Standardization and Harmonization of Cardiac Troponin I Assays:

Where have we been? Where are we now? Where are we going?

Robert H. Christenson, Ph.D., DABCC, FACB
Professor of Pathology
Professor of Medical and Research Technology
Director, Core Laboratory
University of Maryland School of Medicine

Cardiac Troponin is the Cornerstone

NACB Clinical Guidelines for ACS

2007 Clin Chem and Circulation

Class I

- **Cardiac troponin is the preferred marker for the diagnosis of MI. CK-MB by mass assay is an acceptable alternative when cardiac troponin is not available (Level of Evidence: A).**

- **In the presence of a clinical history suggestive of ACS, the following are considered indicative of myocardial necrosis consistent with MI (Level of Evidence: C):**
  - **Maximal concentration of cardiac troponin exceeding the 99th percentile of values (with optimal precision defined by total CV≤10%) for a reference control group on at least one occasion during the first 24 hours after the clinical event (Observation of a rise and fall in values is useful in discriminating the timing of injury).**

Class 1A Recommendation

Circulation 2002;106:1893-1900

- An early invasive strategy in patients with UA/NSTEMI without serious comorbidity and who have any of the following high-risk indicators:
  - Recurrent angina/ischemia at rest or with low level activities despite intensive anti-ischemic therapy
  - Elevated cTnI or cTnT *Elevations in cTnI (0.1 ng/mL) and cTnT (0.01 ng/mL) provides superior predictive capability.*
  - New or presumably new ST-segment depression

Where have we been?

Need for standardization/harmonization?

Clin Chem 2001;47:431-437

- **10 ng/mL troponin CIT standard material, value-assigned by NIST, was measured by 13 participating cTnI assays, in duplicate:**

Assay	Value 1	Value 2	Mean
1	143.7	135.7	139.7
2	46.9	47.9	47.4
3	19.9	19.9	19.9
4	49.2	50.1	49.7
5	4.3	4.1	4.2
6	12.5	12.6	12.6
7	7.4	8.9	8.2
8	6.0	6.1	6.0
9	16.5	17.4	17.0
10	12.6	13.2	12.9
11	12.6	12.4	12.5
12	18.3	18.9	18.6
13	17.6	16.9	17.3

Mean: 28.1
SD: 36.4
CV: 130%

- **Within assay reproducibility good, <u>but</u> measurement of 10 ng/mL material yielded results that were >30-fold different!**

AACC cTnI Standardization Committee

- Desired endpoint is reducing inter-method variability
- Possible strategies
  - Define common reference material
  - Establish consensus values for cTnI in “real” patient specimens
  - Combination of SRM and patient specimens to standardize and harmonize cTnI assays

Strategies

- Phase 1
  - Obtain wide variety of candidate Standard Reference Materials (cSRM)
  - Evaluate and narrow field of candidate SRMs
- Phase 2
  - Full characterization of remaining candidate SRMs
  - Evaluate commutability and investigate harmonization
  - Select final SRM
- Phase 3
  - Obtain and archive large amount of SRM
  - Fully characterize SRM
  - Validate performance of SRM
- Phase 4
  - Develop protocol for cTnI standardization
  - Assign values to serum pools traceable to SRM 2921
  - Develop reference immunoassay for troponin I

Phase 1

- Obtain wide variety of candidate Reference Materials (RM)
- Evaluate and narrow field of candidate RMs

Candidate Reference Materials (cRMs)

Evaluation of 10 candidate SRMs: Human or recombinant material CIT complex, CI complex or free cTnI

Designation	cTnI form	Original matrix	Final matrix	Provider
A	CI	Liquid	Original	Spectral Diagnostics, Inc., Toronto, Canada
B	CTI	Liquid	Original	Spectral Diagnostics, Inc.
C	CI	Liquid	Original	University of Miami, Miami, FL
D	CI	Liquid then lyophilized	Reconstituted	Spectral Diagnostics, Inc.
E	CTI	Liquid then lyophilized	Reconstituted	Spectral Diagnostics, Inc.
F	Free I	Lyophilized	Reconstituted	HyTest LTD, Turku, Finland
G	CTI	Lyophilized	Reconstituted	HyTest LTD
H	CTI	Liquid	Original	University of Miami
I	CI	Lyophilized	Reconstituted	University of Miami
J	CTI	Lyophilized	Reconstituted	University of Miami

Clin Chem 2001;47:431-437.

Round Robin 1

cTnI Measurement Methods

–Evaluated 13 troponin I assays from US, Japan, Germany, the UK, and France

cTnI measurement system	Manufacturer
Access, 1st Generation	Beckman Instruments, Inc.
Access, 2nd Generation	Beckman Instruments, Inc.
ACS:180	Bayer Corporation
AIA	TOSOH
AxSYM	Abbott Diagnostics, Inc.
Centaur	Bayer Corporation
Dimension RxL	Dade Behring
Immuno 1	Bayer Corporation
Liaison	Byk-Sangtec Diagnostica
Opus, 2nd Generation	Dade Behring
Stratus II	Dade Behring
Triage	Biosite Diagnostics, Inc.
Vitros ECi	Ortho Clinical Diagnostics

Clin Chem 2001;47:431-437.

Conclusion: Round Robin 1

Clin Chem 2001;47:431-437.

The outcome of this study was that to identify two cRMs that demonstrated the best performance.

Phase 2:

Results of AACC cTnI Standardization Committee

- Evaluate two candidate RMs from Phase 1
  - TIC complex (Human)
  - IC complex (Recombinant)
- Full characterization of candidate SRMs
- 15 different cTnI assays
  - Evaluate commutability
  - Investigate harmonization strategies
- Select final Reference Material

Materials for Phase 2 Study

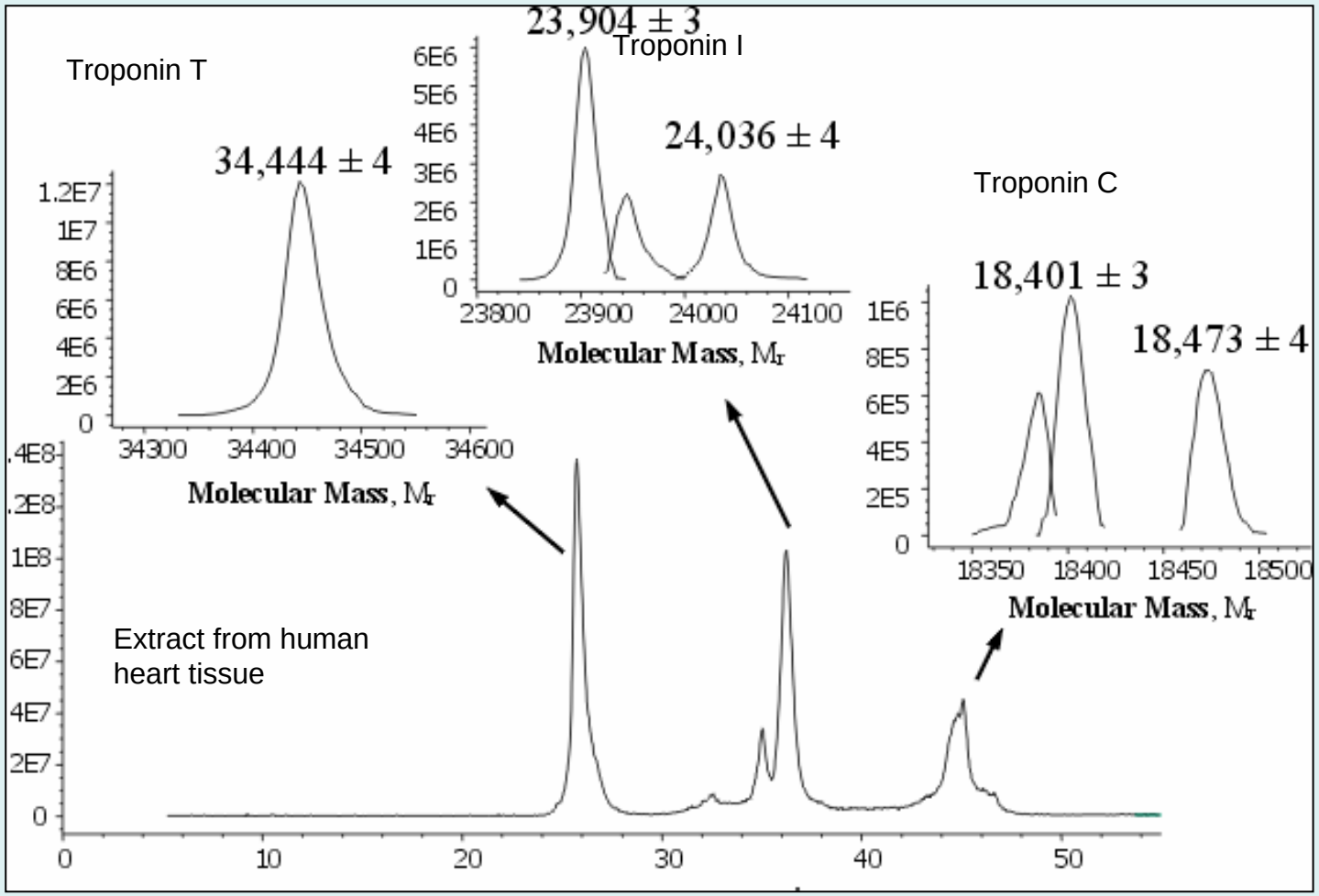
Candidate Standard Reference Materials (cSRMs)

- TIC complex, $cSRM_{TIC}$
- IC complex, $cSRM_{IC}$

Characterized at NIST for purity and concentration.

cTnI-Negative Serum Pool

LC/MS of Cardiac Troponin Complex used in SRM 2921



Six Human Serum Pools

- Pool 1, ~0.4 ng/mL
- Pool 2, ~1.5 ng/mL
- Pool 3, ~15.0 ng/mL
- Pool 4, ~0.4 ng/mL
- Pool 5, ~1.5 ng/mL
- Pool 6, ~15.0 ng/mL

cTnI 2nd Round Robin Participants and Measurement Systems

cTnI Measurement System (15)	Manufacturer (10)
Access	Beckman Coulter Instruments
ACS 180	Bayer Corporation
AIA	TOSOH
Alpha Dx	First Medical Inc
AxSYM	Abbott Diagnostics
Centaur	Bayer Corporation
Dimension RxL	Dade Behring
Immulite 1000	Diagnostic Products Corporation
Immulite 2000	Diagnostic Products Corporation
Immuno 1	Bayer Corporation
Liaison	Byk-Sangtec Diagnostica
Opus, 2nd Generation	Dade Behring
Stratus CS	Dade Behring
Vidas	BioMerieux
Vitros ECi	Ortho Clinical Diagnostics

Commutability Definition:

"degree to which a material yields the same numerical relationships between results of measurements by a given set of measurement procedures applied to those types of material for which the procedures are intended."

European Committee for Standardization (CEN). Draft International Standard ISO/DIS 17511. ISO Central Secretariat, Brussels, Belgium, 2000.

Commutability Assessment

- Calibrate systems with field cTnI methods
- Measure
  - Pools 1-6
  - 1, 5 and 10 ng/mL cSRM samples prepared in negative serum.
- Compare the pool values for each individual cTnI method to each of the other methods
- Calculate regression parameters for each method comparison
  - Method difference for pools (natural materials) determined
- Determine the method difference for the cSRM concentrations
- Compare data from serum pools (natural material) to that of the SRMS with +3/-3 SD criteria
- **Commutability graded as**
  - **YES (1)**
  - **NO (0)**

Degree of Commutability for SRM

A.

	cRM_{CIT} in serum	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	Number of Comparisons Commutable
	A	-	1	0	0	0	1	1	0	0	0	0	1	1	0	0	5
c	B		-	1	0	1	1	1	1	1	1	1	0	1	1	1	11
T	C			-	0	0	0	0	0	0	0	0	0	0	0	0	0
N	D				-	1	1	0	1	1	1	1	0	0	1	1	8
I	E					-	1	0	1	0	0	0	0	0	0	0	2
	F						-	1	1	1	1	0	0	1	1	0	6
M	G							-	1	1	1	1	0	0	0	1	5
E	H								-	1	0	0	0	1	0	0	2
T	I									-	0	0	0	1	0	0	1
H	J										-	1	0	1	0	1	3
O	K											-	1	1	1	1	4
D	L												-	0	0	0	0
S	M													-	0	0	0
	N														-	0	0
	O																

Degree of Commutability is 47 of 105 (45%)

B.

	cRM_{CI} in serum	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	Number of Comparisons Commutable
	A	-	0	0	0	1	0	1	0	0	1	1	0	0	0	1	5
c	B		-	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T	C			-	0	0	0	1	0	0	1	0	1	1	0	0	4
N	D				-	1	0	0	1	1	1	1	0	0	1	1	7
I	E					-	0	1	1	0	1	1	0	0	0	1	5
	F						-	0	0	0	0	0	0	0	0	0	0
M	G							-	1	0	1	0	1	0	0	0	3
E	H								-	1	1	1	0	1	0	1	5
T	I									-	1	1	0	0	0	1	3
H	J										-	1	0	1	0	0	2
O	K											-	1	1	1	1	4
D	L												-	1	1	0	2
S	M													-	1	0	1
	N														-	0	0
	O																

Degree of Commutability is 41 of 105 (39%)

Clin Chem 2006;52:1685-1692

Harmonization

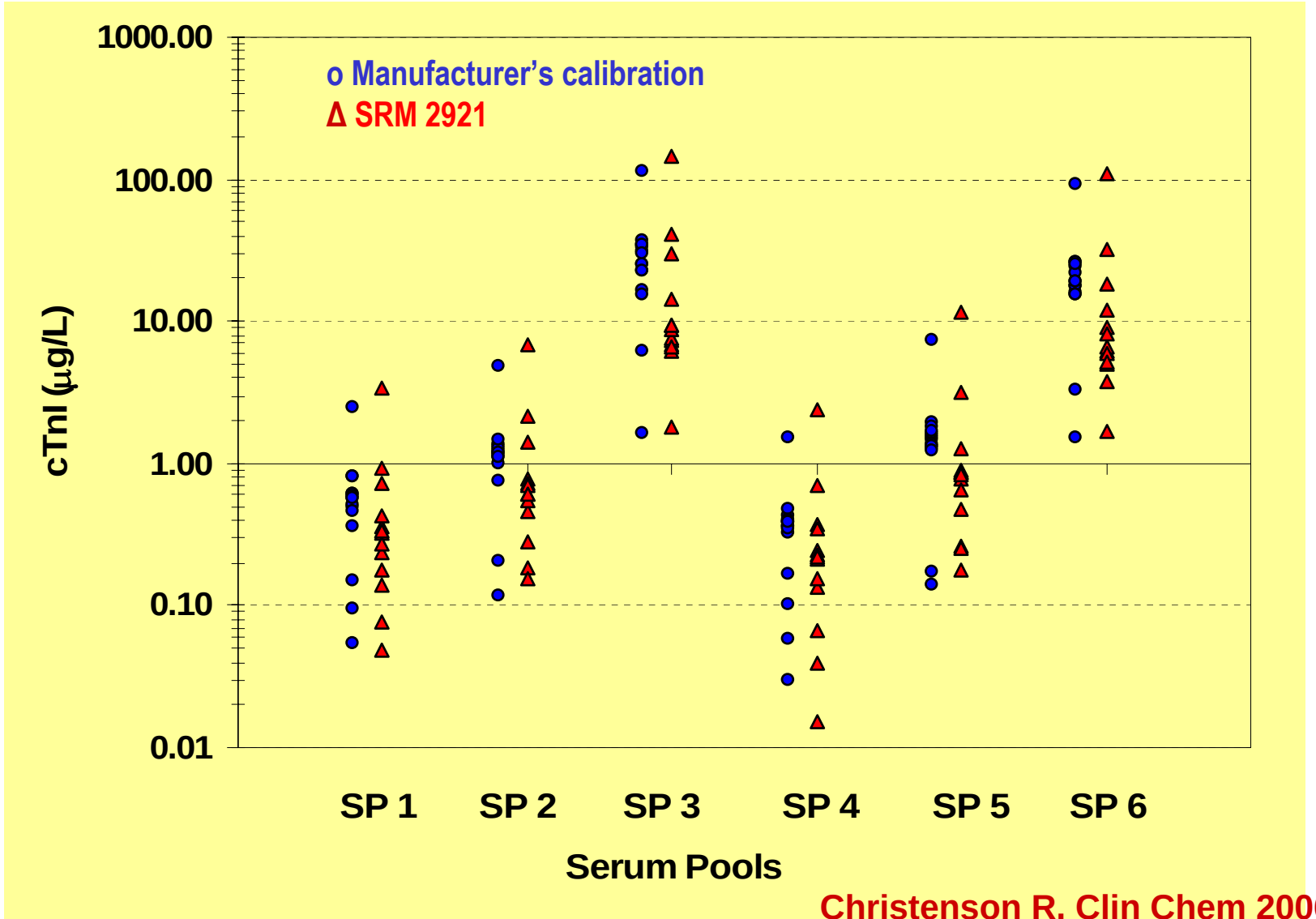
- Agreement between cTnI measurements performed by different methods in the biological matrix (patient serum).

Harmonization: Present State

- Systems calibrated with manufacturers' calibrators and instructions
- Systems calibrated with cSRM
- 6 serum pools measured with each system
- Determine inter-method variability

AACC cTnI Standardization Phase 2

Use of SRM 2921 as the common calibrator did not improve cTnI standardization

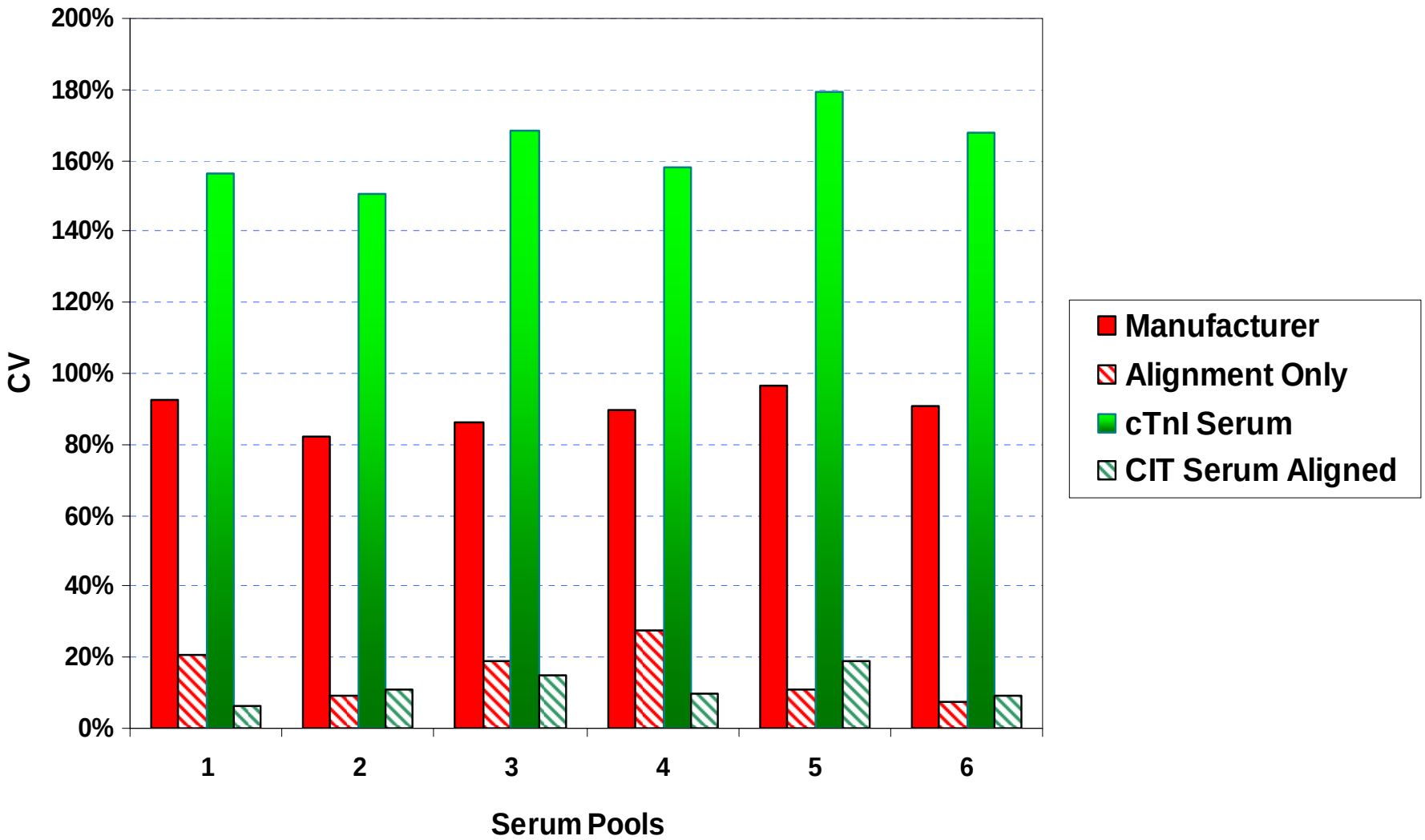


Christenson R. Clin Chem 2006

Harmonization by Alignment

- Calibrate methods (field and cRMs)
- Measure 6 serum pools with each system
- Designate “common comparison” system
- Regression analysis to yield parameters
- Align each serum pool with regression parameters
- Determine inter-method variability of aligned results for each pool

Harminization of cTnI Measurements is Possible



Certification of SRM 2921

- Quantification of troponin I in the troponin TIC complex performed using two methods, LC coupled to amino acid analysis (calibrated using SRM 2389 for SI-traceability) and LC/UV using a purified troponin I primary standard.

- Extensive structural characterization performed using LC/MS for molecular mass and structural heterogeneity evaluation of the intact protein molecules and MALDI/MS on peptide digests of the troponin T, troponin I, and troponin C subunits


2921
2921
Cardiac
2921
2921

Where are we now?

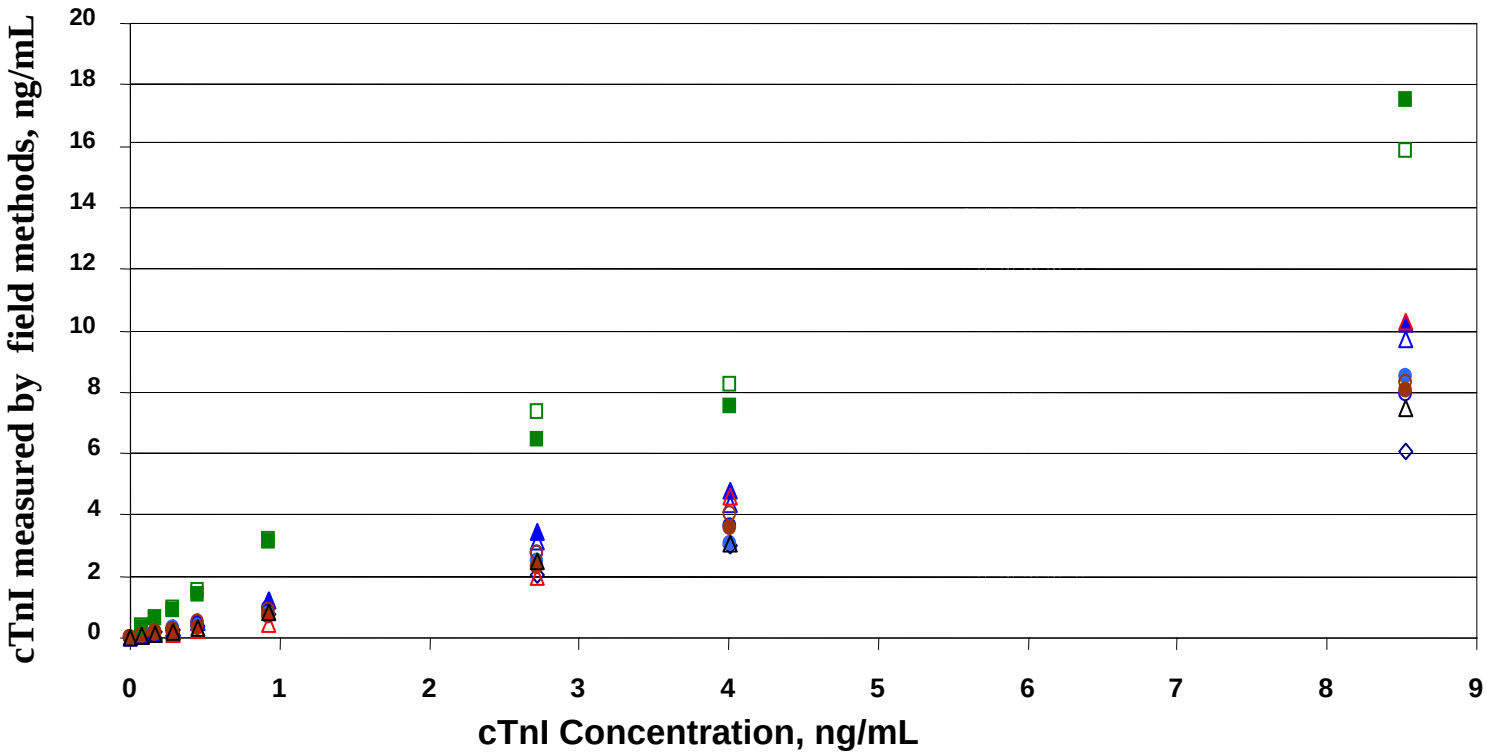
Phase 3

- ***Standard Reference Material 2921***
  - Vials were obtained from NIST, Gaithersburg, MD.
- ***Patient Pools***
  - Eight (8) patient pools were prepared at various cTnI concentrations in accordance with CLIS guidances.
- ***Recovery: Standard Addition Experiment***
  - cTnI positive serum pools were spiked with RM 2921 to gravimetrically produce SRM 2921 target values of 0.5, 1, 2, and 4 ng/mL of cTnI.
- ***Strategy for Stabilizing cTnI Assays with SRM 2921***
  - Characteristic Factor Determined for each cTnI method
  - Validation of RM 2921 Characteristic Factors

Methods Included in Phase 3

Measurement System	Manufacturer
Access 2	Beckman Instruments, Inc
ACS 180	Bayer Corporation
ADVIA Centaur	Bayer Corporation
ARCHITECH System 135	Abbott Diagnostics, Inc.
AxSYM cTnI ADV	Abbott Diagnostics, Inc.
Dimension RxL	Dade Behring, Inc.
IMMULITE	Diagnostic Products Corp
IMMULITE Turbo	Diagnostic Products Corp
Stratus CS	Dade Behring, Inc.
Vidas	BioMerieux
Vitros ECi / ECiQ	Ortho Clinical Diagnostics

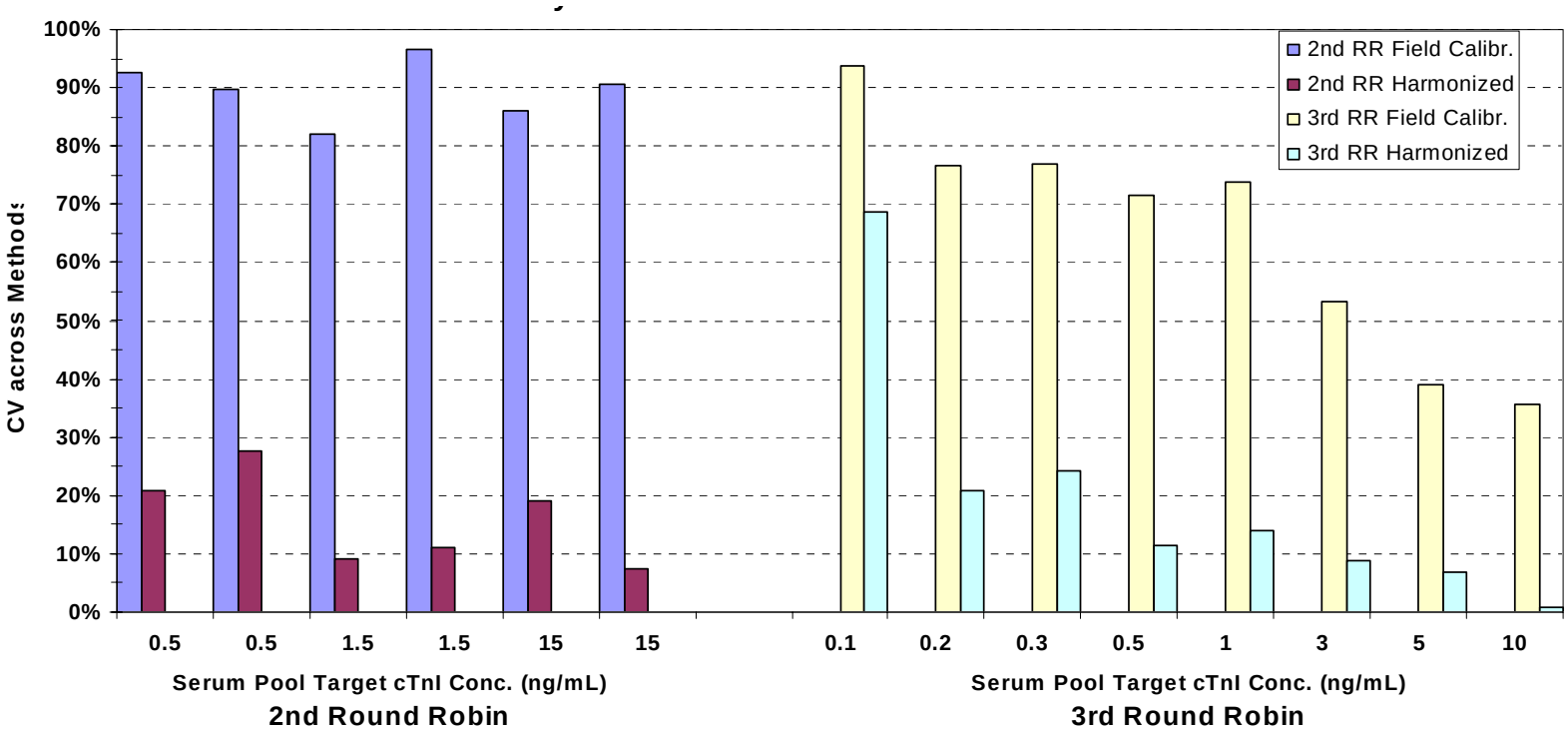
Scatterplot of the 8 serum pools assayed by field methods



After Harmonization

cTnI	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6	Pool 7	Pool 8
Assay	**ng/mL**	**ng/mL**	**ng/mL**	**ng/mL**	**ng/mL**	**ng/mL**	**ng/mL**	**ng/mL**
1	0.08	0.18	0.24	0.42	0.87	2.48	2.48	7.38
2	0.09	0.15	0.25	0.42	0.93	2.52	3.53	7.44
3	0.11	0.17	0.26	0.45	0.95	2.43	3.37	7.53
4	0.04	0.15	0.32	0.47	0.86	2.76	3.33	7.44
5	0.07	0.16	0.25	0.44	0.92	2.59	3.47	7.44
6	0.11	0.20	0.26	0.41	0.78	2.40	3.68	7.42
7	0.00	0.09	0.23	0.45	1.22	2.70	3.19	7.45
8	0.05	0.12	0.23	0.43	1.03	2.77	3.21	7.46
9	0.08	0.18	0.27	0.45	0.84	2.45	3.59	7.44
10	NA	NA	0.47	0.59	0.75	1.97	3.90	7.38
11	0.12	0.20	0.27	0.42	0.92	2.59	3.16	7.56
Mean	**0.07**	**0.16**	**0.28**	**0.45**	**0.91**	**2.52**	**3.47**	**7.45**
CV	**69%**	**21%**	**24%**	**11%**	**14%**	**9%**	**7%**	**1%**

Variability Across cTnI Methods



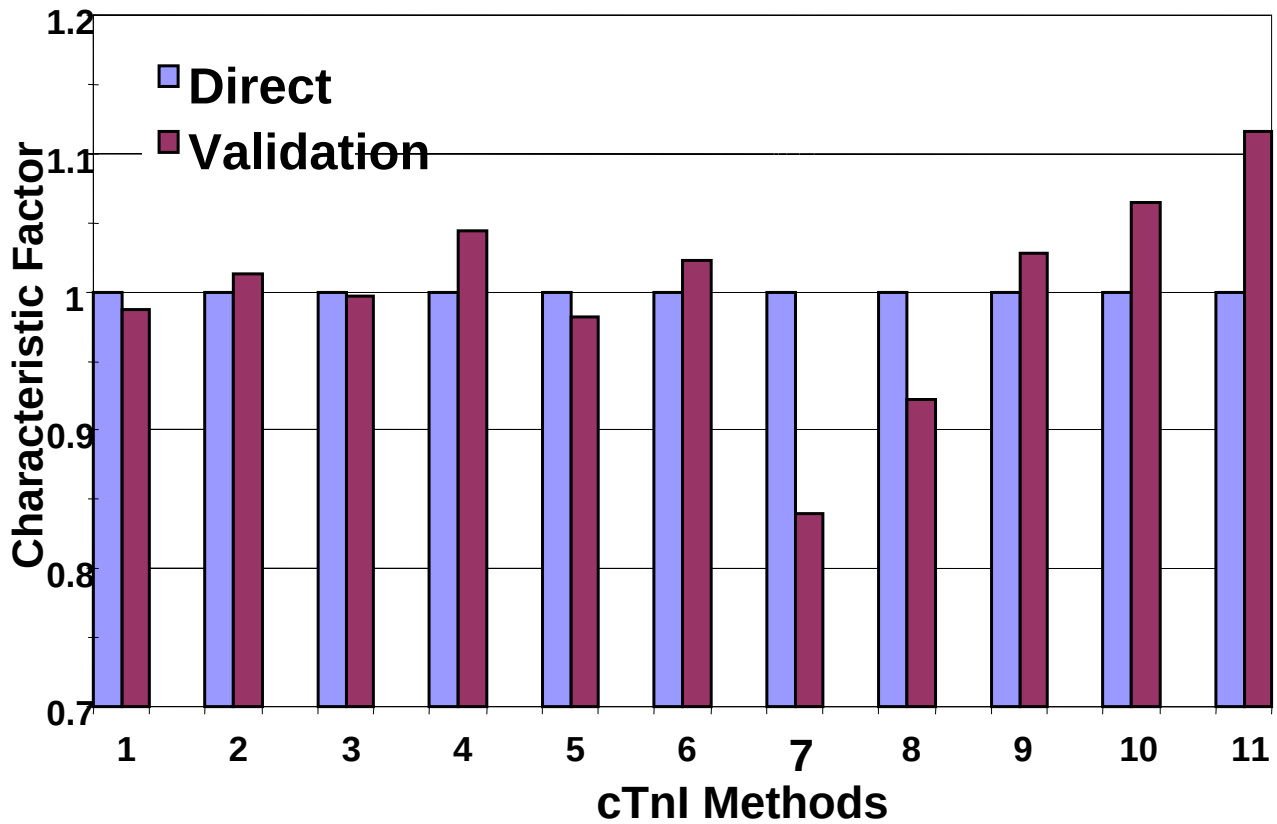
Assure Stability of cTnI Methods

- Characteristic Factor
  - Analytical response of methods to SRM 2921 concentrations
- Calculation:
  - SRM 2921 theoretical value/ measured value
- Characteristic Factor should be a constant

Characteristic Factor (CF) for SRM 2921

cTnI Methods

	1	2	3	4	5	6	7	8	9	10	11
CF	1.67	5.99	6.11	2.96	1.64	2.69	4.52	3.61	3.42	0.57	1.70
Validation	1.65	6.07	6.09	3.09	1.61	2.75	3.79	3.33	3.51	0.61	1.90

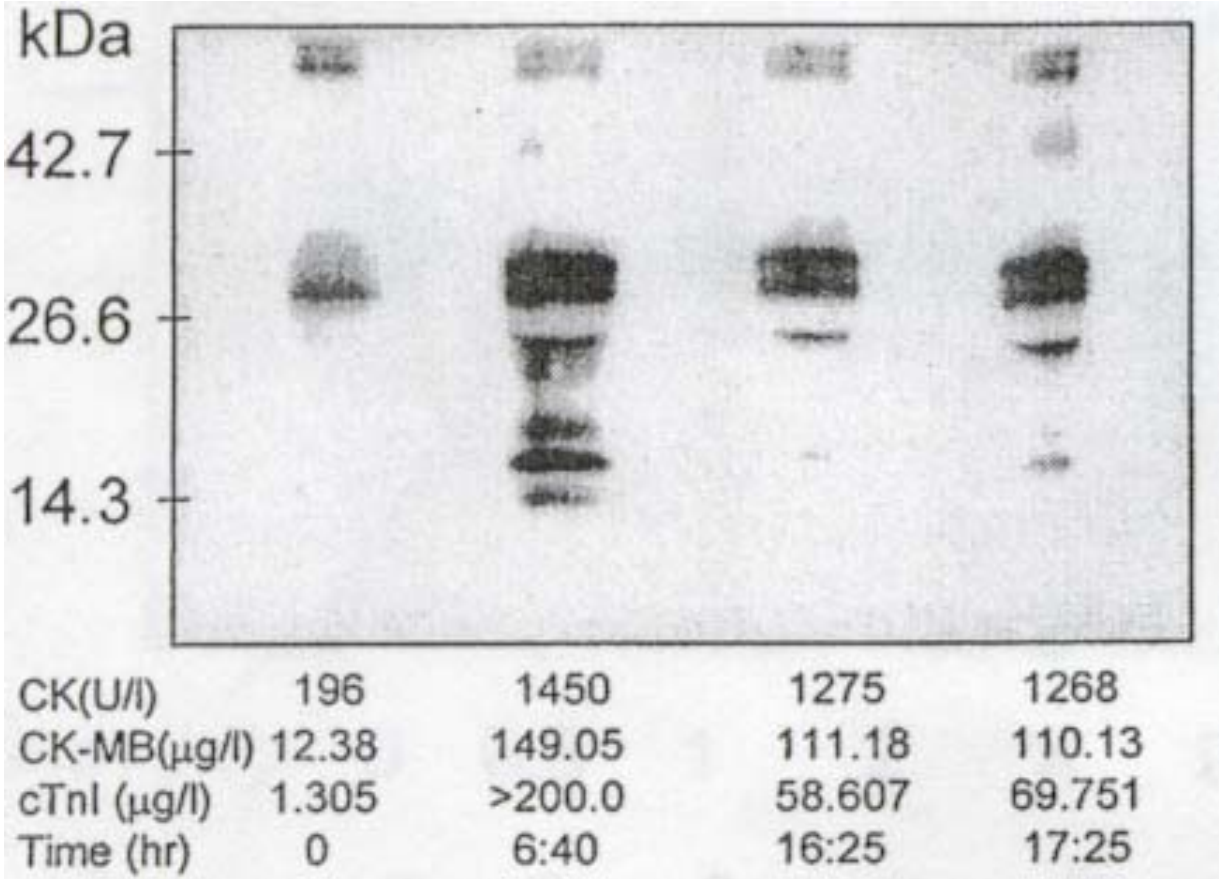


Where are we going?

Metabolism: cTnI is Heterogeneous Analyte

Circulation 2000;102;1221-1226

Western Blot: Reactivity with cTnI Antibody



Phase 4

Goal: To improve standardization of clinical cTnI measurement through the development of a serum-based reference material

Tasks:

- Development of reference measurement procedure for serum cTnI
- Produce pooled serum reference materials (multi-level)
- Evaluate commutability of reference materials and effectiveness to improve inter-assay comparability
- JCTLM submission and approval

Development of a Reference Measurement Procedure

Objective: investigate the use of a critically-evaluated immunoassay as a higher-order measurement procedure

- Immunoassay development a collaboration between NIST(USA) and NPL (UK) with guidance on cTnI antibodies by HyTest Ltd.
- Immunoassay calibrated using NIST SRM 2921
- Clinical expertise and assay evaluation samples provided by the IFCC's Committee for the Standardization of Markers of Cardiac Damage (C-SMCD)
- Interlaboratory evaluation of the cTnI immunoassay to be undertaken as a pilot project of the Bioanalysis Working Group of the CCQM

Summary

Where have we been? Where are we now? Where are we going?

- cTnI showed ~30-fold difference in methods
- Candidate Reference Materials examined
- SRM 2921 characterized
- Commutability issues: ~50% of methods
- Harmonization possible using serum pools
- cTnI is a heterogeneous analyte
- International effort for standardization using serum pools traceable to SRM 2921

Thank you!

rchristenson@umm.edu