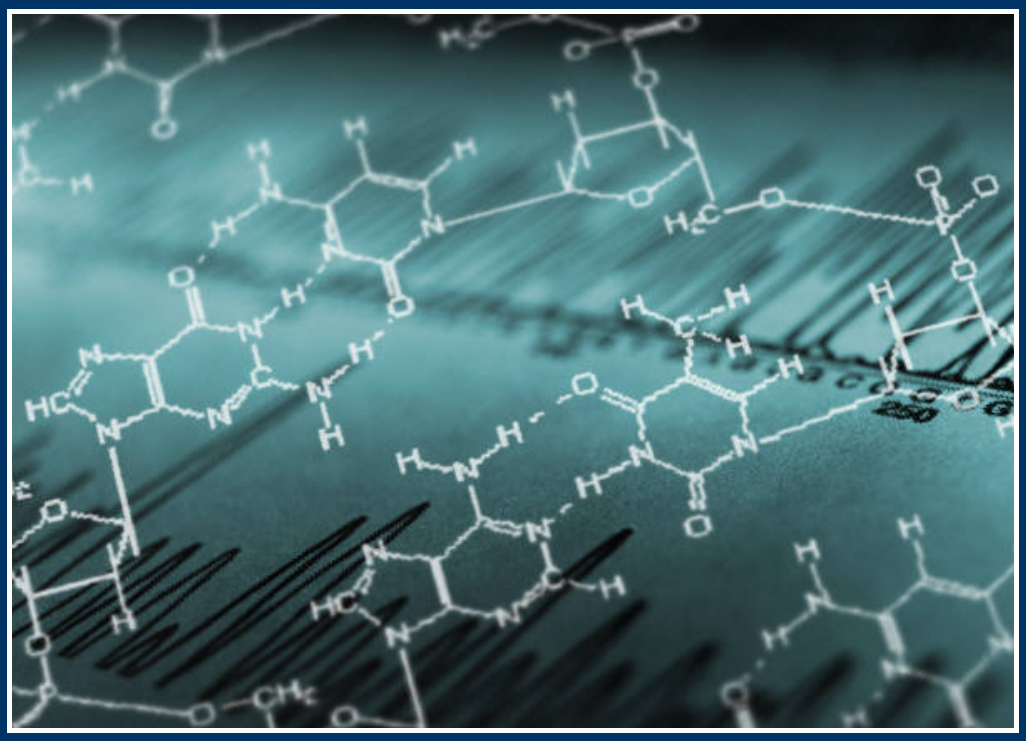
Final Report

Study of Measurement Service and Comparison Needs for an International Measurement Infrastructure for the Biosciences and Biotechnology: Input for the BIPM Work Programme



Rapport BIPM-2011/02 4 March 2011

Final Report

Study of Measurement Service and Comparison Needs for an International Measurement Infrastructure for the Biosciences and Biotechnology: Input for the BIPM Work Programme

Deliverable P3-D6

LGC authors:

John Marriott
Gavin O'Connor
Helen Parkes

Date: **4 March 2011**

Rapport BIPM-2011/02
Report Number: LGC/R/2011/123

Executive Summary

This version of the report follows the publication of a draft report in September 2010, which was distributed to stakeholders for comment. Feedback was received from several organisations and this final report incorporates changes made in the light of these comments.

The report comprises the results of a study on the Measurement Services and Comparison Needs for an International Measurement Infrastructure for the Biosciences and Biotechnology. It was undertaken to provide input to the BIPM's future programme of work as well as being a useful reference for National Metrology Institutes (NMIs). The work carried out for the study included: a review of the published literature on roadmaps and strategies for bio-measurement; a review of the activities and strategy of the CCQM Bioanalysis Working Group; a series of interviews undertaken with representative NMIs that had bio-measurement programmes, selected organisations from the biotechnology industry, the FDA and the IFCC. The aim of the study was to identify the required and expected measurement services, international comparisons and measurement R&D which are needed to underpin the comparability of bio-measurements over the next 10 years. Although the literature review of roadmaps and strategies was broad, later parts of the study focused on protein and nucleic acid measurements for the Healthcare sector as these were the areas of most interest to the BIPM.

For proteins, the measurement services, international comparisons and collaborative R&D needed to underpin the comparability of bio-measurement identified in the report included:

- Support for fundamental metrology, aimed at making protein measurements traceable to the SI
- Improving the comparability of measurement results from different analytical platforms
- The development of measurement services for complex proteins
- The development and application of new and improved physico-chemical techniques for protein structural characterisation
- The comparability of measurements from highly multiplexed analytical techniques

For nucleic acids, similar requirements have been identified for:

- Fundamental metrology, including a consideration of the most appropriate units for amount of substance
- Qualitative/sequence analysis
- 2nd & 3rd generation DNA sequencing
- Quantitative measurements

Conclusions have been drawn for those areas which have significant strategic and underpinning value in the protein and nucleic acid measurement fields and are therefore most likely to be of interest for BIPM activities. They comprise:

Proteins

1. Requirements for underpinning work to assure the traceability of quantitative measurements of proteins to the SI, including the high accuracy purity determinations and analysis of proteins, peptides and amino acids
2. Strengthening of cooperation between the WHO, IFCC, BIPM and NMIs to ensure a coordinated approach to the migration from IU to SI
3. The development of an analytical approach for the characterisation of more complex proteins, the properties of which are not easily expressed in SI units, but which still involve metrological principles
4. Support for the validation of highly multiplexed measurements, including the provision of traceability for 'horizontal' (performance) standards
5. The development of traceability chains for inherently unstable/dynamic proteins
6. Development of a modified system for 'Calibration and Measurement Capability Claims' which is more relevant to proteins and other bio-entities, given the very large number of analytes and matrices

Nucleic Acids

7. The development of techniques for the treatment of measurement uncertainty in nucleic acid sequence determination
8. Improved Total DNA measurements, including the high accuracy determination of the UV molar extinction coefficient of nucleotides
9. The development of purity assessment methods for improved quantitative RNA measurements
10. The provision of traceability for 'horizontal' reference standards needed for highly multiplexed measurements
11. Co-ordination of the discussion on the metrological implications for the use of counting techniques for quantitative analysis and the most appropriate units for amount of substance
12. The compilation of authenticated international reference databases

Contents

1. Introduction

LGC was contracted[1] to deliver a Study of Measurement Services and Comparison Needs for an International Measurement Infrastructure for the Biosciences and Biotechnology which will provide input for the BIPM's future programme of work. This final report follows on from an original draft report[41], published in September 2010 and distributed by the BIPM to stakeholders for comment. Several organisations responded and the report has been modified in the light of their comments.

The main part of study comprised two reviews and a series of interviews. One of the reviews looked into the published roadmaps and strategies for bio-measurement and the other reviewed the CCQM BioAnalysis Working Group's (BAWG) activities and plans. The interviews were conducted either through 'face-to-face' meetings, or via a 'teleconference' with selected measurement organisations (primarily National Measurement Institutes (NMIs) with activities in bio-measurement), bio-industry and regulators.

The main aims for collecting the information were to determine:

1. Measurement services required to establish an International Measurement Infrastructure for the Biosciences that:
    - Are expected to be delivered and/or developed in the next 3-5 years and 5-10 years by NMIs, or other organisations developing measurement standards, or methods for the biosciences
    - Are required by industry and other identified stakeholders over the next 3-5 and 5-10 years
2. International comparisons that are required to demonstrate the degree of equivalence of the measurement services that are, or will be developed and delivered
3. R&D activities necessary for the development of higher metrological order measurement standards and methods for the biosciences

The report reviews the information collected in order to provide input on the future requirements for BIPM laboratory activities and to be a useful reference for NMIs developing programmes in bio-measurement. Although in the early parts of the study, when roadmaps and strategies were reviewed, the coverage of bio-measurement was broad, later focus was given to protein and nucleic acid measurements as these were the fields that the BIPM indicated would be of highest interest to their programme at the start of the study.

The reviews of roadmaps and strategies are given in Section 2 and the CCQM's Bio-analysis Working Group (BAWG) strategy in Section 3. Section 4 covers the results of the interviews with the measurement organisations, followed by those of industry and the regulators in Section 5. A detailed analysis, which identifies the measurement services, the likely international comparisons needed to establish the global equivalence of bio-measurements and the measurement R&D required to develop the measurement services needed in the longer-term is given in Section 6. This focuses on protein and nucleic acid measurements, i.e. the fields of most interest to the BIPM. However, cell measurements are also covered and there is a brief section on emerging nanobiotechnology. Finally, Section 7 draws some conclusions regarding the areas of work that are strategic and underpinning, and which are most likely to be of interest to the BIPM for their programme. Again, the focus is on protein and nucleic acid measurements.

2. Review of Roadmaps and Strategies

2.1. Introduction

This section comprises a review of the relevant publications followed by an analysis of their content and conclusions relating to the measurement service requirements, particularly those which will establish measurement traceability. Details of published roadmaps and strategies were identified through consultation with LGC's metrologists working in the field and contacts at NIST and KRISS. This was supplemented by a formal literature search undertaken by LGC's Business Information Centre, which searched Medline, CAPlus and Biosis, supplemented by a Google search and a search of the websites of organisations connected with metrology.

The review is structured on geographic lines with sections on the U.S./America, Europe, the Far East/Australasia and a section relating to broad-based International organisations.

2.2. U.S./America

NIST

NIST has invested a considerable amount of effort over the last few years in consulting stakeholders in the U.S. about their measurement needs in the bioscience field.

A report[2] on a conference 'Strategy for Health Care through Bio and Information Standards and Technologies', 24-25 September 2007 organised by NIST and the Biotechnology Council focused on a strategy for healthcare through bio and information standards and technologies. The conference participants agreed that the current economic models in the U.S. and the research strategies were insufficient either to develop new diagnostic and therapeutic approaches, or to reverse the increasing trend of health spending, whilst meeting expectations for quality of life. In 2002, healthcare spending in the U.S. was almost $2 trillion and is projected to grow to $4.1 trillion by 2016 accounting for around 20 % of GDP compared to 16 % in 2007, by which time the U.S. Government will be paying almost half of the costs. There was a call for the U.S. to formulate a 20-year economic healthcare strategy for bio and information technologies to enable the country to capitalise on the opportunities which would be forthcoming from the exploitation of 'systems medicine', in contrast to the traditional reductionism approach to medicine. From an industry perspective, the decreasing rate of new innovations in therapeutics and diagnostics over the last 20 years, despite spending $trillions on R&D, was a cause for concern.

The attendees felt that a paradigm shift in healthcare delivery was only possible with the implementation of biological and information technologies and appropriate standards. The implementation of these technologies and standards required a long-term economic strategy that facilitated the identification and implementation of the most promising and necessary technologies and their validation and standardisation needs.

In the Pre-Conference Workshop the topic of a 'Personalised Medicine World' was discussed. This would make use of bio-measurement, bio-imaging, bio-informatics, genomics, proteomics and metabolomics to provide preventative and diagnostic care tailored to an individual's genomic profile. There was agreement that complex biomarkers would play a significant role in disease discovery and treatment. Such biomarkers would comprise complex 'fingerprints' and their analysis and interpretation would necessitate a systems approach to biology. Thus, the future of medicine would be transformed by creating technologies that made it possible to digitise this vast amount of information and, through the use of computers, display the integrated and analysed information in a form that doctors and patients could understand. Dr Leroy Hood, one of the pioneers of gene and protein sequencing, predicted that at least 50 organ-specific proteins from blood plasma/serum would be the biomarkers of the future, with the ability to determine the status of the 50 major organs of the human body. Also discussed were disease signatures, requiring thousands of measurements. A disease signature is a unique health status descriptor comprising quantitative and qualitative measurements of bio-molecules and other markers which are analysed by computers to understand the differences between a healthy biological system and the pre-diseased, or diseased state. The types of measurement which will be required include:

- Fluctuations in gene expression (mRNA and proteins)
- Appearance of new gene expression products

- Alterations in how proteins, DNA, RNA, sugars and lipids interact
- Changes in protein structure (e.g. phosphorylation)
- Changes in how existing proteins function or how a new disease-related protein operates
- Changes in cellular function and activity

Such measurements will present several significant challenges, including the need for the highly multiplexed measurements to be comparable over a patient's lifetime in order to be able to compare current signatures against the patient's own 'normal' signature. This will require a measurement infrastructure to enable measurements to be traceable to recognised reference points.

An economic analysis[3] of the technology infrastructure needs of the U.S. biopharmaceutical industry was prepared for NIST by RTI International in November 2007. It concluded that 25 %-48 % of the $884 million spent on R&D infrastructure and up to 22 % of manufacturing costs, totalling $335 million, could be saved within 5-10 years if specific improvements to the technology infrastructure were made. Key improvements relating to bio-measurement included:

- Bio-imaging – standardisation of image labelling procedures
- Biomarkers – an improvement in the sensitivity in detection of protein expression levels; traceable standards for immunoassay biomarkers; standardisation of protocols for generating gene expression results; standardisation of methods and tools for quicker validation of technology platforms; standardisation of statistical methodologies for data analysis in biomarker validation studies
- Gene expression – development of reference materials that mimic the biological complexity of tissue and blood samples; sample quality standards for storage and degradation assessment; systems, data and analysis mechanisms to benchmark microarray performance; calibration tools and techniques for scanning equipment; standard calibration curves for genes as well as standard control techniques
- Manufacturing – development of on-line measurement methodologies and industry standard QA/QC measures; reference standards analogous to cellular materials for future production of cell and gene therapies

A broader-based conference[4] 'Accelerating Innovation in 21st Century Biosciences: Identifying the Measurement, Standards and Technological Challenges' was co-organised by NIST in October 2008 to discuss the global measurement and standards challenges to innovation in key bioscience areas covering: agriculture, bio-energy, environment, biopharmaceutical manufacturing and medicine. Also discussed were 'hot topics' including: agriculture viability, antibiotic and antiviral drug resistance, environmental remediation, environmental bioterrorism monitoring, marine versus terrestrial sources of bio-energy, personalised medicine, stem cell therapy and synthetic biology. For each area, the broad challenges and barriers were identified and prioritised and the key measurements needed for support were listed. The priority measurement topics for medicine were:

- Development of consensus approaches and markers for sample integrity. A lack of consensus regarding the best approaches to sample preparation, as well as a lack of markers for sample integrity, is impeding progress towards the goal of personalised medicine.
- Understanding clinical and biological phenotypes (the observable characteristics produced as a result of genetic and environmental factors and their interaction). This is needed to better comprehend the causes and development of disease, identify targets for therapy and improve trial design
- Develop phenotype standardisation, outcome measures (morbidity, mortality, and quality of life), disease definitions (the attributes which must be measured to assess the disease) and disease activity (dynamic disease symptoms) to support improved disease identification and management.
- Develop reference materials and standardisation across platforms. Measurement is not standardised across platforms, producing misleading results and interpretations. The solution is to make biological measurements traceable to an independent reference material, which cannot change.

Following their extensive consultation, NIST summarised the measurement challenges to innovation in the biosciences and, consequently the critical roles for the organisation in an overview publication[5]. The strategy considered the need to address the barriers to bioscience innovation in: healthcare, bio-manufacturing, bio-

energy, environment, food/agriculture and homeland security/forensics. The vision developed for NIST's role in the biosciences was that of providing a co-ordinated state-of-the-art measurement science, technology and standards infrastructure with the capability to anticipate and meet emerging needs. The main drivers for their Bioscience Programs were the economic and societal impacts of biotechnology; U.S. publicly traded biotechnology companies have a market capitalisation of $410 billion and advances in the biosciences are critical to improving healthcare, with the future promise of personalised medicine giving better diagnosis and management of disease. NIST has consequently made the support of biosciences a top strategic priority. Total bioscience funding at NIST in 2008 was $58 million, with healthcare accounting for more than half the total spend. Within healthcare, diagnostics accounted for the majority of the expenditure. The key targets in healthcare were advanced multiplex measurement technologies and advanced bio-computing. A priority is to measure, in cells and blood, thousands of nucleic acids, proteins and metabolites that comprise disease signatures, which is a necessity for the advent of personalised medicine.

An initial draft of a more detailed review[6] of the unmet measurement needs for healthcare has been published by NIST, which includes its roadmap for addressing the measurement barriers. The report highlights the lack of adequate standards and comparable measurements for: *in vitro* diagnostics and medical imaging biomarkers, predictive toxicology for drug safety, medical device materials biocompatibility and genetic testing, which are having a major adverse affect on healthcare innovation and costs. In the U.S. 70 % of medical decisions are based on lab test results, yet only about 10 % of the 700 most common tests have internationally recognised reference methods underpinning their performance. In addition, the new multi-parametric analytical capabilities needed to support personalised approaches to medicine will require the underpinning science and standards to capitalise on the innovations. The roadmap does not specify timescales, but is a comprehensive list of topics which could be incorporated into NIST's future work programme.

The roadmap comprises two main sections:

I. Standards and Technology for Increased Quality in Current Generation Biomedical Measurements for Diagnostics and Therapeutics

II. Standards to Support Next Generation Healthcare Measurements – Tools to Support Discovery and Utilization of "Disease Signatures"

Section I lists specific project proposals covering the area and includes:

- Laboratory Medicine: current IVD technology suffers from a lack of reference standards. Areas of need include higher order reference methods and reference materials for clinical analytes:
    - non-peptide hormones e.g. serotonin, melatonin, dopamine and leukotrienes
    - serum proteins e.g. C-reactive protein, HER-2-nu
    - nucleic acids including: virus copy number, microRNA identity and quantity
- Standards to support medical imaging (standards for medical diagnostic imaging modalities, standard materials and methods for radiation therapy, new standards for quantitative nuclear medicine imaging, optical measurement standards to quantify appearance factors, bone density measurement standards, standards for molecular imaging)
- Standards to support molecular pathology (the use of immunohistochemistry in pathology sections to identify diseased tissue e.g. through the presence of HER-2 proteins on the surface of breast cancer cells)
- Standards and technology for biopharmaceutical manufacturing (host cell protein analysis, protein aggregation, multiplex measurement tools for cell production & processing, 'Quality by Design' validation services, standards for 3D protein structure measurement, standards for post-translational modification of proteins, validation of viral clearance methods, improved functional assays for protein therapeutics, stabilisation of manufactured proteins)
- Standards and methods to support measurements of nano-drug safety (measurement science, standards, technology and measurement services are needed for the determination of nano-material parameters such as: chemical composition, size, final product quality, location in biological materials and adulteration)
- Standards to support small molecule drug manufacture: there is a need for updated standards and practices to improve quality control and safety
- Standards for advanced radiation therapy

- Standards to support cell-based therapeutics (methods for accurate cell quantification, methods to identify terminal differentiation markers, methods to determine status of key signalling pathways controlling differentiation, methods to track the fate of injected cells)
- Standards for regenerative medicine (tissue scaffolds, tissue production, process monitoring, safety and efficacy, preservation of final product)
- Standards for gene therapy – small interfering RNA (siRNA) is one of the new types of gene therapy product, which represents a difficult measurement challenge because of its size and difficulty to sequence. Measurement science, standards, technology and services are needed for the determination of siRNA parameters such as size, sequence, distribution and concentration once injected into targeted cells
- Standards to support nanotoxicology measurements

Section II comprises a multiphase programme to establish the measurement infrastructure necessary to develop the requisite reference methods, reference materials and standard data to support the discovery of: the molecular basis for health and disease, new drugs and therapeutics, disease signatures. The highest priority is given to: RNA, proteins and metabolites in cells and blood, intermolecular interactions between biomolecules and next-generation DNA sequencing technologies. Thus additional measurement science, standards and technology are needed for:

- DNA measurements (Next-generation DNA sequencing, epigenetic genomic modifications, fidelity of DNA amplification, multiplex DNA amplification, DNA storage, measurement of low-abundance genomes)
- RNA measurements (copy number quantitation, identification and quantitation of microRNA, siRNA quantitation, RNA storage, real-time monitoring of RNA levels in cells)
- Protein measurements for disease signatures (quantitative, multiplexed measurements (up to 2 500 species), elucidation of disease proteome, measurements and standards for determination of protein structure, standards for protein function measurements, protein storage)
- Metabolite measurements (advanced multi-modal multiplex measurement technologies, imaging techniques for metabolomics)
- Cell and tissue measurements (standards for cell and tissue sample integrity, standards for stem cell characterisation, standards for single cell measurements, models for quantifying diverse cell populations, standards for quantitative histopathology, standards for measurement of environmental effects on cells, standards for measuring secreted proteins from cells, cell imaging reagent standardisation, intermolecular interaction imaging methods, standards for new imaging modalities, standards for sub-cellular imaging, standards for multimodal imaging, improved reagents for real-time intracellular molecular events, single molecule imaging of cells)

Congressional Committee Hearing

Other useful sources of information from the U.S. on bio-measurement are the statements to the House of Representatives Committee on Science and Technology Hearing on the role of reference standards in biologics R&D, 24 September 2009. These include:

- Statement[7] from Dr Willie May, NIST, which details the five key measurement issues for biopharmaceuticals that NIST plans to address. These are: the assessment of structural sameness (development of QA standards for measurement methods for post translational modifications and 3D structure), predicting adverse immune response (better understanding of the aggregation process), comprehensive understanding of the complex inner workings of cells (better understanding of the genetics and biochemical networks, systems biology, of cells used in bio-reactors), predicting drug function and toxicity (more accurate characterisation of the human cell types most often used in toxicity assays) assessing contamination from manufacturing processes and packaging (QA standards for measurements used to detect and quantify potential contaminants)
- Statement[8] from Dr Steven Kozlowski, FDA, which lists three specific properties of therapeutic proteins needing improved measurement: post-translational modification (particularly glycosylation), 3D structure (improved ability to measure and quantify and detect misfolding) and protein aggregation (measure and quantify different types)

- Statement[9] from Dr Anthony Mire-Sluis, Amgen, which argues that standardising immunogenicity testing would be a key step to improving the development and approval of biologics. He also pointed out that the early development of standards and standard methods was important for efficient biopharmaceutical development. This is because sound and rigorous measurement methods, ideally before expensive clinical studies are started, facilitate the optimisation of the product or process, thus maximising its chance of success
- Statement[10] from Dr Patrick Vink, Mylan, which proposes that a biologic's reference material and monograph be deposited with NIST by the originator. In return he proposed that the original manufacturer would have a period of exclusivity for non-patent data

US Department of Energy

In a broader context, but one which highlights some key analytical challenges, the U.S. Department of Energy's Genomics: GTL Roadmap[11] and 2008 Strategic Plan[12] are useful references. GTL is a major programme which aims to use systems biology (the holistic analysis of the complex biological networks and interactions) research to understand how genomes are translated into functional responses through a multi-scale exploration comprising: genes, proteins, molecular interactions, cellular function, microbial communities and ecosystems. Key research technology and methodology development areas include analytical "omics". This comprises transcriptomic, proteomic and metabolomic analyses which identify and measure the abundance and fluxes of key molecular species indicative of an organism or community activity at specific points in time. It requires the global analysis of RNA transcripts, proteins, metabolites and signalling molecules.

Mass spectrometry is seen as the most broadly applicable tool for large-scale identification of macromolecular complexes, but the additional development of quantitative techniques is essential. In particular, in order to identify changes in protein abundances caused by cellular perturbation, it is important that the quantitative data have associated levels of uncertainty. The use of stable isotope labelling for high precision quantification of the proteome and absolute abundance measurements is highlighted.

For metabolomics, mass spectrometry and NMR spectroscopy are commonly used. NMR has the advantage of being non-invasive and non-destructive, but suffers from limited sensitivity. However, recent developments with hyperpolarisable gases, such as xenon have the potential to increase the sensitivity by a factor of 20 000.

The downscaling of standard analytical methods using microfluidic technologies is important for making the analyses low cost and microarrays have become a standard technology for high-throughput gene expression analysis. However, data from global microarray analysis must be validated with lower throughput, more conventional methods, such as real-time PCR.

National Institute of Health (NIH)

The NIH has charted a 'roadmap'[13] for medical research in the 21st century, focused on a vision for a more efficient and productive system of medical research. It identifies key opportunities in three main areas:

1. New Pathways to Discovery
2. Research Teams of the Future
3. Re-engineering the Clinical Research Enterprise

From a measurement viewpoint, the 'New Pathways to Discovery' holds the most interest. It states that: "Future progress in medicine will require a quantitative understanding of the many interconnected networks of molecules that comprise our cells and tissues, their interaction and their regulation". Some of the programmes and projects supported by the initiative which have high relevance to measurement include:

- Epigenomics; developing standardised platforms, procedures and reagents
- Genotype-Tissue Expression; analysing global RNA expression within individual tissues and correlating with genetic variation that can be identified as expression quantitative trait loci
- Structural Biology; structure determination of complicated membrane proteins and their complexes
- Human Microbiome Project; comprehensive examination of microbial communities using advanced DNA sequencing technologies and their role in human health and disease
- Library of Integrated Network-based Cellular Signatures; high-throughput screening approaches to develop a 'library' of molecular signatures that describe the response that cells elicit when perturbed

- Nanomedicine; the development of new tools to probe and manipulate nanoscale biological structures
- National Technology Centres for Networks and Pathways; a programme focused on the dynamic measurement of biological systems

Canada

A Roadmap[14] produced by Industry Canada, which highlights the difficulties facing the biopharmaceutical industry, gives a good overview of the technical challenges associated with drug discovery and development.

For drug discovery, advances in DNA sequencing and ultra-high-throughput screening have reduced the gene discovery bottleneck and the current rate limiting steps are target validation and target identification. Metabolomics, RNA interference and chemical genomics are being used to address bottlenecks in target validation (i.e. the determination that a protein target is crucial to the disease process, that modulating the target will have a therapeutic effect and that any such effect will be dose-dependent). Precise measurements of metabolite concentration are required to underpin systems biology by providing insights into the interconnected molecular pathways. The lack of high-throughput analytical tools and methods for the analysis of glycosylated proteins, which have been targeted extensively for drug discovery, makes it difficult to define their molecular structures and monitor how they interact with receptors. Glycolsylation is a common type of post translational modification to proteins which is key to their function as it determines binding to other species, thus affecting properties such as activity or half-life. Glycosylated proteins present several difficult analytical challenges because of their inherent complexity namely the linkage, branching and composition of the polysaccharide side chains, the existence of a variety of isoforms and the presence of differently glycosylated variants. Hence, the released glycan pool for analysis contains a range of structures. Enzymatic treatment followed by NMR and mass spectrometry have been used to determine the specific sequences contained in the sugar chains, but high-throughput tools for determining the sites of carbohydrate attachment are still lacking.

Better multiplexed assays are required for multiple cellular targets and processes for high-content screening, which identifies those drug candidates most likely to succeed. For clinical trials, ultra-sensitive accelerator mass spectrometry is used to measure pharmacokinetic (PK) properties in human trials using microgram quantities of lightly labelled drug candidates. Many platforms used for biomarker analysis in research are insufficiently robust or reliable and are too expensive for use in the clinic.

Also, the National Research Council of Canada is currently in Phase IV of its major Genomics and Health Initiative[15] which covers 2008-2011. The major programmes within Phase IV comprise:

- Patient-Specific Virtual Reality Systems for Surgical Oncology
- Biorenewable Oil for Food and Fuel
- Heart Disease: Better Tools for Better Treatment
- Biochips for Diagnosis and Understanding of Human Diseases
- The Identification of Proteins Targeting the Tumour Microenvironment for Therapeutic and Diagnostic Applications

The Biochips programme is aimed at creating rapid point-of-care diagnostic services, with the ability to quantify, analyse and detect very small amounts of specific markers for pathogens, including DNA.

2.3. Europe

EU Seventh Framework Programme

The EU Seventh Framework Programme[16] for research and technological development runs from 2007 to 2013 and includes four main specific programmes, of which the 'Cooperation' programme supports research activities in key scientific and technology areas. There are ten themes within the 'Cooperation' programme. Of those, the themes of most interest to bio-measurement are 'Health' and 'Food, Agriculture & Fisheries and Biotechnology'. The 'Health' theme budget is €6.1 billion and one of the three pillars is 'Biotechnology, Generic Tools and Technologies for Human Health'. The main objective is to catalyse the development of new research tools for modern biology, with a focus on new technologies for:

- Sequencing

- Gene Expression, Genotyping and Phenotyping
- Structural and Functional Genomics
- Bioinformatics and Systems Biology

European Metrology Research Programme (EMRP)

A major review[17] of the measurement needs which will have a strategic impact for Europe was undertaken by Euramet during the formulation of the new European Metrology Research Programme.

As a preliminary to the formulation of the programme, a series of road mapping exercises was undertaken in February 2006. The initial roadmaps[18] for the bio-measurement area were developed by the Euromet *ad hoc* Biotechnology Working Group. They cover health, bio-threats and bio-manufacturing and are reproduced in Figs. 1-3.

Fig. 1: Euromet Bio-Measurement Roadmap for Health

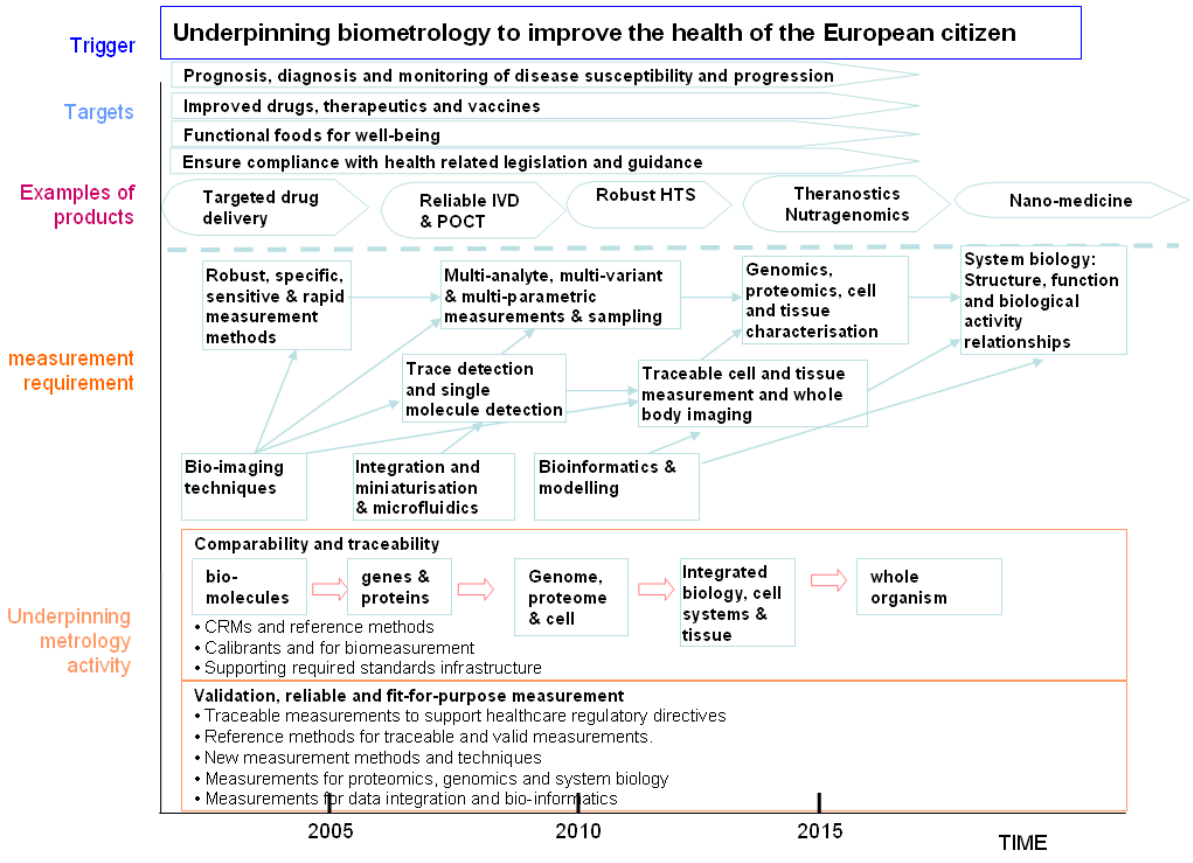


Fig. 2: Euromet Bio-Measurement Roadmap for Safeguard from Bio-Threats

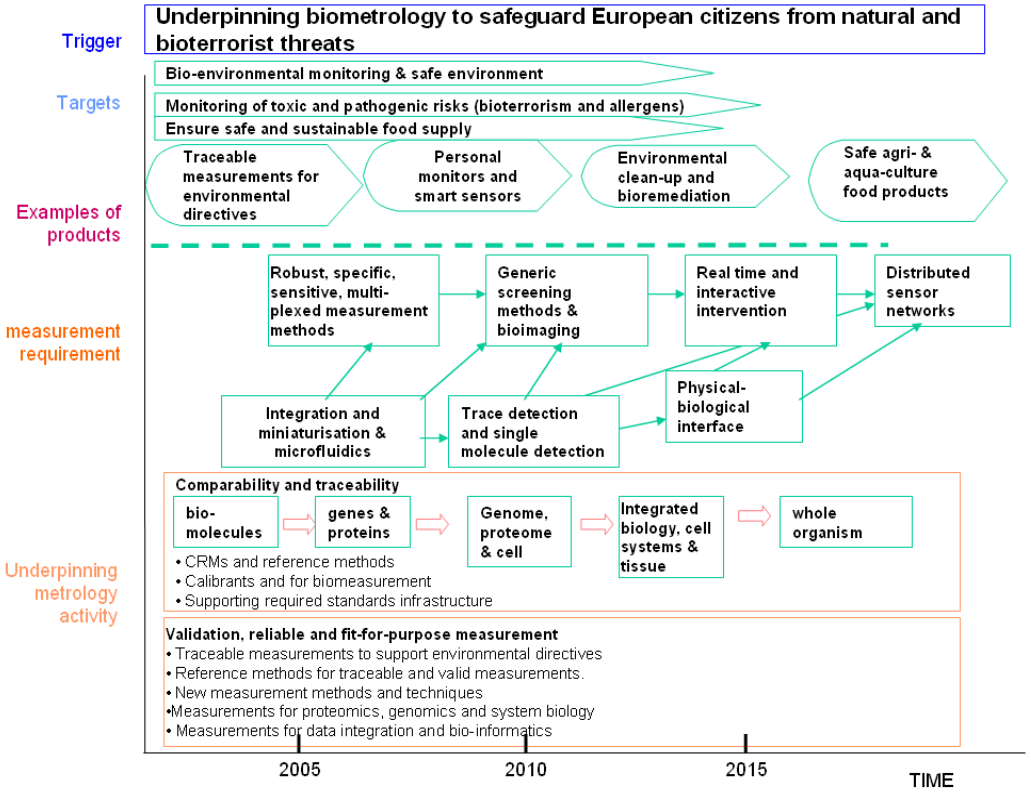
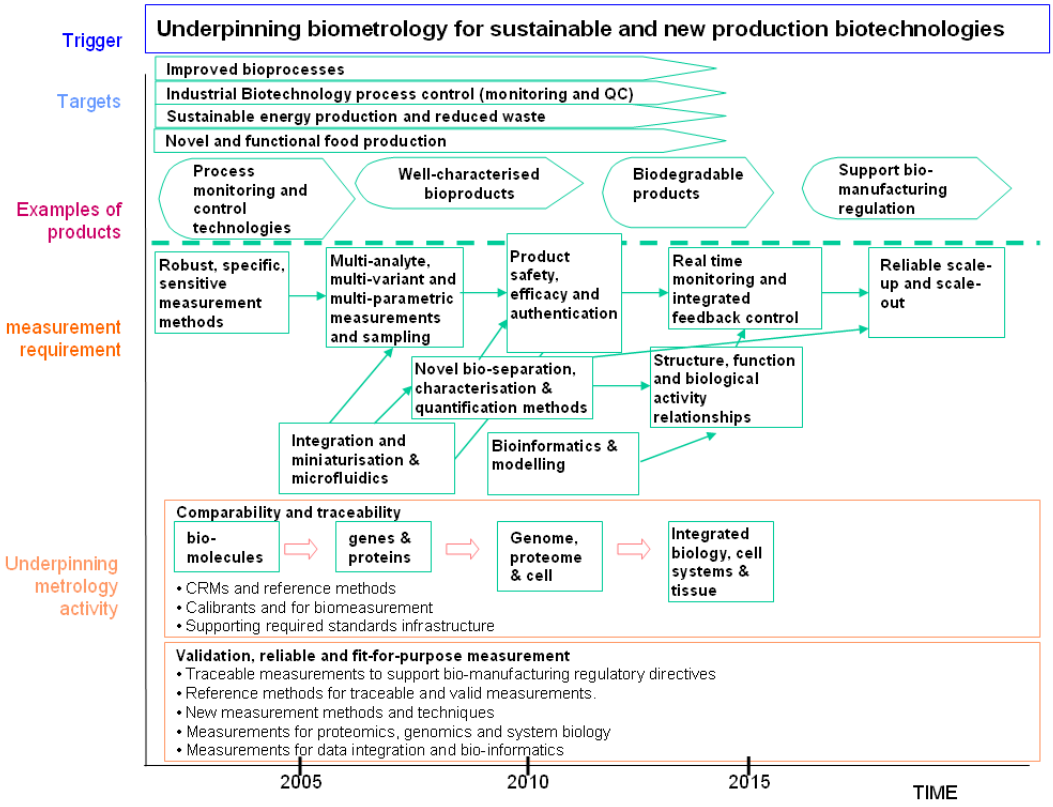
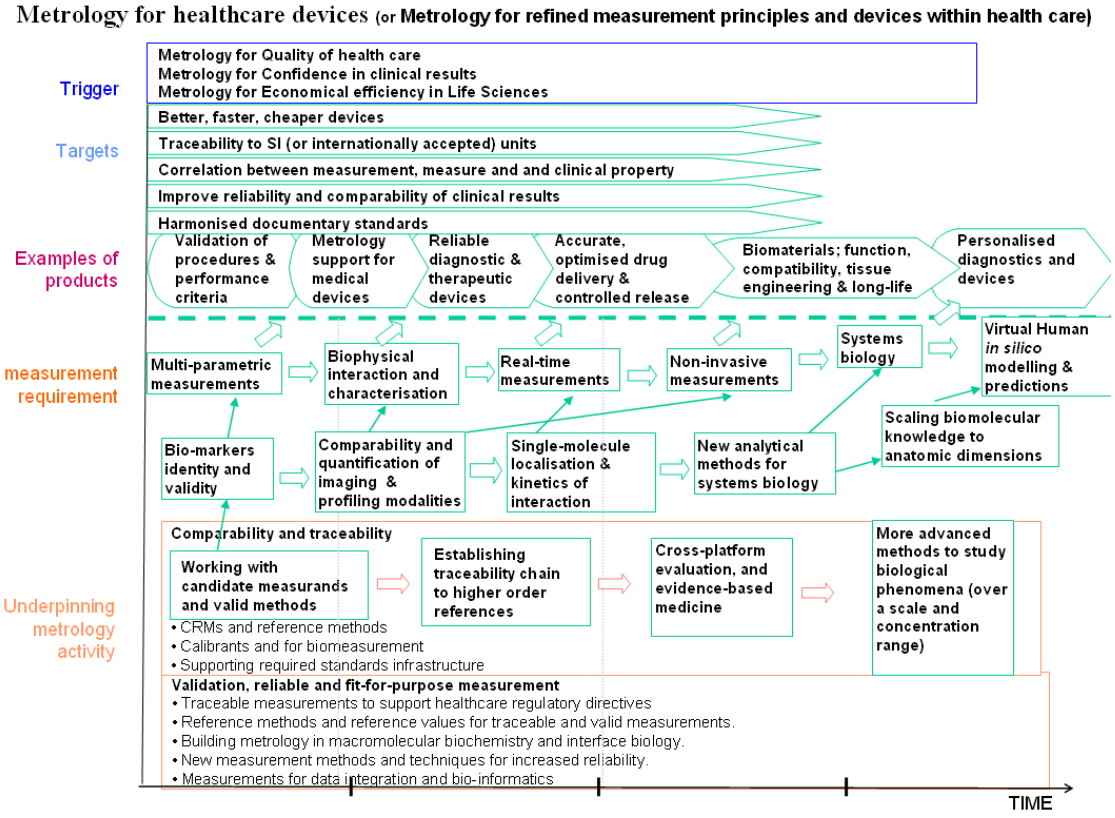


Fig. 3: Euromet Bio-Measurement Roadmap for Biotechnology Production



A roadmap, developed a couple of months later by the INTMET Life Sciences Working Group for the EMRP, on metrology for healthcare devices is shown in Fig. 4.

Fig. 4: INTMET Roadmap for Healthcare Devices



The main measurement requirements identified by the INTMET group were:

- Single molecule localisation and kinetics of interaction
- Scaling bio-molecular knowledge to anatomic dimensions (link to virtual human)
- Bio-physical interaction and characterisation
- Non-invasive measurements
- Real-time measurements
- Multi-parametric measurements
- Biomarkers

The measurement capabilities required to be addressed by the EMRP were identified using the concept of 'Grand Challenges'. One of these covers 'Health' and elements of the programme relevant to this area were:

- Reference measurement procedures and reference materials of a higher order as recommended by the Joint Committee for Traceability in Laboratory Medicine (JCTLM). Examples of immediate need include: C-reactive Protein, Prostate Specific Antigen, Cardiac Troponin, Human Growth Hormone
- The 'virtual human' which refers to a model of the human anatomy and functions as a comprehensive reference standard for manufacturers of medical instrumentation, medical R&D, modelling and training.
- Quantitative diagnostics including: imaging, microscopy and multimodal measurement procedures. A substantial part of the research challenge is related to the development of mathematical models and tools and software

- Diagnostic and therapeutic instrumentation: metrological research on e.g. NMR and ultrasound to improve their quality with respect to reliability, validity, comparability and patient risk and to drive innovation towards real-time and non-invasive measurements, or towards individually optimised drug delivery

In addition, under 'R&D for Emerging Metrology', bio-metrology (the creation of a sound international basis for accurate and reliable comparable bio-measurements) is recognised as being critical for three key areas:

- Healthcare: prognosis, diagnosis, monitoring of disease susceptibility and progression; improved drugs, therapeutics and vaccines; functional foods and compliance with health related legislation and regulation
- Bio-production: improved bioprocesses; industrial biotechnology process control for monitoring and QC; bio-fuels for sustainable energy production; biodegradable products; novel food production
- Security: monitoring of toxic and pathogenic risks including allergens and bio-terrorism; traceable measurements for environmental directives; environmental clean-up; bioremediation; safe 'agri' & aqua culture

The research topics comprising the EMRP range from developing reliable and fit-for-purpose measurement methods for health-marker molecules i.e. simple biological molecules such as nucleic acids through to novel measurements of proteins and their interactions. It envisages that advances in bio-metrology will feed into and facilitate the emerging field of systems biology and, in particular, data integration and bio-informatics in order to aim for the ultimate goal of whole organism metrology i.e. providing the connection between genomics and functionality.

UK

In the UK there has been a recent analysis[19] of government policy objectives and the interventions required by the National Measurement System (NMS) to support these. In the Healthcare and Medicines sector, the NMS will provide a traceable metrology infrastructure that underpins the delivery of safe and cost-effective human and animal welfare provision and support the removal of technical barriers to innovation. The main drivers of change in this sector and the proposed NMS interventions are:

- Demographics: the NMS will play a leading role in providing nationally and internationally traceable standards to underpin evidence-based risk assessment of new technologies required to address changing demographics. One planned outcome will be a significant increase in the application of early diagnosis technologies for a range of diseases which comply with the EC's *In Vitro* Diagnostics Directive
- Health Economics: the NMS will assist in the delivery of safe and cost-effective healthcare by providing the necessary metrology infrastructure to minimise the time taken and cost to bring new pharmaceutical products to market and ensure effective National Health Service (NHS) clinical decisions. NMS intervention will include: introducing metrology earlier in the drug development pipeline; facilitating new approaches to replace animal testing; developing metrology to underpin regulation associated with new therapies, such as regenerative medicine; improving the speed, reliability and cost of diagnostics
- Well-being – increasing the level of quality of life: the NMS will provide standards and metrology to underpin preventative screening and help in the development of new approaches to 'stratified medicine' (see below)
- Globalisation: the NMS will underpin the rapid development of innovative new technologies associated with pandemic preparedness and play a key role in ensuring that products can be traded securely across borders. NMS intervention will include the development of metrology and standards to underpin the trade in pharmaceutical products and combat the trade in counterfeit healthcare products

Following on from this analysis, the National Measurement System strategy[20] has been detailed and Programme Roadmaps[21] published. The NMS Strategy document gives a high level overview of the proposed strategy. Its priorities are: delivering world-class measurement science, providing measurement leadership and responding to National Challenges namely Global Competitiveness, Business Innovation, Energy Sustainability, Healthcare, Digital Economy and Security. The main bio-measurement elements of the strategy are:

- Trace chemical and biological analysis and cellular and molecular imaging technologies e.g. cellular and nanoscale metrology and high accuracy quantitation of complex chemical and biological systems,

such as the detection of trace levels of target DNA in a background of DNA using digital PCR and the use of LCMS for analysing host cell impurities in biopharmaceuticals

- Provision of measurement and characterisation methods to support the development of bio-fuels
- Facilitating 'High Quality Care for All' by underpinning the assurance of consistent delivery across the NHS through good measurement practice. In particular, there is a strong drive within the Health Service to accelerate patient diagnosis through the development of 'point of care' testing (POCT). Following the introduction of the EU *In Vitro* Diagnostic Directive there is a requirement for clinical laboratory measurements to be traceable to a certified reference material of higher order. To date the focus has been on laboratory measurements, rather than POCT. Work is being undertaken to address the measurement issues specific to POCT and develop calibration procedures to establish a line of traceability to the laboratory-derived results
- Enable new drugs, therapies and assistive technologies to be brought to the market quicker and at lower cost through the provision of metrology that underpins regulation. There is recognition that array-based technologies could have the potential to speed up drug development by determining safety biomarkers, which predict drug toxicity. However, there is still an issue over the comparability and reproducibility of data, which severely restricts their use in a regulatory-driven environment. Several international consortia including: the Microarray Quality Control Project (MAQC)[22], the External RNA Controls Consortium (ERCC - an *ad hoc* group hosted by NIST of about 50 companies, universities and federal laboratories to develop materials and tools that will be used to establish the performance of DNA microarrays and other quantitative experiments that measure gene expression) and the Clinical Laboratory Genomic and Genetic Standards (CLGGS - a voluntary organisation of scientists engaged in the development of standardised and harmonised best practices for microarray-based clinical assays) have been set-up to tackle the problems. The overall aim of the NMS work in this field is to support these efforts through the development of performance indicators, quality metrics and reference datasets
- Support the reduction in animal testing through the validation of new types of testing protocol. *In vitro* cell-based assays are currently the best alternative to costly animal testing. However, they present many disadvantages in terms of key performance characteristics and are often associated with high levels of variability. Emerging technologies such as *in vitro* predictive toxicology combined with novel approaches, such as 3D culture systems or the use of stem cells offer real potential to reduce *in vitro* variability and increase process performance. Work will demonstrate the robustness and increased reliability of a 3D liver cell culture as a predictive toxicology model by assessing the sources of measurement variation in key parameters which underpin its performance
- Develop measurement protocols and standards to enable infectious disease detection technologies to be deployed and utilised with confidence. The early diagnosis of disease is a major objective of modern healthcare and molecular techniques are particularly suited to this challenge. However, such applications require the ability to measure extremely small quantities of minority nucleic acid sequences in a background of similar material, pushing the current techniques, such as whole genome amplification (WGA) and 'digital' PCR, to the limit. The work will be undertaken with clinical chemists and diagnostic manufacturers and will develop QC tools such as performance indicators and guidelines for data handling and analysis, thus enabling more robust, early detection methods for the genetic changes leading to conditions such as chronic myeloid leukaemia, colorectal cancer and ovarian cancer
- Provide methods and standards for trace detection of biological agents in the field
- Develop techniques to reliably identify counterfeit pharmaceutical products in the field. Advances in mass spectrometry have enabled the detection of counterfeit goods and the tracing of a sample to its origin using subtle difference in isotopic composition. However, before the technique can be used with confidence in court, validated methods, calibration strategies and calibrants, and reference databases are required. The work will establish a calibration capability that provides absolute isotope ratio data, will assess the best approach for establishing the basis for identifying the authenticity and origin of key materials in collaboration with the pharmaceutical industry and regulators, and will assess the available statistical tools to validate the analytical measurement.

The NMS Programme Roadmaps, published in May 2009, provide a general guide to the direction of the NMS Programmes to 2015. The most relevant are the roadmaps for proteins, genes, cells and tissues and nano-biotechnology, which are reproduced in Figs. 5-8. They were produced to feed into the NMS strategy consultation document. The public consultation is now closed and the NMO is considering the views expressed and will provide a response.

Fig. 5: UK NMS Roadmap for Proteins

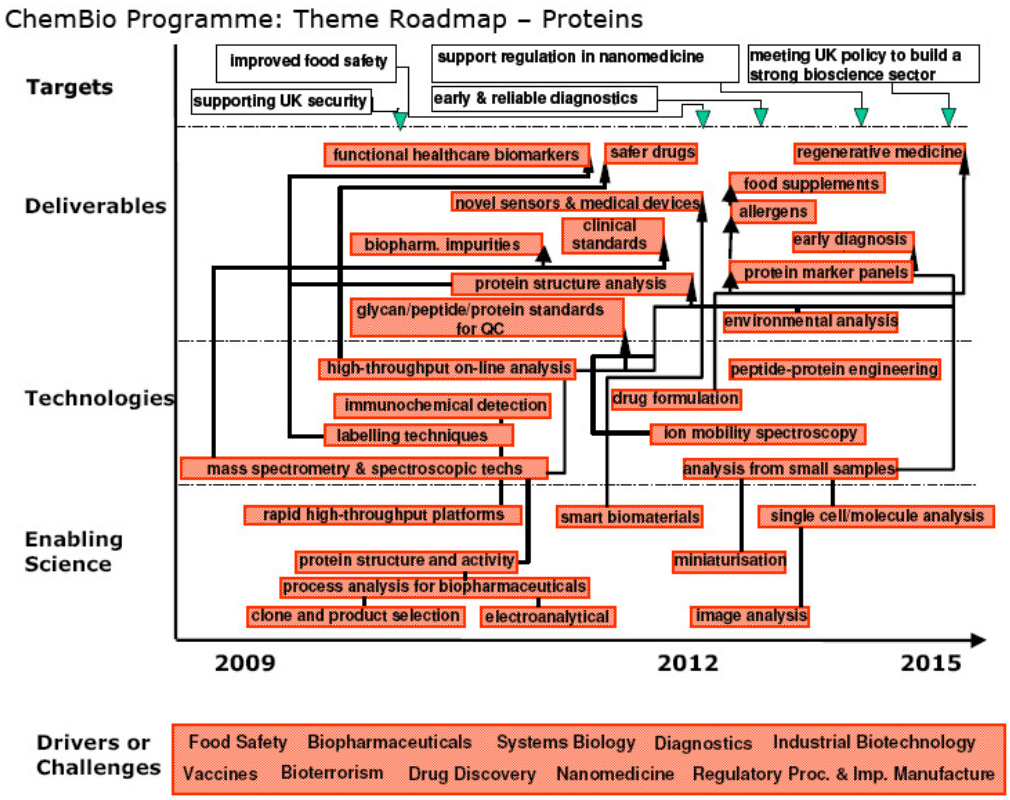


Fig. 6: UK NMS Roadmap for Genes

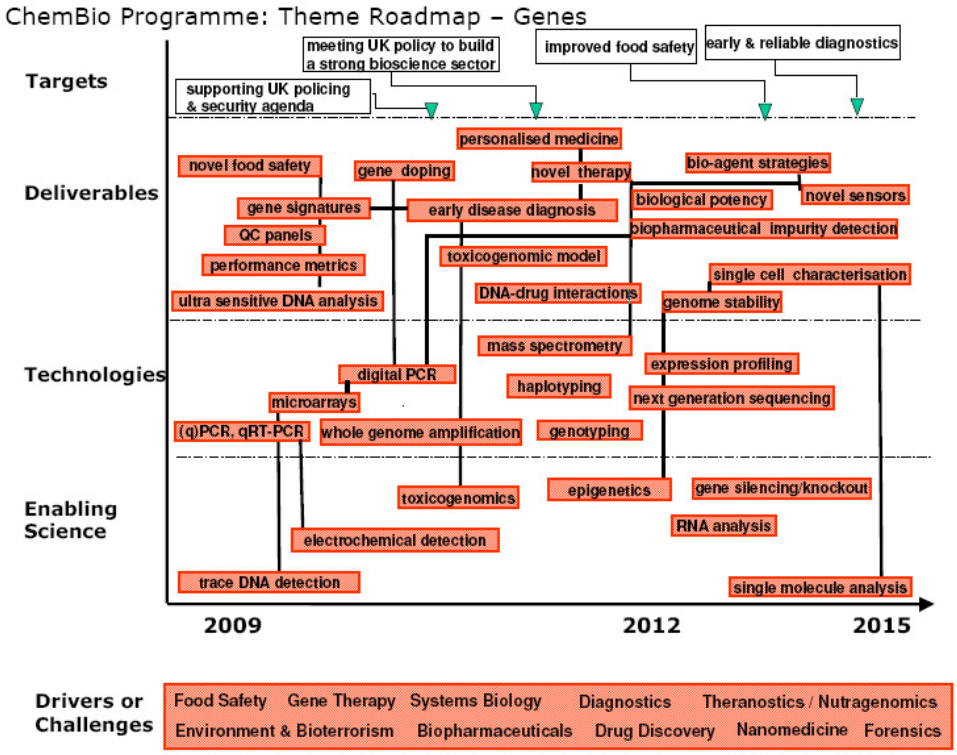


Fig. 7: UK NMS Roadmap for Cells & Tissues

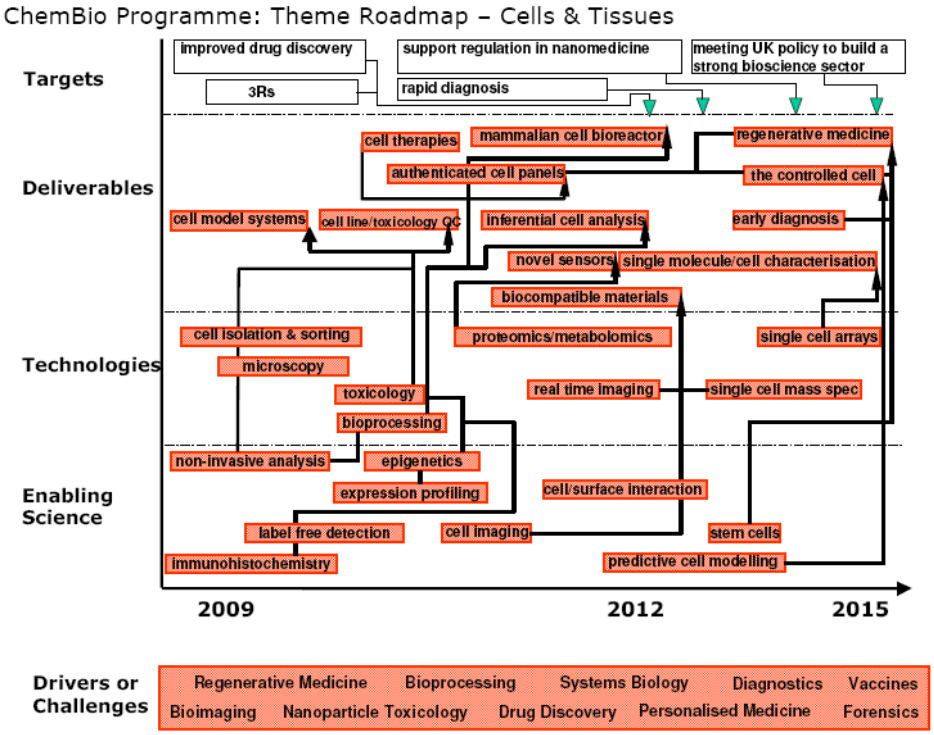
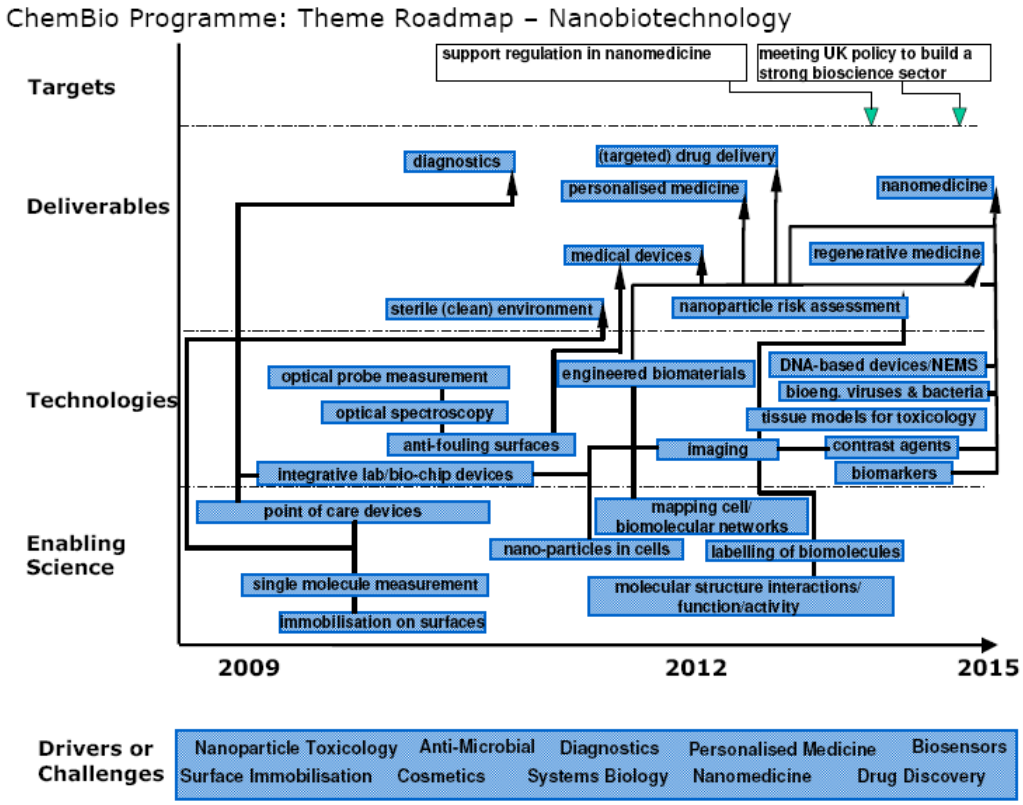


Fig. 8: UK NMS Roadmap for Nanobiotechnology



Another UK Government publication[23] of relevance is a report on opportunities for biotechnology in a low carbon economy. Although there are not many explicit references to bio-measurement, it does contain a recommendation for a standard for calculating the bio-based content of a product.

In a broader context, a review[24] of the UK's bioscience has been published, followed by the Government's response[25]. The review recognises that the adoption of 'personalised medicine' may be too expensive an option, but that 'stratified medicine' which targets groups of people with the same genetic characteristic should be taken forward. This enables clinicians to identify patients for whom a treatment may prove effective and those in whom side effects may occur, and therefore not to expose those patients to the treatments. The impact of stratifying diseases can be applied to clinical trials as well as disease management, with the potential to develop treatments targeted for specific patient populations and thus help address the high attrition rate seen in drug development. The measurement challenges rest mainly in the development of robust, low cost genomic analysis and the development of validated biomarkers.

Key documents, which outline the UK Technology Strategy Board's support for innovation in the Biosciences[26] and Healthcare[27] for 2009-2012, include brief considerations of standards and metrology, the bio-measurement components of which are summarised at the end of each of the following two paragraphs.

The Biosciences Strategy has three priority areas: Genomics, Industrial Biotechnology and 'Agri-food'. In Genomics, high-throughput analytical technologies are identified as being one of the priority technologies relevant to the exploitation of genomics, with applications in: medicines and diagnostics, livestock improvements, bio-energy production, environmental remediation, enzymes and new crop varieties and yield improvements. For Industrial Biotechnology, discovery tools including microbial and gene screening, enzyme activity assays and cell-based bioassays are included in the priority technologies list. In 'Agri-food', diagnostics including: nucleic acid analysis, biomarkers and antibodies, chip technology, multiplex detection, biosensors, automated sampling and rapid liquid chromatographic techniques are considered priority technologies. There is a short section on Standards and Metrology which highlights the potential need for standards to determine the permissible levels of bio-fuel components.

The Medicines and Healthcare Strategy identifies the challenges for this sector in providing for a growing and ageing population namely: disease prevention and proactive management of chronic disease, earlier and better diagnosis of disease and highly effective treatments that are tailored to patients' needs. The strategy includes a

section on metrology and standards. This highlights the need for improvements to current measurement technologies for biological drugs and the development of metrology for 'point of care' testing and new applications relating to 'stratified medicine'.

2.4. Asia-Pacific

There appear to be few published references to bio-measurement roadmaps and strategies for the Asia-Pacific region. However, some information has been provided by the Chinese and Korean measurement institute, which is detailed below, together with a short reference to Australia's strategy. In a regional initiative, the Chinese, Japanese and Korean measurement institutes have established the Asian Collaboration on Certified Reference Materials (ACRM) to jointly develop high quality CRMs. It includes an *ad hoc* working group on CRMs for bio-analysis and has jointly characterised an insulin CRM.

China

China has produced a 5-year plan for the biosciences, but this is not publically available. However, Dr Wang Jing, Deputy Director, Division of Biological, Energy and Environment Measurement, National Institute of Metrology, China (NIMC) gave an interview on the bio-measurement elements of the bio-industry development plan for the Chinese journal, Dialogue[28] and has provided some additional information on the strategy of the NIMC.

As part of its role, NIMC carries out basic bio-metrological research and has the responsibility for developing, enabling and maintaining the national bio-measurement system. Key tasks of the biological division are to address bio-measurement traceability and techniques for assuring the comparability of bio-measurement through the provision of reference measurements and reference materials.

The bio-metrology research focuses on:

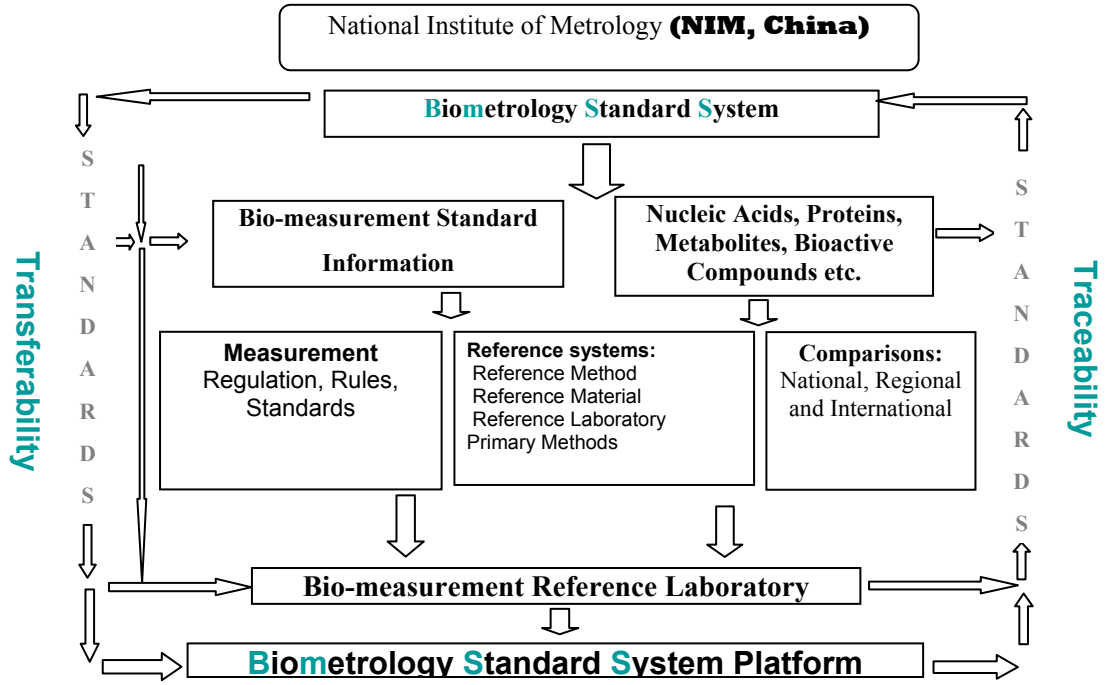
- Nucleic Acids
- Proteins
- Cells
- Bioactive Compounds
- Metabolites for clinical diagnostics
- Microbiology
- New drug safety evaluation

Key projects undertaken for the National Science & Technology Pillar Program in recent years include:

- Establishment of a national resource sharing platform for biochemical measurement standards
- Research on measurement standards for nucleic acids and proteins
- Study of the key techniques in bio-safety measurement traceability and comparability
- Development of food safety related reference materials such as: GMOs, vitamins, peptide and protein molecular weight references, *Bt* series protein, paralytic shellfish poisoning (PSP) in mussel, diarrhetic shellfish poisoning (DSP) in mussel, ginkgolic acids in methanol, bioactive compounds in dietary supplements.
- Development of clinical reference materials such as: bovine serum albumin, glycated haemoglobin, C-peptide, insulin

A portfolio of reference methods and standards databases for nucleic acids, proteins, cells, bioactive compounds, metabolites and microorganisms has been set up. Over 50 biological CRMs have been developed in the last 5 years. A major task of its medium and long-term science and technology development strategy (2006-2020) is to establish a national standard, measurement and testing system. NIMC has been establishing an elementary bio-metrology standard system – see Fig 9. In addition, a National Technical Committee for Bio-metrology (TCBM) was established in July 2007.

Fig 9: China's Bio-metrology Standard System Platform



In 2009 NIM hosted a Conference on China's Bio-metrology Development to determine the priority challenges in five fields: Food Safety, Medicine and Medical Equipment, Forensic Evidence, Microbiology and Biological Agriculture & Marine Biology.

Priority Challenges in Food Safety

- Molecular standard/reference materials (nuclear acid, protein, etc.)
- Reference materials for drug metabolism
- Matrix reference materials (including GM crops, GM food, food borne pathogens, etc.)
- Limit value reference materials of prohibited pesticides

Priority Challenges in Medicine and Medical Equipment

- Establishment of instrument calibration protocols and standards
- Assurance of sequencing precision
- Overcoming matrix effects
- Establishment of national and/or international standards and reference materials
- Measuring enzyme activity
- Equipment and techniques for biomarker detection together with relevant CRMs

Priority Challenges in Judicial Appraisal

- Investigation of reference materials for the hereditary attributes of the Chinese population
- Forensic identification
- Methods for toxicological analysis
- Identification of genetic/epigenetic markers relevant to physiological characteristics
- Establishment of instrument calibration rules and standards
- Establishment of a traceability and transferability system

Priority Challenges in Microbiology

- Analysis and measurement technology for pathogen detection
- Measurement standards/reference materials
- Viability, toxicity, homogeneity and specificity of microorganisms
- Identification/quantification for complex microbial flora

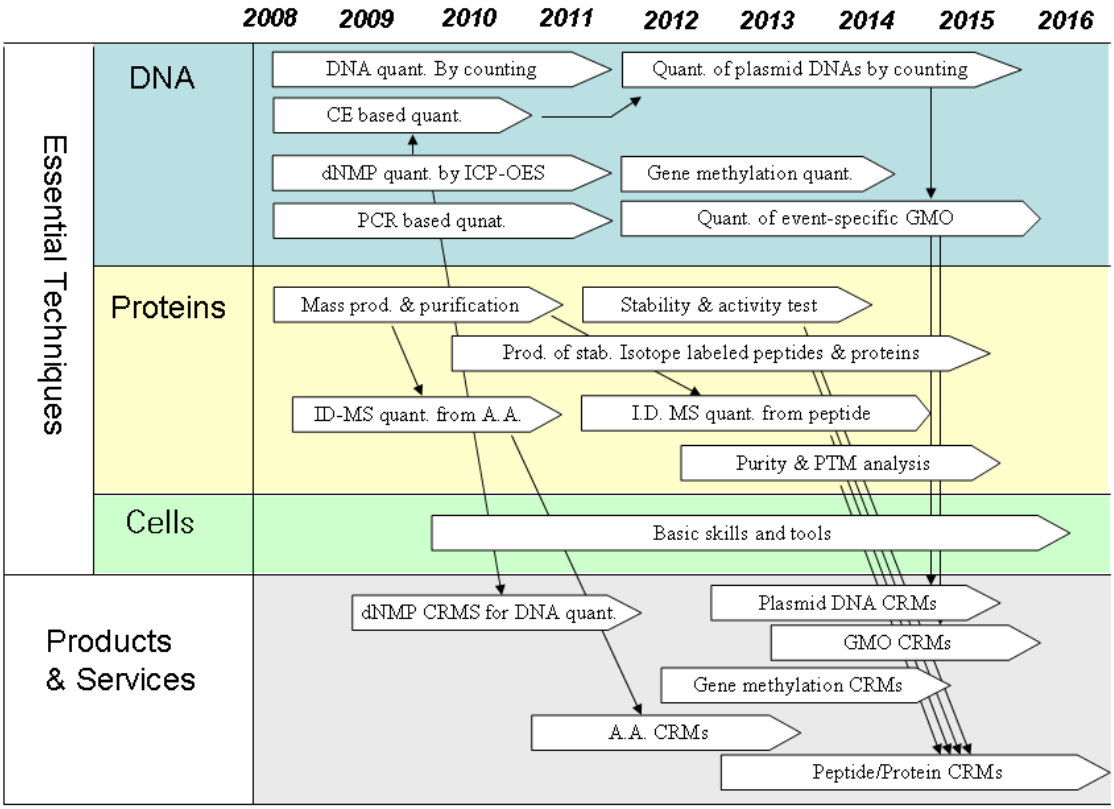
Priority Challenges in Biological Agriculture & Marine Biology

- Matrix reference materials for GMOs
- Standard techniques and reference materials for the evaluation of the safety of GMOs
- Reference materials for biological pesticides, veterinary drugs and bio-feed.
- Reference materials for marine bioactive compounds, marine toxins
- Identification of the marine species in seawater samples

Korea

The Center for Bio-analysis at KRISS has developed a roadmap[29], see Fig. 10. It covers work in DNA, protein and cell analysis and the development of products and services from these, which comprise mainly certified reference materials.

Fig 10: Roadmap for Bio-measurement Developed by KRISS



A more general set of roadmaps[29], covering technologies and products for biotechnology has been developed by the Daejon Techno R&D Consortium in Korea. These give an insight into where the bio-measurement priorities in Korea may lie. The technologies and development timescales comprise:

- **Biopharmaceuticals – Technologies for:**
    - Lead Compound Mining (2006-2013)
    - Biomarker Discovery & Development (2006-2011)
    - Gene Expression & Regulation (2006-2011)
    - Therapeutic Antibodies (2006-2011)
    - Gene Delivery (2011-2016)
    - Therapeutic Cell lines (2008-2016)
    - Evaluation of Safety, Efficacy and Validation (2006-2011)
    - Preclinical & Clinical Trials (2008-2014)
    - Formulation and Mass Production Processes (2009-2014)

- **Biomolecular Diagnostics – Technologies for:**
    - Biomarker Discovery & Development (2006-2012)
    - DNA Amplification & Detection (2006-2012)
    - Protein Detection (2006-2012)
    - Bio-chip and Sensors (2008-2015)
    - Universal Health Care (2006-2013)

- **Food Supplements – Technologies for:**
    - DNA Recombination (2007-2009)
    - Mass-scale Fermentation & Extraction (2010-2013)
    - Metabolic Engineering (2013-2015)
    - Bio-transformations (2008-2011)
    - Gene Expression & Regulation (2011-2014)
    - High-throughput Separation & Purification (2007-2011)
    - Preclinical & Clinical Trials (2011-2013)
    - cGMP (2009-2012)

Australia

In the Australian Government's Innovation Report 2005-06[30] there is mention of the development of a framework for biological measurement by NMIA Australia, but this has not been published. Its aim was to help ensure valid approaches to bio-measurement and underpin all areas of lifescience.

2.5. International

A Council Task Force on Biotechnology[31] was recently set up by ISO to analyse its priority areas of interest, including measurement and characterisation. The Task Force made broad recommendations for ISO work relating to techniques of analysis on standards for data representation and management, statistical methods and methods of analysis for: genomics, proteomics, metabolomics, molecular imaging and stem cell analysis. The BIPM was identified as a potential partner for work in these fields. Recommendations were also made for the consideration of standards for the activity of enzymes and the performance of biocatalysts and the aggregation, glycosylation and folding of proteins. It also recommended work on validation and calibration covering: guidelines, reference data, reference databases and reference materials for 'horizontal' applications.

As a pointer to the growing importance of biopharmaceuticals, the market research company Evaluate Pharma has estimated[32] that the proportion of the top 100 drugs comprising biologics will increase from 28 % in 2008 to 50 % in 2014.

3. Review of BAWG Strategy

3.1. Introduction

This section outlines the strategy of the CCQM BioAnalysis Working Group (BAWG), as documented to May 2010, and gives a summary of the likely areas for bio-measurement comparisons. The information collated is based on presentations and documents tabled at BAWG meetings, discussion and comments minuted at BAWG meetings and e-documents and e-discussion on the CCQM BAWG WIKI. It therefore represents a consensus of the evolving strategy of the BAWG group and not the personal views of any single NMI or BAWG participant.

3.2. Background

The CCQM BioAnalysis Working Group was originally established in 2002. Its Terms of Reference, Scope and Strategy Roadmaps were defined, discussed and agreed by the BAWG participants at CCQM BAWG, 7 April 2005 and have been regularly reviewed and updated[33].

3.2.1. Terms of Reference for BAWG

- To establish global comparability through bio-analytical reference measurement systems of the highest possible metrological order comprising:
    - Traceability to the SI, where feasible, or to other internationally agreed units
    - Reference Methods
    - Certified Reference Materials
    - Uncertainty estimates for the whole process
- To develop, improve and validate those systems through pilot studies prioritised in response to demands of end-users (e.g. healthcare, food, pharmaceutical, forensic) and regulation (e.g., ICH, FDA, EU IVDD)
- To establish comparability of NMIs and designated National Expert Laboratories through prioritised Key Comparisons
- To integrate BAWG activity with that of international stakeholders such as WHO, CODEX, JCTLM in order to harmonise bio-analytical reference measurement systems
- To acknowledge that the immaturity of bio-metrology entails a pragmatic step-wise approach, and 'fit for purpose' levels of metrology

3.2.2. BAWG Scope

The agreed scope of studies and activity of the CCQM BAWG is in the area of bio-analysis and bio-measurement where:

- Bio-analysis covers large macromolecules where the target measurand is of biological origin (including, but not limited to, genes, proteins, cells) in a biological measurement context
- Bio-measurement includes, but is not limited to, the identification and quantification of the macromolecule in complex matrices and mixtures relevant for functional activity.
- Biological measurand, the quantity subjected to measurement, may not easily be defined.
- Direct and indirect measurement(s) and inferences are included.

3.2.3. Strategy Roadmaps

A series of roadmaps were developed by the BAWG (2005-2006) to help define and prioritise areas for pilot studies and Key Comparisons (KCs) to support NMI capabilities in the key areas of nucleic acid, protein and cell bio-measurements. They focused on the key building blocks for measurement studies required to demonstrate capability and develop Reference Measurement Systems (RMS). Each had its limitations, as briefly noted below, but allowed a strategic approach to study design and discussion.

Fig. 11: BAWG Roadmap for Gene Measurement

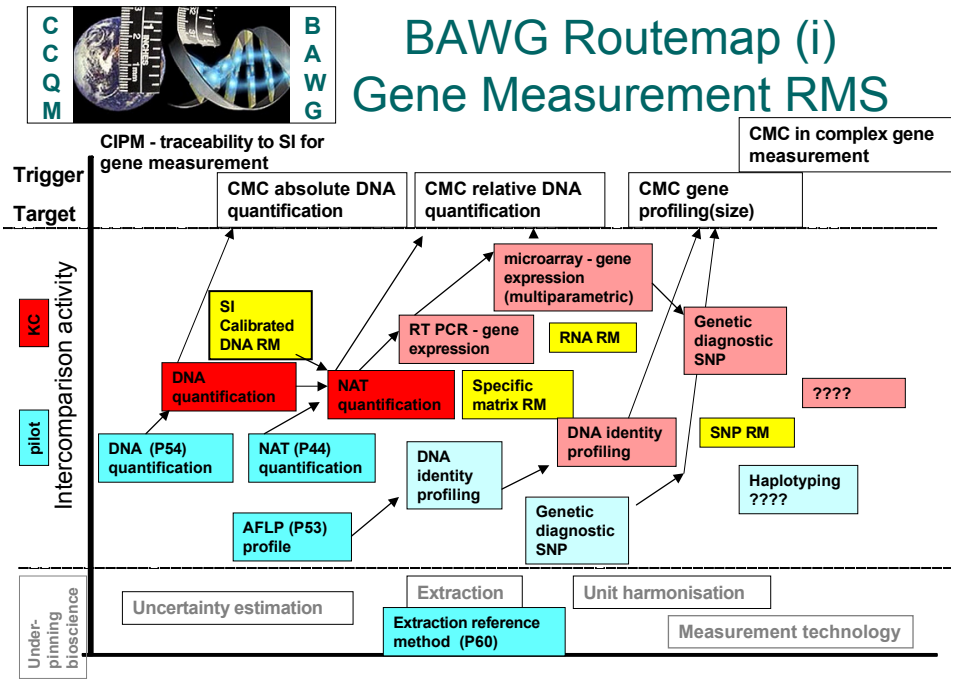
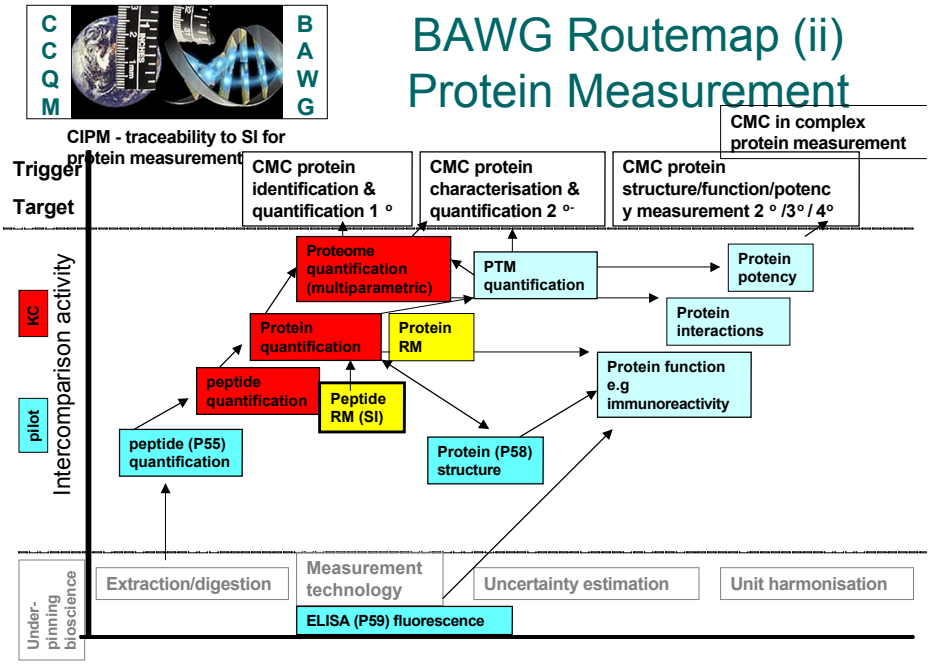


Fig. 12: BAWG Roadmap for Protein Measurement



Notes:

The protein is not inert and the measurand is matrix dependent

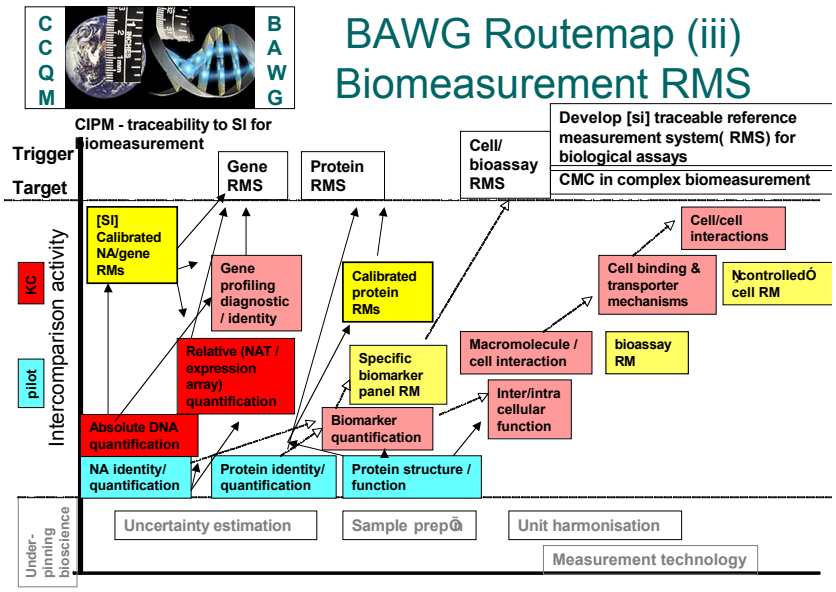
The quantification of total protein activity/function not as simple as the sum of the component amino acids

SI traceability (to the mole) is within reach for absolute quantification, but the traceability of relative/ratio measurements is more difficult to foresee

SI traceability (to the katal) is already established for simple enzyme reactions with Michaelis-Menton kinetics

Structure/function/interaction measurements are key to activity and efficacy determinations; defining the traceability chain is challenging.

Fig. 13: BAWG Bio-measurement Reference Measurement System Roadmap



Notes:

- Many bio-measurements rely on the quantification of a 'panel' of relevant macromolecular biomarkers as a surrogate for the measurand via complex multi-parametric determinations
- Cell based bioassays include determining the chain of events (e.g. receptor binding + signal transduction + gene expression regulation + protein synthesis + modification of metabolic pathway etc.). The definition of the measurand and traceability present major challenges
- Physico/biochem measures of bioassay/cell function in development are key to anchoring traceability (e.g. to gene/protein reference measurement systems)

These roadmaps focus on studies required to underpin key measurement competences and not specific applications, which is in common with the developing strategies of other CCQM Working Groups. With the rapid development of new measurement approaches and technologies in the bioscience sector, reviewing and updating is required on a regular basis. This is in progress (see below). However, the roadmaps currently remain valid tools for the BAWG; the essential building blocks remain, and are still used for informing activity and strategic planning for nucleic acid, protein and cell studies, until the completion of the strategic review, which is covered in the next section.

3.3. BAWG Strategy Review

A major review and discussion of BAWG strategy was covered in the 14th meeting of the CCQM BAWG held during November 2008.

The motivation for this review was to:

- Define the overall direction
- Identify the principles for making decisions as a group
- Define the functional plans
- Identify the gaps
- Identify the stakeholders

It was hoped that a clearly defined strategy would manage the expectations of both the BAWG participants and the BAWG stakeholders.

Discussion was focused on how to develop a strategy that helps to determine the study proposals to undertake. Key elements to take into account were:

- Which principles should be applied?
- The number of interested participants?
- Which CMCs to support?
- The clarity of the measurement problem?
- The maturity of the measurement problem?

Possible frameworks for updating the roadmaps were also discussed:

- Identification of the technical areas that make up the BAWG portfolio
- Enumeration of the measurement challenges
- Identification of the opportunities for BAWG work
- Identification of the critical applications of interest to the BAWG stakeholders
- Description of the measurement process for the application
- Identification of the relevant technical areas in the BAWG portfolio
- Identification of the critical studies and comparisons needed to establish international comparability

Overall it was clear that different NMIs had different drivers for participation in the CCQM BAWG, and therefore had different expectations with respect to studies required. There was a consensus that the studies should support participating NMIs strategies for developing traceability to underpin:

- Stated CMCs
- CRM production
- Method/instrument validation and calibration
- Benchmarking bio-measurement capabilities

Prioritisation should focus on internationally relevant studies.

In order to continue the development and articulation of the BAWG strategy a series of sub-groups were set up in key bio-measurement areas, with nominated individuals co-ordinating and reporting on activity:

1. Nucleic Acids (Kerry Emslie, NMIA)
2. Proteins (David Bunk, NIST)
3. Cells and Tissue (Maria-Paola Sassi, INRIM)
4. Epigenetics (Carole Foy, LGC)
5. Nanobiotechnology (Alex Knight, NPL)
6. Polysaccharides (Chris Jones, NIBSC)

These groups were charged with considering the particular area of bio-measurement:

- Regulatory & other drivers
- Key applications
- Measurement requirements
- Services underpinned
- Identification of the basic building blocks for studies and RMs

The outputs will help identify potential areas for study based on NMIs' strategic and stakeholder requirements, in order to deliver traceability.

A decision making group was also formed to collate the input from these groups and to determine criteria for prioritisation. This ongoing process has been facilitated through emails, a WIKI workspace and focused discussion at BAWG meetings. The discussion below reflects the current 'state of play' with respect to strategy development by each of these groups.

3.3.1. Nucleic Acids

This is the most 'mature' area of activity in the BAWG, and the only area in which a Key Comparison has been undertaken. There are significant regulatory, quality of life and economic drivers for accurate and traceable measurement in this area, particularly in healthcare, diagnostics and food & feed trade. There is very active participation by most BAWG member NMIs that take part in nucleic acid measurement and RM development.

Table 1: Completed, Ongoing and Planned Nucleic Acid Studies

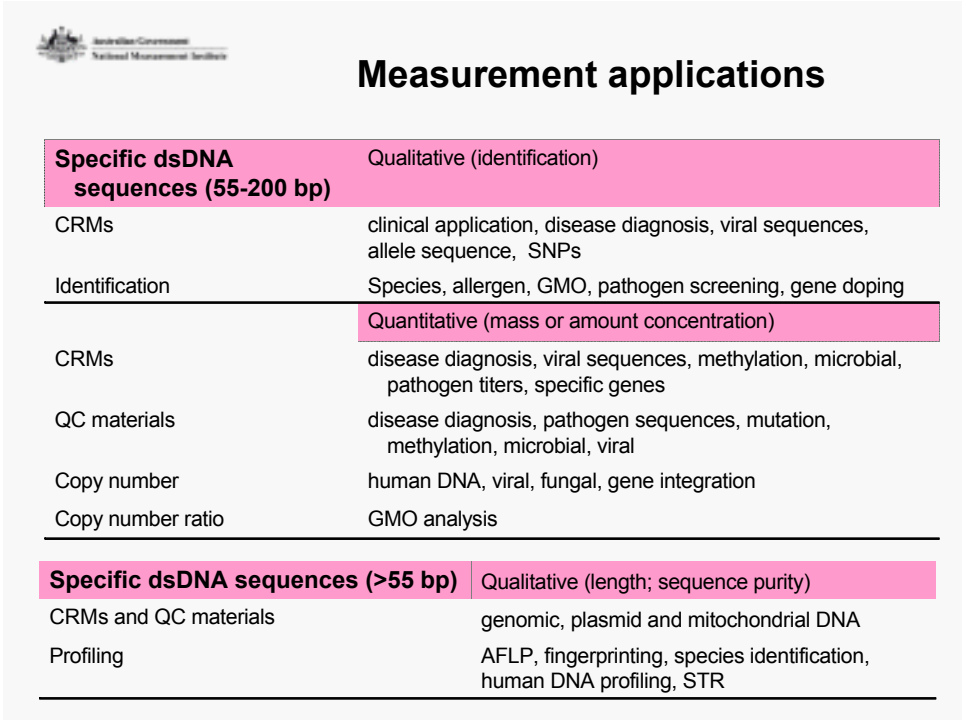
Ref.	Title	Co-ord Labs	Start Year	Status (May 2010)
CCQM-K61	Quantitative PCR	LGC/NIST	2007	Complete, KCDB approved
CCQM-K86	Relative quantification of genomic DNA fragments extracted from a biological tissue	IRMM	2010	Approved, study materials shipping
CCQM-P44	DNA Quantification	LGC/NIST	2002	Complete & reported
CCQM-P44.1	Q-PCR (Repeat)	LGC/NIST	2004	Complete & Reported
CCQM-P53	DNA Profiling	NMIA	2003	Complete & Reported
CCQM-P54	DNA Primary Quantification	LGC	2004	Complete & Reported
CCQM-P54.1	DNA Quantification (Repeat)	LGC	2006	Complete & Reported
CCQM-P60	DNA extraction – Reference Method	IRMM	2004	Complete & Reported
CCQM-P103	Measurement of multiplexed biomarker panel of RNA transcripts	LGC/NIST	2001	Complete, Report in progress
CCQM-P103.1	P103 & multiple transcripts	LGC	2010?	Planned
CCQM-P113	Relative quantification of genomic DNA fragments extracted from a biological tissue	IRMM	2008	Complete & Reported

In developing an ongoing strategy in this area the review group has tried to consider novel applications, emerging technologies ('digital' PCR, next generation sequencing etc.) and the measurement applications of interest to the majority of NMIs.

In furthering this strategy for nucleic acid measurement, determining which measurement building blocks were required in this rapidly evolving area of measurement and which studies would have the most impact, a survey of participants (17 NMI respondents) was carried out and the results analysed (March-April 2010). The survey covered, nucleic acid measurement applications, measurement requirements and technologies employed. A summary of the analysis, given as PowerPoint slides at the CCQM BAWG 17 Meeting (Document BAWG/10-33) by the co-coordinator/reporter of the group (Kerry Emslie, NMIA), is shown in Figs. 14, 15 and 16 below.

They capture the survey feedback with respect to the nucleic acid measurement interests of the BAWG participants, categorised according to the 'measurand' and the area of application.

Fig. 14: Applications for Specific Sequence dsDNA Measurements

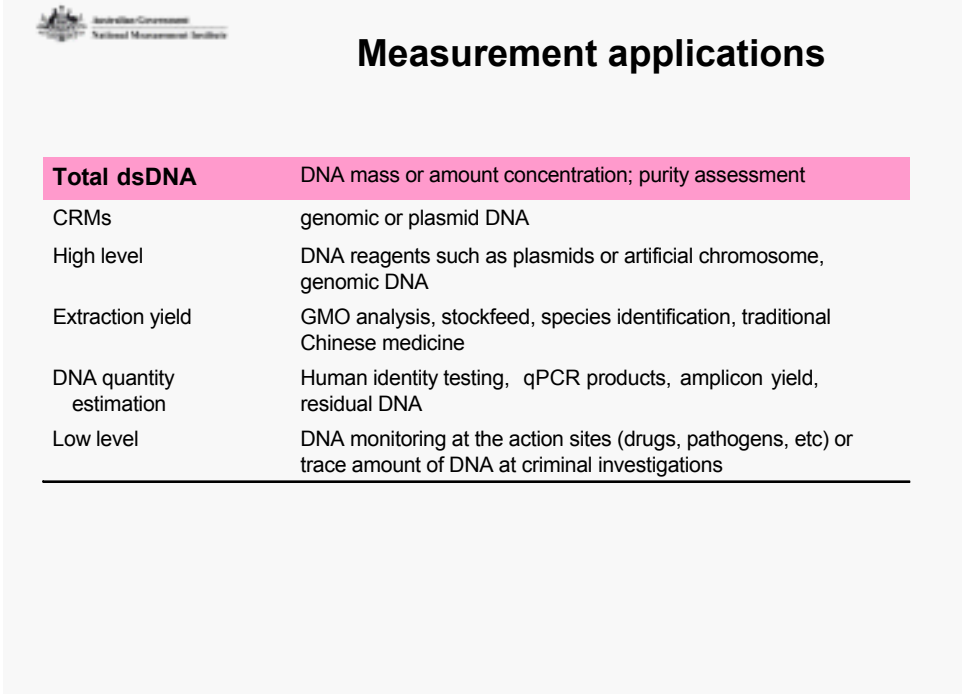


Measurement applications

Specific dsDNA sequences (55-200 bp)	Qualitative (identification)
CRMs	clinical application, disease diagnosis, viral sequences, allele sequence, SNPs
Identification	Species, allergen, GMO, pathogen screening, gene doping
	Quantitative (mass or amount concentration)
CRMs	disease diagnosis, viral sequences, methylation, microbial, pathogen titers, specific genes
QC materials	disease diagnosis, pathogen sequences, mutation, methylation, microbial, viral
Copy number	human DNA, viral, fungal, gene integration
Copy number ratio	GMO analysis

Specific dsDNA sequences (>55 bp)	Qualitative (length; sequence purity)
CRMs and QC materials	genomic, plasmid and mitochondrial DNA
Profiling	AFLP, fingerprinting, species identification, human DNA profiling, STR

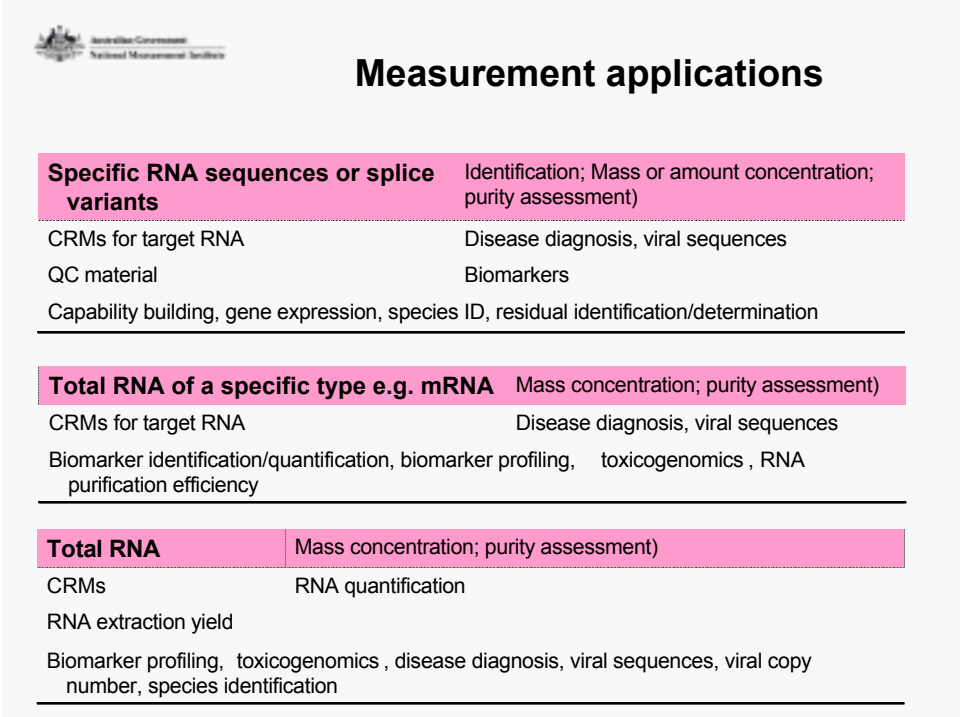
Fig. 15: Applications for 'Total' dsDNA Measurements



Measurement applications

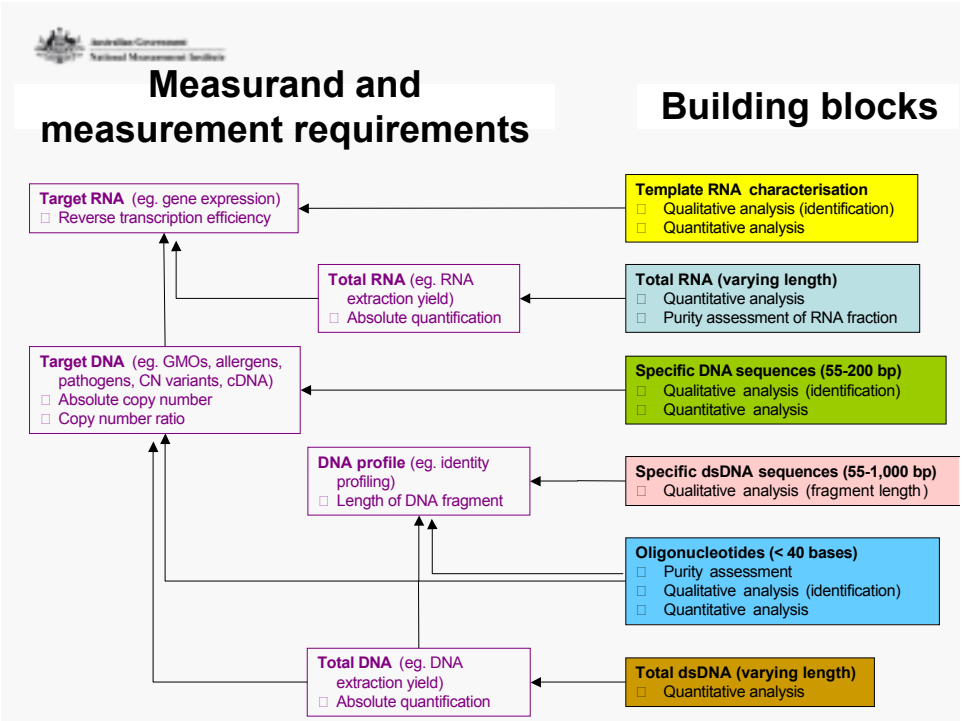
Total dsDNA	DNA mass or amount concentration; purity assessment
CRMs	genomic or plasmid DNA
High level	DNA reagents such as plasmids or artificial chromosome, genomic DNA
Extraction yield	GMO analysis, stockfeed, species identification, traditional Chinese medicine
DNA quantity estimation	Human identity testing, qPCR products, amplicon yield, residual DNA
Low level	DNA monitoring at the action sites (drugs, pathogens, etc) or trace amount of DNA at criminal investigations

Fig. 16: Applications for RNA Measurements



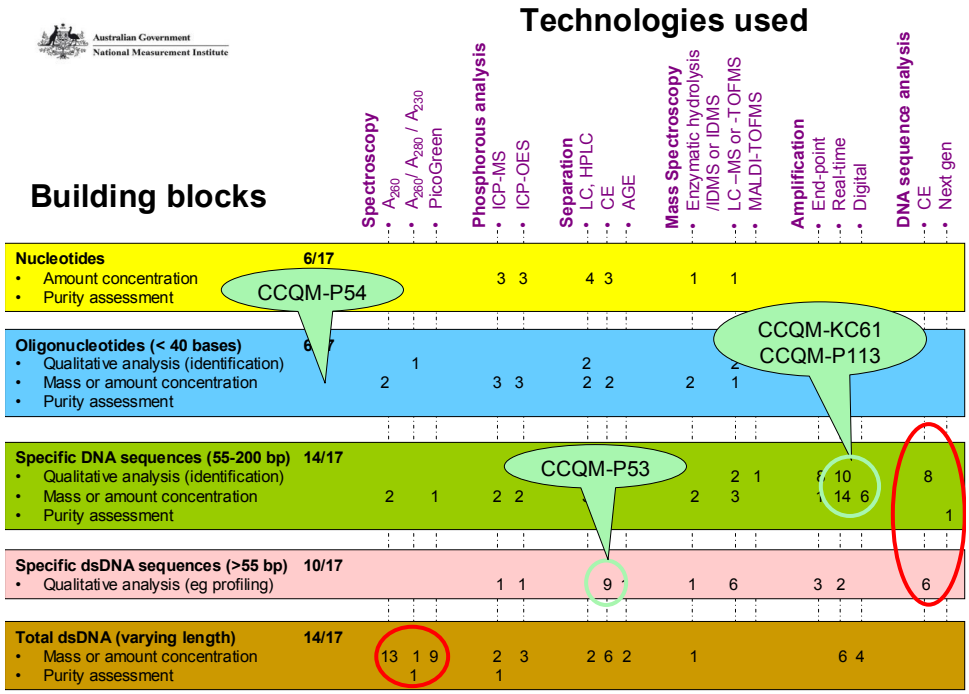
More detail on measurement requirements and route map 'building blocks' of measurement capability were mapped out to identify basic building blocks for studies and RMs, as illustrated in Fig. 17 below.

Fig. 17: Route Map of Nucleic Acid Measurement Capability



In addressing the question "in which areas should new pilot studies be focused?" an analysis of the survey results mapping previous studies onto NA measurement requirements was presented and discussed:

Fig. 18: Analysis of CCQM Studies and NA Measurement Requirements



Using the 17 survey responses, the gap analysis of studies performed (e.g. ringed in red in Fig. 18) mapped against NMI interests and requirements highlighted a couple of significant areas requiring studies to support traceability:

1. Specific DNA sequence measurements:
    a) qualitative (e.g. profiling)
    b) quantitative
        i. identification i.e. “are we measuring the right thing?”
        ii. mass
        iii. purity assessment
2. Total DNA (both ds & ss) measurement
    a) mass or amount concentration
    b) purity assessment

A particular issue was raised with respect to the problems of traceability for nucleic acid measurements; “what corresponds to purity assessment?” In chemical metrology, a key element in determining the ‘primary’ traceability for the amount of substance involves characterising, or at least verifying, the purity of calibration standards. There is not a clear view on what is needed to get a ‘pure’ calibration standard for nucleic acids. It has been proposed that an inter-comparison is required to address the basic problem of characterising an allegedly pure nucleic acid reference material (e.g. nucleic acid sequence) to the extent necessary to get its associated uncertainty down below 1 % or to the required level for calibration.

Studies in support of sequence-based measurement traceability were also thought to be important.

A discussion on studies required in support of NMI’s Calibration & Measurement Capabilities (CMCs) indicated that a very generic approach, based on identified ‘building block’ competences, may not be sufficient to adequately support a CMC e.g. in quantitative PCR. A range of comparisons that cover e.g. quantification of multiple NA targets (both DNA and RNA) from a variety of matrices (blood, tissue, food, environment etc), and at a range of concentrations may be required. This will need to be broken down in to a series of studies that,

when taken together, could potentially support broad CMCs. Examples include viral load measurement in a clinical sample; bacterial quantitation in an environmental sample; BCR-ABL (a leukaemia fusion transcript) quantification in blood etc.

Looking to emerging measurement requirements, the accuracy and comparability of next generation sequencing and structural genomics measurements such as rearrangements, duplications, loss and gain of sequences etc. were also identified as being of potential interest.

3.3.2. Proteins

Activities and studies in protein measurement have been on the BAWG agenda for several years. To date the peptide and protein studies carried out have to some degree represented the two ends of the metrology spectrum:

1. Amount of substance determinations, developing from small molecule metrology approaches – deriving traceability
2. Method comparability/validation studies – with limited metrological rigour

Table 2: Completed, Ongoing and Planned CCQM Protein Studies

Study	Title	Co-ord. labs	Start date	Status (May 2010)
CCQM-P55	Peptide/protein quantification	LGC/PTB /NIST	2004	Complete-reported
CCQM-P55.1	Peptide / protein quantification (repeat)	LGC/PTB /NIST	2008	Sample distribution
CCQM-P58	Fluorescence in ELISA	NPL/NIST	2004	Complete-reported
CCQM-P58.1	Fluorescence in ELISA (Stage 2)	NPL/NIST	2007	Sample preparation
CCQM-P59	Protein structural measurements by CD	NPL	2006	Complete-reported
CCQM-P59.1	Protein structural measurements by CD (repeat)	NPL	2008	Complete-reported
CCQM-P101	Glycan Species measurement in digested glycoprotein mixture	NIBSC /USP/NPL	2007	Complete – report in progress
	Peptide mapping / profiling for impurity measurement in complex biopharmaceuticals	NIBSC/ LGC	2010/11	Planned
	Amylase activity measurement	NIMC	2010/11	Planned
	hGH quantification	LGC/PTB/ IRMM/NPL	2011/12	Planned
	CRP quantification	LGC/PTB/ IRMM/NPL	2011/12	Planned

Protein Measurement Applications

The BAWG strategy aims to carry out metrologically valid studies demonstrating traceability of relevance and value to key stakeholders, particularly in the healthcare community, the clinical and biopharmaceutical sectors. There are key regulatory and stakeholder drivers for measurement comparability in this area e.g. FDA, JCTLM, IFCC, ICH and EMEA. Most NMIs have interests across different sectoral applications including:

- Biopharmaceutical impurities
- Cellular proteomics
- Food allergens
- Nanotoxicity
- GM crop research
- Cultivar/species identification/profiling
- Biomarker discovery
- Peptide and Protein CRMs for clinical diagnostics

In an initial survey of BAWG participants for interests and requirements in protein measurement (October-November 2009) NMIs (10 NMI respondents) reported the above sectoral applications were of interest to stakeholders for protein services.

A number of different technologies were applied for protein measurement including:

- MS (MS/MS of peptides; MS of intact protein) – 9/10
- Immunoassay (uniplexed and multiplexed) blotting – 6/10
- Flow cytometry – 2/10
- Microfluidics (Bioanalyser) – 2/10
- Affinity separations – 3/10
- Gel electrophoresis (1D, 2D, Western) – 5/10

Protein Measurement Requirements

On the basis of the discussion and review of the initial survey, the protein survey template was modified to try and identify common measurement capabilities/building blocks required for the key properties of proteins that are subject to measurement. The preliminary responses (May 2010) give a more detailed indication of the types of studies required to support NMI protein comparability and are summarised in Table 3:

Table 3: Protein Measurement Requirements

Measurement Type	Application	Fundamental Measurement	Measurement Approach
Qualitative: Protein Identification	CRMs for proteomics	Identification of proteins in a complex mixture	Proteolysis + LC-MS/MS; multiplexed immunoassay
	Process and product related impurities in biopharmaceuticals	Identification of proteins in a complex mixture	Proteolysis + LC-MS/MS; multiplexed immunoassay; (m)LC-MS of intact proteins

	Cellular proteomics	Identification of proteins in a complex mixture	Proteolysis + LC-MS/MS; multiplexed immunoassay; flow cytometry
	Allergenic proteins in food	identification of proteins in a complex mixture	proteolysis + LC-MS/MS; multiplexed immunoassay
	Post-translational modifications (glycan + glycosilation + deamidation + oxidation + others)	Identification of proteins in complex matrices	Gel-based detection, LC-MS, LC-MS/MS, MALDI-MS/MS
Qualitative: Structural Characterization	3D protein structure characterization	Evaluate conformation of a single, purified protein	CD; FT-IR; NMR; hydrogen/deuterium exchange LC-MS/MS
	Glycan analysis of biopharmaceuticals	Evaluate protein/glycan structure of a single, purified protein	Affinity separation + mass spectrometry, fluorescence LC /MS, multiplexed bead arrays (affinity)
	Post-translational modification (non-glycan) of biopharmaceuticals	Evaluate protein structure of a single, purified protein	Proteolysis + LC-MS/MS, multiplexed bead arrays (affinity)
	Glycan analysis of clinically-relevant proteins	Evaluate protein/glycan structure of a single, purified protein	Affinity separation + mass spectrometry, fluorescence LC /MS, multiplexed bead arrays (affinity)
	3D protein structure characterization	Evaluation of the conformation of single, purified protein	Hydrogen/deuterium exchange with LC-MS/MS or CE-MS
	Separation of folding states	Evaluation of the conformation of single, purified protein	Liquid chromatography approaches (SCX, SEC); CE
	Glycan analysis	Structural characterisation of purified glycans and glycan analysis on a single purified protein	LC-MS/MS; CE-MS
Quantitative: Amount of Substance Determination	CRMs of clinically-relevant proteins	Quantify protein(s) in a complex mixture	Proteolysis + LC-MS/MS; immunoassay, amino acid analysis
	Process and product related impurities in biopharmaceuticals	Quantify protein(s) in a complex mixture	Proteolysis + LC-MS/MS; (multiplexed) immunoassay; Western blotting
	Quantification of post-translational modifications (glycan + others)	Quantify relative amount of modification relative to unmodified	Proteolysis + LC-MS/MS; (multiplexed) immunoassay; Western blotting

	Cellular proteomics	Quantify protein(s) in a complex mixture	Proteolysis + LC-MS/MS; nanofluidics; flow cytometry
	Allergenic proteins in food	Quantify protein(s) in a complex mixture	Proteolysis + LC-MS/MS; (multiplexed) immunoassay
	Nanotoxicity	Quantify protein(s) in a complex mixture	Proteolysis + LC-MS/MS; (multiplexed) immunoassay
	Traceable peptide quantification	Assigning mass fraction of "purified" specific peptide in a buffer solution	Hydrolysis + GC-MS/MS
	Traceable protein quantification	Assigning mass fraction of "purified" specific protein in a buffer solution	Proteolysis + LC-MS/MS; (uniplexed and multiplexed) immunoassays; flow cytometry
	Relative protein quantification (biopharmaceuticals and proteomics)	Assigning relative differences in protein concentrations	Proteolysis + LC-MS/MS (iTRAQ, ICAT, MeCAT); LC-ICP-MS
	Traceable quantification of proteins in biological fluids	Quantification of Protein(s) mass fraction in a complex matrix	Immunoaffinity or SEC or SCX +LC-MS/MS;
	QC materials for proteomics	Quantification of Protein(s) mass fraction in a protein mixture	Proteolysis + LC-MS/MS; (uniplexed and multiplexed) immunoassay
	Cellular proteomics	Quantification of Protein(s) mass fraction in a protein mixture	Proteolysis + LC-MS/MS; (uniplexed and multiplexed) immunoassays; flow cytometry
	Metalloprotein speciation	Quantification of Protein(s) mass fraction in a protein mixture	Proteolysis + LC-MS/MS; LC-ICP-MS
Quantitative: Avidity or Activity Determination	Antibody characterization	Evaluate binding characteristics of protein:antigen complex	Proteolysis + LC-MS/MS; SPR; Western blotting; flow cytometry; multiplexed bead arrays

Gaps & Potential Study Requirements Identified to Date

In discussing the initial survey responses, a number of issues were debated:

- Should the group consider metrology for identification/qualitative measurements?
- Should the group concentrate on purity assessments and profiling?
- Although there is a focus on mass spectrometry-based protein measurements to deliver traceable services by the majority of NMIs, it was agreed that, in common with the nucleic acids group, the group should not base strategy on technologies but on type of measurement (property) required, e.g. purity of proteins; protein interactions; 'intactness' of proteins; degree of oligomerisation or

aggregation; structural determination (not necessarily just secondary structure); problems of matrix effects.

- There was recognition that 'amount of substance' determination for proteins was only a starting point, and that establishing traceable measurement for other 'properties' would be very challenging, but a clear stakeholder requirement, particularly for the clinical, biopharmaceutical, and diagnostic sectors. However, progress is already being made by participating NMIs on establishing traceable structural measurement using CD (CCQM-P59) and novel mass spectrometry (H-Deuterium Exchange and Ion Mobility MS) approaches.

Strategic discussion on protein measurement building blocks, and the CCQM-P55 study, have identified that a further clear requirement to underpin peptide and protein measurement studies is needed for well characterised, traceable pure amino acids. The group has had some discussion of the relevance of mass balance measurements for peptide purity as compared to amino acid/MS measurements. The latter approach has been preferentially adopted by the majority of BAWG participants to date, particularly where sample size has been limited, which is typically the case for biopharmaceutical and clinical measurements. Studies which demonstrated comparability with emerging calibrants were thought to be of significant value.

Whilst much of the protein measurement work is research based, with a longer potential time horizon for CMCs, there is an immediate need for a comparison on the value assignment of a clinically relevant protein target in serum – but value assigned on the basis of the clinically relevant 'property'. The suggested studies on human growth hormone and C-reactive protein represent such comparisons.

3.3.3. Cells & Tissues

There is now a very significant cell biology expertise and interest among the BAWG NMI and expert laboratory participants. A very active strategy sub group in this area has concentrated on a structured exploration from a metrology perspective of what could constitute 'traceable' cell measurement.

There are a number of significant sectoral drivers for comparable cell-based measurements:

- Biopharmaceutical industry
- Medical diagnosis
- Pharmacopeia
- Regenerative medicine
- Immuno response
- Foods (microbiology)
- Fundamental research

The variability in cell measurements and the lack of standards and measurement uncertainty determinations in the area have been highlighted as concerns, particularly by the developing biopharmaceutical and regenerative medicine industries in demonstrating product quality, safety and efficacy in regulatory submissions (see Section 5.5.3 and the NIST and NMS bioscience roadmaps[6, 21]).

The strategy review by participating NMIs has identified the most significant measurement requirements as:

- Cell quantification
- Cell authentication
- Cell sub-populations analysis
- Cells viability/proliferation
- Viability/Apoptosis ratio
- Cell differentiation
- Cell lineage
- External matrix characterization in function of the cells response
- Tissues metrology requirements

- Microbiology requirements

The particular measurement services requested of NMIs related to:

1. Quantitation of cells in suspension/cells adhered
2. 3D measurements of cells and tissue

The group identified important capabilities (underpinned by RMs) in cell measurement, as outlined in a strategy presentation update at CCQM BAWG 17 (Document: BAWG/10-34) illustrated in Table 4 below:

Table 4: Measurement Methods and Calibration Standards for Cells

Measurement Method	Calibration Standard
Fluorescent Microscopies	Fluorescent Standards
Non-labelled Microscopies	Spectroscopic Standards Nano-microstructured Standards
Flow Cytometry	Bead Standards
Colorimetric Methods	Optical Standards

Potential Study Requirements Highlighted to Date

The group has taken a very pragmatic approach to cell measurement studies and in addressing the issues of measurement traceability. In line with the strategy, the initial studies will focus on very basic quantitative cell measurements, in order to establish the capabilities of the NMIs in this field. Relevant quantitative measurements include: quantifying cells according to cell density, geometry and population distribution; specific phenotype; vital cells. Initial studies identified are:

- CCQM-P102 'Quantification of Cells with Specific Phenotype Characteristics' (Document BAWG/10-22)
- CCQM-P123 'Number and Geometric Property of Cells Adhered on a Solid Substrate' (Document BAWG/10-25, 26)

Table 5: Ongoing Cell Studies

Study	Title	Co-ord labs	Start date	Status (May 2010)
CCQM-P102	Quantification of cells with specific phenotypic characteristics (flow)	NIBSC/PTB /NIST	2004	Sample distribution
CCQM-P123	Cell quantification on solid substrate	INRIM/LGC /NIST	2010	Sample preparation

3.3.4. Epigenetics

Epigenetics is an emerging scientific field. An epigenetic trait is a stable heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence. Chromatin (DNA and the protein histones around which it is wrapped), is the template for all eukaryotic genetic information. Epigenetics involves modifications (e.g. methylation) which impact genome structure, regulate gene activity and affect gene

expression but do not alter the base sequence of DNA and therefore cannot be measured/detected by simple nucleic acid measurements.

A growing body of evidence suggests that a wide variety of illnesses and behaviours may be linked to such epigenetic mechanisms. These include various cancers, cognitive dysfunction, and autoimmune diseases. Researchers are exploring drug therapies to change the epigenetic profiles of cancer cells and are developing novel diagnostics based on epigenetic profiles.

There is an increased focus on the importance of bio-measurements in this area, as distinct from nucleic acid measurement, hence the consideration in the developing BAWG strategy.

Epigenomic factors which could be considered within BAWG include:

- DNA modification (e.g. methylation)
- Histone modification (e.g. methylation, phosphorylation and acetylation)
- Non-coding RNAs
- Non-histone DNA binding proteins

A preliminary consideration of the types of study to underpin capabilities in epigenetic measurements discussed in a strategy session (Document BAWG/09-35) has identified the following potential approaches and detection technologies:

Approaches

- Global or Genome-wide: CCQM-P94 Quantification of DNA Methylation
- Gene Specific: CCQM-P94.1 Quantification of DNA Methylation
- Site Specific

Detection Technologies

- miRNA
    - Profiling – arrays and sequencing
    - Quantification – real-time PCR, 'digital' PCR
- DNA Methylation
    - Microarrays
    - Bisulfite conversion followed by PCR
    - Bisulfite sequencing
    - Antibody detection
    - DNAmethyltransferase assays
    - Methylation sensitive restriction digestion followed by PCR
    - Mass spectrometry
    - HPLC and CE
- Protein Binding and Modification
    - Chromatin Immunoprecipitation (ChIP) – on-chip and sequencing
    - Mass spectrometry
    - HPLC and CE
    - Immunoassays
    - FISH

Preliminary thinking on potential studies required to support the accuracy and traceability of epigenetic measurements has highlighted the following issues which would need to be considered in BAWG study design:

- Lack of well characterised reference materials to support studies:
    - Whole genome DNA methylation studies
    - Gene specific DNA methylation studies
    - Site specific DNA methylation studies
- Relative merits of synthetic vs. genomic reference materials:
    - Lack of robust and accurate methods to characterise either material
- Many technical challenges and significant contributions to measurement uncertainty and study material stability which would need to be identified and controlled e.g.
    - Incomplete methylation of DNA controls
    - Incomplete bisulfite conversion of non-methylated DNA
    - PCR amplification bias of un-methylated sequences (richer in A/T bases)

Table 6: Completed and Ongoing Epigenetic Studies

Study	Title	Co-ord labs	Start date	Status (May 2010)
CCQM-P94	Quantification of DNA methylation	KRISS	2006	Compete-Reported
CCQM-P94.1	Quantification of DNA methylation	KRISS	2008	Results submission

The ongoing CCQM-P94/94.1 pilot study series on quantification of DNA methylation has further highlighted the difficulties and issues in designing suitable studies in this area. For example there are both qualitative (is gene x methylated or non-methylated in a particular sample?) and quantitative considerations (what is the level of methylation of gene x in a cancer tissue vs. a "normal" tissue?) - absolute or relative.

Challenges include:

- What is the measurand?
- Methylated cytosine to non-methylated cytosine ratio, (or cytosine to thymine ratio after bisulfite conversion)?
- Methylated gene sequence to non methylated gene sequence ratio (i.e. species/haplotype measurements)?
- As not all methylated genes are fully methylated what are the criteria for calling 'methylated' or 'non-methylated' species?

Although this group has made an initial assessment of needs in this area, a full survey of NMI requirements to support epigenetic measurement capabilities has not yet been carried out, and the issues raised by the initial study in this area has indicated a cautious approach to the design of studies will be required.

3.3.5. Nanobiotechnology

As progress in the manufacture and characterization of nanoscale materials continues to accelerate, a growing list of stakeholder needs has arisen, including specifications and tests needed to support nanoscale measurement and characterisation as well as how this new technology will impact health, safety and the environment.

From the NMI perspective, the most significant current driver for inclusion of nanobiotechnology within the scope of BAWG activity is ISO TC 229 which was formed in mid-2005 to progress standardisation in the field of nanotechnology and develop standards for terminology and nomenclature; measurement and instrumentation,

including specifications for reference materials; test methodologies; modelling and simulation; science-based health, safety, and environmental practice.

The BAWG strategy in this area is still very much in development and there has not as yet been a survey of NMI requirements. The discussion to date has been primarily focused on exploration of the bio-measurement issues, particularly with respect to nanoparticles. However, two recent workshops have contributed to BAWG strategic thinking in this area:

1. The CCQM BAWG held a joint discussion meeting with the CCQM SAWG (November 2009) to highlight some of the measurement research being carried out at NMIs at the bio/surface interface, including bionanotechnology. For example: nanotoxicology measurements, biomolecular immobilisation, molecular imaging measurements
2. The strategy leaders of the BAWG bionanotechnology strategy group chaired the bionanotechnology session in a recent CCQM Metrology for Nanotechnology workshop (February 2010). Key issues were discussed in this session which highlighted measurement and standards issues and requirements in this area, including:
    - Physicochemical characterisation of nanoparticles is critical to the field. Poorly characterised nanoparticles will give rise to artefacts when used in biological assays. This has been seen several times in the literature.
    - There is a need to characterise nanoparticles in biological media, for example cell culture medium, blood plasma, etc. This is relevant for nanotoxicology and *in vitro* assays as well as for nanomedicine (e.g. drug delivery). For example, do the nanoparticles aggregate in such media?
    - The characterisation, quantification and identification of proteins and other biomolecules bound to nanoparticles is important for understanding their interactions with, and effects, on biological systems.
    - There is a need for reference materials; the OECD stewardship programme is a useful start, but is not focused specifically on the bionano area. For example, positive and negative controls for nanotoxicology would be extremely useful.
    - There is a need for international coordination and comparisons on nanotoxicology - e.g. the development of *in vitro* cell-based assays.
    - Discussion has focused too much on nanotoxicology and seeing nanoparticles as a problem; there should be more emphasis on the useful applications of nanoparticles. However, many of the same measurement challenges are important in both.

3.3.6. Polysaccharides

There has been no significant activity in this area. A preliminary consideration of possible measurement applications undertaken by the review team leader identified a number of measurements based on monosaccharides etc. which lie more within the scope of the organic analysis group. Other measurements identified e.g. glycan/glycoprotein have been incorporated into the protein measurement area. No specific studies have, as yet, been undertaken or identified in this area.

4. Interviews with Measurement Institutes

4.1. Introduction

A total of 22 National Measurement Institutes and other measurement organisations that had activities in bio-measurement were contacted for the Study with the aim of determining the need for measurement services to support the biosciences, the international comparisons needed and the measurement R&D required to support a global infrastructure for bio-measurement. Of those institutes contacted, 20 agreed to participate, representing the majority of NMIs that have a bio-measurement programme. Also included in this section on Measurement Institutes was the IFCC in its practical role on measurement harmonisation. The interviews were undertaken either through 'face-to-face' meetings or via teleconferences. Details of those interviewed are given in Annex I.

Interview forms were developed to provide a framework for the sessions and to help with the capture and analysis of the information provided. The forms are shown in Annex II. Due to the nature of the interviews and the limited time available to carry them out, not all the questions were answered by every institute. Nevertheless, the quality and quantity of the information collected enabled a good, broad picture of the profile, activities and needs of the institutes.

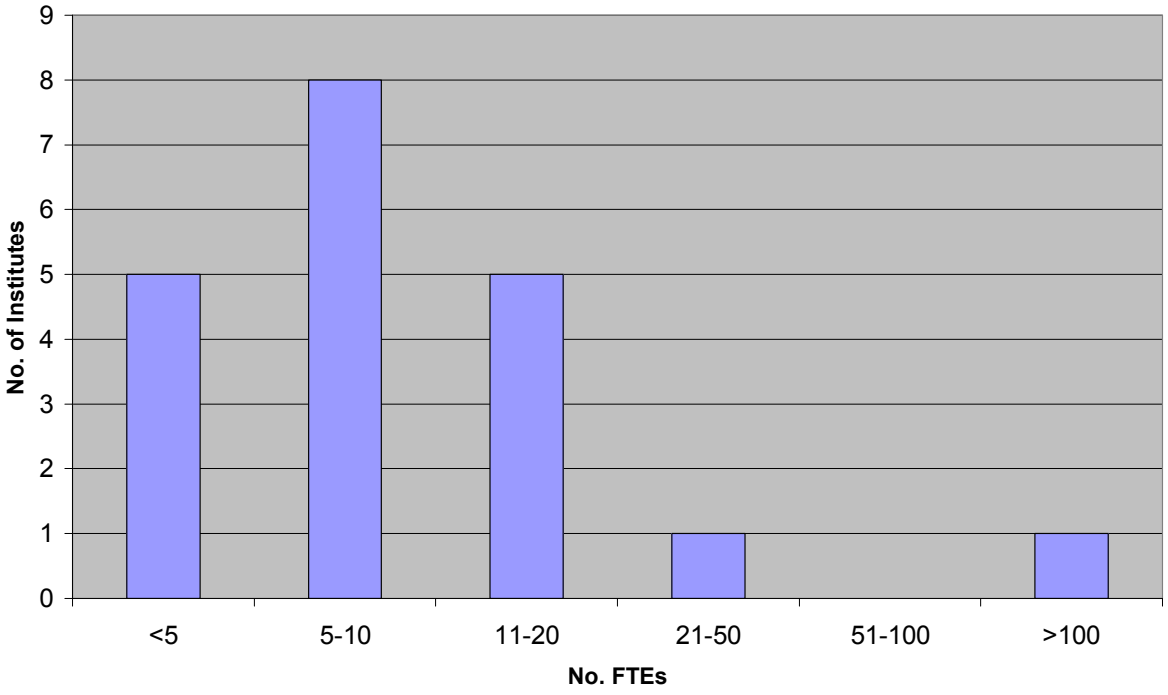
The first part of the questionnaire sought to establish a profile on the types of measurement being undertaken, the sectors supported and the type of service provided. There were also questions concerning the amount of effort involved, the length of time the institute had been involved in bio-measurement activities and the anticipated growth of their work. The second section of the form probed the level of an institutes activities with the BIPM's Bioanalysis Working Group (BAWG) and their requirements for studies to support the Calibration and Measurement Capabilities (CMCs) they wish to claim for Appendix C of the CIPM Mutual Recognition Arrangement (MRA). Later sections probed activities relating to protein and nucleic acid measurements and the future measurement services which will need to be developed.

4.2. Profile of the Institutes

4.2.1. Staff, Time and Growth

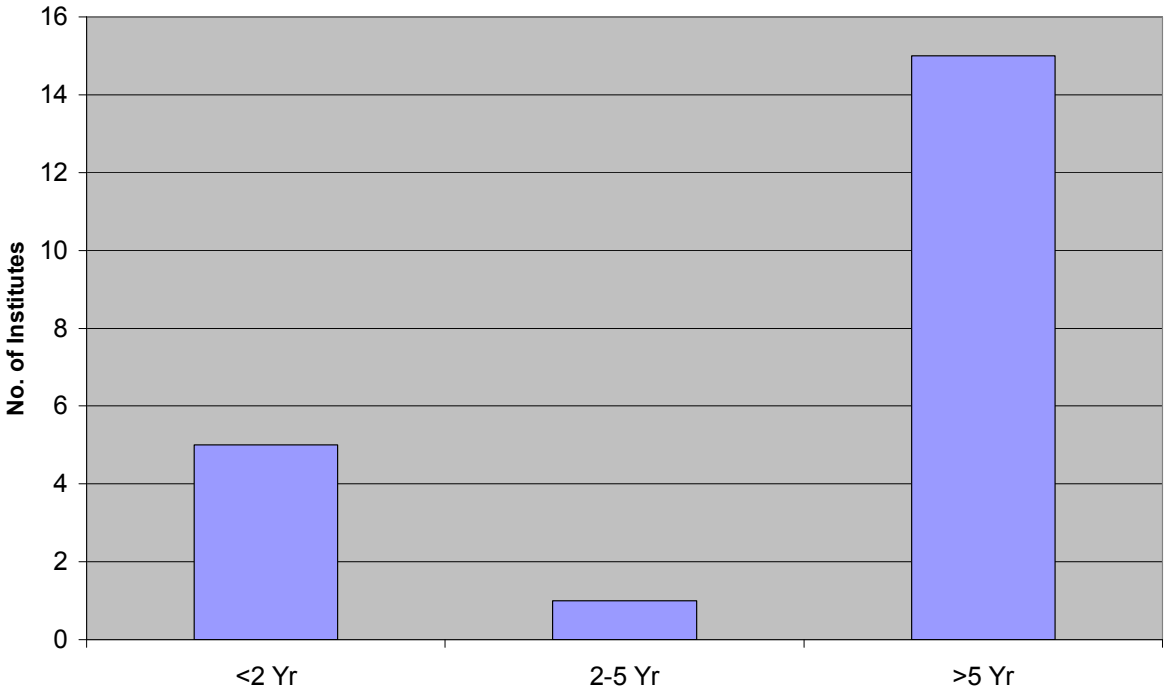
Profiles of the number of full time equivalents staff (FTEs), the length of time that the institute had been involved in bio-measurements and the anticipated growth in their bio-metrology activities obtained from the interviews are summarised in Figs. 19 - 21.

Fig. 19: Staff Equivalents for Bio-Metrology



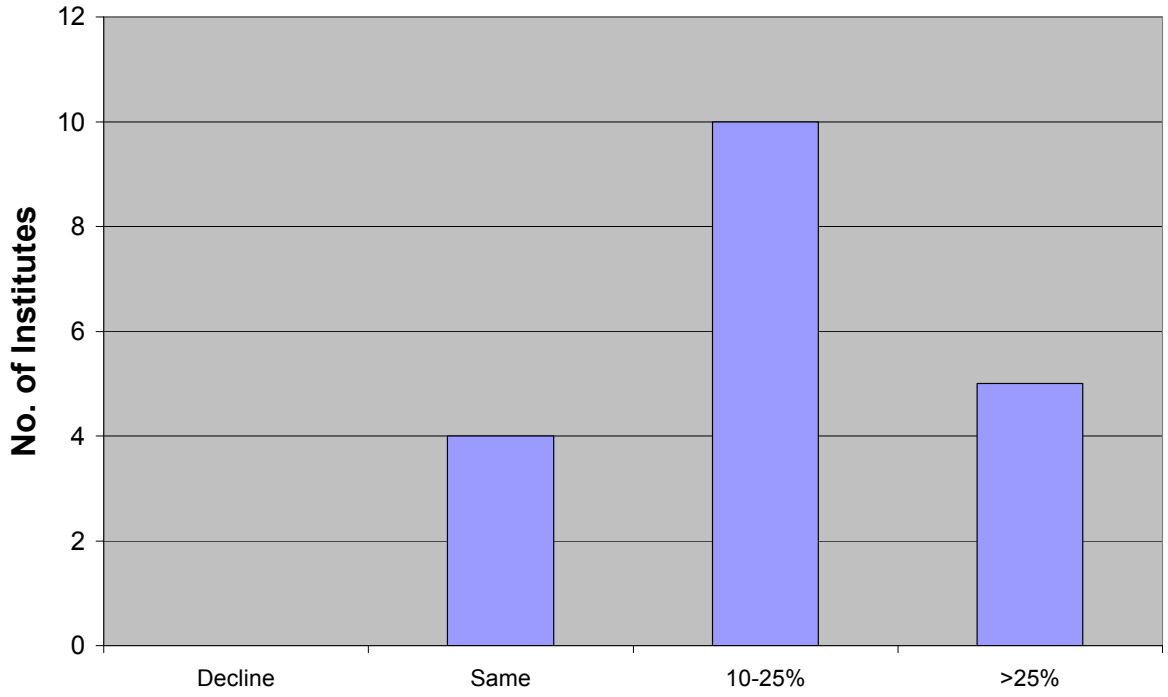
As can be seen from Fig. 19, most institutes have a relatively modest number of staff assigned directly to bio-metrology activities. NIST was the only institute to have large numbers of staff working in bio-metrology, with over 100 full-time staff dedicated to the field.

Fig. 20: Time of Involvement in Bio-Measurement



The majority of institutes have well-established bio-measurement programmes, although there are several which have only recently embarked on activities in this field.

Fig. 21: Growth in Bio-measurement Activities



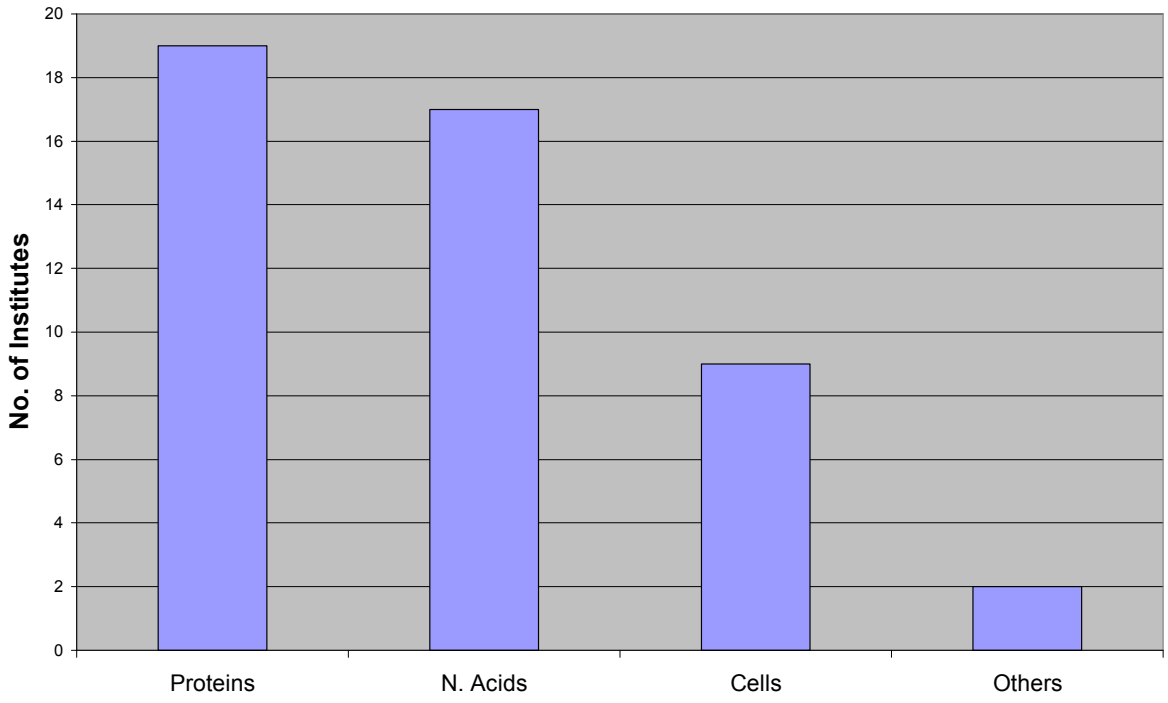
Most institutes anticipate growth in their bio-measurement activities over the next few years. However, some foresee little growth, primarily because of the pressure on government finances. Even for those where government funding may be restricted, some of the institutes affected are still looking to grow by expanding their metrological service offerings to the healthcare and biotechnology sectors. Drivers cited for the growth were:

- New national and international regulations in healthcare such as: the introduction of the EU IVDD Directive for diagnostics and ISO15189 for clinical labs
- The growing demands from the diagnostics industry for higher order reference standards and value assignments
- Support for the growing biopharmaceutical industry
- Demand for measurements relating to food safety, including GMO and allergens detection and measurement
- Other reasons were: work to support forensics, regenerative medicine and to address nanotoxicity

4.3. Type of Work and Sectors

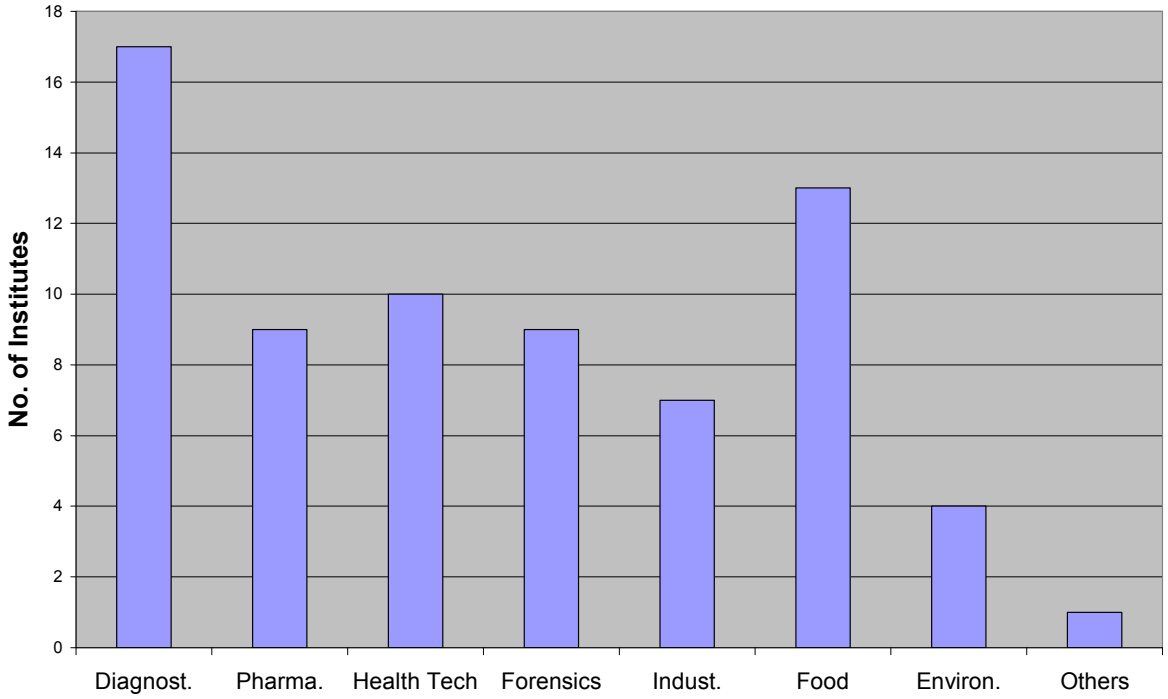
Fig. 22 shows the distribution of the main type of work being undertaken by the NMIs and Fig. 23 the sectors to which the work relates.

Fig. 22: Main Type of Analysis Undertaken by the NMIs



Of the 21 institutes interviewed, 19 had significant work programmes on protein analysis and 17 were involved in nucleic acid analysis. Work on cells was less widespread, with less than half of the institutes having significant activity in this field.

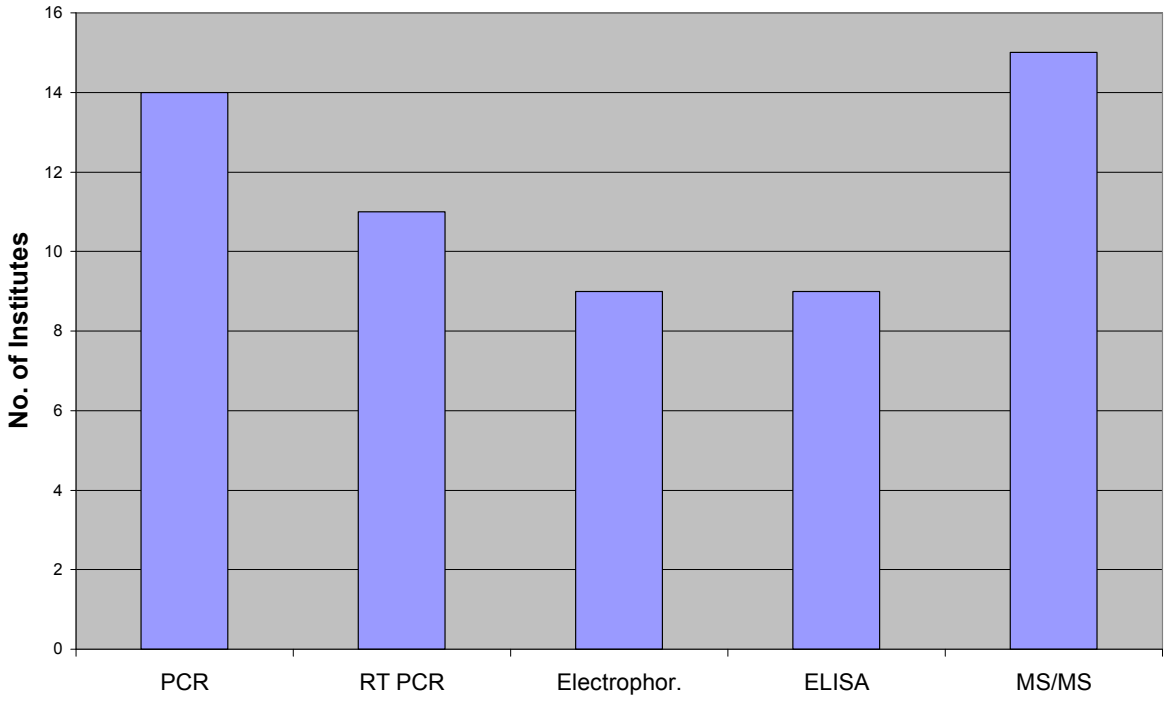
Fig. 23: Target Sectors for the Work



Work for diagnostics and clinical chemistry was the most popular sector, with the vast majority of institutes undertaking work for this area. Other areas of healthcare, notably pharmaceuticals and health technologies have reasonable support, but are not as prominent as diagnostics. Food is also well represented, with over half the institutes supporting this sector and forensics is also reasonably-well represented. In contrast there are few institutes undertaking work directly for the environmental sector.

A wide range of technologies are used by the institutes to perform the work. Fig. 24 charts the most popular.

Fig. 24: Most Common Analytical Technologies

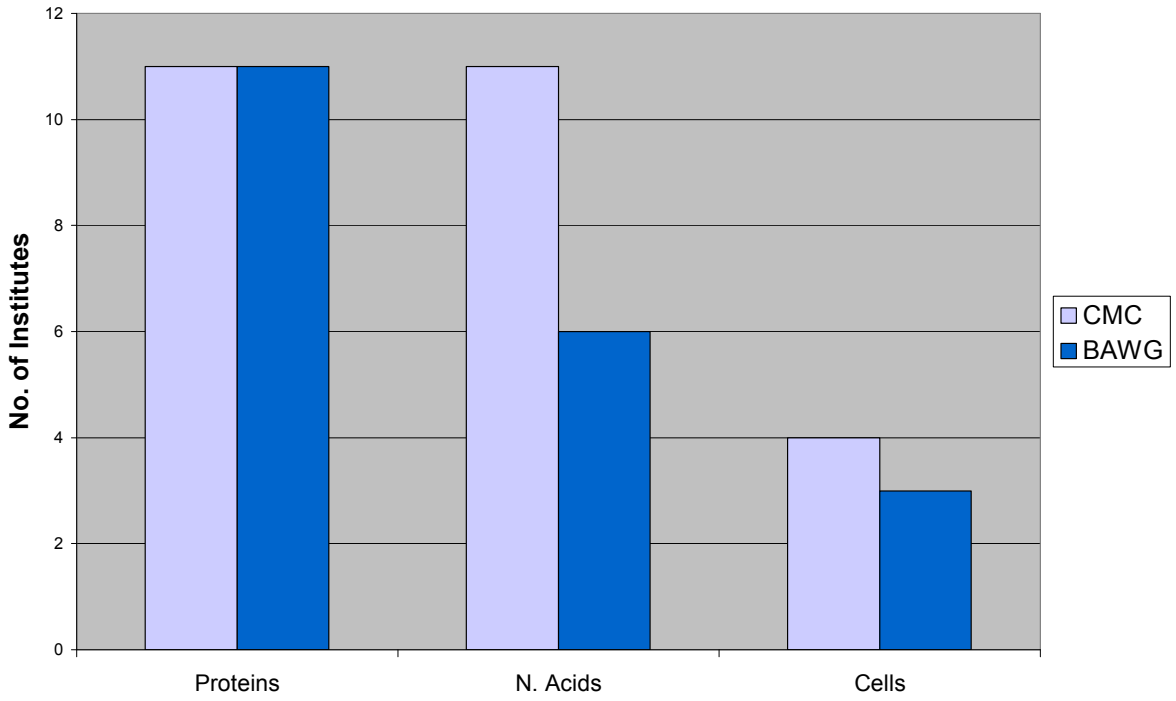


The main outlet channels for the institutes' work are reference materials, reference methods and publications. Very few institutes undertook calibrations to support bio-measurements.

4.4. CMCs and BAWG Studies

Fig. 25 summarises the main areas of bio-measurements where the NMIs aim to have 'Calibration and Measurement Capabilities' (CMCs) listed in Appendix C of the CIPM MRA and areas where they would envisage leading a BAWG study.

Fig. 25: Types of CMC and Supporting Study



In line with the findings in Fig. 22, both proteins and nucleic acids were the dominant areas for measurement claims, although nucleic acids were not as popular in terms of the number of institutes considering leading BAWG studies.

Suggestions for future BAWG studies included:

- Proteins: high accuracy quantitation, peptide mapping, amino acid purity, amino acid quantification, protein interactions, ELISA measurements, activity, protein heterogeneity
- Nucleic Acids: DNA mass concentration, DNA copy number, RNA copy number, DNA sequence, fraction of methylated cytosine
- Cells: flow cytometry, cell counting, cell FACS analysis

4.5. Key Measurements

In commissioning the Study, the BIPM requested a focus, based on previous reports on NMI activities (Document CCQM/06-41) on Healthcare, particularly diagnostics and therapeutics involving protein or nucleic acid analysis. Therefore, the questions at the core of the interviews were concentrated in these areas. They were designed to determine the key measurements undertaken by the NMIs, the main measurement services provided and required by the NMIs and their views on future requirements. This section summarises those measurements, which were of primary interest to the institutes in order to support their reference material and reference method development.

4.5.1. Proteins

Many of the NMIs highlighted their work on identification and quantitation of proteins relevant to diagnostics and clinical chemistry. In most cases, guidance on the candidate requirements for diagnostics was taken from priorities identified by the IFCC, or national associations of clinical chemists.

Specific proteins highlighted by the institutes in the interviews are listed in Table 7, although this represents a selection, rather than an exhaustive list of all proposed activities.

Table 7: Protein Measurements for Diagnostics and Therapeutics

Area	Analyte
Diagnostics	
Cancer Markers	CA 19-9, CA 125, Prostate Specific Antigen, Carcinoembryonic Antigen
Cardiac Markers	Brain Naturietic Peptide (BNP), pro-BNP, Atrial Naturietic Peptide (ANP), Troponins I & T, Creatine Kinase, Apolipoprotein B100
Immunological	Immunoglobulins A, E, G & M
Diabetes	Hb1Ac, Insulin, Glucagon, Glucose Oxidase, Albumin in Urine
Blood Coagulation	Coagulation Factors II, III, V, VII, VIII, IX, X; Activated Partial Thromboplastin
Endocrine	Thyroid Stimulating Hormone, Angiotensin, Somatotropin
Kidney	Cystatin C
Liver	Alanine Aminotransferase, Aspartate Aminotransferase, γ-Glutamyltransferase
Viral	Anti-Hepatitis B Core Antigen, HIV-2 Antibody, H1N1 Antibody
Bacterial/Infection	Anti-Meningococcal Serotype MAB, Antistreptolysin, C-Reactive Protein
Blood Serum	Serum Albumin, α-Fetoprotein, Ferritin, Transferrin, α-Globulins (e.g. α1-Antitripsin, α2-Macroglobulin), β2-Microglobulin
Miscellaneous	Alkaline Phosphatase, Lactate Dehydrogenase, α-Amylase, Allergenic Proteins
Therapeutics	
Cytokines	Interferons, Interleukins (IL-2, IL-4), Granulocyte Colony Stimulating Factor
Recombinant Proteins	Somatropin, Monoclonal Antibodies
Host Cell Proteins	Relevant to *E. coli* and Chinese Hamster Ovary

Some key comments and issues relating to the analysis of proteins captured during the interviews included:

- For diagnostics, the need for commutable protein matrix reference materials, with a fit-for-purpose uncertainty, rather than purified substances, because pure proteins are generally not good calibrants for clinical diagnostic tests. However, the latter are needed as calibrants to provide traceable value assignments for the former. There is also a need for multi-analyte panel materials at clinically relevant concentrations. Over the next 10 years representative mixtures of proteins in a suitable matrix e.g. plasma will be required. Ideally, representative mixtures of 'normal' and 'disease' states will be needed, such as simulated 'disease' and 'normal' standards produced by varying the proportions of the markers. These could be used to evaluate statistical classifiers to identify the disease state, based on biomarker profile, according to the detection platform.
- For therapeutic proteins, there are three broad areas of interest, with increasing analytical complexity:
    1. Peptide hormones are the simplest because they are non-glycosylated and are relatively well-defined. However, they still present some major analytical challenges including problems with the solubility of some of the constituent peptides and measuring their water content.
    2. Cytokines, which are used as ancillary materials for the production of cell and tissue-based therapies, are a little more challenging than the peptide hormones as they frequently have post translational modifications. Traditionally the interferons have been characterised using a potency test and one of the major challenges is to move this to an amount of substance measurement. For the interleukins e.g. IL-4, the main analytical challenges are to characterise the material before use

in tissue culture and to measure the residual amount in the therapeutic product. A 'Gold Standard' method (i.e. a universally accepted best current practice) for the latter would be useful for validating ELISA-based measurements, which are the current method of choice in the field. Other similar products of interest are IL-2 and Granulocyte Colony-Stimulating Factor (GCSF). Because they are in widespread use, there would be a lot of leverage in improving the consistency and traceability of these measurements and a well-planned study, which examined the main reasons for the variability of ELISA results using model systems, would be beneficial.

3. As monoclonal antibodies (MABs) are of similar structure the development of a set of reference methods would be beneficial. Key analyses include: an identity test, characterisation of the glycosylation, potency (the only approved binding assay for potency approved by the FDA is for a MAB), degradation (de-amidation and loss of the C-terminal lysine), purity (HPLC), sizing and aggregation (the FDA specify analytical ultracentrifugation).

- Characterisation and quantitation of proteins by mass spectrometry, starting with well-defined proteins and moving on to those with a higher degree of complexity. To underpin the work, high accuracy amino acid purity determinations and inter-laboratory comparisons on their quantification are needed. Currently, NMIJ is focusing on making pure substance standards as a traceability source. For this purpose, precise knowledge of the analyte is needed, which in some cases can be very ambiguous. In the short term, they will have developed analytical methods for CRMs in clinical matrices, because such materials will be required to establish the traceability chain with the clinical labs. They have already developed a RM for C-Reactive Protein in a buffer and over the next three years will be looking to produce one for insulin in serum and other protein hormones. They have recently produced pure amino acid standards for isoleucine, phenylalanine, valine and proline, which will help to provide a fundamental underpinning for protein analysis.

- The purity assessment of proteins is a major challenge and, for high accuracy absolute protein quantification, there is a need to link the measurement back to the fundamentals by quantifying the constituent peptides and amino acids, where, issues of purity determination again arise. NMR could be a way forward for this. For very high accuracy quantitation by IDMS, it is advantageous to get the internal isotopically labelled standard into the measurement process as early as possible in order to reduce the analytical errors at all stages of the measurement. There are initiatives under way to produce ^{13}C and ^{15}N substituted proteins by growing genetically modified *E. coli* on labelled growth media.

- Post translational modifications must be determined and characterised to a high level for biologics, which presents major analytical problems, because even subtle changes can influence efficacy or immunogenicity. High-level characterisation is also needed for antibody probes used for protein analysis. Hence there are significant efforts to develop reference materials, standards and reference data for protein structure characterisation for biologics quality control and to develop instruments and protocols for ensuring that follow-on biosimilars are structurally and functionally the same as their brand name counterparts. NIST is working on a peptide and a glycan database. It is developing a rapid method for glycan analysis which absorbs the protein onto nanoparticles whereby the glycans are still accessible to a colorimetric lectin analysis. This will be correlated with a mass spectrometric reference method.

- Progress is being made on the development of 'Horizontal' standards to validate the performance of instruments and methods especially for highly multiplexed analysis platforms. Early work in this area includes a panel of six non-human proteins of varying physico-chemical properties (i.e. size, hydrophobicity, isoelectric point, and post-translational modifications), with a dynamic range of five orders of magnitude. Measurement issues (e.g. reproducibility, cross-reactivity, dynamic range, homogeneity and stability) associated with the protein panel have been characterised. The generic standards serve as a functional indicator of platform performance

- Techniques for characterising the protein structure and correlating this with activity are being developed in the EMRP ClinBioTrace project[34], which is also developing methods for the absolute quantification of hGH. For PTMs, a more complex series of quantitative glycosylation reference standards is being developed to enable accurate relative quantification of different PTMs within a typical recombinant antibody product.

- In the area of work on the development of standards to ensure the accuracy and comparability of techniques used to determine immunogenicity NIST, in particular, is looking to put together a reference method for measuring immunogenicity. It is investigating optical flow micro-imaging for the

measurement of aggregates and anticipates having a physical reference standard within 3-5 years. It will be sent out for 'round robin' evaluation

- Supporting host cell protein (HCP i.e. protein impurities originating from the cells used to produce the biopharmaceutical) measurements through:
    - The development and validation of an improved sensitivity LCMS method for the quantification of HCP in biopharmaceutical products
    - The exploration of the quantification of intact proteins by LCMS
    - The development of standards relevant to HCP quantification through work with a sample representative of the host cell proteins such as a CHO culture medium antigen sample. This requires the characterisation of the sample and developing a method able to identify the proteins contained in the sample when spiked at ppm level relative to the active product in a solution containing an antibody and undertaking the relative quantification of those proteins

Development work identified for the longer term, in order to support future technology included:

- Addressing the measurement challenges presented by the need for highly multiplexed measurements such as protein-based gene expression analysis measurements using microarrays, 2nd generation immunoassays and physically-robust mass spectrometers. High throughput protein measurements, frequently at 'Point of Care', will be needed to support personalised medicine. The validation of biomarkers from highly multiplexed measurements is a particular problem. For microarray measurements, there is a need to improve their performance with respect to target sequence detection and relative quantification. For the multiplex bead platform, there is a need to improve the quantification through better calibration of the fluorescent beads. Other challenges include ensuring that the analysis is not biased for certain types of sequence and coping with the dynamic range needed to analyse all the constituents. It will also be necessary to develop systems for the validation of technologies which generate massive data sets to ensure that the clinical conclusions are valid.
- Stem cell therapy: measurement services supporting procedures for the release criteria of the product, including the characterisation of any unwanted activity, for example, that leading to carcinogenesis.
- Patient autologous therapy; this involves removal of material from the patient, followed by modification and re-introduction. Examples include the tissue culture of cells to help wound healing or the reintroduction of bone marrow stem cells following radiation treatment. One of the main problems is the detection of adventitious organisms: fungi, yeast, bacteria etc. particularly when it is not possible to know in advance what is being looked for. One approach may be for rapid testing using a surrogate test, possibly HPLC based, which looks for evidence of the contaminants' metabolites. A key challenge is how to ensure with a high degree of confidence that all adventitious contamination has been detected in autologous therapy products.

Issues raised which could be the subject of international collaboration were:

- The definition of the measurand for the more complex proteins of clinical significance where there are several isoforms. There was a call to develop NMR for better structural characterisation of proteins and to help define the measurand.
- The need to characterise primary, secondary and tertiary structure of proteins if activity is to be determined adequately by physico-chemical techniques rather than by bioassays. This then leads to the issue of values derived from bio-assays expressed in International Units and SI values determined by physico-chemical techniques. By definition the uncertainty of a reference standard assigned using the IU approach is zero, whereas the measurement uncertainty assigned to an SI derived standard using conventional metrological procedures may be too high to be used as a reference standard. Hence, the question is how to use a metrologically-based approach to improve the determination of immunological and biological functions, such as the activity of an antibody standard? Strict adherence to a reductionist approach is probably not the answer. A systems/complexity approach which uses metrological techniques, when it can, is a possible way forward. Also, in view of the potential confusion if measurements are expressed in both SI and IU, there was a strong call for high-level international discussions to be held to smooth any transition.
- 'Gold Standard' methods are required against which field-based 'Point of Care' measurements can be calibrated. However developing such methods takes time and the funding to undertake the exercise is frequently not forthcoming.

- Development of new traceability chains in difficult areas such as highly multiplexed analysis and for biologics, where the instability/dynamic nature of the analyte presents problems.
- Given the potentially huge number of reference materials that would be required to assure conventional traceability, there is a need to consider whether this is a viable approach for assuring the comparability of the majority of bio-measurements. Alternative approaches need to be considered e.g. those based on competency and generic method suitability assessment using 'horizontal' type reference materials.
- Obtaining the raw material for certain diseases e.g. Lupus is problematic because once diagnosed the patient is treated and material is not available from that patient. Other ways of producing this type of material need to be developed.
- The purity assignment of proteins, peptides and amino acids is a major challenge. Collaborative international work on the purity assignment of key bio-standards e.g. insulin or hGH and their key peptides and amino acids, would be very welcome as this would underpin important protein measurements. In addition, determining the sources of error from different techniques for value assigning proteins e.g. mass balance, elemental N, qNMR would be very useful, again using insulin, or hGH as an example.
- Improvements to the reliability of ELISA techniques, by examining the main causes of variability and validating against a reference method.

Suggestions for international comparisons which would help to improve the global comparability of protein measurements included:

- Comparison of quantitative protein measurements using different instruments and commercial kits.
- Purity studies on amino acids and peptides required for high accuracy protein quantification.

4.5.2. Nucleic Acids

There was widespread interest from many of the institutes interviewed in the qualitative (sequence) and quantitative analysis of DNA and RNA. Key measurement work being undertaken included:

- Development of sequence based reference materials for genetic diseases such as Huntingdon's Disease, Cystic Fibrosis, Fragile X, Factor V Leiden, Hereditary Haemoglobinbiopathy and cancer genetics such as BRCA1&2, APC Gene and HNPCC Gene.
- Trace DNA for cancer and pre-natal diagnostics and host-cell DNA impurities: reference materials being considered include a 'trace material' certified for copy number (e.g. 70 copies/µl) and a material comprising trace amount of male DNA in a female DNA background. For host-cell DNA analysis, the development and characterisation of a relevant quantitative DNA reference standard is required; key host cells being the commercially important *E. coli* and Chinese Hamster Ovary (CHO).
- Gene specific epigenetics: the ratio of methylated to un-methylated cytosine in the relevant gene
- Integrated/multiplexed rapid DNA analysis: the development of best practice guidelines, quality metrics, standards and acceptance criteria for assessing and quality controlling system performance of rapid DNA diagnostic devices such as disposable chips for molecular diagnostics (e.g. sequencing, STR profiling, genotyping and expression profiling). This will be done through an investigation of the performance and 'fitness-for-purpose' of rapid DNA testing systems in development, compared with current centralised 'gold-standard', laboratory based methods
- Measurement of viral load and viral sequences e.g. for HIV, cytomegalovirus and hepatitis. 'Digital' PCR will be used to give high accuracy quantitation. Priorities will be determined by clinical need, advised by professional bodies such as the Association for Molecular Pathology.
- RNA gene expression measurements: in the medium term, there is a need for RNA reference materials to support gene expression analysis. Reference materials are being developed for microarray and real-time PCR measurements. Some have already been produced and incorporated into commercial gene expression microarrays. Work is being undertaken with the External RNA Controls Consortium (ERCC) to produce a standard to help improve the quality of gene expression analysis, both with respect to sequence detection and relative quantification. Multiplex beads are frequently used for this type of analysis. NIST is investigating the fundamentals of the fluorescent measurement in order to develop better methods of calibrating the beads. They are looking into quantifying the fluorescence

radiance using MESF (Molecules of Equivalent Soluble Fluorophore). Reference beads can then be used to calibrate the response of the flow cytometer used for the measurements.

- DNA length measurements: INMETRO aims to develop a length measurement RM and for activity, a fluorescent standard. As a first action they are preparing a homogeneous dsDNA sample for AFM imaging studies with other NMIs. The objective is to get a consensus value on DNA length.

Measurement services being developed for the longer term include:

- 3rd Generation DNA sequencing, i.e. very high throughput analysis without the need for amplification, producing long read lengths: because the technology is moving so rapidly, it is difficult to predict what standards will be required, but they are likely to address the quality assurance needs for the fidelity of the sequencing and a mechanism for checking the models for extracting the sequence data i.e. the bioinformatics aspects.
- siRNA gene knockout therapy and microRNA to modulate gene expression and control disease, including cancer. Measurement issues include: quantifying the modulation and the effect on non-target genes, determining the fate of the siRNA, including its transport into the cell and its time in the cell
- Gene therapy: characterisation of the vector e.g. engineered adenovirus

Measurement issues and challenges mentioned, which would benefit from international collaboration were:

- The absence of an agreed paradigm for sequence analysis and quantitative DNA standards. There are currently two basic approaches to producing quantitative DNA standards, such as standards for Epstein-Barr Virus, which are used to determine infectivity. One method is to transfect cells and grow these. The other, closer to the SI approach, is to produce a plasmid and insert it into bacteria, so that the number of copies can be determined from the number of bacteria. Commutability of the standard is a key issue
- Defining the measurand; selecting the most appropriate form for the 'reference material' i.e. from purified target DNA through to organism containing target DNA (e.g. from purified viral DNA to virus); demonstrating commutability and applicability of the chosen reference material
- R&D on the purity assessment of RNA for quantitative RNA measurements
- R&D and studies on the molar absorption coefficients of nucleotides to underpin 'Total DNA' measurements
- The development of internationally recognised databases of authenticated reference sequences e.g. for medicinal herbs
- The development of a broader range of genomic standards for forensics which cover a wider range of racial groups than the current NIST standards
- Work on overcoming the matrix effects which hamper the DNA analysis of blood and tissue samples
- Understanding the metrological implications of copy number and 'counting' techniques for DNA quantification and their relationship to the mole

Suggestions for studies were:

- A study on trace DNA quantification using 'digital' PCR
- Studies on the absolute quantification of trace level plasmids or artificial chromosomes
- Study on the molar absorption coefficient of nucleotides

4.5.3. Other Measurements

Although the focus of the interviews was on protein and nucleic acid measurement, several institutes mentioned work in other areas, primarily cell-based measurements. The main points raised in the interviews were:

- Measurements on live cells using flow cytometry
- Supporting the development of cell-based assays for toxicity evaluation through:
    - The development of a set of procedures for the tiered testing and characterisation of immortalised or primary cells encompassing: cell viability, cell authenticity; cell functionality

    - the development of a panel of well-characterised authenticated cell lines of interest to industry
    - the organisation of a CCQM pilot study which addresses one of the key measurement requirements of the ‘standard cell’
- The use of systems biology to develop cell-based screening techniques for the evaluation of biologics
- The determination of molecular fluxes, particularly in Chinese Hamster Ovary cells
- The development of standard cell lines

5. Measurement Needs of Industry & Regulator's Comments

The measurement needs of the diagnostics and biopharmaceutical industry were ascertained through a set of nine interviews; five via face-to-face meetings and four over the telephone with industrial organisations. The help of Dr Vincent Delatour, LNE, who conducted the interviews with the French-based companies, is gratefully acknowledged. The interviews probed their use of measurement, particularly proteins and nucleic acids and their views of the future requirements for bio-measurements. The interview form is given in Annex III.

The FDA and the IFCC were also interviewed to capture the views of the regulators and professional bodies. They were asked about the issues they encountered with protein and nucleic acid measurements and the measurement challenges they foresaw in the future. The interview form is shown in Annex IV.

5.1. Profile of the Industrial Organisations

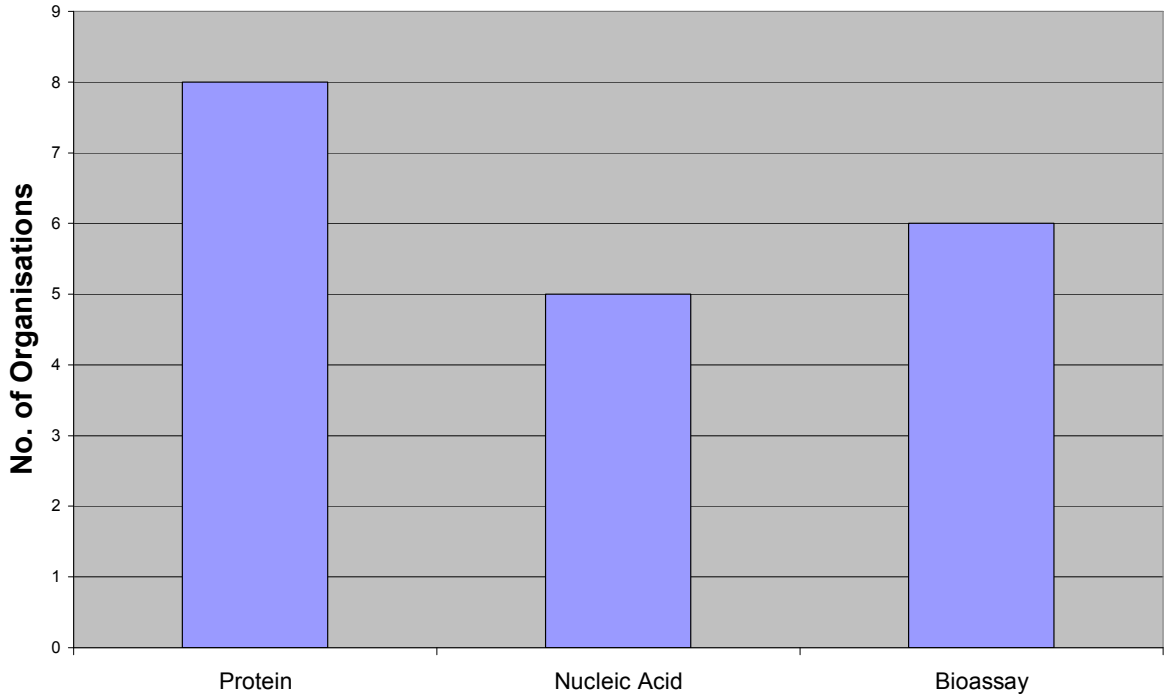
The majority of the organisations interviewed had >$1 billion of business reliant on bio-measurements and a profile of the activities of the organisations is shown in Table 8. Further details on each of the companies interviewed are given in Annex I. One of the companies interviewed, a European biopharmaceutical manufacturer, was willing to collaborate, but requested anonymity, so their details have not been listed.

Table 8: Activities of Industrial Organisations

Organisation	Bio-Pharm R&D	Bio-Pharm Production	Diagnostics R&D	Diagnostics Production
Abbott Diagnostics			X	X
Biocon	X	X		
Dr Reddys	X	X		
Lonza Biologics	X	X		
European Biopharmaceutical Manufacturer	X	X		
AGEPS	X	X		
BioRad			X	X
Diagnostica Stago			X	X
LFB	X	X		

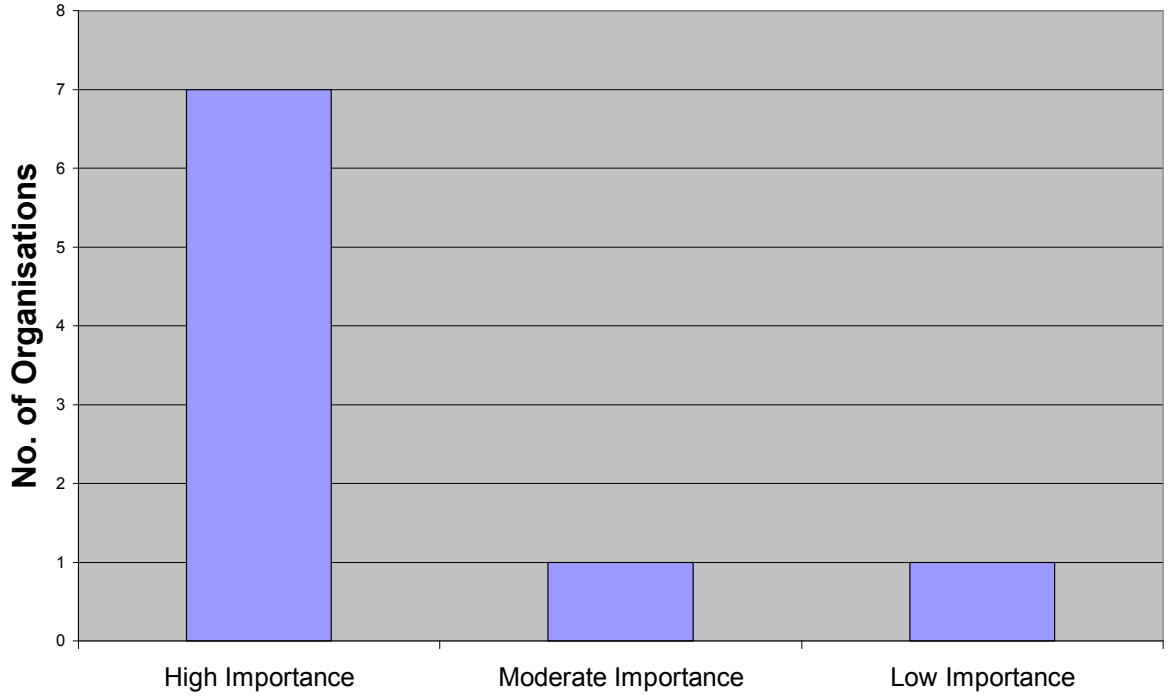
The type of bio-measurements being undertaken by the organisations interviewed is shown in Fig. 26.

Fig. 26: Type of Measurement



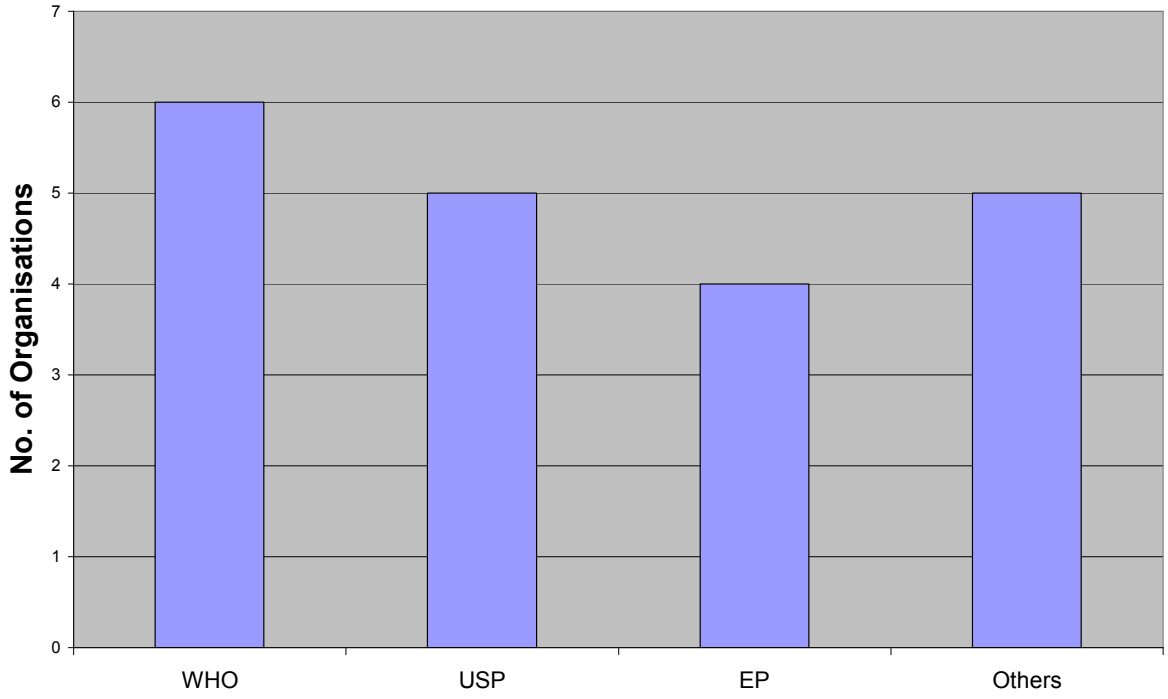
Although most of the organisations considered it to be very important that their measurements had global recognition – see Fig. 27, only two of the organisations said that they were in regular contact with their NMI.

Fig. 27: Importance of Global Measurement Comparability



An indication of the source of their reference standards is given in Fig. 28.

Fig 28: Sources of Reference Standards



The 'Other' sources of reference standards mentioned were: NIST, IRMM, LGC, ATCC, NCCS India. One of the companies also mentioned sourcing standards from the IFCC network. Two of the biopharmaceutical companies had significant resources for producing their own internal standards. This is driven by the substantial need for reference materials from their laboratories.

Although the number of organisations covered by the interviews was insufficient to get a comprehensive picture of the bio-measurement needs of the industry, the organisations selected did provide a valuable insight into the measurement activities and requirements of major diagnostics and biopharmaceutical companies.

5.2. Diagnostics Industry Needs

Diagnostic products can be split into three main areas: Laboratory Diagnostics, Molecular Diagnostics (nucleic acid analysis) and Patient POC devices (e.g. hand-held glucose monitoring devices for diabetes care).

The Laboratory Diagnostics are primarily immunodiagnostics i.e. they use antigen/antibody reactions to detect conditions such as: infectious disease, cancer and cardiovascular disease. There are also some screening tests e.g. for viral antigens. The outcome of the measurement depends on antigen-antibody interactions and their specificity. For a given target protein, different antibodies targeting different protein epitopes can give different results, particularly if the protein is comprised of a mixture of different isoforms. This is not a very satisfactory position and can result in different test kits giving different results, either through lack of traceability, or incomplete understanding of the measurand.

The Molecular Diagnostics devices detect genetic mutations, e.g. HER-2 and viral disease such as HIV, HPV and Chlamydia. PCR is used for HIV-1, HBV, HCV, and Chlamydia/Gonorrhoea tests; real time PCR for hepatitis and HIV-1 viral load measurements and DNA FISH for HER-2 detection.

When the target protein is well-characterised, the best way of validating diagnostic kits is to use mass spectrometry as the reference method. The next best approach is to participate in External Quality Assessment round robins. However, even for this type of proficiency test, there is a preference for a reference value to have been assigned by mass spectrometry. Higher order reference materials are used whenever possible, however, these are not always available; in particular, a good standard for troponin is required. hCG used in pregnancy tests and also as a cancer marker has a WHO standard, but this is not pure. It would be beneficial to have a purer and better characterised standard. Also hepatitis and HIV antibodies would benefit from better purity and

characterisation. In some instances, such as for viruses, it is difficult to see how a reference material could be made available.

The global comparability of bio-measurements is viewed as being very important. In this respect, moves such as the EU IVD Directive and the definition of the traceability chain in ISO 17511 are seen as being the way forward to improve the comparability of diagnostic tests. In addition, ISO 15189, which specifies the quality management system requirements for medical laboratories, is being adopted widely around the world for accreditation and it calls for traceability and uncertainty estimates of the measurements. A future driver for better test comparability is the development of electronic patient care records in the US, where it is crucial that tests undertaken on patients at different times and in different centres be comparable, in order to follow the progression of disease and treatment.

International efforts to develop reference measurement procedures for common immunoassays would be very welcome and troponin would be a good starting point. The structural characterization of proteins, including structural heterogeneity, is key to defining the measurand properly (e.g. glycan analysis, specificity against isoforms). Again, international efforts to characterise clinically important proteins and standardise procedures would be very welcome.

Regarding units of measurement, in the US, amount of substance measurements of proteins are generally expressed in what is termed 'conventional units' e.g. glucose is reported in mg/dL, as opposed to mmol/L and enzymes in IU/mL.

For the future, multiplexed, 'black box' assays are likely to come into common use. Analytical challenges include how to make these devices failsafe and self-calibrating/self diagnosing.

5.3. Diagnostics from a Regulator's Perspective

The major issue with protein measurements is the difference in results produced by different measurement platforms. This is particularly acute for immunoassays where the devices can detect different epitopes or different isoforms. Thus, different results can be produced from different kits.

For nucleic acid measurements, there are two emerging diagnostic uses, the measurement of viral load and the detection of RNA transcripts, used as tumour markers. For the former there is a need to improve the accuracy of the quantitation.

There are many instances of persistent analytical problems; some of the key ones are:

- D-Dimer and other degradation products of fibrin. These are used as markers for detecting blood clots. There are three types of test, each giving different results. Their importance is likely to increase as it is likely that Point of Care (POC) tests will be introduced and they will be used in the doctor's surgery.
- Autoantibody assays: there is no standard for polyclonal antibodies in human serum, which means that it is difficult to be sure what antigens are being measured in this type of assay.
- Cancer biomarkers e.g. CA15-3 for breast cancer, CA125 for ovarian cancer, PSA for prostate cancer. There is only one reference standard available for protein biomarkers compared with over 20 diagnostic tests which use protein biomarkers, which leads to unacceptably variable results.
- C-Reactive Protein and Troponin are also being introduced as POC tests, to be used by the physician in the surgery, hence their importance is growing and high quality reference materials for improving the traceability of the results would have a high impact. A high order metrological standard for Troponin I is required for the newer, high sensitivity Troponin assays.
- A key emerging protein biomarker is BNP (Brain Natriurectic Peptide), which is coming into use as a cardiac marker.
- Transcription standards for BCR-ABL, a tumour marker, are also important and there is a consortium working on this.
- Another key protein, whose use will increase significantly, is procalcitonin. This is used for measuring bacterial infection and can distinguish between viral and bacterial infection. It is particularly important for distinguishing chest infections and will be used as a POC assay, to enable the physician to decide whether or not to prescribe antibiotics (viral infections do not respond to antibiotics). It is important to help limit the inappropriate prescription of antibiotics.

Measurement standards are the key to improving the quality and reliability of diagnostic assays. There was general frustration from regulators at the unavailability of reference materials for key tests and the length of time that it appears to take to develop a reference material. They regard this as a major issue.

There was some concern regarding the possibility of having two types of standard; one which was based on amount of substance and the other, a WHO-type of standard based on activity via a bioassay. It was felt that this could lead to some confusion and recommended that the WHO should be involved in discussions on the introduction of amount of substance-based assays.

The regulators saw the main future trend as being the introduction of multiplex protein measurements. The view was that microarrays were doing a good job now for the qualitative measurements; however, it was felt that they would not be useful for quantitative analysis. They envisaged that mass spectrometry would become the method of choice for this type of measurement. The development of a performance indicator type of standard and measurement protocols would be needed to support the analysts. In particular 'horizontal' type reference materials should be developed to enable the performance assessment and validation of mass spectrometric assays for multiplex protein measurements.

5.4. Diagnostics from IFCC Perspective

Current IFCC Committees include:

- Molecular Diagnostics (interested in the development of well-defined, but low level reference materials for use as positive and negative controls)
- Plasma Proteins (standardisation through the use of suitable reference materials)
- Reference System of Enzymes (including work with IRMM on Aspartate Aminotransferase and Alkaline Phosphatase)
- Traceability in Laboratory Medicine
- Reference Intervals and Decision Limits

The current IFCC Working Groups include those working on: HbA1c, TSH and 'free' thyroid hormones, HbA2, Carbohydrate Deficient Transferrin, Cystatin C, Albumin, Pregnancy-Associated Plasma Protein A (PAPPA), hGH, Insulin, Troponin-I, Autoantibodies.

IFCC envisages a key role for the NMIs in helping companies implement traceability correctly and to provide value assignments, validate platform assays and assign reference values to external QA schemes. Support is also required for ISO 15195 accreditation.

There is a lack of internationally accepted 'anchors' for traceability and clinical comparability, with no control at the end of the traceability chain. There are three sectoral frameworks: EU, U.S. and Japan, but they do not meet, agree or co-ordinate. For example, there are three different programmes for HbA1c which give different results with no international comparability. The end-user needs confidence and hence international agreements and co-ordination are required.

Clinical guidelines drive the need for quantitation where the therapy or diagnostic/clinical action is dependent on the reported value e.g. BNP, TSH. There are insufficient reference materials for quantification.

Molecular diagnostics "are theoretically important" but have not yet realised any clinical applications. Currently there is a qualitative approach, but there is a need to move to quantitative measurements.

In the future there will be a need for RMs for multi-analyte detection in a clinical matrix for use in clinical laboratories. Currently there are only 6 or 7 enzymes in a purified form. Also, the FDA may apply a metrology traceability concept in future guidance/regulation. At present there is no metrological traceability in U.S. regulations, traceability being to the oldest known method, the 'predicated' method.

5.5. Biopharmaceutical Industry Needs

Four companies, with major activities in biopharmaceuticals, were interviewed in face-to-face meetings. They comprised two Indian-based bio-similar manufacturers, Biocon and Dr Reddys, a European-based biopharmaceutical manufacturer and a major European contract supplier of biopharmaceuticals, Lonza. A French-based biopharmaceutical group, LFB, who manufactures plasma-derived medicinal products and is developing monoclonal antibodies, was interviewed by telephone. It enabled a reasonably balanced picture of the bio-measurement needs in proteins, nucleic acids and cells/bioassays to be built-up.

5.5.1. Proteins

Proteins are analysed using N terminal and C terminal sequencing, MS peptide maps, CD, NMR, elemental analysis, Keldjhal nitrogen analysis (although there is a move away from this), amino acid analysis and Karl Fischer determination of water. All traceable analysis for well-defined species such as insulin and hGH is related back to amino acid analysis and elemental nitrogen. Concerning the issue of the SI and IUs, for many recombinant proteins there is now a well established link between the amount of certain preparations and activity. Therefore the determination of amount of substance would meet both requirements. For example, both insulin and hGH have set mg-IU conversions. Most products are sold in mg quantities. However a caution was expressed that some insulin analogues have very different activities and in these instances it is activity (IU) that is important. For new products, where a WHO reference standard has not been developed, biological activity is expressed simply in 'Units' (U).

There is a tendency to undertake two basic sets of measurements: QC for batch release and more sophisticated analysis for process development. For QC the main focus is on identification, purity and activity. Identity can be through a charge measurement method and HPLC fingerprint. Purity measurements comprise measuring attributes of the main component namely: % main product, % low MWt material, % aggregates and impurity determination - host cell proteins, host cell DNA and leachates. The maximum levels of these are set by dose. Endotoxins are measured, but this is probably the most concise methodology of all the tests. Activity and strength are also determined. For process development, more sophisticated analytical techniques are employed, in particular LC/MS for the determination of primary and secondary structure.

For biopharmaceutical product characterisation, a wide range of properties are determined: purity, identity, amount, PTMs - oxidation, reduction, aggregation, charge, glycan profile, de-amidation etc. The techniques used include mass spectrometry, peptide mapping, mass comparisons and CD. For process monitoring, HCP is determined using ELISA, metabolites are measured off-line and enzyme profiling is also carried out. Immunoglobulins, coagulation factors and pro-inflammatory cytokines are analysed using ELISA.

For biosimilars, all properties prescribed by ICH[35] and EMEA[36, 37] biosimilar guidelines are measured namely: sequence, amount, activity, PTMs, aggregation. Typical techniques used and accepted as validated by regulators are ELISA (activity & quantification), disulfide bridge mapping, NMR, peptide mapping (using MS). The guidelines on EMEA regulations for biosimilars are generally sound and practical, but 'similarity' is a difficult concept for efficacy, safety & quality measurements. PTMs can be determined, but is the PTM critical to activity, if differences are found? The situation for biosimilar characterisation and comparability should improve as techniques improve and there is a greater harmonisation of techniques employed for critical measurements. SPR has been found to be valuable for interaction analysis.

Problems and issues encountered, which require the development of measurement services and support include:

- The analysis of post-translational modifications using different mass spectrometric platforms. Each instrument manufacturer has a different routine for the auto mass selection which can give rise to different results. As this technique becomes more mainstream, this could lead to comparability problems. Well-characterised protein reference standards would be useful for 'calibrating' the different instrument platforms.
- Water determination by different Karl-Fischer techniques can be a problem. Standards for the moisture content of biopharmaceutical products would be very useful, but how to stabilise such a standard would be a major challenge.
- Pure amino acid calibrators are needed to underpin the key amino acid analysis for product characterisation, but the currently used internally sourced standards do not have a robust uncertainty assignment. However, NMIJ has just released a set of four amino acid standards.
- The method dependent variability of glycan profiling and other PTMs; this is an important analysis for biosimilars and it is difficult to judge whether the measured variability is due to the product or to the analysis. There is a lack of consensus on the 'best methods'. Standards for glycan profiling would be very useful in this respect plus international harmonisation of 'best practice' for PTM analysis. Also standards and reference methods for aggregates, oxidation and de-amidation. Standards for enzyme digestion are required so that the efficiency of de-glycosylation can be measured.
- Primary and secondary protein structure can be measured with SI traceability. However, improvements are required in tertiary structural measurements for proteins; there are too few methods.
- Development of MS technology: peptide mapping cannot discriminate small changes e.g. in oxidation state of the amino acids and the size limitations when trying to characterise whole protein structure.

- Further development of structural methods for tertiary protein structure measurements e.g. HDX

5.5.2. Nucleic Acids

Nucleic Acid measurements were not used widely by the companies interviewed, the main exception being QPCR for host cell DNA determination. Here there are some issues with trace detection, spike recovery and matrix problems. However, once host cell DNA levels have been established in a production process and, if it is shown not to be a patient issue, then this analysis is not undertaken very frequently.

PCR is used for viral genome searches. There is a need for a reference standard for CMV; there used to be one, but this has not been replaced.

Qualified or validated methods are required for mutation, sequence and motif analysis.

5.5.3. Bio-assays/Cells

Bioassay measurements are product dependent and cover measurements such as cytotoxicity and proliferation as well as amount and activity. For bioassays, there is no consensus on activity reporting/data analysis, so there are issues over measurement uncertainty. Regulators (typically with small molecule backgrounds) are 'over optimistic' on the level of uncertainty that is attainable when setting bioassay acceptance criteria and specification. There is a requirement for a common approach to avoid problems e.g. with misunderstandings of uncertainty of cytotoxicity data. Bioassays show very great variability and there is need for assurance that a bioassay for a biosimilar is truly measuring safety, efficacy etc. There are no reference methods or standards, which would aid comparability and improve confidence in bioassay validation, but, if developed, these would have to be very product specific.

There is a general shift from ELISA-based activity measurements to cell-based methods, initially using binding assays, but there is a move towards more sophisticated tests for potency which also look at cell response as well as a binding assay. Cell-based assays, which are being introduced, partly to reduce the amount of animal testing, are prone to problems with reproducibility and comparability. Each test is specific to the particular molecule being analysed, but it was felt that there would be merit in looking at every aspect of the measurement process generically to determine the analytical variability of each step and see how this could be reduced.

There is an increased use of flow cytometry for cell counting and calibration of flow cytometers would be a good area for this type of investigation. For cell line characterisation and cell banking, measurements undertaken include: titres, stability and gene copy number, a key measurement for making master cell banks.

5.5.4. Reference Standards & Measurement Services

Many biopharmaceutical manufacturers produce most of their own reference standards and have a special department for producing, characterising and distributing these. They consider it crucial that all measurements for production, clinical evaluation and release are comparable. In addition, the correlation between the measurement results in documentation of the drug product and the clinical evaluation is required by the authorities. They would find it useful to have some well-characterised reference materials for the main classes of proteins e.g. one for IgGs. There is currently collaboration on a NIBSC/WHO activity to make a single traceable standard for insulin. It is hoped that this will be adopted by all pharmacopoeias. hGH is already considered traceable to the WHO materials.

Regarding measurement traceability, just stating and understanding/accepting the associated measurement uncertainty would be a step forward for the industry as a whole. Currently there is a standards symposium being organized by USP to help inform regulators and industry about measurement uncertainty. Once informed the next steps will be to look at the methods and standards required to reduce the uncertainty to acceptable levels.

The biopharmaceutical manufacturers observe that there are very few relevant biological standards for the biosimilar market. The problem is that biosimilars have to be comparable with the innovator API in formulation, so to produce them, the commercial product has to be reversed engineered. RMs for glycan PTMs can be obtained from Ludger. They can source MS peptide calibrants from commercial suppliers, but the quality is variable and dependent on the supplier.

5.5.5. Future/Longer-Term Requirements

It is anticipated that the miniaturisation of the current analytical techniques, an increase in analytical throughput and the broader application of these techniques in the QC laboratory and 'at-line' will be implemented and that

mass spectrometry will become more important, but this requires more automation. Biosimilar manufacturers find some technologies, such as lectin arrays too costly to be adopted in their QC labs.

Size exclusion UPLC is one area of considerable interest at the moment. 5 or 6-plex measurements are the current focus of development, with highly multiplexed analysis becoming important for the longer term. Although there is some R&D being undertaken on the applications of systems biology to process optimisation, it was felt that this will probably not be applied for at least 5-10 years. One issue is how to handle and data-mine the huge amount of data coming from the measurements.

There was some concern that the inclusion of uncertainty may cause regulators to narrow product specifications and acceptance criteria. This was perceived as being potentially very costly to the industry, so education may help in acceptance and hasten SI-traceable approaches. The harmonisation of measurement units is seen as being one of the major challenges. There is also concern about the potential non-harmonisation of new pharmacopeia standards e.g. the Chinese compendium. If these standards are not harmonised with the others it will require industry to have different acceptance criteria for this region; something they wish to avoid.

There is a desire to introduce more physico-chemical based techniques to replace some bioassays and ELISA; ideally with single step assays and no matrix effects! There is a need for more and better structural methods for tertiary protein structure measurements.

Greater regulation of biosimilars is envisaged; USP monographs are in preparation and new U.S. regulation is imminent. Lower detection levels will be required as the regulators are pushing the boundaries.

6. Analysis

6.1. Introduction

This section analyses the information gathered from the reviews of roadmaps and strategies and the BAWG activities and the series of interviews held with measurement organisations, industry and regulators. The analysis is primarily aimed at identifying the measurement services, studies and measurement R&D needed to underpin the comparability of bio-measurements, which would be of interest to the BIPM in formulating its future programme of work.

Roadmaps & Strategies

The review of roadmaps and strategies found that detailed analyses of the needs for bio-measurement and strategies for meeting these needs had been published by NIST in the US, Euramet in Europe and the NMS in the UK. It was apparent that the sector with the greatest need for improved bio-measurements was Healthcare, particularly diagnostics and drug development.

For diagnostics, the European IVDD Directive, with its requirement for the traceability of the measurements to a reference material of high metrological order, was a major driver. One of the key drivers for new diagnostic measurements was the ageing population, with the need for the earlier detection and diagnosis of disease. A major driver for therapeutics was the role that improved measurement technologies and measurement infrastructure could play to address the worrying decline in productivity of new drug development. For existing biopharmaceuticals, the need to demonstrate the 'equivalence' of follow-on biologics, or biosimilars with respect to efficacy and toxicity when compared to the original product, was driving a significant amount of work on validated methods for detailed protein characterisation.

CCQM BAWG

The CCQM Bioanalysis Working Group (BAWG) was founded in 2002 with the aim of helping to establish global comparability of bio-measurements through bio-analytical reference measurement systems of the highest metrological order and establishing the comparability of NMIs and National Expert Laboratories via prioritised Key Comparisons (KCs). It developed a series of roadmaps in 2005-2006 to help define and prioritise pilot studies and KCs in the key areas of nucleic acid, protein and cell bio-measurements, focusing on key building blocks for measurement studies required to demonstrate capability. The studies were aimed at underpinning key measurement competences, not specific applications. A major review of the BAWG strategy was undertaken in 2008, which resulted in the formation of several sub-groups in key measurement areas namely:

- Nucleic Acids
- Proteins
- Cells and Tissues
- Epigenetics
- Nanobiotechnology
- Polysaccharides

Interviews

Information gathered from the interviews showed that the majority of NMIs with activities in bio-metrology had been working in the area for some time, although there was a significant number that had recently started work in this field. Their work was divided into the development of measurement services for introduction over the next 5 years, primarily reference standards and reference methods, supported by R&D which aimed to provide a basis for future measurement services (>5 years) to address the new measurement challenges posed by advancing technology and capabilities.

The growth forecast by most institutes was in response to the importance of the recent developments in biotechnology and the impact that this was likely to have on healthcare. Diagnostics were the main target for the work of the NMIs, with food being the second most popular area. Healthcare technologies and biopharmaceuticals also featured prominently and work to support forensics was significant.

Almost all the institutes interviewed were working on proteins and the majority had work programmes on nucleic acids. Less than half those interviewed had activities covering cells/bioassays. Proteins were also the most dominant measurement area for the NMI's CMC claims and BAWG studies followed by nucleic acids, with cells/bioassays again being the least prominent. The most cited analytical technology was mass spectrometry, with PCR being the most frequently cited technology for nucleic acid analysis.

Interviews with diagnostic and biopharmaceutical manufacturers confirmed that protein measurements were key. For those biopharmaceutical manufacturers interviewed, cell/bioassays were currently more important than nucleic acid measurements and there may be a case for arguing that this field of measurement is under-represented in the current NMI portfolios. However, it may also represent the forward focus of the NMIs work, with an anticipation that nucleic acid analysis will continue to grow in importance and potentially displace some more conventional cell analysis e.g. for microbial identification and quantitation, although the physical/chemical, rather than biological origin of the work of the majority of NMIs is also a significant factor for this bias.

The BIPM requested that the main focus of the study be the identification of programmes in support of protein or nucleic acid measurements for healthcare; this is a good choice, as it is broadly aligned with the major interests of the NMIs. This position is also supported by the needs of the diagnostics industry and the desire of the regulators to have global comparability of measurement underpinned by the traceability of the results to higher order reference standards. In the light of this, the analysis has concentrated on proteins and nucleic acids, with applications in the diagnostics and biopharmaceutical sectors. These are analysed in Sections 6.2 and 6.3. However, some comments have also been made on cells, which are covered in Section 6.3, followed by a short Section 6.4 with comments on the emerging bionanotechnology field. Imaging technology is an area that was identified as being of significant importance to bio-measurement, but was not further developed in the study as priority had been given to activities related to protein and nucleic acid measurements. However, because of its importance and rapid advances, this remains an area of high impact from metrology projects. Although imaging has not figured much in the work of the BAWG to date, this may change as there have been recent discussions with the Surface Analysis Working Group (SAWG) regarding possible collaboration on surface imaging[40].

6.2. Proteins

The analysis of proteins presents major measurement challenges, particularly when the analysis progresses to more complex proteins and to highly multiplexed analysis. It has been forecast[32] that 50 % of the top 100 drugs will be biopharmaceuticals by 2014. Characterising these therapeutic proteins presents a major analytical challenge, but good characterisation is required to avoid adverse effects caused by either a changing production environment or the substitution of a brand drug with a generic, follow-on biologic. Significant problems exist in the measurement of post-translational modifications, three-dimensional structure and protein aggregation, all of which can influence the protein product's immunogenicity, safety and efficacy and limit its shelf-life.

The BAWG strategic position on proteins is not as well-advanced as that for nucleic acids. There is a continuing debate on how to demonstrate meaningful measurement traceability to stakeholders and the studies carried out so far have been at either end of the metrology spectrum: amount of substance determinations on simple analytes, based on small molecule approaches, or method comparability/validation studies, with limited metrological rigour.

Based on the results of two surveys, the Protein sub-group has identified four main types of protein measurement of interest to the NMIs to support their measurement services. These are:

- Qualitative: Protein Identification
- Qualitative: Structural Characterisation
- Quantitative: Amount of Substance Determination
- Quantitative: Avidity or Activity Determination

With the exception of the last point, mass spectrometry techniques dominate the measurement approaches by the NMIs. However, there was agreement that the BAWG should not base its strategy on technologies, but on the type of measurement required. In this context, progress is being made by the NMIs on the use of CD for protein structural measurements (CCQM-P59). There was recognition that 'amount of substance' determination for proteins was only a starting point and that establishing traceable measurements for other properties would be very challenging. However, there was a clear stakeholder need, particularly for the clinical, biopharmaceutical and diagnostic sectors. The sub-group identified that there was a strong requirement to underpin peptide and protein measurement studies with well-characterised, traceable pure amino acids. Also, there was an immediate

need for an international comparison on the value assignment of a clinically relevant 'property' for a protein target in serum.

Five main areas have been identified where measurement services provided by the NMIs can underpin the comparability of protein measurements. These are:

- Fundamental metrology applied to proteins i.e. the development of primary reference standards for amino acids, peptides and proteins and efforts to aid the transition from IU to SI units for amount of substance
- Improvement in the comparability of measurement results from different platforms, particularly for diagnostic measurements
- The development of measurement services for complex proteins – those containing post-translational modifications, which significantly affect the biochemistry, e.g. glycans
- The development and application of new and improved physico-chemical techniques for protein structural characterisation enabling a better definition of the measurand for complex proteins and linking these to function
- Assuring the comparability of measurements from highly multiplexed analytical techniques

Each of these is analysed in turn in the sections below. The likely areas for measurement services and international comparisons in the next 5 years are identified together with the collaborative R&D required to support a global improvement in measurement comparability and develop new measurement services for the 5-10 year horizon.

6.2.1. Fundamental Metrology

Linking protein measurements back to the SI is the most robust way of ensuring the global comparability of measurements across space and time. The NMIs have a lead role in effecting this by providing the key reference points, especially through the development of Primary Reference Measurement Procedures and Primary Calibrators, which are at the pinnacle of the traceability chain described in ISO 17511 for *In Vitro* Diagnostic Devices. However, because of the complexity of many proteins, this is by no means a trivial task; it will not be achieved quickly, if ever for all protein measurements. Nevertheless, practical steps are being taken by the NMIs to start the process, namely the work being undertaken by the CCQM BAWG on peptide and protein quantification and in the EMRP ClinBiotrace project[34].

The high accuracy analysis and purity determination of amino acids, peptides and proteins to support the development of primary standards are key to this effort. The work spans several techniques including isotope dilution mass spectrometry, qNMR and methods for high accuracy water determination. A proposed way forward is to undertake detailed work on a model 'simple' protein, good examples being insulin and hGH. With supporting international comparisons on high accuracy protein quantification, amino acid purity and amino acid quantification, this will facilitate the development of primary standards for the proteins and their constituent peptides and amino acids. The work will have considerable leverage in underpinning protein measurements because the primary calibrants can be used to assign the values of other protein reference materials in clinical matrices at clinically-relevant levels. As a first step towards this underpinning, four 'pure' amino acid standards have recently been produced by NMIJ.

Several organisations interviewed raised concerns that a successful outcome of the above work could lead to the potential confusion with properties expressed in two different units, IU and SI. Consequently, there was a strong desire from industry, regulators and measurement institutes for close co-operation with the WHO and the BIPM to ensure that there is an orderly transition from IU to SI, as improved physico/chemical techniques enable protein structure and quantity to be measured precisely enough for the determination of protein activity. This will require major effort led by the three key international bodies, WHO, BIPM and IFCC.

Table 9: Measurement Services Linked to Fundamental Metrology

Service	Remarks
Primary quantitative amino acid standards	Four 'pure' amino acid reference materials recently produced by NMIJ namely Isoleucine, Phenylalanine, Valine, Proline. A broader range of primary amino acid reference standards is needed in addition to the ones developed by NMIJ. Possible ones are: Alanine, Leucine, Proline, Histidine, Lysine, Argenine and Glycine.
Primary quantitative peptide standards	Purified peptides are being produced by PTB as part of the ClinBiotrace EMRP project and are being quantified by Exact Matching IDMS.
Primary quantitative protein standard	Primary standards for protein hormones e.g. insulin, hGH
Isotopically (^{13}C and ^{15}N) substituted proteins for high accuracy IDMS measurements on primary protein standards	Produced by growing genetically modified *E. coli* on labelled growth media.

Table 10: Potential International Comparisons Linked to Fundamental Metrology

Comparison	Remarks
High Accuracy hGH Quantification	Possible BAWG study for 2011/2012
High Accuracy C-Reactive Protein Quantification	Possible BAWG study for 2011/2012
Traceable Peptide Quantification	Assigning the mass fraction of a 'purified' specific peptide in a buffer solution is being discussed by BAWG
Traceable Protein Quantification	Assigning the mass fraction of a 'purified' specific protein in a buffer solution is being discussed by BAWG
High accuracy amino acid purity determination	Suggested by NMIJ and NPL in order to underpin high accuracy protein quantitation and primary reference standards

Collaborative R&D Requirements

- Methods for the purity assignment of key bio-standards e.g. insulin, hGH
- Discussion on the transition from IU to SI Units with relevant international bodies

6.2.2. Consistency of Results Across Measurement Platforms

The inconsistency of results produced by different measurement platforms, whether it is with immunoassays, where different devices detect different epitopes and isoforms, or different mass spectrometers with different algorithms to interpret the data, was highlighted as an issue by both industry and the regulator. The regulators identified many instances of persistent analytical problems, some key ones being: D-dimer, autoantibody assays, cancer biomarkers (e.g. CA15-3, CA125, PSA), C-reactive protein, troponin, BNP, procalcitonin. To ensure consistency, it is important that the measurand is defined and the units in which the measurement is expressed are common. The lack of certified reference materials of a higher order for the calibration of *in vitro* diagnostic (IVD) Devices is being addressed through the work of the Joint Committee for Traceability in Laboratory Medicine (JCTLM). NMI priorities have been established in collaboration with the stakeholders and measurement services are needed in the short term (0-3 years) for: non-peptide hormones such as serotonin, melatonin, dopamine, leukotrienes and serum proteins such as C-reactive protein, troponin I, HER-2 and

metalloproteins. In the medium term (3-5 years) it is anticipated that protein reference materials of improved accuracy will be introduced by using isotope-dilution mass spectrometry (IDMS) analysis of proteins labelled by extracting them from bacteria produced on isotopically-labelled growth media.

The development of high order reference materials and 'gold standard' measurements based on mass spectrometry can be time consuming and expensive and R&D is needed to determine how this could be reduced. Ideally the reference materials should be in a relevant clinical matrix i.e. 'secondary calibrators' rather than pure substances and linked back to the SI using the primary calibrators, the subject of the previous section.

At the trace level, the amount of host cell protein (HCP) is strictly regulated. It is more difficult to remove impurities from biologics than from small molecule drugs and major problems are associated with their presence. Improved techniques, such as LC/mass spectrometry with enhanced sensitivity using antibody-based enrichment techniques are being developed. In the medium-term, reference standards for HCP quantification will be needed.

Table 11: Measurement Services Linked to Providing Consistency across Platforms

Service	Remarks
Commutable 'higher order' reference materials with 'fit for purpose' uncertainty in a clinical matrix to support diagnostic measurements assigned using 'gold standard' methods, especially mass spectrometry.	This is a major task as there are a huge number of potential protein analytes. In most instances, the NMIs take advice on priorities from professional and other key bodies in the clinical field e.g. IFCC, German Medical Practitioners Council, Association of Molecular Pathologists, National Cancer Institute. The priority analytes cited during the interviews can be found in Table 7.
Reference standards for host cell proteins at ppm level, relative to the active product, certified by IDMS	Relevant to the main bio-process cell types namely *E. coli*, CHO
Standards for moisture content of biologics	Requested by industry, but will be difficult to develop

Table 12: Potential International Comparisons Linked to Consistency across Platforms

Comparison	Remarks
Peptide Mapping/Profiling for Impurity Measurements in Complex Biopharmaceuticals	BAWG Study planned for 2010/2011 on hGH
Identification of Proteins in a Complex Mixture	BAWG discussing potential studies of relevance to proteomic CRMs, process and product-related impurities, allergenic proteins in food and cellular proteomics
Relative Protein Quantification for Proteomics	BAWG participants have research activity in this area which could lead to a study based on proteolysis and LC tandem MS
Quantification of Proteins in a Biological Fluid	BAWG participants have research activity in this area which could lead to a study based on immunoaffinity, or size exclusion chromatography or strong cation exchange + tandem MS

Collaborative R&D Requirements

- Study using model systems to examine the main reasons for the variability of ELISA-based measurements
- Explore alternative approaches to the development of specific reference materials in order to assure the traceability of protein measurements e.g. based on competency and method suitability assessment using 'horizontal' reference materials

- Determination of sources of error from different techniques for the value assignment of proteins (e.g. mass balance, elemental N, qNMR) using insulin or hGH as a model system

6.2.3. Complex Protein Measurements

The definition of the measurand for more complex proteins and for those which comprise several isoforms is a problem. For biopharmaceuticals, post-translational modifications need to be determined to a high level because even subtle changes can influence efficacy, safety and immunogenicity. One of the key post-translational modifications is glycosylation, which determines the protein's activity and clearance rates and thus has profound clinical significance. Analytical approaches are still being developed for their characterisation and the biopharmaceutical industry has a strong need for reference standards to support the analysis. In the short-term there is a need for sets of glycan reference standards and guidance on the analytical techniques. In the longer term, there is a need for a more complex set of quantitative glycosylation reference standards which would help to validate analytical procedures e.g. for recombinant antibodies.

For therapeutic proteins it makes sense to meet the metrological challenge by progression from 'simple' to more complex ones namely from peptide hormones (well-defined, non-glycosylated) to cytokines (frequently have PTMs) to monoclonal antibodies. The most complex proteins are unlikely to succumb to a classical traceability chain back to the SI using primary methods and alternative approaches to improve the comparability of the measurements, using metrological techniques where appropriate, need to be developed. For instance, collaborative R&D on the characterisation of monoclonal antibodies, which are used in both diagnostics and as therapeutics and are of a similar structure, would be very beneficial. Such work would encompass the development of reference methods for identity, potency, degradation, purity, sizing and aggregation.

The instability/dynamic nature of biologics standards makes it difficult to develop a practical traceability chain and there were requests for collaborative R&D work on this from several of the organisations interviewed. For those areas where the complexity of the analytes means that the 'physico-chemical/reductionist' approach is not appropriate there is a need to explore how metrological techniques can be profitably used in a 'systems-based' approach.

Table 13: Measurement Services Linked to Complex Protein Measurements

Service	Remarks
Quantitative Glycosylated Reference Standards	To enable accurate relative quantification of different PTMs within an antibody product
Standards and reference methods for oxidation and de-amidation	Requested by biopharmaceutical industry
Development of a glycan database	Being undertaken by NIST and other organisations such as the Glycobiology Institute
Rapid method for glycan analysis based on colorimetric lectin analysis	This is being developed by NIST and will be validated by mass spectrometry.

Table 14: Potential International Comparisons Linked to Complex Protein Measurements

Comparison	Remarks
Qualitative PTM (glycan, glycosylation, de-amidation, oxidation)	For possible consideration by BAWG, following identification by their Protein Strategy Group
Qualitative Glycan Analysis of single purified protein	For possible consideration by BAWG, following identification by their Protein Strategy Group
Quantification of PTM modified protein relative to unmodified	For possible consideration by BAWG, following identification by their Protein Strategy Group

Collaborative R&D Requirements

- Development of an international consensus on the 'best methods' for PTM analysis with related guidance documents
- Development of traceability chains, which incorporate stability uncertainty
- Development of schemes for making traceable bio-measurements where traceability to the SI is unlikely to be possible, including alternatives to the 'reductionist' approach to protein measurements; development of a more 'systems' based approach incorporating metrological principles

6.2.4. Structural Characterisation

This comprises the development of improved physico-chemical techniques for the full characterisation of protein structure. This could involve development of techniques such as HDX, Ion Mobility Spectroscopy (IMS), NMR, Circular Dichroism (CD), optical flow micro-imaging. Much of this work is currently in the research phase and measurement services have yet to be defined. Collaborative European research (EMRP ClinBioTrace[34]) is under way for characterising protein structure using HDX and IMS and correlating this with activity. For CD some initial BAWG studies have been undertaken (CCQM-P59 and CCQM-P59.1) to address the comparability between laboratories in CD spectroscopy and to determine the measurement uncertainties. It is interesting to note that although X-Ray Diffraction is the 'classical' technique for protein structural determination, this was not a prominent technique in the NMIs work programmes.

Most large proteins are prone to aggregation, which severely limits the lifetime and safety of biopharmaceuticals, unless stored correctly. Present methods for measuring aggregation based on size exclusion chromatography have significant limitations and there has been a call for improved methods. Methods such as light scattering, CD, optical flow micro-imaging, IMS and ultrasound are currently being evaluated. When accomplished, the required measurement services in this area can be defined.

Table 15: Measurement Services Linked to Protein Structural Characterisation

Service	Remarks
Reference Method and Standard for Aggregation	Work being undertaken by NIST includes value assignment by optical flow micro-imaging. Electrospray IMS could also be a useful technique for this measurement.
Reference Standards and Reference Data for Protein Structure Characterisation	Aimed at ensuring that follow-on biologics are structurally and functionally the same as the 'original' drug. Also for helping to determine structural variants.

Table 16: Potential International Comparisons Linked to Protein Structural Characterisation

Comparison	Remarks
3D Structure Characterisation of a Single Purified Protein	Characterisation being undertaken as part of the EMRP ClinBioTrace project. To be considered by BAWG; techniques including CD, FT-IR, NMR, H-Deuterium Exchange LC MS/MS
Separation of Folding States of a Single Purified Protein	To be considered by BAWG; techniques include LC (Strong Cation Exchange, Size Exclusion Chromatography) and CE

Collaborative R&D Requirements

- Development of NMR for structural characterisation of proteins to enable a clear definition of the measurand

- Antibody characterisation – the development of techniques such as SPR, HDX, IMS to determine antigen interaction and correlate this with activity

6.2.5. Highly Multiplexed Measurements

Highly multiplexed analysis of proteins, RNA and metabolites in human serum will be required for the realisation of personalised medicine, which is being given high priority in the developed world. Estimates suggest that around 2 500 protein measurements may be required to support this. In addition the measurements will need to be comparable over the patient's lifetime to compare 'current' signatures with the patient's 'normal' signature, which requires the measurements to be traceable to recognised reference points. Current programmes, which will lead to new measurement services, cover microarray analysis and mass spectrometry. In the short-term these will address the discovery and validation of biomarkers and in the long-term, full 'disease signatures' requiring the simultaneous, quantitative measurements of thousands of species.

Microarrays currently do a reasonable job for qualitative measurements, but improvements are needed for quantitative measurements, in the short-term for clinical measurements, but in the longer term to measure accurately the change in concentration necessary for determining the molecular fluxes required for systems biology. NMIs are working on ways of improving fluorescent measurements and calibration of fluorescent beads in order to improve the quantitative performance of these measurements.

New measurement services are needed in the short-term for mass spectrometric proteomic analysis, to address the poor repeatability of these measurements. These will comprise reference standards to be used in concert with improved computational tools, which assist in the identification of clinically relevant biomarkers and also improve the comparability of cross-instrument measurements. In the longer term, the development of highly multiplexed mass spectrometric techniques is the probable answer to the accurate multiplex analysis of clinical samples. Performance indicator, 'horizontal' type reference materials, which are particularly relevant to relative measurements, need to be developed to support these highly multiplexed measurements. These should be in clinical matrices at clinically relevant concentrations and, ideally, simulate 'normal' and 'disease' states. It is the NMIs role to ensure that these standards have the required traceability.

Table 17: Measurement Services Linked to Highly Multiplexed Measurements

Service	Remarks
Calibration of Fluorescent Beads – quantifying fluorescence radiance	To improve quantitative performance of multiplexed measurements using fluorescence detection
Simulated 'Disease' and 'Normal' protein biomarker profiles in a clinical matrix e.g. plasma	Used to evaluate statistical classifiers for disease to support personalised medicine
'Horizontal' Reference Standards for multiplexed immunoassays	To validate the performance of highly multiplexed analytical platforms and help standardise data generated from different laboratories and on multiple platform technologies. Some existing commercially available proteomics standards are of questionable quality and are tailored for mass spectrometry and electrophoretic applications, and are not extended for use with immunoassays.

Table 18: International Comparisons Linked to Highly Multiplexed Measurements

Comparison	Remarks
Multiplexed, Traceable Protein Quantification	Assigning mass fraction of purified specific proteins in a buffer solution being considered by BAWG

Collaborative R&D

- Work to ensure highly multiplexed analyses are not biased for certain species e.g. size, hydrophobicity, isoelectric point, post-translational modifications and can cope with the required dynamic range; a minimum of 5 orders of magnitude.
- Develop systems for validating technologies which generate massive data sets to ensure that clinical conclusions derived from them are valid

6.3. Nucleic Acids

The measurement building blocks identified by the BAWG Nucleic Acid sub-group for studies and reference materials were:

- Nucleotides: amount, concentration, purity
- Oligonucleotides (<40 bases): sequence/identification, mass or amount concentration, purity
- Specific DNA Sequences (55-200 bp): sequence/identification, mass or amount concentration, purity
- Specific dsDNA Sequences (55-1 000 bp): qualitative analysis (fragment length)
- Total dsDNA (varying length): mass or amount concentration, purity
- Template RNA Characterisation: sequence/identification, quantitation
- Total RNA (varying length): quantitation, purity

Discussion on the type of studies required in support of the NMI's CMCs suggested that a very generic approach, based on identified 'building block' competences, may not be sufficient to support a CMC e.g. in quantitative PCR. A range of comparisons that cover a variety of matrices and concentrations may be required.

A gap analysis of the survey responses highlighted that qualitative (profiling) and quantitative specific NA sequence measurements and mass or amount concentration and purity of total NA were significant areas for future studies. Studies in support of sequence-based measurement traceability were also considered important. With respect to emerging measurement requirements, accuracy and comparability of next generation sequencing and structural genomic measurements, such as re-arrangements, duplications, loss and gain of sequences were identified.

A specific issue was raised regarding the traceability of nucleic acid measurements, in particular what corresponds to a 'classical' purity assessment? It was proposed that a comparison was required to address the problem of characterising allegedly pure nucleic acid reference material to the extent necessary to obtain its associated uncertainty down to the level required for calibration.

Although nucleic acid analyses are not used as widely in clinical analysis and biopharmaceutical production as protein measurements, analytical techniques are developing rapidly and nucleic acid measurements are replacing conventional analysis in areas such as pathogen identification and viral load determination. The fields where new measurement services are needed have been divided into four areas:

- Fundamental NA Metrology
- Qualitative/Sequence Analysis
- 2nd & 3rd Generation DNA Sequencing
- Quantitative Measurements

These are discussed in the following sections:

6.3.1. Fundamental Metrology

The ultimate goal for quantitative nucleic acid metrology is to anchor the measurements firmly to the SI units, in particular expressing the 'amount of substance' in terms of the mole. Early attempts to achieve this employed a variety of high accuracy analytical techniques including the use of ICP/OES to determine phosphorus content, thus quantify the nucleotides and IDMS to quantify either the nucleotides or nucleosides. These techniques are still relevant for oligonucleotides up to about 50 bases.

Recent work in this field has utilised 'digital' PCR, which is essentially a molecular counting technique. A recent paper[38] reviews the metrological principles for the use of identifying and counting techniques. Given the technical possibility of counting DNA elements, there is a need for a detailed consideration of the units of measurement arising from such techniques. Is the mole the most relevant unit or should the results be obtained in terms of 'copy number'? In addition, a key part of the measurement is to ensure that the entity being measured has been correctly identified and, in the case of nucleic acids, one key element is determining sequence purity.

Table 19: Measurement Services Linked to Fundamental NA Metrology

Service	Remarks
'Pure' calibration standard for nucleic acids	Material prepared for CCQM-P103 was a step towards this but larger quantities of material and more rigorous characterisation would be needed in order to produce a calibration standard
Measurement Protocols and Reference Standards for Digital PCR/Counting Techniques	Actively being considered by several BAWG NMIs, in particular by NMIA

Table 20: Potential International Comparisons Linked to Fundamental NA Metrology

Comparison	Remarks
Characterisation of 'pure' nucleic acid material	Being discussed by BAWG
Absolute quantitation of trace level plasmids or artificial chromosomes	Study suggested by KRISS

Collaborative R&D

- R&D on the sequence fidelity of DNA measurements e.g. PHRED score[39] and ways of treating its measurement uncertainty
- R&D on techniques for RNA purity assessment to facilitate quantitative RNA measurements. There is no clear view as to what is needed to produce a 'pure' calibration standard for NAs. Next Generation Sequence approaches could be a good way of determining RNA purity using deep sequencing techniques
- Examining the metrological implication of the use of 'counting' techniques for nucleic acid quantification and the appropriate units for expressing the results namely mole or copy number.

6.3.2. Qualitative/Sequence Analysis

A range of reference materials and sequence databases are required covering viruses and pathogens e.g. HPV, CMV; cancer markers e.g. BCR-ABL for leukaemia; a broader range of genomic standards for forensics; reference database for herbal medicines.

Table 21: Measurement Services Linked to Qualitative NA Sequence Analysis

Service	Remarks
DNA Length Measurement Standard	Length assigned using AFM technology with potential applications for determining the number of repeat units for diagnosis of Fragile X syndrome and characterising specific DNA-protein binding sites, which are key to the understanding of gene expression
'Best Practice' Guidelines, Quality Metrics and Standards for rapid, POC DNA testing systems	Aimed at devices such as disposable chips for diagnostics.
Sequence-based Reference Standards for Genetic Diseases	Examples: Huntington's Disease, Cystic Fibrosis, but specific priorities will be advised by the relevant professional bodies e.g. AMP. Other priorities listed by Eurogentest include Fragile X, Factor V Leiden and Hereditary Haemoglobinopathy.
Sequence-based RMs for Cancer Diagnostics	Examples, BRCA1 & 2, APC Gene and HNPCC gene.
Sequence-based Reference Standards for Viral NA	Example: HPV 1-16 panel.
RNA Reference Materials to Support Gene Expression Analysis	To support sequence detection for highly multiplexed technologies, especially the discrimination of closely related transcripts.

Table 22: Potential International Comparisons Linked to Qualitative NA Analysis

Comparison	Remarks
Specific DNA Sequence Measurements (e.g. panel of analytes for profiling)	Being considered by BAWG following findings of the Nucleic Acid Working Group

Collaborative R&D

- Development of a database of authenticated reference sequences in relevant areas such as herbal medicines

6.3.3. 2nd & 3rd Generation DNA Sequencing

The technology for whole genome sequencing is advancing rapidly and the time and costs are being reduced. For the full exploitation of this technology, it is important that the reliability of the sequence data is assured. Although the need for measurement services here is likely to be strong, currently the measurement organisations do not appear to have incorporated a significant amount of work into their programmes. However, it is anticipated that in the medium term there will be requirements for new measurement services to support this technology, not least the provision of reference sequence data and materials to validate the measurements, especially the fidelity of the sequencing.

Table 23: Measurement Services Linked to 'Next Generation' DNA Sequencing

Service	Remarks
Reference Standards for 'Next Generation' Sequencing	Defining the standards needed is still at a very early stage of development. They are likely to address the quality assurance needs for: sample preparation, the fidelity of the sequencing and a mechanism for checking the models for extracting the sequence data. Two examples under consideration are: mixtures of bacteria for metagenomic analysis and RNA sequences for gene expression profiling.

Table 24: Potential International Comparisons Linked to 'Next Generation' DNA Sequencing

Comparison	Remarks
Accuracy and Comparability of 'Next Generation' Sequencing	Indentified as being of potential interest for BAWG programme by the NA Working Group. CCQM-P103.1 study may be extended to incorporate 'Next Generation' Sequencing.

Collaborative R&D

- Research into the approaches for ensuring the comparability of 3rd Generation DNA sequencing
- Use of Next Generation Sequencing to detect structural genetic changes such as: rearrangements, duplications, loss & gains of sequences

6.3.4. Quantitative Nucleic Acid Measurements

There is a need for the development of reference materials for the measurement of viral load for e.g. HIV, HBV, HCV and studies into the molar absorption coefficient of nucleotides to help with quantitative measurements of 'total' NA.

As was the case with proteins (Section 6.2.5), there is a need to move from highly multiplexed qualitative assays, based on microarrays, to quantitative assays and similar efforts are being made to support this. Included in the NA work is the development of gene expression biomarker panels to serve as 'horizontal' standards for method validation.

Also included in this section is 'Epigenetics'. This is an emerging field relating to a stable heritable phenotype derived from changes in a chromosome without alterations in the DNA sequence e.g. through DNA methylation. The BAWG Epigenetic sub-group has made an initial assessment, but a full survey of the NMI's needs to support epigenetic measurements is yet to be carried out. The initial assessment highlighted the Epigenetic factors which could be considered for BAWG activities:

- DNA modification (e.g. methylation)
- Histone (the protein around which DNA is wrapped) modifications (e.g. methylation, phosphorylation and acetylation)
- Analysis of non-coding RNAs
- Analysis of non-histone DNA-binding proteins

The first studies in this area (CCQM-P94/94.1) have centred on the quantification of DNA methylation.

Table 25: Measurement Services Linked to Quantitative NA Measurements

Service	Remarks
Quantitative Reference Materials for Sequence Specific NAs	Example: BCR-ABL for Leukaemia diagnosis. Standards are under consideration by KRISS & LGC
Standards for Fidelity of DNA Amplification	A possibility would be a DNA reference material with a certified sequence which could be used to assess the fidelity of PCR enzymes during amplification.
Quantitative NA Reference Standards for Determining Viral Load	Being developed for diseases such as CMV. Other examples are: HIV, HBV and HCV. There are many requirements for standards. Priorities are being advised by bodies such as the AMP

Isotopically Labelled Modified Nucleotide Bases	Being developed by NIST for assessment of DNA damage and repair, caused by oxidative stress.
RNA Reference Standards to Support Gene Expression Analysis	NIST and LGC are working with ERCC on a standard to help improve the quality of gene expression analysis with respect to both sequence and relative quantification.
siRNA standards for Gene Therapy – size, sequence and concentration in tissues	For the longer term, because of the measurement challenges presented by their size and difficulty in sequencing.
Quantitative Reference Standards for Host Cell DNA	Standards relevant to *E. Coli* and CHO at the appropriate levels
Quantitative Reference Standard for Measurement of Foetal DNA in Maternal Blood	Being considered by LGC; areas under consideration include a 'trace material' certified for copy number (e.g. 70 copies/µl) or a material consisting of trace amounts of specific DNA in female DNA background

Table 26: Potential International Comparisons Linked to Quantitative NA Measurements

Comparison	Remarks
Measurement of Multiplexed Biomarker Panel of Multiple RNA Transcripts	Planned CCQM-P103.1 study for 2011
Relative Quantification of Genomic DNA Fragments Extracted from a Biological Tissue	Approved CCQM-KC68 to start 2010
Quantitative Specific Sequence DNA Measurement	Includes identification, mass concentration and purity assessment. The need was highlighted by BAWG Nucleic Acid Working Group. A GMO target study has already been completed and a diagnostic-relevant study is now being considered e.g. BCR-ABL or a viral sequence target.
Quantification of DNA Methylation	Required for epigenetics. KRISS-led P94 and P94.1 studies are complete. Further challenges which need to be addressed include the ratio of methylated to un-methylated cytosine bases in the relevant gene.
Total DNA Measurement	Includes mass, or amount concentration and purity assessment. Need highlighted by BAWG Nucleic Acid Working Group

Collaborative R&D

- R&D to address problems with matrix affects e.g. blood or tissue for gene specific quantitation of genomic DNA
- High accuracy determination of the UV Molar Absorption Coefficient of nucleotides, which would underpin routine DNA measurements
- Quantifying the modulation and effect on non-target genes for siRNA gene knockout therapy
- Developing an agreed paradigm for quantitative DNA standards – using transfected cells or plasmid insert; there is a debate between genomic standards derived from mass ingredients or tissues vs. synthetic standards derived from plasmids

6.4. Cells

For cell-based testing, emergent technologies such as *in vitro* predictive toxicology, which combines high-throughput reproducible screens with toxicogenomic, proteomic metabolomic and *in silico* techniques, have the potential to circumvent the use of animal tests and speed up drug development. Such methods are still being perfected, but they are likely to be progressively introduced. Measurement services for the technique in the medium-term include the development of stem cells that can be harnessed as reference lines of an 'infinite' source of genetically identical cells for use in the tests. Reference standards having well-characterised toxicity profiles for performance testing set-ups and procedures will be needed. A long-term measurement service need is the provision of a panel of well-characterised immortalised, or primary cell lines which represent the 'controlled cell' to which other cell measurements can be compared. Ideally, the measurement of the controlled cells characteristics should be tied to the SI, or other recognised units, thus enabling the traceability of measurement to these units with the aim of significantly improving cell-based measurement comparability, which is currently very variable.

New cell-based therapeutics include stem cell therapy and tissue engineered products. The regulatory controls for these products are still being defined and the regulators have called for the development of new measurement science, standards and technology and other measurement services to assess the viability and function of cells used for therapeutic services. For stem cells, critical measurements include the identification of differential markers to ensure that the cell is of the desired phenotype. This will require the provision of standard phenotypes for differentiated cells. In the short-term, standards for benchmarking optical imaging and flow cytometry used to identify and count cells are needed.

Almost all tissue engineered products incorporate a scaffold, or biomaterial, designed to mimic the extracellular matrix and support the viability of the cells to be replaced. In addition, as products become more complex, there may be a requirement for the cells to grow and differentiate within the product. The measurement of both the scaffold and cells within the product present some formidable analytical challenges. Industry is looking for innovative methods for assessing the intrinsic variability in the active cellular component of the product and for characterising product quality. In the short to medium term there is a need to develop a standard model of a tissue engineered product against which the developing analytical techniques can be objectively assessed.

Work of the BAWG sub-group has concentrated on what would constitute a 'traceable' cell measurement. The variability in cell measurements and the lack of standards and measurement uncertainty determinations have been highlighted as concerns by the biopharmaceutical and regenerative medicine industries in demonstrating product quality, safety and efficacy in regulatory submissions.

The particular measurement services requested by NMIs related to the quantitation of cells in suspension/cells adhered and 3D cells measurement in tissue. In response to this, the sub-group has decided to focus on cell quantitation. Specific areas for studies are:

- Quantify cells number/area, geometry and population distribution
- Number and geometric property of cells adhered on a solid substrate (CCQM-P123)
- Quantify a specific phenotype/matrix
- Quantification of cells with specific phenotypic characteristics
- Quantify 'living' cells
- Quantify the concentration of a molecular ligand

6.5. Nanobiotechnology

The BAWG strategy in this area is in the early stages of formulation and a NMI survey of requirements has yet to be undertaken. Initial discussions have focused on nanoparticles and two CCQM workshops have helped to develop the thinking. Areas where there are requirements for future measurement services include:

- Characterisation of nanoparticles in biological media
- Characterisation of proteins and other biomolecules bound to nanoparticles
- Reference materials e.g. positive and negative controls for nano-toxicology
- International comparisons on nano-toxicology

7. Conclusions

The Conclusions focus on the areas of work on proteins or nucleic acids, identified during the study, which are likely to have significant strategic value in underpinning and ensuring the comparability of these measurements. These are the areas which are likely to be of most interest to the BIPM. However, comments from stakeholders on an earlier draft have highlighted the importance of imaging technologies. They were not included because this area was not of direct or immediate interest to the BIPM in its initial programme, nor has it featured strongly in the BAWG activities to date, although this may change as active discussions on imaging are under way in collaboration with the SAWG. Therefore, in view of its importance and its inclusion in several NMI's R&D programmes, it should be re-considered in any future BIPM studies or programmes.

Proteins

1. Requirements for underpinning work to assure the traceability of quantitative measurements of proteins to the SI.

   This should be initiated on 'simple' and well-characterised proteins, such as insulin, or hGH. It comprises high accuracy measurements and purity determinations on amino acids, peptides and proteins using IDMS, qNMR and high accuracy water determination. NIMJ have already produced four high purity amino acid standards, but more are needed as these are key high purity protein standards

2. Strengthening of cooperation between the WHO, IFCC, BIPM and NMIs to ensure a coordinated approach to the migration from IU to SI.

   Advances in physico-chemical techniques and the metrology support work described above will enable a greater number of protein measurements to be made traceable to the SI, with the advantage of improved consistency of measurement over time and space. There is a strong desire from industry, the regulators and the NMIs for a co-ordinated approach to the migration from the IU to the SI to avoid potential confusion.

3. Development of an approach involving metrological principles where traceability to the SI is unlikely to be feasible for the more complex proteins.

   Although conventional traceability back to the SI will be feasible for well-defined proteins, for more complex protein measurements, where there are clinically significant post translational modifications and where isoforms and activity are being measured, this will not be feasible for many years. Work is needed to develop an alternative approach to examine how the use of metrological principles can be applied in these cases, possibly linking it to the use of new techniques such as HDX, IMS, optical flow micro-imaging for structural characterisation. Studies to support measurement comparability for post translational modification analysis are also required.

4. Validation of highly multiplexed measurements.

   Highly multiplexed measurements of proteins in clinical matrices will be crucial for the support of 'personalised medicine'. Approaches need to be developed, including the use of 'horizontal' reference standards and simulated diseased and non-diseased states to validate the methods to ensure that the measurements are not biased and that the conclusions derived from the processing of the very large datasets generated are clinically valid.

5. Development of traceability chains for inherently unstable proteins.

   The instability/dynamic nature of many proteins of medical significance, including biopharmaceuticals makes it difficult to devise a practical traceability chain. Work is required to develop an approach which takes into account stability uncertainty and addresses problems such as the shipping of samples for measurement comparability studies.

6. Development of a modified system for 'Calibration and Measurement Capability Claims' which is more relevant to proteins and other bio-entities.

   Because of the sheer number of proteins of clinical and therapeutic significance and the time and resources required to develop reference standards, it is unlikely that there will be a standard for each one and that the established procedure of registering Calibration and Measurement Capability Claims by NMIs will be feasible for many bio-measurements. Therefore thought needs to be given to alternative ways of assuring the validity of all protein measurements including the development of an alternative approach for 'Bio-Measurement Claims'.

Nucleic Acids

7. Development of techniques for the treatment of measurement uncertainty in NA sequence determination.

   Sequence is a 'nominal' property (i.e. the property has no magnitude) which raises issues regarding metrological traceability. For conventional Sanger sequencing, approaches to assessing sequence fidelity based on PHRED score have been developed, which would make the measurement traceable to the nucleotide reference. Comparable approaches need to be developed for 2nd and 3rd Generation sequencing.

8. Improved 'Total' DNA Measurement.

   Detailed, underpinning work is needed for Total DNA measurements, including mass, or amount concentration, purity assessment and the high accuracy determination of the UV Molar Absorption Coefficient of nucleotides to support the UV measurements commonly used to determine 'Total' DNA.

9. Quantitative RNA Measurements.

   In order to underpin quantitative RNA measurements, work is required to develop methods for purity assessment and determine what is needed to produce a 'pure' calibration standard for nucleic acids. Methods based on Next Generation sequencing are probably the best approach to nucleic acid purity determination.

10. Highly Multiplexed NA Measurements.

    In parallel with protein measurements, similar efforts are needed, such as the development of 'horizontal' reference standards to ensure that the measurements are not biased and the validation of data processing protocols.

11. The metrological implications for the use of 'counting' techniques for quantitative analysis.

    The advent of 'digital' PCR and the application of 'copy number' have raised questions relating to the use of 'counting' techniques and whether copy number, rather than the mole would be a more appropriate unit for amount of substance in this field. Co-ordinated international discussions to resolve the metrological implications are needed.

12. Authenticated International Reference Databases.

    There is a need for authenticated international reference DNA databases for selected areas e.g. herbal medicine. An organisation is required to co-ordinate and host the databases. Issues to be addressed include the identification of the definitive sequence characterising the sample identity and the appropriate quality control criteria.

8. References

1. BIPM Invitation to Tender Ref. AO/BIPM/CHIM/2009/015
2. Report on the Strategy for Health Care through Bio and Information Standards and Technologies Conference, 24-25 September 2007, NIST
3. Economic Analysis of the Technology Infrastructure Needs of the US Biopharmaceutical Industry, NIST Planning Report 07-1, November 2007
4. Conference Report: Accelerating Innovation in 21st Century Biosciences: Identifying the Measurement, Standards and Technological Challenges, NIST Special Publication 903034, July 2009
5. Measurement Challenges to Innovation in the Biosciences: Critical Roles for NIST, March 2009, NIST
6. Draft Report: Measurement Science and Measurement Standards to Support Innovation in Healthcare, NIST, June 2009
7. Prepared Statement and Testimony of Dr Willie E. May, NIST before the U.S. House of Representatives Committee on Science and Technology Hearing on 'The Potential Need for Measurement Standards to Facilitate the Research and Development of Biologic Drugs', 24 September 2009
8. Statement of Dr Steven Kozlowski, FDA before the U.S. House of Representatives Committee on Science and Technology Hearing on 'The Potential Need for Measurement Standards to Facilitate the Research and Development of Biologic Drugs', 24 September 2009
9. Testimony of Dr Anthony Mire-Sluis, Amgen Inc. before the U.S. House of Representatives Committee on Science and Technology Hearing on 'The Potential Need for Measurement Standards to Facilitate the Research and Development of Biologic Drugs', 24 September 2009
10. Prepared Statement and Testimony of Dr Patrick Vink, Mylan Inc. before the U.S. House of Representatives Committee on Science and Technology Hearing on 'The Potential Need for Measurement Standards to Facilitate the Research and Development of Biologic Drugs', 24 September 2009
11. DOE Genomics: GTL Roadmap, August 2005, US Department of Energy
12. DOE Genomics: GTL 2008 Strategic Plan, September 2008, US Department of Energy
13. National Institute of Health Roadmap
14. The Canadian Biopharmaceutical Industry Technology Roadmap: Challenges and Innovative Solutions, 2006, Industry Canada
15. National Research Council Canada Genomics and Health Initiative Phase IV, 2008-2011
16. European Union Seventh Framework Programme, 2007-2013
17. European Metrology Research Programme: Outline 2008, November 2008, Euramet
18. Personal Communication, Helen Parkes, LGC
19. Analysis of Government Policy Objectives and NMS Interventions, March 2009, UK National Measurement Office
20. The National Measurement System Strategy, May 2009, UK National Measurement Office
21. National Measurement System Programme Roadmaps, May 2009, UK National Measurement Office
22. The Microarray Quality Control (MAQC) Project, FDA, Nov 2006
23. Maximising Opportunities from Industrial Biotechnology in a Low Carbon Economy, May 2009, UK Department for Business, Enterprise and Regulatory Reform
24. The Review and Refresh of Bioscience 2015, Jan 2009, UK Department for Business, Enterprise and Regulatory Reform
25. Government Response to Refresh and Review of Bioscience 2015, May 2009, UK Department for Business, Enterprise and Regulatory Reform
26. Biosciences Technology Strategy 2009-2012, September 2009, UK Technology Strategy Board

27. Medicines and Healthcare Strategy 2009-2012, October 2009, UK Technology Strategy Board
28. Dialogue: Biomeasurement: A Brand New World in Scientific Field
29. Personal Communication from Dr Sang-Ryoul Park, KRISS
30. The Australian Government's Innovation Report 2005-06
31. Council Task Force on Biotechnology, August 2009, ISO AIC048-09
32. World Preview 2014, May 2009, EvaluatePharma
33. "CCQM Bioanalysis working group: Terms of Reference and Routemap to the future" - www.bipm.org/wg/CCQM/BAWG/Restricted/April_2007/BAWG07-24.pdf
34. European Metrology Research Programme Joint Research Project T2.J.11 'Traceability of Complex Biomolecules and Biomarkers in Diagnostics – Effecting Measurement Comparability in Clinical Medicine'
35. ICH Harmonised Tripartite Guideline Q5E
36. EMEA Guideline CHMP/42832/05
37. EMEA Guideline CHMP 49348/05
38. Price G., and De Bievre P., *Accred Qual Assur,* 2009, **14**(6), 295-305
39. Rousseau F., *et al., Clin Chem Lab Med,* 2009, **47**(11), 1343-1350
40. CCQM BAWG Minutes of Meeting 16, Brazil, November 2009
41. Study Report: Report Number: LGC/RT/2010/064, 17 September 2010

9. Acronyms and initialisms

ACRM Asian Collaboration on Certified Reference Materials

AFM Atomic Force Microscopy

AMP Association for Molecular Pathology

ANP Atrium Naturietic Peptide

APC Adenomataous Polyposis Coli Tumour Suppressor Gene

API Active Pharmaceutical Ingredient

ATCC American Type Culture Collection

BAWG BioAnalysis Working Group of the CCQM

BCR-ABL An oncogene fusion protein comprising BCR and ABL associated with chronic myeloid leukaemia

BIPM Bureau International des Poids et Mesures

BNP Brain Natriuretic Peptide – marker for cardiac disease

Bp Base Pairs

BRCA Human Tumour Suppressor Gene

CA 125 Cancer Antigen 125 – a tumour biomarker

CA 15-3 A marker for breast cancer

CA 19-9 A marker for bowel and pancreatic cancer

CCQM Consultative Committee for Amount of Substance – Metrology in Chemistry

CD Circular Dichroism spectroscopy

CE Capillary Electrophoresis

ChIP Chromatin Immunosuppression

CHO Chinese Hamster Ovary – cells used for production of recombinant therapeutics

CLGGS Voluntary organisation developing best practices for microarray-based clinical assays

CMC Calibration and Measurement Capabilities for Annex C of the CIPM MRA

CMV Cytomegalovirus – a type of herpes virus

CODEX Commission created by the UN's Food & Agriculture Organisation and World Health Organisation to develop food standards

CRM Certified Reference Material

CRP C-Reactive Protein

DNA Deoxyribonucleic Acid

dNMP Deoxyribonucleoside monophosphate

dsDNA Double Strand DNA

DSP Diarrheic Shellfish Poisoning

ELISA Enzyme-Linked Immunoabsorbent Assay

EMEA European Medicines Agency

EMRP European Metrology Research Programme

ERCC External RNA Control Consortium – *ad hoc* group developing RNA transcripts for microarray performance assessment

FACS™ Fluorescence Activated Cell Sorting – a specialised form of flow cytometry

FDA U.S. Food & Drug Administration

FISH Fluorescent *In Situ* Hybridisation – used to detect specific DNA sequences on chromosomes

FTE Full Time Equivalent

FT-IR Fourier Transform Infrared spectroscopy

GCSF Granulocyte Colony-Stimulating Factor – a cytokine stimulating bone marrow to produce granulocytes

GDP Gross Domestic Product

GMO Genetically Modified Organism

GTL U.S. Dept. of Energy program using microbial genome data for the investigation of microbes

HbA2 Gene coding for the α-chain of haemoglobin

Hb1Ac Glycated Haemoglobin – used to measure plasma glucose concentration

HBV Hepatitis B Virus

hCG Human Chorionic Gonadotropin – marker for pregnancy and certain tumours

HCP Host Cell Protein

HCV Hepatitis C Virus

HDX Hydrogen-Deuterium Exchange – mass spectrometric technique for characterising protein structure

HER-2 Human Epidermal Growth Factor Receptor 2 – protein associated with aggressive breast cancer

hGH Human Growth Hormone (Somatotropin)

HIV Human Immunodeficiency Virus

HNPCC Gene associated with rectal cancer

HPLC High-Performance Liquid Chromatography

HPV Human Papillomavirus – can cause genital warts and lead to cervical cancer

ICAT Isotope-Coded Affinity Tag

ICH International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use

ICP-MS Inductively-Coupled Plasma Mass Spectrometry

IDMS Isotope Dilution Mass Spectrometry – high accuracy MS technique using isotopically labelled internal standards

IFCC International Federation of Clinical Chemistry and Laboratory Medicine

IL-2 Interleukin 2; a type of cytokine

IL-4 Interleukin 4; a type of cytokine

IMS Ion Mobility Spectroscopy

INRIM Istituto Nazionale di Ricerca Metrologica, Italy

IRMM European Commission's Institute for Reference Materials and Measurements

ISO International Standards Organisation

iTRAQ Isobaric tag used for absolute protein quantification by mass spectrometry

IU International Unit – measurement of the amount of substance based on bio-activity

IVD *In Vitro* Diagnostic

IVDD European *In Vitro* Diagnostic Devices Directive

JCTLM Joint Committee for Traceability in Laboratory Medicine

KC Key Comparison

KCDB BIPM Key Comparison Database

KRISS Korea Research Institute of Standards and Science, the Republic of Korea

LC Liquid Chromatography

LCMS Liquid Chromatography and Mass Spectrometry - hybrid analytical technology

LGC UK designated NMI for chemical and biochemical measurements

LNE Laboratoire National de Métrologie et d'Essais, France

MAB Monoclonal Antibody

MALDI A 'soft' ionization technique used in mass spectrometry

MAQC Microarray Quality Control Project

MeCAT Metal Coded Tagging Technology

MESF Molecules of Equivalent Soluble Fluorophore

miRNA MicroRNA – a type of non-coding RNA active in gene regulation

mRNA Messenger RNA

MS Mass Spectrometry

NA Nucleic Acid

NCCS National Centre for Cell Sciences, India

NHS National Health Service, UK

NIBSC National Institute for Biological Standards and Control, UK

NIMC National Institute of Metrology, China

NMS National Measurement System, UK

NMI National Measurement Institute

NMIA National Measurement Institute, Australia

NMIJ National Metrology Institute of Japan

NMR Nuclear Magnetic Resonance Spectroscopy

NPL National Physical Laboratory, UK

OECD Organisation for Economic Co-operation and Development

PAPPA Pregnancy-Associated Plasma Protein A

PCR Polymerase Chain Reaction – technique used to amplify DNA fragments as a precursor to detection

POC Point of Care Testing

PHRED Score used to characterise the quality of DNA sequences

PK Pharmacokinetic

PSA Prostate-Specific Antigen – a marker for prostate cancer

PSP Paralytic Shellfish Poisoning

PTB Physikalisch-Technische Bundesanstalt, Germany

PTM Post-Translational Modification

QA/QC Quality Assurance/Quality Control

QPCR Quantitative PCR

qNMR Quantitative NMR

RM Reference Material

RMS Reference Measurement System

RNA Ribonucleic Acid

SAWG BIPM Surface Analysis Working Group

SCX Strong Cation Exchange

SEC Size-Exclusion Chromatography

siRNA Short-strand RNA used to ‘silence’ specific genes

SPR Surface Plasmon Resonance – non-label technology used for measuring biomolecular interactions

STR Short Tandem Repeat – used in DNA profiling

TCBM Chinese National Technical Committee for Biometrology

TSH Thyroid-Stimulating Hormone

UPLC Ultra-Performance Liquid Chromatography –uses smaller particle size column packing material

USP U.S. Pharmacopeia

UV Ultraviolet

WHO World Health Organization

Annex I: Participating Organisations and Staff Interviewed

Measurement Institutes

CDRI

Central Drug Research Institute (Council of Scientific and Industrial Research, India) specialising in pharmaceutical-related/drug research

Dr Dinesh Dikshit

Dr GK Jain

AK Saxena

CENAM

NMI of Mexico

Dr Yoshito Mitani

Dr Melina Pérez

GLHK

Government Laboratory of the Hong Kong Special Administrative Region (HKSAR) of the People's Republic of China

Dr Della Sin

Dr CS Mok

Mr BKK Cheung

Dr KY To

Ms Christina Li

HAS

Designated Institute of Singapore

Dr LeeTong Kooi

Ms Tan Wan Loo

INMETRO

NMI of Brazil

Lilian Costa

Danielle Cavalcanti

Celso S'antanna

Andrea Martinny

Gustavo Menezes

INRIM

NMI of Italy

Maria-Paola Sassi

KRISS

NMI of South Korea

Dr Sang-Ryoul Park

LGC

Designated UK NMI for Chemical and Biochemical Measurements

Julian Braybrook

Carole Foy

Gavin O'Connor

Helen Parkes

LNE

NMI for France

Phillippe Charlet

Vincent Delatour

NIBSC

UK National Institute for Biological Standards and Control

Adrian Bristow

Chris Jones

Chris Burns

NIMC

NMI for China

Wang Jing

Wu Liqing

Fu Boqiang

Zhang Ling

Dong Lianhua

Sheng Linghui

NIMC (continued)

Mi Wei

Liu Yingying

NIMT

NMI for Thailand

C Chainarong

V Duangkamol

W Kanjana

N Kanokwan

P Chaiwat

NIST

NMI for U.S.

Laurie Locascio

Anne Plant

Steven Choquette

Miral Dizdar

John Butler

Steve Wise

Karen Phinney

Mike Tarlov

John Small

Keena Scott

Michael Amos

NMIA

NMI of Australia

Dr Laurie Besley

Dr Lindsey Mackay

Dr Kerry Emslie

NMIJ

NMI of Japan

Koichi Chiba

NMISA

NMI of South Africa

Jayne de Vos

Desiree Prevoo

Sara Prins

NPL

NMI of the UK

Alan Brewin

Alex Knight

Adrian Horgan

NPLI

NMI of India

Prabhat Gupta

Prof. RC Bundhani

Dr Bansi Malhotra

Dr AM Biradar

Ranjana Mehroira

PTB

NMI of Germany

Bernd Guettler

USP

US Pharmacopeia

William Koch

Tina Morris

Kevin Hool

Mike Ambrose

Industry

Abbott Diagnostics

Diagnostics Division of U.S. Drugs Company

David Armbruster

AGEPS

France-based Healthcare Support Company specialising in the supply and distribution of drugs and medical devices and the implementation of clinical trials.

Sandrine Roy

Biocon

A major Indian Drug Company specialising in Biopharmaceuticals & Bio-similars

Dr Laxmi Adhikary

BioRad Laboratories

European operations of the U.S. Diagnostics Company

Dr Claude Giroud

DiagnosticaStago

French manufacturer and formulator of a broad range of reagents and analytical instruments for haemostasis

Emmanuel Jouvent

Frederick Esteve

Dr Reddy's

India's second largest Pharmaceutical Company

Darshan Koticha

Natasha Shanker

Sandhay Kumaraswamy

Samir Kulkami

Saranya Sivakumar

Anjali Mahajan

Venkata Yeturu

Hari Prasad Reddy

European Biopharmaceutical Manufacturer

Collaborated in the interviews, but has requested anonymity

Interviewed five staff involved in quality, analytical support, product support and reference materials

LFB

French producer of plasma-derived medicinal products

Dr Sebastien Bagot

Lonza Biologics

Division of Lonza specialising in the contract manufacture of biopharmaceuticals

Mike Davies

Regulator & Professional Body

FDA

U.S. drug regulator

Elizabeth Mansfield

Robert Becher

Abraham Tzou

Sally Hojvat

Donna Roscoe

Loise Magruder

Joshua Levin

Pat Reeves

Nisar Pampori

Karen Bijwaard

Tamara Feldblyum

Uwe Scherf

Martin Ruta

IFCC

International Federation of Clinical Chemistry and Laboratory Medicine

Prof Mauro Panteghini

Annex II: NMI Interview Form

Measurement Service and Comparison Needs for an International Measurement Infrastructure for the Biosciences and Biotechnology: Interviews with NMIs and Other Measurement Organisations

Details

Organisation:

Location:

Person(s) Interviewed:

Position:

Email:

Tel:

Interviewer:

Date:

Remarks:

Introduction

LGC have been contracted by the BIPM to provide input to their proposed new Bio-measurement programme. A major part of this input will be obtained through interviews with key organisations in the field. BIPM complements the work of the National Measurement Institutes (NMIs) by undertaking work which requires a long-term strategic commitment and which can include the organisation of Key Comparisons to underpin measurements in the biosciences.

In order to help define their Bio-measurement work programme, the BIPM are particularly interested in answers to the following questions:

1. Measurement services required to establish an International Measurement Infrastructure for the Biosciences that are expected to be developed and delivered in the next 3 – 10 years by NMIs or other organisations developing measurement standards or methods for the biosciences
2. International comparisons that are required to demonstrate the degree of equivalence of the measurement services that are, or will, be developed and delivered
3. Research and development activities necessary for the development of higher metrological order measurement standards and methods for the Biosciences

The information obtained from the interviews will be analysed and a draft report of the study will be placed on the BIPM website and distributed to stakeholders for comment. Account will be taken of the comments and a final report produced.

Questionnaire

Section 1: Profile of the Organisation

1.1 How long has your organisation been involved in Bio-measurements?

< 2yr ☐ 2-5 yr ☐ >5yr ☐

Remarks:

1.2 What types of Bio-measurement are regularly undertaken by your organisation?

Proteins ☐

Nucleic Acid ☐

Cells/Bioassay ☐

Others (Please Specify)

Remarks:

1.3 What are the main analytical techniques you use to undertake Bio-measurements in your laboratory?

Remarks:

1.4 To which sectors does your work primarily relate?

Healthcare –Diagnostics/Clinical ☐

Healthcare – Therapeutics ☐

Health Technologies

– Tissue Engineering ☐
- Regenerative Medicine ☐
- Drug Delivery ☐
- Implants ☐

Forensics ☐

Industrial Biotechnology

- Bioprocessing ☐
- Bioenergy ☐

Food

- GMOs ☐
- Food Safety ☐
- Nutraceuticals ☐

Environment ☐

Others (Please Specify)

Remarks:

1.5 How many staff (Full Time Equivalents) are currently engaged in Bio-measurement activities?

<5 ☐ 5-10 ☐ 11-20 ☐ 21-50 ☐ 51-100 ☐ >100 ☐

Remarks:

1.6 What proportion of the above effort relates primarily to metrological activities?

Remarks:

1.7 What have been the main outputs from your Bio-measurement activity:

Reference Materials ☐ Publications ☐ Reference Methods ☐ Calibrations ☐

Others (Please Specify)

Remarks:

1.8 What growth in your organisation's Bio-measurement activities do you anticipate over the next 5 years?

Decline ☐ Same Level ☐ 10-25% Increase ☐ >25% Increase ☐

Remarks:

1.9 If your activity is likely to increase significantly, which areas are expanding and what are the main drivers?

Remarks:

Section 2: CCQM Activities

2.1 Which areas of CMCs do you envisage claiming over the next 10 years?

Proteins ☐ Nucleic Acids ☐ Cells/Bioassays ☐

Others (Please Specify)

Remarks:

2.2 What types of study will be required to support your CMCs, or your position as an NMI?

Remarks:

2.3 Does your organisation envisage leading any BAWG studies in the next 3 – 10 years?

Remarks:

If so, in which areas?

Proteins ☐ Nucleic Acids ☐ Cells/Bioassays ☐

Other (Please Specify)

Remarks:

Section 3: Metrology Activities Relating to Protein and Nucleic Acid (NA) Measurements

3.1 Is your organisation involved in the development of Reference Materials, Reference Methods or in providing Value Assignment Services for protein or NA materials?

If so, for which sectors?

Diagnostics/Clinical ☐ Therapeutics ☐ Food ☐ Environment ☐ Bio-processing

Forensics ☐ Others (Please Specify)

Remarks:

3.2 <u>Diagnostics/Clinical</u>:

If your organisation is planning to support the Diagnostics/Clinical sector with Reference Materials, Reference Methods or Value Assignment Services, please answer the following:

3.2.1 What are the key types of protein or NA analysis which need to be supported by higher order Reference Materials, or Reference Methods?

Remarks:

3.2.2 What are the main measurement issues/challenges in the key types of analysis?

Remarks:

3.2.3 How does your organisation intend to help to address these issues over the next 10 years?

Remarks:

3.2.4 What properties (or quantities) of proteins or nucleic acids are you interested in value assigning (e.g. amount, structure, sequence, activity etc) and why?

Remarks:

3.2.5 Have you or are you planning to submit nominations for the JCTLM Database of higher order reference materials, methods and services in support of the IVD industry in the bio-measurement field?

Remarks:

3.2.6 What are the underpinning requirements which would need long-term support on an international level, such as regular inter-laboratory comparisons, or competence demonstration?

Remarks:

3.2.7 Do you envisage any scheme for classifying the types of protein and NA measurement required for diagnostics that would help to define which Reference Materials or inter-laboratory comparisons would be key for assisting a range of measurements?

Remarks:

3.2.8 What broad types of protein Reference Materials will be required by the diagnostics/clinical sector over the next 10 years?

Remarks:

3.2.9 What inter-laboratory comparisons will be needed to support the assignment of these Reference Materials to ensure that the assigned values have international acceptability?

Remarks:

3.2.10 What are the long-term R&D activities or inter-laboratory comparisons that are needed to underpin protein or NA measurements for diagnostics/clinical analysis which you will not be able to undertake or organise, but would benefit from?

Remarks:

3.2.11 Which are the main organisations that you are collaborating with in this field?

Remarks:

3.2.12 What new technologies do you see influencing the diagnostics/clinical area over the next 10 years?

Remarks:

3.2.13 What do you see as the main measurement issues influencing the traceability and consistency of results from the new technologies you envisage?

Remarks:

3.3 Therapeutics

If your organisation is planning to support the Therapeutics sector with Reference Materials, Reference Methods or Value Assignment Services relating to proteins or NAs, please answer the following:

3.3.1 What are the key types of protein analysis which need to be supported by higher order Reference Materials, Methods or Value Assignment Services?

Remarks:

3.3.2 What are the main measurement issues/challenges in the key types of protein or NA analysis?

Remarks:

3.3.3 How does your organisation intend to help to address these issues over the next 10 years?

Remarks:

3.3.4 What properties (or quantities) of proteins or nucleic acids are you interested in value assigning (e.g. amount, structure, sequences, activity etc) and why?

Remarks:

3.3.5 What are the underpinning requirements which would need long-term support on an international level, such as regular inter-laboratory comparisons, or competence demonstration?

Remarks:

3.3.6 Do you envisage any scheme for classifying the types of protein and NA measurement required for therapeutics, which would help to define which Reference Materials or inter-laboratory comparisons would be key for assisting a range of measurements?

Remarks:

3.3.7 What broad types of protein or NA Reference Material will be required by the pharmaceutical industry over the next 10 years?

Remarks:

3.3.8 What inter-laboratory comparisons will be needed to support the assignment of these Reference Materials to ensure that the assigned values have international acceptability?

Remarks:

3.3.9 What are the long-term R&D activities or inter-laboratory comparisons that are needed to underpin protein or NA measurements for bio-pharmaceutical analysis which you will not be able to undertake or organize, but would benefit from?

Remarks:

3.3.10 Which are the main organisations that you are collaborating with in this field?

Remarks:

3.3.11 Do you envisage any new type of bio-therapeutic over the next 10 years for which new types of protein or NA measurement will be required?

Remarks:

3.3.12 What do you see as the main measurement issues influencing the traceability and consistency of results from the new types of measurement that you envisage?

Remarks:

Annex III: Industry Interview Form

Measurement Service and Comparison Needs for an International Measurement Infrastructure for the Biosciences and Biotechnology: Interviews with Industrial Organisations and Trade Associations

Details

Organisation:

Location:

Person(s) Interviewed:

Position:

Email:

Tel:

Interviewer:

Date:

Remarks:

Background

The BIPM is the International Weights and Measures Organisation tasked through a diplomatic treaty, the Convention of the Metre, with ensuring world-wide uniformity of measurements and their traceability to the International System of Units (SI). Historically the activities of the BIPM were exclusively based on physical measurements and the International Standard for the kilogram is housed in the BIPM's laboratories in Sèvres, near Paris. Recently they have undertaken a programme of work on chemical measurements and they are now looking to introduce a programme on bio-measurements. The aim of the programme is to support efforts being undertaken by several National Measurement Institutes (NMIs) and other measurement organisations to assure the global comparability of bio-measurements, through traceability to the SI. This is clearly not a trivial task and may not be achievable in every case.

As a first step, the BIPM want to identify areas of potential activity for them to undertake in protein and nucleic acid (NA) measurements applied to healthcare, both diagnostics and therapeutics. These areas were selected on the basis of their potential for overcoming some of the measurement comparability issues, by making them traceable to the SI, and of their importance to human wellbeing. The BIPM have contracted LGC (the UK NMI for chemical and biological measurements to undertake a study to provide input for a proposed BIPM work programme on bio-measurement. The BIPM are particularly interested in answers to the following questions:

1. Measurement services required to establish an International Measurement Infrastructure for the biosciences that are expected to be developed and delivered in the next 3 – 10 years by NMIs or other organisations developing measurement standards or reference methods for the biosciences
2. International comparisons between NMIs that are required to demonstrate the degree of equivalence of the measurement services that are or will be developed and delivered
3. Research and development activities necessary for the development of higher metrological order measurement standards and methods for the biosciences

Of special interest are areas where there are difficulties in achieving comparable results and the issues associated with harmonising the measurements, in particular where the concepts of metrological traceability will be beneficial and applicable, as well as progress towards physico-chemical characterization of biologicals and value assignment of their properties in SI units.

The information obtained from the interviews will be analysed and a draft report of the study will be placed on the BIPM website and distributed to stakeholders for comment. Account will be taken of the comments and a final report produced.

Questionnaire

Section 1: Profile of Your Organisation

1.1 What are the main activities of your organisation, which relate to bio-measurement?

Bio-pharmaceutical Discovery and Development ☐

Bio-pharmaceutical Production ☐

Bio-pharmaceutical Standards ☐

Diagnostic Devices Research and Development ☐

Diagnostic Devices Production and Supply ☐

Clinical Testing Methods Development ☐

Others (Please Specify)

Remarks:

1.2 What is the value of your business which is reliant on bio-measurements?

<$10M ☐ $10-$100M ☐ $100M-$1000M ☐ >$1000M NA ☐

Remarks:

1.3 Do you have any direct contacts with your NMI, either for Reference Materials, or Calibration Services for bio-measurements?

Yes ☐ No ☐ Don't Know ☐

Remarks:

1.4 What are the main sources of your biological standards?

WHO ☐ USP ☐ EP ☐ Others (Please State)

Remarks:

Section 2: Use of Measurement

2.1 What types of bio-measurement are regularly undertaken by your organisation?

Proteins ☐ Nucleic Acid ☐ Cells/Bioassay ☐

Others (Please Specify)

Remarks:

2.2 What properties of these systems (e.g. amount, structure, sequence, activity etc) do you measure and what techniques are used for the measurements?

Proteins:

Remarks:

NAs:

Remarks:

Cells:

Remarks:

Others:

Remarks:

2.3 For which areas of application are the measurements made?

Therapeutics – Drug Discovery and Development ☐

Therapeutics – Production and Quality Control ☐

Diagnostics – Lab-based measurements ☐

Diagnostics – Point of Care measurements ☐

Others (Please Specify)

Remarks:

2.4 Which types of protein or Nucleic Acid (NA) analysis give significant problems regarding the comparability of results?

Remarks:

2.5 For these problem areas, what steps could be taken to improve the comparability of the results?

Remarks:

2.6 Are Reference Materials or Reference Methods of Analysis available for the problem analyses?

Remarks:

2.7 Do you require quantitative protein or NA measurements (absolute, total, specific)?

Remarks:

2.8 Which properties of proteins and NAs do you measure in SI units?

Remarks:

2.9 Are there properties of proteins and NAs that are currently measured in non-SI units where progress in physico-chemical characterization techniques could allow SI traceable values to be obtained in the near future? Please give examples.

Remarks:

2.10 How important is it for the results of your measurements to be comparable on a Global scale?

Remarks:

2.11 Given the ICH Quality Guideline requirements with respect to reference standards, what are the most critical areas of protein or NA analysis where higher order Reference Materials or Reference Methods are required to ensure the global comparability of results?

Remarks:

2.12 Can you identify any underpinning measurement R&D which will be required to support the comparability of proteins or NA measurement?

Remarks:

Section 3: Future Requirements for Bio-Measurements

3.1 What do you envisage will be the most critical new protein or nucleic acid analyses to support your business?

Remarks:

3.2 What are the main measurement challenges and issues associated with these new measurements?

Remarks:

3.3 Do you foresee any new regulations or guidance documents (ICH, FDA etc.) which could impact significantly on the type of protein or NA analysis and the level of traceability required?

Remarks:

3.4 What new measurement technologies do you envisage emerging for bio-pharmaceutical analysis over the next 10 years and what would be the likely measurement issues associated with these?

Remarks:

Annex IV: Regulator Interview Form

Measurement Service and Comparison Needs for an International Measurement Infrastructure for the Biosciences and Biotechnology: Interviews with Regulators

Details

Organisation:

Location:

Person(s) Interviewed:

Position:

Email:

Tel:

Interviewer:

Date:

Remarks:

Background

The BIPM is the International Weights and Measures Organisation tasked through a diplomatic treaty, the Convention of the Metre, with ensuring world-wide uniformity of measurements and their traceability to the International System of Units (SI). Historically the activities of the BIPM were exclusively based on physical measurements and the International Standard for the kilogram is housed in the BIPM's laboratories in Sèvres, near Paris. Recently they have undertaken a programme of work on chemical measurements and they are now looking to introduce a programme on bio-measurements. The aim of the programme is to support efforts being undertaken by several National Measurement Institutes (NMIs) and other measurement organisations to assure the global comparability of bio-measurements, through traceability to the SI. This is clearly not a trivial task and may not be achievable in every case.

As a first step, the BIPM want to identify areas of potential activity for them to undertake in protein and nucleic acid (NA) measurements applied to healthcare, both diagnostics and therapeutics. These areas were selected on the basis of their potential for overcoming some of the measurement comparability issues, by making them traceable to the SI, and of their importance to human wellbeing. Of special interest are areas where products run into difficulty because of unconvincing analytical data or lack of standards to support the data, or where product release is hampered by irreproducible measurements. The BIPM have contracted LGC (the UK NMI for chemical and biological measurements) to undertake a study to provide input for a BIPM work programme on bio-measurement. The BIPM are particularly interested in answers to the following questions:

1. Measurement services required to establish an International Measurement Infrastructure for the biosciences that are expected to be developed and delivered in the next 3 – 10 years by NMIs or other organisations developing measurement standards or methods for the biosciences
2. International comparisons between NMIs that are required to demonstrate the degree of equivalence of the measurement services that are or will be developed and delivered
3. Research and development activities necessary for the development of higher metrological order measurement standards and methods for the biosciences

The information obtained from the interviews will be analysed and a draft report of the study will be placed on the BIPM website and distributed to stakeholders for comment. Account will be taken of the comments and a final report produced.

Questionnaire

Section 1: Profile of the Organisation

1.1 Which of the following areas does your organisation regulate?

Drug Manufacture and Supply ☐

Diagnostic Devices ☐

Others (Please Specify)

Remarks:

1.2 What is the jurisdiction of your regulation?

Remarks:

1.3 What types of Bio-measurement are needed to demonstrate compliance with regulations in the field that you regulate?

Proteins ☐ NA ☐ Cell/Bioassays ☐

Others (Please Specify)

Remarks:

Section 2: Measurement Issues for Proteins and Nucleic Acids (NAs)

2.1 What do you see as the main issues associated with protein or NA measurements in the areas that you regulate?

Remarks:

2.2 Are there any specific instances where issues with the comparability of results are giving rise to persistent problems?

Remarks:

2.3 What have been the major consequences of non-comparable results for proteins and NAs

Remarks:

2.4 How are measurement standards, or reference materials or reference methods helping legislative requirements to be met? Please give specific examples.

Remarks:

2.5 Have you encountered specific instances where the lack of measurement standards, or measurement traceability has led to problems in meeting regulations? Please give details

Remarks:

2.6 Can you identify areas where properties of proteins and NAs are being expressed in both SI and non-SI units (e.g. IU) and where this is leading to significant impact on measurement comparability?

Remarks:

Section 3: Challenges for the Future

3.1 What new issues concerning protein, or NA measurements do you foresee arising over the next 10 years?

Remarks:

3.2 What is your view on the new types of Reference Material that will be required in the next 10 years?

Remarks:

3.3 Can you identify any underpinning protein or NA R&D work which will be needed over the next 10 years to ensure the comparability of measurement for these types of analysis?

Remarks:

3.4 Do you foresee introducing any new regulation which would impact significantly on the type of protein or NA analysis required?

Remarks:

Section 4: Support from International Metrology

4.1 Do you have contact with your NMI and, if so on what basis?

Remarks:

4.2 To what extent have you been involved in the development or specification of Reference Materials or Reference Methods to underpin legislative requirements?

Remarks:

4.3 Have you had any direct contact with the BIPM?

Remarks:

4.4 What are you views on the key roles that the International Measurement Standards Community should undertake to help ensure the comparability of bio-measurements?

Remarks:

Imprimerie Centrale
15, rue du Commerce
L-1351 Luxembourg
ISBN 13 978-92-822-2239-3
Achevé d'imprimer : mars 2011
Imprimé au Luxembourg