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Centro Interdipartimentale per la Riferibilità Metrologica in Medicina di Laboratorio (CIRME)

under the auspicies of the

IFCC International Federation of Clinical Chemistry and Laboratory Medicine

JCTLM The Joint Committee for Traceability in Laboratory Medicine

3rd International Scientific Meeting

STANDARDIZATION OF PROTEIN BIOMARKER MEASUREMENTS: NEW INITIATIVES FOR REFERENCE MEASUREMENT SYSTEMS

Milano, 17 November 2009

Current approaches for standardization of cardiac troponin I measurements

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Centro Interdipartimentale per la Riferibilità Metrologica in Medicina di Laboratorio (CIRME)

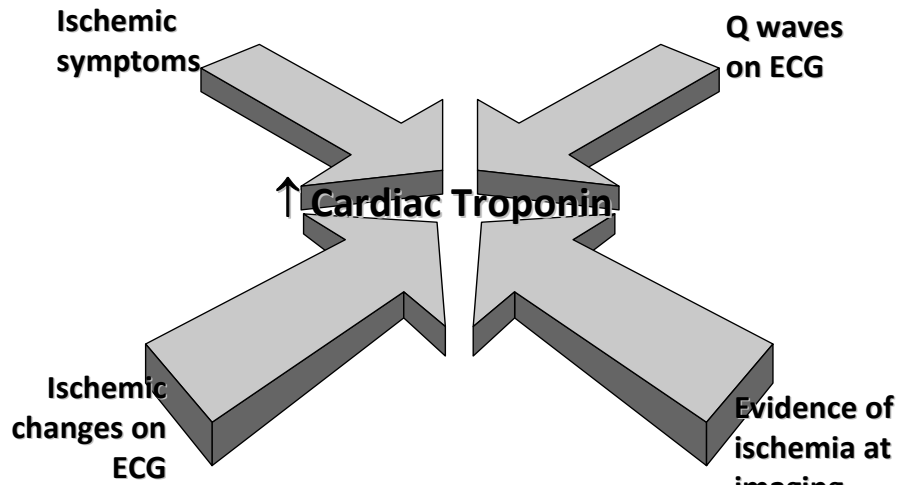
Mauro Panteghini

University of Milan Medical School

Centre for Metrological Traceability in Laboratory Medicine

Milano, IT

ESC-ACCF-AHA-WHF Universal Definition of Myocardial Infarction



Circulation 2007;116:2634

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The benefit of standardization of troponin measurements

Interchangeability of results over time and space would significantly contribute to improvements in healthcare, since results of clinical studies undertaken in different locations or times could be universally applied

Standardize clinical decision limits (i.e., cutpoints for intervention)

↓

Effective application of evidence-based medicine

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List of available quantitative cTnI assays

- Abbott AxSYM
- Abbott Architect
- Abbott i-STAT
- Beckman Access
- BioMerieux Vidas
- Bio-Rad BioPlex 2200
- Brahms Kryptor
- DiaSorin Liaison
- Innotrac AIO!

- Inverness (Biosite) Triage
- Mitsubishi Pathfast
- Ortho Vitros ECi
- Radiometer AQT90 Flex
- Randox Evidence
- Response Biom. RAMP
- Siemens Centaur
- Siemens Dimension RxL
- Siemens Immulite
- Siemens Stratus CS
- Tosoh AIA 21 & 600 II

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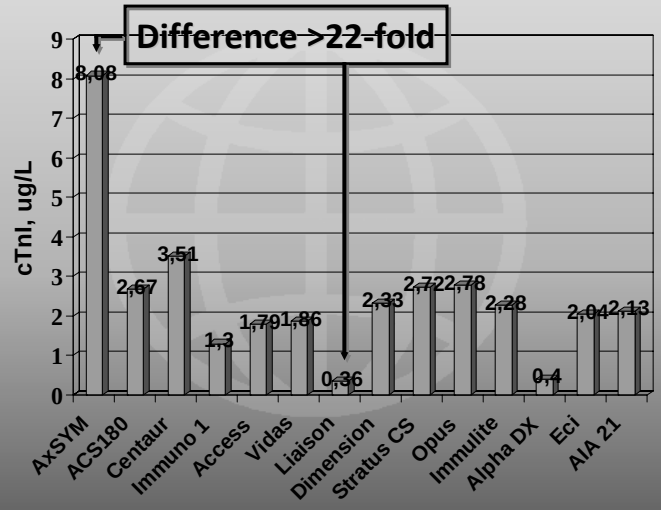
Mean troponin I concentrations in pool H



International Federation Of Clinical Chemistry And Laboratory Medicine

Panteghini M et al., Clin Chem 2004

99th centile decision limits of commercial troponin I assays as stated by manufacturers

Company/platform/assay (generation)	99th centile, µg/L
Abbott AxSYM ADV (2nd)	0.040
Abbott Architect	0.028
Abbott i-STAT	0.080
Beckman Access AccuTnI (2nd)	0.040
BioMerieux Vidas TnI-Ultra (2nd)	<0.010
Innotrac Aio!	0.025
Inverness Biosite Triage	<0.050
Mitsubishi Chemical PATHFAST	0.029
Ortho Vitros ECi (2nd)	0.034
Response Biomedical RAMP	<0.100
Radiometer AQT90	0.023
Siemens Centaur TnI-Ultra (2nd)	0.040
Siemens Dimension RxL (2nd)	0.070
Siemens Immulite 2500 STAT	0.200
Siemens Stratus CS	0.070
Siemens VISTA	0.045
Tosoh AIA (2nd)	<0.060

Difference ~20-fold

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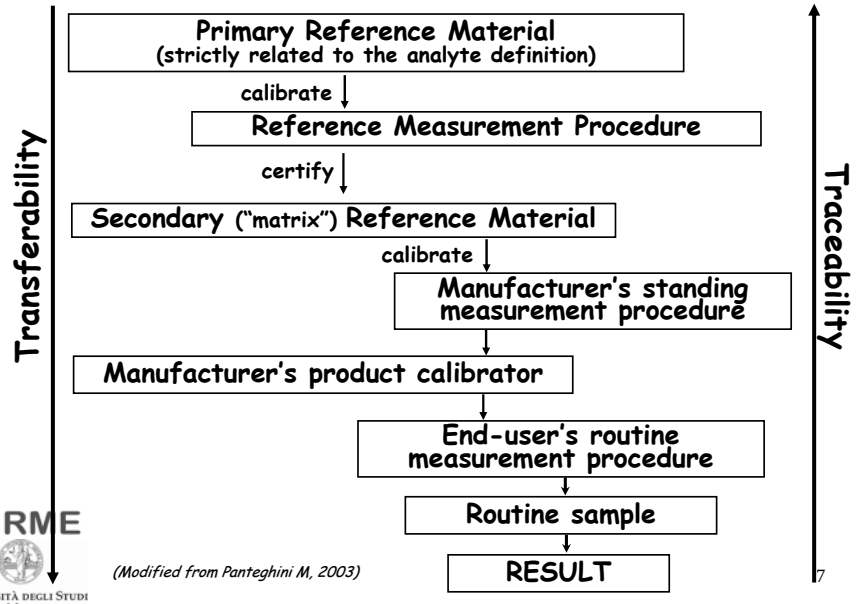
Hypothetical Reference Measurement System for Cardiac Markers



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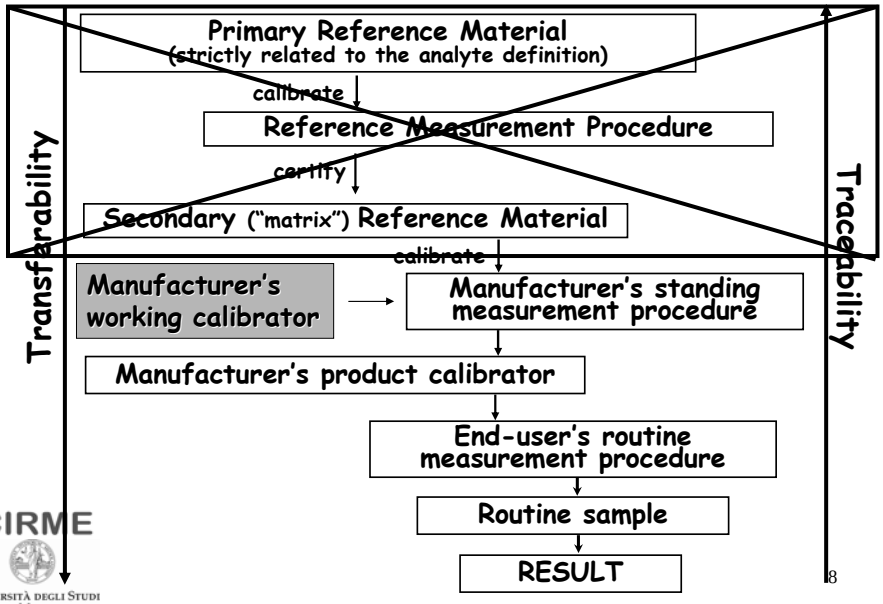
(Modified from Panteghini M, 2003)

Hypothetical Reference Measurement System for Cardiac Markers



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Components of a Working Reference Measurement System

- Clear definition of the analyte to be measured in human samples
- Reference measurement procedure(s) which specifically measures the analyte as defined
- Primary and secondary (commutable) reference materials
- Reference measurement laboratories, possibly collaborating in a network

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Panteghini M, Clin Biochem Rev 2007

Definition of the analyte "Cardiac Troponin I"

It should be decided whether it refers to:

> a mixture of different forms, i.e. free and complexed with troponin C and T, or to only one prevalent form;

> composition classes
(in terms of oxidation, phosphorylation, etc.);

> content classes
(in terms of % of phosphorylation, etc.).

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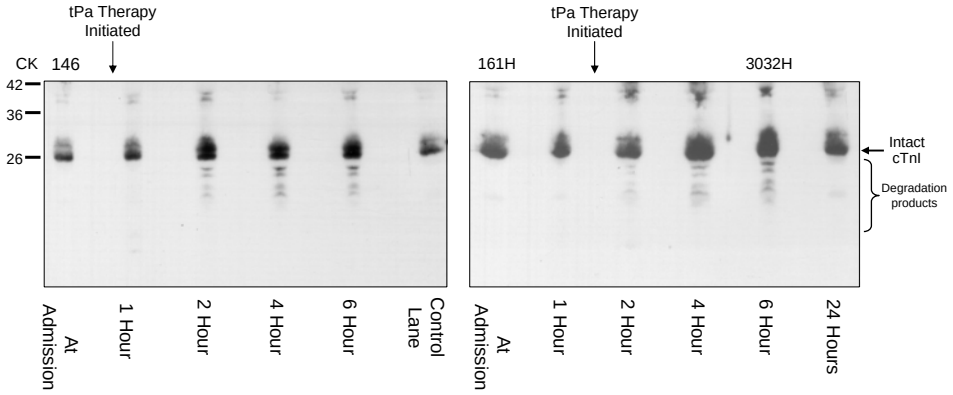
Panteghini M, Clin Chem Lab Med 2004

Troponin I Degradation Products in Serum of Patients with AMI



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Courtesy of J.E. Van Eyk

When is a heterogeneous analyte more like a SI-traceable quantity?

Answer:

When we can find a structural "common denominator" that eliminates the structural heterogeneity present in the intact analyte

Such "common denominators" could be *specific amino acids* of the protein analyte or *peptides* derived from it.

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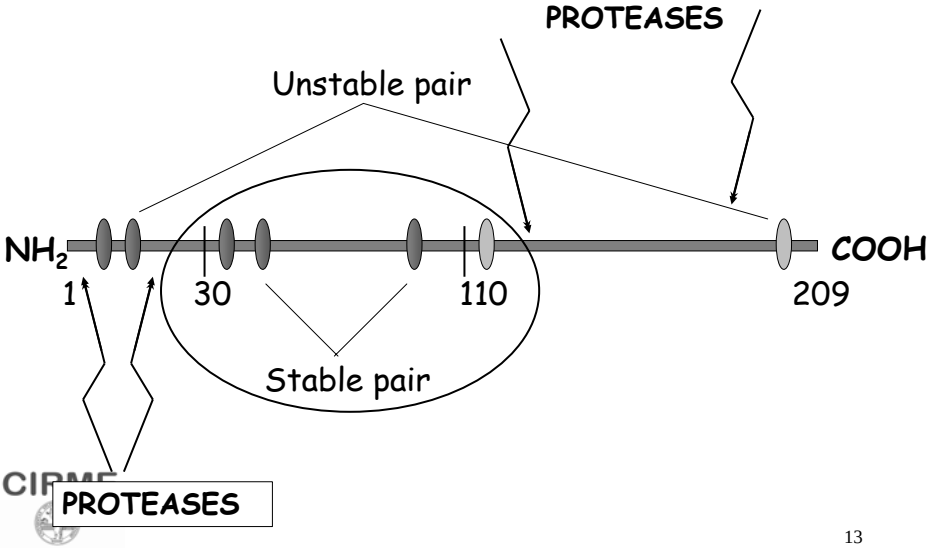
Cardiac troponin I molecule



"Quality Specifications for Cardiac Troponin Assays"
Mauro Panteghini, Willie Gerhardt, Fred S. Apple, Francesco Dati, Jan Ravkilde, and Alan H. Wu
Clin Chem Lab Med 2001;39:174-8

Recommendation

Antibodies used for the development of reliable cardiac troponin I assays should preferably recognize epitopes that are located in the stable part of the molecule and are not affected by complex formation (such as ICT) and other in vivo modifications

cTnI Assay System	Antibody specificity: a.a. residues	
Abbott AxSYM/Architect	MAb1 (capture) MAb2 (capture) MAb3 (detection)	24-40 87-91 41-49
Beckman Access AccuTnI	MAb1 (capture) MAb2 (detection)	24-40 41-49
BioRad BioPlex 2200	MAb1 (capture) MAb2 (capture) MAb3 (detection)	31-34 41-47 88-94
Diasorin Liaison	PAb1 (capture) MAb2 (detection)	27-39 80-110
Innotrac AIO	MAb1 (capture) MAb2 (capture) MAb3 (detection)	41-49 190-196 137-148
Mitsubishi Pathfast	MAb1 (capture) MAb2 (detection) MAb3 (detection)	41-49 71-116 163-210
Ortho Clinical Diagn. ECi	MAb1 (capture) MAb2 (capture) MAb3 (detection)	24-40 41-49 87-91
Randox Evidence	MAb1 (capture)-MAb2 (detection)	87-91
Siemens Dimension/Stratus CS	MAb1 (capture) MAb2 (detection)	27-32 41-56
Siemens ADVIA Centaur	MAb1 (capture) MAb2 (capture) PAb3 (detection)	41-49 87-91 27-40
Siemens Immulite 2500	MAb1 (capture) MAb2 (detection)	24-40 80-110
Tosoh AIA	MAb1 (capture) MAb2 (detection)	41-49 87-91

IFCC

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IFCC

Antibody specificity according to the IFCC recommend.

Panteghini M
Clin Chim Acta 2009

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"Quality Specifications for Cardiac Troponin Assays"
Mauro Panteghini, Willie Gerhardt, Fred S. Apple, Francesco Dati, Jan Ravkilde, and Alan H. Wu
Clin Chem Lab Med 2001;39:174-8

Recommendation

The assays should be calibrated against the material representing the natural and major form of the antigen present (as a complex) in blood after tissue release.

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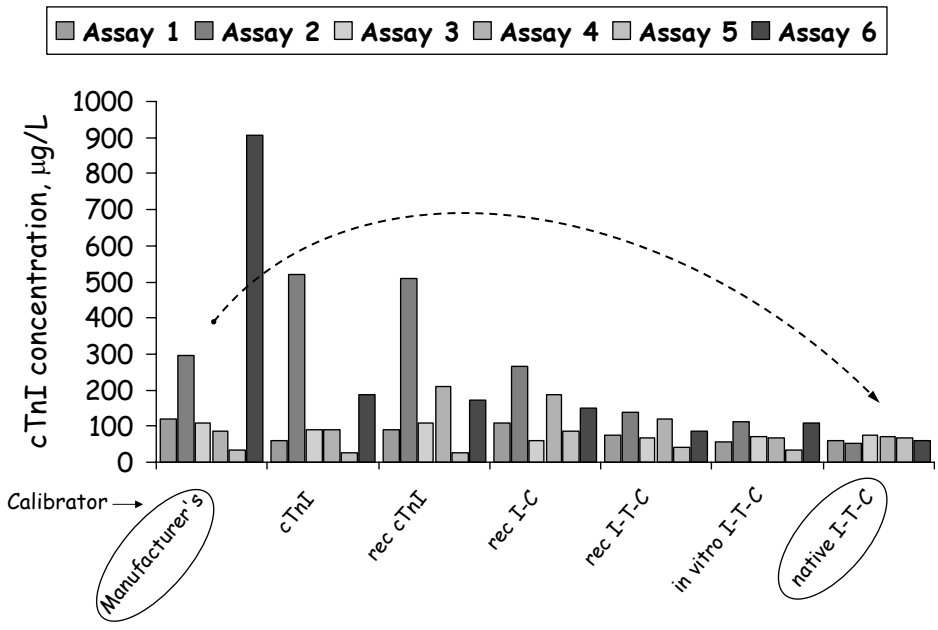


Katrukha A et al., 1999

AACC TnI Committee (in conjunction with NIST and IFCC):

Development of SRM 2921 cardiac troponin complex

Process steps

1. **Acquire samples of candidate reference materials (#10)**
2. **Characterize materials by mass spectrometry (NIST)**
3. **Conduct round-robin exercises with assay manufacturers**
4. **Evaluate results and select the best material suitable for standardization**
5. **Characterize the selected material as to different troponin forms present (NIST)**
6. **Value assign concentration of cardiac troponin I (NIST)**

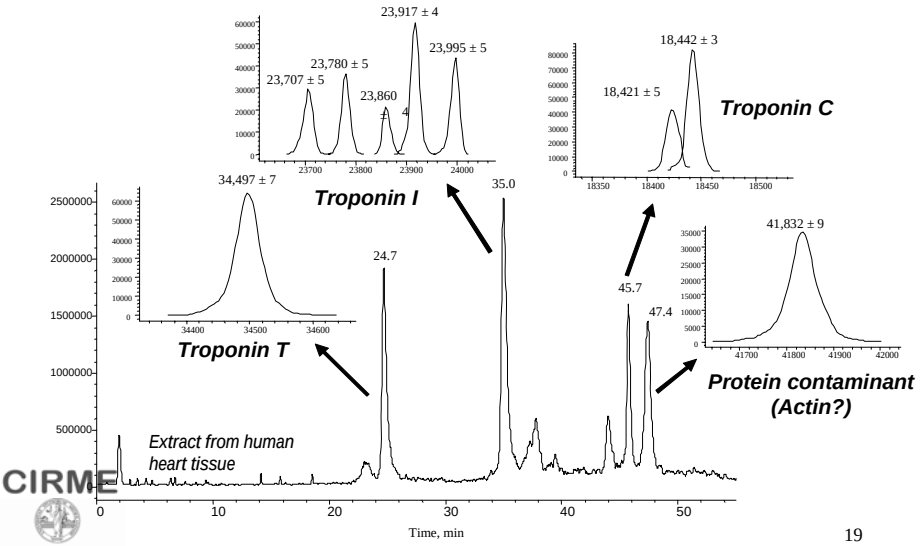
National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2921

Human Cardiac Troponin Complex

LC/MS analysis of SRM 2921



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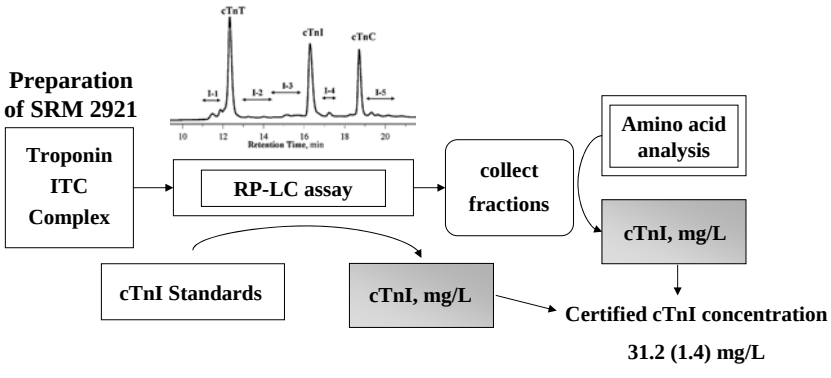
Certification analysis of NIST SRM 2921



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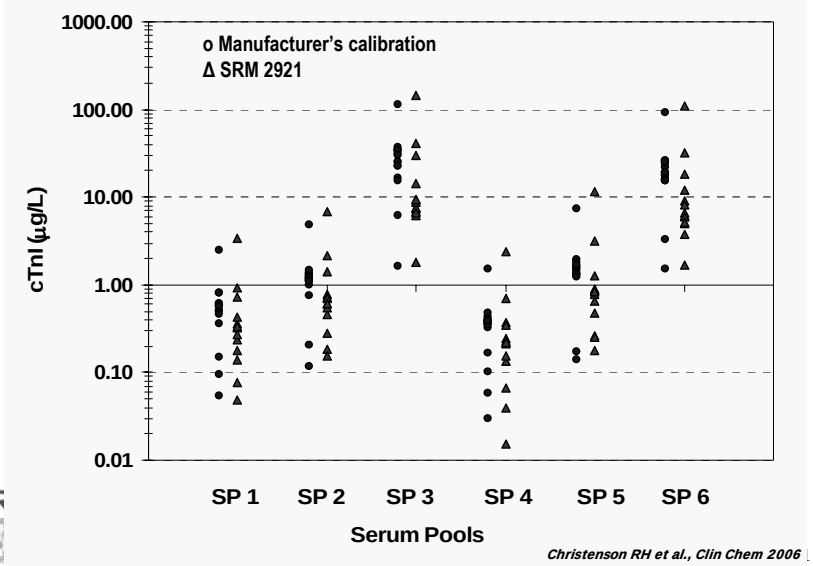
Bunk DM & Welch MJ, Clin Chem 2006

Panteghini M, Bunk DH, Christenson RH, et al, Clin Chem Lab Med 2008

Use of SRM 2921 as common calibrator did not improve cTnI comparability



Christenson RH et al., Clin Chem 2006

NIST SRM 2921
Primary reference material

Pure analyte (human purified protein) with values assigned by mass determination/calculation.

This reference material can be only a surrogate for the analyte measured in human samples, representing only an "average" condition.

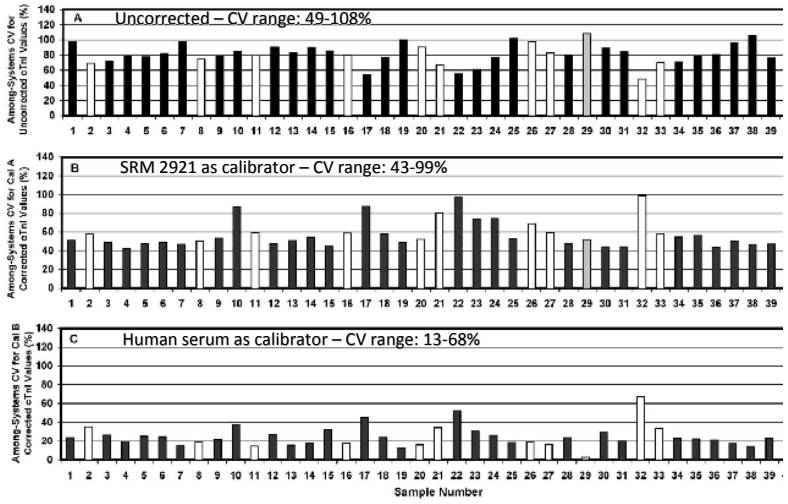
Commutability of Reference Materials

- **Ability of a material to show interassay properties similar to those of human samples**
- **Only commutable materials can be used for direct value assignment to manufacturers' calibrators, having great importance to ensure an unbroken traceability chain**

Hierarchy of Reference Materials for Immunoassays

- **Primary reference material: pure analyte (recombinant or human purified protein), with values assigned by mass determination/calculation**
- **Secondary reference material: matrixed, with values assigned by a reference procedure against the primary material**
  - **Pool of human sera spiked with the corresponding purified antigen**
  - **Pool of human sera containing the corresponding antigen ("native") in detectable concentrations**

Dati F, Panteghini M, Apple FS et al, Scand J Clin Lab Invest 1999
Panteghini M, Bunk DH, Christenson RH et al, Clin Chem Lab Med 2008



Tate JR et al., Clin Chim Acta 2002

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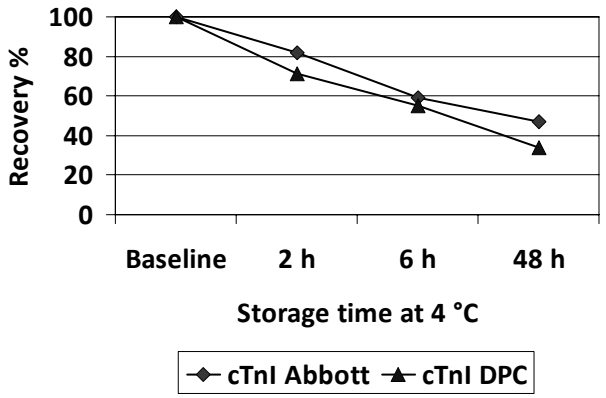
Dati F, Panteghini M, Apple FS et al, Scand J Clin Lab Invest 1999
Panteghini M, Bunk DH, Christenson RH et al, Clin Chem Lab Med 2008

Time-dependent instability of cTnI in human pools spiked with NIST SRM 2921



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Cobbaert CM et al., Clin Chem 2008

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Dati F, Panteghini M, Apple FS et al, Scand J Clin Lab Invest 1999
Panteghini M, Bunk DH, Christenson RH et al, Clin Chem Lab Med 2008

Candidate cTnI Commutable Secondary Reference Materials

- 3 pooled cTnI positive serum samples from AMI subjects with cTnI around clinically relevant concentrations (multi-level: high ≈10 μg/L, medium ≈1 μg/L, low ≈0.1 μg/L)
- Production of at least an estimated 5-year supply for each pool (~5000 vials)
- Assessment of homogeneity and stability

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Requirements for a Higher-Order (Reference) Immunochemical Procedure for cTnI

- Non-commercial sandwich-based ELISA:
  - based on mAbs directed against epitopes that can be considered stable from the point of view of stereochemical inhibition of the binding
  - comparable antibody specificity with the last-generation commercial assays (invariant part of the molecule)
  - optimised for standard assignment, rather than ultra-sensitive detection (dynamic range: 0.1 to 10 μg/L)
  - calibrated with NIST SRM 2921
- Thorough definition of assay characteristics including:
  - antibody specificity
  - immunoreactivity to cTnI forms present in serum
  - detection limit and uncertainty

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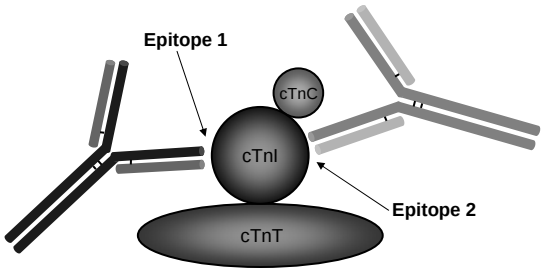
Panteghini M, Bunk DH, Christenson RH et al, Clin Chem Lab Med 2008

The higher-order “reference“ microplate-based ELISA for cTnI



capture

1 ADGSSDAAREPRPAPAP IRRRSSNYRAYATEPHAKKKSK ISASRKLQLKT 50

detection

51 LLLQIAKQELEREAEERRGEKGRALSTRCQPLELAGLGFAELQDLCRQLH 100

101 ARVDKVDEERYDIEAKVTKNITEIADLTQKIFDLRGKFKRPTLRRVRISA 150

151 DAMMQALLGARAKESLDLRAHLKQVKKEDTEKENREVGDWRKNIDALSGM 200

201 EGRKKKFES 209

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IFCC WG-TNI Pilot Study: AIMS

1. To compare the candidate immunoassay reference measurement procedure for cTnI with commercial assays;
2. To preliminarily evaluate the commutability and stability of candidate secondary reference “blended” serum pools for cTnI.

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IFCC WG-TNI Pilot Study: Participating Laboratories and Assays

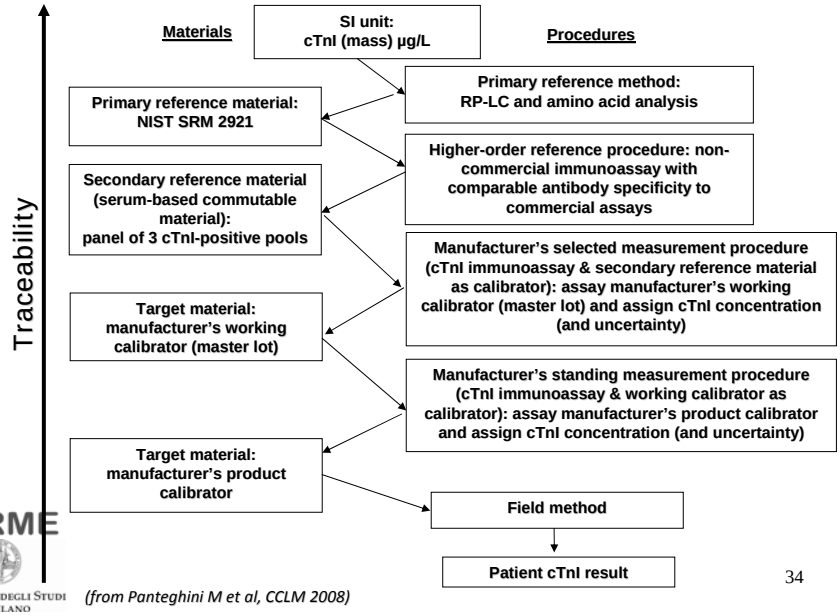
Co-ordinating Laboratory (Baltimore) - to select appropriate samples, and with NIST, to prepare serum pools

ELISA RMP: UK NPL & US NIST

Diagnostic Industry – Instruments:

- ABBOTT – AxSYM, Architect, i-STAT, New gen assay
- BECKMAN COULTER - Access
- bioMerieux – Vidas
- MITSUBISHI CH. – Pathfast
- OLYMPUS AMERICA INC - AU3000i
- ORTHO-CLINICAL DIAGNOSTICS - Vitros ECi/ECiq
- SIEMENS – Centaur XP, Centaur CP
- SIEMENS – Immulite 2000, Immulite 2500
- SIEMENS – Stratus CS, Dimension RxL, Vista, ExL
- ROCHE DIAGNOSTICS – E 170

Suggested approach for the standardization of cTnI measurements through traceability implementation to the reference measurement system



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(from Panteghini M et al, CCLM 2008)

Summary

- ~20-fold differences among cTnI method values leading to result discrepancies and frustration to clinicians
- Lack of cTnI standardisation despite introduction of the primary reference material SRM 2921 (complexed ITC in buffer) due to (non)commutability issue
- Standardization requires a reference measurement system to link higher-order reference methods and reference materials to routine calibrators and procedures used in clinical laboratories ('unbroken traceability chain')

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