SRM 2921 reference material for cTnI: Lights and Shadows

November 30, 2011

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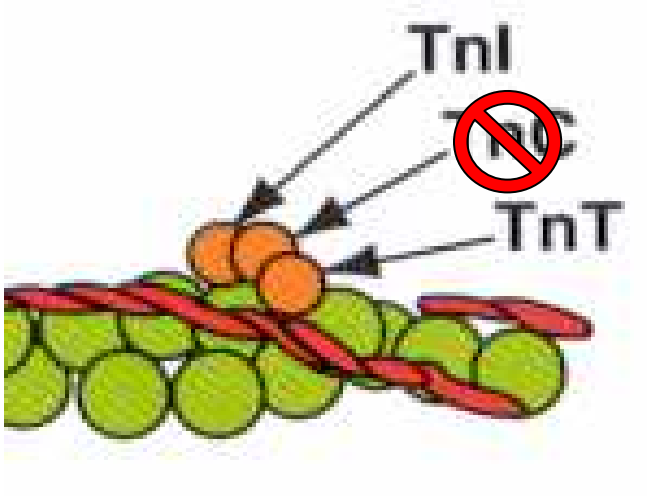
When troponin is increased think heart

Cardiac isoforms in blood



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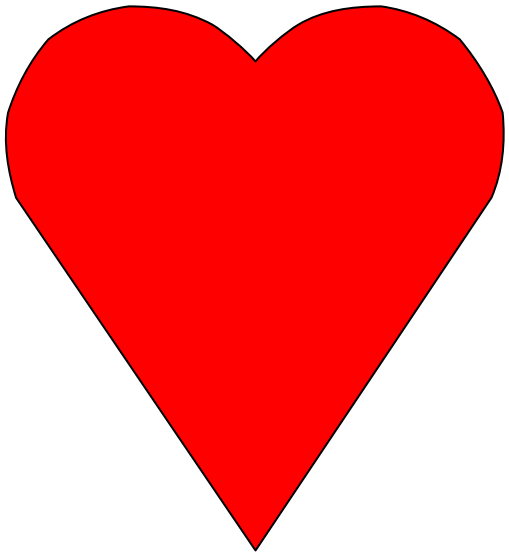


NACB Clinical Guidelines for ACS

2007 Clin Chem and Circulation

Class I

Patients with suspected ACS should undergo early risk stratification based upon an integrated assessment of symptoms, physical exam findings, ECG findings, and biomarkers (Level of Evidence: C).

Case

- 29 year-old patient presents to the Emergency Department
- hCG level is 10,000 U/L
- What is the diagnosis?

It’s a man, baby.

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

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:**

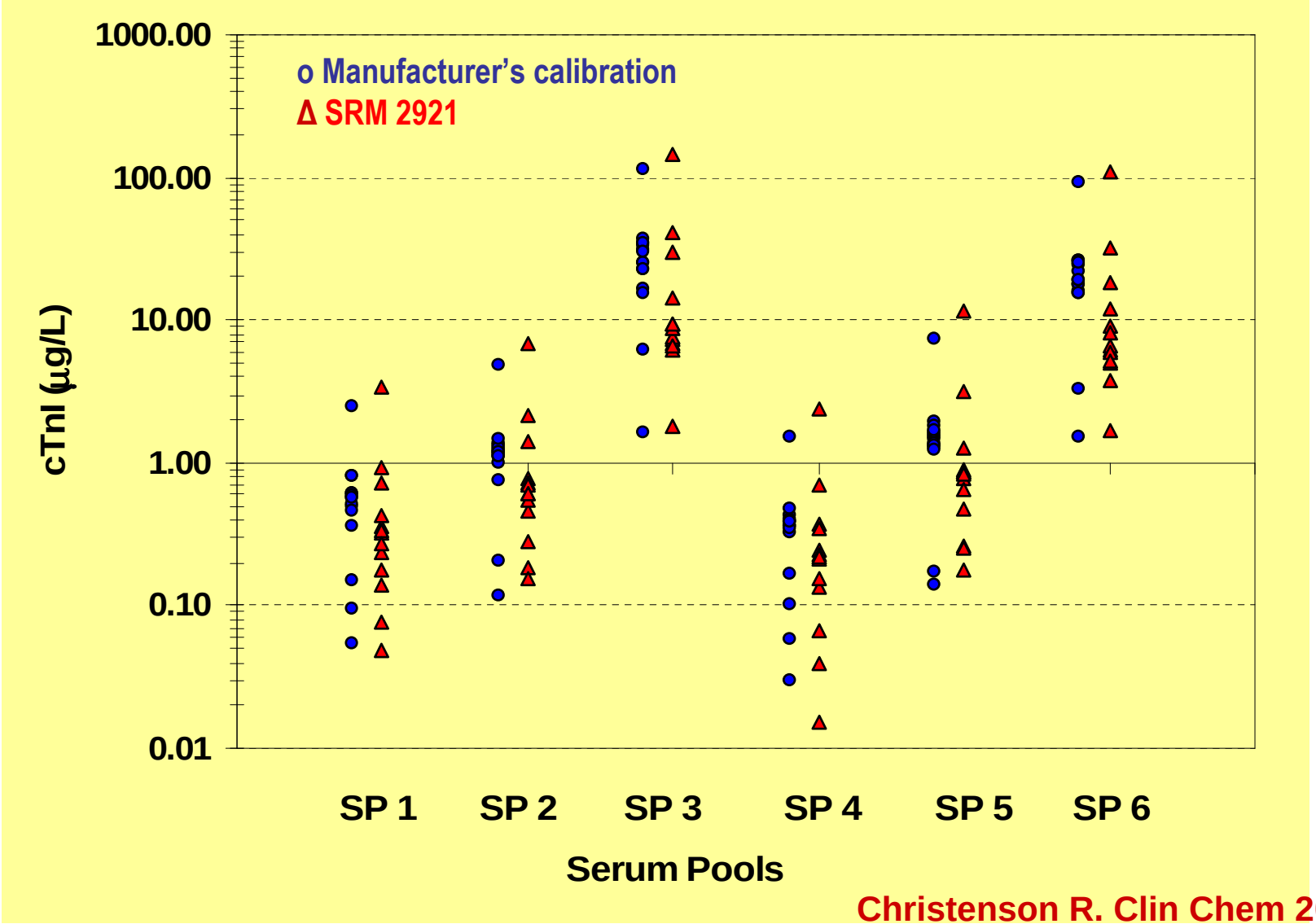
Assay	Value 1	Value 2	Mean
1	143.7	135.7	139.7
2	46.9	47.9	47.4
3	19.9	19.9	19.9
4	49.2	50.1	49.7
5	4.3	4.1	4.2
6	12.5	12.6	12.6
7	7.4	8.9	8.2
8	6.0	6.1	6.0
9	16.5	17.4	17.0
10	12.6	13.2	12.9
11	12.6	12.4	12.5
12	18.3	18.9	18.6
13	17.6	16.9	17.3

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!**

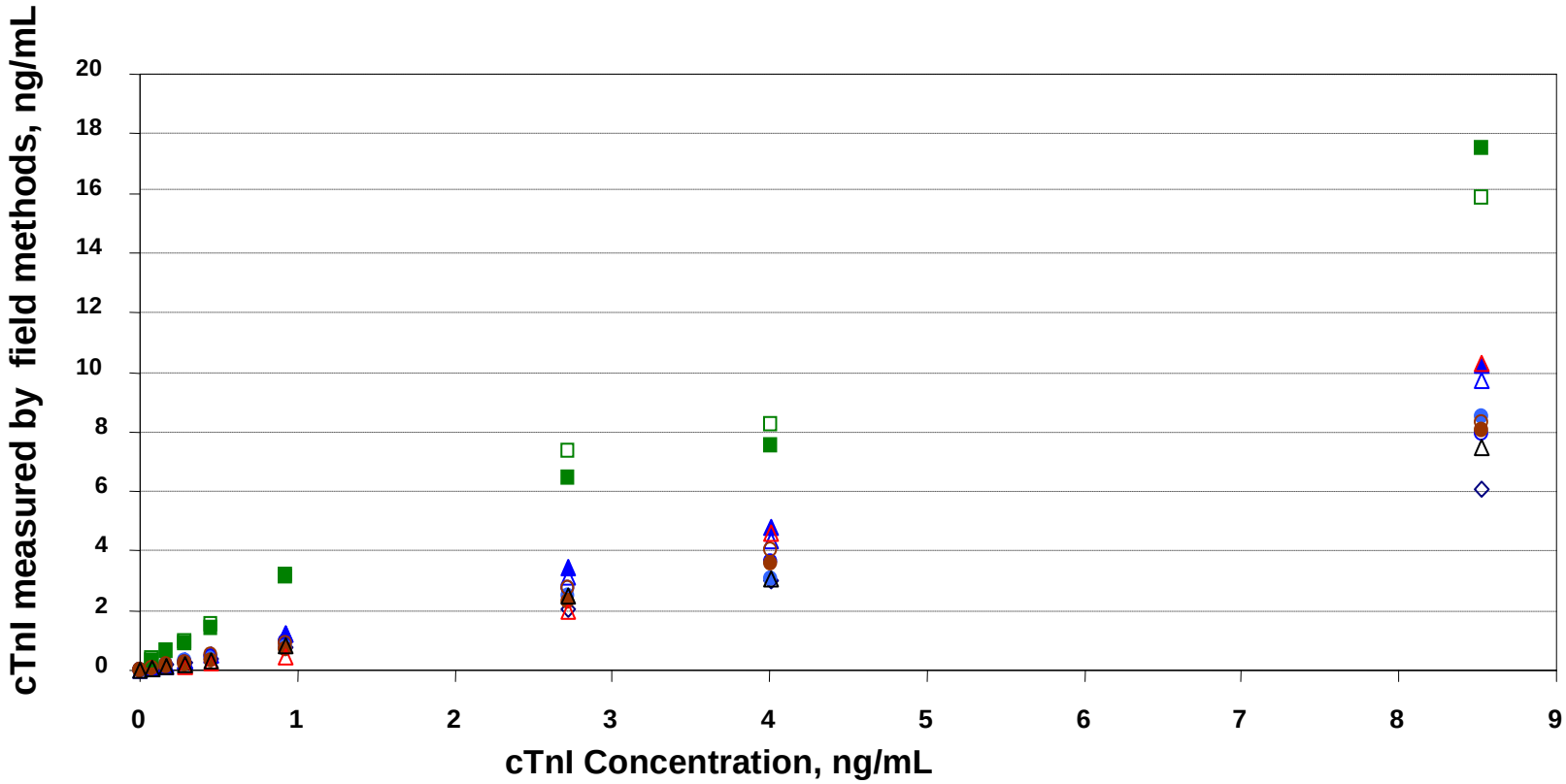
AACC cTnI Standardization Phase 2

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



Christenson R. Clin Chem 2006

Scatterplot of the 8 serum pools assayed by field methods



Universal Definition of Myocardial Infarction

Joint ESC/ACCF/AHA/WHF Task Force

Circulation. 2007 Nov 27;116(22):2634-53.

Criteria for acute myocardial infarction

..rise and/or fall of cardiac biomarkers (preferably) troponin

- ECG changes indicative of new ischaemia [new ST-T changes or new left bundle branch block (LBBB)];
- Development of pathological Q waves in the ECG;
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

The delta used for interpretation of acute MI must exceed the natural biological variance plus the analytical variability.

- Most use baseline & follow-up 1 to 6 hours later. *N Engl JMed 2009; 361: 868–77.*
- Values of 15–20% suggested as delta *Circulation 2007; 115: e356–75.*
- Delta threshold of 46% for increasing pattern or 32% for a decreasing pattern *Clin Chim Acta 2009; 401: 170–4.*
- Values 117% 0 & 3 hours, and 243% for 0 & 6 *Clin Chem 2010; 56: 642–50.*
- delta of 20% *J Clin Pathol 2008; 130: 964–8.*

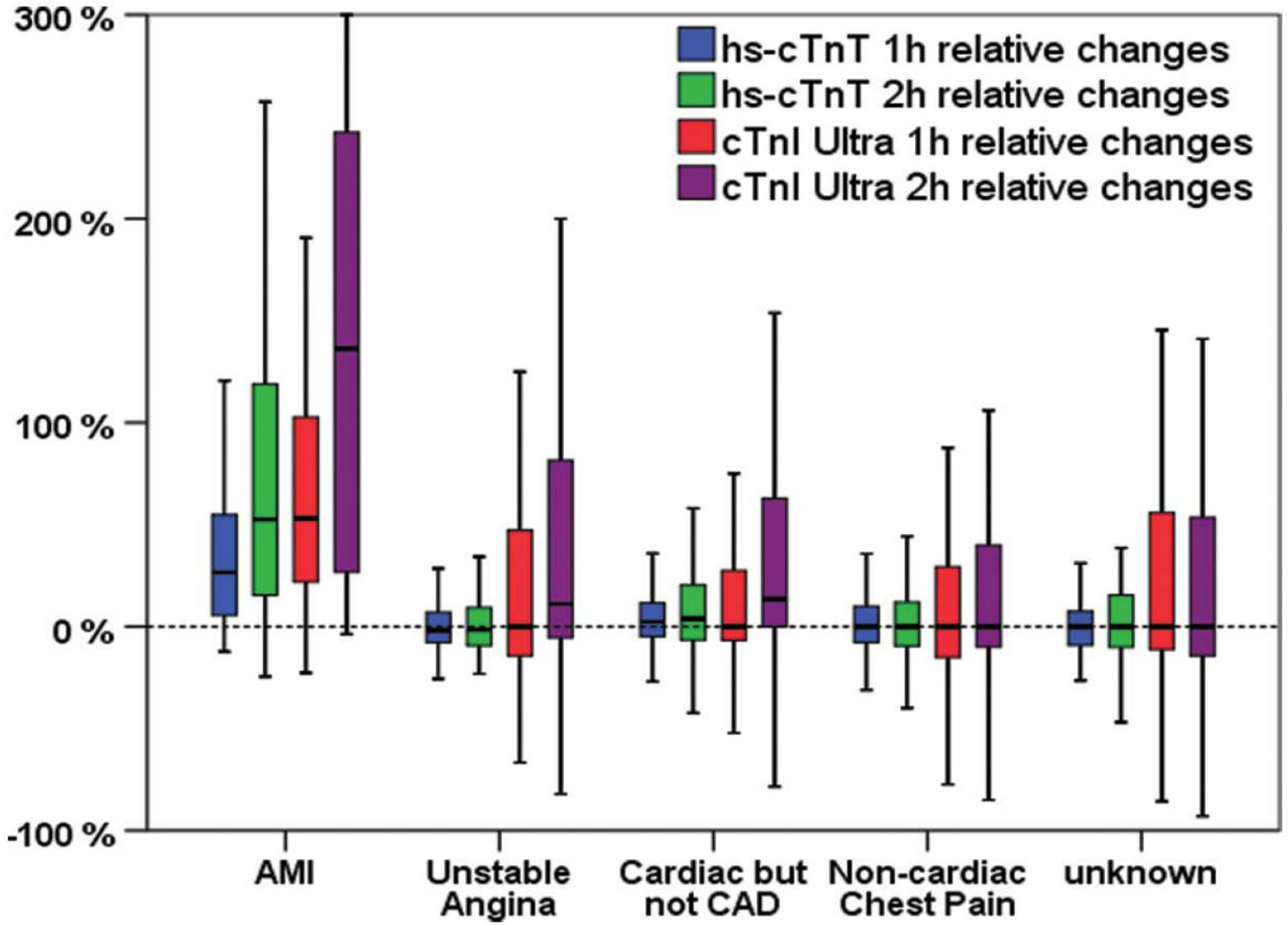
Coronary Heart Disease

Utility of Absolute and Relative Changes in Cardiac Troponin Concentrations in the Early Diagnosis of Acute Myocardial Infarction

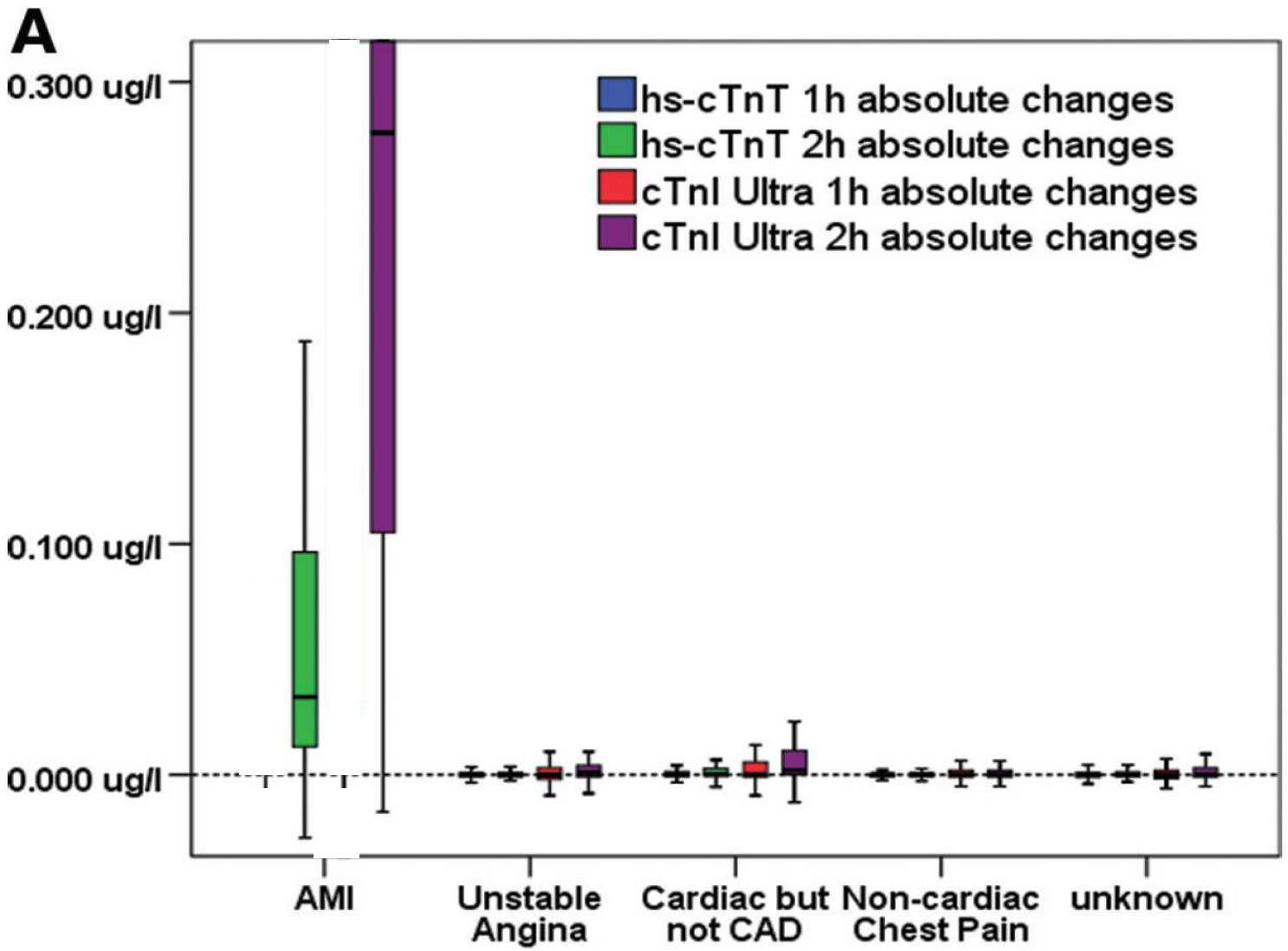
Tobias Reichlin, MD*; Affan Irfan, MD*; Raphael Twerenbold, MD; Miriam Reiter, MD; Willibald Hochholzer, MD; Hanna Burkhalter, MD; Stefano Bassetti, MD; Stephan Steuer, MD; Katrin Winkler, MD; Federico Peter, MD; Julia Meissner, MD; Philip Haaf, MD; Mihael Potocki, MD; Beatrice Drexler, MD; Stefan Osswald, MD; Christian Mueller, MD, FESC

Circulation 2011, 124:136-145

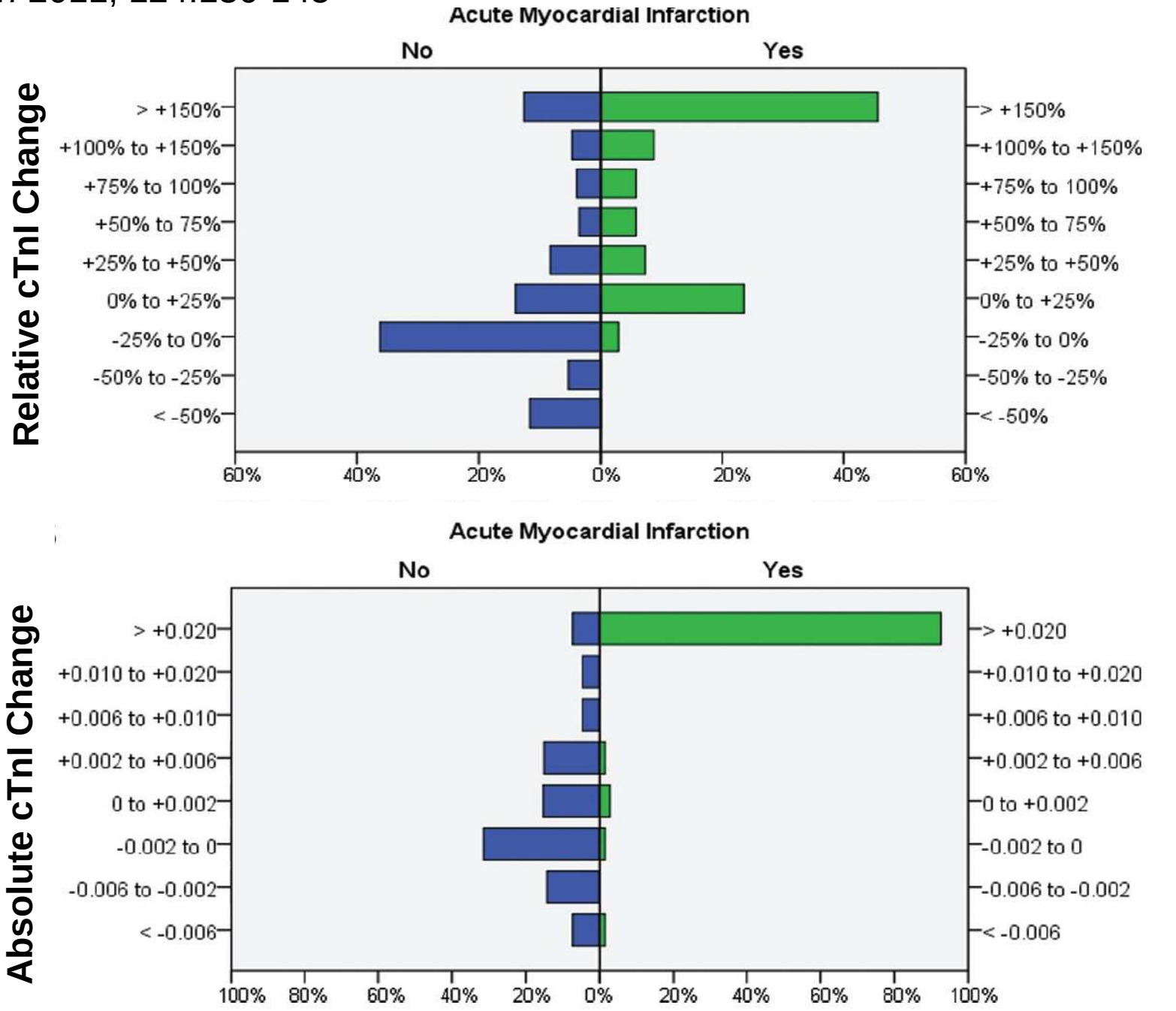
Circulation 2011, 124:136-145



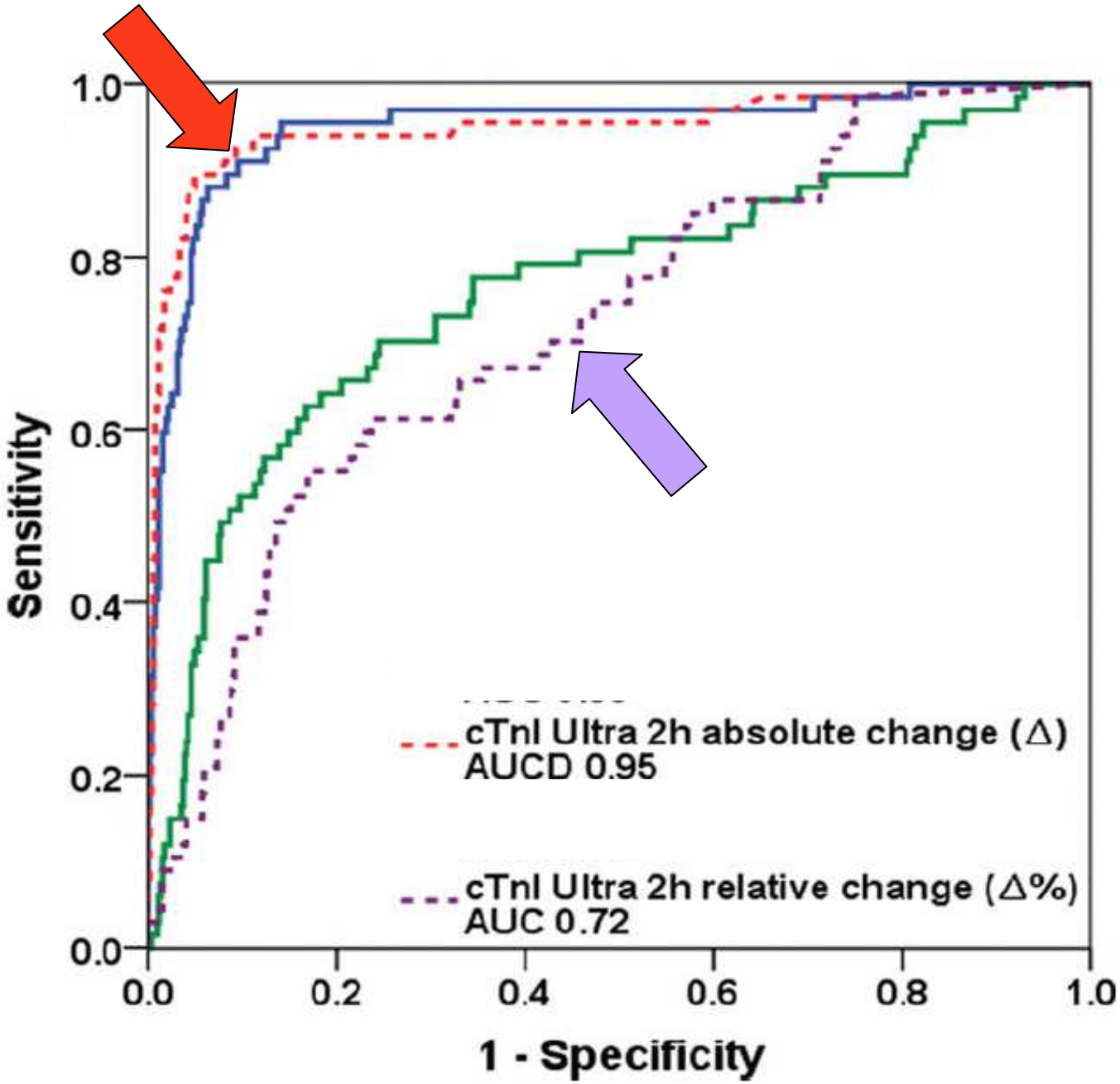
Circulation 2011, 124:136-145



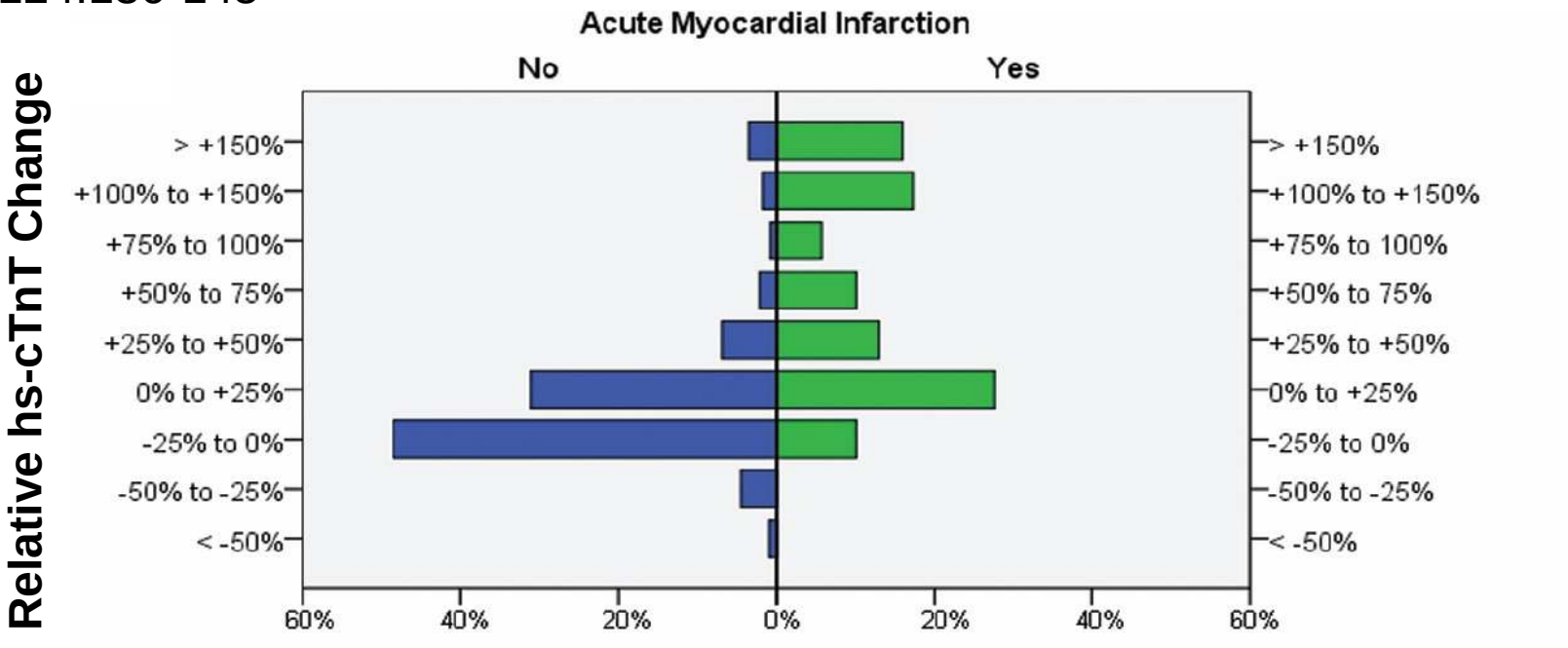
Circulation 2011, 124:136-145

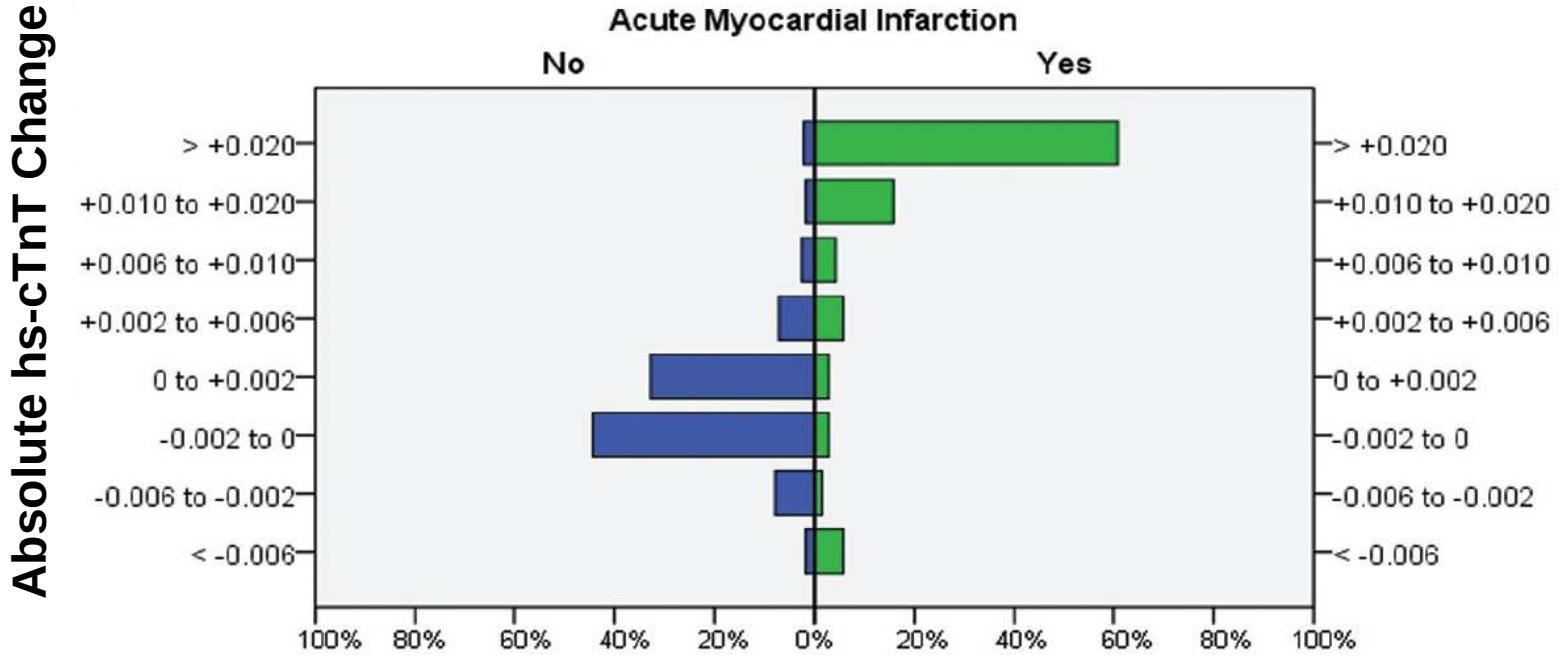


Circulation 2011, 124:136-145

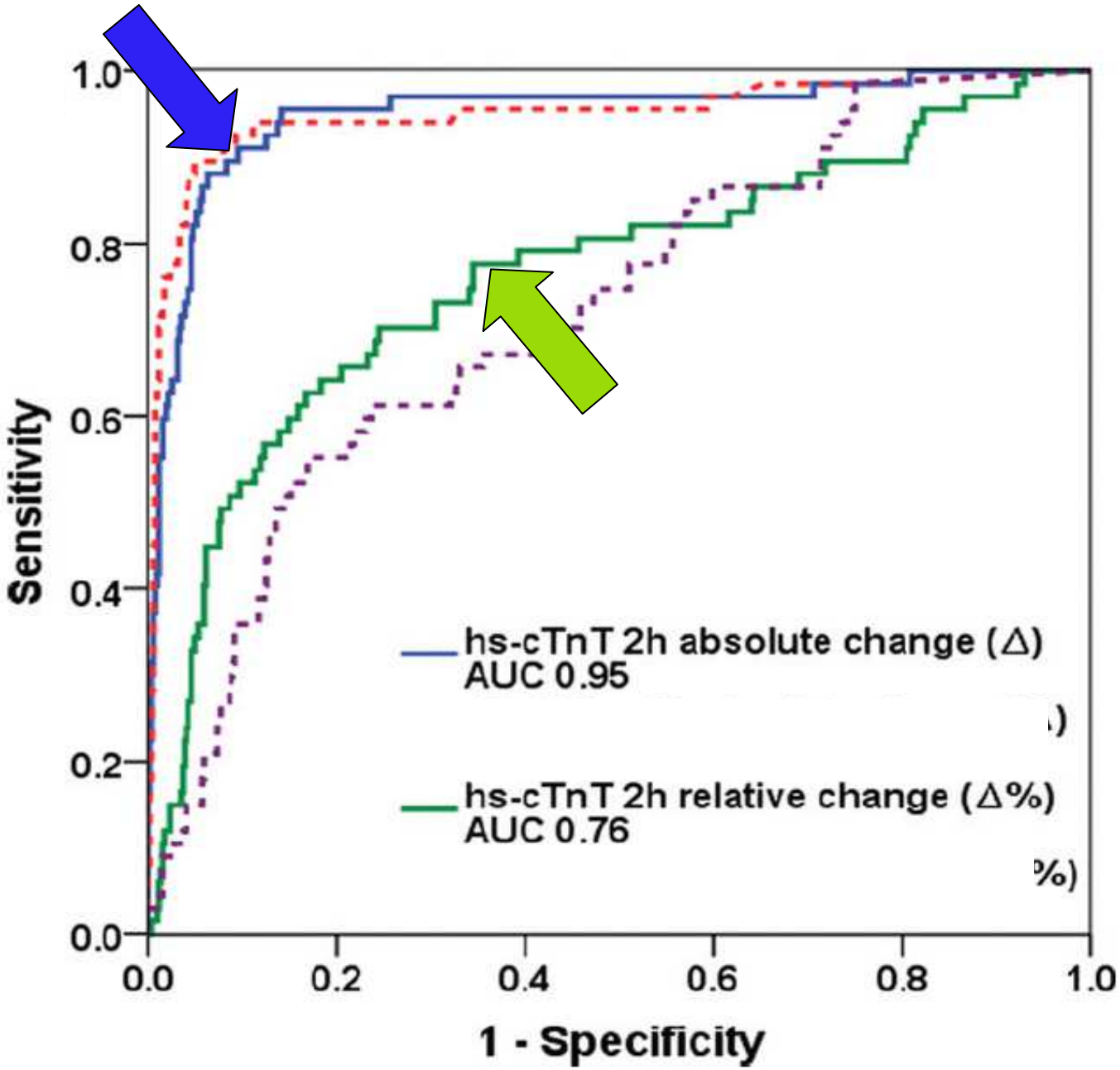


Circulation 2011, 124:136-145





Circulation 2011, 124:136-145



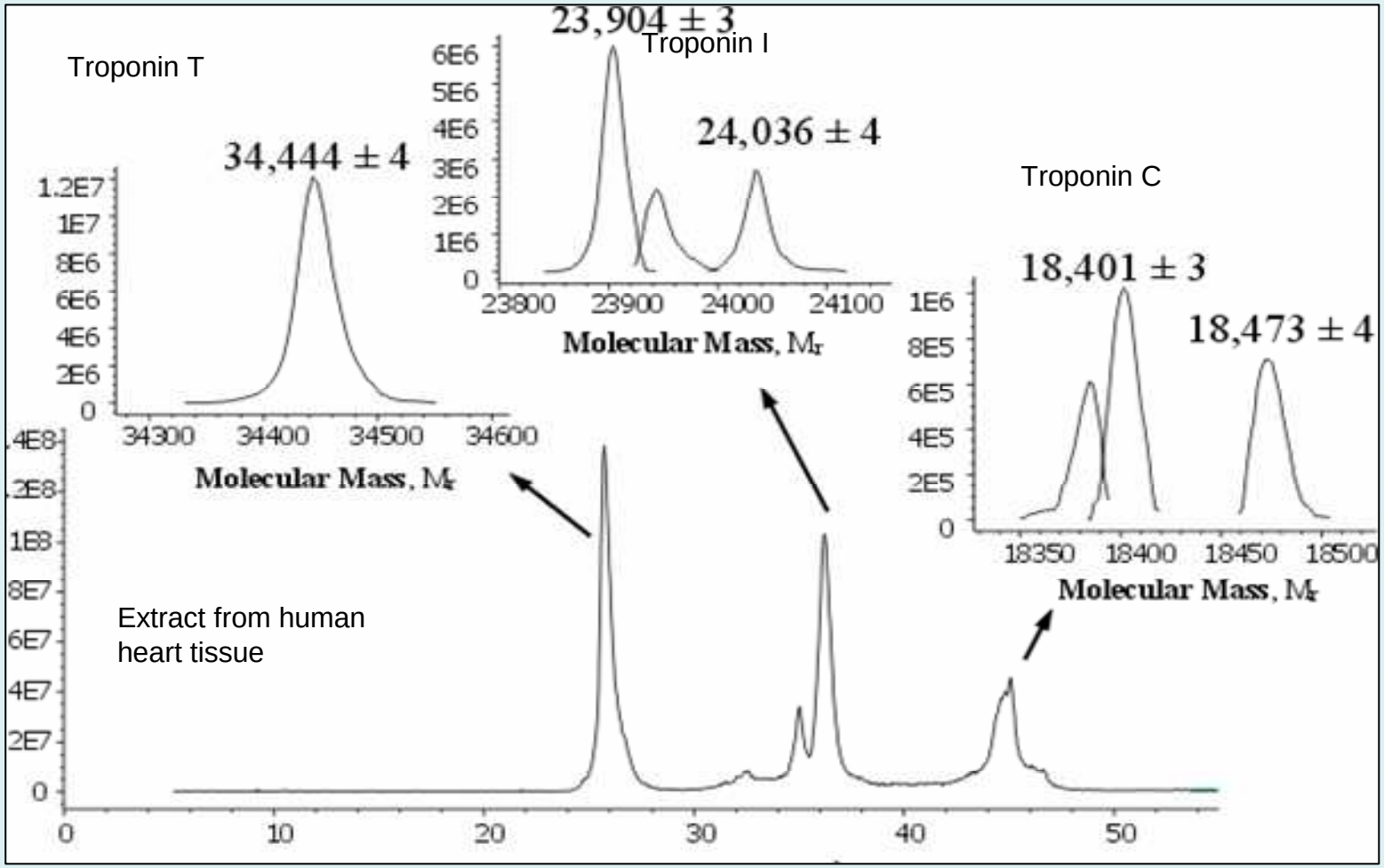
cTn Reference Material

Candidate Standard Reference Materials (cSRMs)

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

Characterized at NIST for purity and concentration.

LC/MS of Cardiac Troponin Complex

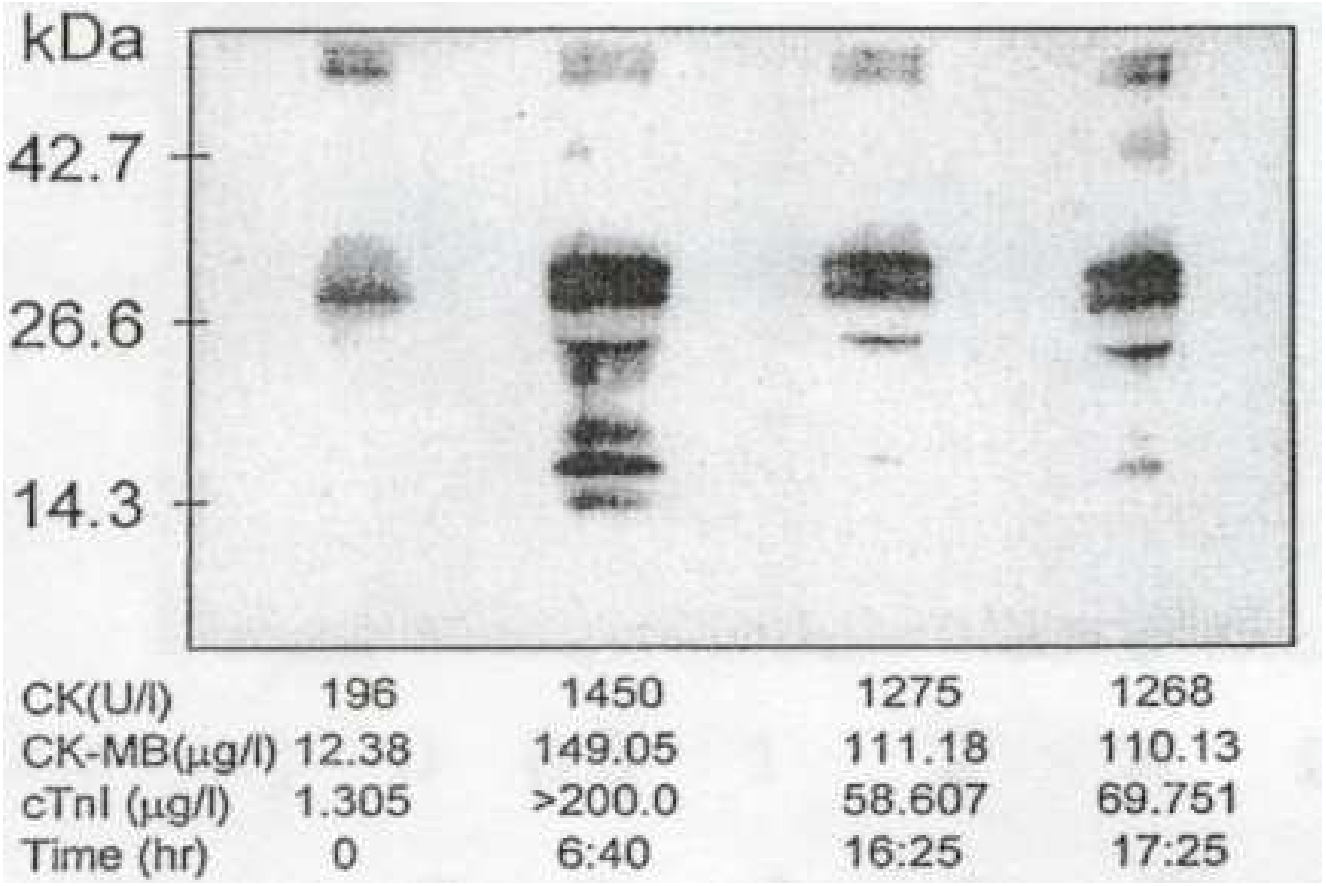


Certification of SRM 2921

Metabolism: cTnI is Heterogeneous Analyte

Circulation 2000;102;1221-1226

Western Blot: Reactivity with cTnI Antibody



Time-Dependent Instability of Cardiac Troponins in Human Plasma Spiked with NIST Reference Material 2921

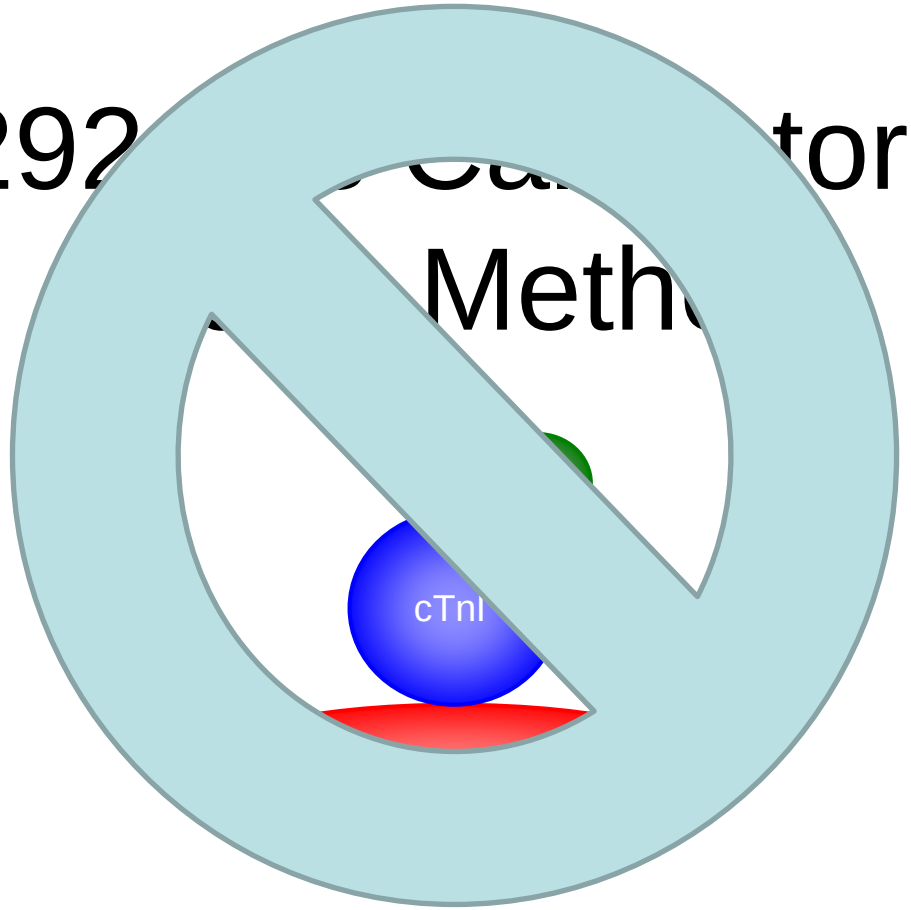
C.M. Cobbaert
C.W.Weykamp
E.C.H.J. Michielsen
H. Baadenhuijsen
M.P. van Dieijen-Visser

Clinical Chemistry 54:12: 2078–2079 (2008)

Clinical Chemistry 54:12: 2078–2079 (2008)

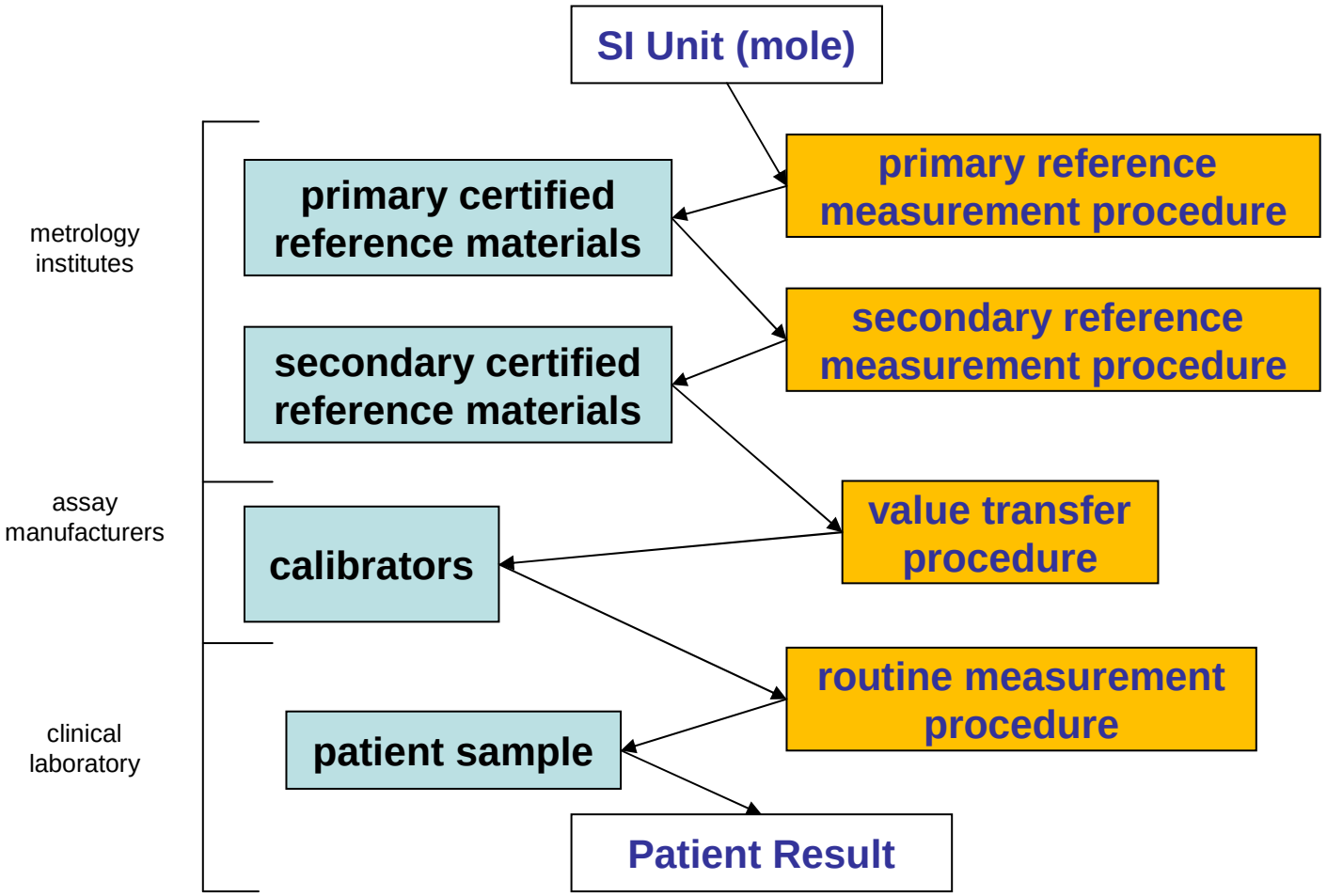
Samples	Storage time at 4 °C			
	Baseline	2 h	6 h	48 h
cTnI Abbott, μg/L (% recovery)				
1 ACS pts, native cTn	3.98 (100)	3.95 (99)	3.87 (97)	3.72 (93)

SRM 292 tor For Field Meth

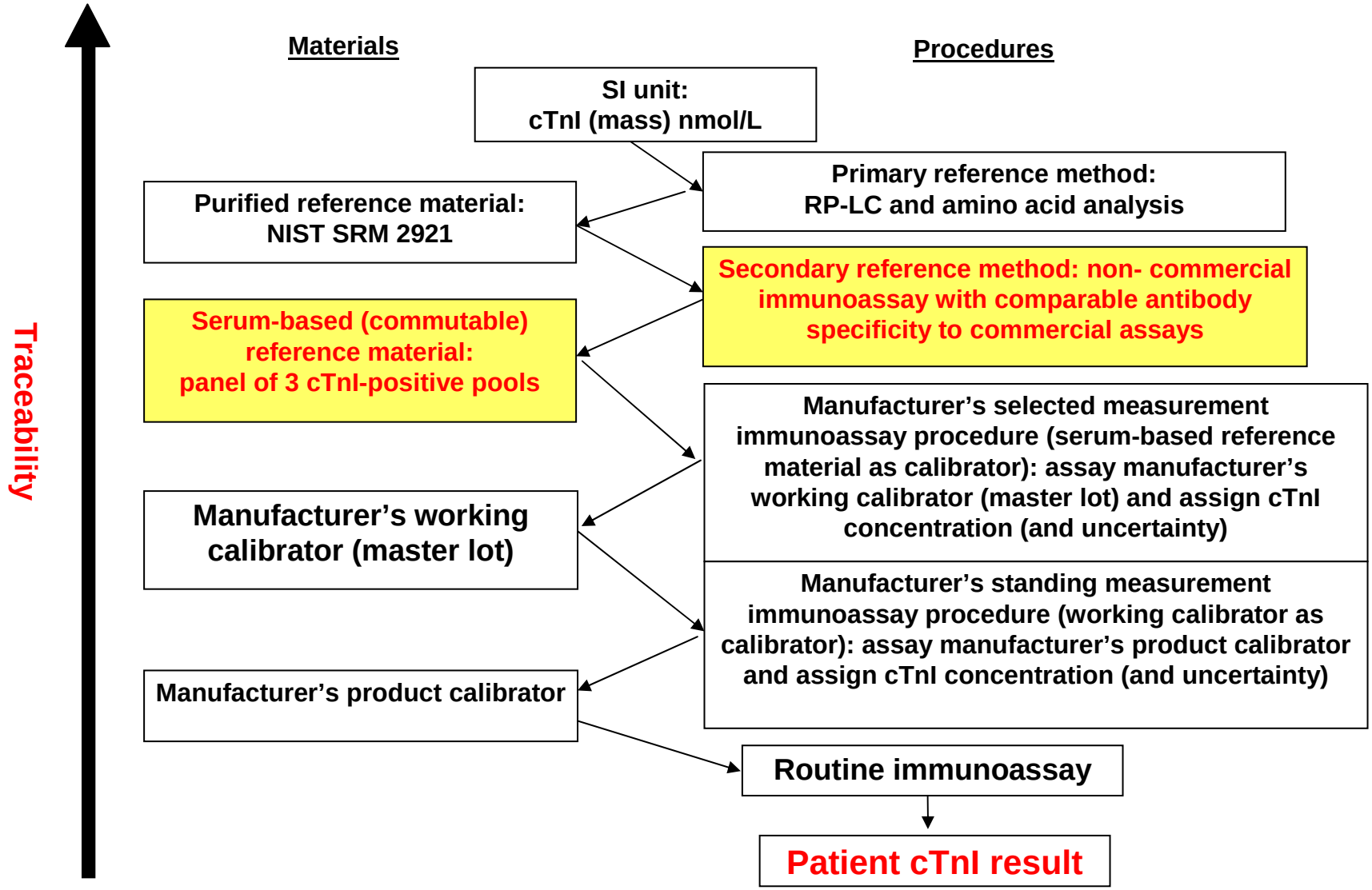


1000 X NO!

Metrological Traceability in Clinical Chemistry



*Adapted from ISO 17511



Tate et al. Pathology 2010; 42: 402-8

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.

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 Assessment

- Calibrate systems with field cTnI methods
- Measure
  - Patient Pools
  - Candidate RM samples prepared in cTnI negative serum.
- Compare the pt pool values for each individual cTnI method to each of the other methods
- 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.

		c	T	N	I		M	E	T	H	O	D	S			
	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																

TIC complex Degree of Commutability is 47 of 105 (45%)

B.

		c	T	N	I		M	E	T	H	O	D	S			
	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																

IC complex Degree of Commutability is 41 of 105 (39%)

Clin Chem 2006;52:1685-1692

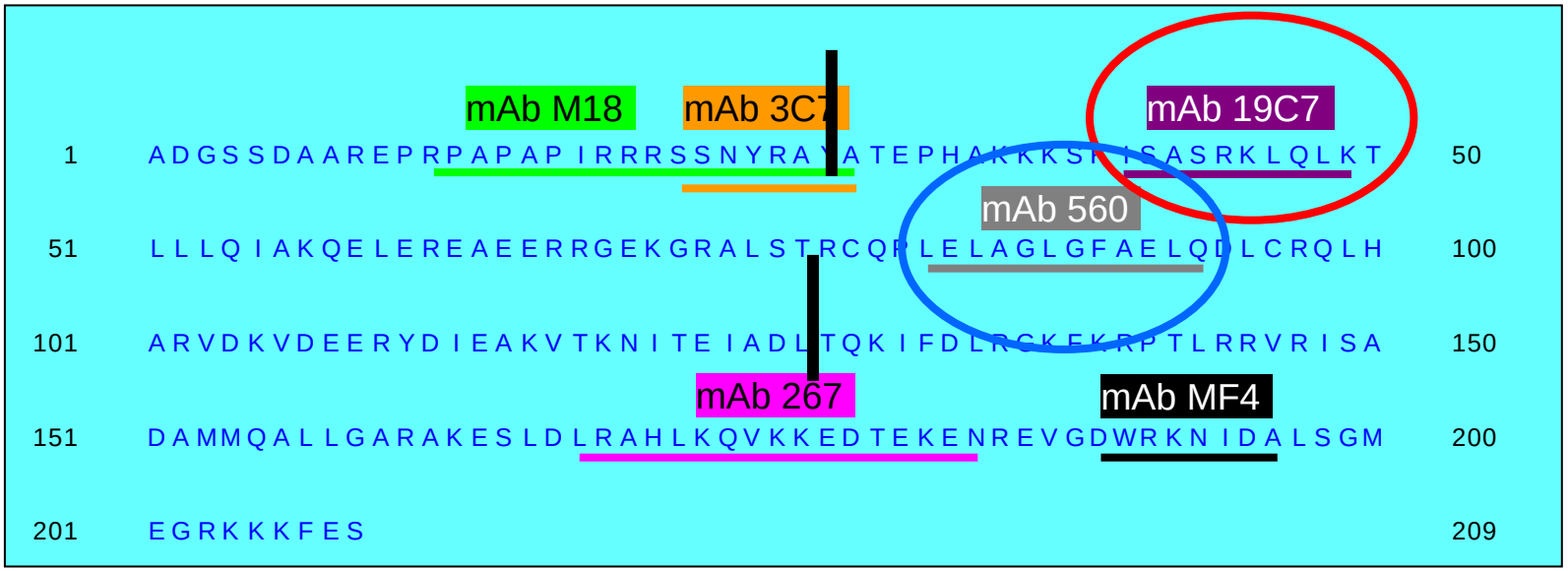
Autoantibodies against Tn?

Tn autoantibodies potentially capable of interfering with cTn assays are found in 5-20% of individuals with or without established cardiac disease.

- Clin Chem 2003;49:1095-104.
- N Engl J Med 2005;352:98-100
- Clin Chem 451 2005;51:839-47.
- Ann N Y Acad Sci 2009;1173:67-74.
- Clin Chem 2009;55:1592-3.

cTn Autoantibodies commonly target the stable mid-fragment amino acid residues (30-110)

Eriksson S, Junikka M, Pettersson K. Clin Biochem 2004;37:472-80.



IFCC Table
Troponin Assay Characteristics

Commercially available assays - Company/ platform(s)/ assay	LoB# (ng/L)	LoD* (ng/	99th % (ng/L)	%CV at 99th %	10% CV (ng/L)	Risk Stratif	Epitopes recognised by Antibodies	Detection Tag
Abbott AxSYM ADV	20		40	14.0	160	Yes	C 87-91, 41-49; D 24-40	ALP
Abbott ARCHITECT	<10		28	14.0	32	Yes (No US)	C 87-91, 24-40; D: 41-49	Acridinium
Abbott i-STAT	20		80	16.5	100	Yes	C: 41-49, 88-91; D: 28-39, 62-78	ALP
Alere Triage SOB	50		NAD	NA	NA	No	C: NA; D: 27-40	Fluorophor
Alere Triage Cardio 3 (r)	10		NAD	17.0 (at 20)	NA	No	NA	Fluorophor
Beckman Coulter Access Accu	10		40	14.0	60	Yes	C: 41-49; D: 24-40	ALP
bioMerieux Vidas Ultra	10		10**	27.7	110	No	C: 41-49, 22-29; D: 87-91, 7B9	ALP
Mitsubishi Chemical PATHFAST	8		29	5.0	14	No	C: 41-49; D: 71-116, 163-209	ALP
Ortho Vitros ECi ES	12		34	10.0	34	Yes	C: 24-40, 41-49; D: 87-91	HRP

Troponin Assay Characteristics

Research assays - not commercially available	**LoB[#] (ng/L)**	**LoD* (ng/**	**99th % (ng/L)**	**%CV at 99th %**	**10% CV (ng/L)**	**Risk Stratif**	**Epitopes recognised by Antibodies**	**Detection Tag**
Beckman Coulter Access hs-cTnI	2.0		8.6	10.0	8.6	NA	C: 41-49; D: 24-40	ALP
Nanosphere VeriSens hs-cTnI	0.2		2.8	9.5	0.5	NA	C: 136-147; D: 49-52, 70-73, 88, 169	Gold-nanoparticles
Singulex hs-cTnI	0.09		10.1	9.0	0.88	NA	C: 41-49; D: 27-41	Capillary flow fluorescence

<http://www.ifcc.org/index.asp?cat=Scientific_Activities&scat=Troponin_Assay_Analytical_Characteristics&rif=4&dove=1>

Summary

- The issue of standardization / harmonization is clinically important!
- The nature of circulating cTnI is heterogeneous
- SRM 2921 (ITC complex) has been characterized
- cTnI methods
  - Earlier methods ~30-fold difference
  - Later generation differences much smaller, ~2- to 3-fold
- Commutability issues: ~50% of methods
- Harmonization possible using serum pools
- Impact of Tn Autoanitbodies?
- International effort for standardization using immunoassay, serum pools, all ***traceable*** to SRM 2921.

Thank you!

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