Metrological Traceability and Assay Standardization in Laboratory Medicine

CIRME

UNIVERSITÀ DEGLI STUDI DI MILANO

STRESA, ITALY

May 24, 2013

EUROMEDLAB MILANO 2013

19-23 MAY

Standardization of cTnI: Is there a light at the end of the tunnel?

Jill Tate
Pathology Queensland
Brisbane, AUSTRALIA

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JCTLM
Joint Committee for Traceability in Laboratory Medicine

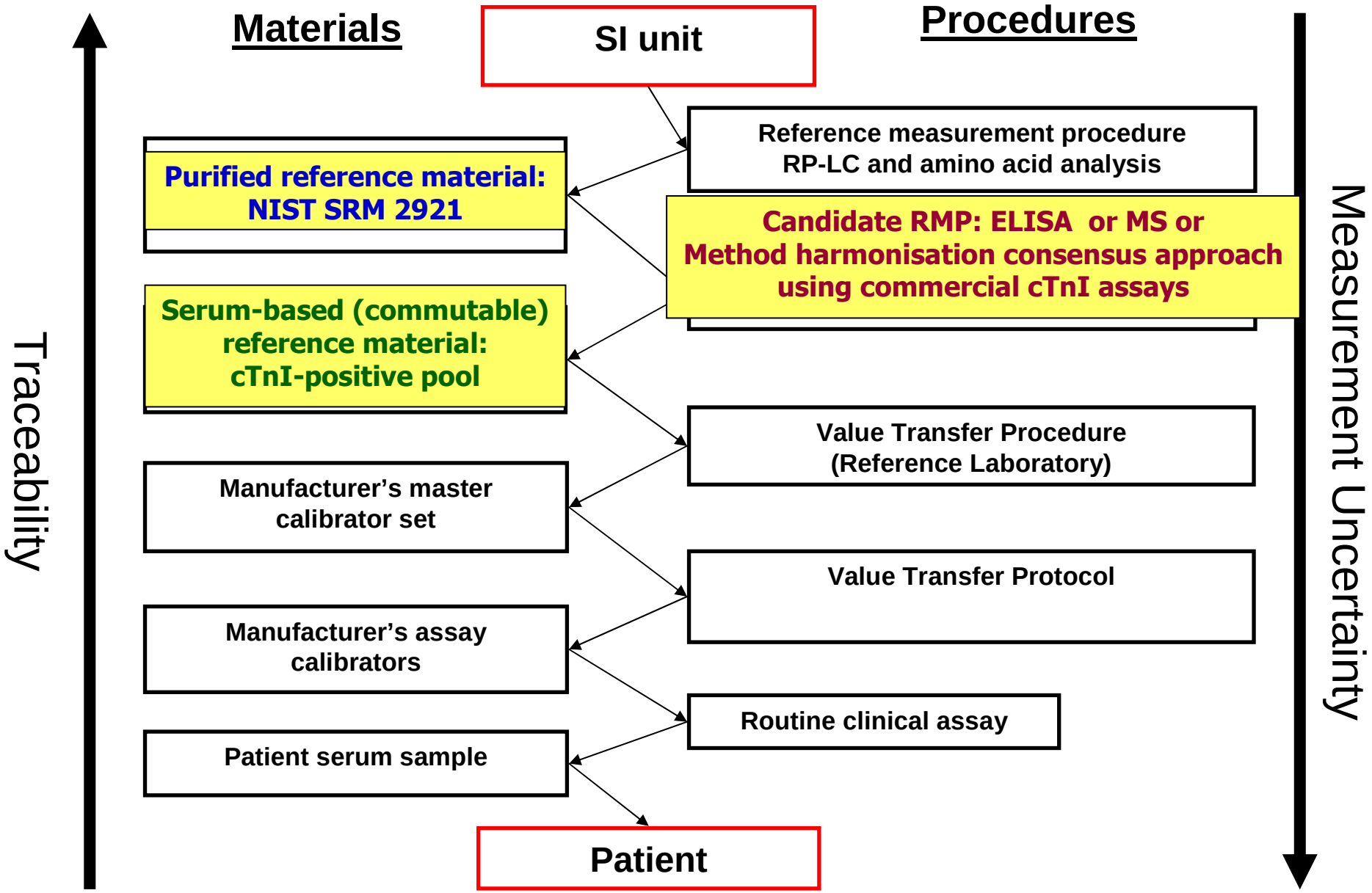
EFCC
EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE

ifcc
International Federation of Clinical Chemistry and Laboratory Medicine

Talk Outline

- Background
- IFCC cTnI Pilot Study
  - Serum pool as surrogate SRM
  - Harmonisation capability
  - Commutability across all assays
  - Stability
- Next steps
  - Preparation of candidate SRM
  - Value assignment and uncertainty budget
  - Value transfer to manufacturer's calibrator
  - Harmonisation / commutability testing phase

cTnI reference measurement system



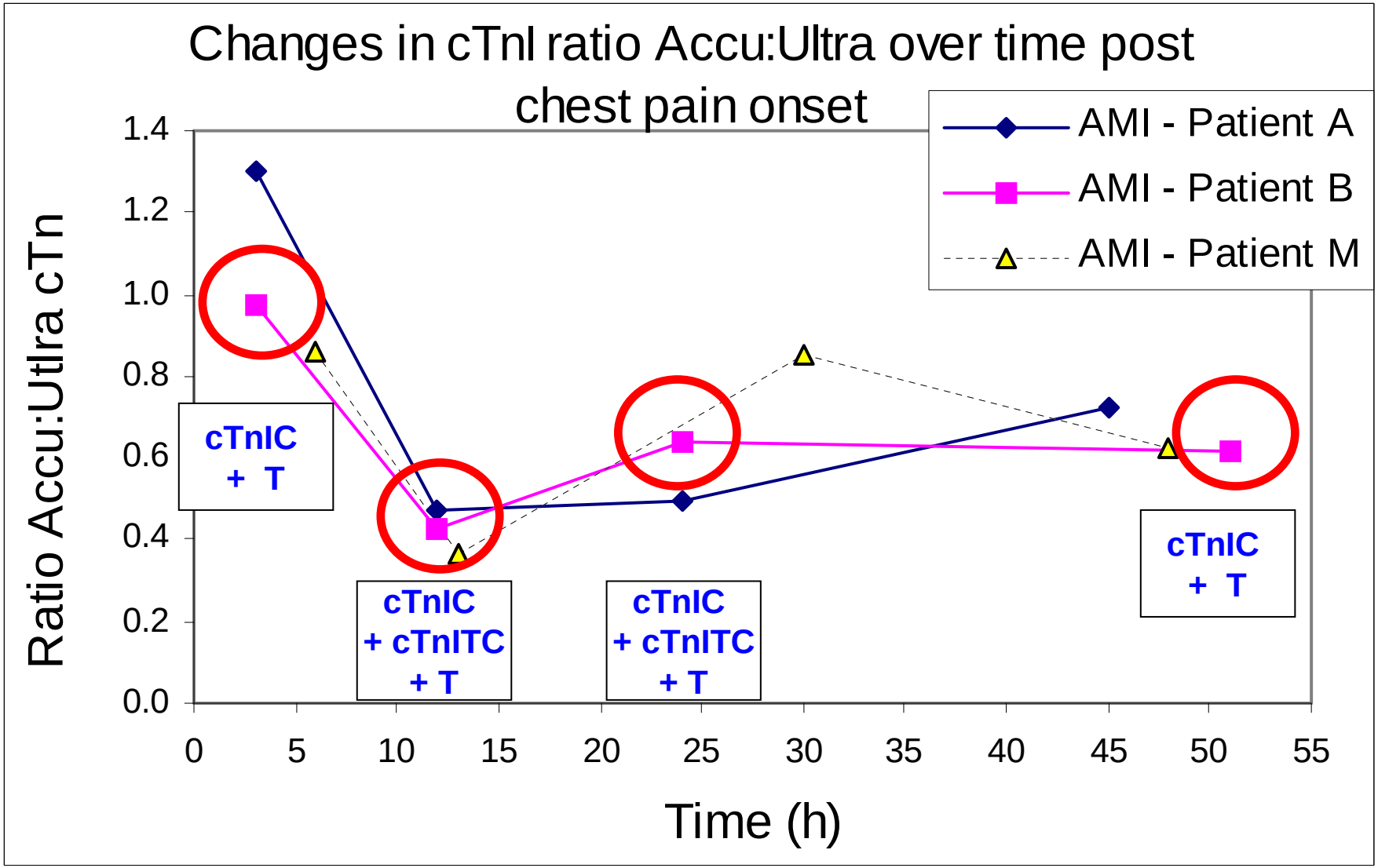
Reference materials for cTnI

- NIST SRM 2921
  - Purified ICT complex
  - Value assignment: RP-LC & amino acid analysis
  - Not commutable in ~50% commercial assays
- Serum-based certified SRM
  - Commutable in all commercial assays
  - Lack of interferences
    e.g. cTnI autoantibodies, heterophile antibodies
  - Stable over long-term
  - Standardised procedures in place for value assignment and value transfer to manufacturers' master calibrators

Requirements for equivalent cTnI measurements

- Measurand is defined
  - unique, invariant part of molecule common to all components of the mixture present in serum
- Antibody specificity is defined
  - Abies preferably recognise epitopes located in the stable part of cTnI molecule
  - all plasma cTnI forms have equal reactivity or the difference in reactivity is not clinically relevant
- Assays are capable of being harmonised
- SRM is commutable across majority of assays
- Manufacturers have calibration traceability to SRM

cTnI isoforms and assay recognition



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

PROOF of PRINCIPLE: Serum pools as SRM for cTnI

- Pools to consist of a blend of clinically relevant cTnI forms and act as “surrogate SRM for cTnI” rather than reflecting the cTnI composition of each individual clinical sample
- Pools are commutable with patient samples covering the clinical cTnI concentration range
- Pools lead to equivalent cTnI measurement values

cTnI Pilot Study in 2010-2012: AIMS

- Validation of the immunoassay reference measurement procedure for cTnI
- Current status of commercial cTnI assays
- Assessment of the commutability of "blended" serum pooled cTnI candidate reference materials
- Evaluation of the stability of serum reference materials for cTnI

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cTnI Pilot Study Samples

- Collection of samples from >90 patients with suspected AMI
  - 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
  - Aliquotted within 4 h of collection & stored at ≤ -70 °C
- Preparation of pools & sample kits at NIST
- Testing by NIST and Diagnostic Industry (NPL)
  - January to May 2012 (1 lab in December 2012)

Participating Laboratories

- **Beckman Access (AccuTnI)**
- **Biomerieux VIDAS TnI Ultra**
- **Siemens ADVIA Centaur (Ultra)**
- **Siemens Immulite 2000/Xpi**
- **Siemens Dimension EXL w/LM**
- **Siemens Stratus CS**
- **PATHFAST cTnI (PF 1011-K)**
- **PATHFAST cTnI-II (PF 1101-K)**
- **OCD Vitros 5600**
- **Roche Elecsys cobas e601**
- **Roche Elecsys cobas e411**
- **Siemens Immulite 1000 TPI**
- **Siemens Dimension Vista**
- **Siemens Dimension RxL**
- **Abbott Architect STAT hsTnI**
- **Abbott Architect i2000SR**
- **Abbott AxSYM cTnI-ADV**
- **cRMP (at NIST)**

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 and 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-screened for cTnAAs – none detected
  - all participating labs also screened an aliquot.

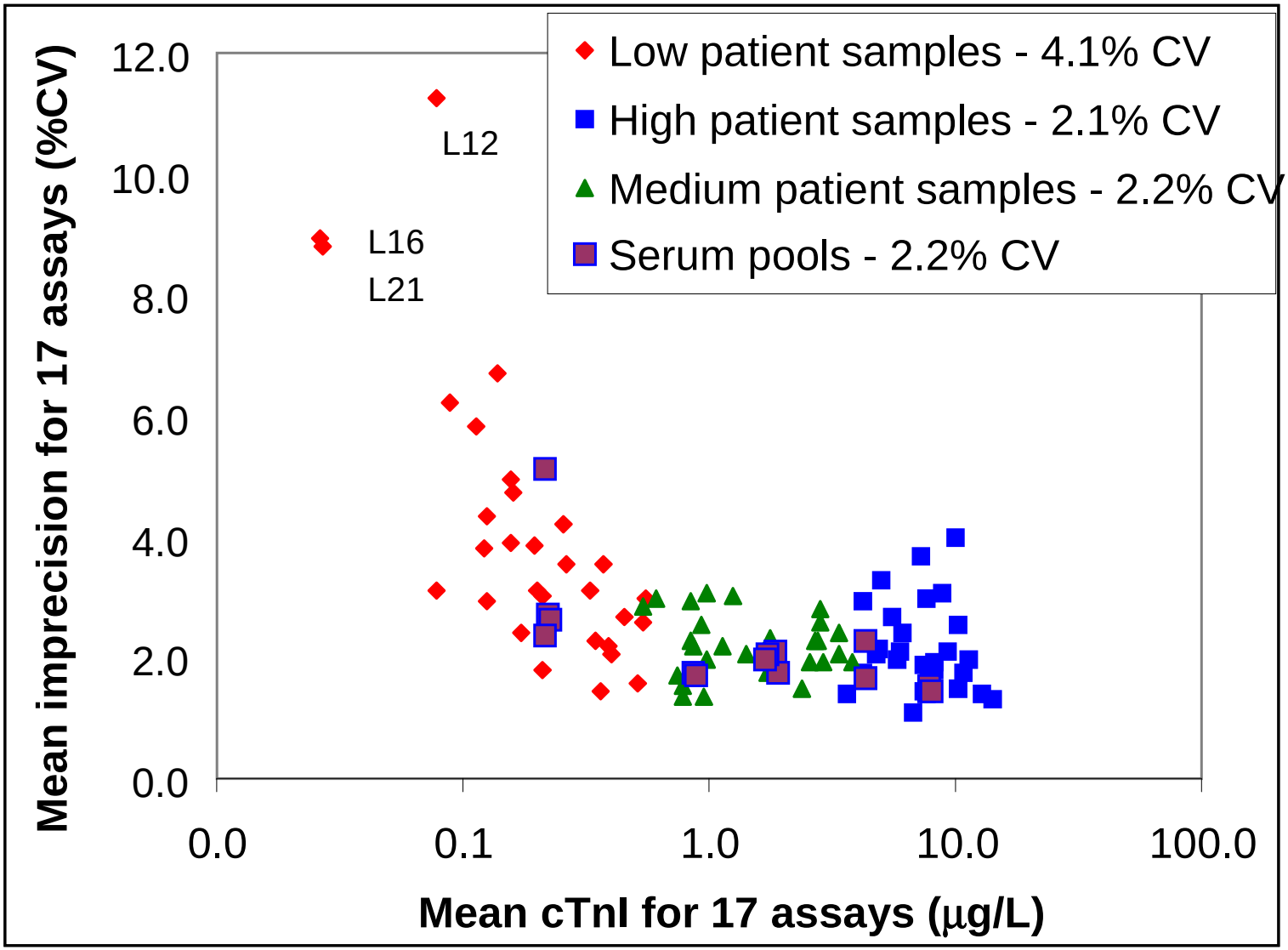
cTnI Candidate Serum Pools

Pool	Description
A	18 low cTnI patient samples pooled using volumes which ranged from 1.25 mL to 8.0 mL
B	21 medium cTnI patient samples pooled using volumes which ranged from 1.5 mL to 6.0 mL
C	21 high cTnI patient samples pooled using volumes which ranged from 0.75 mL to 10.75 mL
D	28.0 mL **Pool A** and 7.0 mL **Pool C**
E	14.0 mL **Pool C** and 21.0 mL **Pool B**
F	4.0 mL **Pool C** and 36.0 mL **Pool NORM**
G	4.0 mL **Pool B** and 36.0 mL **Pool NORM**

cTnI Pilot Study: data analysis and results

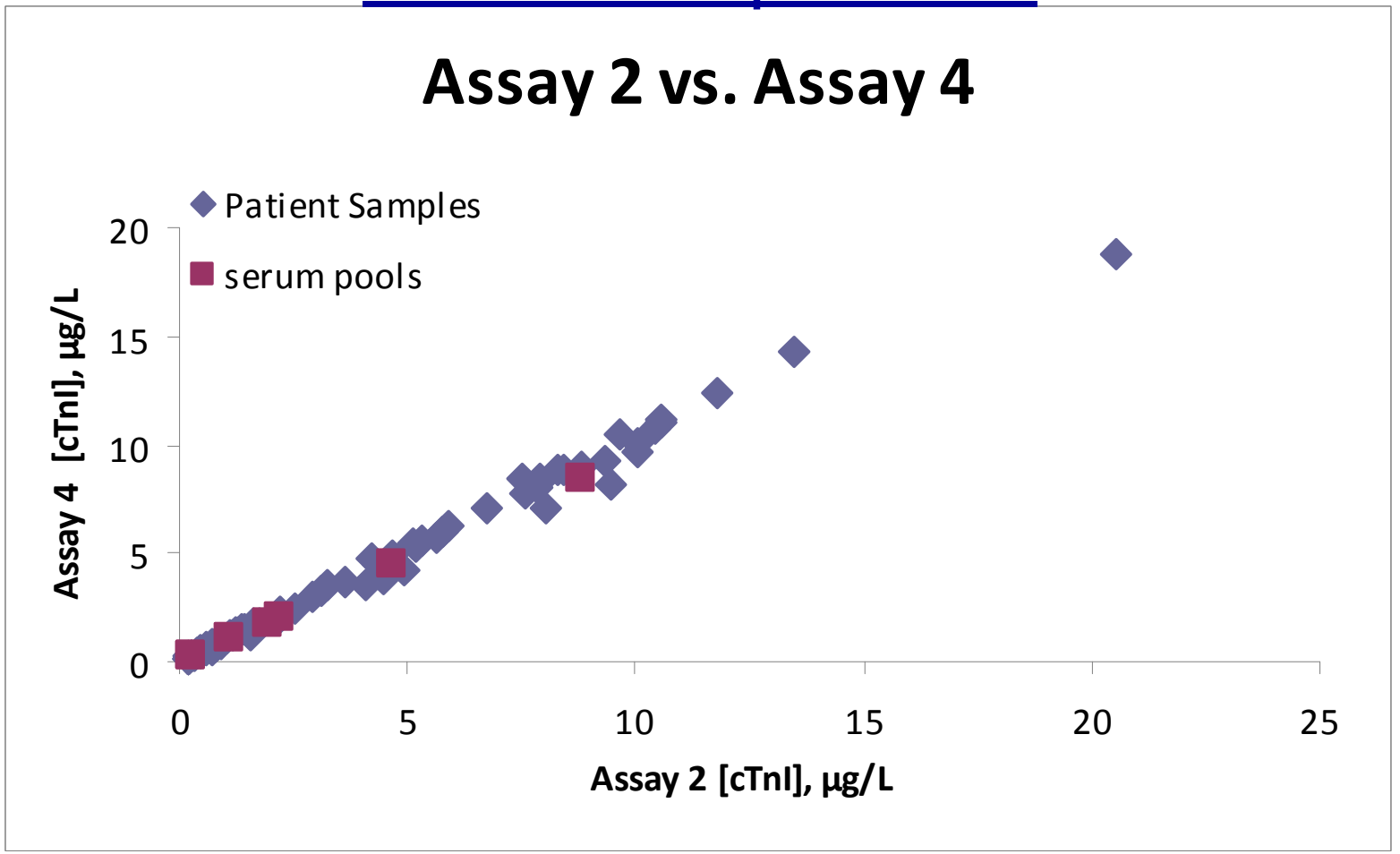
- Imprecision
  - Duplicate measurements for 90 patient samples and 7 duplicate vials of pools
- Current status of commercial cTnI assays and cRMP
  - Between-method variation
- Commutability
  - Pools vs 90 patient samples
- Harmonisation capability
  - Between-method agreement

cTnI Pilot Study - imprecision



Commutability Assessment of Pools

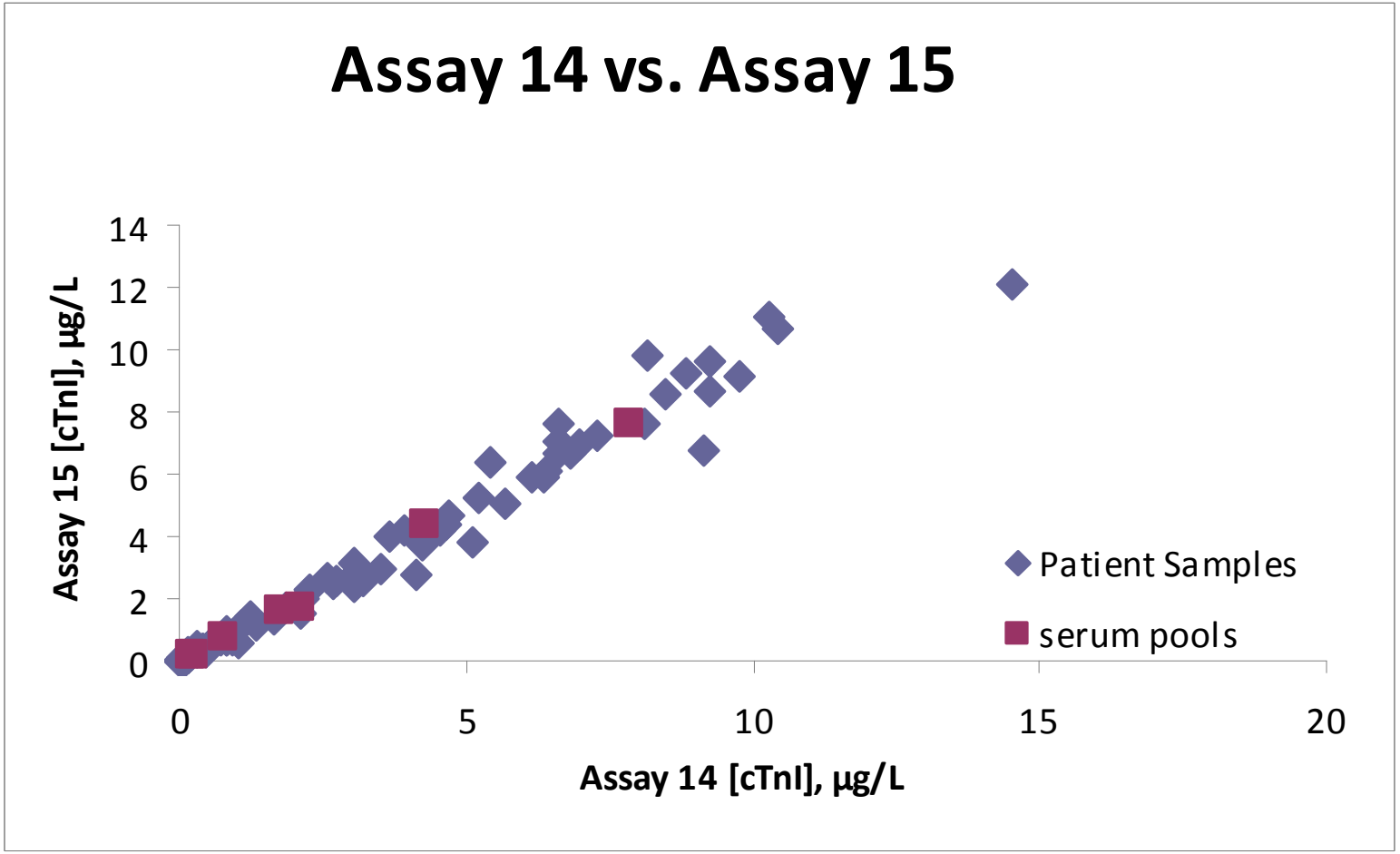
Paired comparisons



Assay 2 and 4 from same manufacturer

Commutability Assessment of Pools

Paired comparisons

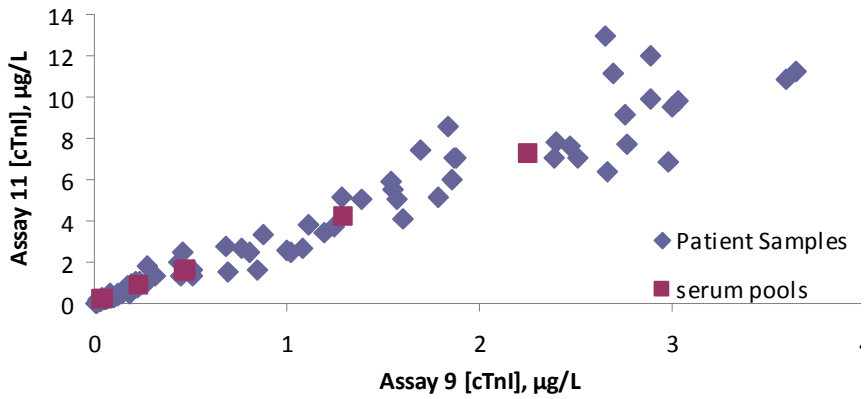


Assay 14 and 15 from different manufacturer

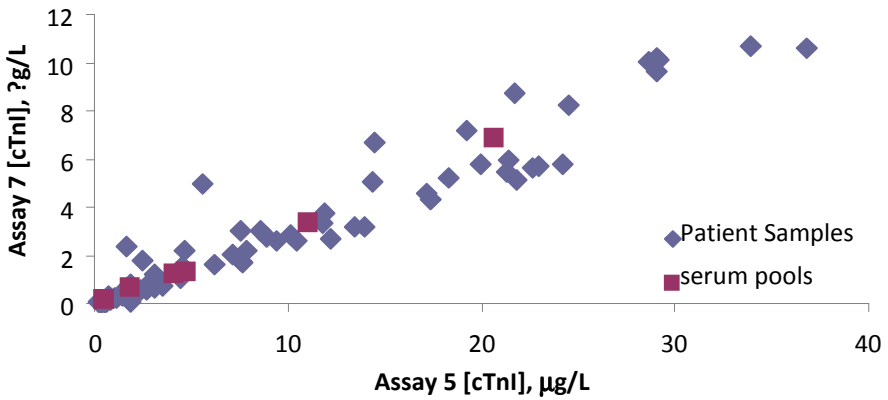
Commutability Assessment of Pools

Paired comparisons

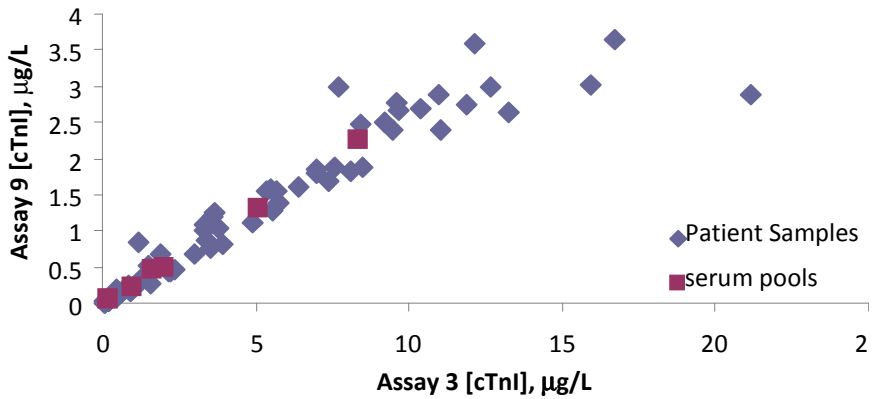
Assay 9 vs. Assay 11



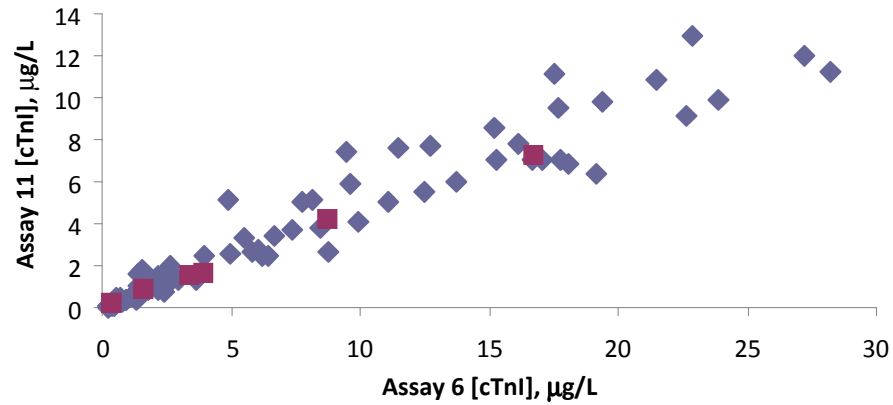
Assay 5 vs. Assay 7



Assay 3 vs. Assay 9



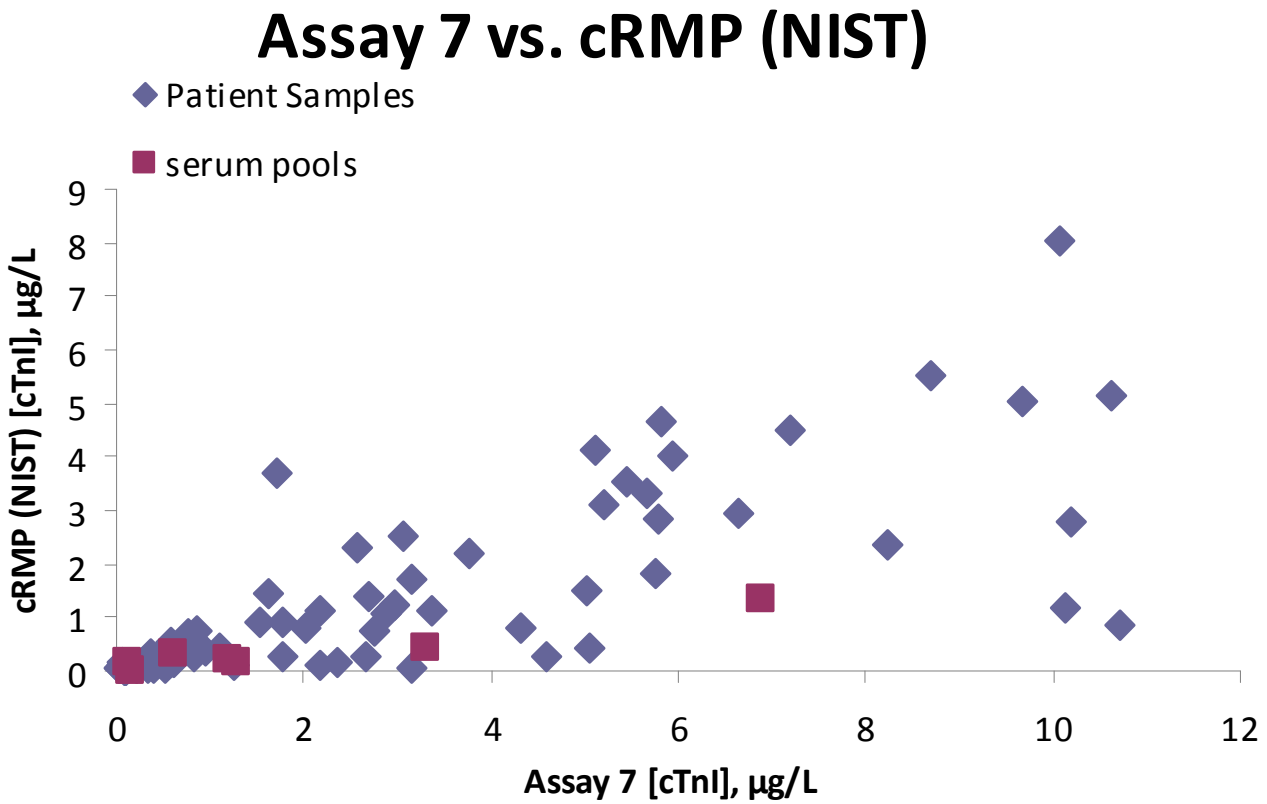
Assay 6 vs. Assay 11



Most of the 136 paired comparisons looked like these

Commutability Assessment of Pools

Paired comparisons



Nearly all paired comparisons of the cRMP vs. commercial assays looked like this

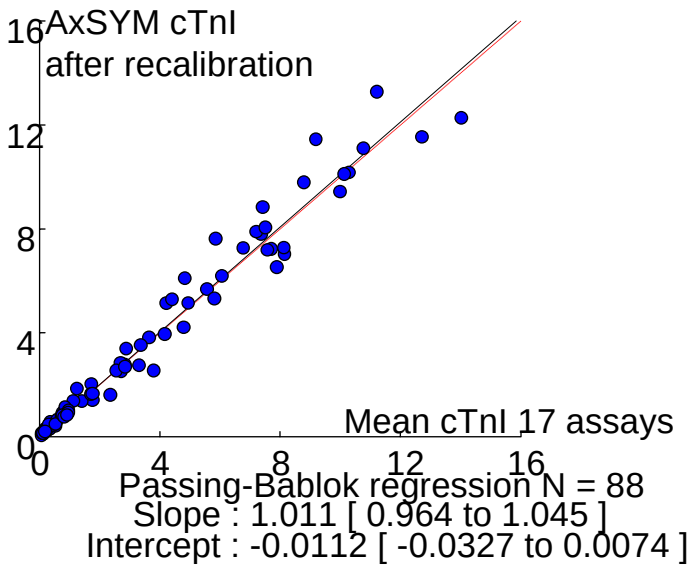
Current status of cTnI assays in 2012

- For commercial assays ~10-fold difference in concentration between assays
- cRMP shows poor correlation with all routine assays
- Passing-Bablok analysis indicates overlap of the 95% confidence intervals of the regression slopes of patient samples and all pools indicating that all the serum pools are commutable for all routine assays
- PCA also indicates pools are commutable

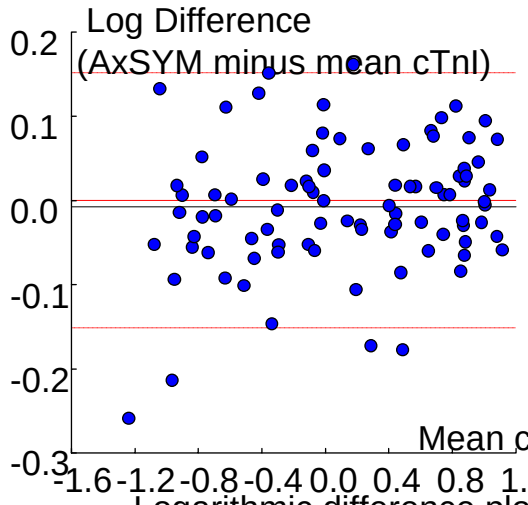
Data analysis of cTnI harmonisation

- Slope correction was determined for each assay
  - using Passing-Bablok regression analysis against mean cTnI for 17 assays for 90 patient samples
- Mathematical recalibration/recalculation was applied
  - correction factor (CF) determined as [1/regression slope]
  - recalculated cTnI = measured cTnI x CF
- Between-method agreement (CV) post recalibration for:
  - all 17 assays
  - 16 assays (1 assay excluded)

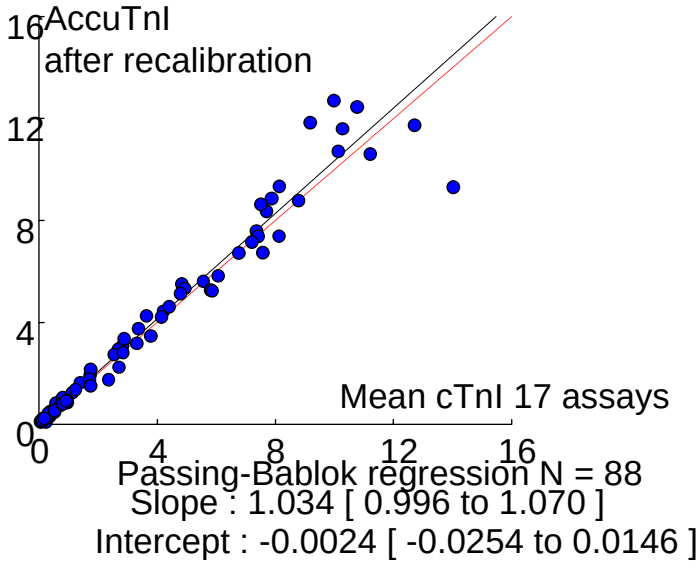
cTnI post recalibration



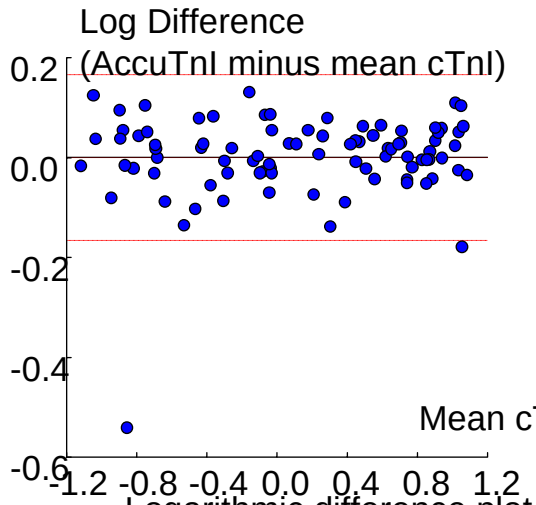
Passing-Bablok regression N = 88
Slope : 1.011 [0.964 to 1.045]
Intercept : -0.0112 [-0.0327 to 0.0074]



Logarithmic difference plot N = 88
Mean difference : -0.00726 [-0.0234 to 0.00887]

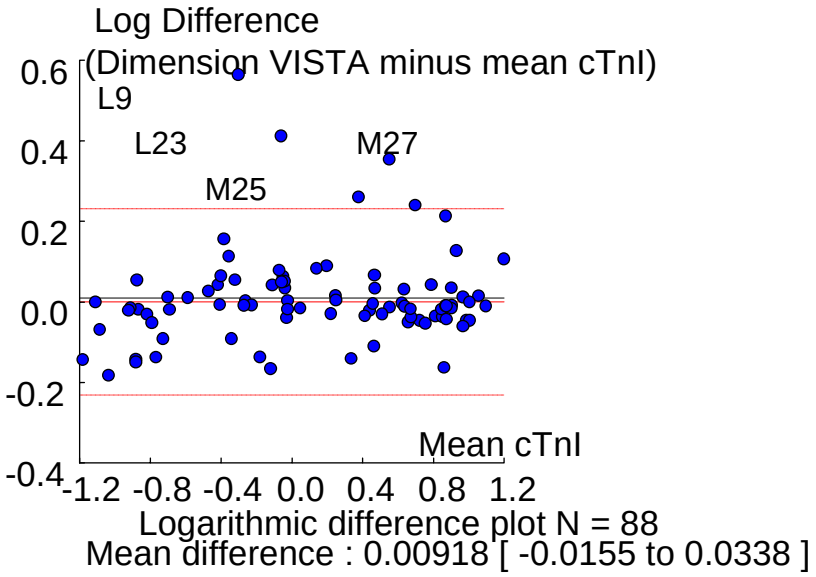


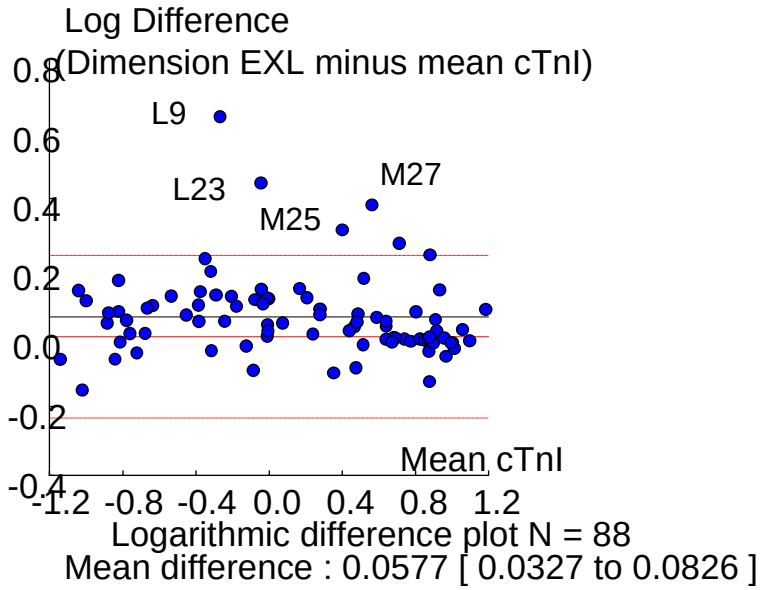
Passing-Bablok regression N = 88
Slope : 1.034 [0.996 to 1.070]
Intercept : -0.0024 [-0.0254 to 0.0146]

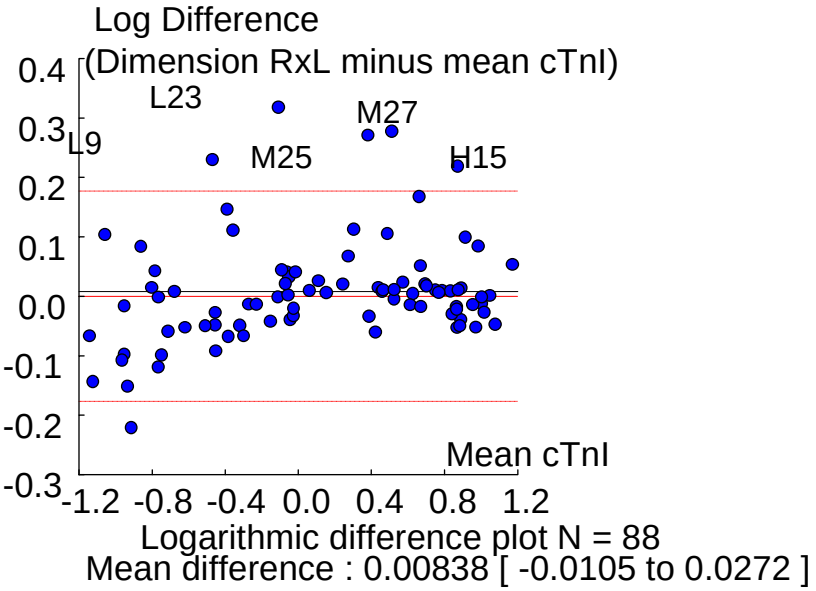


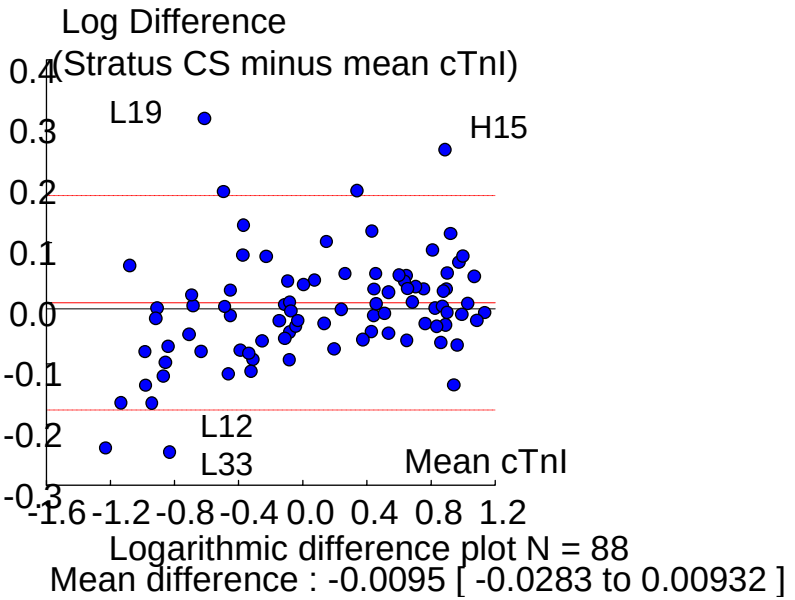
Logarithmic difference plot N = 88
Mean difference : 0.000835 [-0.0168 to 0.0185]

Assays with same antibody specificity

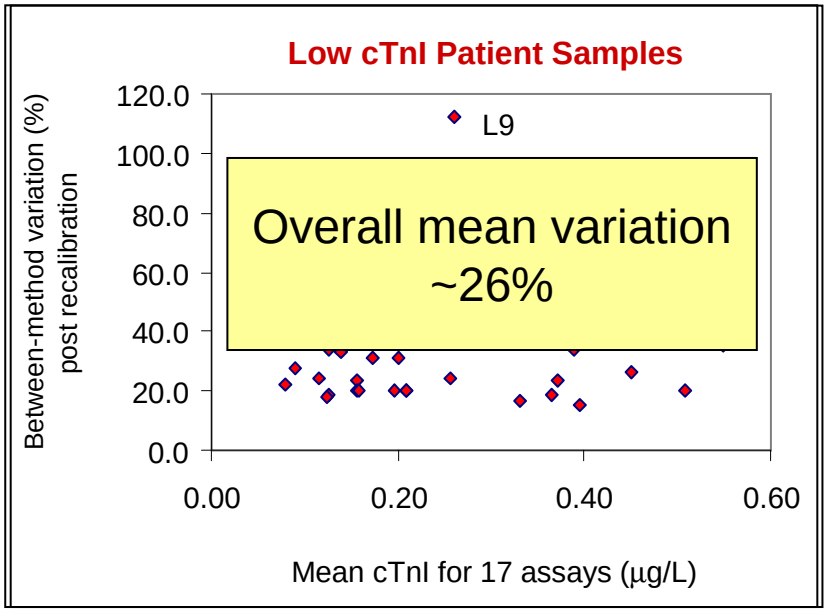


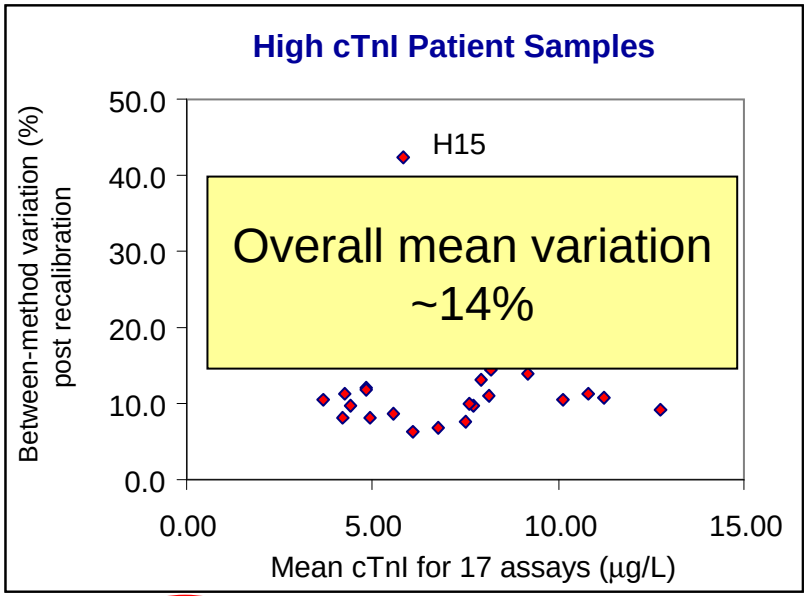


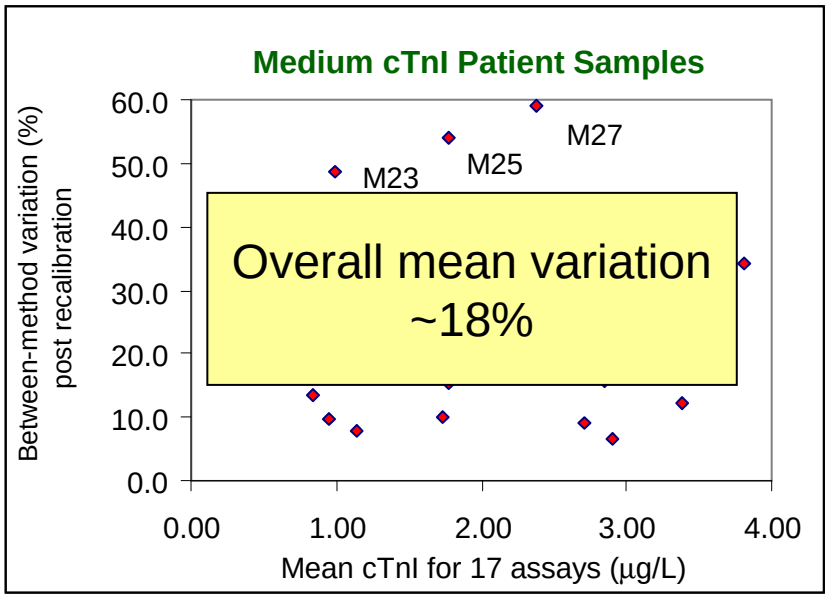


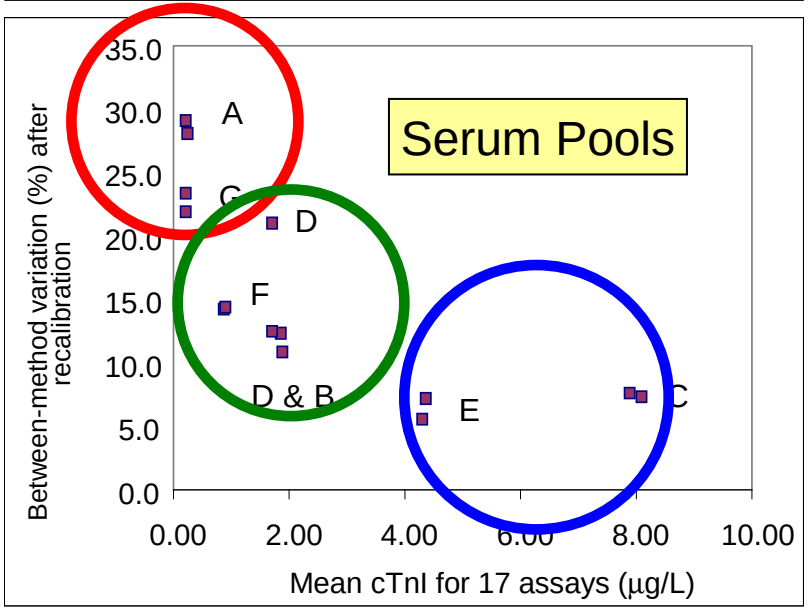


cTnI harmonisation post recalibration

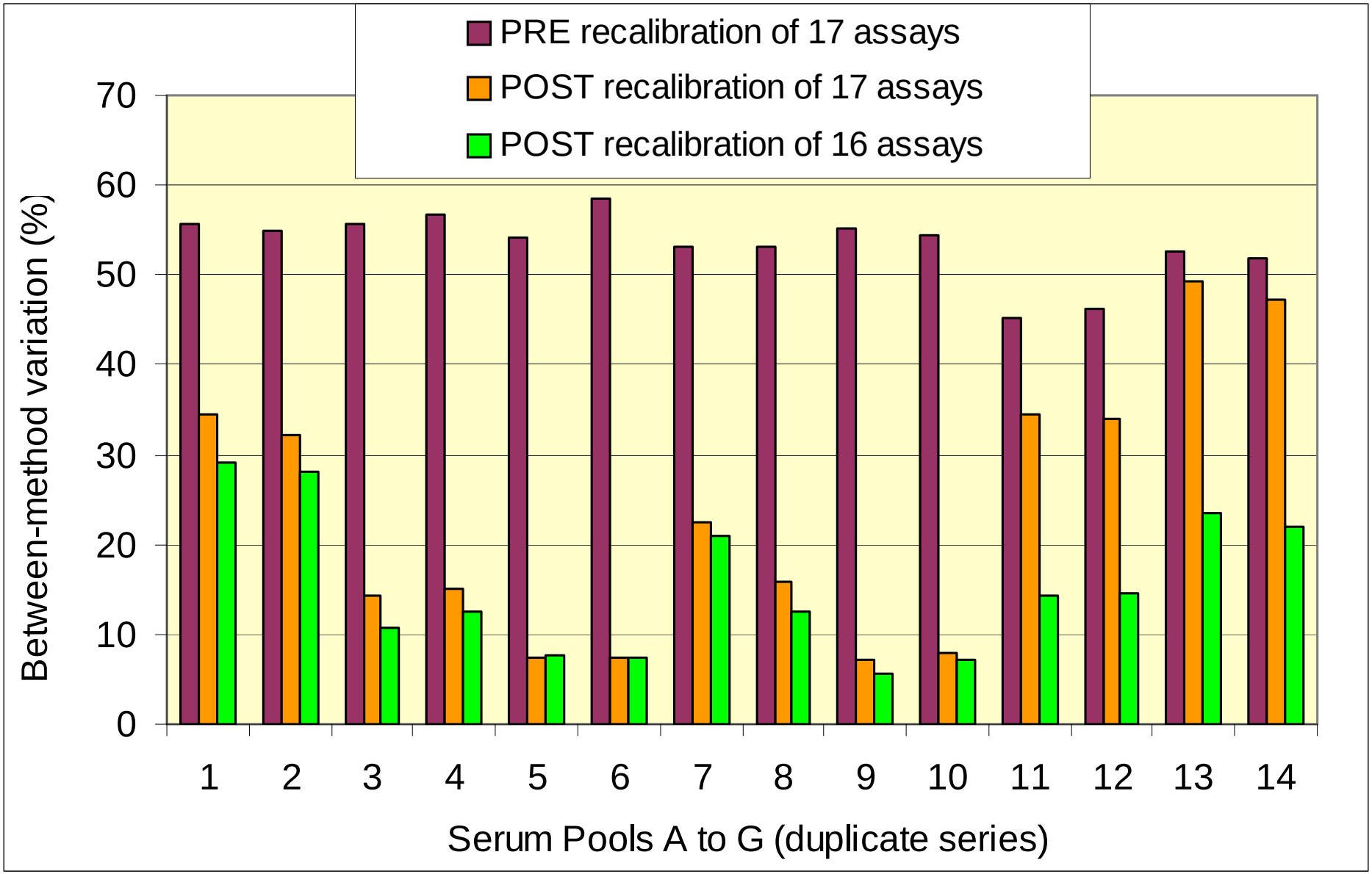








cTnI between-method agreement



cTnI Pilot Study: CONCLUSIONS

- Serum pools behave better than most of the patient samples with lower inter-assay variability
- All serum pools are commutable with all routine assays
- Some assays correlated to the mean value better than other assays
- A high between-assay correlation for some assays from same manufacturer
- After calibration differences are removed method agreement was ~8 to 15 %CV in range 1-8 µg/L cTnI

Next Steps

- Production of SRM for cTnI
- Value assignment and commutability testing of SRM
- Uncertainty budget determined for SRM
- Value transfer to manufacturers' master calibrators
- Phase 3: harmonisation testing in a round robin

Production of SRM 2922 for cTnI

- Minimum of 20 patient serum samples (min. vol 20 mL each)
- cTnI in range 5-20 μg/L
- Stored at ≤ -70 °C
- Prepare a serum pool from
  - ≥20 patient sera (min. vol 610 mL) and
  - Dilute 5-fold with normal pooled human serum (min. vol 2,440 mL)
- Aliquot diluted serum pool (0.5 mL) into 2 mL PP vials to be stored at ≤ -70 °C
- 6,000 vials to be stored at NIST

Consensus value assignment for cTnI

- Method harmonisation consensus approach using all commercial cTnI assays
  - mean or weighted mean value
- Use another panel of individual patient samples to confirm correlation at the time of value-assignment measurements
  - similar to the pilot study but scaled down
  - fewer patient samples and narrower concentration range
- Also use calibrant samples prepared from dilutions of SRM 2921 in cTnI negative serum to “re-calibrate” the manufacturers’ data sets of the patient serum panel

Performance criteria for cTnI: measurement uncertainty

Performance goal	Imprecision goal	Bias goal	Total error goal *
Minimum	7.3%	21.6%	36%
Desirable	4.9%	14.4%	24%
Optimum	2.4%	7.2%	12%

CVintraindividual 9.7%; CVinterindividual 56.8%

* TE = Bias goal + 1.96xCVa

Panteghini M. In: Laboratory and Clinical Issues Affecting the Measurement and Reporting of Cardiac Troponins: A Guide for Clinical Laboratories. Alexandria: AACB; 2012. p. 53-61.

Value transfer to manufacturers' calibrators

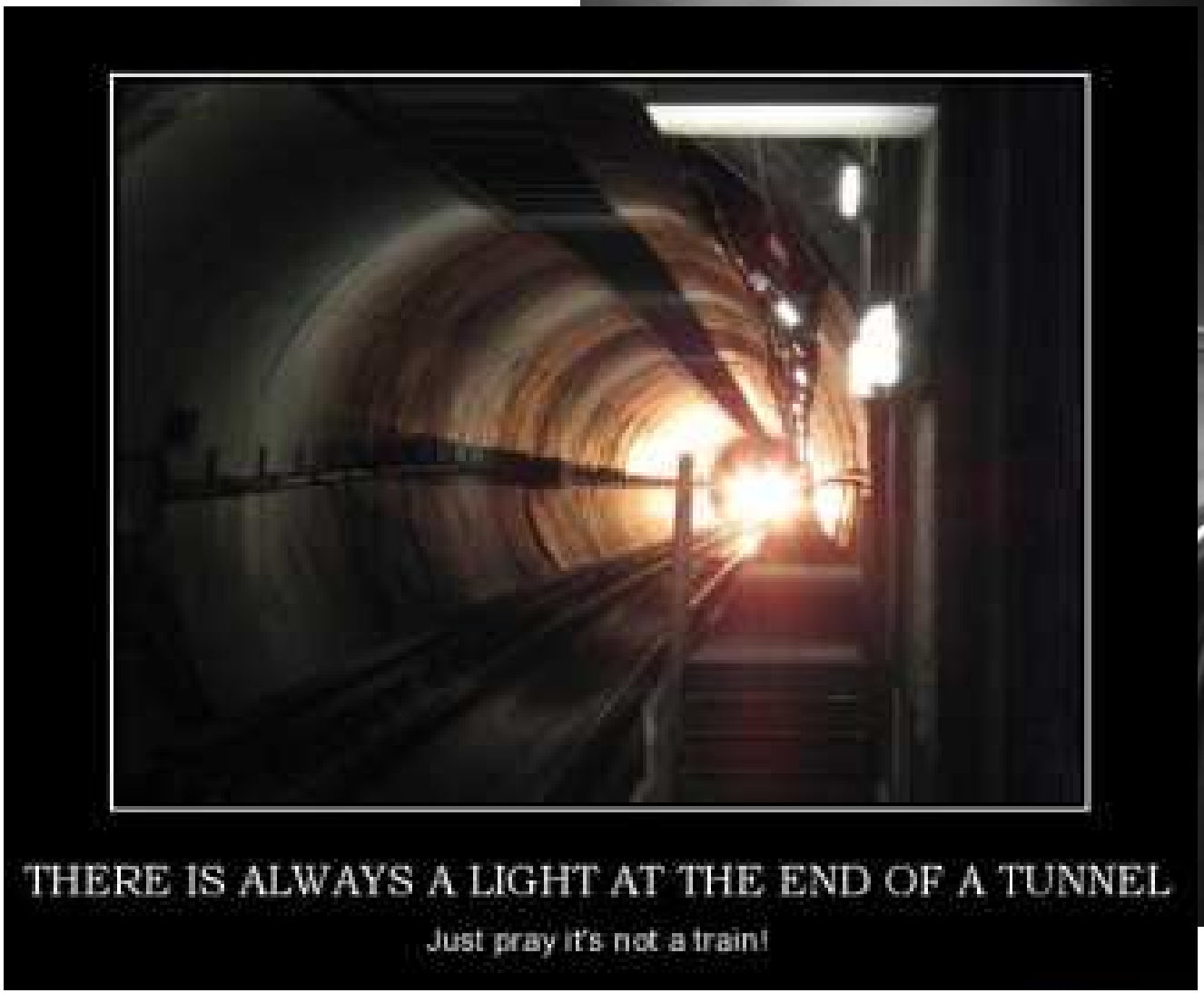
- Compare with value transfer of cystatin C ERM-DA471/IFCC
- Consensus method process uses a standardised value transfer RMP consisting of dilutions of master calibrator for cTnI and candidate SRM
  - Within and between day runs
  - Number of replicates to depend on a predetermined precision goal
- Phase 3 Round Robin:
  - Harmonisation testing using patient samples

IFCC WG
Standardization 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 that participated in cTnI Pilot Study

ABBOTT DIAGNOSTICS
BECKMAN COULTER
BIOMERIEUX
MITSUBISHI CHEMICAL MED CO
ORTHO-CLINICAL DIAGNOSTIC
ROCHE DIAGNOSTICS GmbH
SIEMENS DIAGNOSTICS
NIST
NPL


THERE IS ALWAYS A LIGHT AT THE END OF A TUNNEL
Just pray it's not a train!