STANDARDIZATION OF CARDIAC TROPONIN I: THE ONGOING INTERNATIONAL EFFORTS

CIRME

UNIVERSITÀ DEGLI STUDI DI MILANO

MILANO, ITALY

NOVEMBER 30, 2011

Secondary reference materials for cTnI: The way forward

Jill Tate
Pathology Queensland
Brisbane, AUSTRALIA

CIRME
UNIVERSITÀ DEGLI STUDI DI MILANO

JCTLM
Joint Committee for Traceability in Laboratory Medicine

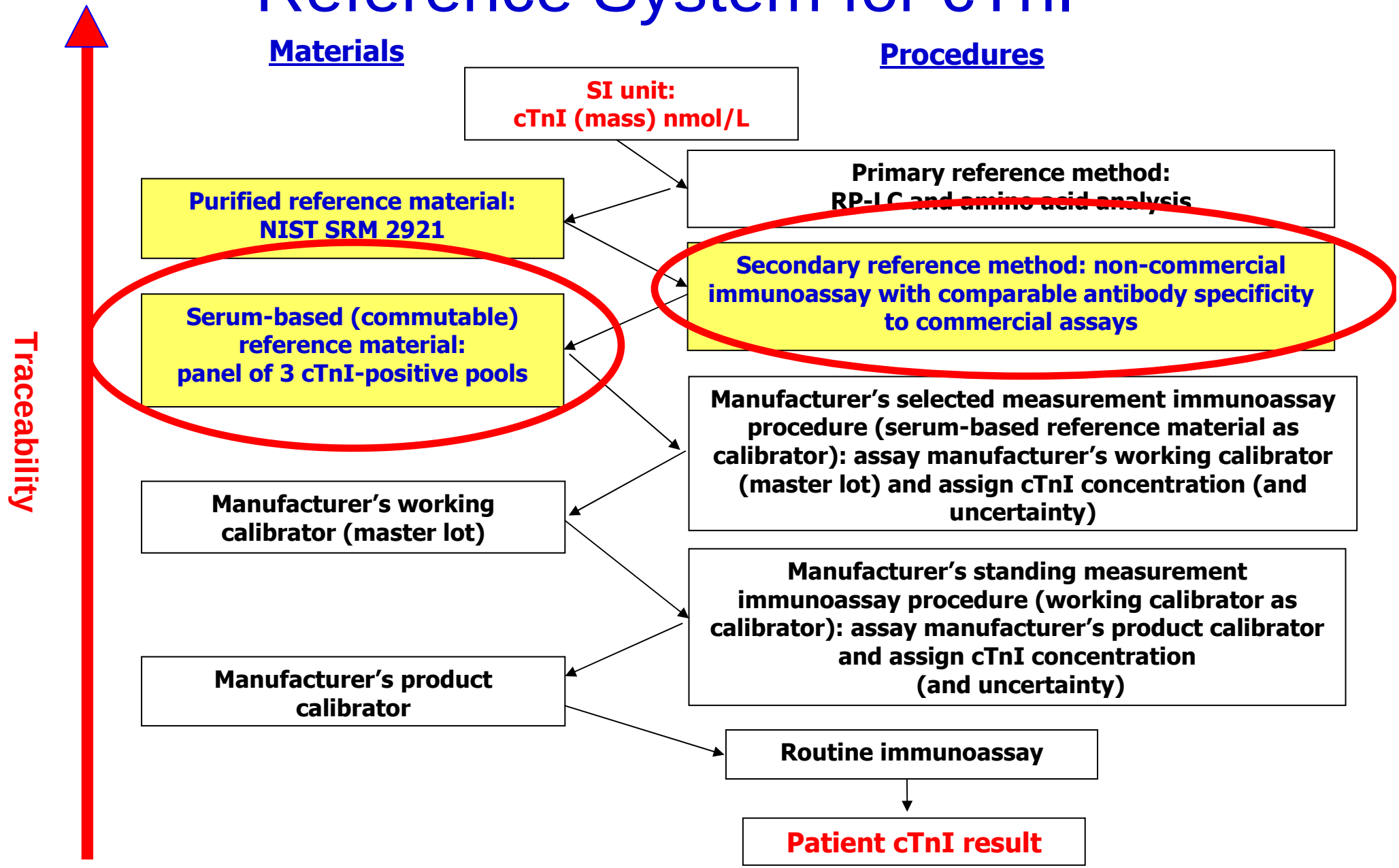
EFCC
EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE

ifcc
International Federation of Clinical Chemistry and Laboratory Medicine

Talk Outline

- Reference measurement system for cTnI
- Reference materials for cTnI
- Characterisation of secondary RM
  - Harmonisation ability
  - Composition
  - Commutability
  - Stability
- Value assignment
- Value transfer to manufacturer’s calibrator.

Reference System for cTnI



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

Reference materials for cTnI

- NIST SRM 2921
  - Purified ICT complex
  - Well-characterised
  - Value assignment by RP-LC & amino acid analysis
    - **31.2 (1.4) mg/L and traceable to S.I.**
  - Not commutable in ~50% of commercial assays
- Serum-based commutable material
  - Complex matrix
  - Sourced from AMI / ACS patients
  - Either serum pools or a panel of individual patient sera
  - Requires characterisation
  - Value assignment by candidate secondary RMP.

Secondary reference material for cTnI

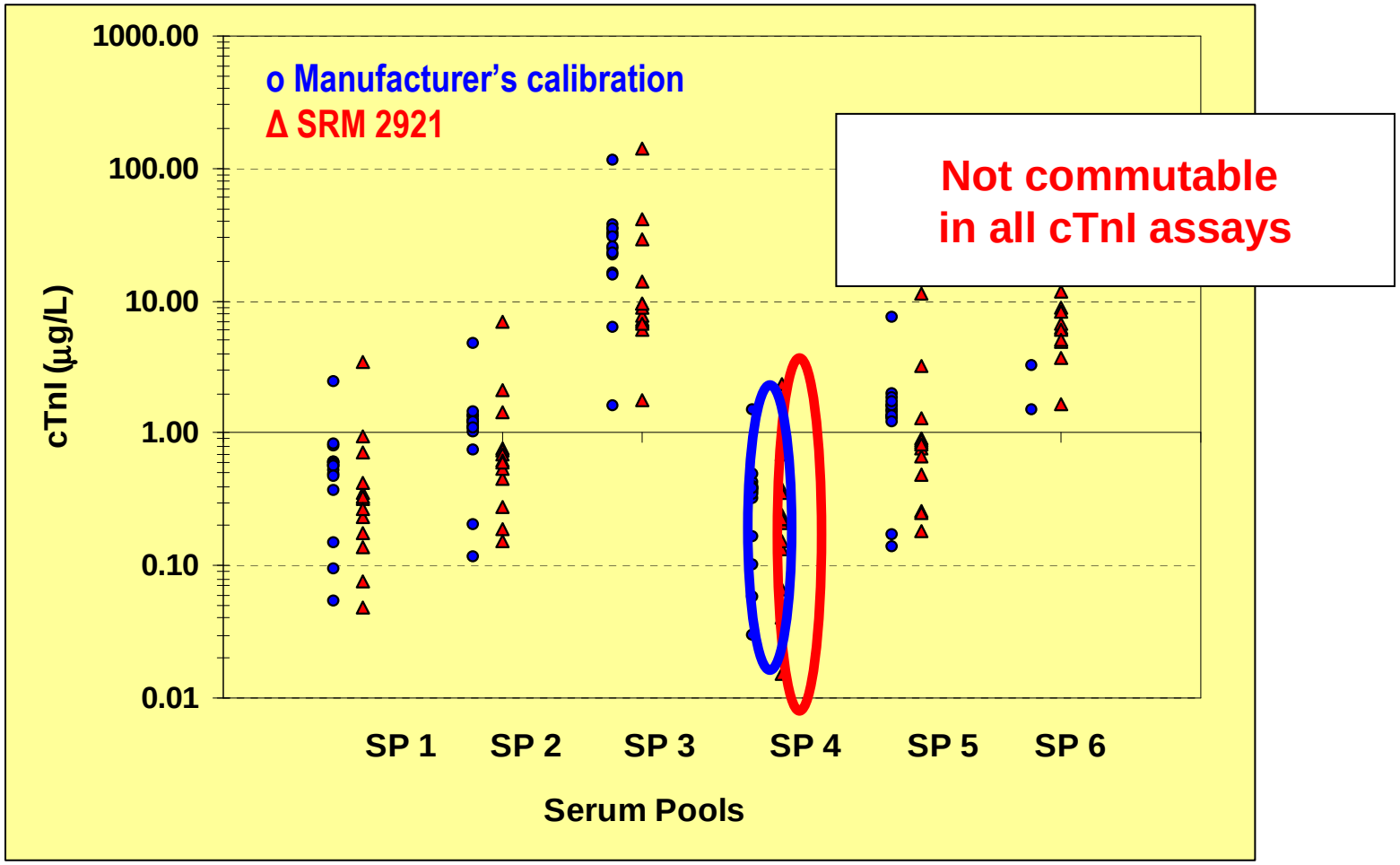
- Secondary calibrators can be used by IVD medical device manufacturers to calibrate their selected or standing measurement procedures for assigning quantity values to in-house master or commercial product calibrators

The Goals:

1. Three cTnI concentration levels:
   - **0.1 µg/L, 1.0 µg/L, 10 µg/L**
2. ≈ 5 liters of pooled serum at each cTnI concentration level to yield at least a 5-year supply of the reference material
3. Value assignment and traceability to the S.I.

AACC cTnI Standardisation Study

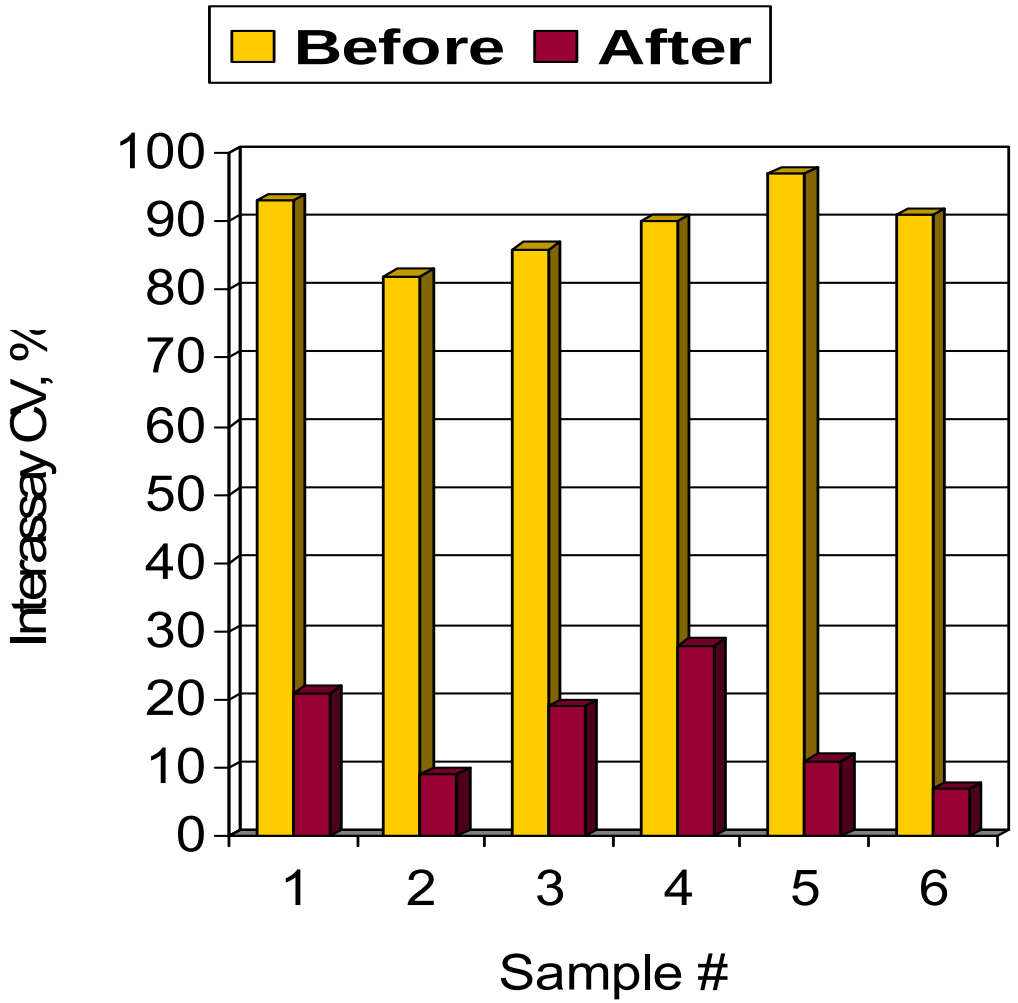
NIST SRM 2921 ternary ITC complex



Christenson RH et al. Clin Chem 2006; 52: 1685-92

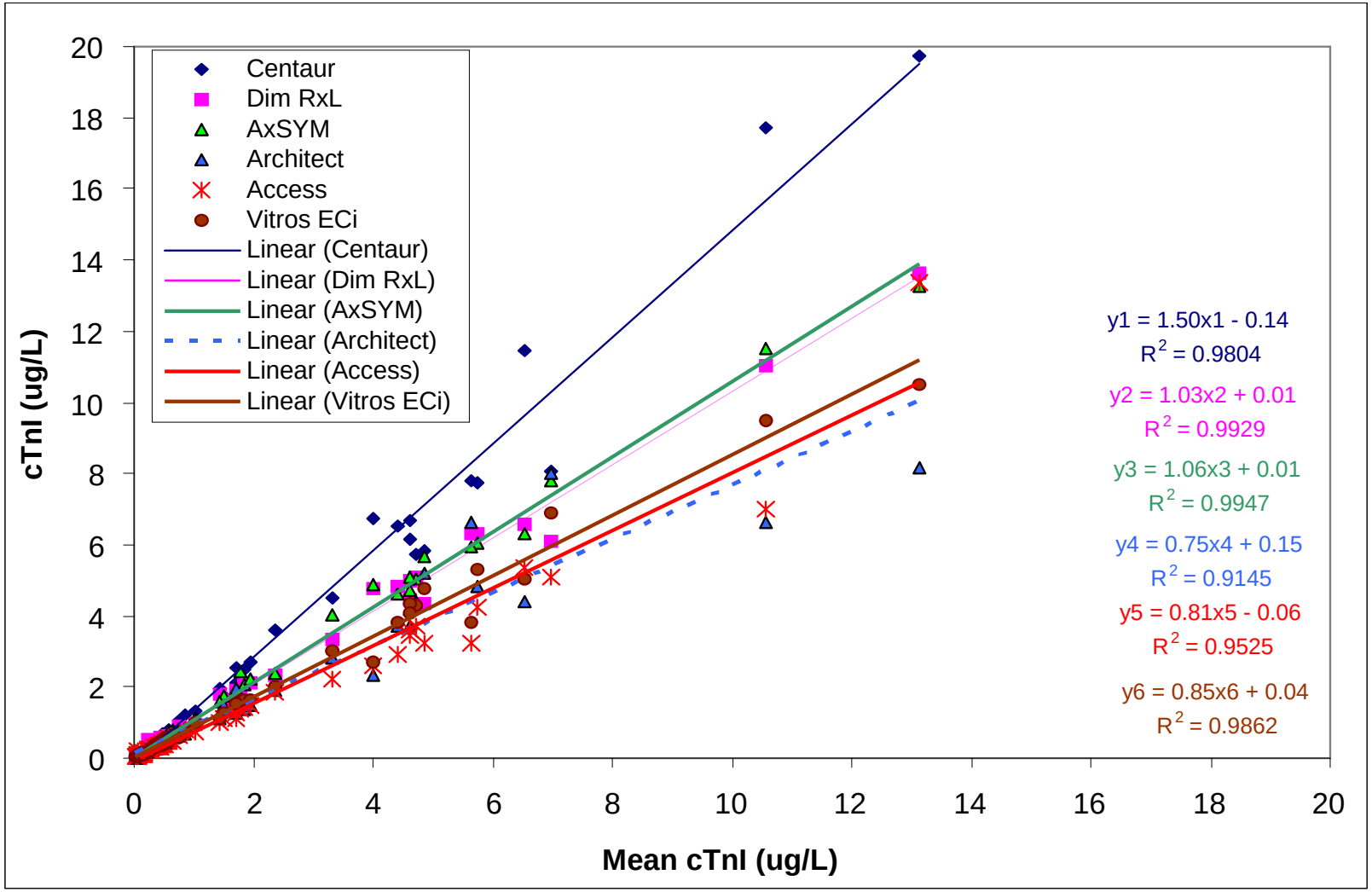
cTnI Harmonisation by Alignment

1. Calibrate methods
2. Measure 6 serum pools with each of the 15 cTnI systems
3. Designate “common comparison” system
4. Regression analysis to yield parameters
5. Align each serum pool with regression parameters
6. Determine inter-method variability of aligned results for each pool



Christenson RH et al. Clin Chem 2006; 52: 1685-92

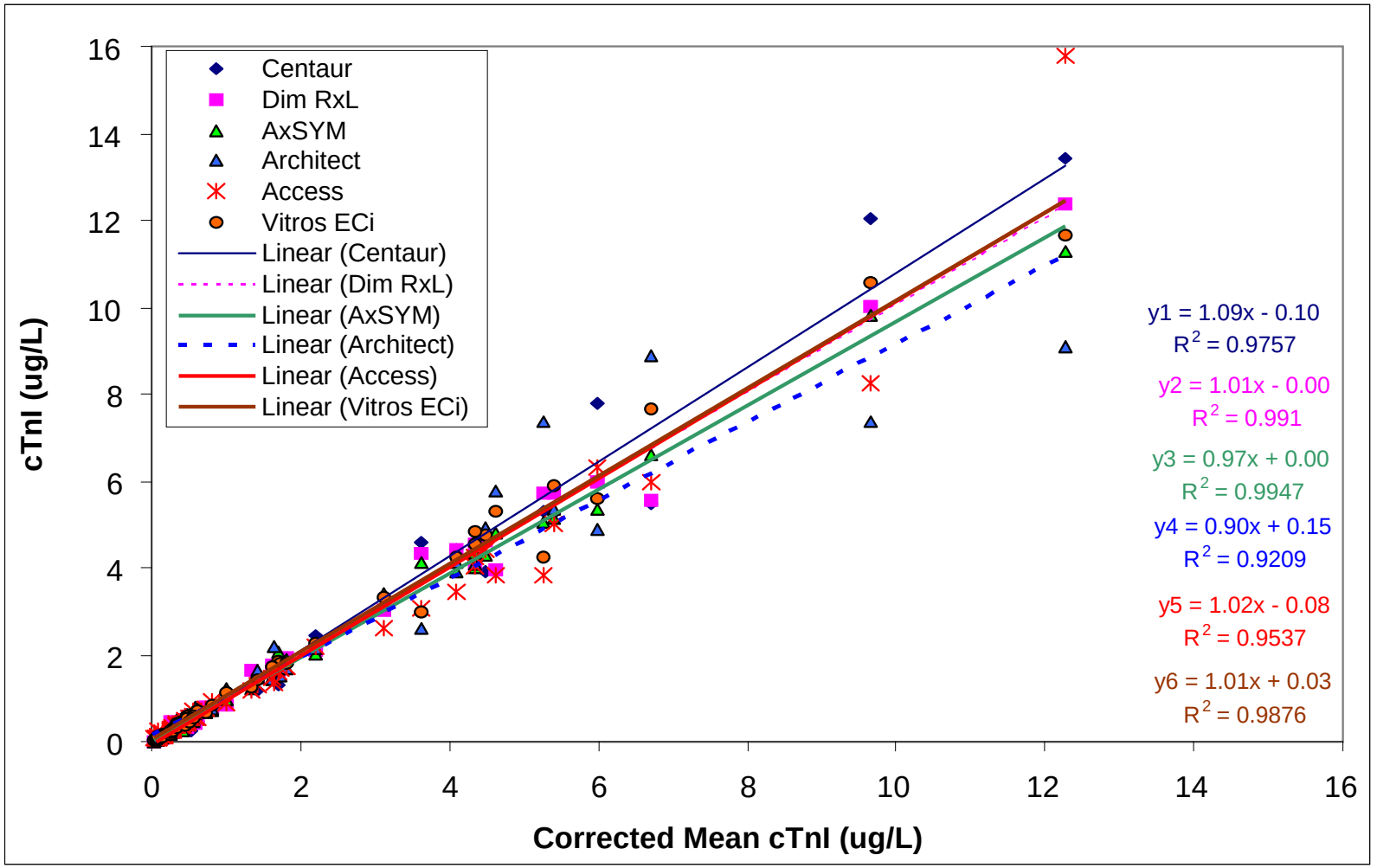
cTnI assays 2006



Tate J. Clin Chem Lab Med 2008; 46: 1489-500

Post realignment against cTnI-positive serum

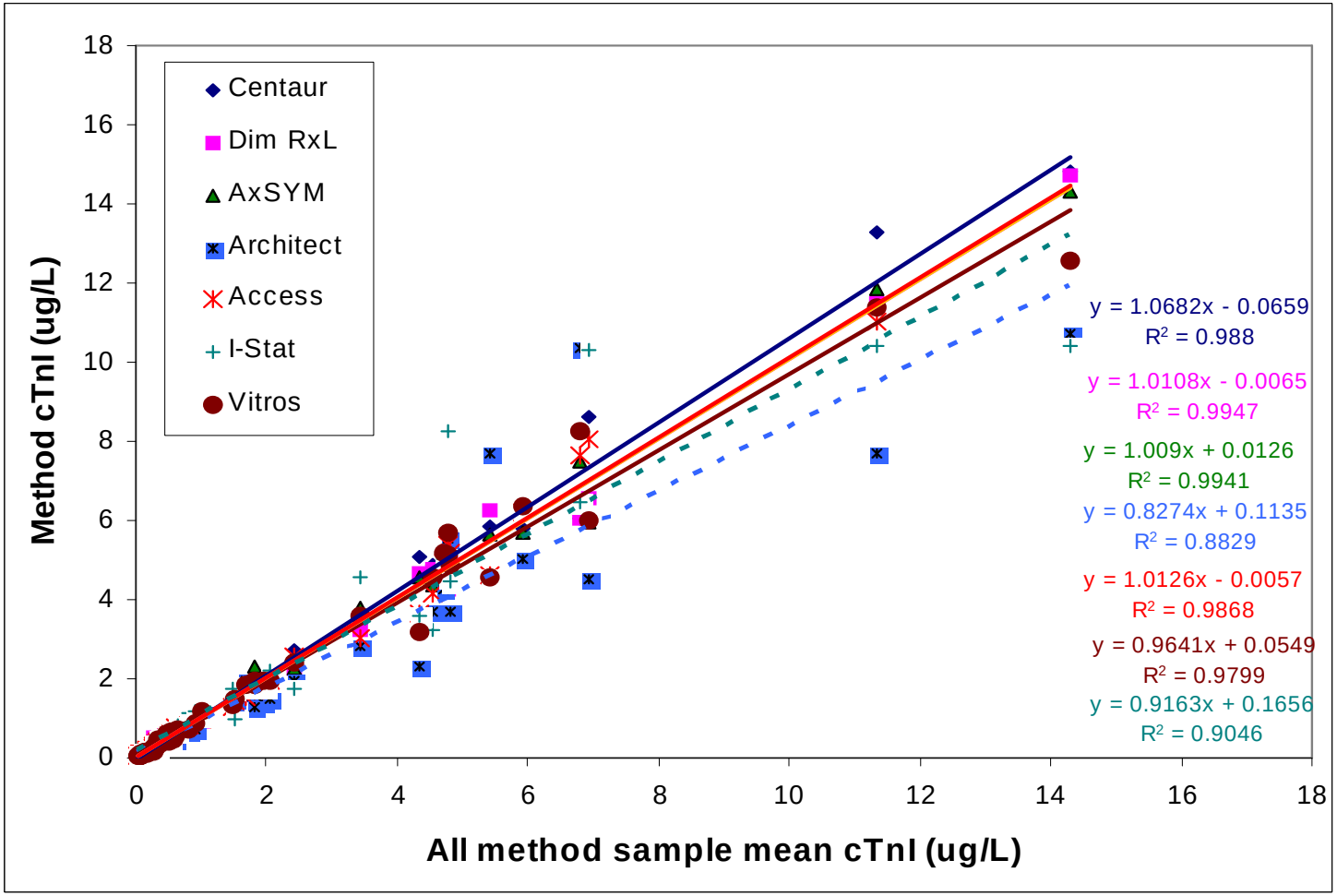
Normalisation factor applied - All method median for MI serum



Tate J. Clin Chem Lab Med 2008; 46: 1489-500

Post realignment against all method mean (patient panel)

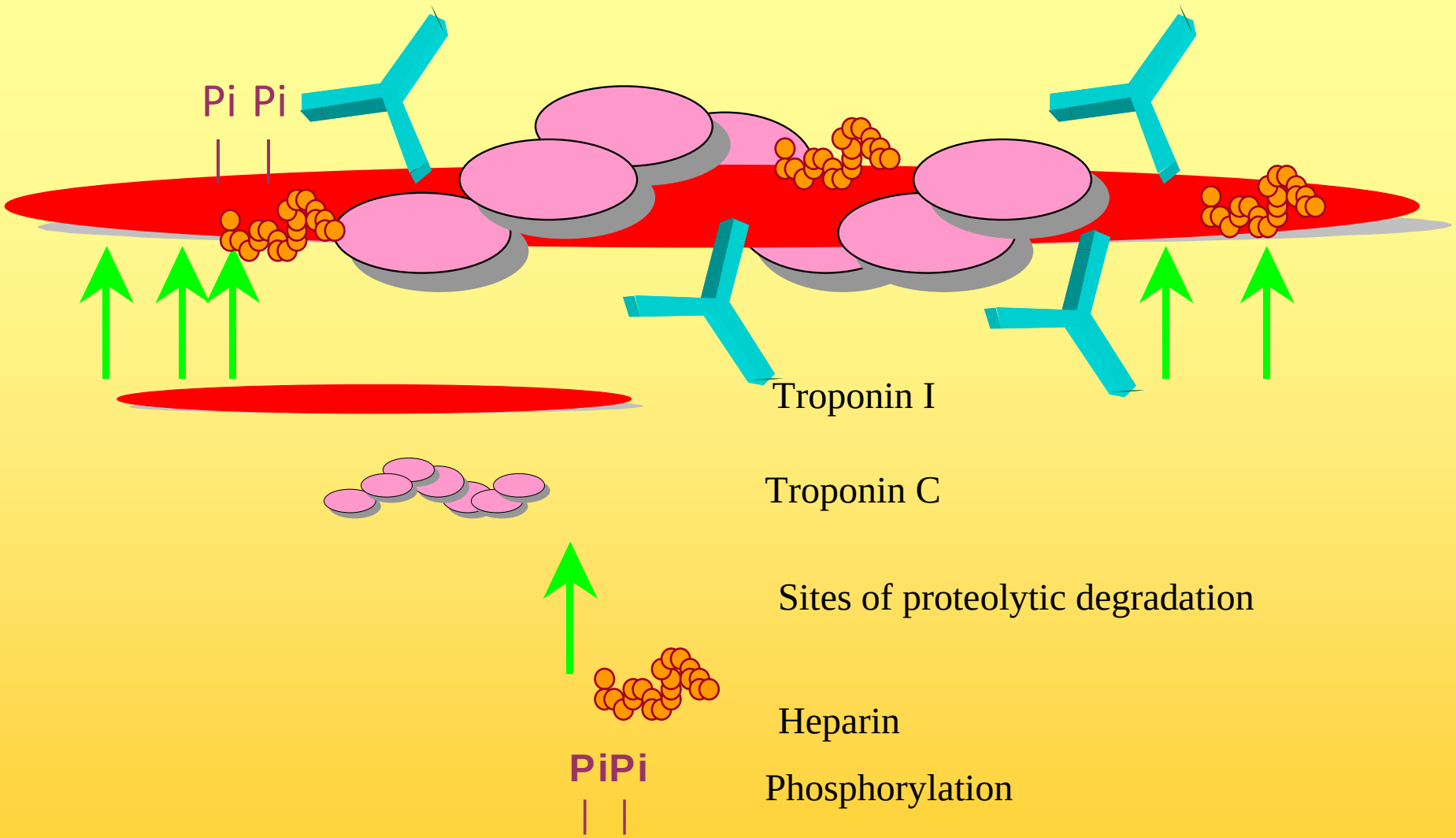
Post mathematical recalibration - All method sample means



Calculations by Dietmar Stöckl

Secondary reference material – Issues to address

- Definitions
  - measurand
  - antibody specificity
- Potential biases from
  - non-commutability
  - cTnI isoforms and non-equimolar reactivity
  - interferences e.g. cTnI autoantibodies, HetAbs
  - instability
- Harmonisation capability
  - what about traceability at low cTnI concentrations?



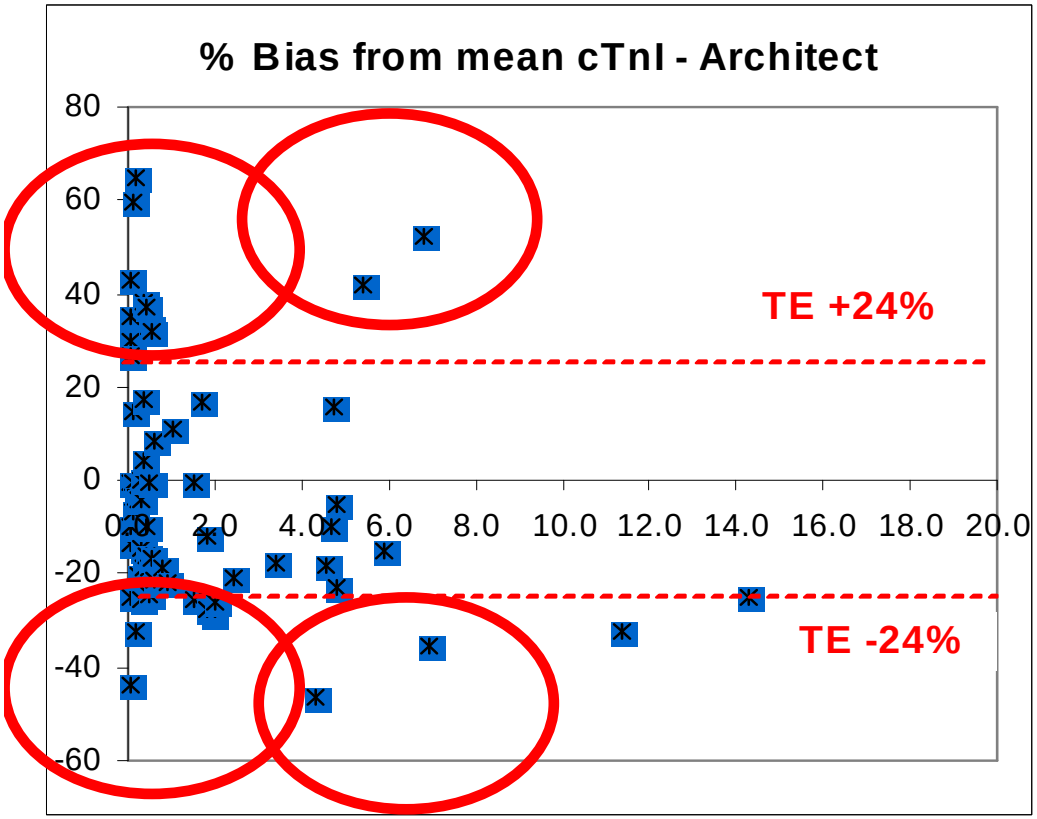
Other chemical modifications – phosphorylation, oxidation, reduction, N-acetylation

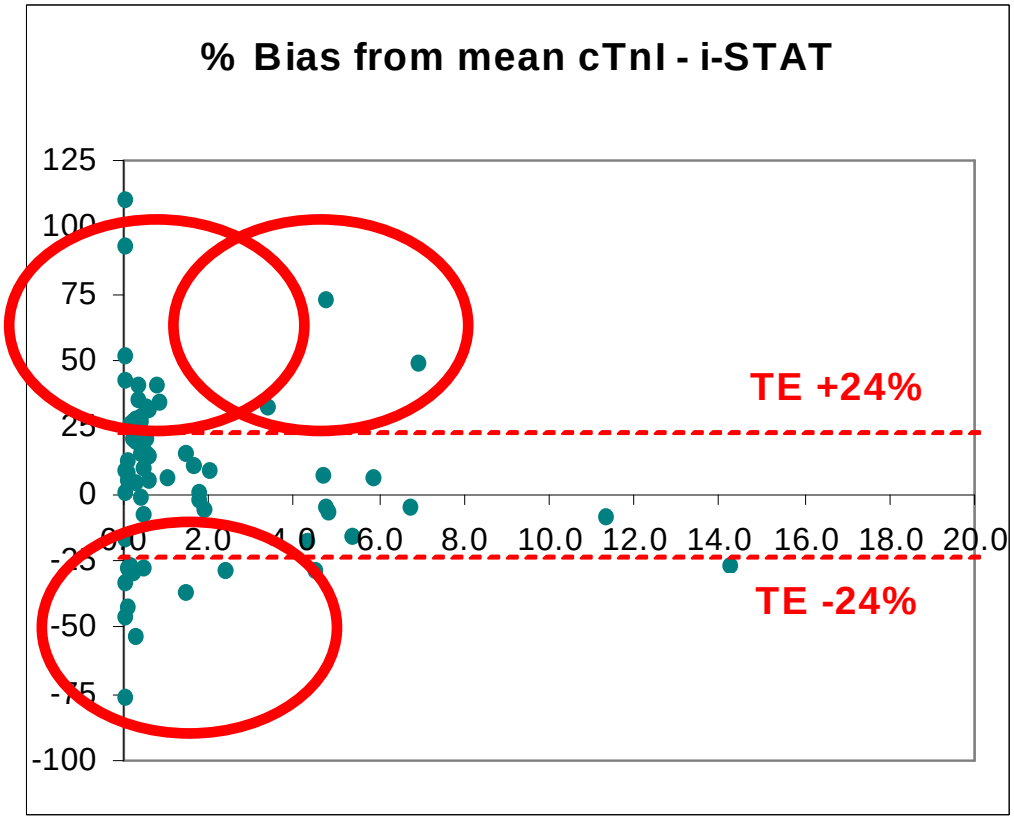
Figure kindly supplied by Dr Alexey Katrukha

Definitions

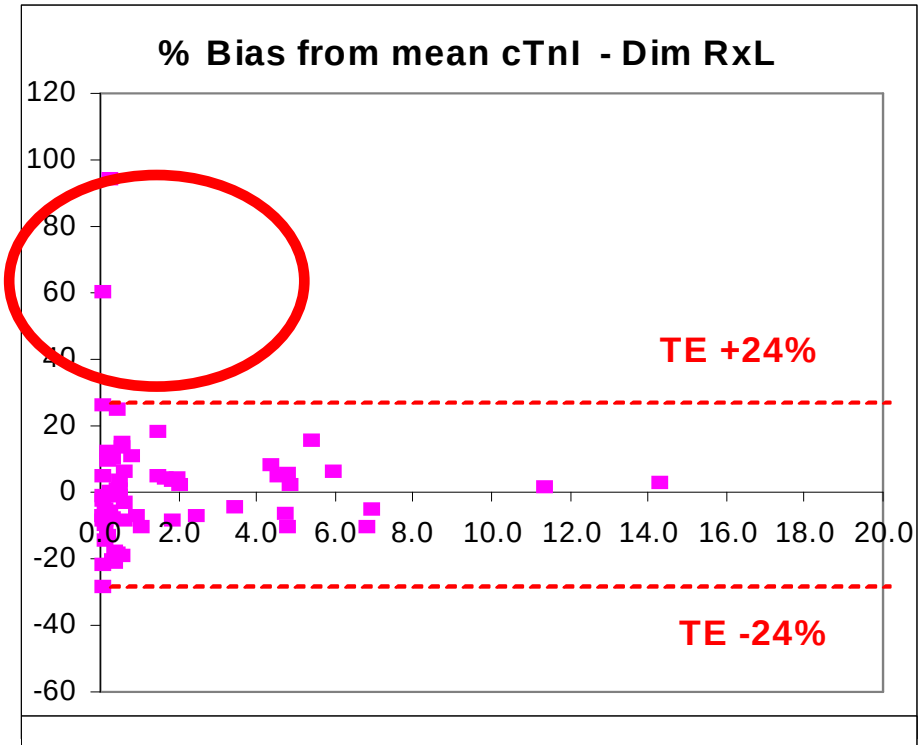
- cTnI measurand
  - unique, invariant part of molecule common to all components of the mixture present in serum
    - **i.e. a specific amino acid sequence**
- Antibody specificity
  - antibodies should preferably recognise **epitopes that are located in the stable part of the cTnI molecule** and not be affected by complex formation (such as ICT) and other in vivo modifications - Panteghini M et al. Clin Chem Lab Med 2001
- Caveat
  - **all plasma cTnI forms** are **measured** by current assays either **equally**, or the difference in reactivity is not clinically relevant.

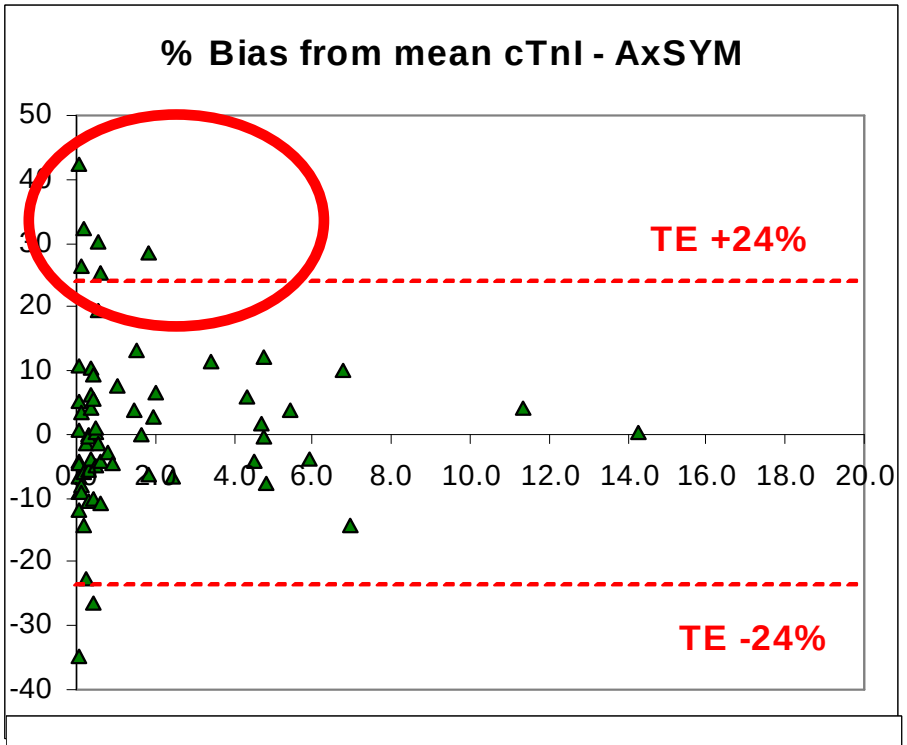
Potential biases between methods

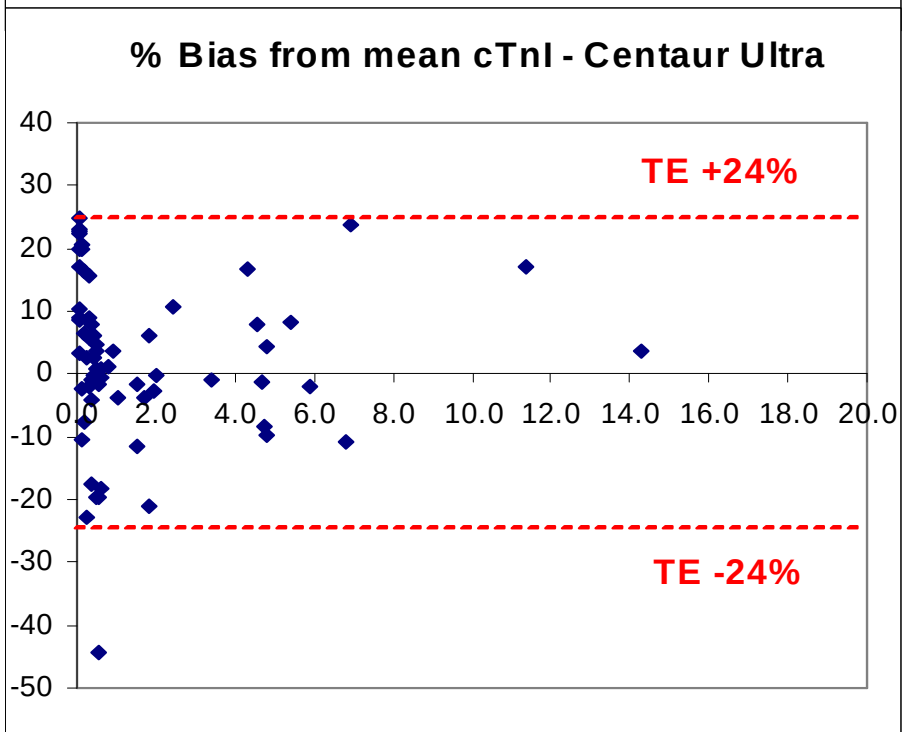


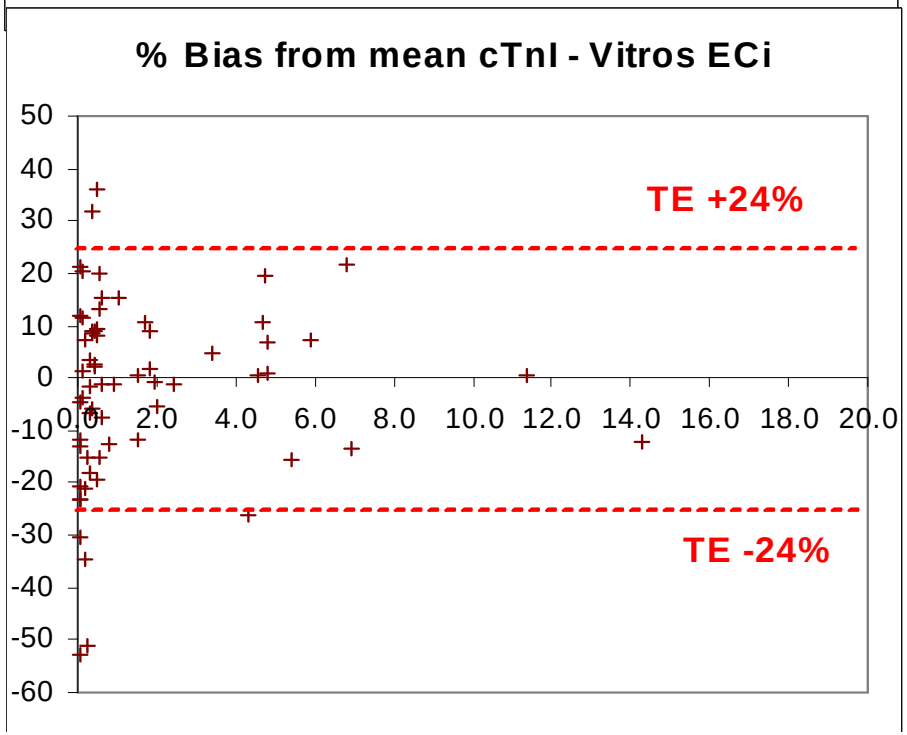


Analytical performance goals for cTn I measurements using routine methods based on biological variability data for cTnI (CVi 9.7 %; CVg 56.8 %) obtained by Wu A et al.
Total Error = bias goal + (1.96 x imprecision goal).


% Bias from mean cTnI - Dim RxL
120
100
80
60
40
20
0
-20
-40
-60
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
TE +24%
TE -24%


% Bias from mean cTnI - AxSYM
50
40
30
20
10
0
-10
-20
-30
-40
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
TE +24%
TE -24%

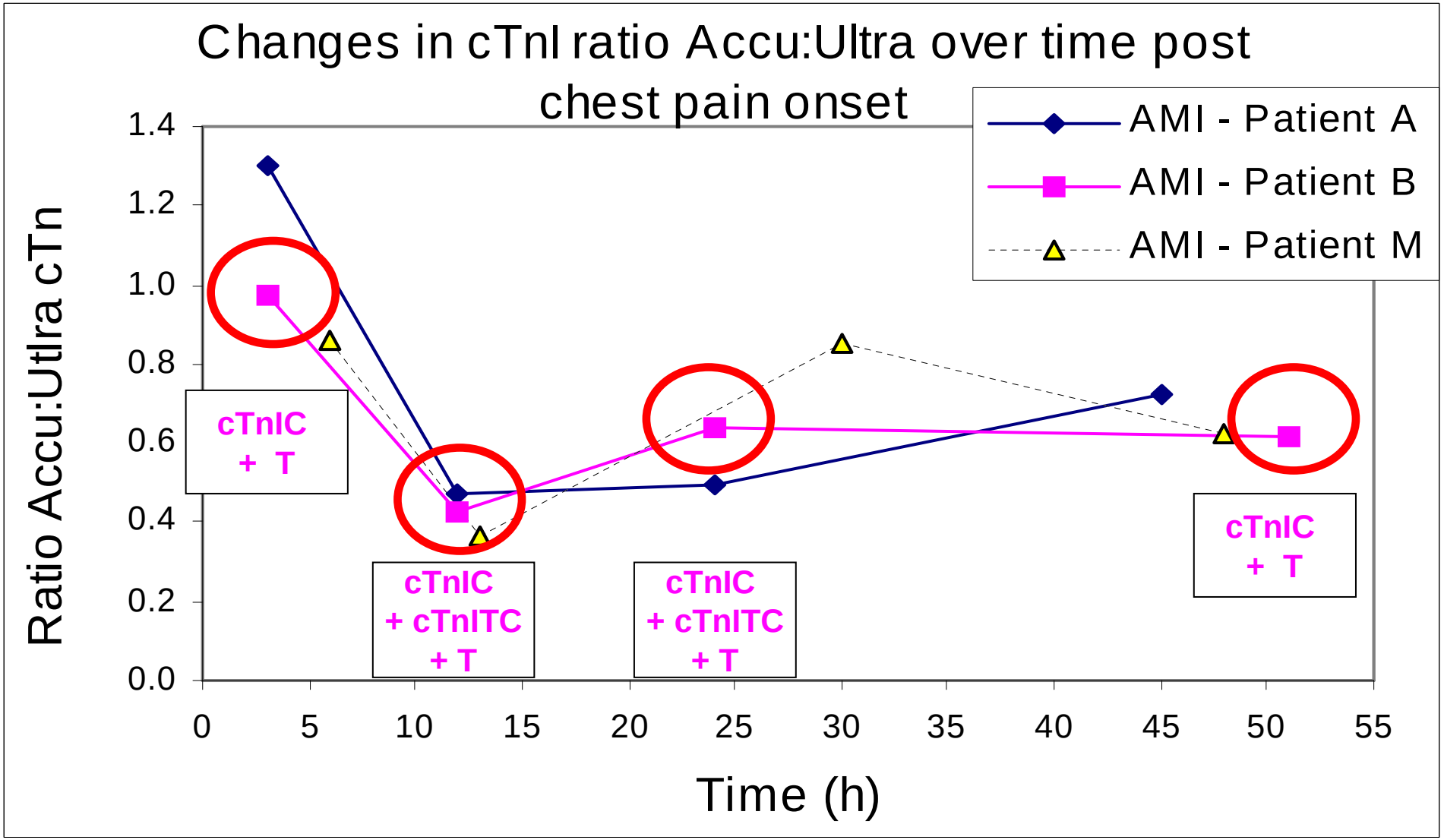

% Bias from mean cTnI - Centaur Ultra
40
30
20
10
0
-10
-20
-30
-40
-50
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
TE +24%
TE -24%


% Bias from mean cTnI - Vitros ECi
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
TE +24%
TE -24%

cTnI plasma forms and assay recognition

cTnI Assay	cTnIC vs. free cTnI vs. cTnICT	Phosph. cTnI vs. native I in cTnICT	Oxidised & reduced forms	Protease degraded or heparin added	Literature Reference
Beckman Coulter Access cTnI	Equimolar	Equimolar	-	-	Uettwillger-Geiger et al Clin Chem 2002
Abbott I-STAT cTnI	Equimolar (± 95%)	Equimolar (± 95%)	Equimolar (± 95%)	-	Apple et al CCA 2005
BioSite Triage cTnI	93-125% recovery for IC, IT, ITC		93-125% recovery for oxid I & red I.	-	Package insert
Radiometer AQT90 FLEX cTnI	cTnIC 1.62-fold higher vs. free cTnI	1.22-fold higher	-	Degraded cTnI 9.46% vs. intact cTnI. Heparin (10 IU/mL) 1.24-fold higher	Eriksson et al Clin Chem 2005
cRMP ELISA 1+1 cTnI	Equimolar cTnIC vs. free cTnI	Insensitive	-	Degraded cTnI similar response to intact. Heparin insensitive	Noble et al CCLM 2010

cTnI plasma forms and assay recognition



Bates KJ et al. Clin Chem 2010; 56: 952-8

Pools as surrogate reference material

69 patients:	AccuTnI 0.05-20 (µg/L)	Accu: Ultra	Accu: Archit	Accu: AxSYM	Accu: Vitros	Accu: Dim	
Mean ratio		**0.66**	**0.99**	**0.95**	**0.93**	**0.96**	**-**
Ratio range		**0.4-1.2**	**0.5-2.6**	**0.5-3.0**	**0.7-1.7**	**0.5-3.0**	**-**

AACC Pools	AccuTnI (µg/L)	Accu: Ultra	Accu: Archit	Accu: AxSYM	Accu: Vitros	Accu: Dim	Accu: Stratus
SP1	0.09	0.9	1.3	1.3	1.7	1.5	1.2
SP2	0.17	0.9	1.0	1.0	1.3	1.2	0.9
SP3	0.22	0.8	0.7	0.9	1.1	1.2	0.8
SP4	0.37	0.7	0.9	0.8	1.1	1.1	0.8
SP5	0.74	0.6	1.0	0.8	0.9	1.1	0.8
SP6	2.05	0.7	0.8	0.8	0.8	0.9	0.8
SP7	3.03	0.7	1.0	0.8	1.0	0.8	0.8
SP8	6.05	0.6	0.7	0.8	0.8	0.8	0.7
Mean ratio		**0.74**	**0.93**	**0.89**	**1.09**	**1.07**	**0.84**
Ratio range		**0.6-0.9**	**0.7-1.3**	**0.8-1.3**	**0.8-1.7**	**0.8-1.5**	**0.7-0.9**

AACC information from R Christenson on behalf of AACC Standardisation Study; Australian study

cTnI Reference Material Development

Aims of IFCC WG-TNI 2010/2011 Pilot Study:

1. Evaluate performance of ELISA-based cRMP using pooled and individual patient sera
   - 30 each at low, medium, and high cTnI concentrations vs. commercial cTnI assays
2. Evaluate pooling protocols for individual patient sera using commercial cTnI assays
   - minimise need for cTnI-positive sera samples by pooling cTnI-positive with normal serum
   - evaluate commutability of pools
   - evaluate stability of pools.

ifcc International Federation of Clinical Chemistry and Laboratory Medicine

cTnI Pilot Study Samples

- Samples from 90 AMI / ACS patients
  - cTnI concentrations in range ≈0.05-20 µg/L
  - Collected from patients up to 72 h post presentation
- 30 samples per low, medium and high level
  - ≈20 mL serum (≈50 mL blood) collected per patient
  - Aliquoted within 4 h of collection & stored at ≤ -70 °C
- Testing by NIST, NPL, Diagnostic Industry:
  - Method comparison (cRMP vs. 23 commercial cTnI assays)
  - Current status of cTnI method agreement
  - Commutability (patient samples vs. serum pools)
  - Stability.

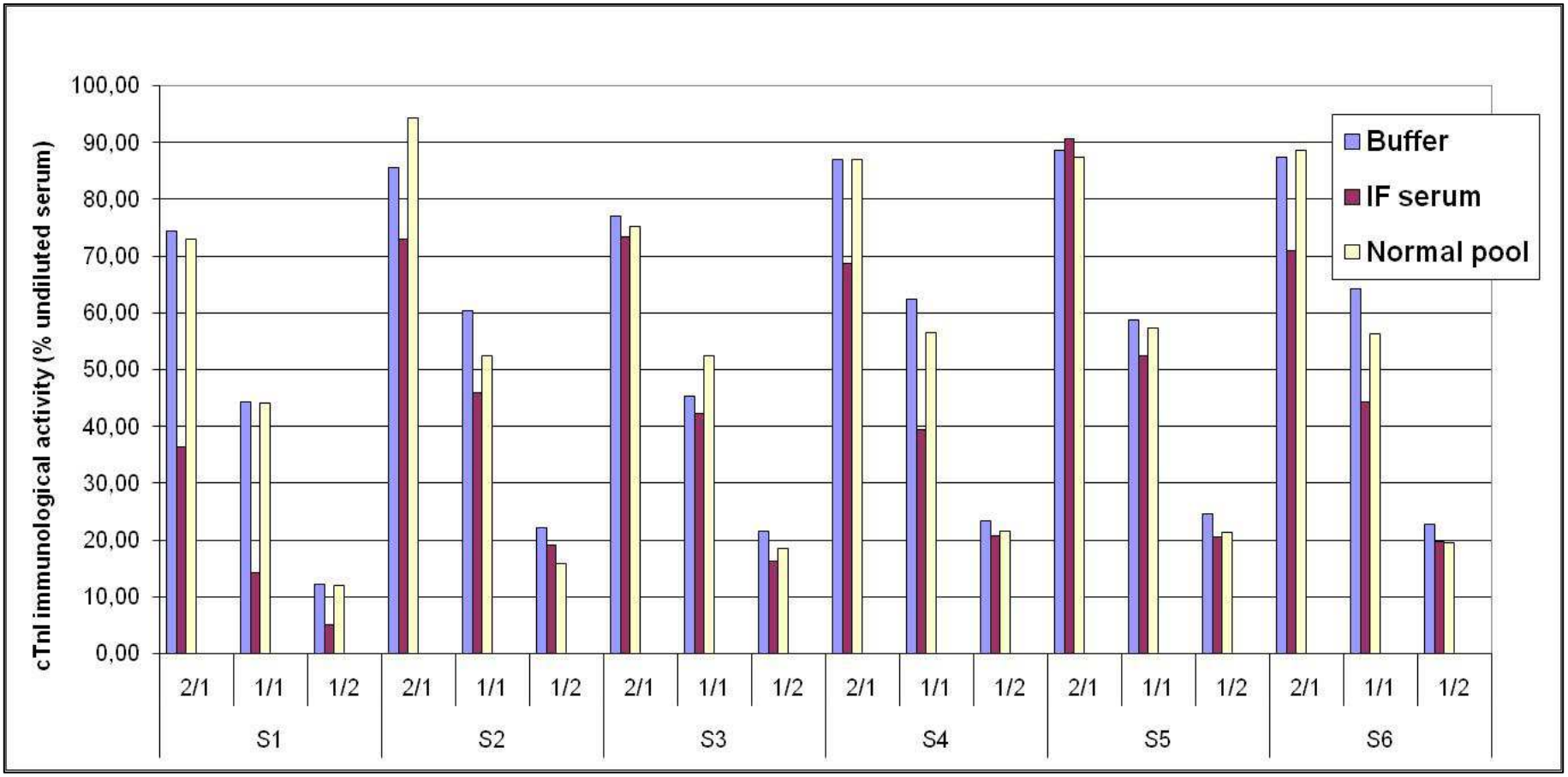
ifcc International Federation of Clinical Chemistry and Laboratory Medicine

Preparation of Serum Pools

- Patient pools prepared in three ways by:
  - addition of individual cTnI-positive native patient samples
  - dilution of high cTnI concentration pool with low & medium concentration pools
  - dilution of high & medium pools with a normal pool
  - final concentration range ≈ 0.2-10 µg/L
- Normal Pool
  - 500 mL pool from ~5-10 female donors (<30 y, BMI <25, & no reported history of heart disease)
  - pre-screen for cTnAAs, HAMA, HetAbs
  - all participating labs to also screen an aliquot.

ifcc International Federation of Clinical Chemistry and Laboratory Medicine

Interference Testing of Normal Pool



Serum dilution: AMI serum : Diluent – 2:1, 1:1, 1:2
Hytest internal assay using mabs to central region (sensitive to cTnAAs)
Conclusion: Normal pooled serum suitable for IFCC study.

Data kindly supplied by Dr Alexey Katrukha

Pilot Study - interference testing

- Testing for cTnI AutoAntibodies
  - Add Normal pool to cTnI-POSITIVE sample in stated proportions (1:3, 1:1, 3:1) and test for recovery
- Testing for cTnI Heterophile Antibodies
  - If Normal pool has a cTnI value above assay 99th percentile concentration proceed to test for HetAbs
    - using heterophile blocking tube
    - by dilution (1:1, 1:2) in assay diluent.

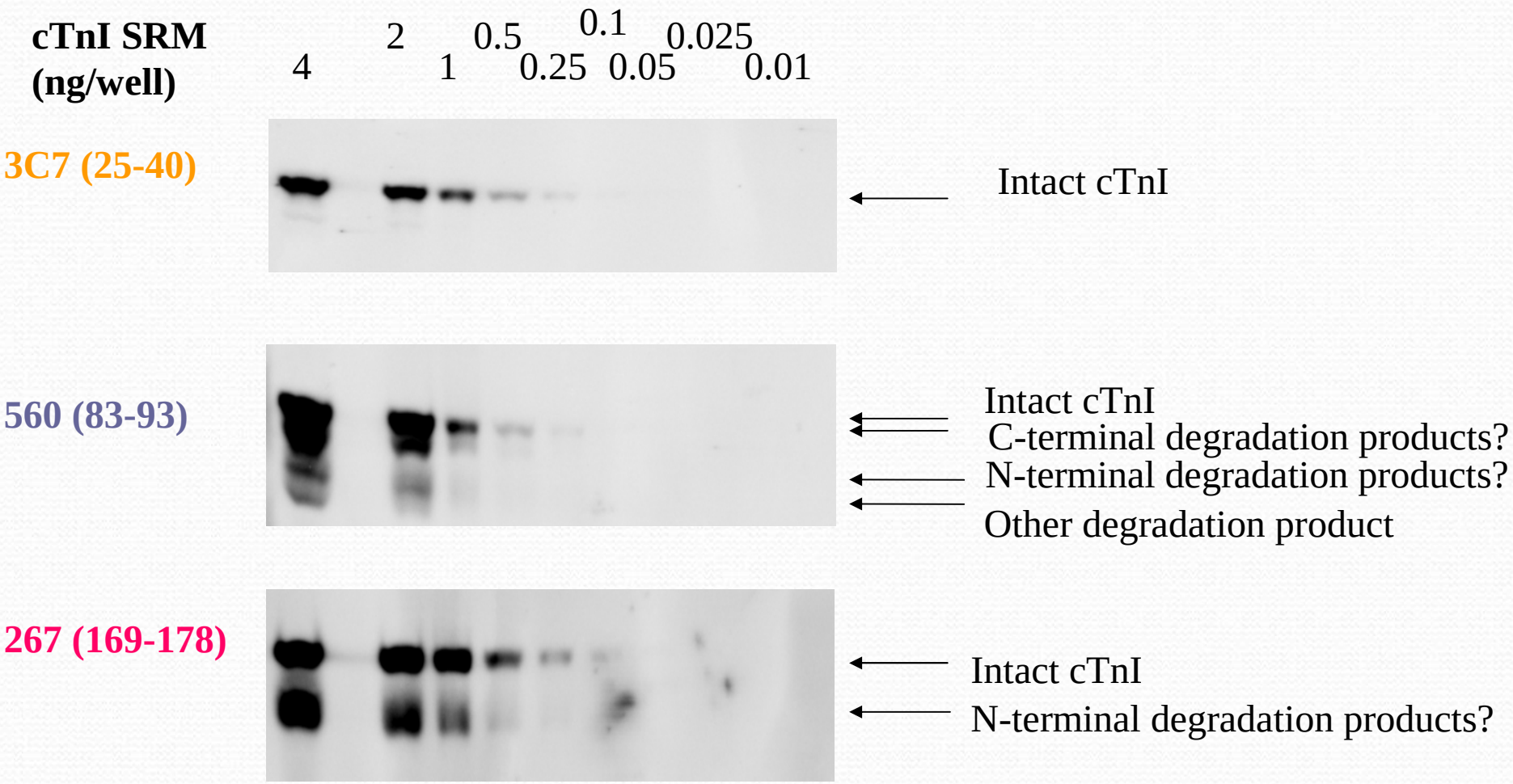
ifcc International Federation of Clinical Chemistry and Laboratory Medicine

cTnI Stability Study

- To test effect on cTnI stability of adding a cocktail of protease/ phosphatase inhibitors
  - cRMP and commercial cTnI assays
- To assay 2 serum pools (low and high levels) spiked with inhibitor cocktail along with unspiked pools & determine recovery
- Isochronous Stability Study to be done by NIST
  - High pools stored at +5 ℃, -20 ℃, & -80 ℃ for t ime intervals ranging from a one day to one month to be assayed on a single platform.

ifcc International Federation of Clinical Chemistry and Laboratory Medicine

cTnI Stability Monitoring - SRM 2921



He H-J, Lowenthal MS, Cole KD, Bunk D, Wang L. Clin Chim Acta 2011;412:107-11

cTnI Quantification in Positive Serum

cTnI spike in (ng)	-	-	-	-	-	-	0.1	0.25	0.5	1	2
Normal serum (100 µL)	+	-	-	-	-	+	+	+	+	+	+
Positive serum (µL)	-	50	100	200	400	-	-	-	-	-	-

Positive serum **SRM 2921**

1. The cTnI concentration in the positive serum pool is ~2.0 µg/L (10-20 % error)
2. cTnI in positive serum has degraded more than that in SRM
3. Immunoprecipitation coupled with fluorescent Western blot analysis can serve as the validation tool for high cTnI concentration pools in the stability study.

He H-J, Lowenthal MS, Cole KD, Bunk D, Wang L. Clin Chim Acta 2011;412:107-11

Commutability of serum pools

- A material is commutable when it gives the same result as a patient sample with the same cTnI concentration
- Study aims to compare serum pools and patient samples by both candidate secondary RMP and routine cTnI assays
- Determine if cTnI concentrations of 7 pools fall within the prediction interval measured by two measurement procedures (one of these is cRMP) for 90 native patient samples.

Commutable if same as patients

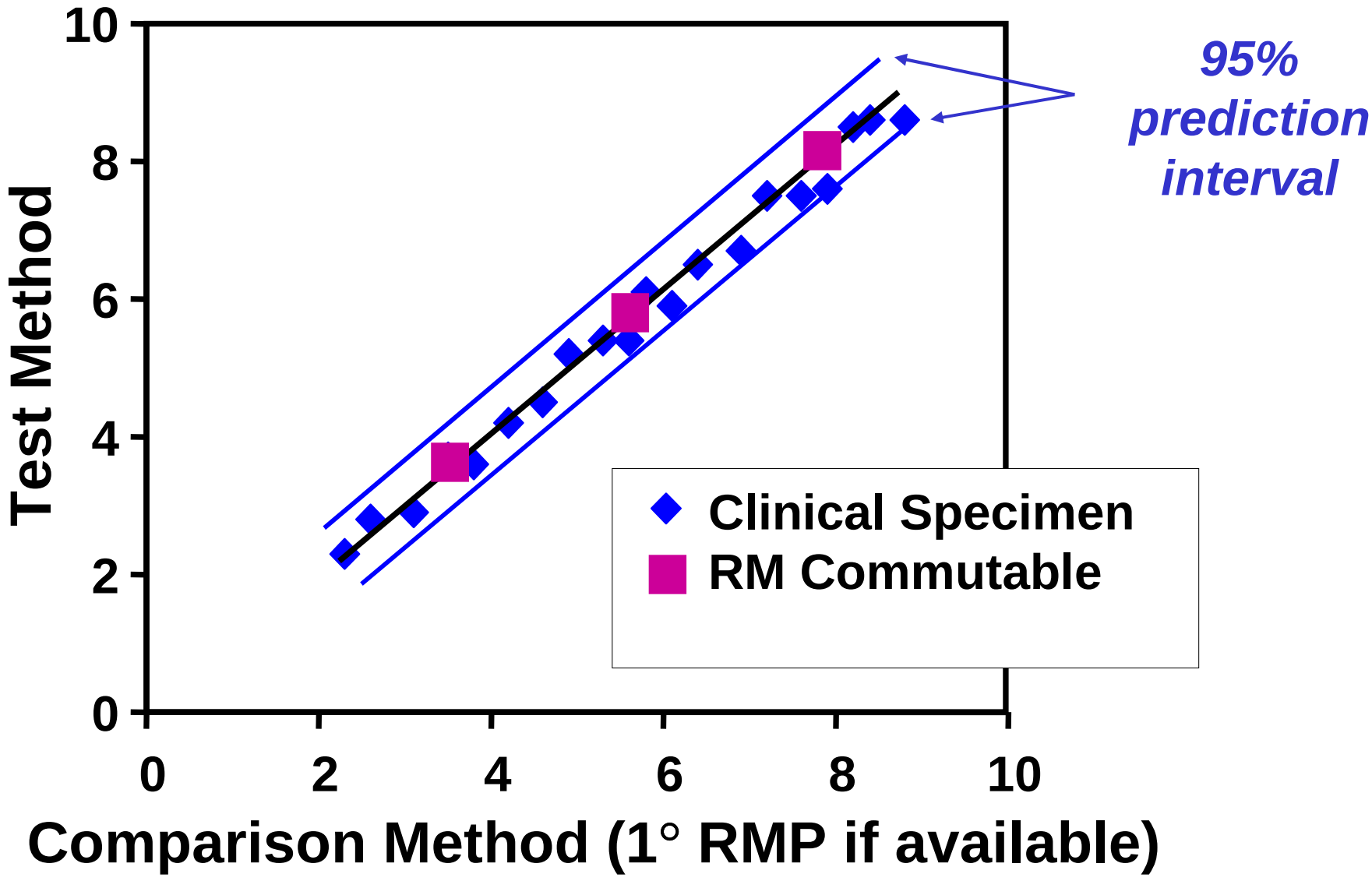


Figure kindly supplied by Dr Greg Miller

Not commutable if different to patients

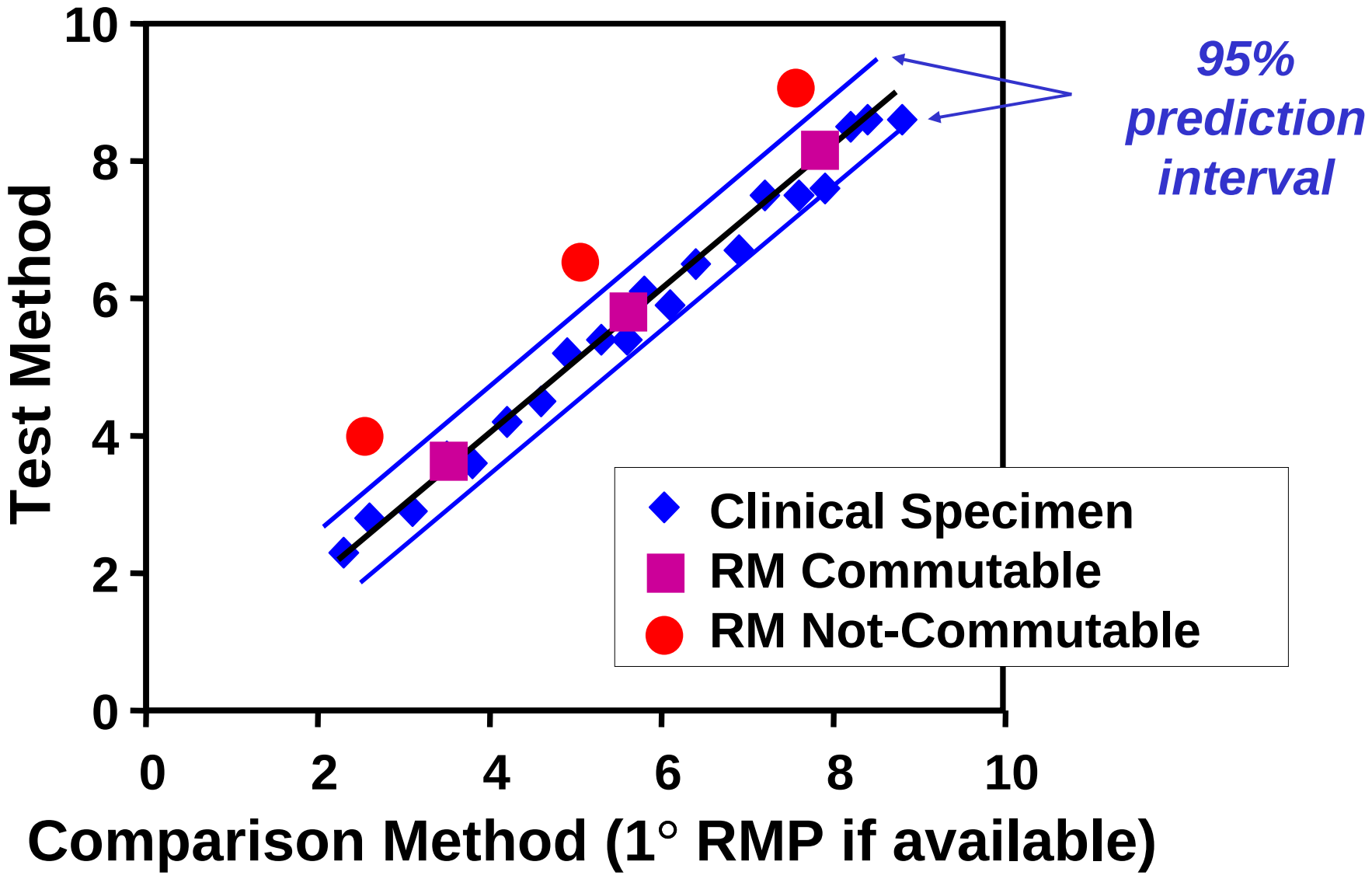


Figure kindly supplied by Dr Greg Miller

Commutability testing requires optimal assay performance

- Imprecision
  - internal QC throughout run(s)
  - duplicate sample measurements
  - quadruplicate Pool (A-G) measurements
- Linearity
  - curvature in curves will make it difficult to assign commutability
  - assess with Residuals plot
- Exclusion of outlier samples e.g.
  - sub-optimal assay performance
  - possible isoform differences
  - Normal pool gives an interference in an assay
  - further testing of specific samples shows the presence of interference.

Commutability testing of cTnI serum pools

1. Assay performance: precision, linearity, sample biases
2. Method comparability before and after exclusion of outlier samples
3. Determine allowable prediction interval (95% C.I., 90% C.I., clinical limits) for acceptable commutability
4. Deming regression analysis (weighted) and/or Relative Residual Analysis or Correspondence Analysis.

ifcc International Federation of Clinical Chemistry and Laboratory Medicine

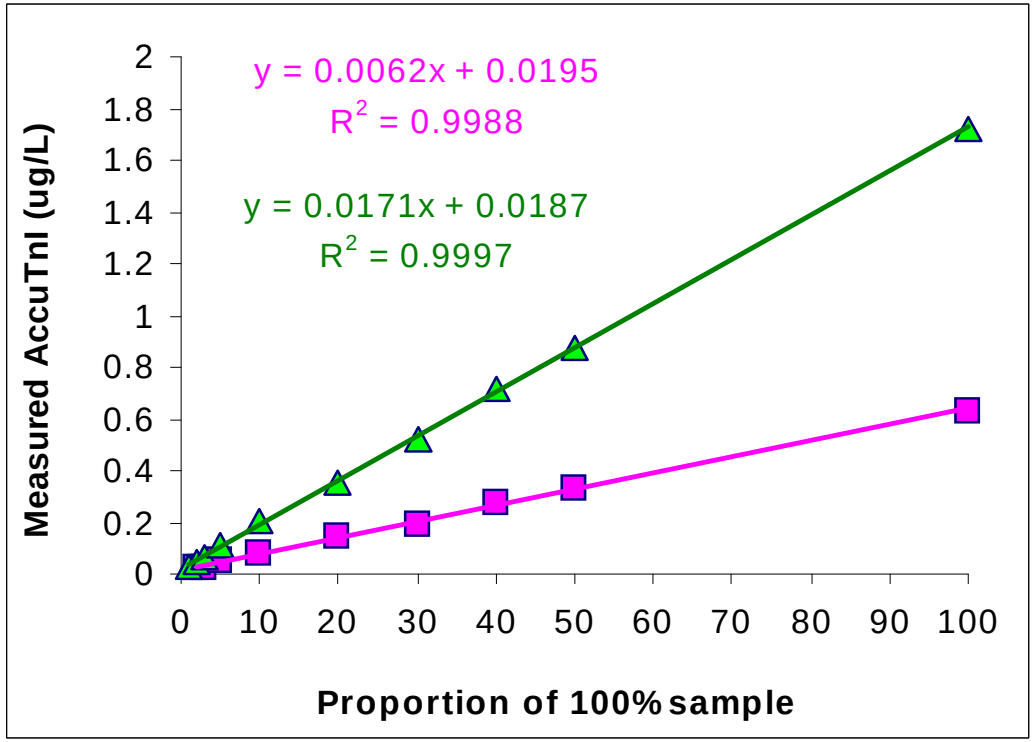
Value assignment of secondary RM: different models

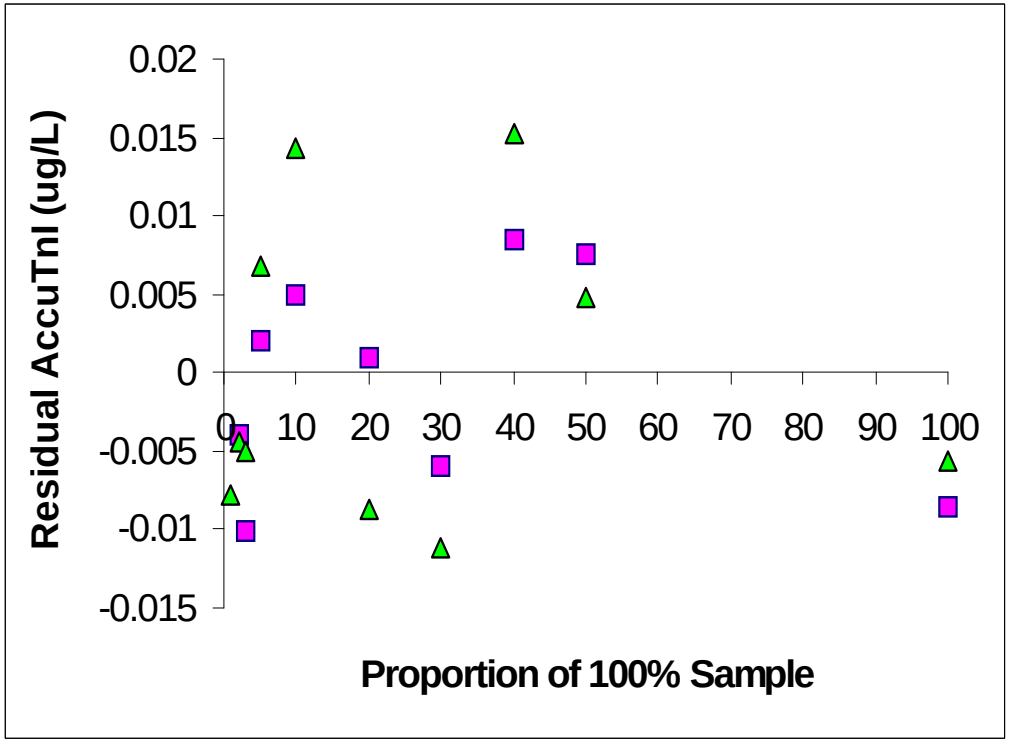
1. A candidate secondary immunoassay-based higher-order measurement cTnI procedure (ELISA)
   - Calibrated with NIST SRM 2921
   - Traceable to higher-order method and to S.I.
   - Independent method traceable to SRM 2921
   - Standardisation approach
2. Mathematical recalibration
   - All method mean (or trimmed) is surrogate RMP
   - Harmonisation approach
3. Consensus method process
   - Use multiple commercial methods
   - Requires traceability to SRM 2921 and commutable assays.

Value transfer to manufacturer's calibrators and to low cTnI levels

- Compare with value assignment of CRM-470 (ERM-DA470/IFCC) – Blirup-Jensen S et al. CCLM 2008
- Consensus method process uses a standardised value transfer RMP consisting of dilutions of pure material (SRM – gravimetric dilution) and candidate reference material
  - Slope and y-intercept within acceptable limits
  - Imprecision within- & between-run within acceptable limits.
- Value transfer to low cTnI concentration using a dilution series and specified dilution protocol e.g. hs-CRP CRM
  - Linear (R^2 >0.99) & residuals plot has no bias.

Value transfer to low cTnI concentration – linear dilution





Patient sample & NIST SRM 2921 diluted in normal serum pool

Diluted patient sample measured: 0.020 – 0.631 µg/L (LoD 0.01 µg/L)

NIST SRM 2921 expected range: 0.010 – 1.00 µg/L

R^2 >0.99

Residuals plot shows no discernible trend suggesting scatter of points about the line is random

IFCC WG
Standardisation of Troponin I

WG-TNI Membership	
Name	***Affiliation***
J Tate (Chair) (AU)	IFCC
J Barth (UK)	ACB
D Bunk (US)	NIST
R Christenson (US)	AACC
A Katrukha (FI)	HyTest Ltd.
M Panteghini (IT)	CIRME
R Porter (UK) J Noble (UK)	NPL
H Schimmel (BE)	IRMM
L Wang (US)	NIST
I Young	IFCC SD Liaison

Labs Participating in cTnI Pilot Study

ABBOTT DIAGNOSTICS
BECKMAN COULTER
BIO-RAD LABORATORIES
BIOMERIEUX
MITSUBISHI CHEMICAL MED CO
ORTHO-CLINICAL DIAGNOSTICS
RANDOX LABORATORIES LTD
ROCHE DIAGNOSTICS GmbH
SIEMENS DIAGNOSTICS
NIST
NPL