CCQM P58.1:
Metrology for Clinical Sciences

Diagnostic Formulation Meeting, 24th Feb
James Noble
Development of a Reference Immunoassay for cTnI

- Cardiac Troponin I (cTnI) is an important marker for the diagnosis of ACS and risk stratification
- The NPL metrology study is linked to the IFCC initiative for standardization and traceability of cTnI measurements:
  - Drivers:
    - No reference method for value assignment – MS not sensitive enough to determine clinically relevant levels (ng/L – μg/L).
    - Analytically more sensitive assays being developed to enhance initial diagnosis – the need for improved assay performance and comparability.
Stakeholders and Project Drivers

Quality of Life; Improved cTnl measurement comparability

Develop cTnl RMP + associated standards

IVD Directive (98/97/EU): Traceability for in vitro medical diagnostic devices

Metrological methods applied to other programs: ChemBio, TSB and NIRD

Assign cTnl Standards

IFCC WG-cTnl

CCQM - BAWG

NIST

NPL

International Metrology Community:
- NIBSC (WHO)
- BAM + PTB (Germany)
- IRMM (Euro)
- VNIIM (Russia)
- NMIJ (Japan)
- NIM (China)
- ISS (Italy)
- KRISS (South Korea)
- NMIA (Australia)

Formulation: UK industrial Input
IFCC: Traceability for cTnI Measurements (1)

- Multiple manufactures of cTnI assays – each with own standards and antibodies with different epitope specificities.
  - Need to standardize cTnI measurements to facilitate intercomparison of clinical studies.
  - Traceable cTnI SRM displays limited commutability therefore:
    - Preparing secondary standard from AMI patients.
    - Develop immunoassay RMP to support secondary standards.
    - Characterise and prepare antibodies for RMP.
    - Evaluate the effectiveness of this approach through round robin studies.
IFCC: Traceability for cTnI Measurements (2)

• Purified cTnI SRM not suitable for standardizing all measurements:

BIPM: Metrology and the Development of a Immunoassay Reference Measurement Procedure

• ‘The task of the BIPM is to ensure world-wide uniformity of measurements and their traceability to the International System of Units (SI)’.
  – Reference System to be SI traceable.
  – The cTnI immunoassay RMP will be used by metrology institutes, reference laboratories and industry to assign concentrations to reference materials and manufacturers ‘master calibrators’.
  – World-wide comparability through CCQM studies to evaluate robustness of RMP.
  – RMP to be developed as a non-proprietary open access assay system that can be run in various laboratories using non-specialist equipment/techniques.
Proposed Reference Measurement System for human cTnI

- Materials
  - Purified CIT standard – NIST 2921
  - Serum-based commutable reference materials for cTnI
  - Target material e.g. manufacturer’s working calibrator
  - Patient samples

- Procedures
  - Reference measurement procedure: LC/MS and Amino Acid Analysis
  - Reference immunoassay method
  - Manufacturer’s selected reference measurement procedure
  - Manufacturer’s standing measurement procedure to value assign the target material

Slide prepared by Jill Tate, Royal Brisbane and Women’s Hospital, QLD, Australia.
cTnI – Standardization of a Heterogeneous Protein Analyte

• Compared to small organic and inorganic molecules the standardization of proteins is more complex:
  – Chemical heterogeneity:
    • Post-translational modifications
    • Protease digestion
  – 3D Structure
  – Protein complex formation
  – These issues can be patient, sampling/processing and time dependent

• The measureand cTnI can therefore be classed in terms of multiple isoforms:
  – RMP should display equal reactivity to all cTnI isoforms
Measurement Claim P58.1:

- The measurand ‘cTnI’ will be defined by the presence of both epitopes (fully characterised and mapped) that reside within the stable region of the molecule.
- Proposed Measurement Claim:
  - To determine the concentration of human myocardial infarction marker cTnI in the range of [ng/L-μg/L] using a defined non-competitive ELISA protocol with a controlled uncertainty.
Development of the cTnI Immunoassay RMP

• NPL has experience in immunoassay metrology running previous CCQM intercomparison studies.
  – Through internal research and intercomparison studies the sources of uncertainty in assigning cTnI concentration with this method will be quantitated.
  – NPL will be involved in the reference material assignment and commutability studies to assess the utility of this approach.
  – USP Protein A reference Immunoassays

• Current funding round is supporting the development and robustness analysis of the cTnI RMP
Cause and Effect Diagram showing Sources of Uncertainty

- Other effects ($f_{\text{other}}$)
  - Specificity
  - Interferences
  - Tertiary and quaternary structure

- Imprecision ($C_{\text{uncorr}}$)
  - Variation in non-specific binding
  - Sample Volume
  - Variation in phosphatase activity
  - Reagent Volume(s)
  - Variation in phosphatase binding

- Correction ($f_{\text{corr}}$)
  - Preparation of standards
  - Value of SRM

- Sample effects ($f_{\text{sampler}}$)
  - Homogeneity
  - Loss (recovery, or dilution) or contamination
  - Stability

- Statistical estimation

Performance criteria for the Secondary Reference ELISA

- Dynamic range to include the expected three (0.1 – 10 μg/L) serum standards and cTnI cut-off (0.05 μg/L).
- Display equal reactivity with cTnI isoforms and orthogonal methods.
- Multiple combinations of antibodies directed to epitopes both within and outside stable region tested.
Further Biomarker Standardization Work

- Validate RMP immunoassay and commutability of the cTnI standards.
- Characterise troponin complex and its interaction with selected antibodies for RMP.
- Develop methods to characterise the cTnI measure and (St George’s).
- Links to the JRP11 project: Traceability of Complex Biomolecules and Biomarkers in Diagnostics – Effecting Measurement Comparability in Clinical Medicine
- If successful can the cTnI standardization approach be applied to other complex protein biomarkers.
Acknowledgements

- Organisation + Input
  - BAWG
  - cTnI WG
  - Robert Porter NPL Simon Attree NPL
  - Lili Wang: NIST Maurice Cox NPL
  - David Bunk NIST Elaine Gray NIBSC
  - Alexei Katrukha HyTest Adrian Bristow NIBSC
  - Heinz Schimmel IRMM
  - Jill Tate IFCC

- Publications:

Thanks to the UK Government for funding the project through the Chemistry and Biology NMS Programme.