

# National Measurement System

2004-2007 Measurements for Biotechnology Programme

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## Summary

The Measurements for Biotechnology (MfB) programme forms part of the National Measurement System (NMS). It aims to provide a sound international basis for accurate and reliable measurements, which underpin the development and exploitation of biotechnology by industry in the UK, to increase user confidence and to support the formulation of policy. Its objective is to improve the comparability of measurements at interfaces key to the exploitation of biotechnology – between discoverer and developer, between small company and large company and between company and regulator. MfB, launched in 2001, is the first comprehensive attempt by the National Measurement System to tackle the measurement challenges of the biosciences.

MfB is executed in three-year phases, and this document sets out the content proposed for the period April 2004 to March 2007, following wide consultation and responding to the advice of an independent DTI Working Group. The numerous suggestions for technical work were distilled into projects under four themes:

**Gene measurement**, where the programme inherits six projects from the sister programme in Valid Analytical Measurement (VAM), building on a strong record of achievement in primary methods, standards, harmonisation and validation, culminating in international collaboration.

**Protein measurement**, which is critical to the exploitation of genome knowledge and yet is limited across its whole operational sequence: sample extraction; separation; identification; quantification and determination of structure and function.

**Cell-based technology**, where greater confidence in measurement would lead to an accelerated reduction in animal testing and more effective drug screening, but where the principles of comparable measurement are difficult to apply.

**Product characterisation**, which is particularly demanding for complex biomolecules and a continuing focus of regulatory concern.

In those themes the programme builds upon the successful start of MfB, addresses the reasons for poor measurement comparability and develops and disseminates better approaches. A fifth **knowledge transfer** theme connects the programme with the wider stakeholder community, leads in developing international activity in biomeasurement and seeks to raise the profile of measurement quality.

This document describes the projects fully and sets them in context.

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## 1 CONTEXT AND BACKGROUND

The Measurements for Biotechnology (MfB) programme represents the acknowledgement by the National Measurement System (NMS) that comparable measurement is central to securing the benefits of biotechnology for wealth creation and the quality of life. The formulation of MfB 2004 – 2007 sought to consolidate upon the start of the programme and to respond to any significant changes in the industrial requirements, drivers and context for improved measurement. MfB will continue to:

- Help to pull biotechnology from the science base into industry
- Develop generic reference methods and measurement standards for UK industry;
- Maintain the UK lead in developing an international framework for bioscience measurement
- Help to base regulation more securely in measurement science
- Attract strong industrial collaboration and partnership;
- Transfer knowledge effectively to industry, particularly to small companies

The key changes in the drivers for improved measurement can be summarised simply. The bulk of the industrial activity in biotechnology still focuses on pharmaceutical markets, with an impetus increased by the success of the human genome project. It is becoming clearer that the benefits of genome knowledge will be realised only through a greatly increased understanding of protein science, and that measurement of protein concentration and structure are central. Functional proteomics is becoming more prominent in the discovery of small-molecule drugs, through identifying key protein pathways and ways to disrupt them. Emerging biotherapies and more complex biopharmaceuticals are expected to begin to challenge the market dominance of small-molecule drugs. Balanced regulation of R&D employing stem cells has given the UK a competitive advantage, but the centre of gravity of interest in stem cell technology is still in the science base.

There is no slackening of societal pressure to reduce animal testing. Applications of biotechnology in the agri-food chain are still inhibited by the disputes over the safety and public acceptability of genetically modified organisms, where clarity is being sought through the GM Science Review. Interest in genetic testing is increasing, with consultations [BioBank, Human Genetics Commission, White Paper '*Our Inheritance, Our Future*'] informing policy debates concerning privacy, consent and quality of testing. These debates might improve the competitive environment for UK diagnostic

companies, provided that the cultural barrier to private sector diagnostic services to the National Health Service can be lowered.

For DTI, encouraging small companies and transferring knowledge from the science base remain central, and fostering biotechnology remains a priority; the DTI report '*Competing in the Global Economy: the Innovation Challenge*' (December 2003) promised action to foster innovation in ways directly relevant to biotechnology. The BIA/DTI/DoH Biotechnology Innovation and Growth Team report (November 2003) recommended initiatives to which biomeasurement is central, most notably in clinical trials and in bioprocessing. For DEFRA and the Food Standards Agency, the question of quality of research and surveillance in the biosciences is higher among the agenda, following the well-publicised case of mistaken identification of sheep brains. And as more biopharmaceutical products move closer to the market, the debate over effective regulation intensifies. In promoting a risk-based approach to pharmaceutical regulation, the US Food and Drug Administration has launched a Process Analytical Technology initiative. Internationally, the debate on positioning efforts to work towards mutually acceptable measurement in biotechnology is gaining momentum.

## **2 PROGRAMME STRUCTURE**

### **2.1 Themes**

The following themes are present in the 2004 – 2007 programme.

- **Gene measurement**
- **Protein measurement**
- **Cell-based technology**
- **Product characterisation**
- **Knowledge transfer**
- **Programme management & development**

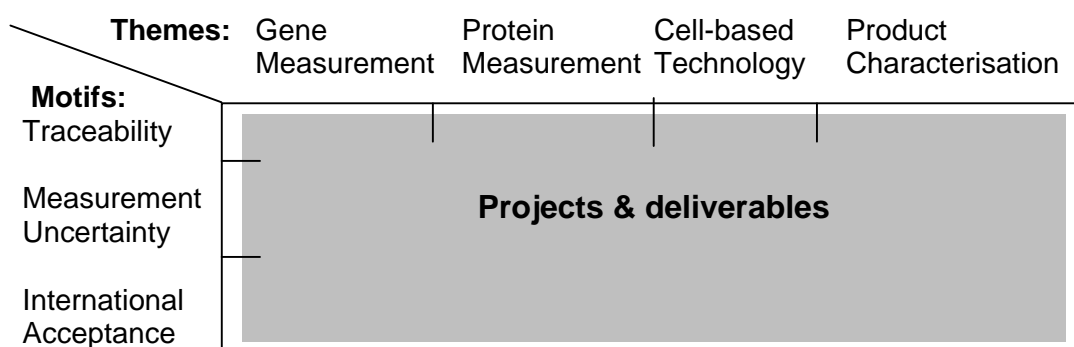
There are three significant areas of current activity that a biomeasurement programme must acknowledge. First, there is **bioinformatics**, where there is a huge national and international effort to facilitate the collection and use of bioscience data through information technology. Since MfB aims to improve the quality of those data, close connection between particular MfB projects and relevant bioinformatics initiatives is necessary, but we are clear that bioinformatics *per se* is not measurement and not, therefore, a programme theme. Second, it is clear that MfB

must acknowledge the inescapable variability of the biosciences and that projects should employ relevant **statistical approaches** to experimental design and interpretation. Close connection should be established and maintained between MfB and NMS activity in software validation. And third, the MfB cell-based technology projects should acknowledge **in silico testing**, which some expect to supplant cell-based tests in the longer term. That approach is a substitute for measurement, however, and therefore inappropriate for MfB.

## 2.2 NMS Motifs and biotechnology

The extension of the reach of the NMS from physics, through chemistry, to biology is an extension into disciplines that are more empirical and less quantitative. It strains the limits of the conceptual basis of metrology, and many chemists and bioscientists doubt its relevance to their disciplines. The challenge for the NMS in biotechnology is to adapt the concepts and language of metrology appropriately – to find effective ways of introducing the key motifs of the NMS, namely traceability, measurement uncertainty and mutual international acceptance. These motifs are the aspects that distinguish MfB from the plethora of other programmes in biotechnology. We propose that the MfB programme should be structured and managed so as to emphasise the motifs and to ensure that contractors and sub-contractors unfamiliar with the NMS give them sufficient attention. The following matrix structure, distinguishing theme from motifs, will facilitate:

- a strategic overview of motif deliverables from individual projects
- the appraisal of proposals for their NMS relevance
- balanced effort between themes and across motifs
- evaluation of the development of MfB as the reach and impact of the motifs grow.



Thanks in good measure to the VAM programme, the NMS motifs are beginning to be established and accepted in nucleic acid measurement, underpinning the **Gene Measurement** theme of MfB. Six DNA projects will be transferred from VAM to MfB, consolidating and developing that process (see page 5). For the **Protein Measurement** theme the motifs represent relatively novel ideas. In identifying and quantifying proteins and in the **Product Characterisation** theme it should be possible to build on the progress of the Chemical VAM programme using primary methods and reference materials. Applying the motifs to the measurement of secondary and tertiary protein structure is uncharted territory. The **Cell-based Technology** theme - the first attempt to bring metrology to the study of living organisms – challenges the scope of the motifs.

For the ‘international acceptance’ motif, MfB is very well positioned to lead in the development of an international infrastructure for the mutual acceptance of biotechnology measurements. The debate is in its earliest stages, but the UK and the US have led in identifying the central issues. Collaborative projects will flow from this initiative, and need to be accommodated within MfB.

### **2.3 Programme balance**

Finally in this section, the formulation needs to reflect comments made on the balance of the programme. There is consistent advice that MfB should maintain an appropriate balance between exploring emerging technology and improving the comparability of current technology. Similarly, a balance has been advised between the timescale of project deliverables; there need to be some ‘low hanging fruit’ where an impact can be shown during programme life and also some more challenging, longer term objectives. And, given that MfB is in its infancy, the issue of continuity was identified where the current programme has clearly made a promising start.

## **3 OVERVIEW OF PROGRAMME THEMES AND PROJECTS**

### **3.1 Gene measurement (GM)**

The NMS has already made significant progress in introducing the concepts and supporting the practice of metrology in nucleic acid measurement. The VAM programme can point to a strong record of achievement in primary methods, standards, harmonisation and validation, culminating in international collaboration. DTI has decided to transfer to the MfB programme a suite of six VAM nucleic acid projects directed towards consolidating that success and moving forward. The six projects address two VAM sub-themes, namely 'standards & performance indicators'

and 'comparability, quality & interpretation'. They were formulated in response to the requirements and views of an end-user community wider than that of MfB, including those conducting nucleic acid measurement relating to environmental regulation, food safety, agriculture, forensic investigation and the identity of people and products. The MfB Gene Measurement theme is therefore already populated with a significant volume of technical work, centrally relevant to the MfB aims and objectives. Full rationales and descriptions of the projects identified below can be found in the Chemical VAM Public Release Programme document at [www.dti.gov.uk/nms/prog/new/vamc0306prv.pdf](http://www.dti.gov.uk/nms/prog/new/vamc0306prv.pdf).

- International Standards & Performance Indicators
- A Primary Method for DNA Quantitation
- Development of Standard Units to Measure Gene Expression
- Specificity Standards and Performance Indicators for Arrays
- Comparability and Consistency of Genetic Measurements
- Critical Data Analysis for Low Level DNA Measurements

The core MfB thrust of moving leading-edge biotechnology forward from the science base adds further requirements, reflecting innovative approaches to measurement. Since the gene measurement theme was populated by six projects before the MfB consultation began, it was not emphasised in discussions with industry during formulation. Nevertheless there was significant support for an additional project on the validation of emerging technologies for genetic analysis

Current attention in genotyping is focused on the analysis of haplotypes, a term describing clusters of closely spaced variations (deletions, inversions, insertions, single nucleotide polymorphisms). The importance of haplotype determination in mapping for disease susceptibility, therapeutic response etc is clear from the initiation of the HapMap project, a US National Institute of Health 3-year international effort worth \$100 million to build a complete haplotype map of the human genome. Determination of haplotypes is time consuming and expensive when analysing large populations, and often requires the help of computational and statistical procedures. A variety of novel technologies for performing haplotyping in a simpler and more cost-effective manner have recently been reported. In the UK there is the potential for the exploitation of haplotyping (BioBank, Genetic Reference Laboratories, Genetic Knowledge Parks), but only if the technologies are robust and validated. Panels of

reference samples of known haplotype are scarce, yet would be of great value for use when conducting genetic analyses and developing and validating assays.

### **3.2 Protein Measurement (PM)**

Proteomics is the comparative analysis of the variation of the population and activities of an individual's proteins. There are high hopes for proteomics as the royal road to drug target identification, product discovery and lead optimisation for pharmaceuticals in the 21<sup>st</sup> century. But limitations of measurement ensure that current activity in proteomics only scratches the surface of its potential for post-genome industry. The measurement of protein populations is central to capturing major prizes for biotechnology, but the relevant technologies are in their infancy. Proteomics must deal with inevitable problems of limited and variable sample material, sample degradation, a vast dynamic range, a plethora of post-translational modifications, inherent variety in tissue, developmental and temporal specificity, and disease and drug perturbations. All of these "sample" difficulties add to the proteomics challenge.

Protein measurement faces problems throughout the whole operational sequence from sample extraction, through separation, identification and quantification to the determination of structure and function. All measurement approaches are challenged, including mass spectrometry, 2D gel electrophoresis, protein micro arrays, surface plasmon resonance, fluorescence, two hybrid protein interactions and phage display. Both absolute and relative measurements are important. Determining the post-translational modifications of proteins, such as phosphorylation and glycosylation, is a further challenge. There is scope to apply new methods to the measurement of the complex mixtures that constitute the active components of the cellular machinery. There are particular needs for rapid identification of variants or deviations from a known sequence or structure, for the high-throughput quantification of a known protein in a complex mixture, for the integration of new technology into existing systems and for standards to foster the comparability of protein measurements over time. When protein measurement is improved for drug discovery, breakthroughs also become possible in point-of-care diagnostics, which in turn require validation. More effective and comparable protein measurement would contribute centrally to the better exploitation of biotechnology, not only in health-related sectors but also in the agri-food chain and in environmental applications.

Proteomics is the subject of significant academic and industrial R&D, which will doubtless lead to technical advances and refinements to existing techniques in the

short term. The NMS projects will need to be positioned carefully and to be responsive during their life to extra-programme developments in technology.

Consultation yielded 36 candidate MfB targets in protein measurement, suggested by 20 organizations. The requirements with the strongest industrial endorsement could be captured in projects addressing two major sub-themes.

- **Optimisation of proteome identification and quantification**

Robust, reproducible ways to identify and quantify the protein complement of cells are still some way off. The most widely used analytical instrumentation in proteomics can analyse proteins across 3-4 orders of magnitude of protein concentration. Since cellular proteins range over about 6 orders of magnitude of concentration, and serum proteins over some 10 orders, there is a formidable measurement challenge in characterising a significant percentage of the total proteome. The accuracy of quantitative data is also an issue in proteomics. Protein identity most commonly relies upon proteolytic digestion into peptides followed by mass determination, with greater confidence given by fragmentation patterns. As more complex protein mixtures are employed this is progressively more challenging and plagued by false positives. In addition, new approaches to proteome quantification, such as protein microarrays raise many new issues in seeking comparable quantification.

- **Optimised characterisation of higher-order structure and interactions of proteins**

Despite the recognised significance of post-translational modifications for biological function, their study has been limited by the complexity of measurement methods. The full extent and functional importance of protein modifications has yet to be fully realised. Complete characterisation of post-translational modifications in large populations of proteins is still considered a significant challenge but a fruitful area for proteomic analysis

Fundamental to drug discovery and diagnostics is the determination of the interactions between a protein target and a probe molecule [protein, DNA or small molecule]. A wide range of techniques is used to analyse these processes. However, there is a current lack of comparability between platforms and of standardization within platforms - a major limitation in the exploitation of many methods.

### 3.3 Cell-based Technology (CT)

Cell-based technologies are important for the whole span of biotechnology R&D. A balanced policy for stem cell research has given the UK a competitive edge. Cell-based measurement is central to high-throughput drug target screening, to studies of drug absorption, distribution, metabolism, and excretion, to toxicity-testing, to lead selection and optimisation, to biopharmaceutical production and to tissue engineering. There is a real need to develop cellular systems that provide a viable alternative to animal testing, not only to respond to continued societal concerns but also to facilitate the early to middle stages of pharmaceutical and biomaterials development. To achieve this, further progress is needed to improve regulatory, industry and public confidence in cell-based testing methods. Companies are wary, however, of encouraging regulatory demands for more tests of doubtful value. National and international programmes have made significant progress, but confidence in cell-based tests needs to be increased.

There are specific drivers for better cell-based tests. A key requirement is to understand the causes of the inconsistencies in the behaviour of different cell lines used in bioassays. Pressures towards mechanism-based risk assessment demand better models for toxicity testing, especially for products of novel types. And there is considerable industrial concern over the prominence of bioassay in the regulatory control of potency of biopharmaceuticals. Since the assays are extremely variable there needs to be major improvement in accuracy and precision in bioassay data, and the development of valid alternatives.

Consultation yielded 18 candidate targets in cell-based technology for MfB projects, suggested by 17 organisations. The requirements with the strongest industrial endorsement could be captured in three projects, under two sub-themes.

- **Validity of cell-based assays**

A meaningful cell-based assay requires cells in the appropriate functional state for the test. The requirement is for the validation of existing methods or for novel methodologies to identify cell-lines and to test for cellular functionality and suitability for a specific measurement procedure. These methodologies might exploit the

identification and characterization of markers unique to individual viable cell-lines or identify standard markers for relative measurements between different cell-lines. They might be applied to comparing the differences between primary and secondary cell-lines. This work should focus on human cell-lines but it is hoped that the techniques will be applicable to cells or tissues from other species. The need is to apply a multi-parametric approach to determine critical factors influencing functionality and stability.

- **Bioproduct safety assessment**

The extreme variability of current bioassays (typically 25-50%) does nothing for industrial confidence in their use. They are, however, a regulatory requirement for control of the potency and efficacy of bioproducts. Alternative tests, more robust and with demonstrable clinical relevance, are clearly needed. Despite the volume of current preclinical testing, toxicity is still the cause of the majority of failures in stages 1-3 clinical trials. There is particular industry interest in improved models for predictive toxicology of biotechnology products (e.g. DNA vaccines and viral gene therapy products), to improve regulatory confidence prior to clinical trials. A recent report from the International Life Sciences Institute Technical Committee on Application of Genomics to Mechanism-based Risk Assessment highlighted the significant potential for the application of genomic and proteomic approaches to toxicity determinations, but recognised that there are considerable reproducibility issues.

### **3.4 Product Characterisation (PC)**

Biopharmaceuticals are complex products and the control of their safety, quality and efficacy is demanding. Two regulator-led initiatives are significant for MfB. First, the concept of the 'well-characterised biological' seeks to promote the development and use of sophisticated physico-chemical techniques to capture those key structural features of the biomolecules that give rise to their functionality and to provide a valid body of data to complement the measurements of biological activity. Progress is being made and there is growing confidence that, with the correct validation, physicochemical measurement will become sufficiently robust to secure regulatory support and to generate direct correlations with data from bioassay and animal testing. Indeed, there are growing numbers of biopharmaceutical products marketed with batch releases regulated solely by physico-chemical testing. Second, the concept of 'process analytical technology' promotes the use of an IT-based systems

approach to process control in pharmaceutical manufacture, enhancing process understanding and assisting in identifying and controlling critical points. In the UK the Centre for Process Analysis and Control Technology occupies this territory. Biopharmaceuticals are excluded from current FDA guidance on process analytical technology, but the industry sees significant potential for microheterogeneous products. Measurement devices that can be placed at- or on-line support the systems approach, but will require validation.

Methods for the characterisation of the identity, structure, function and quantity of complex biological products come under increasing pressure when used for product regulation in biotechnology and pharmaceuticals industries. Many techniques are used to characterise biological products and more are being developed, but few are proven to provide comparable data. Because validation is often carried out on a product specific basis, there is little broad applicability of methods.

Most of the widely used methods for biological analysis, such as circular dichroism, nuclear magnetic resonance, optical spectroscopies and mass spectrometry, are stretched in characterising and quantifying large, complex molecules or mixtures. For instance, the identification of post-translational modifications and the determination of immunogenicity of therapeutic proteins are very demanding for existing technologies but they are essential for the product to gain regulatory approval. This, combined with the perception of these techniques and their IT data analysis as “black boxes”, leads to the reliance on comparative methods for data analysis, which are viewed poorly by both industry users and regulators. In addition, there have been few attempts to compare results of physico-chemical methods, or to identify the method of choice for specific classes of biological products, to validate methods and to foster comparability of measurement. Small, inventive companies frequently rely upon contractors in the science base for such specialized measurements, but harbour doubts over quality and comparability. Moreover, since those companies seek large company partners to develop and market products, a failure to use mutually acceptable means of characterisation constitutes a barrier to innovation.

MfB 2001 – 2004 included projects on techniques newly emerging from the science base research that could become significant in characterising biologicals. The rationale was that early testing and validation would facilitate that process. The 2003 consultation made it quite clear that the overwhelming need in industry was for projects directed towards the solution of current measurement-based problems in

regulatory conformance. For that reason, there is no 'emerging methods' sub-theme in the proposed 2004 – 2007 programme. There is every prospect, however, that novel techniques will be employed in investigation of the current measurement problems.

Consultation yielded 20 candidate targets for MfB projects in product characterization, suggested by 15 organisations. The requirements with the strongest industrial endorsement could be captured in five projects, under three sub-themes.

- **Validation of established and alternative methods to support regulatory and industry confidence**

Biopharmaceuticals are complex products, whose safety and efficacy are very sensitive to details of structure and composition [such as post-translational modification, micro-heterogeneity, impurity profiles]. It is not uncommon for batches of expensive product to be rejected or even withdrawn from the market due to inadequacies in product characterisation at those levels. The methods currently employed are stretched by the difficulties of analysis and there is a clear need for validation, for the identification and dissemination of best practice and for close liaison with companies and regulators. Quantifying process- and product-related impurities in biopharmaceuticals and improving methods for characterising micro-heterogeneity in biopharmaceuticals are the most significant requirements.

- **Development and validation of methods for the characterization of higher order structure**

The concept of the 'well-characterised product' has been debated and explored technically for some 5 years, but still has no settled place in the regulatory context. The ideal is to use physico-chemical methods to characterize the functionality of biomolecules but confidence in that relationship would need to be increased enormously before established regulatory bioassay tests could be abandoned. For new products, physico-chemical tests face fewer hurdles and there is active debate on their utility. A clearer understanding of the validity of methods for characterizing higher order structure of biomolecules would contribute centrally to the establishment of guidelines for their uptake, developed by regulators and industry. Similarly, limitations in methods for the characterization of the factors underlying the relation between protein structure and function will be a target of MfB.

- **Application of process analytical technologies to biopharmaceutical products and processes**

The Food & Drug Administration's initiative 'Process Analytical Technology' looks to measurement devices that can be placed at- or on-line to support a systems approach to risk-based regulation of pharmaceutical manufacture. Interest has focused mainly upon relatively gross processing characteristics (tablet composition, cleaning validation, blending, powder uniformity, drying, particle size). The specific interest for MfB lies in validation of the at- or on-line measurement of the molecular and cellular characteristics that feature elsewhere in the programme.

### **3.5 Knowledge transfer (KT)**

Knowledge transfer in MfB faces a central challenge. The concepts and language of metrology are foreign to biotechnology, and many biotechnologists doubt their relevance. The technical projects of MfB seek to develop and demonstrate the value of the concepts of metrology. MfB will be effective only if there is a complementary, cross-programme effort in knowledge transfer, acknowledging the immaturity of metrology for much of the biosciences, and seeking:

- to build communities of interest in comparable measurement,
- to disseminate practical guidance through advice and training
- to capitalise upon the UK lead in international initiatives in biometrology,

### **3.6 Programme Management & Development (PMD)**

Work under this theme is necessary to ensure that the projects are co-ordinated and managed effectively in order to deliver a seamless, value for money programme. The project aims to ensure that full account is taken, in programme monitoring, appraisal and review, of cross-theme issues and of the development and uptake of the NMS motifs. It will include also a trend-spotting surveillance to inform the further development of the programme, so that formulation of the next round is informed by continual input from the user community, and an independent impact evaluation.

## **ANNEX 1 PROJECT DESCRIPTIONS**

### **GM7 Standardisation requirements for SNP genotyping/haplotyping measurements**

#### **BACKGROUND AND RATIONALE**

Genotyping of single nucleotide polymorphisms (SNPs) has evolved over recent years and is now widely used for a multitude of applications including the identification of disease susceptibility and drug response in individuals. Industrial exploitation of these applications in the design of new drugs and diagnostics has grown rapidly over the past few years and such approaches are now core to drug discovery and development and to the pharmaceutical and diagnostics industries. However, recent findings have indicated that the analysis of the grouping and interactions of SNPs in haplotype blocks may be more informative. Current pharmaceutical company attention in genotyping is focusing on the analysis of such haplotypes, a term describing clusters of linked variations. Such information about genetic variation can help companies design tests that will predict which patients are most likely to benefit from specific drugs. The importance of haplotype determination in mapping for disease susceptibility, therapeutic response etc is clear from the initiation of the HapMap project, a NIH 3-year international effort worth \$100 million to build a complete haplotype map of the human genome. Indeed exploitation of rapidly emerging genotyping/haplotyping technology is already resulting in the development of diagnostics in this area. A number of companies, including UK based Sciona, have recently initiated the marketing of genotyping/haplotyping based consumer products.

In the UK there is the potential for exploitation of both genotyping and haplotyping measurements giving both economic and quality of life benefits (BioBank, Genetic Reference Laboratories, Genetic Knowledge Parks, pharmaceutical and biotechnology companies), but only if the technologies used are robust and validated. As part of the evaluation and validation process the assessment of panels of samples of known status is required. Panels of reference samples of known genotype/haplotype are scarce, yet would be of great value in developing and validating assays, in conducting genetic analyses and in ensuring measurement comparability.

This project is a logical extension of current MfB and VAM projects to develop and produce panels of standards to improve the comparability of genotyping technologies. Previous projects have focussed on the production of standards for array based measurements, however, non-array based genotyping/haplotyping technologies also require reference standards and performance indicators and this could benefit from being carried out alongside the current development of array based genotyping standards. Development of reference standards of known genotype/haplotype status will fulfill the requirements for the current genotyping market and also the newly emerging but rapidly expanding haplotype market.

#### **AIMS AND OBJECTIVES**

- To investigate the requirements for developing a reference panel of genotype/haplotype-validated samples.
- To develop a preliminary genotype/haplotype reference panel and demonstrate feasibility and utility as a measurement performance indicator /QC tool

#### **MAIN ACTIVITIES**

- Liaison with the relevant biotechnology company and healthcare stakeholders to assess requirements
- Screening panels of samples for selected genotypes/haplotypes.
- Identification and preliminary evaluation of a panel for developing into a generic reference panel
- Disseminating recommendations to the relevant industrial and healthcare scientific communities

#### **DELIVERABLES**

- A review evaluating the feasibility of producing a generic genotype/haplotype reference panel of samples
- A panel of samples identified for development into a generic reference panel and demonstration of feasibility as a measurement QC tool
- Proactive knowledge transfer through publication and dissemination to industry, through supplying the MfB Hub with material for publicity and through participating in programme-wide events

## **PM1 Development of validated procedures and reference standards for the high confidence analysis of complex protein mixtures.**

### **BACKGROUND AND RATIONALE**

Robust, reproducible and validated ways to fractionate, identify and quantify individual proteins in a cellular proteome or in complex protein formulations, are still some way off. The most widely used procedures can quantify proteins across 3-4 orders of magnitude of concentration. Since the concentration of cellular proteins ranges over about 6 orders of magnitude, and serum proteins over some 10 orders, there is a formidable challenge in identifying and quantifying a significant proportion of a total proteome. Optimisation of each stage of the analysis is essential in order to yield the maximum information from a sample.

Efficient and robust **separation** methods are particularly important for the analysis of complex protein mixtures. Currently, two separation methods are dominant. The classical 2D-gel electrophoresis can provide relative quantification and can easily separate post-translationally modified proteins, but it is labour-intensive, making high throughput and automation difficult, and membrane/hydrophobic proteins are often under-represented. Recent advances in coupling 2D-liquid chromatography with mass spectrometry (2DLC-MS) yield an alternative approach that is very sensitive and potentially able to detect low-abundance proteins. It is also easy to automate, enabling high throughput, and allows several different strategies to obtain reliable relative quantification. There is a clear industrial demand for a quantitative comparison of the technologies, and for the development of a reference material to enable inter-laboratory assessment and process optimisation, as evidenced by an international web-based debate in October 2003.

Mass spectrometry has become the technique of choice for the high throughput **identification** of proteins. Successful identification of a protein depends usually upon matching the accurate mass of proteolytic peptides generated from an analyte with those from a database. The variability associated with the measurements has not been evaluated. Database searching of protein digest mass spectra is too uncertain for the unambiguous identification of proteins. Protein digestion typically produces a peptide mixture with a very broad dynamic range of abundances, with only a few peptides present at an abundance appropriate for mass measurement with high accuracy. An alternative approach, namely peptide sequencing by MS<sup>n</sup> increases the complexity of the proteomic experiment, requiring further investment in computing power, instrumentation and operator expertise. Industry wants to understand the accuracy and precision of mass spectrometry, and to clarify the level of confidence of protein identification. MfB 2001 – 2004 included the VIMMS project, developing a good practice guide for accurate MS mass measurement through critical inter-laboratory comparisons between some 50 laboratories in industry and the science base. MfB 2004 – 2007 needs to build upon that success through drawing the user community into a collaborative improvement of procedures in progressively more demanding areas of complex protein profiling and proteomics. This should provide an independent assessment of the current measurement capability of different types of mass spectrometer and develop a measure of the associated identification uncertainty.

The accuracy of **quantitation** continues to be an issue in protein measurements. Methods of relative quantitation are evolving at a great pace but are often highly selective and may introduce a measurement bias. The analysis of more complex protein mixtures becomes progressively more challenging and plagued by misidentifications. Currently there are no appropriate standards for the absolute quantitation of proteins. The international metrology community has targeted the establishment of fully characterised traceable standards, tied to a reference method. The requirement for traceable protein reference standards is current also in the work of the Joint Committee for Traceability in Laboratory Clinical Medicine (JCTLM), which has initiated a review of standards for industry that comply with the EU In-vitro Diagnostics Directive. Moreover, biopharmaceutical industry acknowledges that mass spectrometry has become the established routine means of confirming protein identity and seeks traceable protein standards against which to calibrate in-house proteins. The current quantitative proteomics MfB project has also highlighted gaps in the traceability chain. Peptide standards need further characterisation and more rigorous methods of protein digestion are required.

This project represents an integrated approach to the development and validation of procedures for the optimised separation, high confidence identification and high accuracy quantification of proteins from complex mixtures. It will contribute significantly to the international biometrological effort by developing internationally relevant reference procedures and standards, in the emerging area of complex protein measurement. It is important that the work supported by the NMS focuses on areas where the metrological approach can make a real impact. These encompass the development of reference standards to assist in proteome analysis, and high accuracy techniques to underpin quantitative measurements. Thus the project will focus on three main areas of work:

- Validated procedures and reference samples for the optimisation of proteomic analyses.

- Standards for accurate mass measurements to facilitate high confidence protein identification.
  - Techniques for high accuracy protein quantitation that realise high quality international standards.
- Other NMIs have now embarked on similar protein quantitation projects to address some of these issues as well as that of the low-level detection of intact protein and digestion products. The UK needs to maintain its leading role in this international effort to ensure value for money in global biometrology and to ensure harmonisation of protein quantitation efforts.

#### AIMS AND OBJECTIVES

- To identify and develop optimised and validated strategies for separation, high confidence identification and quantification of proteins in proteomic samples and in complex protein formulations
- To develop appropriate reference procedures, standards and measurement uncertainty determinations to support high complexity protein measurements
- To collaborate with the international biometrology community to secure a global harmonisation of the approach

#### MAIN ACTIVITIES

- Identification of a complex eukaryotic proteome and a complex protein formulation as model systems
- Qualitative and quantitative comparison of two optimised multi-dimensional separation techniques, with respect to: proteome representation, classes of proteins detected, dynamic range, ease and speed.
- Production of a reference sample that has been characterised, both qualitatively and quantitatively, using the two separation systems.
- Theoretical evaluation of the sequence coverage and mass accuracy required for the unequivocal identification of proteins over a broad range of peptide m/z values.
- Development and comparison of MS methods for the accurate and reproducible assignment of peptide and protein ions in complex samples, over a broad range of m/z values and abundance.
- Development and validation of methods for the traceable low-level quantitation of intact protein and peptide standards, and partial hydrolysis products
- Working with other key NMIs (NIST, PTB, AIST) to aid the assessment of new strategies for robust protein and proteome measurements.
- Lead in design and implementation of a CCQM comparison of protein/peptide standard quantification by MS, working towards the assignment of a traceable quantity to a protein reference material.

#### DELIVERABLES

- A robust strategy for the selection and optimisation of a “fit for purpose” multi-dimensional separation technique for the MS analysis of a given complex protein mixture validated through an inter-laboratory comparison with industrially relevant samples
- A validated reference sample to be used by the proteomics/protein analytical community to benchmark and optimise relevant multidimensional separation techniques
- A comparison of MS<sup>n</sup> sequencing and high accuracy mass fingerprinting for the identification of components in complex proteome samples
- A standard peptide mixture that can be used for accurate mass measurement over a broad m/z range validated through an inter-laboratory comparison
- Improved robust and traceable methods, with full uncertainty estimates, for the absolute quantification of peptides in complex matrices.
- The assignment of a traceable quantity to a protein reference material.
- A CCQM international inter-laboratory comparison on protein/peptide quantification
- Scientific publications and presentations at appropriate scientific and industrial meetings and at relevant stakeholder workshops
- Proactive knowledge transfer through publication and dissemination to industry, through supplying the MfB Hub with material for publicity and through participating in programme-wide events

## **PM2 More comparable measurement of higher-order structure and interactions of proteins**

### **BACKGROUND AND RATIONALE**

The measurement of the interactions between proteins, proteins and nucleic acids and proteins and small molecules is core to (bio)pharmaceutical drug discovery, lead optimization, quality control, and medical diagnostics. There are many platforms that use the measurement of binding parameters to probe the activity of proteins or protein populations. Techniques range from the relatively low-throughput, such as Isothermal Calorimetry (ITC), Surface Plasmon Resonance (SPR), and Analytical Ultracentrifugation (AUC), to higher-throughput, multiplexed approaches, such as ELISA's and protein microarrays. The lack of available data concerning the reliability or comparability of data arising from these techniques is a major barrier to exploitation, particularly for regulation, which would be addressed within this project. This would complement work within the NMS Photonics Programme (2004-7) to establish a measurement infrastructure for SPR.

High throughput approaches to protein interaction measurement, such as protein microarrays, require the immobilization of target proteins on a substrate. This can have a major impact on the data obtained and consequently the validity of the interpretation. Often these methods rely on fluorescently labelling protein populations for visualisation and quantitation, which can introduce bias to relative quantitation. In addition new approaches are emerging that use novel fluorescence methods such as fluorescence lifetime measurements to detect biological events. The NMS Optical programme (2004-7) is developing an infrastructure to support these novel fluorescence techniques but biologically relevant standards are required to allow the Biotechnology industry to access this infrastructure.

Many interaction measurements use internal controls to ensure internal data comparability, but there are relatively few controls that can be applied across different platforms. Furthermore, these controls tend to be very strong molecular interactions and so not representative of the interactions under analysis. There is a pressing industrial requirement for the development of well-characterised standards which represent a range of different binding parameters, and that can be applied across platforms.

One factor controlling protein interactions, and protein function is the post-translational modification (PTM) of the protein. Consequently the characterisation of PTMs is key to the exploitation of proteomics, for example in the development of therapeutic agents. The commonest PTMs of proteins are glycosylation (the addition of carbohydrates) and phosphorylation. Each type of PTM presents different technical measurement challenges. Existing techniques are labour-intensive and low-throughput, disadvantages in a process-linked QC regime. New approaches based on mass spectrometry, optical spectroscopy, and arrays show promise, but require development and validation. The Beacon project in MfB 2001–2004 has made a promising start in addressing these problems, in collaboration with end users, and merits further development.

As measurement methods mature the need for consistency and comparability between different laboratories becomes paramount to ensure confidence in a method. Fluorescence and circular dichroism measurement are two techniques which have been identified as significantly important for International comparability, and pilot studies under the Bioanalysis Working Group of CCQM are planned to determine the sources of error in these measurements and to establish appropriate calibration techniques and controls to ensure wider uptake of the techniques and regulatory acceptance of the data.

### **AIMS AND OBJECTIVES**

- To improve the comparability of protein interaction measurement parameters.
- To identify optimal strategies for fluorescence labelling and immobilisation of protein populations to improve the consistency of fluorescence based protein measurement.
- To develop improved approaches to the characterisation and quantitation of post-translationally modified proteins, particularly therapeutic protein products.
- To lead international CCQM pilot studies for comparability in protein-based fluorescence and circular dichroism measurement.

### **MAIN ACTIVITIES**

- Experimental comparison of several techniques for the measurement of protein interactions to determine the cross-platform comparability of key parameters, and identify the key sources of measurement uncertainty.
  - Comparison of protein labelling mechanisms to identify the best strategies to enable accurate relative quantitation while maintaining protein function.
  - Investigation of the effect of surface and attachment chemistries on the measurement of protein interactions and activities in representative systems.
  - Development of a set of interaction standards for use as controls in protein-interaction measurement platforms, which may be applied across different platforms.
  - Review approaches to the characterisation of post-translational modifications of proteins identifying emerging techniques and recommending approaches to their validation
  - Experimental evaluation of different approaches to the quantitation of PTMs, considering sensitivity, relative quantification accuracy, ease of use, and speed of assay.
  - Develop and test a set of fluorescence standards for intensity linearity, fluorescence lifetime, fluorescence polarisation, and other system performance characteristics of fluorescence based protein measurement systems.
  - Lead and conduct two CCQM International Pilot Studies on the comparability of two key protein measurement techniques: fluorescence and circular dichroism.
- **DELIVERABLES**
  - A best practice guide to the measurement of protein interaction parameters, identifying strengths and weakness of different approaches, and the level of confidence in comparing data from different platforms.
  - A set of “interaction” standards suitable for use in a wide variety of protein interaction measurement platforms.
  - A guidance document for the immobilisation of proteins on different substrates to improve the performance of multiplexed protein interaction measurement platforms.
  - A guidance document comparing the advantages and disadvantages of different fluorescent labelling strategies for the visualisation and quantitation of protein populations, with an emphasis on the maintenance of protein activity.
  - A set of fluorescence standards for protein-based measurements, which are compatible with existing and emerging fluorescence measurement techniques (such as lifetime), which are targeted at the most commonly used labelling technologies.
  - A critical evaluation and validation of existing technologies and a report on emerging strategies for characterising and quantitating post-translationally modified proteins focussing on limits of detection, sensitivity, accuracy and reproducibility.
  - A report on international comparability in protein-based fluorescence and circular dichroism measurement presented to the Bioanalysis working group of CCQM.
  - Proactive knowledge transfer through publication and dissemination to industry, through supplying the MfB Hub with material for publicity and through participating in programme-wide events.

## CT1 Improved comparability of current industrial *in vitro* tests for toxicity and bioavailability

### BACKGROUND AND RATIONALE

The importance of *in-vitro* methodologies for assessing toxic potentials and bio-availability and for reducing the costs and reliance on animal tests in regulatory testing are now universally acknowledged. In order to reduce adverse drug reactions pharmaceutical manufacturers seek new compounds that are non-toxic, metabolised safely and effectively and cleared from the body effectively. Even a highly potent material cannot be marketed unless it fulfills these criteria. Cell-based *in vitro* assays have now become the tests of choice for such primary screening for the safety and biometabolism of new compounds. Indeed such tests are now prevalent in the early stages of product development within the biotechnology and pharmaceutical communities, and are receiving increasing attention from bodies such as the FDA, the OECD and the ICH. However, while the economic and ethical advantages of *in-vitro* testing are clear and compelling, some key barriers to up-take need to be addressed if the full potential of the approach is to be realised. One of these barriers relates specifically to the quality and reproducibility of measurements; issues that are at the cornerstone of NMS activities to support UK industry and to move towards international harmonisation and inter-comparability. *In-vitro* tests are for the most part associated with very high coefficients of variation and are notorious for performing differently between laboratories and even between individual testers. This lack of comparability does nothing to foster:

- Regulatory acceptance, since data variability provides statistical difficulties in defining comparability with *in-vivo* data
- The development of *in-silico* approaches to evaluating safety and bioavailability, since these would benefit from the high volume of data that *in-vitro* approaches can provide, assuming that consistent test outputs are achievable
- Widespread up-take, since the level of variability can be sufficiently large to question the interpretation of screening data.

FDA guidance advocates the use of particular cells in this context, but gives no prescription for cell handling. Many factors impact on cell function including cell source, culture conditions, storage conditions, operator and other environmental factors. A systematic approach to identifying and addressing the sources of uncertainty of *in-vitro* measurements is overdue, and will assist industry in maximising the value of current *in-vitro* methods and assist regulators in establishing relevant protocols and guidelines.

### AIMS AND OBJECTIVES

- To improve the reliability and reproducibility of high value cell based assays
- To establish approaches and guidelines for wider use in test optimisation and validation.

### MAIN ACTIVITIES

- Working with industry and regulatory partners to identify high value cell-based methods for studies of inter-laboratory comparability and measurement uncertainty
- Conducting 'round robin' cell-based assays with increasing degrees of freedom to ascertain the key steps contributing to measurement uncertainty
- Developing controls, standards and protocols to minimise the variability of identified "key steps"
- Producing guidelines of best practice in the performance of the identified tests, and on the validation and optimisation of *in-vitro* methodology based on experimental and statistical evidence.
- Repeating the round robin using the guidelines produced to determine their effectiveness in improving test outputs.

### DELIVERABLES

- A generic approach to improving the reproducibility of cell based methods
- Defined critical points of variability in high value *in-vitro* assays
- Biomarkers, reference standards and protocols to support enhanced assay comparability
- Guidelines for enhancing assay performance and minimising data variability
- Develop a framework for an industrial PT scheme for *in vitro* tests.
- Proactive knowledge transfer through publication and dissemination to industry, through supplying the MfB Hub with material for publicity and through participating in programme-wide events

## **CT2 Validation of fit-for-purpose approaches for the design of new *in vitro* tests**

### **BACKGROUND AND RATIONALE**

It is confidently predicted that the ultimate scope of use of *in-vitro* methods will be defined to a large extent by the degree to which suites of assays can be developed and applied to mimic the fate and effect of chemicals and biochemicals within humans and in the environment. The drivers for *in-vitro* (and *in-silico*) methods are certainly real and compelling. Consumer pressures will continue to influence regulation (such as the EU cosmetics directive) to reduce the use of animals in product registration and testing. And the economics of product discovery and development will be greatly improved by screening methods that provide early, effective and reliable indicators of performance and safety at low cost. The usefulness of *in vitro* tests as predictors of *in vivo* behaviour depends often on their ability to replicate living biochemical processes and pathways. Thus in order to model complex biological processes, such as organ-specific toxicity, bioavailability and immunogenicity, the key pathways and markers associated with a particular effect need to be identified and replicated within a cell based system. These requirements bring measurement challenges in identifying appropriate markers and then testing for these compounds in-situ as a prelude to assay performance. Guidelines covering the fit-for-purpose design and conduct of these measurements will thus assist greatly in the development of new and important relevant tests for industrial and regulatory up-take. This project should build on the validation framework developed employing hepatotoxicity models in the current MfB programme

### **AIMS AND OBJECTIVES**

- Further develop and validate the measurement approaches for biomarker identification and monitoring in the context of *in-vitro* screening developed in MfB 2001-2004
- To confirm the approach using three novel *in vitro* models to ensure accurate replication of *in vivo* processes.
- To identify relevant biomarkers and/or reference standards that may be used routinely to indicate fitness for purpose of cell-based models

### **MAIN ACTIVITIES**

- Defining 2-3 novel and important cell based methods/disease models in consultation with relevant industrial stakeholders
- Identifying relevant biomarkers and/or reference standards which correlate the relevant *in vivo* process with the *in vitro* model
- Validating the selected biomarkers and/or reference standards in routine test procedures to ensure fitness for purpose of the cell model
- Preparing detailed protocols to transfer to other users

### **DELIVERABLES**

- Guidelines to assist new test development, optimisation and validation
- Validated tests with protocols for research and industrial use
- Biomarkers and/or reference standards that ensure fitness for purpose of cell models
- Proactive knowledge transfer through publication and dissemination to industry, through supplying the MfB Hub with material for publicity and through participating in programme-wide events

### **CT3 Improved reliability of mechanism-based risk measurements for toxicity profiles**

#### **BACKGROUND AND RATIONALE**

Despite the volume of current preclinical testing, toxicity is still the cause of the majority of failures in stages 1-3 clinical trials. There is particular industry interest in improved models for predictive toxicology of biotechnology products (e.g. DNA vaccines and viral gene therapy products), to improve regulatory confidence prior to clinical trials.

Progress in genomic research has resulted in the ability to study the expression of thousands of genes simultaneously using microarrays. Toxicological research can now be performed with DNA microarrays to redefine the pharmaceutical testing paradigm. The use of toxicogenomics, together with metabolomics and proteomics, will provide an understanding of the complex mechanistic pathways of toxicity, will identify molecular biomarkers capable of predicting toxicity early in drug development and will provide biomarkers of toxicity, efficacy and exposure in preclinical and clinical trials. Toxicogenomics promises a substantial impact across the entire discovery-development-clinical drug development pipeline.

However, although microarrays show huge potential benefits, there are still some major challenges to overcome. A recent report from the International Life Sciences Institute (ILSI) Technical Committee on Application of Genomics to Mechanism-based Risk Assessment highlighted their potential, but recognised many reproducibility issues. There is some way to go before scientifically and medically accurate conclusions can be drawn irrespective of the platform used by the experimenter.

One of the main problems is a lack of standardisation of the technology, both in the platforms used in data analysis. In addition, there is a lack of information regarding the reproducibility of data generated from arrays. The current MfB programme includes an investigation of these issues with a strong network of interested array users, whose initial findings will be published shortly. The FDA has highlighted as a major priority the need to establish quality metrics for microarray data. These may include the identification of relevant probes that provide consistent data across platforms or other important parameters, such as the quality of the starting template or the efficiency of labelling. A standardised framework for the experimental annotation of array-based toxicogenomic studies is being introduced (MIAME/Tox), but its focus is solely upon experimental details and data recording. There is still a need to evaluate and manage the variability that can be inherent in such measurements. A greater understanding of the impact of experimental variables on the conclusions drawn from toxicogenomic data and a framework for standardisation will be required in order to maximise the potential of the technology. This project develops and extends the array comparability work successfully undertaken in MfB 2001-2004.

#### **AIMS AND OBJECTIVES**

- To increase confidence in conclusions drawn from toxicogenomic measurements through guidance on minimising the impact of various sources of uncertainty and variability on data comparability.
- To develop a panel of quality metrics to provide objective performance measurements for validating and standardising toxicogenomic array based measurements
- To build on existing array analysis framework and initiatives instigated in the previous MfB programme to improve confidence in microarray based measurements.

#### **MAIN ACTIVITIES**

- Toxicogenomic data analysis employing both extensive data generated on relevant toxic response pathways in current MfB project and liver cell model/primary hepatic cultures or others if available
- Evaluate reproducibility of toxicity profiles using expression arrays targeted to genes involved in cell proliferation, cell growth arrest/senescence and inflammation, apoptosis and necrosis.
- Identify and develop quality metrics necessary to improve confidence in measurements
- Co-ordinate activity with key bioinformatics initiatives such as MIAME-Tox

#### **DELIVERABLES**

- Best practice guidelines
- Published Quality metrics for toxicogenomic data interpretation, endorsed by relevant industrial and regulatory stakeholders
- Workshop for industrial and regulatory stakeholders on validation and standardisation of microarray toxicogenomic experiments
- Proactive knowledge transfer through publication and dissemination to industry, through supplying the MfB Hub with material for publicity and through participating in programme-wide events

## **PC1 Detection and quantitation of process- and product-related impurities**

### **BACKGROUND AND RATIONALE**

Biopharmaceutical products are produced by fermentation using either eukaryotic or microbial cells grown in complex media. Crude preparations from fermentation contain a large number of protein contaminants derived from the expression cells (known as host cell proteins (HCPs)). Some of these HCPs, with similar physicochemical properties to the product, co-purify with the product through several steps during the production process. They may be toxic, immunogenic, or be enzymically active. Similarly trace levels of host cell DNA (HCD) may also contaminate the final product. The quantitation of host cell contaminants that remain in the product following purification is a major concern of the biopharmaceutical manufacturing industry, and the associated regulatory authorities. The Biopharm Subgroup of the Pharmaceutical Analytical Sciences Group (PASG) identified the measurement of host cell contaminants as an area of significant industry-wide concern.

Regulatory guidance on host cell contaminants analysis is somewhat vague. ICH guideline Q6B suggests immunochemical approaches using anti-HCP antibodies in either an immunoblot or ELISA assay. Immunological assays depend upon polyclonal antiserum raised against partially purified HCPs, only some of which will be present in the final product. Even if these criteria are met, then the immunoblot is at best semi-quantitative and has low sensitivity while the ELISA is quantitative it is unclear what is being measured. Other possible approaches include separation of proteins by SDS-PAGE and identification by a variety of staining methods or HPLC analysis, or a combination of these methods. Current measurement of HCD includes radioactive dotblot analysis which although sensitive is very slow and has safety implications for the analyst. Alternative approaches include nucleic acid amplification but this has not been fully validated. These approaches currently lack regulatory support. There is pressing industry demand and a regulatory requirement to validate existing approaches to host cell contamination characterisation and quantitation, ultimately leading to industry-wide best practice to this problem, as well as the need to generate new generic approaches to host cell contaminant analysis, potentially using validated proteomics technologies.

### **AIMS AND OBJECTIVES**

- To provide validated methods of characterising and quantitating host cell protein and DNA contamination in biopharmaceutical products in concordance with ICH guidelines.
- To develop new approaches to the characterisation of host cell contaminants in biopharmaceutical products, tested and validated on commonly used expression systems.

### **MAIN ACTIVITIES**

- Undertake a thorough review of current industry practice in host cell contaminant characterisation and quantitation to identify generic approaches based on current methodology.
- Validate these generic approaches with respect to sensitivity, specificity, compliance with current guidelines, fitness for purpose, and breadth of application.
- Review potential new proteomics/genomics-based approaches to the characterisation of host cell contaminants, identifying the most appropriate for further development.
- Validate these new methods with respect to sensitivity, specificity, compliance with current guidelines, fitness for purpose, and breadth of application.
- Undertake a round-robin comparison study to ensure the transferability of these methods approaches, and to provide confidence in the results.
- Work with the industry and regulatory bodies to ensure that the methods and best practices are widely adopted in support of current regulatory practice.

### **DELIVERABLES**

- A review of existing methods and new proteomics-based approaches to the measurement of host cell contaminants.
- Development of commonly applicable methods and associated best practice to the characterisation of host cell contamination, based on at least one currently applied approach, and one emerging method.
- Validation data to support the adoption of best practice by industry and regulators alike.
- Proactive knowledge transfer through publication and dissemination to industry, through supplying the MfB Hub with material for publicity and through participating in programme-wide events

## **PC2 Validation of CE/ LC/ MS coupled methods for improved micro-heterogeneity discrimination**

### **BACKGROUND AND RATIONALE**

Biopharmaceutical products make new and challenging demands of biological measurement, since their safety and efficacy are very sensitive to details of structure and composition. Of particular significance in this respect are batch-to-batch variations in the micro-heterogeneity (e.g. glycosylation, amidation, oxidation, disulphide bonds) that can accompany biopharmaceutical manufacture. The nature of the characterisation of micro-heterogeneity required for regulatory approval is the subject of debate with the producers. Regulatory guidelines from the FDA (CDER and CBER), ICH, EMEA indicate the types of analytical information expected for a regulatory submission, but refrain from prescribing methods. Industry and regulators have to arrive at mutually acceptable measurement through balancing the requirements for “appropriateness” (as required by ICH Q6B) and for cost effectiveness, routinely applicability and GLP compliance. This debate needs to be informed more fully by an understanding of the fitness for purpose of conventional and more novel measurement, especially for quantitative analysis.

The methods currently employed are stretched by the difficulties of analysis and there is a clear need for validation, for the identification and dissemination of best practice and for close liaison with companies and regulators. Although gel based electrophoretic methods and immunoassay are still commonly used within the industry, it is becoming accepted that capillary electrophoresis (CE) and liquid chromatography (HPLC), coupled to information rich or sensitive detection (such as fluorescence and mass spectrometry), have the potential to become the cornerstones of biopharmaceutical characterisation. The Biopharma Subgroup of the Pharmaceutical Analytical Sciences Group (PASG) has recently identified the potential benefit that more widespread adoption of CE and LC measurement would offer its members in meeting regulatory requirements, and has instigated a working group to discuss the issue. Judicious use of the most appropriate techniques and methods in CE and HPLC can characterise even complex ingredients including low-level impurities. Thus, the efficiency of response to regulatory requirements will be greatly enhanced by gaining a better understanding of the richness and fitness-for-purpose of the different modes and methods in CE and HPLC analysis, a fact that is becoming acknowledged by both regulators and industry.

### **AIMS AND OBJECTIVES**

- To enhance the mutual acceptance of micro-heterogeneity characterisation through increasing the confidence of industry and regulator in the fitness for purpose of capillary electrophoresis (CE), liquid chromatography (LC) and coupled fluorescence and mass spectrometry (MS)
- To develop guidance to assist regulatory compliance and the establishment of quality control procedures.
- To disseminate the results actively by drawing industry and regulator partners into contact with the project and by using web based dissemination tools.

### **MAIN ACTIVITIES**

- Consultation with stakeholders to identify information on micro-heterogeneity characterisation required for product registration.
- Comparison of the quantitative performance of CE (fluorescence and MS) and LC (fluorescence and MS) for chosen applications with the traditional methods
- Develop a panel of relevant glycoforms/isoforms of defined mass and heterogeneity as a reference standard for assessment of microheterogeneity measurement capability
- Liaising closely with the PASG
- Disseminating the outputs through publication, a focused workshop and seminar, industrial trade associations and links with regulatory authorities.

### **DELIVERABLES**

- A validated “route map” (endorsed by at least 2 organisations) for microheterogeneity characterisation of selected biopharmaceuticals
- A report outlining protocols developed and results of comparative evaluation of techniques disseminated to industrial and regulatory stakeholders
- Production of a panel of appropriate heterogeneity reference standards
- A cosponsored practical workshop for biopharmaceutical QC managers, with speakers from the consortium, to raise awareness of CE/LC/MS
- Proactive knowledge transfer through publication and dissemination to industry, through supplying the MfB Hub with material for publicity and through participating in programme-wide events

### **PC3 Strengthening quality control of biopharmaceutical products through the establishment of structure-function relationships**

#### **BACKGROUND AND RATIONALE**

The introduction of recombinant protein pharmaceuticals and the growth of the biopharmaceutical industry are driving changes in biopharmaceutical quality control. A significant development is the concept of the “well specified product”, where the product rather than the manufacturing process is the target of regulation. In this approach, a battery of physico-chemical methods is used to determine the acceptable variance between production batches and the material used in clinical trials. To predict clinical efficacy of different batches of the biopharmaceutical in patients, the data from the clinical trial is correlated with the data provided by bioassays and physico-chemical methods.

Regulatory authorities require bioassay data to demonstrate the specific ability of biological products to achieve their intended biological effect. Potency is the quantitative measure of the biological activity of a pharmaceutical/biopharmaceutical product. This is an essential product characteristic that requires the use of a measurement method that can be correlated with certain aspects of the clinical response of the patient to the product. The clinical trial provides the “gold standard” measure of product efficacy and safety, however it is unfeasible to repeat a clinical trial for each product batch. The present strategy is to develop bioassays that can be used to measure different biological responses to the product. Bioassays traditionally use whole animal, isolated tissues and organs or cultured cell models, with industry favouring the use of cell culture models. The biopharmaceutical industry has highlighted the fact that current bioassay measurements are often very imprecise, poorly reproducible and for some products, extremely difficult to develop.

For many biopharmaceutical products it has been proposed that it should be possible to establish a relationship between the structure and biological activity, which would enable the potency to be established by physico-chemical means alone. This has been achieved for products such as insulin, and one or two monoclonal antibody products. This approach requires robust structure-function relationships to be established based on precise bioassay data and extensive structural characterisation – improvements need to be made to both physico-chemical methods and bioassays in order to underpin this approach. The “well specified product” concept is a long-term regulatory goal, and work is required to underpin the later generic application of this approach.

Many QA bioassays measure a late stage response of the cell to the product such as proliferation or death. A vast range of factors determines cell behaviour, all of which can increase the uncertainty on the bioassay. During product development it is therefore preferable to establish a bioassay that measures an early stage response to the product, for instance receptor phosphorylation or modifications of signalling cascade molecules. Early response bioassays are that they are rapid - data can be available in minutes rather than days. Binding of a product to its cell surface receptor is not of itself necessarily a measure of the biological activity of the product. However, correlation of data from studies of product interaction with cell surface receptors with data from studies of functional cellular responses can lead to development of an assay based on product-receptor interaction that can be followed by physico-chemical methods.

Early stage bioassays and appropriate physicochemical assays have the potential to show less measurement uncertainty than late stage bioassays while still giving an accurate measure of product activity and potency for quality assurance. This project will start to develop this concept, provide guidance for the development and conduct of early stage cell-based assays, and thereby support for the “well specified product” concept.

#### **AIMS AND OBJECTIVES**

- To underpin the use of the “well specified product” concept for product quality control and regulation.
- To establish a portfolio of physico-chemical and biological assays with sufficient sensitivity that can be used to provide meaningful structure-activity relationships for biopharmaceutical products.
- To identify the current sources of uncertainty in cell based bioassays used to measure the potency of biopharmaceutical products, and develop guidelines to reduce uncertainty.
- To investigate the relative merits of early and late stage readouts and correlate in appropriate model systems.

#### **MAIN ACTIVITIES**

- Review the structural information provided by a key selection of physico-chemical methods, with focus on those sensitive to factors affecting receptor or ligand binding.
- Establish the sensitivity of the key physico-chemical methods to the detection of structural changes in the model system.

- Carry out a review of the use of cell based bioassays in biopharmaceutical quality control and identify the major contributions to measurement uncertainty, focussing on generic factors.
- Select an industrially relevant cell culture based bioassay and attempt to correlate results from early and late stage readouts and compare the uncertainty of the measurements.
- Correlate key physico-chemical data with cell-based bioassay data to form a structure-function relationship for a model system, and produce guidance for the establishment of structure-function relationships for biopharmaceutical products.
- Knowledge transfer activities to ensure that the measures are recognised by the regulators and industrial users.

#### DELIVERABLES

- Demonstration to industry and the regulators of a structure-function relationship for a complex biopharmaceutical product.
- Support for the industrial and regulatory drive towards the “well specified product” concept.
- Recommendations of key methods important for structure-function relationships for future validation.
- Recommendations for the design and conduct of early stage cell-based bioassays that reduce the uncertainty in these measurements.
- Guidance documents underpinned by experimental evidence and statistical analysis.
- Interaction with industry and regulators to ensure relevance of outputs and uptake by the user community.
- Proactive knowledge transfer through publication and dissemination to industry, through supplying the MfB Hub with material for publicity and through participating in programme-wide events

## **PC4 Development and validation of higher order structure measurement methods to support ICH guidelines**

### **BACKGROUND AND RATIONALE**

The characterisation of higher-order structure of biopharmaceutical products is a key requirement for their regulatory approval. The current guidelines (International Committee for Harmonisation Q6B) recommend circular dichroism (CD) as the appropriate method for higher-order structure determination. It is widely recognised that the use of this method has limitations, and work within the current MfB programme has sought to ameliorate these problems. The success of this initial work has produced a vibrant UK industry and regulator led community that is committed to the adoption of best practice. There are now initial steps towards expanding this into an international effort to standardise CD data, through the establishment of a CCQM approved pilot study to evaluate the reliability and comparability of CD measurements used to support regulation. The international scope of this study is vital since the regulation of biopharmaceutical products shows increasing international harmonisation.

Across the biopharmaceutical industry, the measurement of protein concentration is recognised as a major problem for product characterisation, and a major source of uncertainty in protein structure measurement. Industry lacks confidence in the accuracy of all current methods to measure protein concentration. There is a pressing requirement to establish the fitness for purpose of these approaches particularly when applied to Quality Control.

While CD provides essential information about protein structure, there are a number of alternative approaches to the characterisation of higher order structure, for instance those based on vibrational spectroscopy. These techniques often provide complementary information to CD – at a recent CBER/IABS meeting (June 2003, NIH, Bethesda), the FDA called for the introduction of new methods for higher-order structure characterisation to show product comparability in biopharmaceutical quality control. This highlights the pressing requirements for the evaluation and validation of these methods to ensure their uptake and application by industry and the regulators.

### **AIMS AND OBJECTIVES**

- To promote best practice in circular dichroism to support ICH guidelines, and encourage the development of international standardisation for CD measurement.
- To improve the accuracy of higher-order structure measurement through a reduction in the uncertainty associated with protein concentration measurement.
- To gain acceptance of new approaches for the characterisation of higher order structure of biopharmaceutical products.

### **MAIN ACTIVITIES**

- Maintaining and expanding the CD users community in the UK through appropriate knowledge transfer and training events.
- Undertaking a comparative study of methods for the measurement of protein concentration focusing on accuracy, reproducibility, dynamic range and cost-effectiveness.
- Organising and leading the CCQM pilot study in CD measurement under the BAWG.
- Coordinating the generation of a reference database of protein CD spectra that is compliant with recognised protein information databases.
- Reviewing new approaches to the characterisation of higher-order structure of biopharmaceutical products.
- Selecting a technique for validation, and evaluate the performance of the technique, particularly in the ability to distinguish structural differences between biopharmaceutical product batches.

### **DELIVERABLES**

- Proactive knowledge transfer campaign in the UK, including the continuation of the annual CD-user community meetings, and training for the next generation of industrial QC analysts.
- Adoption of best practice for CD measurement, by industrial users and the regulatory community.
- Report on the comparability of protein concentration measurement methods.
- Report on the UK-led CCQM pilot study on CD measurement and comparability.
- A database structure for the curation of CD spectra designed to be linked to a publicly accessible protein structure database.
- Report on alternative approaches to the characterisation of differences in higher-order structure between batches of biopharmaceutical products.
- The availability of validated approaches to the characterisation of higher-order structure of biopharmaceutical products, complementary to CD, and recognised by industry and by regulators.
- Proactive knowledge transfer through publication and dissemination to industry, through supplying the MfB Hub with material for publicity and through participating in programme-wide events

## **PC5 Development & validation of non-invasive methods for product and process characterisation**

### **BACKGROUND AND RATIONALE**

The FDA began in 2001 to promote the concept of Process Analytical Technology (PAT) as an IT-based systems approach to process control in pharmaceutical manufacture, enhancing process understanding and assisting in identifying and controlling critical points. The PAT initiative looks to measurement devices that can be placed at- or on-line to support a systems approach to risk-based regulation of pharmaceutical manufacture. Interest has focused mainly upon relatively gross processing characteristics (tablet composition, cleaning validation, blending, powder uniformity, drying, particle size), and biopharmaceuticals have not featured strongly. The industry believes that the PAT concept will come to be fostered for biotechnology, however, and endorses proposals for work in preparation.

MfB interest in PAT should focus on the molecular characteristics addressed elsewhere in the programme, such as aggregation, crystallisation or protein folding. Transferring measurement techniques from a highly skilled laboratory environment to routine at-line or on-line use is demanding, and history shows that few techniques have been rugged enough. Careful assessment of reliability and validity of candidate methods is necessary. To be applicable to at-line and on-line measurements the device should possess the following characteristics: low cost, non-invasive, easy to use, able to operate through vessels, fast (real time) data acquisition, and applicability to raw samples (no cumbersome sample preparation). Acoustic spectroscopy is one of the very few techniques that meets these criteria, and is the target technique for the project. Its potential for QC monitoring for bioproducts has already attracted considerable industrial interest and participation under MfB 2001-2004. In order to test the utility of a candidate device, access is necessary to a library of samples with a separately characterised variance reflecting changes in process conditions. A successful device will need to be shown to detect variations that correlate well with differences in end-product quality.

### **AIMS AND OBJECTIVES**

- To establish a well-characterised reference library of samples representing process changes at a level that needs to be controlled
- To validate an acoustic spectrometry device and produce a set of statistical criteria to ensure batch conformity and consistency.
- To transfer the technology /knowledge to industry

### **MAIN ACTIVITIES**

- Preparing batches of biological compounds under different conditions to form a test library
- Testing acoustic spectrometry for its ability to detect credible process changes, using the library set.
- Statistically validating the results to determine batch consistency/conformity:
- Identifying and addressing validation barriers to transferring methods to a QC environment.
- Liaison with the PAT interest group of the PASG.

### **DELIVERABLES**

- A biological library for evaluation of at-line/on-line devices
- Validation of an acoustic spectrometry device
- Proactive knowledge transfer through publication and dissemination to industry, through supplying the MfB Hub with material for publicity and through participating in programme-wide events

## **KT1 Building a biomeasurement community**

### **BACKGROUND AND RATIONALE**

Biology is, by tradition, an empirical and semi-quantitative science in which the concepts of metrology - such as traceability, measurement uncertainty and mutual acceptance - have had little purchase.

Biotechnology forces a marriage between biology and the more quantitative sciences of chemistry and physics, and sharpens the requirement for comparable biomeasurement. In order to disseminate the messages of metrology, to communicate the measurement solutions emerging from the MfB technical projects and to encourage identification of key issues for measurement in the biosciences, effort is needed to build and sustain a community of interest in the subject.

The composition of the community of interest in biomeasurement that MfB needs to build follows from the positioning of the programme. MfB addresses measurement issues important at the interfaces between the science base, companies and regulators in a research-led context. The pharmaceutical sector is the most immediate beneficiary, but the generic improvements in measurement comparability will have the potential for dissemination into the food and environment sectors. The key recruits to the biomeasurement community are science-base researchers seeking to commercialise their discoveries, research staff in companies seeking to take biotechnology products to market and regulators concerned to control the safety, quality and efficacy of the products. Regulatory compliance is a central issue for biotechnology. An acknowledgement of regulatory constraints and the associated demands of measurement needs to inform even the earliest steps towards the market. KT activities at the regulatory interface are important for MfB.

### **AIMS AND OBJECTIVES**

- To promote the cause of better measurement for biotechnology
- To grow and sustain an active network of researchers, industrialists and regulators with an interest in fostering comparable biomeasurement.
- To inform that network of developments in sounder biomeasurement
- To secure comment and information from the network relevant to the development of the programme.

### **MAIN ACTIVITIES**

- Maintenance of the MfB website, established in the 2001 – 2004 programme, containing up-to-date information on all MfB projects and news on relevant policy and technical developments elsewhere.
- Regular publication of an MfB Bulletin, seeking to raise awareness of the importance of comparable measurement and including contributions from the developing community
- Sustaining a programme of promotional activities including articles in relevant literature and presentations at conferences and exhibitions etc

### **DELIVERABLES**

- An MfB website containing current information on the programme and relevant news from elsewhere
- An active database of MfB contacts, distributed regularly to MfB contractors
- A regular MfB Bulletin distributed free to MfB contacts
- A programme of promotional activities

## **KT2 Advice and training**

### **BACKGROUND AND RATIONALE**

This project concerns the 'nitty gritty' of bridge building between the biotechnology community and the motifs of sound measurement – traceability, measurement uncertainty and mutual international acceptance. The concepts are so foreign to most biotechnologists that a significant effort will be needed to guide and advise on their adoption. Since MfB focuses on a research-oriented measurement community, the programme's efforts need to target mainly the science base and research users of biomeasurement techniques. It is salutary to note that the education and training projects of the VAM programme have found these targets to be among the most resistant to the message of sound analytical science. MfB can exploit the VAM training material in introducing the basic concepts of measurement quality, but MfB can add most value – and can best secure the attention of its target audience – by focusing upon the measurement techniques featured in the technical programme.

University researchers adhere to peer review as the sole guarantee of quality and are culturally averse to quality procedures. They miss the crucial point that adherence to the principles of measurement quality is the equivalent of peer review for the analytical community. Quite apart from the wastefulness of poor measurement, this undervaluing of measurement quality has specific damaging effects, of which three merit mention. First, the excitement of the 'rush to application' of novel biomeasurement techniques often diverts attention from the requirement for validation. Second, research students are prepared poorly for a world of employment in which measurement quality will be of increasing importance. And third, the opportunity is missed to engender more confidence among companies in technology that university researchers seek to transfer to them. Countering those tendencies is clearly consistent with the MfB mission, but it will be necessary to work with the grain of university perceptions, and to be seen to offer advantage to leaders of university research groups.

In an important complementary step, DEFRA and the FSA, working closely with others, introduced in 2003 a code of practice for the proposal and conduct of commissioned research projects, many of which require biomeasurement. Since the piper's paymaster calls the tune, DEFRA laboratories, Research Associations and the institutes of BBSRC and NERC will adopt the code, but neither BBSRC nor NERC is applying this measure of quality control in its 'responsive mode' support for university research. DEFRA and FSA are prepared to collaborate actively with MfB advice and training activities.

### **AIMS AND OBJECTIVES**

- To increase understanding of how attention to measurement quality can enhance technology transfer, competitiveness in seeking research contracts and the employment prospects of students.
- To develop and provide materials to disseminate the message of measurement quality in academic research training
- To pilot training courses in traceable biomeasurements, following feasibility study

### **MAIN ACTIVITIES**

- Production of guidance documents, accessible via the website, giving background information and briefings on progress with the adoption of the NMS motifs.
- Organisation of workshops and seminars on themes and motifs, from a cross-programme perspective, and on the measurement implications of regulation
- Working at the interfaces between the science base, companies and regulators to identify relevant case studies and presenting them to academic research groups and to Research Councils etc
- Lobbying influential officials in funding bodies and Government departments to acknowledge measurement quality in biotechnology policy and programmes
- Working with identified academic research group leaders to develop and pilot a training course in traceable biomeasurement
- Complementing the E&T activities of the VAM programme by strengthening the biotechnology input and by capitalising upon relevant VAM material and expertise

### **DELIVERABLES**

- Updated guidance documents on the NMS motifs and their adoption
- A programme of workshops and seminars, in partnership with industry organisations
- A portfolio of case studies demonstrating the benefits of sound biomeasurement
- Seminars, workshops, meetings for academic research groups and research funding bodies
- A pilot training course for research students
- Perceptible influence in raising the profile of sound measurement in broader biotechnology policy and programmes

## **KT3 International Leadership**

### **BACKGROUND AND RATIONALE**

Interest in the biosciences is taking off in the international metrology community. And the attention of international organisations whose activity is underpinned by biomeasurement is focusing increasingly upon the need for more comparability. The UK and US National Measurement Institutes are more expert in the biosciences than most, and have taken the lead in the international metrology community. The high profile thus secured has led to a demand for participation in wider international gatherings, all of which promise to impact significantly upon UK interests in industrial development and the quality of life. A significant effort will be needed to contribute effectively.

The UK heads the Bioanalysis Working Group of the CCQM, and led an influential 'thinkshop' in April 2003, which made significant headway towards:

- determining the critical points in key biomeasurements which would benefit from international effort on reference method and reference material development
- giving international NMI participants a greater understanding of biomeasurement
- defining a CCQM biometrology programme that addresses "real life" bioanalytical challenges in response to the demands of industry and regulation
- identifying a rational approach to developing a biomeasurement infrastructure and to assigning priorities to candidate biometrology pilot studies and projects

Taking that initiative forward is clearly of central importance to the MfB programme.

Contributions to wider international activities are more responsive to requests for advice and participation in meetings. They offer, nonetheless, excellent opportunities to disseminate the message of sound measurement and to influence developments in line with UK interests, following consultation. The most significant current fora are:

The Joint Committee on Traceability in Laboratory Medicine (JCTLM)

ISO Committees

CEN Committees

WHO

### **AIMS AND OBJECTIVES**

- To maintain the UK lead in the CCQM Bioanalysis Working Group (BAWG)
- To capitalise upon that lead by establishing an international programme to develop a global biomeasurement infrastructure in line with UK interests.
- To connect the UK biomeasurement network effectively with that programme
- To represent UK interests at wider international fora where biomeasurement is important and to ensure that their deliberations take due account of metrology principles.

### **MAIN ACTIVITIES**

- Maintaining continual contact with BAWG members, by means including an informal newsletter, so as to enhance commitment to the international programme and to sustain the UK lead.
- Attending and managing BAWG meetings
- Consulting with the UK biomeasurement network in order to inform international discussions, and reporting developments via the MfB website
- Advising and contributing to the activities of wider international organisations upon whose interests biomeasurement impinges.
- Briefing NMSD and officials in OGDs on developments relevant to policy

### **DELIVERABLES**

- Formulation of an international programme in biomeasurement reflecting UK requirements and connected to the UK biomeasurement network.
- Representation of UK and MfB interests at international discussions where biomeasurement is significant, and follow-up with specific actions
- Communication of relevant developments to Government and the user communities

