems

- List of standard reference materials
  - Primary
    - Pure substance
  - Secondary
    - Serum, plasma, urine
    - Access trueness of calibrators
    - Quality control

Single molecular Structure

- Isotope Dilution mass Spectroscopy (IDMS)
- Substitute C14 for C12
- Example: Estradiol-17β



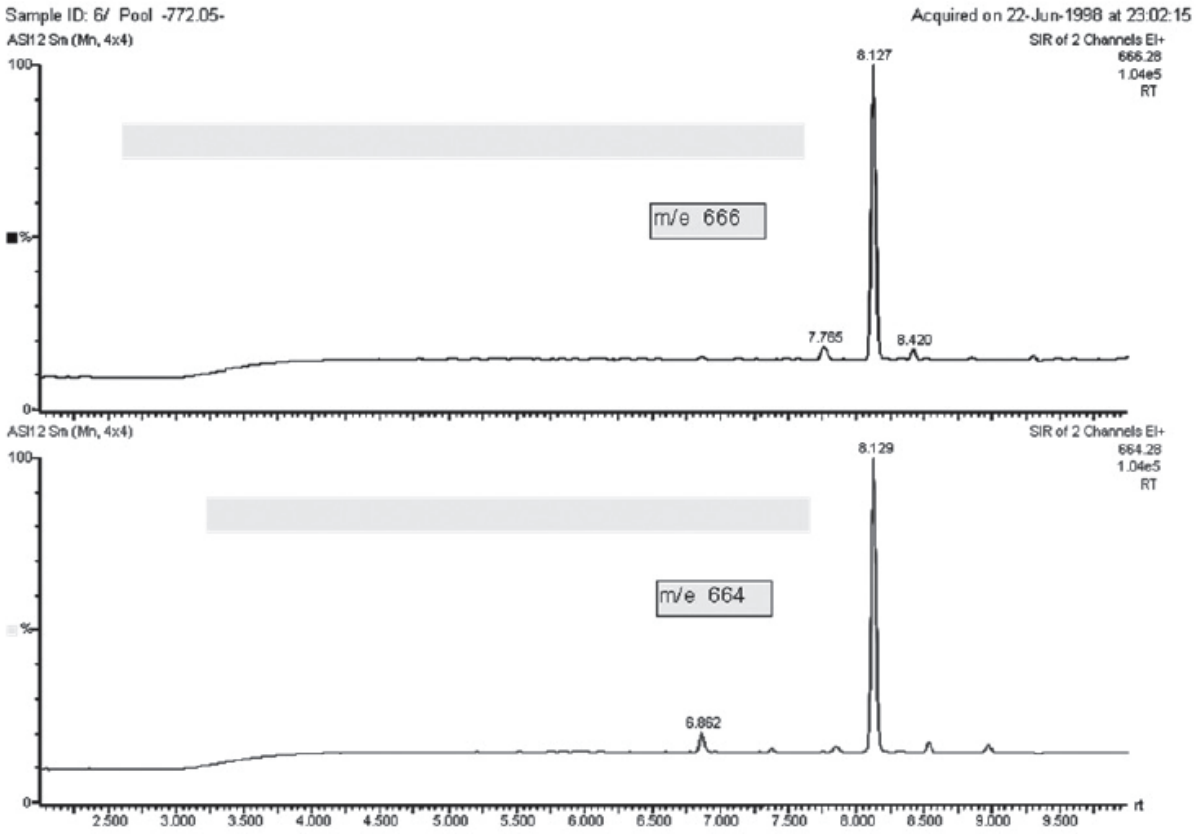
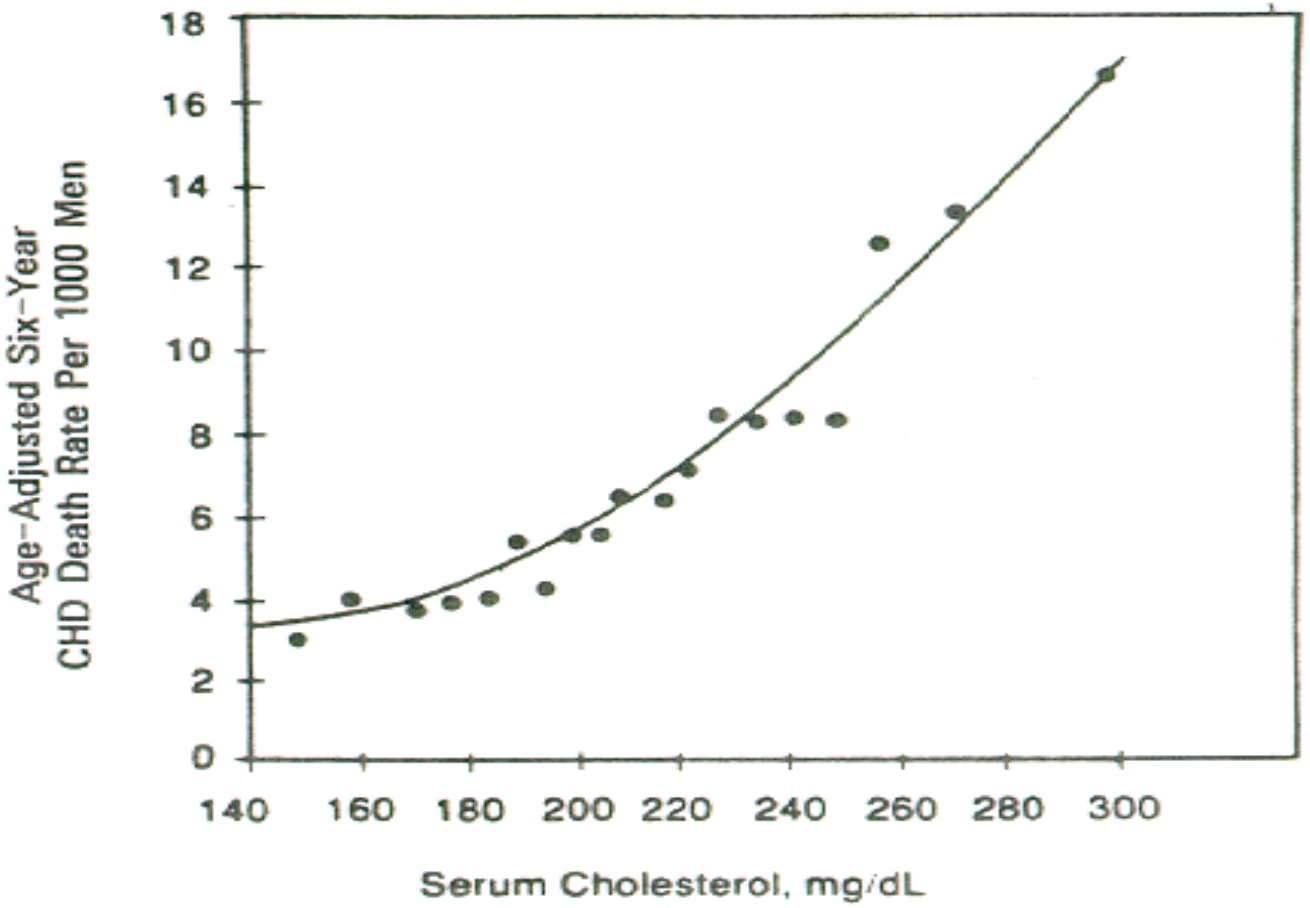


Figure 2. IDMS the selected ion recording at m/z 664 and 666 after processing a serum sample and formation of the Heptafluorobutyric ester derivative for measurement of estradiol-17β.
Siekmann L. Clin Biochem Rev 28, 149-154, 2007

Cardiovascular Biomarkers

- Screening biomarkers-people at risk for disease
  - Cholesterol, LDL, HDL, hsCRP
- Diagnostic biomarkers-people suspected of having disease
  - TnI, TnT, CPK, CK-MB, BNP, NT-BNP
- Prognostic biomarkers-patients with previously diagnosed diseases
  - TnI, CK, BNP, urinary albumin/Creatinine ratio, hsCRP

SERUM CHOLESTEROL : CHD Death



From Gotto AM Jr, et al. Circulation 81:1721-1733, 1990

Cholesterol

- National Cholesterol Education Program
  - NHLBI 1985
  - Educate medical community
  - Risk factors for CHD
  - Medical decision points 200 and 240 mg/dl
  - Complex molecular structure
  - Use of processed materials impacted by matrix effects

Matrix

- Sample Matrix
  - All components of sample except analyte
- Matrix effect
  - Influence of sample matrix on measured value
  - Chemical
    - Due to analytical method
  - Physical
    - Viscosity
    - Surface tension
    - Patient sample differs from control
  - Distinguish between specificity and matrix effect
    - Different epitopes for immunoassays

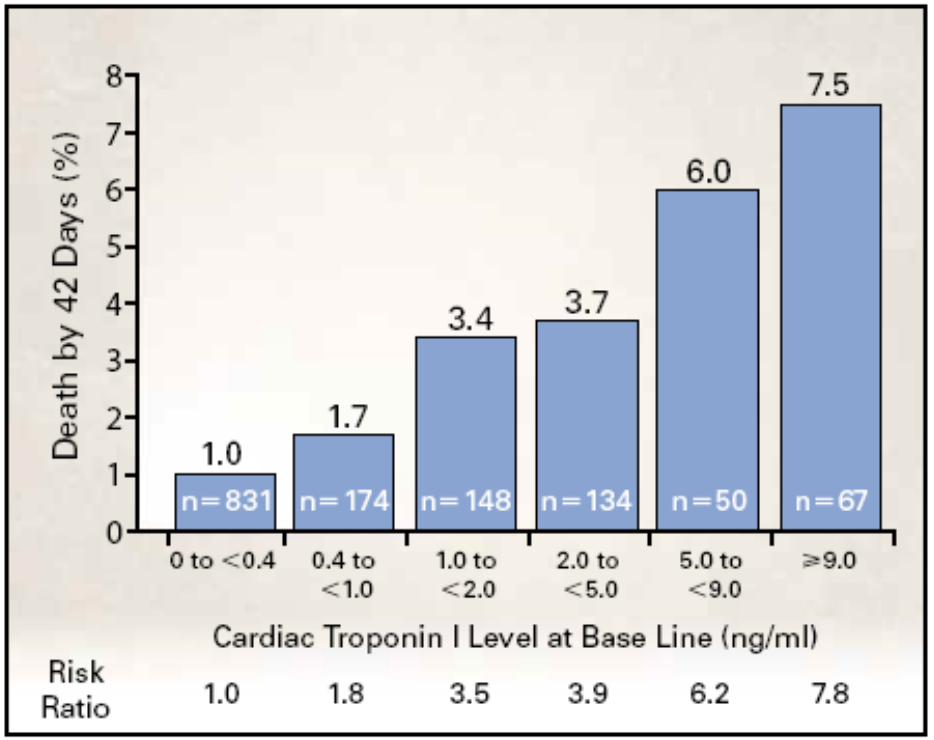
Cholesterol

- Cholesterol Reference Method Laboratory Network – CDC 1990
  - Reference services to manufacturers
  - Based on fresh samples from patients
  - Minimum 40 patient samples in duplicate by test and reference method
  - Sample stability protocol
  - Total cholesterol
  - HDL cholesterol
  - LDL cholesterol

www.cdc.gov/labstandards

Current Status: cTnI and cTnT

- Cardiac troponin I and T are released during cellular injury of the heart
- cTnI and cTnT have generally replaced CK/CK-MB as preferred markers of cardiomyocyte necrosis
- Because of their high sensitivity and near specificity cTnI and cTnT were adopted as preferred biomarkers for diagnosis of MI



Antman E. M. NEJM 346,2079-2082 (2002)

Complex molecular Structure

- Troponin I and T
  - Cardiac biomarkers released during cellular injury of the heart
- NIST collaborated with IFCC and AACC to develop a Standard Reference Material (SRM)
- Several SRM's with different forms of Troponin were evaluated by commercial manufacturers of IVD assays
- A SRM with Troponin I, T and C had assay performance similar to patient samples and SRM 2921 was released in 2004

Certification of SRM 2921

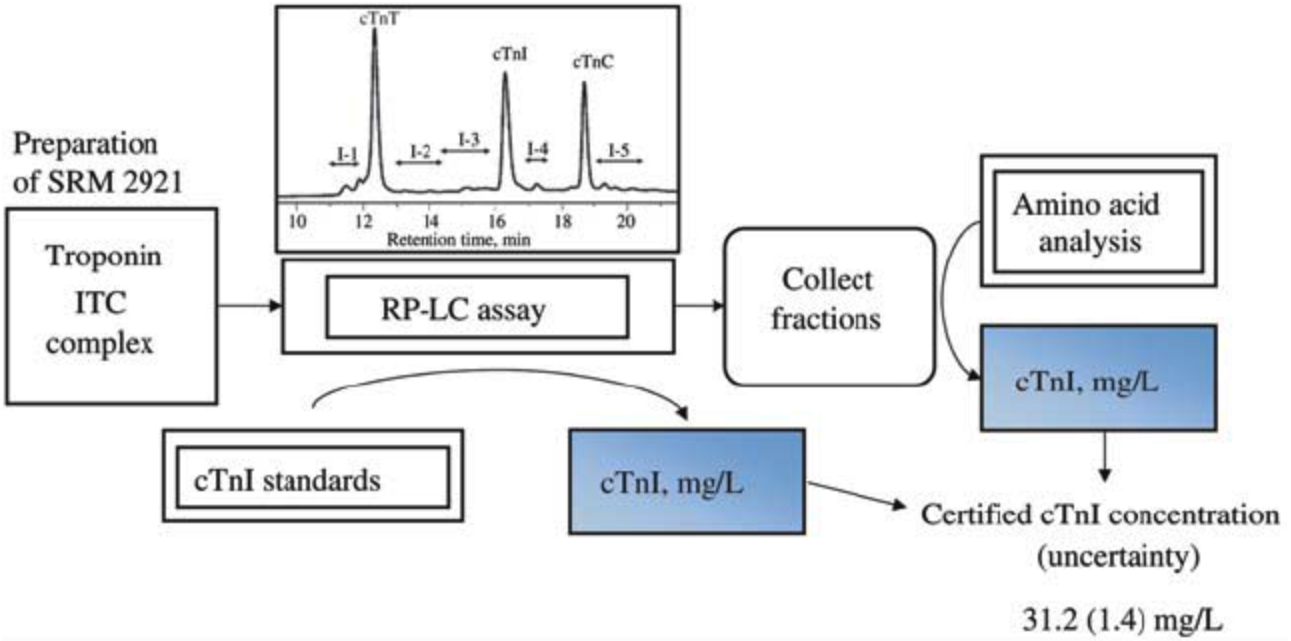
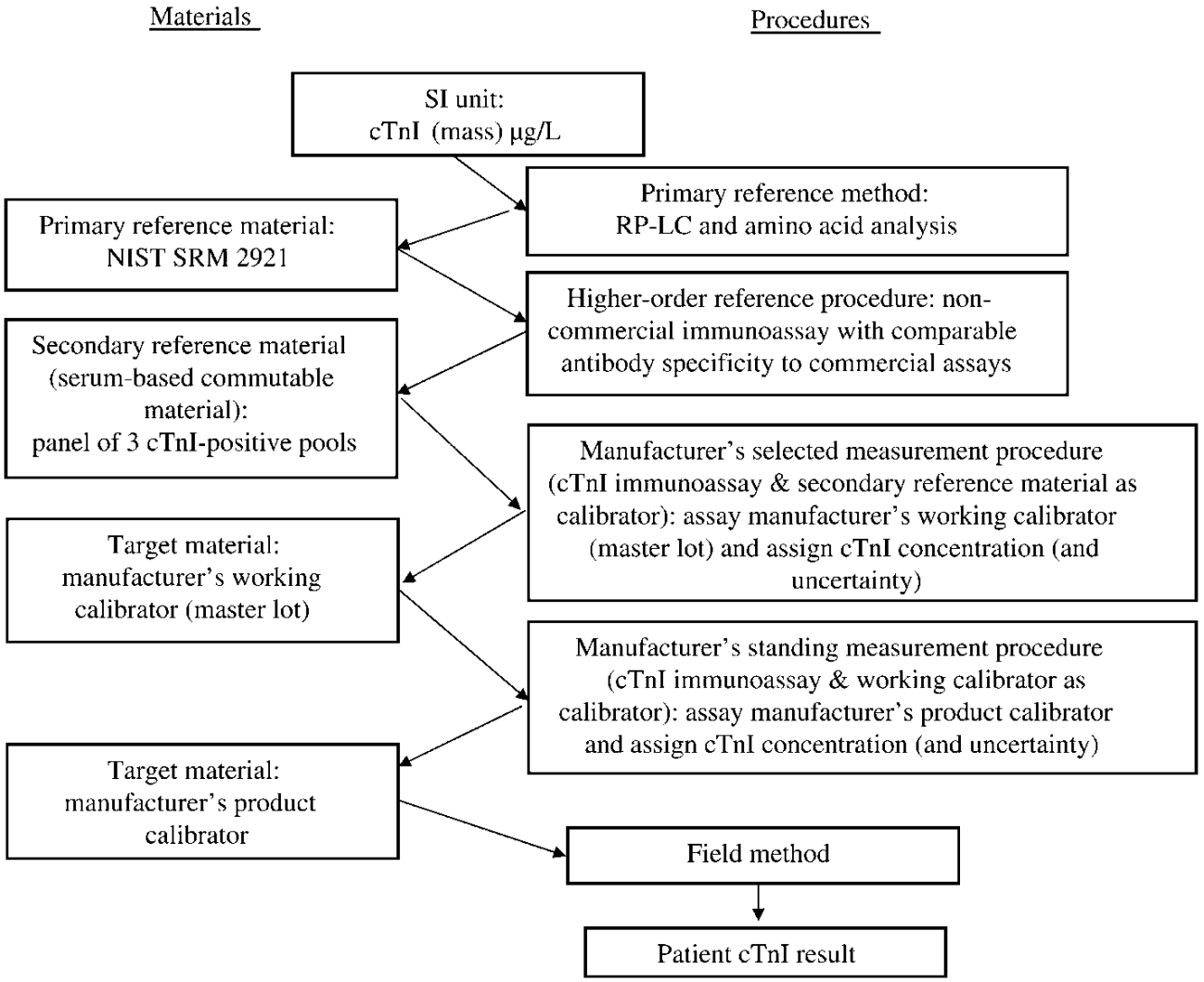


Figure 1 Steps of the certification analysis of the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2921 – human cardiac troponin complex. RP-LC, reversed-phase liquid chromatography; cTnI, cardiac troponin I.

Panteghini et al, Clin Chem Lab Med 46, 1501-1506, 2008

Standardization of Cardiac Troponin



Panteghini et al, Clin Chem Lab Med 46, 1501-1506, 2008

Commutability

- Equivalence of results for different methods
- Method specific
- Normal/Diseased samples
- No measurement bias between selected Reference Measurement Procedures
- Different labs

Laboratory Standardization

- Results are accurate
- Independent of:
  - Reference Measurement Procedures
  - Location
  - Time of testing

Laboratory Quality Assurance

- Establishes Standard Operating Procedures
  - Sample Handling
  - Instrument Calibration/Validation
  - Assay Protocols
- Administrative Requirements
  - Record Keeping
  - Data Evaluation
  - Internal Audits
- Corrective Actions
  - Documentation
  - Responsible Employees

Standardization Program

- Reference measurement procedures
- Reference materials
- Lab results traceable to Reference Materials and Reference Measurement Procedures

Center for Disease Control

- Customized quality assurance and standardization programs
  - Reference materials
  - Proficiency testing
  - Training
  - Guidelines
  - Consultations
  - “Most free of charge”

www.cdc.gov/labstandards

Worldwide Reference Measurement Services

- 2002- Joint Committee for Traceability in Laboratory Medicine (JCTLM)
  - International Federation of Clinical Chemistry (IFCC)
  - Bureau International des Poids et Mesures (BIPM)
  - International Laboratory Accreditation Cooperation (ILAC)
- Develop and maintain a database
  - Certified reference materials, measurement methods/procedures
  - Worldwide laboratories providing reference measurement services

JCTLM Biomarker Categories

- Blood Gases
- Coagulation factors
- Electrolytes
- Metabolites/substrates
- Non-electrolyte metals
- Nucleic acids
- Vitamins/micronutrients
- Blood grouping
- Drugs
- Enzymes
- Microbial serology
- Non-peptide hormones
- Proteins
- Other compounds

JCTLM Database

- 200 certified reference materials for 130 measurands
- 125 reference measurement methods/procedures for 76 IVD biomarkers
- Designed to meet ISO 15194 and 15193 criteria
  - Must be verified by manufacturer

Summary

- Biomarkers
- Reference Measurement Systems
  - Measurand
  - Measurement procedures
  - Reference Materials
- Matrix Effects
- Commutability
- Laboratory Standardization
- Measurement Services