BIOANALYSIS: THE FINAL FRONTIER FOR METROLOGY!

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Abstract: The importance of biotechnology for wealth creation and the quality of life is widely acknowledged, and measurement plays an indispensable role in research, development and regulation for its safe and sustainable innovation and exploitation. Biomeasurements are complex, with a progression in difficulty and complexity from genes, through proteins to cells and tissues. While there are significant challenges in meeting the technical requirements for bioanalytical measurement techniques, there is also a requirement for their parallel validation to ensure accuracy, analytical robustness and fitness for purpose. The development of appropriate analytical standards and a biometrology infrastructure is also a priority. This paper will review some key biomeasurement challenges and specific research projects within the UK national measurement system addressing these issues (www.vam.org.uk/biomeasurement./ www.dti.gov.uk/nms/prog/newbiotech.pdf).

The importance of biotechnology for wealth creation and the quality of life is widely acknowledged, and measurement plays an indispensable role in research, development and regulation for its safe and sustainable innovation and exploitation. The human and other genome and proteome mapping programmes have had a very significant impact on driving forward technological advances in the analytical biotechnology arena, particularly in response to the demands for rapid, high throughput genomic, proteomic and cellular analysis. There are a number of emerging technologies that address these demands and offer tremendous opportunities for genetic screening and complex multivarient analysis in multigene systems. The challenge for speed, high throughput and automation potential has been taken up by DNA microarrays, which are extensively used in gene discovery and SNP analysis and protein arrays are emerging. Increasingly MALDI-TOF and other mass spectrometric methods are being applied to rapid DNA sequencing, SNP analysis and oligonucleotide analytical QA, as well as proteome analysis. Significant demands are also being placed on the rapidly growing biopharmaceutical industry in bioproduct characterisation for regulatory approval, entailing a number of biochemical and biophysical measurements. While there are significant challenges in meeting the technical requirements for bioanalytical measurement techniques, there is also a requirement for their parallel validation to ensure accuracy, analytical robustness and fitness for purpose. The development of appropriate analytical standards and a biometrology infrastructure should also be a priority.

TARGET OF MEASUREMENT	WHAT IS MEASURED
NUCLEIC ACID	**Sequence** of bases **Length** of base sequence **Amount** [quantification]
PROTEIN	**Identity**, through aminoacid / peptide fragment sequence **Amount** [quantification] **Size –** peptide fragment size, mass **Function –** receptor, signal transduction, binding **Activity –** enzyme catalysis, antibody affinity **Structure –** primary through quaternary
CELL / TISSUE	**Identity –** cell typing, profiling, growth characteristics **Quantity –** cell counting **Size –** cell sorting **Viability –** growth / response **Cellular functionality –** gene expression, metabolism **Interactions –** adhesion, recognition, toxicity

Measurement in the biosciences presents an even greater challenge for the identification and application of appropriate metrology systems than chemical measurements. There is a steep gradient of difficulty, from measurements at the level of the gene, through the protein to the cell.

And the scope and complexity of biomeasurement summarised above is only part of the story. There are additional challenges of measurement in dynamic systems, where metabolic pathways are interdependent, where subtle processes of molecular recognition and interaction are occurring and where protein denaturation and post-translational modification are possible.

The National Measurement System [NMS] is the technical and organisational infrastructure that ensures a consistent and internationally recognised basis for measurement in the UK. It enables organisations to make measurements competently and accurately, and to demonstrate their validity. It ensures that the UK's measurement system is co-ordinated and developed in harmony with those of other countries. Under the UK's Valid Analytical Measurement (VAM) programme (www.vam.org.uk/biomeasurement), LGC has been identifying recent developments in "DNA measurement" technologies which may have significant impact on the future of bioanalytical measurements through addressing these analytical demands, and investigating validation issues in their routine application. More recently (2001) the UK Department of trade and Industry (DTI) took the policy decision to address bioscience measurement issues in the NMS more comprehensively, through a programme in Measurement for Biotechnology (www.dti.gov.uk/nms/prog/newbiotech.pdf).

The Measurements for Biotechnology Programme aims to enable 'better measurements for biotechnology' by improving and strengthening

measurement science in areas and technologies of importance to UK industry. The programme should increase the ability of UK companies to exploit the biotechnology emerging from the science base - improving the accuracy, reliability and integrity of data obtained using the novel measurement technologies.

The Measurements for Biotechnology programme has been formulated to:

- Concentrate at the frontiers of biotechnology to facilitate commercial exploitation of biotechnology emerging from the science base;
- Undertake R&D to support the provision of reference methodology and measurement standards for technologies and processes that are of generic benefit to UK industry;
- Enable the UK to play a leading role internationally in the development of a framework in metrology for biosciences through active participation in international fora;
- Provide leadership in improving the climate for innovation by strengthening the relation between measurement science and regulation, developing standards and reference materials;
- Attract strong industrial collaboration and partnership;
- Ensure effective knowledge transfer of results from the NMS to industry, particularly small biotechnology companies.

Within the programme four priority technical themes have been identified at the frontier of biotechnology, where there are rapid developments in measurement technology that is critically important in exploiting the biotechnology emerging from the science base, namely:

- **Microarray-based testing**, which is central to product discovery and testing. These techniques are characteristically multiplexed and high-throughput, exploiting sensitive and specific biological recognition in microarrays. Microarrays represent the future of measurement in many aspects of biotechnology – in genomics and proteomics, in protein/drug and lipid/drug interactions - but there is no consensus about the technology. There are significant doubts over how representative and uniform are the immobilisation of probes and the binding and labelling of targets. The sensitivity of the signal, usually fluorescent, is a concern. This theme aims to develop approaches to improve the comparability of data from microarrays, cross-connecting with IT-based initiatives in harmonisation
- **Proteomics and genomics**, the focus of much scientific interest and commercial investment. The NMS has already directed significant effort, in the VAM programme, towards fostering comparability of **nucleic acid measurement**, focusing upon primary methods, standards, harmonisation and validation. **Protein measurement** is even more demanding, because the 'proteome' is continuously changing and protein structure is complex. Absolute and relative measurements are important, and determining the glycosylation pattern a particular challenge. The established approach to protein measurement demands separation – usually by 2D electrophoresis – and identification – usually by mass spectrometry. The limitations of the approach are well known: 2D gel electrophoresis is difficult to carry out, and fails with very significant groups of proteins [hydrophobic, high molecular mass, low abundance, highly charged]; identification of proteins takes mass spectrometry to the limits of its scope. Very accurate MS measurements can, in principle, analyse a complex protein mixture without separation, but the approach has some limitations, and measurements are relative. Quantification techniques are emerging, but validation studies and reference standards are required. This theme aims to focus on the development and promulgate valid methodology for comparable proteome measurements.
- **Cell-based testing**, the way forward in assessing the effectiveness of candidate products, and central to reducing the number of animal tests. The reduction in the use of animal testing of candidate drugs and chemicals is widely acknowledged as a necessary target. Cell-based testing has long been seen as the alternative. National and international programmes have made significant progress, but substantial barriers remain before many cell-based tests command sufficient confidence. The key difficulties are the standardisation of techniques that rely upon consistent behaviour of cell-lines, which are inherently difficult to control and reproduce, and the compatibility of

cell-based approaches with multi-sample, automated screening. Measurements for biotechnology at the highest conceivable metrological level have no value unless the biological components of the measurement are authentic, be they cells, tissues, clones, nucleic acid or proteins (antibodies). Ensuring the authenticity of the biological resource is no light task since biological material is inherently - and, if living - continuously variable. Assuring authenticity at genotype level is straightforward. The more challenging task is to test for cellular functionality, through monitoring the level of expression of key genes and post-translational modification of proteins, for example. Comparable biotechnology measurement demands comparability of the biological components**.** This theme aims to develop approaches to assure the authenticity and assess the variability of cell-lines used in tests of effects of candidate drugs or of food allergens etc., and to encourage the reliable provision of human and animal cells and tissues

- **Methods for biomolecular characterisation**, increasingly important in gaining regulatory approval for marketing a bioproduct, and a necessary part of traceable measurement in proteomics and genomics. The regulatory community, led by the FDA, is seeking to increase the use of physico-chemical characterisation in the control of the quality of biological products, initially in approving batch releases. Most of the candidate methods [e.g. circular dichroism, FTIR, mass spectrometry] are stretched in characterising and quantifying the large, complex molecules. There has been little attempt to compare results of physico-chemical methods, to identify clearly the method of choice for specific classes of biological products, to validate methods and to foster comparability of measurement. This theme aims to: stretch the limits of key techniques [mass spectrometry, circular dichroism, capillary electrophoresis, HPLC] and validate their use at higher molecular mass and with complexes; to evaluate the relevance of emerging techniques to characterisation for regulatory purposes and to develop and disseminate means of ensuring measurement comparability in the use of the priority methods for regulatory purposes.

- A further theme on developing concepts of **uncertainty budgets in trace biological measurement** focusing mainly on the interface between biotechnology and regulation has also been identified**.** The driver for trace biological measurement is often legislative. The trace measurement topic is characterised by a conflict between the regulator's desire - often compounded by public perception - to press for control at the lowest detectable level and the realities of measurement technology and the system under study. Often missing from this debate is consideration of the central metrological concept of measurement uncertainty. This theme aims to: identify the main contributors to uncertainty in identification and quantification in selected trace biological measurements, to reduce those contributions; to make the concept of measurement uncertainty more central to the regulatory debate; and to define criteria and procedures for the production of reference materials to enhance the comparability of those measurements.

This talk will review the complexity of biomeasurement and discuss progress in the BioVAM and Measurements for Biotechnology programmes towards addressing comparability issues, and developing a metrology infrastructure in these exciting but challenging areas of bioanalysis.