Traceability of complex biomolecules and biomarkers in diagnostics

EURAMET
European Association of National Metrology Institutes

The need for the project

The International Federation of Clinical Chemists (IFCC) and the Joint Committee for Traceability in Laboratory Medicine (JCTLM) have highlighted the need to develop reference measurement systems to provide traceable values for complex biomolecules, such as disease state protein biomarkers. This should also enable in vitro diagnostic (IVD) and clinical measurement comparability and improve diagnostic efficiency and reliability.

However, few reference measurement procedures exist for protein biomarkers and biomolecular measurements and consequently standards can be affected by multiple parameters that need to be considered in the establishment of a traceability chain.

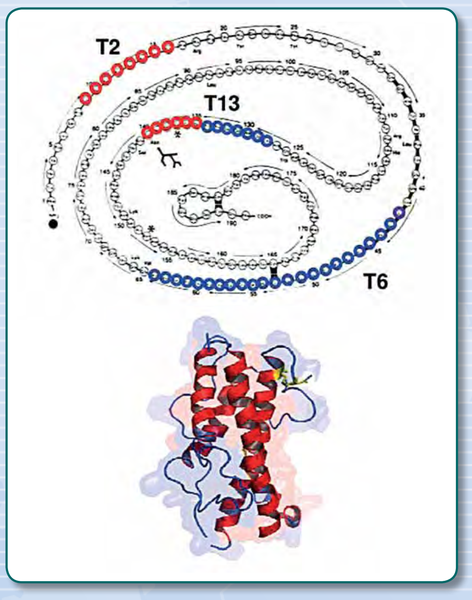
The critical measurement is not the total amount of a protein biomarker but the quantity of 'active/functional' component. A protein's structure, folding state and interactions with other proteins/ligands all define its activity and can influence the measurement result and its diagnostic 'clinical' value.

This project aimed to address these issues by developing measurement procedures for complex protein biomarkers in order to provide SI traceable results.

Technical achievements

The project's most significant achievement is the successful demonstration of the feasibility of developing reference measurements, that deliver SI traceable values and reference materials for two clinically important biomarkers; human growth hormone (hGH) and C-reactive protein (CRP).

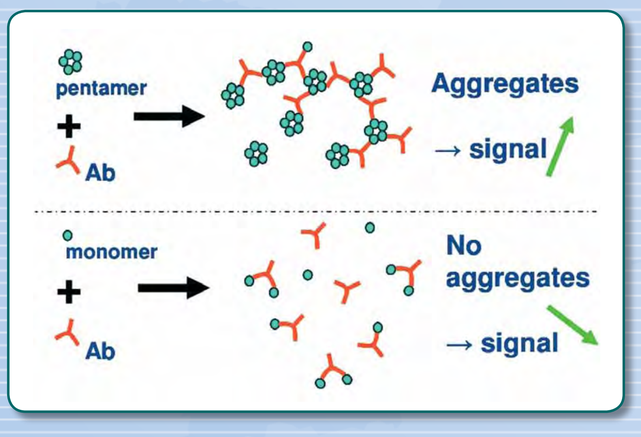
The project developed, validated and published strategies for the application of isotope dilution mass spectrometry (IDMS) methods for the quantification of hGH and CRP in purified materials and serum matrixes.



Amino acid sequence highlighting tryptic peptides used for quantification, and ribbon structure of Human Growth Hormone (22kDa form).

Development of robust and selective enzymatic digestion and cleanup protocols enabling assignment of SI traceable values for hGH in serum by IDMS using isotopically enriched peptides and proteins as internal standards.

Method development and optimisation for isoform profiling and quantification of oligomeric and aggregation state which has influenced immunoactivity in hGH and CRP reference preparations.



The impact of CRP oligomeric state on immunoassay response.

Evaluation of advanced mass spectrometry-based techniques for the elucidation of protein structure:

- Hydrogen Deuterium Exchange (HDX) in combination with proteolysis mass spectroscopy and Ion Mobility Mass Spectroscopy (IMS) were optimised to assess structural differences in the currently available reference standards for hGH
- the potential for HDX and IMS for quantification of protein folding states and protein ligand interaction was demonstrated
- the significant influence of sample preparation, heterogeneous isoforms and structures on clinical immunoassay response for both hGH and CRP was demonstrated

Input to standardisation & regulation

The World Health Organization (WHO) has expressed an interest in the project's multiparametric protein quantification providing SI traceable values for value assignment of relevant WHO International Standards for biologicals. Project results may be acknowledged in future revisions of ISO TC212 *'Clinical laboratory testing and in vitro diagnostic test systems'* documentary standards.

Supporting the clinical community

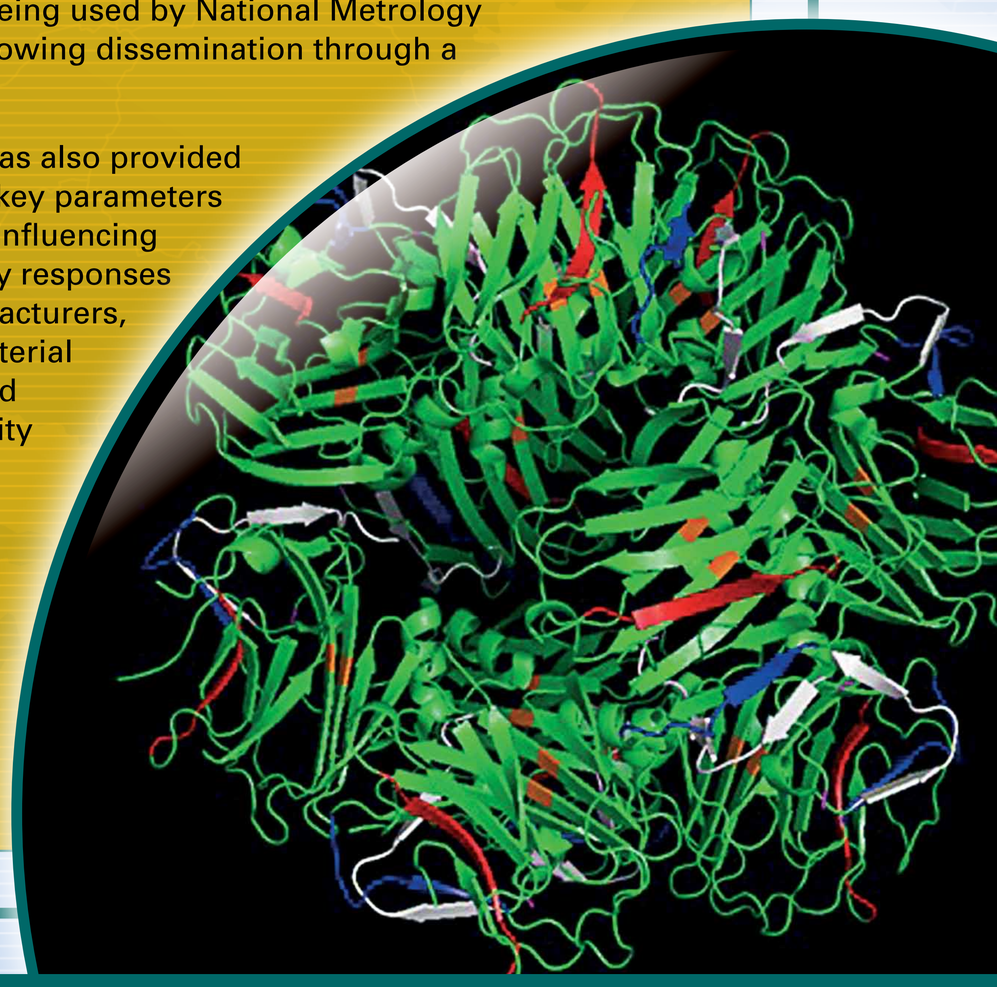
Engaged with key clinical and metrology stakeholders, including the IFCC, JCTLM and Consultative Committee for Amount of Substance (CCQM) BioAnalysis working group.

The project's JRP-Partners participated in the IFCC Scientific Division, IFCC hGH and plasma proteins working groups. The results of the project have input in to IFCC guidance, clinical test kits and the organisation of clinical commutability studies.

New reference methods

The IDMS method developed for protein quantification is currently being used by National Metrology Institutes following dissemination through a CCQM study.

The project has also provided guidance on key parameters identified as influencing immunoassay responses to IVD manufacturers, reference material producers and external quality assurance organisers.



Ribbon structure of pentameric form of C-reactive protein.

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