NPL
National Physical Laboratory

Development of an immunoassay-based measurement procedure of higher metrological order for cTnI

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National Measurement System

Analytical requirements of a RMP

1. Comparable cTnI specificity to commercial assays
2. Acceptable assay precsion
3. Calibration against purified primary reference material (NIST SRM 2921)
4. Ability of the assay to be unaffected by interferences
5. Technical validation and transferability in a laboratory network

Tate et.al. Pathology 42(5), p402-8.

cRMP: Intended use

- Higher order immunological procedure (not for routine analysis).
- Reference laboratory network – assign values to secondary reference materials
- Open access - published assay procedure

Assay Principle

- Measurand – stable form of cTnI, aa residues 30-110:
  - Using 1 + 1 assay format, hcTnI specific clones targeting postulated epitopes 41-49 and 83-93.
  - Assumes an equimolar response for all ‘stable’ forms of cTnI released upon myocardial cell death.
  - Sandwich ELISA, using enzymatic amplification.

cRMP: Assay Criteria

- Precision at required range:
  - Serum standards tentative: 0.1, 1 and 10 µg/L
  - Required precision 3-5 % CV.
- Assay to be repeated in various reference labs:
  - Not aligned to a single assay manufacturer
  - Reagents commercially available, ideally from multiple suppliers (except antibody clones)
  - To be performed on standard plate readers
- Traceability chain
  - Traceable to SRM 2921

cTnI form Specificity of cRMP

cRMP analysed with cTnI diversity kit:

A). Mass corrected data ~ equal. Limited complex influence.

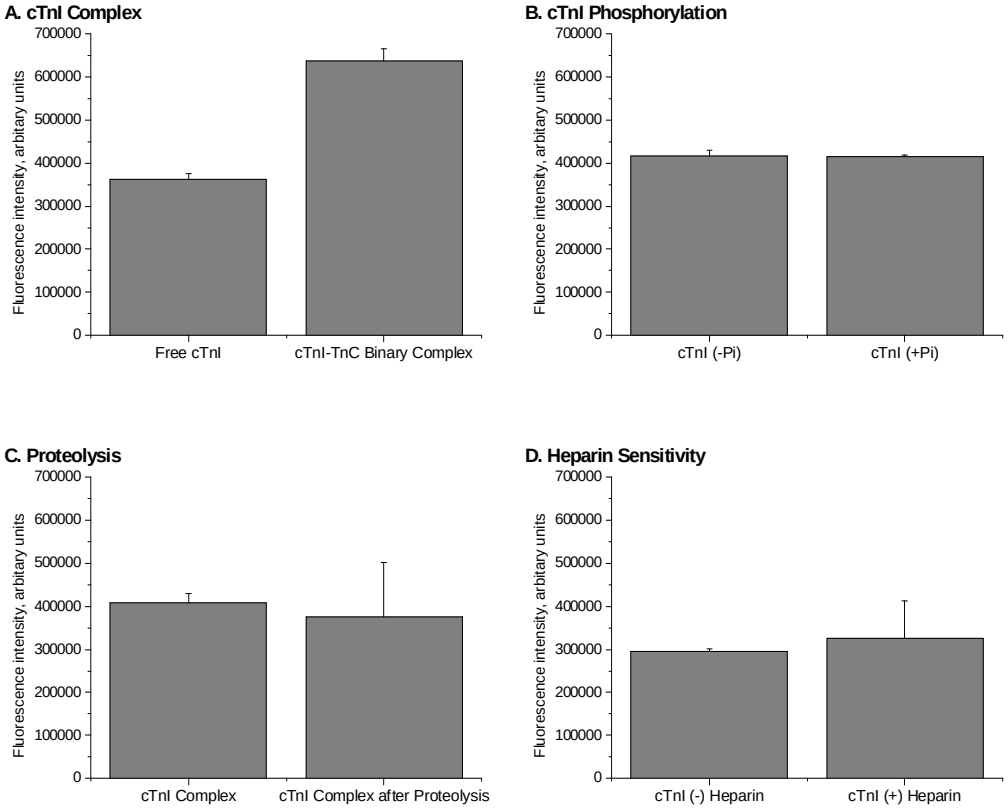
B). Phosphorylation insensitive

C). Proteolysis treated cTn, similar response – detection of stable region.

D). No apparent heparin sensitivity

Limited cTnI form bias:

however analysis does not take into account all possible cTnI forms present



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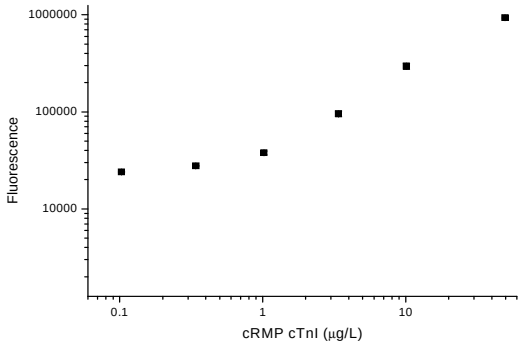
Comparison of Fluorescence and Chemiluminesence (1)

- Open assay format – non-specialist equipment.
  - Fluorescence: plate readers standard laboratory equipment, fluorescent substrate 4-MUP multiple suppliers.
  - Chemiluminescence: many dual function plate readers, substrates limited to a few commercial suppliers.

Comparison with Commercial Analyzer

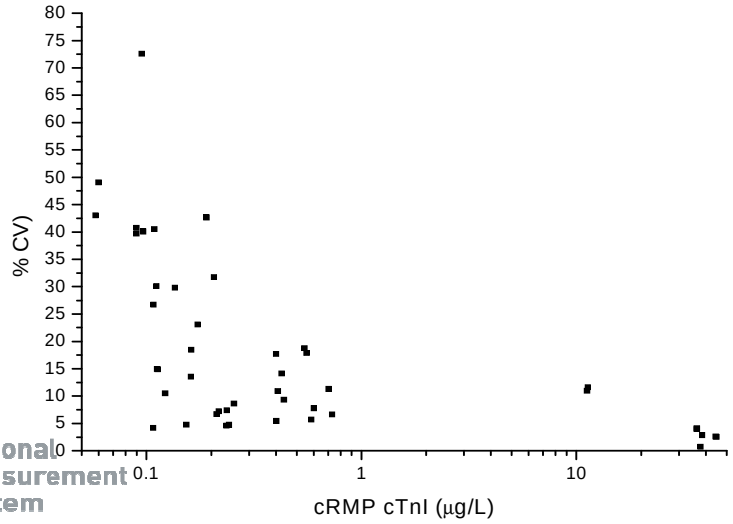
- Anonymous leftover serum samples tested on cRMP
  - Compared to Centaur (Ultra) data
  - Upon collection and measurement on Centaur samples frozen and stored at –20 °C, then 80°C – tested on cRMP within 2 months.
  - Limited volume (< 1 ml) (Test volume 50 µl)
  - SRM used as calibrant, spiked into pooled male patient serum (< 0.05 µg/L) from a commercial source.
  - No pre-selection of samples, no interference data from Centaur

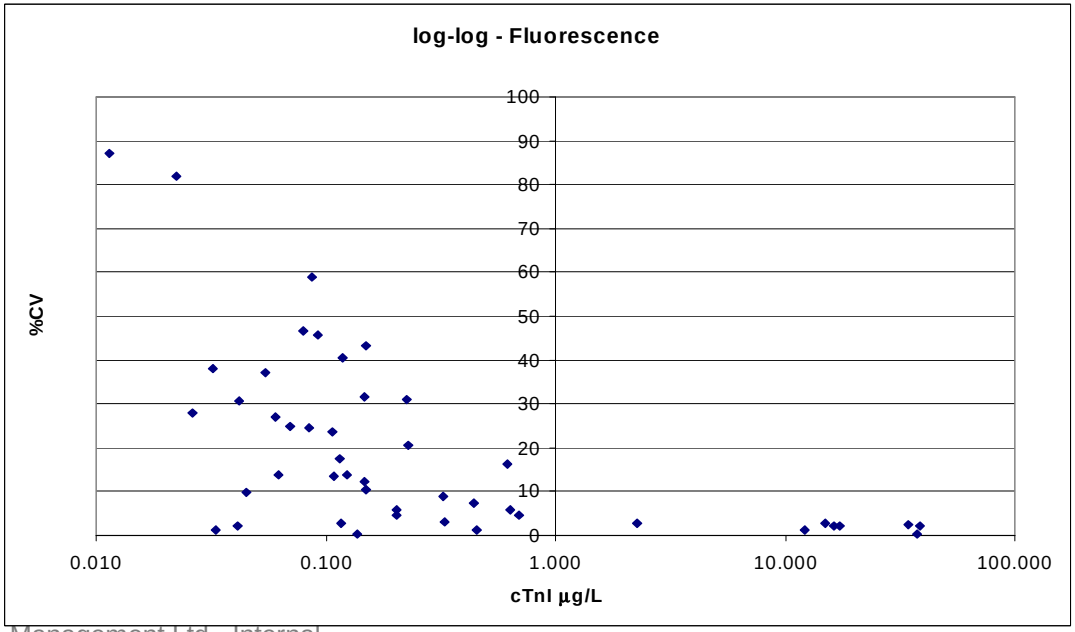
Fluorescence Assay



Limited dynamic range

Fluorescence (log-log)





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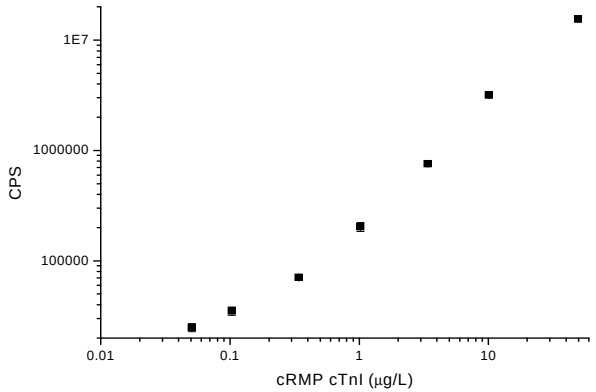
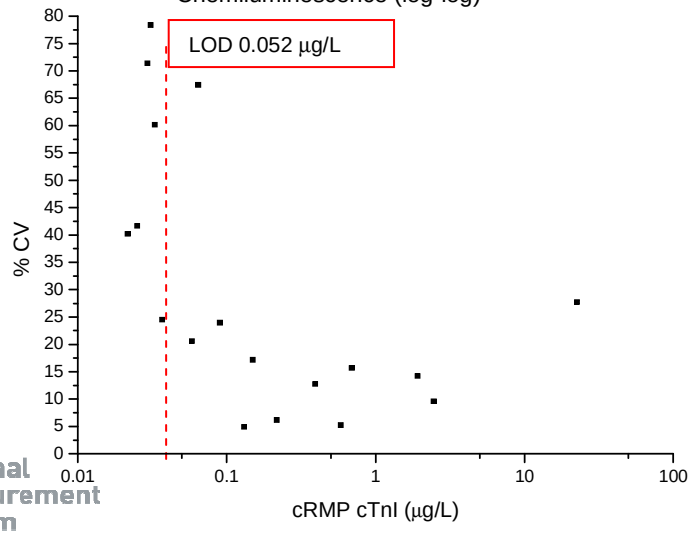
Chemiluminescence Assay



Chemiluminescence (log-log)



Improved dynamic range compared to fluorescence

Large intra assay error (>25 % CV).

Appears random

% CV too high for cRMP

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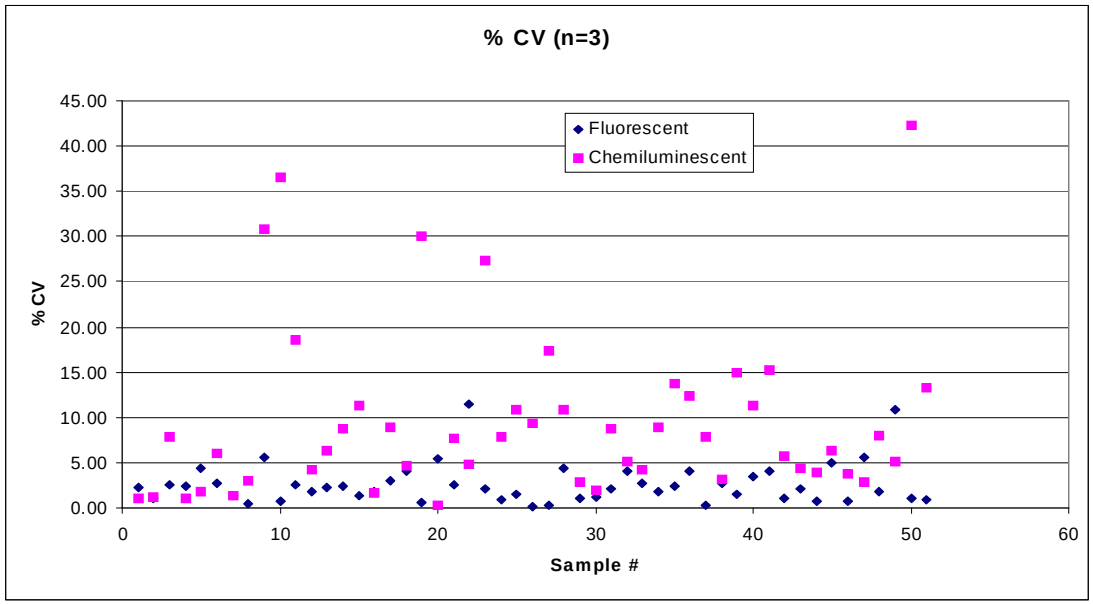
Comparison of error profile



Error associated with raw PMT count data:

- Patient samples analysed using same protocol.
- Chemiluminescent assay format more prone to error:
  - Memory effect
  - Stray light
  - Interferences
  - Crosstalk

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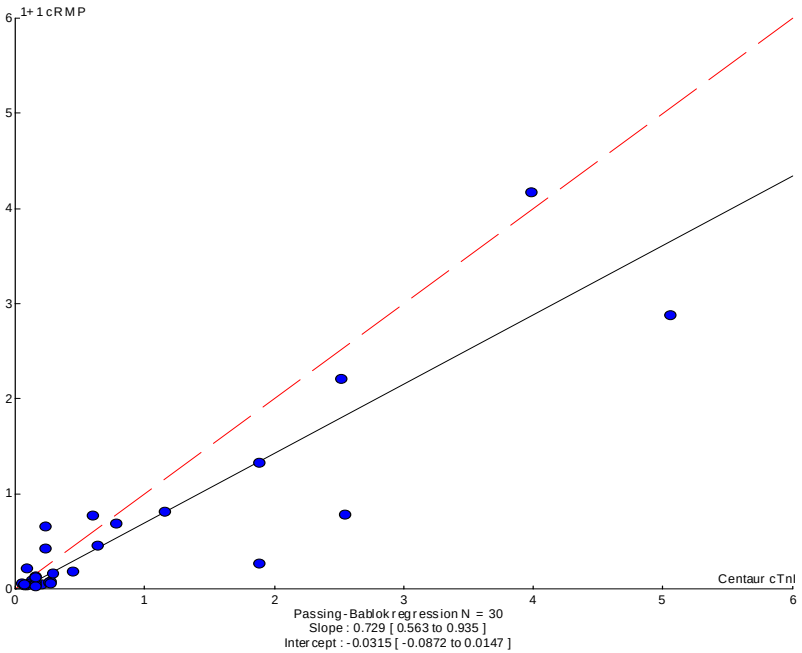
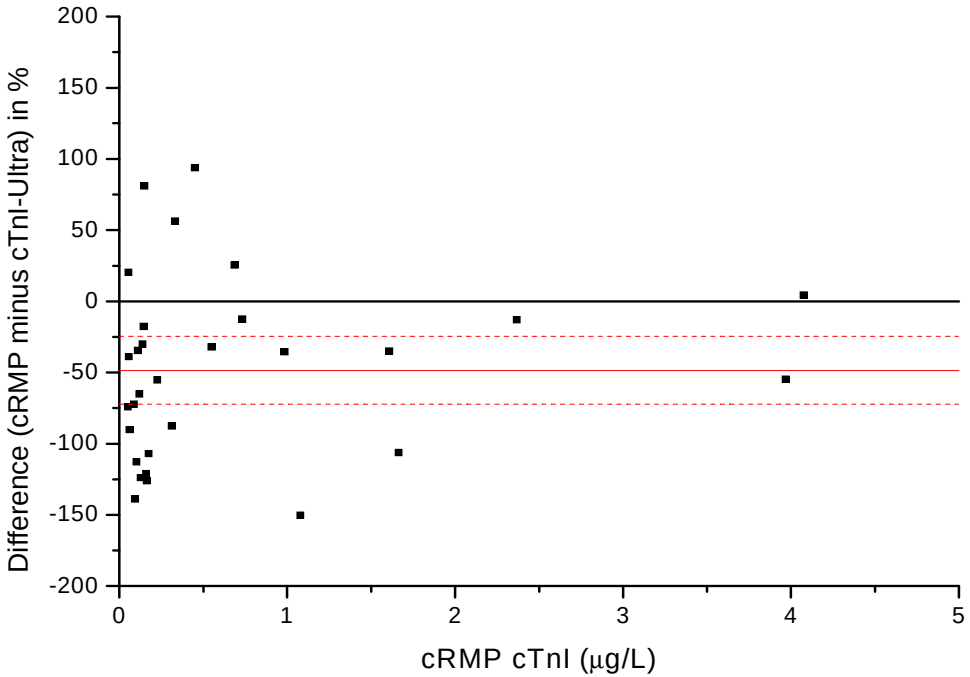
Comparison of cRMP and Centaur (1)





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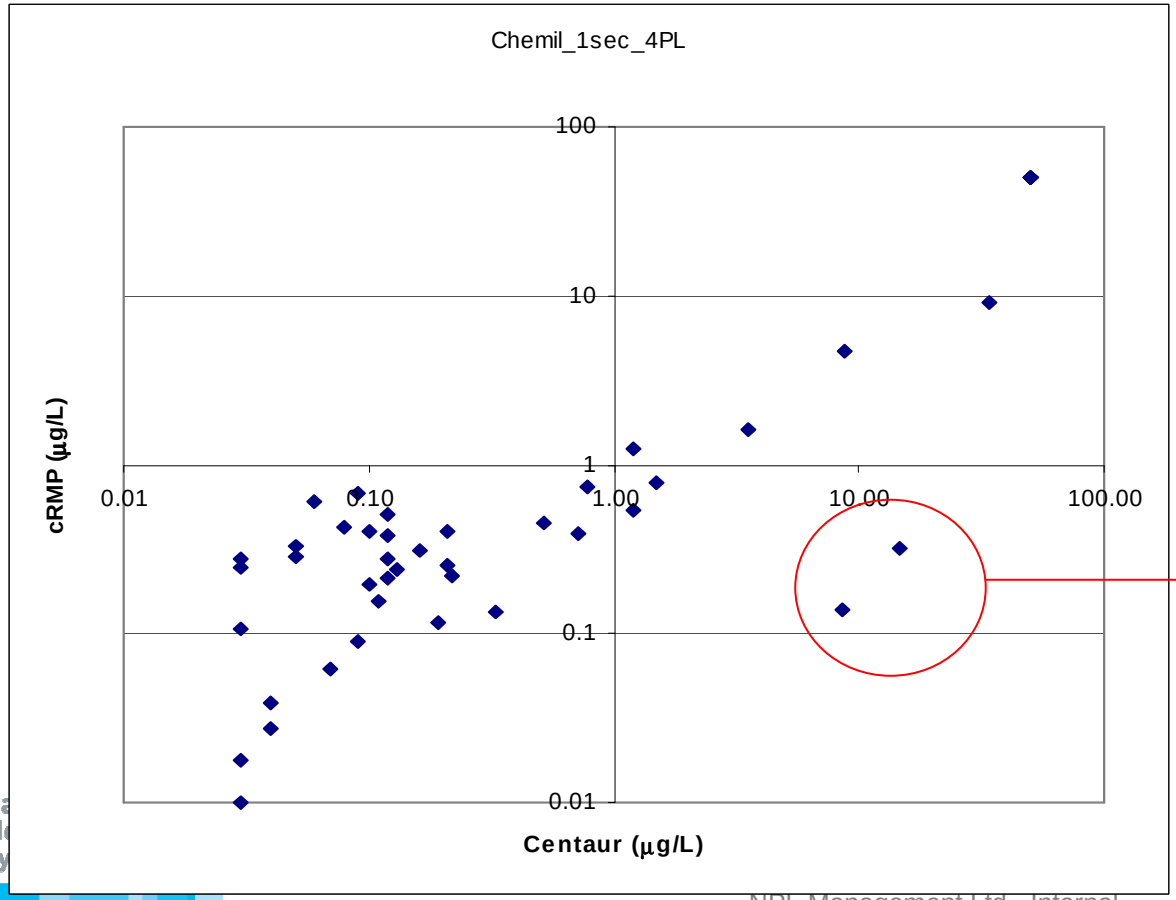
Comparison of cRMP and Centaur (2)



Assay Interference?

Optimization of the Fluorescent Assay

- Aim: Reduce LOD to <50 ng/L, currently > 100 ng/L.
- Current Approaches:
  - Optimise assay conditions – limited success.
    - Reduce NSB associated with background in patient serum samples.
  - Use a HRP enzymatic amplification
    - Fluorescent substrates HPAA, Amplex Red.

Improved sensitivity and precision

- Fluorescence:
  - Use different assay format, 2+2, antibody clones, or conjugate
  - Time-resolved fluorimetric (Eu-labelled antibodies)
  - Two assay formats to cover required range (high + low)
- Chemiluminescence:
  - Improve intra-plate variation
  - Source different reagents and plates
  - Dedicated reader

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