Proposed National Measurement System Programme for Chemical and Biological Metrology (2009-2012)

Public Comment Document

November 2008

NPL National Physical Laboratory

Department for Innovation, Universities & Skills

LGC Setting standards in analytical science

National Physical Laboratory
Hampton Road, Teddington, Middlesex, TW11 0LW

LGC
Queens Road, Teddington, Middlesex, TW11 0LY

We gratefully acknowledge the financial support of the UK Department for Universities,
Innovation and Skills (National Measurement System)

Approved on behalf of the Managing Director, NPL
by Alan Brewin, Business Leader, Analytical Science Team

Approved on behalf of LGC
by Julian Braybrook, Head of Measurement Research & Development

Executive Summary

This document has been produced for Public Consultation. It describes a portfolio of potential activities, brought together under common technical themes, that have been worked up in response to the measurement challenges identified during the early stages of the formulation process. Such activities are destined to form part of the Department for Innovation, Universities and Skills (DIUS) Chemical and Biological Metrology Programme, 2009-2012, one of a portfolio of Programmes supporting the UK National Measurement System (NMS). All identified activities are in line with the developed Programme strategy document.

The document provides a brief overview of the common technical themes undergoing formulation at this time - Bioanalysis, Organic analysis, Inorganic and Speciation analysis, Surface analysis and Trace analysis. Two additional activities prepared in response to specific user needs have been placed under the Nanobiotechnology theme. This overview is followed by Annex 1, which sets out each proposed activity in the form of a standard template indicating:

- The measurement needs addressed by the proposed activity, which are the result of extensive consultations held between May and October 2008 with the relevant user communities, and analysis of relevant roadmaps, government reports and market research reports;
- The expected impact to be achieved, particularly the impact on quality of life, innovation and economic benefits;
- The sub-projects that make up the activity; and
- The proposed deliverables within each sub-project.

This Public Comment Document is subject to a 6-week public consultation period. Following this period, there will be a Decision Conference in early December 2008 to determine the final content of the Programme. Following that meeting, the prime contractors of NPL and LGC will develop the document further to create the Final Programme document. At this final stage the start and end dates for each deliverable will be finalised.

Those wishing to comment are advised to review the outlines in Annex 1 with the following question in mind -

Do the activities proposed:

- Meet the short to medium term measurement needs of the UK?
- Enable identified sectors to perform in the competitive global economy?
- Support regulatory needs and standards requirements?

Furthermore, all parties wish to encourage participation in the Programme, whereby interested organisations may collaborate with NPL and LGC; this may extend the scope of the activities through co-funding ‘in kind’ and/or in cash.

Please forward your comments to michael.adeogun@npl.co.uk or julian.braybrook@lgc.co.uk as indicated in the respective project outlines. Please be good enough to quote the relevant project number or title when doing so.

Acknowledgements

We gratefully acknowledge that the work to create this report was funded by the DIUS NMS Chemical and Biological Metrology Programme. The editors are indebted to the many contributors to this report from the user communities already consulted and staff within the Quality of Life Division of NPL and the Research and Technology Division of LGC.

Contents

1. Introduction to the Chemical and Biological Metrology Programme

The Chemical and Biological Metrology Programme forms one part of a larger portfolio of Programmes funded by the Department for Innovation, Universities & Skills (DIUS) as part of the UK National measurement System (NMS).

More specifically, the Chemical and Biological Metrology Programme is an NMS Knowledge Base Programme which underpins some of the most challenging chemical, physical and biological measurements being made in the UK that are important to the UK's industrial competitiveness and quality of life. The Programme is concerned with the realisation and maintenance of measurements and standards for the determination of the quantity of matter, for which the mole is the SI unit. In addition, the programme maintains and develops the UK primary measurement capability and calibration facilities.

The Programme started on 1 April 2007 as a result of the merger of much of the work carried out under the former Valid Analytical Measurement Chemical and Physical (VAM-C and VAM-P) and Measurements for Biotechnology (MfB) Programmes. It represents both chemical and physical metrology projects that started in October 2006 and biometrology projects that started in April 2007. This Programme has established expertise and facilities at NPL and LGC that are recognised as centres of excellence for gas analysis, surface analysis, micro-/nano-particle measurement, mass spectrometry and molecular and cell biology, both within the UK and internationally.

The Chemical and Biological Metrology Programme directly addresses many of the priority issues in the UK, such as human health, food safety, climate change and its mitigation, protection of the environment, detection and prevention of security threats and nanoparticle safety, whilst also underpinning innovation in key, high added value manufacturing sectors such as biotechnology, pharmaceuticals, regenerative medicine, plastic electronics and alternative fuels.

Overall, the principal aims of the NMS Chemical and Biological Programme are aligned with those of the NMS programme portfolio in general, namely:

- To ensure valid and traceable analytical measurements in the UK, thus helping to improve the quality and comparability of measurements, enhance UK competitiveness and support regulatory needs
- To coordinate the UK's measurement system with those of our trading partners and other countries.

At present, the Programme is split into six main strategic priority themes that are aligned with the Bureau International des poids et Mesures (BIPM) CCQM Working Groups, which bring together international experts as advisers on scientific and technical measurement matters, namely:

- Bioanalysis (Genes, Proteins, Cells and Tissues) - BAWG
- Gas analysis - GAWG
- Inorganic and Speciation analysis - IAWG
- Organic analysis - OAWG
- Particles and electrochemistry analysis - EAWG
- Surface analysis – SAWG.

To optimize delivery of technical work and gain maximum synergies, projects in the six themes are divided between nine technical sub-themes plus three additional sub-themes, which address interfacial science, namely Environmental Technologies, Nanobiotechnology and Trace Analysis (see Figure 1.1).

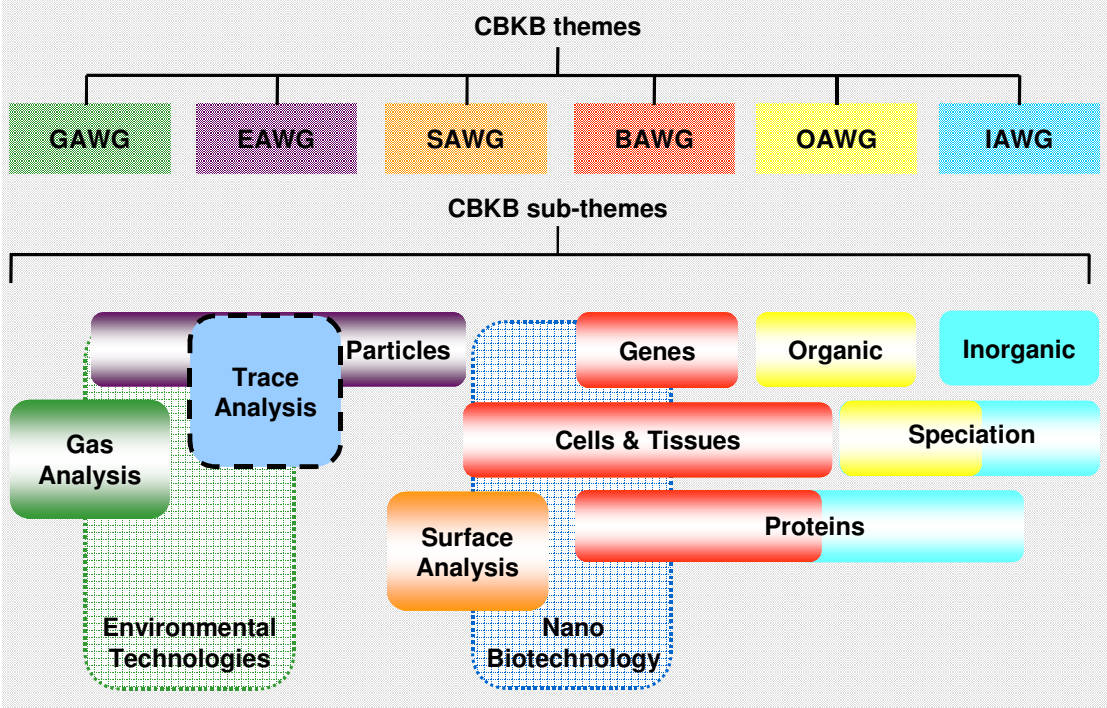


Figure 1.1: Schematic diagram of the Themes within the Chemical and Biological Metrology Programme

The main contractors for the Programme are the National Physical Laboratory (NPL) and LGC, two of the UK National Measurement Institutes (NMIs). The contractors have formulated proposals under the Surface Analysis, Trace Analysis, Bioanalysis, Organic Analysis, Inorganic and Speciation Analysis and Nanobiotechnology themes, on behalf of the NMS, to around 120% of the anticipated budget. The proposed activities identified by each contractor are available in Annex 1 of this document for public comment from 3 November to 12 December 2008.

The proposed activities are intended to enable strategically identified sectors of UK Industry to be competitive and world leading. This is done by providing a programme of focused measurement work that produces significant scientific, social or economic impact for UK Plc.

2. Programme Themes

During the summer and autumn of 2008 there has been an intensive effort to both understand and develop solutions to the measurement problems posed in the identified theme areas. This work involved the analysis of over 300 documents that included Government and independent reports, roadmaps and research council and other organisational strategy documents and discussions with over 100 organisations. Many of the measurement needs had already been captured in the Programme strategy document completed in June 2008. The additional opportunity provided by the formulation process allowed supplementary needs to be identified too through consultation with stakeholders to refine the measurement needs and reduce them to a manageable set of project proposals. Initial assessment was made based on the following two criteria:

- Impact
    - Economic
    - Quality of life
    - Leading-edge science
    - Standards & technical regulation
    - International influence
- Collaboration/Partnership
    - Co-funding/Industry support
    - Cross programme potential.

Fit with the UK Technology Strategy was considered in terms of potential to facilitate innovation and address market failures, bearing in mind that the Chemical and Biological Metrology Programme sits as a knowledge-based programme.

The resulting proposed activities outlined in Annex 1 were then developed. The following sections provide the context of each theme and an overview of its constituent projects. More detailed information about each project is presented in Annex 1.

2.1. Bioanalysis Theme

Extending the reach of the NMS from physics, through chemistry, to biology draws in disciplines that are more empirical and less quantitative. This strains the conceptual basis of metrology and so the challenge for NMS bioanalysis is to adapt these concepts appropriately – to find effective ways of introducing the key NMS motifs of traceability, measurement validation and uncertainty, and mutual international acceptance. These are the aspects that distinguish the Chemical and Biological Metrology Programme from the many other biotechnology programmes.

Theme aims

The Bioanalysis theme aims to provide a sound international basis for accurate and reliable measurements, which underpin the development and exploitation of biotechnology, principally through the healthcare industry, in the UK. The drive is to develop generic reference methods and measurement standards for UK industry and improve measurement comparability at interfaces key to the exploitation of bioanalysis – between discoverer and developer, between small company and large company, and between company and regulator. This will help translate bioanalysis from the science base into industry, help base regulation more securely on measurement science and maintain the UK lead in developing an international framework for bioscience measurement.

The theme has five areas of UK priority: biopharmaceutical manufacture; drug discovery; diagnostics; medical devices; and 'bioscience' - long-term biometrology completes the six focus areas for the theme. The proposed activities address challenge-driven priorities through the provision of:

- Reliable methods for low-level detection in complex matrices
- Cell-based models for improved safety and efficacy testing
- Screening methods to predict tissue engineered product safety
- Quantitation for compliance and regulation
- Reference methods and standards for alternative energy sources
- Primary reference methods, higher order reference standards/calibrants and measurement uncertainty, particularly for molecular diagnostics.

The NMS has made significant progress in introducing the concepts and supporting the practice of metrology in nucleic acid measurement. LGC, as the designated National Measurement Institute (NMI) for chemical and biochemical analysis, has demonstrated a strong record of achievement in 'standards and performance indicators' and 'comparability, quality and interpretation', culminating in the lead of international collaboration. However, the core thrust of moving leading-edge bioanalysis forward from the science base adds further requirements which reflect innovative approaches of emergent 'digital' technologies for gene analysis and sequencing, and emerging novel entities with potential for gene regulation as biomarkers of disease conditions and novel therapeutic interventions.

The NMS has similarly done much through LGC's centre of excellence for mass spectrometry to progress peptide and protein metrology practice (critical to exploitation of genome knowledge) through systematic address of robust sample extraction and separation strategies, absolute identification and quantification capability and determination of structure. Further requirements therefore reflect the need to apply traceable protein measurement approaches to priority health issues, e.g. food allergens, whilst addressing the need to link protein structure with function.

Though still relatively in its infancy and viewed as a long-term priority metrology issue within the NMS, there is now a more general awareness of the importance of robust cell measurements and the need to study these systems at the global level and the level of the various biological networks that exist within them. To date, in cell-based technology, where greater confidence in measurement would lead to an accelerated reduction in animal testing, more effective drug screening and potential novel biotherapeutics, the principles of comparable measurement have been difficult to apply. The NMS has however made some notable progress through the efforts of LGC, but there is the need, not only to respond further to continued societal concerns over animal-testing and drug safety, but to facilitate the early to middle stages of pharmaceutical and medical device materials development. Further progress is therefore needed to improve regulatory, industry and public confidence in cell-based testing methods; companies are wary of encouraging regulatory demands for more tests of doubtful value.

However, there are sectors and technologies where the NMS has been involved in only a limited manner to date, namely 'bioscience'. The use of biological processes or biomass to transform raw materials into useful commodities can be more cost effective than traditional chemical methods. Environmental biotechnology exploits biological processes and organisms to monitor, protect and restore the environment – to date the interest lies in phytochemistry. Functional foods and nutraceuticals are areas in which interest is

growing. The measurement issues in these cases remain less well-defined. A limited feasibility study relating to biofuels has been proposed and a cross-NMI consultation, led by LGC, is planned to make clear recommendations for the exact nature of future NMS involvement. Views would be welcomed on these particular issues during the public consultation.

Key drivers

The proposed activities within the theme address a number of high-level drivers. The bioanalysis industry sectors represented are economically hugely valuable to the UK - the Technology Strategy Board has identified the areas of Bioscience, Advanced Materials and High-value Manufacturing to be critical to the UK's transition to a knowledge-based economy. This is especially true in these sector activities where a strong science base offers the potential for innovation, fostering competitive business and enhanced global competitiveness, based on successful translation. Innovation is essential to the UK's future economic prosperity and quality of life - all the sector activities proposed contribute, either directly or indirectly to the health, safety and security of individuals. The activities also underpin active regulatory frameworks through their provision of standards and/or measurement science. Those addressing long-term biometrology are contributing to the development of the SI system to support emerging biomeasurement needs. Bioanalysis is inherently cross-sector and so the majority of the proposed activities will also impact activities elsewhere.

The key technological drivers for improved bioanalysis can be summarised simply. The bulk of the industrial activity in bioanalysis still focuses on pharmaceutical markets although, in recent years, there has been a paradigm shift in the strategies used for drug discovery screening. Successful small molecule leads from candidate screening assays are optimised and tested, frequently in functional cell-based assays to understand parameters such as toxicity. The decreasing product pipeline and threat from 'biosimilar' manufacturing is however testing the traditional face of the industry.

There is no slackening of either legislative or societal pressure to rely solely on cell-based assays. Pressures towards mechanism-based risk assessment demand better models for toxicity testing. As valid alternative assays are not widely available, and those that are have been extremely variable, improvement in the accuracy and precision of the data from new models is a key requirement. Emerging biotherapies and more complex biopharmaceuticals are also challenging the market dominance of small-molecule drugs, but issues related to process optimisation and control remain a challenge. Medical device regulation is being 'merged' with medicinal legislation from the pharmaceutical sector due to the emerging novel advanced therapy medicinal products. Product quality remains an underpinning measurement issue. Molecular diagnostics faces a period of significant change and new challenges, although its generic underpinning technologies have a range of other diverse range of target sectors of application. NHS policy remains aligned with achieving an increased use of point-of-care testing, and early disease state diagnosis and prognosis. Technical developments therefore promise benefit from faster, lower level and more reliable genetic and proteomic testing where early intervention in solving measurement issues will be beneficial. The greater consideration towards sustainability is driving interest in the use of biological systems as alternatives to fossil resources for the production of biofuels and in the generation of renewable energy sources from biomass. The diverse range of feedstocks and process efficiencies pose different measurement challenges – their solutions are therefore relatively undefined at present. The present physical model of SI traceability to the mole fails to connect with biological identity and activity. Most biological characteristics have no traceable units, although the framework for moving towards this is being established within the mutually-accepted roadmaps established by the CCQM Bioanalysis Working Group, led by LGC, with NPL as

rapporteur, on behalf of the NMS. Proposed international biometrology activities within the Chemical and Biological Metrology programme fully meet the strategic direction being set by this Group.

The Projects of this theme are summarised in the following table (the detail being given in Annex 1):

Project no.	Project title
BA1	Industrial consultation and analytical feasibility study for emerging metrology requirements to support biofuels
BA2	Cell phenotype authentication
BA3	Validated in vitro sensitisation models for consumer products
BA4	Reference measurement methods for nucleic acid metrology
BA5	Traceable methods for the characterisation of structurally significant proteins (EMRP JRP11 Clinbiotrace project)
BA6	Evaluation of fitness-for-purpose digital and real-time PCR methods for non-invasive pre-natal diagnostics
BA7	Traceable methodologies for food allergens
BA8	Measurement guidelines for improved genotoxicity testing
BA9	Glycan reference materials
BA10	Development of a framework for standardisation of miRNA measurement
BA11	Reference standard requirements for high throughput sequencing and metagenomics
BA12	Predictive toxicology
BA13	Quality control materials for mass spectrometry-based proteomic analysis
BA14	Development of a robust toxicogenomic-based measurement capability to support REACH legislation
BA15	Characterisation of tissue engineered products (EMRP JRP4 Regenerative Medicine project)

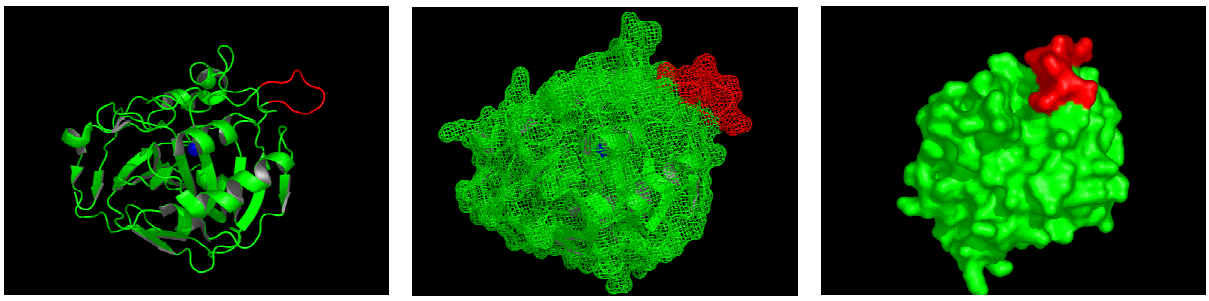


Figure 2.1.1: LGC's recognised centre of excellence for mass spectrometry has developed accurate protein quantitation and structure determination – Here, a specific portion of a thermally-stressed sample shows protein conformational changes.

2.2. Inorganic and Speciation Theme

The inorganic and speciation theme provides a sound international basis for the use of accurate and reliable organo-metallic and non-metal speciation (where the precise chemical binding of elements of interest can significantly affect toxicity or bioavailability), and trace element and isotope ratio measurements in research and industry to underpin the key application areas of food, clinical and environmental analysis in the UK.

Theme aims
The theme has four priority areas: food and feed manufacture; diagnostics/ therapeutics; sustainability; and consumer products. The proposed activities within the theme address challenge-driven priorities through the provision of:

- The first reference standards for absolute carbon isotope ratios traceable to the SI
- Competitive metal/non-metal species identification and distribution
- Quantitative speciation methodologies for characterisation of metallo-drugs and bioconjugates
- High accuracy methods for the determination and identification of metals at or below regulatory limits
- Primary reference methods, higher order reference standards/calibrants and measurement uncertainty.

Figure 2.2.1a shows the timeline for the key technologies for atomic spectrometry measurement. The NMS has made significant progress in introducing the concepts and supporting the practice of metrology involving these technologies. LGC, as the designated National Measurement Institute (NMI) for chemical and biochemical analysis, has demonstrated a strong record of achievement in the provision of primary reference methods, higher order reference standards/calibrants and measurement uncertainty through the lead of international collaboration efforts in the area. LGC is now recognised as an international lead in speciation analysis (see Figure 2.2.1b). The extension to non-metals broadens the scope of application to molecules of biological interest, such as DNA oligonucleotides and peptides. Furthermore, increasing recognition of the role of 'elemental and isotope analysis' in key areas of interest to the UK adds further requirements which principally reflect the emerging role of certain metal species in key drug metabolism pathways and clinical efficacy for disease therapy/protection, and of isotope ratio determination for authenticity and geographical origin for a range of diverse application areas.

The 'roadmap' for this theme and the proposed activities presented reflect this requirement for transition to a higher level of application-based work in keeping with the greater maturity of the elemental analysis work, whilst allowing development of the core 'elemental' capability so as to be able to respond to future chemical metrological challenges.

Key drivers
The proposed activities within the theme address a number of high-level drivers. They are again consistent with the Technology Strategy Board priorities. The strong science base offers the potential for innovation, fostering competitive business and enhanced global competitiveness. All the activities contribute, either directly or indirectly to the health, safety and security of individuals. They also support the industrial and regulatory community by improving measurements and thus regulatory confidence.

Understanding the mechanisms by which products benefit animal and human health is crucial to commercial improvements in product processes and the safety and efficacy (and regulatory approval) of enriched food and feeds, and food supplement products essential to tackling current metal species' deficiencies in the UK diet. The provision of a matrix reference material for product quality control is proposed. Advances in mass spectrometry have made possible the detection of counterfeit goods using trace element analysis and/or subtle differences in both stable and radiogenic isotope composition. Certification of reference standards for absolute carbon isotope ratios traceable to the SI is proposed. Metal drugs are increasingly showing potential medicinal use in the treatment of common disease states. Elucidation of metabolic pathways and traceable measurement capability associated with such compounds, essential for the safety and efficacy of potential novel pharmaceutical products, is proposed. The shift from total metals to reliable information on both the amount and composition of metal-containing species in accumulating plants is of importance for their successful use in removing environmental pollutants from soils and waters for land reclamation and effluent treatment, and for regulatory purposes. Accurate measurement of restricted products in consumer products and wider industrial (e.g. construction) products supports sustainable development by enabling effective waste management and recycling strategies and underpins assurances about human health. The new Waste Electronic and Electrical Equipment (WEEE) and Restriction of Hazardous Substances (ROHS) Directives target the use of certain metal substances in electrical and electronic equipment. Manufacturers test products to supplement material declarations and to verify supply chain declarations of component composition. Enforcement authorities perform testing to verify individual supplier compliance and as part of broader surveillance programmes. The proposed international metrology activities within the Chemical and Biological Metrology programme fully meet the strategic direction of the mutually-accepted roadmaps being established by the CCQM Inorganic Analysis Working Group, led by LGC on behalf of the NMS.

The Projects of this theme are summarised in the following table (the detail being given in Annex 1):

Project no.	Project title
IS1	Certification of reference standards for absolute carbon isotope ratios traceable to the SI
IS2	Elemental tagging/labelling and HPLC-ICPMS for environmental applications
IS3	High accuracy quantification
IS4	Characterisation of a methionine certified reference material
IS5	Simultaneous measurement of sulphur with other metals for bioclinical and food applications
IS6	Quantitative speciation methods for characterisation of a speciated plant reference material
IS7	Speciation methodologies for quantifying DNA-adduct formation with metallo-drugs
IS8	Traceable measurement of vanadium-containing biomolecules
IS9 (see OA4)	Measurements and materials to support ROHS/WEEE legislation and VOC emission control

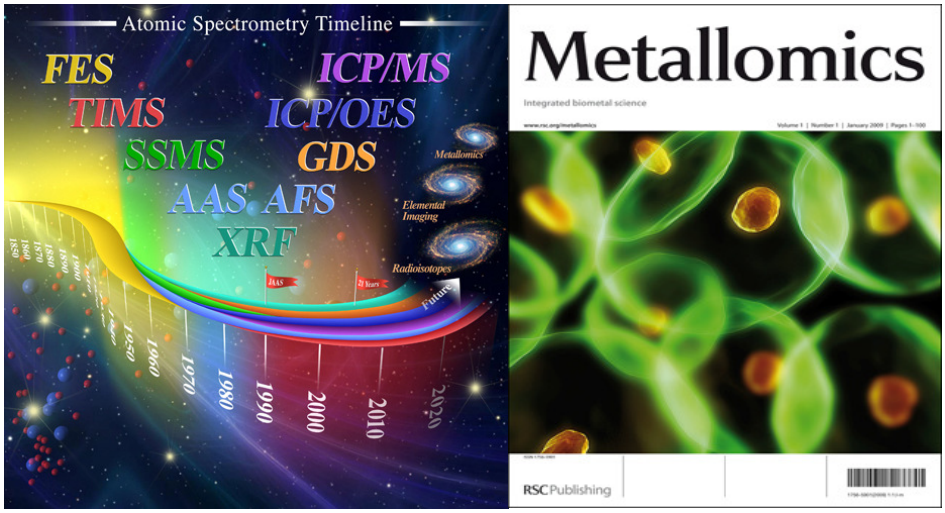


Figure 2.2.1a: Timeline showing new key technologies for atomic spectrometry (picture courtesy of JAAS).
Figure 2.2.1b: LGC's international reputation recognised through invitation to join the international editorial committee for the new RSC Metallomics journal, to be launched in 2009 (picture courtesy of RSC).

2.3. Organic Analysis Theme

The portfolio of proposed activities for the organic theme is focused mainly on mass spectrometry, the dominant technique for the high accuracy determination of trace organics, and underpinning metrology activities (including purity determination) that support the UK's chemical measurement system. The activities provide a sound basis for the uptake, or direct use, of accurate and reliable measurements for application in the key areas of healthcare, security and sustainability.

Theme aims

The theme has four areas of application focus: national security; (clinical laboratory) diagnostics; sustainability; and consumer products. Certified reference material (CRM) development and supply, underpinned by the infrastructure of expertise and competencies to fulfil UK chemical measurement obligations under the CIPM Mutual Recognition Agreement (MRA), features highly. The proposed activities in this theme sustain and develop this important capability, providing materials that support key UK sectors. The proposed activities within the theme address:

- High resolution separation systems for improved quantitative mass spectrometry
- High accuracy methods for the determination and identification of volatile organic compounds at or below regulatory limits
- Primary reference methods, higher order reference standards/calibrants and measurement uncertainty, principally in support of food, clinical laboratory, environmental and consumer legislation.

The NMS has made significant progress in introducing the concepts and supporting the practice of metrology involving these technologies. LGC, as the designated National Measurement Institute (NMI) for chemical and biochemical analysis, has demonstrated a strong record of achievement in the provision of primary reference methods, higher order reference standards/calibrants and measurement uncertainty. LGC is a recognised international player in purity measurements and its proposed extension of the UK

Chemical Calibration Facility capability to establish a UK adiabatic calorimetry capability for enthalpy of fusion of pure substance materials is presented.

The 'roadmap' for this theme and the proposed activities allows development of the core capability so as to be able to respond to future chemical metrological challenges balanced with a progressive evolution to a higher level of application-based work. There are strong connections from previous work on high mass accuracy determination of 'small molecules' and purity measurements.

Key drivers

High quality matrix reference materials are expensive to produce and represent a classic case of market failure. There is no strong driver to provide materials anchored to international reference points, which could be used by a broader range of organisations to achieve more general comparability. Hence, throughout the world, Governments have stepped in to provide funding to ensure that appropriate reference materials are developed for key areas relating to trade, health, food and the environment. The establishment of the European Reference Materials concept helps share resources across Europe and ensures that there is no unnecessary duplication of effort.

The proposed activities within the theme develop this infrastructural capability to address a number of high-level drivers, consistent with Technology Strategy Board priorities. Again the strong science base offers the potential for innovation, fostering competitive business and enhanced global competitiveness. All the activities address safety and sustainability issues. They also support the industrial and regulatory community by improving measurements and thus regulatory confidence.

The pressure for today's laboratories to perform higher throughput analyses, requires novel separation strategies to maintain accurate quantification using LC-MS approaches. By addressing this issue, the proposed activity will support the broad analytical community. Appropriate food RMs are required to ensure food products meet UK legislation. However, there is a paucity of such materials for the main constituents of food materials and for food contaminants. The proposed activities address 'wet' sample packaging issues and provide a priority food contaminant CRM. The need for traceable, high quality clinical CRMs is reinforced by the demands of ISO 15189; the increasing use of LC-MS for organic analyses by hospital laboratories has led the Clinical RM Users Group to identify key clinical analyte/matrix combinations as priority interest. These will be provided by proposed theme activities. Address of 'missing' reference materials, such as priority organics in water, to support UK laboratories implement the Water Framework Directive and regulators to monitor pollution control measures is proposed. As indicated in the 'elemental' theme, accurate measurement of restricted products in wider industrial (e.g. construction) and consumer products, by manufacturers and enforcement authorities alike, under the new Waste Electronic and Electrical Equipment (WEEE) and Restriction of Hazardous Substances (ROHS) Directives supports sustainable development and underpins assurances about human health. EU Regulations addressing existing and new persistent organic pollutants (POPs) limit levels of specified perfluorinated and dioxin-like PCB compounds in food and feeds and in the environment. Current methodologies demonstrate several measurement issues, which are to be addressed by the relevant theme activities to provide industry and regulators with the means to comply with the appropriate EU Regulations. A number of proposed activities will ensure the UK's chemical measurement system is developed appropriately and linked internationally. These include efforts to improve methods for estimating consensus values in proficiency testing, addressing current method bias. The development of a semi-preparative HPLC approach identified within the VAM Programme to produce ultra-pure substances as calibrants for

characterising larger batches of the same measurand so as to ensure traceability of high accuracy measurements. The UK's range of enthalpy of fusion CRMs used by a range of UK industries can no longer be characterised within Norway and it is proposed to establish a UK capability. The proposed international metrology activities within the Chemical and Biological Metrology programme fully meet the strategic direction of the mutually-accepted roadmaps being established by the CCQM Organic Analysis Working Group.

The Projects of this theme are summarised in the following table (the detail being given in Annex 1):

Project no.	Project title
OA1	International metrology measurements
OA2	Traceable methods and reference materials to support clinical laboratory measurements
OA3	Traceable methods and reference materials to support the EU Water Framework Directive
OA4	Measurements and materials to support ROHS/WEEE legislation and VOC emission control
OA5	Evaluation of matrix suppression effects using rapid high-resolution separation systems and on-line sample clean-up strategies on quantitative mass spectrometry detection
OA6	Enthalpy of fusion reference measurements and materials – Feasibility study
OA7	Improved certified reference materials for food analysis
OA8	Improved characterisation and estimation methods for proficiency testing and reference material certification
OA9	International harmonisation of reference measurements for malachite green
OA10	Traceable methods to assign polyfluorinated compound and perfluorinated persistent organic pollutants certified reference materials – Feasibility study
OA11	Improved traceability of pure certified reference materials
OA12	Reference material certification and support
OA13	Traceable values for dioxin-like polychlorinated biphenyls

2.4. Surface Analysis Theme

The successful, modern knowledge-based economy is built on growing innovation for the development of high added-value products that have a strong competitive edge in the global market place. Central to the correct operation and novel properties of many of these products is the surface chemistry. Surface chemical analytical techniques are key to understanding and characterising these surfaces from the microscale to the nanoscale. The frontline industry-favoured techniques for surface chemical analysis are X-ray photoelectron spectroscopy (XPS), static secondary ion mass spectrometry (SSIMS) and atomic force microscopy (AFM). These complementary techniques provide key quantitative information on atoms and molecules at surfaces, providing high specificity, identification of complex molecules and analytical measurement of materials at the

nanoscale (see Figure 2.4.1). There is an increasing need for analytical techniques that operate in ambient environments and the uptake of new innovative techniques, such as desorption electrospray ionisation (DESI) and tip-enhanced Raman spectroscopy (TERS), is growing strongly. The Chemical and Biological Metrology Programme is providing a key role in developing metrology and confidence in these new techniques.

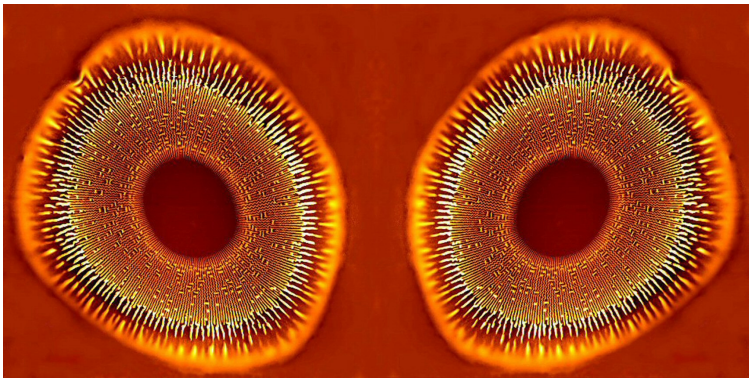


Figure 2.4.1: "Look to the future" – The NPL Surface and Nanoanalysis group is recognised internationally for its excellence and leadership. The internationally award winning image shown here is from work on nanoscale features in molecular nanolayers.

Figure 2.4.2: Pioneering new media to maximise wider benefits and impact. The Surface Analysis virtual laboratory in SecondLife (http://nanoisland.wordpress.com/) allows visitors to access programme outputs, learn about the operation and principles of the techniques through model instruments and provides an introduction to nanoscience.

The Surface and Nanoanalysis group at NPL is recognised as an internationally leading group with a strong track record of impact through publications, books, collaborative research, technology transfer, conferences and workshops, international standards, secondments and scientific leadership in many fora. The group has pioneered the use of new media such as the virtual world, "SecondLife", illustrated in Fig 2.4.2, to maximise impact to a broader community giving wider benefits and an educational platform. Research supported under previous Chemical and Biological Metrology Programmes has led to significant benefits for innovation in both industry and academia. The programme has provided revolutionary new methods, major improvements in measurement reproducibility and repeatability, an infrastructure to ensure valid interpretation and fit-for-purpose measurements and frontier research for challenging measurements at the

nanoscale. The proposed new programme builds significantly on this, developing key measurements needed by industry for innovation and begins the development of strategically important new areas such as nanoparticle characterisation.

Theme aims

The aims of the Surface and Nanoanalysis area are to:

- To support innovation and competitiveness through the development of reliable leading-edge measurement.
- To develop, strategically, the measurement capabilities and infrastructure to meet the needs of knowledge-based industries and for quality of life.
- To ensure that UK measurements are fit-for-purpose, provide international comparability of measurements and ensure that the measurement infrastructure is in place to underpin systems for accreditation and quality control.
- To provide leadership in frontier issues in surface and nanoanalysis measurement in the UK and internationally.
- To conduct key knowledge transfer activities to maximise our impact and wider benefits, including national and international comparisons, representation on international bodies to ensure international uniformity and traceability of UK measurements and to ensure that knowledge outputs are promulgated effectively and impact the key audiences including exploitation of new media.

Key drivers

This theme's projects are designed to address a number of high-level drivers that were identified during the consultation and formulation process and analysis of relevant roadmaps and government reports. A key driver centres on the UK's need to innovate and compete with emerging countries that are characterised by a low-wage and increasingly skilled workforce. The competition is running hard: For example, China and India are determined to improve the quality of their goods and services, increase their rates of innovation, and move into high-value added goods and services. Therefore, the UK must excel at all types of innovation to raise productivity, foster competitive business and meet the challenges of globalisation. Innovation is essential to the UK's future economic prosperity and quality of life.

To support the UK's drive toward a high added value, knowledge-based economy, the Technology Strategy Board has identified core technologies that are critical to the UK's success, including Advanced Materials, Biosciences, Nanotechnology and High-value manufacturing. These areas cover a wide range of industry sectors, including Health and Personal Care, Packaging, Pharmaceuticals, Chemical Additives, Advanced Materials, Aerospace and Defence, Novel Displays and Electronics. The innovative technologies in these sectors are wide-ranging such as drug delivery and controlled drug release systems, anti-fouling implants, medical imaging (contrast agents), high-efficiency detergents, natural-fibre coatings, cosmetics and technical textiles with applications including wound healing, protection from chemical warfare, functionalised microfabricated devices, organic electronics, displays and ink-jet fabrication.

Nanomaterials are front-runner nanotechnologies that are key to many of the high innovation products highlighted above and are of enormous economic importance. Concomitantly, there is an increasing concern over their potential health risks. It is clear that the surface chemistry is a critical parameter for materials, such as nanoparticles, used in innovation: For instance, in nanomedicine, the modification of the surface chemistry for specific organ and tissue targeting, in engineering, or, in transport, and catalysts such as cerium oxide additives used to improve fuel efficiency in combustion engines.

Importantly, a key issue is the determination (if any) of the environmental, health and safety aspects of nanomaterials. The international toxicology community is making significant progress in identifying which parameters of nanoparticles have the strongest effect on mammalian cell toxicity. In general, three key parameters have been identified that can give reliable predications of toxicity; particle size distribution (also aspect ratio), specific particle surface area and surface chemistry/charge. Characterisation of the surface and bulk chemistry of nanoparticles is therefore an underpinning requirement for both innovation and environment, health and safety aspects. The "Surface and NanoAnalysis – Advanced Metrology for Innovation and Quality of Life" theme has emphasis on these knowledge-based industries with an objective to develop the necessary measurement capability and infrastructure. Recent key reports by the DEFRA-funded REFNANO project, the Nanotechnologies Research Coordination Group and the Organisation for Economic Cooperation and Development Working Party on Manufactured Nanomaterials have highlighted the need for the characterisation of nanoparticle surface chemistry.

The Projects of this theme are summarised in the following table (the detail being given in Annex 1):

Project no.	Project title
S1	Surface and nanoanalysis of micro and nanoparticles for innovation and environment, health and safety
S2	Multivariate methods for identification, classification and quantification in spectroscopy and imaging
S3	G-Tip innovation and cluster ion metrology
S4	Quantitative imaging XPS
S5	Organic depth profiling in SIMS and XPS
S6	Analysis of molecular layers – ultrathin layers on polymers and molecular orientation
S7	Characterisation of AFM-probe-chemistry
S8	Novel AFM modes for soft-surface imaging
S9	Measurement of interparticulate interaction
S10a	Ambient and imaging mass spectrometry
S10b	Metrology for innovative ambient mass spectrometries and MALDI
S10c	Assessment of the quantitative attributes of imaging mass spectrometry

2.5. Trace Analysis Theme

There is strong demand for accurate chemical measurements at very low concentration levels for testing compliance with regulatory levels and to detect contamination, especially in the healthcare, pharmaceutical, environmental (inc. air quality) and food industries. In particular, there is a requirement to identify and measure trace components that may be hazardous to human health and the environment.

The Trace Analysis sub-theme underpins a wide range of industrial, governmental and legislative measurement requirements, with work in this area concentrating on three related sub-areas:

- Electroanalytical Chemistry – within the Particles sub-theme;
- Environmental Analytical Chemistry – within the Particles and Environmental Technologies sub-themes;
- Surface Enhanced Raman Spectroscopy (SERS) and related technologies – within the Particles sub-theme.

These subject areas have been recognised for excellence by the recent award of the Royal Society of Chemistry's 34th SAC Silver Medal for the work of the Trace Analysis area. Despite its relatively small size, the area continues to deliver a high level of output and impact – including dissemination via twenty-eight peer-review papers, to date, in the 2006-2009 CBKB Programme cycle and influence via representation on eight international standardisation and national learned-society committees.

Work in the area is centred on measurement issues relating to the sampling, sample preparation and analysis of airborne particles, the use of novel electrochemical devices and techniques for environmental analysis and the development of SERS as a quantitative analytical technique. Synergy exists between the Trace Analysis area and the work in the Gas Analysis sub-theme where techniques for handling particle-laden air are similar to those for handling gas mixtures, and also with the work in the Nano-Biotechnology sub-theme where aspects of a tip-enhanced Raman spectroscopy project are related to the SERS research work.

Theme aims

The aims of the Trace Analysis area are:

- To develop novel, validated miniaturised ion-selective electrodes and electroanalytical techniques for use in in-situ environmental applications.
- To develop validated, traceable methods for the automatic measurement of mercury vapour in ambient air.
- To validate sampling and sample preparation methodologies for key emerging pollutants.
- To provide sampling methodologies and validated compositional measurements of the particulate matter size fractions most relevant to health studies.
- To demonstrate the enhancement, reproducibility and repeatability of functionalised nanoparticle arrays as SERS substrates.
- To disseminate the outputs of this work via peer-reviewed publications, presentations at high-profile conferences, and input into relevant learned society and standardisation committees.

Key drivers

These projects are designed to address a number of high-level drivers that were identified during the consultation and formulation process and analysis of the relevant roadmaps:

- Regulation and legislation. In particular, new and forthcoming EU legislation concerning the composition of different size fractions of particulate matter in ambient air, and the concentration of ambient mercury vapour.
- UK competitiveness. Development of technologies, such as improved SERS platforms, and novel and miniaturised ion sensing devices, in order to provide measurements more cheaply, in-situ, and with a high throughput, to give UK industry a competitive edge in the market place.

- Quality of life. Development of robust measurement strategies to better monitor pollution and provide more certain and more extensive data to protect the environment, inform health studies and aid the work of toxicologists and epidemiologists. Additionally, from the SERS perspective, the underpinning of homeland security is an important contribution to this driver.
- Underpinning measurement science. Fundamental research to advance the state of the art, and stimulate research and development in a variety of areas such as: novel SERS substrates, novel materials for ion selective electrodes, and novel data handling techniques and chemometric procedures for complex analytical measurements.

The Projects of this theme are summarised in the following table (the detail being given in Annex 1):

Project no.	Project title
TA1	Traceability for miniaturised electrochemical devices
TA2	Traceable and validated automatic measurements of mercury vapour in ambient air
TA3	Validated analytical strategies for emerging ambient pollutants
TA4	Accurate compositional studies of size fractionated particulate matter to inform health studies
TA5	Quantitative SERS using optimised substrates and novel platforms
TA6	Measurements of ultra-low permeability barriers for flexible organic electronics

2.6. Nanobiotechnology Theme

Nanobiotechnology is a rapidly advancing area of scientific and technological opportunity that adapts the principles and designs from nature, as well as biological components and materials. It is a highly interdisciplinary field, featuring a close collaboration between life scientists, physical scientists, and engineers. Measurements to support safety assessment of nanotechnology products are included.

Theme aims

The current areas for focus relate to the measurement of nanoparticles to support their application in nanomedicine. Measurement of their structure, activity and biomolecule interaction supports toxicological studies of nanoparticles. Activity within the theme is directed to developing key underpinning metrology to support:

- The development of reliable leading-edge nanoparticle measurements, particularly those identified by the cross-departmental Nanotechnology Research Co-ordination Group (NRCG).
- The quantification, identification, structure and activity of biomolecules at planar and nanoparticle surfaces, from monolayers to single molecule measurements.
- Measurements for nanoparticle toxicology
- International leadership in the standardisation of nanobiotechnology measurements.

Following the recent NPL Programme formulation on the biophysical measurement aspects of the theme aims, LGC now has the opportunity to extend NMS involvement by formulating two complementary activities which address the measurement issues associated with dosimetry and *in vitro* bioassay standardisation.

The proposed activities in this theme will provide a testing strategy that can be used to assess accurately engineered nanoparticle (ENP) dose-response characteristics. In doing so, it will develop a new NMS capability that expands the centre of excellence for mass spectrometry and the growing reputation for cell metrology activities within LGC. They will also produce guidelines for ENP cytotoxicity testing based on assessment of the suitability of standardised toxicity assays for measuring ENPs and their potential for transfer to high throughput screening regimes. This effort will be carried out, in association to that of NPL's efforts (already identified within the Surface Analysis theme of this Programme), with the international collaborative research under EU Framework Programme 7 project (NAPIRA).

Key drivers

The 2004 Royal Society and Royal Academy of Engineering report on the opportunities and uncertainties of nanoscience and nanotechnologies has been instrumental in informing UK Government strategy, resulting in the NRCG and nanotechnology research initiatives via the Research Councils and the Technology Strategy Board to address the lack of established nanobiotechnology industry within the UK and the potential benefits of major developments in this field.

The EU aims to improve the competitiveness of its industry by enabling a knowledge-intensive industry producing high-value products, processes and technologies; nanosciences and nanotechnologies are specified priorities. Furthermore, a recent European workshop reviewing the knowledge gaps within existing research initiatives on the safety of nanoparticles, called for urgent action to:

- Develop validated test methods for nanoparticle toxicity testing.
- Develop rapid, user-friendly, *in vitro* toxicity screens.
- Develop guidelines for nanoparticle toxicity testing.

Understanding ENP safety is essential to the broad acceptance of the technology and to deriving a pragmatic approach to regulatory requirements.

The Projects of this theme are summarised in the following table (the detail being given in Annex 1):

Project no.	Project title
NB1	Nanotoxicology – Dosimetry
NB2	Nanotoxicology – Assay standardisation

Annex 1: Projects by Theme

This Annex contains details of all the proposed activities, presented by theme in the following order:

1. Bioanalysis
2. Inorganic and Speciation Analysis
3. Organic Analysis
4. Surface Analysis
5. Trace Analysis
6. Nanobiotechnology.

Each project description is presented in tabular form. Each project table contains the following elements:

- Abbreviated theme name and Project Number, e.g. Trace Analysis – 'TA1';
- Project Title;
- Project Objectives – a listing of the main aims of the proposal;
- Background and Rationale – text describing the identified user community needs being addressed by the project – these are taken from the priority needs identified by the consultation process and/or the analysis of the relevant roadmaps;
- Impact – text describing the impact that the project as a whole is expected to have on the relevant user communities. In addition, this element describes more specific types of impact that are expected to arise from some part of the project, perhaps a single sub-project or one or more individual products;
- Summary of Technical Work – text describing actual intended work details and an overview of the outputs;
- Deliverables – a listing of the expected project deliverables and any milestones.

BA1					
Project Authors/ Contact	**Authors: Nick Boley/Gavin O'Connor** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Industrial Consultation and Analytical Feasibility Study for Emerging Metrology Requirements to Support Biofuels

Project Objectives

- To inform DIUS strategy on the global and UK specific metrology needs to support legislation for existing and emerging biofuels.
- To establish capabilities for supporting the emerging diversity of biofuels.
- To collaborate with EU/international-supported activities for the production of reference materials to inform biofuel specification.

Background and Rationale

Biofuels and the energy sector are one component of the newly emerging knowledge-based 'bioeconomy'. Considerable attention is being devoted globally to renewable energy supplies and biofuels offer an important addition to supplement/replace conventional fossil fuels.

Biofuels can be categorised as 'first' to 'fourth' generation. Current 'first generation' biofuels are derived from products of conventional food crops (the starch, sugar and oil feedstocks from crops such as wheat, maize, sugar cane, palm oil and oilseed rape). These are converted to biodiesel and bio-ethanol for use as automotive fuels. Given these feedstocks are important food sources, investment has been seen in a broader range of feedstocks (inc. lignocellulose in dedicated energy crops such as perennial grasses, and from forestry, the co-products from food production and domestic vegetable waste biomass – 'second generation' biofuels). 'Third generation' fuels include new feedstocks from novel non-food oil crops, the use of marine organisms and the direct production of hydrocarbons from plants or microbial systems. Synthetic biology offers future potential to produce novel chemicals through re-design of biological pathways or organisms – such 'fourth' generation biofuels are attractive for use within the existing transport infrastructure without conflicting with current engines, supply-line modifications or fuel standards. Such breadth of feedstocks and variety/complexity of processing efficiencies has a large effect on the biofuel quality, a cause of increased concern to industry and end-users alike. The metrology required to support this evolving activity is not well defined or understood internationally.

The EU has set an ambitious target that, by the year 2020, 20% of the EU energy consumption will be from renewable sources. Member States have been set a target requiring 10% of transport fuels to be from renewable sources. Policy frameworks such as the EU Biofuels Directive and Renewable Transport Fuel Obligation (RTFO) for the UK, target 5% of transport fuel supply to be from biofuels by 2010 and 10% by 2020. Fuel standards play a major role in defining the opportunities for biofuels. The revised EU Fuel Quality Directive (98/70/EC, 2007) effectively limits the amount of bioethanol blended with petrol, and biodiesel with mineral diesel, to a max. 5%/vol, to avoid adaptation of the existing car fleet. EN228 (unleaded petrol; EU Auto Oil Directive) sets EU fuel standards for petrol/ethanol; EN14214 (fatty acid methyl esters (FAME); UK EN590; all EU diesel) for diesel/biodiesel. However biofuels have a limited ability to replace fossil fuels whilst conforming to European fuel standards.

A current EU FP7 initiative (Biorema), involving NMIs from the EU, USA and Brazil aims to address these issues by producing two RMs for biofuels for a global market. However, specific consideration for the local source of the biofuel and local environmental conditions will be required, indicating presence/absence, tracing of regional origin and quality of biofuels (including under storage conditions) to be of measurement importance.

With such a complex set of problems and the likely pace of change in the field, this project sets out to:

- consult widely with the community, both locally and internationally, as to the major metrology barriers to trade and innovation.
- establish a core capability for addressing specific issues which may include the provision of traceable standards, labelled materials, and reference methods for the traceable value assignment of priority measurands.
- interact with current EU framework projects by providing analytical measurement to support their goals.

This will enable the development of an informed strategy combined with the capability for the provision of traceable measurement to support the future requirements for reference materials in this field.

Impact

At all levels globally, the main drivers for the development of bioenergy and biofuels are climate change, energy security and rural development. The political motivation to support biofuels arises from each individual driver or combinations thereof. Policies designed to target one driver can be detrimental to another as different biofuels have widely differing environmental, societal and economic impacts.

The UK's consumption of biodiesel has risen 10x in the past two years with its global production quadrupling during 2000-05; in the same time period, the global production of bio-ethanol has more than doubled. The major supplier of UK biofuels is now selling >100mn litres of biofuel/week, 10% of the UK road fuel market.

Obtaining a consensus on the need and direction for the development of traceable methods and standards for biofuels, and the reproducibility and repeatability of industry practice, is essential to extending NMS science input in an appropriate manner that leads to removal of trade barriers, and ensures consumer and environmental protection through the rapid assessment of product origin and quality.

Summary of Technical Work

The consultation, carried out in close association with other interested UK NMI parties, will provide a strategic paper on measurement requirements for biofuels for consideration by the DIUS Measurement Board.

Building on current capabilities for the traceable value assignment of complex organic molecules in volatile solutions, advanced sample preparation, separation strategies and traceable detection methods will be developed for the isolation of biofuel markers (initially for the characterisation of FAMES and ethanol in biofuels to support EU activity). However, the approach developed will be to establish a core capability that enables the analysis of a wide selection of biofuels, thereby taking into consideration future developments and changes in marker profiles as new methods for biofuel production come on-line.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW a consultation report on industry requirements for metrology in the biofuels industry is reported to DIUS.			
2	CEW a core capability, initially for the analysis of two priority markers, has been developed.			
3	CEW the developed approaches have been used to value assign candidate reference materials from the EU FP7 project.			
4	CEW results have been disseminated at relevant conferences and via at least 1 peer-review publication.			
			Total cost	

BA2					
Project Author/ Contact	**Author: Damian Marshall** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Cell Phenotype Authentication

Project Objectives

To develop a robust generic phenotype authenticity test for cell lines, cell banks and cell products, based on cell surface marker expression levels by:

- Development of measurement tools which can be incorporated into QC and publication procedures to ensure the comparability and robustness of data obtained using cell assay systems.
- Proposing an international pilot study for generic cell authenticity testing orchestrated through the bioanalysis working group at CCQM.
- Promoting uptake of cell authenticity practices through collaborations in the international metrology arena.

Background and Rationale

Authentication of cell phenotype is central to ensuring the robustness of data obtained using *in vitro* screening tools. Many of the assays routinely used in pharmaceutical, chemical, cosmetic and diagnostic assays involve the use of cell lines that are differentiated to a desirable phenotype using specialised techniques. At present there are few control procedures to ensure that final cell populations are differentiated fully along the appropriate lineage and that population purity is at acceptable levels to ensure confidence in assay data.

A lack of cell line authentication procedures has been shown in recent years to be one of the key causes for wasted research and lack of both intra and inter laboratory data comparability. A long-term goal of the CCQM Bioanalysis Working Group (BAWG) is to make cell-based measurements more comparable by developing reference measurement system for cell based assays and anchoring as many measurements as possible to the SI or other internationally recognised units. This has led to an initial BAWG pilot study to examine the application/comparability of flow cytometry data for cell type specific phenotypic analysis (P102) as well as examination of collaborative opportunities within the international metrology arena. The development of a generic testing strategy for phenotypic authentication would represent a significant step forward in this area and provide a powerful measurement tool for those scientists utilising cell lines.

Most cell phenotypic analysis involves the use of genomic, transcription profiling and flow cytometry analyses, often used in combination to identify a profile that is specific to a cell type and/or tissue phenotype. This analytically heavy process relies on the availability of lineage specific markers to identify one cell population from another. Breaking away from this individual cell type analysis through the development of a generic authentication procedure would be of huge benefit to cell assay users as it would allow rapid and comparable confirmation of cell phenotypic status and allow population purity levels to be ascertained.

An approach to this problem is the use of common cell surface markers which form part of the immune signature such as the Major Histocompatibility Complex proteins (MHC class I and II), and CD markers and which have the potential to be measured using both rapid qualitative antibody-based measurements, as well as quantitatively using mass spectrometry. The measurement challenge lies in identifying a suitable panel of markers which can be used to generically fingerprint cell lines based on expression patterns, and developing methods to isolate cell samples without damaging cell membrane markers.

Impact

Cell phenotype identification based on surface markers and/or histocompatibility markers would be a powerful measurement tool for cell assay users in general and for companies developing therapies for regenerative medicine. Recent European guidelines on the characterisation of allogeneic cells used in tissue engineering also recommend the phenotypic authentication of cells using histocompatibility markers (CHMP/410869/06).

To ensure the outputs of this project reach the appropriate end user, knowledge transfer will be promoted through links within standards organisation (BSI RGM/1, ISO TC194/7) and commercial links within the UK contract research community. In addition information generated on different cell lines will be disseminated thought established links with cell bank organisations (ECACC, ATCC) and internationally through collaborative metrology studies proposed to the bioanalysis working group at CCQM.

Summary of Technical Work

This project will identify a range of generic cell surface markers which can be used in isolation, or in combination with cell type specific markers, to fingerprint the differentiation status (phenotypic profile) of both cell lines and stem cell models. To ensure translation of the methodologies developed into standard cell biology laboratories, a range of common techniques such as ICC and flow cytometry will be used as measurement tools. To demonstrate the quantifiable expression of the generic cell surface markers a range of cell isolation techniques and protein measurements using mass spectrometry will be applied to each cell model.

Activities:

- Selection of a panel of cell lines and stem cell models for surface marker analysis.
- Determination of cell transcription profiles for each cell line to identify generic cell surface markers.
- Analysis of cell surface marker expression using immunocytochemistry and flow cytometry.
- Development of methods to isolate cell samples with intact surface proteins.
- Investigation into the use of mass spectrometry to quantifiably measure surface marker expression.
- Confirmation of metrology robustness through CCQM pilot study.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW selection of suitable panel of cell lines and stem cell models completed			
2	CEW a range of cell surface markers identified using transcription profiling has been completed			
3	CEW the use of ICC and flow cytometry to measure cell surface marker expression in each cell model has been evaluated			
4	CEW techniques developed to isolate cell samples with intact surface proteins			
5	CEW mass spectrometry assessed to quantify cell surface marker expression			
6	CEW when CCQM pilot study completed			
			Total cost	

BA3					
Project Author(s)/ Contact	**Authors: Damian Marshall/Dan Scott** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Validated *In Vitro* Sensitisation Models for Consumer Products

Project Objectives

To support the UK cosmetics industry in the development of *in vitro* methods to test the safety of new cosmetic compounds in response to the 7th amendment of the EU cosmetic directive through the:

- Development of tools to measure the inherent variability of key marker expression in current cell models for skin and/or dendritic cells.
- Development of a well characterised *in vitro* cell system which can be used for inter-laboratory comparison studies.
- Provision of guidance on the variability of current cell models with recommendation on reducing variability.

Background and Rationale

The cosmetics industry represents one of the key year-on-year growth sectors both in the UK and across Europe, with growth in the UK rising at 4-6% p.a. The Cosmetics, Toiletries and Perfumery Association (COLIPA) estimates the European market for cosmetics to be >£70bn, of which the UK is the third largest market accounting for ~16% share. This market is fuelled by the continual development of new products and formulations which appeal to societal needs. Future development in this market however may be hindered by the implementation of the 7th amendment to the EU cosmetics directive which will initiate an EU marketing ban on animal-tested cosmetics (whole products or individual ingredients) by 2009 or 2013 (depending on toxicological endpoint). These restrictions will be enforced irrespective of whether alternative OECD-approved methods are available with companies facing the prospect of a slowdown in the development of new product formulations due to the lack of validated *in vitro* methods to test new compound ingredients. Despite the immanent implementation of the amended cosmetics directive it is widely recognised that sufficient methods will not be in place when the ban comes into force. The development of robust in-vitro methods to test cosmetic compound safety therefore represents a major challenge to one the UK's key industrial sectors.

One of the major areas of compound testing which lack sufficient *in vitro* tests is skin sensitisation. During the sensitisation process low molecular weight chemicals become immunogenic by covalently binding to skin proteins which are then processed by dendritic cells, and presentation to the T-cells in the local lymph node initiating an immune response. At present, skin sensitisation is measured in-vivo by the mouse local lymph node assay, with no validated alternative in-vitro method being available. In addition, it is recognised that, due to the complexity of the mechanisms underlying this human health endpoint, it is unlikely that a single *in vitro* test will replace the whole animal test. More likely, a combination of *in vitro* tests, each of them covering an essential component of the biological mechanism, will presumably lead to the full replacement of current animal procedures. Developing *in vitro* tests for skin sensitisation is complex and is hampered by the lack of characterised cell systems (cell lines and primary cells) for both skin and dendritic cells. Commercial cell lines are notoriously heterogeneous and sensitive to subtle changes in cell culture conditions (cell confluency, passage number, media batches, inter/intra lab variability etc.). They are also of carcinoma origin and so may not represent normal in-vivo conditions. Primary cells derived from peripheral blood or skin explants are an alternative, but, as with any primary cells, there are difficulties with reliable supply and high inter-individual heterogeneity. The availability of well characterised cell lines and an understanding of the variability that is inherent between the different in-vitro systems would, therefore, be of huge benefit to the cosmetics industry, facilitating in-vitro model validation and providing systems to test the safety of new cosmetic compounds.

Accurate characterisation of *in vitro* cell models presents a major metrology challenge, but the NMS has facilitated development of the TIERed testing approach (CBM Programme, LS3) on the basis of viability, phenotypic authenticity and functional stability using measurement capability present within the NMS.

Impact

The development of robust *in vitro* models for compound testing at the skin surface is needed urgently by the cosmetics industry due to the imminent European-wide ban on the use of animals to test cosmetic ingredients. However, these *in vitro* models will be equally as beneficial to the pharmaceutical sector for testing topically

applied medicine designed to penetrate the skin barrier.

Through consultation with regulatory authorities and UK organisations championing alternatives to animal testing, the development of *in vitro* methods based on improving the robustness of measurement from current cell models is of primary focus. Methods for achieving this have already been developed within the NMS through TIERed testing strategies which have been applied successfully to a range of cancer cell lines to show the parameters under which the cell models can be used. The NMS is therefore ideally placed to facilitate the development of *in vitro* tests based on current cell models which can be used by the UK cosmetics industry to minimise the impact of the ban on animal testing.

The measurement methodologies developed within the NMS will be utilised and disseminated through established links with major UK cosmetic companies, contract research organisations and regulatory authorities to facilitate dissemination of the outputs of this project to the wider cosmetics community. This project will also build upon and support European initiatives.

Summary of Technical Work

This project will use the successful TIERed testing approach for cell characterisation which was developed under the current NMS CBM Programme (project LS3) to measure the variability in the performance characteristics of current in-vitro models for skin sensitisation. This testing strategy will measure cell characteristic at three levels, viability, phenotypic/genetic stability and functional performance in order to define the main areas which lead to cell line variability and poor model performance. In addition this project will also develop measurement techniques to evaluate the fitness for purpose of novel in-vitro skin barrier models which are designed to replace the use of animals in cosmetic, pharmaceutical and biological product development.

Activities

- Evaluation of measurement parameters which define cell model performance based on TIER 1 analysis of cell viability, TIER 2 analysis of cell phenotypic and genotypic stability and TIER 3 analysis of cell function.
- Development of measurement techniques to assess cell characteristics and barrier function of novel 3D *in vitro* skin models.
- Production of guidelines for the use of cell based models for skin sensitisation.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW cell models for skin sensitisation incorporated within the NMI and master and working cell banks generated			
2	CEW cell model performance has been characterised based on TIERed testing strategy approach			
3	CEW a measurement tool to evaluate fitness for purpose of novel *in vitro* 3D skin models has been completed			
4	CEW a guideline for maximising performance of *in vitro* skin sensitisation models has been produced			
			Total cost	

BA4					
Project Author(s)/ Contact	**Authors: Carole Foy/Steve Ellison** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Reference Measurement Methods For Nucleic Acid Metrology

Project Objectives

- To fulfil the UK role in International Biometrology and validate UK reference biomeasurement capability by co-ordination of, and participation in, relevant CCQM studies.
- To develop and validate high-precision and minimally-biased calibration strategies, with uncertainty budgets, suitable for reference biological measurements on nucleic acid and protein targets.

Background and Rationale

In comparison to physical metrology there is a paucity of higher order reference standards and materials maintained by the NMIs for the biological sciences. At the international Joint Committee for Traceability in Clinical Measurements (JCTLM), the IFCC highlighted the need for robust quantitative reference methods and traceable reference materials for clinically relevant biomolecules based on the legislative requirement for reagent traceability and robust measurements for *in vitro* diagnostic (IVD) kit compliance with the IVD Directive and other emerging regulations & guidelines (ISO TC212, CLSI). In the field of diagnostics the emerging model of multiparameter multianalyte testing sets new measurement challenges, particularly the need to assess changes in overall profile of multiple analytes.

The route map for the Bioanalysis Working Group (BAWG) at CCQM directs the work of this group towards the production of key reference measurement systems to underpin future biological measurements. This requires international collaboration in developing and validating reference measurement capabilities for biometrology. To maintain its lead and gain from these studies, the UK needs to develop methods of measurement capable of attaining the uncertainties required and to participate actively in the work of CCQM. Active participation will discharge the UK's biometrology responsibilities in maintaining the SI and assist in validating reference biological measurement methods for use in the UK NMS. This project therefore focuses on the maintenance and development of reference methods and their validation via international comparisons.

Reference measurements need to have substantially smaller uncertainties than routine measurements if the uncertainties associated with calibration or validation are to meet their objectives; typically, a calibration value should have uncertainties at least four times smaller than the uncertainty required by the routine measurement being calibrated. To achieve this for biological measurement remains challenging. Biases arise from, for example, susceptibility to matrix effects and interferences, and commutability problems in reference materials. At very low copy number, precision is poor and 'drop-outs' occur due to the variations in number of copies present – an issue highlighted at the recent JCTLM workshop at AACC for nucleic acid reference measurement. Such events compromise existing calibration methods, biasing low-level measurement.

This project will address these problems by developing and applying improved calibration or measurement strategies. The effects of matrix and some interferences can be reduced by careful internal standardisation methods, including bracketing, standard additions and progressive dilution, which need development to adjust for the non-linear nature of many biomeasurement calibration methods. 'Drop-outs' can be accommodated by maximum likelihood methods which can take proper account of null observations. Optimal distribution of reference values in calibration helps to obtain minimal uncertainties in interpolated values. 'Digital' PCR and microfluidic dynamic array PCR methods allow considerably higher levels of replication and can be made much less sensitive to variable amplification. Additionally, next generation sequencing methods can potentially improve multiple target measurement capability through high levels of replication. All of these strategies are technically feasible given current technological developments, statistical methods and software, but require proof of principle, development and optimisation for practical implementation. The more novel measurement methods in particular will require not only method development but also underpinning studies to establish reliable measurement uncertainty budgets.

Participation in international studies will both continue existing international work and provide opportunity to validate improved methods. Studies in at least the following areas are envisaged:

- Pilot and Key comparisons related to gene expression biomarker measurements based on RNA quantitation. Initial work will continue the existing BAWG Pilot study P103 led by LGC in collaboration with NIST, using a reference panel of synthetic gene transcripts based on the External RNA Controls Consortium (ERCC) standards. The complexity of such multiparametric biomarker measurements

compared to previous studies requires a step-wise approach. Phase 1 (represented by CCQM P103) will be a single transcript target in isolation; Phase 2 will be a single transcript in a complex RNA background; Phase 3 will be multiple transcripts in isolation and Phase 4 will be multiple transcripts in a complex RNA background. A successful outcome to Phase 4 will be followed by a Key comparison study.

- DNA Quantitation using genomic and plasmid reference materials for GM ratio and absolute quantitation. A BAWG pilot study is currently under way within the present programme to test a novel dual-target plasmid reference material for GM ratio measurement; this methodology is expected to move towards Key comparison in 2010. Improved calibration methods for absolute quantitation of DNA will require participation in other quantitation studies.
- DNA Methylation. Methylation is an important modifier of gene activity and extent of methylation is a clinically important measurement. The UK participated in a Pilot study in 2007-8 and is intended to improve methodology to achieve further successful participation in an envisaged Key comparison.

Impact

The standards and measurement systems developed in this project will assist manufacturers to demonstrate compliance of *in vitro* diagnostic kits with the IVDD, in terms of reagent traceability and robust measurements. The work will also be fed directly into the development of CLSI standards on clinical requirements for nucleic acid reference measurement through NMS participation in the JCTLM forum.

Participation in CCQM BAWG work will support the UK's international trading position by demonstrating continued lead of the UK NMS in the field of biological measurement.

Development of improved calibration strategies will assist the production of reference nucleic acid materials, in turn allowing the development of high-accuracy methods for reference biological measurement. This will provide calibration materials to improve metrological traceability at the routine level, and validation materials to improve comparability and support new measurement technologies in the longer term. In addition, through knowledge transfer activities, awareness of new reference measurement provision will be made available to lead improvements in measurement quality and practice of routine laboratories.

Summary of Technical Work

The technical programme will include:

- Coordination of international studies for reference RNA measurements, including completion of Pilot study CCQM-P103
- Implementation and optimisation of candidate high-accuracy calibration strategies for Real Time PCR, including theoretical development, practical demonstration, optimisation of calibration designs, development of uncertainty budgets and comparison of performance with existing methods
- Development of methods for calibration at low levels where stochastic variation in copy number or protein molecule count causes frequent 'non-detects' - maximum likelihood methods are currently expected to provide the most useful approach
- Participation in CCQM BAWG studies coordinated by other institutes, currently expected to include DNA methylation and DNA quantitation
- Implementation and optimisation of highly replicated reference measurement methods, including digital PCR, dynamic arrays and next-generation sequencing, including optimisation of relevant method design
- Theoretical and experimental studies of nucleic acid and protein calibration design to provide optimal precision, resulting in guidelines for calibration designs for reference measurements.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW reports published on at least two completed pilot studies coordinated by the UK in the field of RNA quantitation.			
2	CEW at least one peer-reviewed paper has been published which includes proof of principle for an internally standardised calibration method for Real Time PCR, with demonstration of reduced bias compared to traditional calibration.			
3	CEW implementation of a methodology for unbiased treatment of non-detects at low copy number, including single-laboratory validation has been published for a clinically or commercially relevant application.			
4	CEW reports published showing successful UK participation in at least two further international comparisons in biometrology			

5	CEW one peer reviewed paper has been published which demonstrates the reference measurement potential of a highly replicated DNA quantitation method.			
6	CEW representation at international biometrology meetings such as BAWG helps maintain and develop UK position in international biometrology.			
7	CEW guidance published on calibration designs for optimal precision in reference measurements in non-linear calibrations such as RT-PCR and highly replicated 'digital' PCR methods, including theoretical principles and experimental validation.			
			Total cost	

BA5					
Project Author(s)/ Contact	**Authors: Gavin O'Connor/Helen Parkes** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Traceable Methods for the Characterisation Of Structurally Significant Proteins (Joint EMRP JRP11 *"Clinbiotrace"* Project)

Project Objectives

Development of mass spectrometric approaches for the traceable quantification of protein structural confirmations. This will be achieved through a number of approaches including:

- The use of IDMS methodologies for the traceable quantification of amino acids and peptides.
- Development of digestion protocols for the traceable quantification of proteins.
- Preliminary investigations into the use of protein-ligand interaction hydrogen deuterium exchange for the determination of binding constants and protein structure.

Background and Rationale

The hotly debated, fundamental issue for clinical, healthcare and metrology communities is the establishment of traceability for biologicals, in the same way as for simpler chemical entities by assigning values in SI. Complex biomolecules, such as proteins, have additional chemical heterogeneity (de-amidation, oxidation, isoforms) and plasma-based heterogeneity (complexing to other proteins). So, in general, the concept of 'a unique, homogeneous chemical entity' does not apply to proteins and other macromolecules. Furthermore few reference measurement methods exist and traceability cannot be determined readily.

The requirement for traceability, the "*property of the result of a measurement, whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties*," in clinical measurement is addressed by ISO/EN 17511 'Measurement of quantities in samples of biological origin'. In diagnostics, it is driven by the EU IVD Directive (98/79/EC) where "t*raceability of values assigned to calibrators and control materials for IVD devices must be assured through available reference measurement procedures and/or reference materials of higher order".*

WHO international biological standards and reference reagents, based on arbitrarily assigned international units (IU), remain a pragmatic, 'best-fit' approach to comparisons of quantitative measurements of heterogeneous biologicals. However, this lack of traceability has been identified as an issue in achieving consistent measurement comparability by the laboratory medicine and industrial diagnostics community.

Building on previous research success, delivered under the CBM Programme in developing traceable methods for the quantification of proteins, the project will develop capability for metrological traceability of complex biomolecules, including their structural heterogeneity. This will be achieved by applying previously developed methods for the traceable determination of a proteins primary structure. These will be combined with mass spectrometry based structural measurements, which will be investigated for the quantification of specific epitopes and/or structural motifs which infer biological activity. It matches the EMRP 2007 framework commitment which specifically focuses on health-related metrological activity (TP2 Healthcare Grand Challenge) and which highlights the requirement for the *"expansion of the range of reference measurement procedures and reference materials of a higher order" as recommended by the International JCTLM.*

Impact

The provision of a route to traceable complex protein measurements will enable development of a biometrology framework that encompasses both metrological rigour in developing SI traceability and the real concerns of the clinical/healthcare community, that measurements must be relevant and commutable - IVD and clinical measurement comparability is a value-added exercise that will improve patient care, testing, accuracy, reliability and, in the long-run, reduce costs. Successful development and validation of this functional traceability concept will impact on key stakeholders in biological standardisation including the JCTLM, IFCC, WHO, reference material producers, competent authorities, producers of commercial measuring systems and practitioners of Laboratory Medicine by:

- Studying systematically and raising awareness of factors influencing existing immunoassay measurement comparability, and developing multiparametric traceable potential reference measurement methods for key diagnostic proteins of clinical relevance.
- Informing the stakeholders involved in the SI /IU debate on the need and feasibility of developing traceable clinical measurements for protein structure related "activity" in the *in vitro* diagnostics.
- Enabling the reference material producers to prepare Certified Reference Materials better suited for the calibration of in vitro diagnostic devices and in line with ISO 17511 in order to achieve acceptable comparability of IVDs on individual patient samples which could then be considered for recommendation by JCTLM.

A clear route for knowledge transfer therefore exists.

Summary of Technical Work

The project will be delivered across four NMIs throughout Europe. The UK will be responsible primarily for the mass spectrometry based approaches whilst collaborating with other NMIs who will be responsible for their activity and functional measurements. The work packages are:

- Prioritisation of complex proteins as model proteins for study and isoform characterisation.
- Underpinning quantification of amino acids.
- Investigation of traceable quantification of model protein [isoform] derived peptides.
- Investigation of traceable quantification of a model protein in a complex biological matrix.
- Investigate feasibility of traceable quantification of model protein tertiary structure.
- Demonstration of concept by linking immunoassay/functional measurements with SI traceable approaches to model protein 1^{o} and 3^{o} measurements.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW a fully validated method for determining the mass fraction of individual amino acids with a measurement uncertainty of less than 5% has been developed.			
2	**CEW digestion methods for the equimolar release of the chosen peptides suitable for absolute protein quantification have been assessed.**			
3	CEW an assessment of the production of stable protein solutions standards with a traceable mass fraction and assigned uncertainty has been completed.			
4	CEW target protein concentration, determined by absolute &/or relative MS based procedures, has been assessed.			
5	CEW peptide H/D exchange rate for the relative quantification of the selected proteins structures has been assessed.			
6	CEW peptide H/D exchange rate for the relative quantification of the selected proteins structures in the presence of ligands/ antibodies has been assessed.			
7				
			Total cost	

BA6					
Project Author(s)/ Contact	**Authors: Carole Foy/Malcolm Burns/Dan Scott** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Evaluation of Digital and Real-Time PCR Methods For Non-Invasive Prenatal Diagnostic Testing

Project Objectives

- Provide objective evidence of the 'fitness for purpose' of digital PCR for the detection and quantitation of trace-level fetal DNA/RNA in maternal samples by objective assessment of performance characteristics.
- Contribute towards the UK clinical research and diagnostics industry, by developing methods for improved quality and accuracy of quantitative DNA measurements.
- Objectively compare the limits of detection of digital PCR to other real-time PCR systems, and provide evidence for the biological sensitivity of such approaches in terms of DNA/RNA detection.
- Develop and disseminate guidelines and robust protocols for trace detection of fetal DNA/RNA in maternal blood.

Background and Rationale

Prenatal screening for genetic abnormalities (e.g. Down's Syndrome) currently relies on invasive sampling methods, such as amniocentesis and chorionic villus sampling, where there is a risk to the fetus and potential to induce miscarriage. The discovery that cell-free fetal DNA/RNA is present in the mother's blood offers the possibility to develop non-invasive prenatal diagnostic tests. However, this has proved to be a significant analytical challenge for several reasons:

- Fetal nucleic acid is present at very low levels relative to a large background of maternal nucleic acid
- There are very few fetal specific markers
- Aneuploidy diseases, such as Down's Syndrome, are difficult to discriminate from wild type as they harbour extra copies of apparently normal chromosomes rather than consisting of genetic mutations that can be targeted for analysis.

Successful diagnostic tests from blood samples therefore require methods that can precisely detect trace levels of mutations, as well as very subtle changes in nucleic acid copy number, amidst a background of maternal nucleic acid.

One approach showing promise in this area is digital PCR, where a sample is diluted and partitioned between many hundreds or thousands of individual PCR reactions such that a single molecule or less on average is present in each reaction. Recent advances in microfluidics have facilitated the development of digital PCR by combining microfluidics with PCR to increase levels of replication, throughput and cost efficiency. As digital PCR potentially affords absolute quantitation, it does not suffer from inaccuracies that are often afforded from relative approaches using calibration curves, for example through extrapolation. The effect of different matrices between calibrant/standard and sample may also be minimised when using digital PCR. *In vitro* proof of principle studies have shown that digital PCR has the potential to detect fetal copy number variations (e.g. trisomy 21 for Down's Syndrome) when samples contain minority fetal nucleic acid in mixed fetal-maternal nucleic acid samples.

Another route is to analyse genes that are expressed exclusively in the fetus as these gene transcripts may be present at higher levels in the maternal blood and be more amenable to analysis. Several specific fetally-expressed transcripts that contain diagnostic SNPs within them have already been identified and, as the field progresses, more are likely to follow. These approaches now require validation of the performance characteristics of trueness, precision, detection limit, range, selectivity, effect of replication levels, and measurement uncertainty in order to provide objective evidence of the 'fitness for purpose' of digital and real-time PCR for use in prenatal diagnostics.

This project will validate the applicability of novel digital PCR and dynamic gene expression systems for use in trace-level fetal diagnostics. Of particular interest will be the comparability of digital PCR to other published approaches such as limiting dilutions and standard additions, which can suffer from introduction of manual errors, imprecision, length of time, have limited throughput and large consumable use.

The NMS is well placed to address this issue given the prior experience within its NMI analysing fetal DNA in maternal blood, and standardisation and determination of limits of detection and effect of replication levels for related DNA diagnostic methods such as real-time PCR. The NMS also benefits from excellent, established links with key stakeholders - clinical collaborators and stakeholder networks - such as EuroGentest, CLGGS, CLSI, IFCC, JCTLM and FDA. These links not only provide clinical material but also key insights into the needs of clinical practice. Additional discussions with stakeholders in the NHS genetics testing community and with *in vitro* diagnostics developers have reinforced the need for improved pre-natal testing using non-invasive methods, such as digital PCR and the validation studies proposed in this project. These stakeholders will be key collaborators and help evaluate the applicability of the proposed methods and disseminate the findings throughout the prenatal diagnostic field.

Impact

Validation of digital PCR methods will provide objective evidence for the application of this new technology to prenatal testing. This will impact healthcare and diagnostic stakeholders who will be able to develop and implement new and improved prenatal diagnostic assays capable of gaining regulatory approval. Moreover, this method has the potential to directly benefit mother and unborn child by removing the need to perform invasive sampling methods with their associated risks to the unborn fetus. Realisation of the advantages of this new technology will re-define approaches to trace-level DNA analysis in terms of high sample throughput, minimal sample handling/pipetting, cost effectiveness, biological sensitivity and greater statistical confidence. This will benefit UK metrology, healthcare, diagnostic and regulatory stakeholders by providing a standardised approach for trace-level DNA analysis and demonstration of its applicability to fetal DNA testing.

Summary of Technical Work

Evaluation of digital PCR as a tool for non-invasive prenatal diagnostics and development of robust protocols for trace DNA/RNA detection. This will be achieved in the first instance by spiking-in known DNA/RNA sequences in to complex blood samples. The methods developed will then be transferred to clinical samples obtained from collaborators and the fitness for purpose for detection of a panel of key genetic diagnostic markers evaluated. Measurement parameters including trueness, biological sensitivity, repeatability and robustness will be evaluated and benchmarked against current 'gold standard' DNA/RNA technologies.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW performance characteristics of digital PCR compared to real-time PCR have been evaluated for trace DNA/RNA detection			
2	CEW robust protocols for improved detection and quantification of trace levels of DNA/RNA have been developed			
3	CEW protocols developed in deliverable 2 have been validated on clinical samples for a panel of genetic markers (in association with clinical collaborators)			
4	CEW findings have been disseminated through production of guidance notes, protocols and peer-review publications on improved methods for non-invasive prenatal diagnostics			
			Total cost	

BA7					
Project Author/ Contact	**Author: Gavin O'Connor** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Traceable Methodologies for Food Allergens

Project Objectives

The overall aim of this project will be to develop and deliver traceable measurements for priority food allergens by realising the following objectives:

- To interact with current UK and European initiatives on food allergens to identifying the key measurement issues so as to enable comparability of protein based allergen detection methods.
- To develop protein based traceable reference methods for problematic allergens.
- Establish the feasibility of providing a matrix certified reference material for selected food allergens.

Background and Rationale

Food allergens represent an increasingly important health problem across Europe. The severity of reactions covers a range from mild to life threatening, including anaphylactic shock. It is estimated that up to 8% of children have reproducible adverse reactions to food. In western Europe and the USA most immunological adverse reactions are triggered by a limited number of foods. These foods have been identified and have been the subject of many double-blind placebo control food challenges (DBPCFC), such as the low dose challenge used in the EuroPrevall program (www.europrevall.org), to help establish threshold values at which an adverse response is unlikely.

The concern over the prevalence of food allergens has resulted in strictly enforced legislation, UK Food labelling regulations 1996 (amended 2004) & European directive 2003/89/EC, which requires all pre-packed foods to declare if they contain any of the 14 priority allergens. This has led to the development of a number of test kits (ranging from laboratory based quantitative fluorescent ELISA to qualitative threshold based swabs or dip sticks) which can be used by industry to check for the presence of trace contamination and/or in cleaning plant equipment.

With such an importance based on measurements, in the setting of safety response thresholds, enforcing legislation and the development of high throughput screening technologies it is vital that measurements in the area are comparable. One of the aims of an EU Sixth Framework initiative was the establishment of a network of excellence called MoniQA (Monitoring of Quality Assurance in the Food Supply Chain) (www.moniqa.org). The food allergen working group of MoniQA highlighted *the lack of certified reference materials* and *the need for an improved validation procedure* as major challenges in the search for adequate allergen detection.

The objectives and deliverables of this proposal are aligned with the needs of a community already actively searching for traceable materials and CRMS for a set of fourteen know foods. The exact measurands are not well defined in all cases but in many a target protein is the basis of testing. Therefore it is likely that CRMs assigned with traceable amounts of specific proteins would improve measurement comparability in the sector.

Currently there are very few CRMs for allergenic foods. A number of NMIs have started to address this shortfall with varying success. Currently the activity is concentrated on the provision of stable forms of the allergenic foods (IRMM-481 Peanut test kit, which is not a RM due to homogeneity and stability issues, and the NIST powder dried eggs), but there is a lack of CRMs of allergenic foods at threshold levels in processed foods, which is where the major challenges are arising.

Impact

Methodologies and practices developed under previous NMS Programmes allow traceable value assignment for standard protein solutions. These standards and methods are currently being applied to more complex matrices. Many of the priority food allergens are detected on the basis of known protein constituents of the food allergen (Lysozyme for eggs, Casein & β-lactoglobulin for milk) and therefore the traceable value assignment of these proteins would be a considerable improvement in value assigning CRMs for use by the community.

By linking with current EU and UK activities, such as MoniQA and the Institute of Food Research Allergy Cluster network (www.foodandhealthnetwork.com) & Government Chemist activities, the proposed objectives will address industry needs and a direct request for CRMs. The provision of traceable standards in the area would better inform:

- Clinicians on the validity of data used to establish threshold values.
- Industry on the assessment and comparison of new and innovative methods for the detection of allergens and help in the assessment of the appropriate test kit for their specific needs.
- Enable the enforcement of food labelling legislation providing a recognised standard, developed with the industry, which is traceable to the SI ensuring a reference for contest cases and disagreements.

Summary of Technical Work

The technical work will build on previous expertise developed under the NMS CBM Programmes. The work will involve interaction with legislators, researchers and industry representatives through the MoniQA, Europrevall and Allergy cluster network initiatives and this will help guide decisions on priority allergens and the choice of proteins used to indicate the presence of these allergens. These will also provide a major dissemination route for knowledge, issues highlighted and potential reference materials.

Once the target proteins have been chosen, the first challenge will be the traceable value assignment of a standard material. This will be achieved by traceable amino acid analysis on proteotypic tryptic peptides derived from the allergen protein. These peptides will then be used for the exact matching IDMS analysis of the selected protein. Once a standard has been characterised it will be used to evaluate methods for the isolation of target proteins in a complex food matrix. This will be achieved using the same traceably assigned peptides using IDMS methods. On achieving this the method will then be used to assess the homogeneity and stability of candidate reference materials for use as QC materials, in intra-laboratory proficiency testing schemes and method development.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW a report is produced outlining the selection criteria for up to three proteins from priority allergens.			
2	CEW developed IDMS methods have been applied for the value assignment of purity of up to four peptides per allergen protein.			
3	CEW a report has been published on the developed methodology for the quantitative and traceable analysis of allergen proteins in a complex food matrix. CEW report has been disseminated to industry through active participation in networks (e.g. MoniQA).			
4	CEW preliminary assessment of a candidate matrix CRM for suitable homogeneity and stability has been completed.			
			Total cost	

BA8					
Project Author(s)/ Contact	**Authors: Carole Foy/Ana Pardos-Pardos** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Measurement Guidelines for Improved Genotoxicity Testing

Project Objectives

To help the chemical, pharmaceutical and cosmetics industries reduce animal experimentation through the development of:

- more accurate and robust *in vitro* models and methodologies for genotoxicity testing.
- guidelines on improved approaches for genotoxicity testing.

Background and Rationale

Measuring the human genotoxic potential of a compound is a critical step in assessing the potentially harmful impact of a substance on human health. Current testing strategies use *in vitro* assays as an initial form of testing, followed by *in vivo* assays in the event of a positive *in vitro* result. Unfortunately, *in vitro* genotoxicity testing produces a remarkably high occurrence of irrelevant positive results due to:

- the common cell lines used often having a lack of normal metabolism, impaired p53 function or altered DNA repair capability.
- the common assays used for determining DNA damage, such as the comet, micronucleus and chromosome aberration assays, only give a semi-quantitative and largely subjective determination of DNA damage at a gross cellular level, and are not capable of providing information on specific DNA lesions to determine the underlying mechanisms of genotoxicity.
- the typical use of much higher doses of compounds than may be expected during typical *in vivo* exposure.

This leads to a significant waste of animal studies. The drive to understand underlying mechanisms of genotoxicity and to reduce (and eventually replace) animal experiments which is critical to the successful implementation of several recent EU Directives such as REACH and 7th amendment to the Cosmetics Directive, will be reliant upon the development and validation of more accurate and reliable prediction assays.

Improving the cell systems used for genotoxicity testing is key to improving assay reproducibility - guidance from ECVAM states that cell systems which are p53 and DNA-repair proficient, have defined Phase 1 and Phase 2 metabolism covering a broad set of enzyme forms, and are used within the context of appropriately set limits of concentration and cytotoxicity, offer the best hope for reduced false positives.

Alongside improved cell models, improved analytical approaches also need to be developed and validated to give a more accurate, sensitive, quantitative, informative and reliable indication of the genotoxic impact of a compound at multiple relevant doses. Comparative Genomic Hybridisation (CGH) technologies have been proposed as possible alternatives to the micronucleus and chromosome aberration tests and PCR-based amplification techniques to identify site-specific mutations, strand breakage, loss and gain of material and base lesions have also been proposed. DNA sequencing may also prove a useful tool for assessing DNA damage at a global level. However, the feasibility of novel tools and robustness, sensitivity, reliability and relevance of the other technologies for directly assessing DNA damage has yet to be established.

A further alternative approach to the generic, non-specific DNA damage methods is the measurement of specific DNA damage markers using mass spectrometry-based techniques. A specific advantage of such an approach is that, once identified, specific DNA nucleobase lesions can be quantified accurately at very low (fmol) levels. With the provision of traceable pure standards and isotopically-labelled internal standards, this quantitation can be carried out with greatly improved accuracy. However, it requires the identification of specific target analytes and ultimately a panel of them whose combined analysis gives a true reflection of the total DNA damage rate. Nonetheless, undertaking an exploratory study of this new approach could bring dramatic benefits where more robust and sensitive measurements are required.

Impact

The Pharmaceutical Analysis Steering Group (PASG) has recently set up a Genotoxicity Working Group in response to the high level of industrial interest in the development of improved models and methods. This project will feed its developments and outcomes directly into this Group through active NMS participation within

it and will therefore effect a more significant impact on the UK pharmaceutical, chemical and cosmetic companies who are in critical need of improved *in vitro* genotoxicity assays to comply with newly implemented legislation. Improved assays would also contribute towards the reduction, refinement and replacement of animals for safety testing, directly resulting in significant savings in both time and money.

Strong working relationships build up over previous programmes with key industry stakeholders and current ties with ECVAM and regulators such as MHRA, EMEA and OECD will help ensure that the relevant authorities are aware of the project and act on relevant outputs in a timely and effective manner.

Summary of Technical Work

- Evaluation of improved *in vitro* cell-based models for genotoxicity testing.
- Identification of up to 5 model genotoxic compounds for assessment (in conjunction with related CBM nanotoxicology, toxicogenomics and cosmetics projects).
- Identification of the main nucleobase lesion associated with each of the model compounds from deliverable 2 and assessment of the performance of mass spectrometry-based techniques applied to them.
- Evaluation and development of alternative molecular approaches (e.g. CGH, PCR and sequencing) for measuring genotoxicity.
- Comparison of key alternative approaches to current approaches, such as comet and micronucleus assays.
- Production of measurement guidelines for improved genotoxicity testing.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW alternative cell models for genotoxicity testing have been assessed.			
2	CEW alternative approaches for identifying and measuring DNA damage have been assessed. This should include assessment of at least 1 mass spectrometry and 2 molecular approaches.			
3	CEW recommendations and guidelines for introduction and implementation of improved genotoxicity testing strategies have been produced. CEW the project outputs have been transferred to industry and other interested parties, as appropriate.			
			Total cost	

BA9					
Project Author/ Contact	**Author: Paula Domann** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Glycan Reference Materials

Project Objectives

To provide industry with robust analysis strategies for glycoprotein characterisation and quantification through:

- Expanding existing traceable glycan reference materials to encompass more complex glycosylation (highly sialylated species), yeast and plant glycosylation (xylose and alpha-1,3 linked fucose species).
- Developing the best practise guide to encompass new analysis strategies including those relating to plant and yeast glycosylation.
- Supporting database development to allow the rapid sequencing of glycans by CGE-LIF.

Background and Rationale

Glycosylation is viewed by the biopharmaceutical industry as the key post-translational modification that affects the biological activity, biological half-life, immunogenicity and stability of recombinant proteins.

Industry guidance exists as to which methods are 'fit for purpose' for glycoprotein characterisation at various stages in product development in the form of the 'best practice guide' completed in 2007 and available online. This is currently being extended to include novel and emerging techniques and an initial set of traceable glycans standards is being developed to support such measurements. This will provide an anchor point to allow relative quantification and comparison between methods, still highlighted as a major issue by HUPO[1].

The use of plants and yeast to produce pharmaceutical and industrial proteins offer a number of benefits over conventional cell-based systems in terms of lower costs with increased flexibility in scale and storage. However many products require human-like glycosylation to be of maximum effect and avoid triggering allergenic responses[2]. Although a number of solutions have been applied to achieve this[3], specific measurement issues remain, namely:

- For plant glycans containing alpha-1,3 linked fucose (which are resistant to cleavage by PNGase F) certain structures are missed if analysed using classical routes.
- The presence of plant specific monosaccharides such as xylose can cause immunogenic responses.
- Yeast glycosylation results in high percentages of mannose species.

Draft EMEA regulatory guidelines[4] to be implemented this year reflect the concerns regarding non-human-like glycosylation in these systems, with new submissions requiring in-depth qualitative and quantitative data to be provided. However, without advances in current technology, cross validation of platforms and the availability of suitable standards, this will be difficult to achieve and may act as a barrier to product development.

This project aims to address these measurement issues by expanding the set of methods in the 'best practice guide', and the range of traceable reference materials, to incorporate more complex and plant-derived glycosylation. To support these measurements a database of glucose unit (GU) values for the analysis of glycans using the CGE-LIF technique will be incorporated into an external, established glycobase database, thereby allowing the identification of unknowns in a reduced timeframe.

Impact

After the USA, Britain's pharmaceutical companies' market share is more than all its European competitors combined. The value of UK pharmaceutical exports in 2007 was £14.6 bn[5], from which bio-pharmaceuticals accounting for 10% of the total drug sales and 30% of the products in the pipeline today[6]. The market for therapeutic monoclonal antibodies is one of the most dynamic sectors within the pharmaceutical industry. It is predicted that the worldwide market for therapeutic and diagnostic monoclonal antibodies is expected to reach $26 million (€21.2 million) by 2010[7]. Key to this growth is the need for development procedures to be refined and it is therefore essential to support UK industry in maintaining its lead position in this area. Reliable orthogonal analysis methods and supporting reference standards for complete glycoprofiling will improve product characterisation and control, improve the safety and efficiency of these biopharmaceutical products. The availability of traceable standards will improve measurement confidence, speed up validation and allow robust evaluation of new innovative technologies.

1. Wada Y. *et. al.* Glycobiology, 17(4), 411–422, 2007; 2. Transgenic Res, 16, 147-161, 2007; 3. J.Experimental Botany, 49(3), 26,

1463-1472; 4. EMEA/CHMP/BWP/48316/2006; 5. http://www.abpi.org.uk/statistics/section.asp?sect=1; 6. http://www.bioprocesst website.org/about.htm; 7. http://www.drugresearcher.com/Research-management/Worldwide-antibody-market-to-reach-26-billion

Summary of Technical Work

New methods and the exact reference materials to be produced will be agreed with the project Steering Group, following which the standards will developed in conjunction with an expert SME in the field. New methods for inclusion in the best practice guide will be reviewed and incorporated into the on-line guide. The CGE-LIF database will be developed in conjunction with the established database partner, using the established standards to validate the system. An instrument manufacturer will provide instrument loan and consumables. Industry will act as a test laboratory for the system. A series of publications and workshops will be carried out to promote the findings.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW new methods added to the guide suitable for the analysis of glycoproteins produced in transgenic plants			
2	CEW expanded glycan reference material panel includes complex sialylated glycans and glycans specific to transgenic plants.			
3	CEW CGE-LIF GU unit database freely available on the glycobase website. CEW glycan library standards available to support glycan characterisation. CEW new database promoted at one international conference and through two publications.			
4	Knowledge transfer activities: At least two peer-review publications, two workshops have been delivered t attendee satisfaction; and the 'best practice guide' has been updated.			
			Total cost	

BA10					
Project Author(s)/ Contact	**Authors: Carole Foy/Ramnath Elaswarapu** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Development of a Framework for Standardisation of miRNA Measurement

Project Objectives

To develop a framework for standardisation of miRNA measurement that helps UK biotechnology and healthcare industries to exploit recent developments in miRNA analysis. This will be achieved through:

- Evaluation of method performance for miRNA profiling technologies across multiple platforms.
- Development of robust methods and reference standards for interrogating processes within and between platforms.

Background and Rationale

Recently discovered microRNAs (miRNAs) are small, non-coding regulatory RNAs which are 20-23 nucleotide long, known to modulate gene expression at the pre- and post-transcriptional level in many species. So far hundreds of miRNAs have been identified and their number is ever increasing. In humans, conservative predictions indicate that up to 30% of the genes may be regulated by miRNAs and are known to control a wide variety of cellular functions including development, metabolic pathways, cell proliferation and differentiation, disease development and cell death.

As such, analysis of miRNA is opening up new avenues for developing novel therapeutic interventions and disease diagnostics. A recent survey of miRNA researchers worldwide (Select Biosciences Industry Research, 2008) shows that ~40% of researchers are now deploying this technology for cancer studies and it is estimated that the global market will grow rapidly to US $330 million in worldwide revenues by the year 2010.

The small size of miRNAs, the varying level of sequence conservation between miRNA species, their relatively low abundance in cells, the presence of miRNA precursors in the background and the ability of some miRNAs to regulate the expression of multiple genes is making analysis of miRNA challenging. Microarray, real-time PCR and high throughput sequencing approaches are currently being developed for their analysis. However, the field is very much in its infancy and reliable, robust methods and standards have yet to be developed and fully validated. Typically miRNA profiling involves a series of complex steps which are highly sensitive to technical manipulations. Factors impacting on assay performance include quality of RNA, selection of reverse transcription strategies and kits, PCR and assay detection systems and detection limits of platforms. Though several miRNA microarray kits have been launched recently in the market, there has been little effort to standardise various procedures for within or cross-platform comparisons.

This project focuses on developing robust methods and standards which can be used as reference materials for interrogating performance of processes within a platform (dynamic range, sensitivity, specificity, reproducibility etc) as well as cross-platform comparison and sample handling/extraction issues.

The NMS is well placed to make a significant impact in this area having previously developed improved methods, metrics and standards for complex sample handling, extraction and analysis, and having been actively involved in microarray and real-time PCR standardisation. Panels of performance indicators for array based measurements developed in previous programmes are currently being extended for use with multiple types of microarray and real-time PCR assays. These standards could also be extended to the field of miRNA analysis and contribute to this project. Outputs from these previous projects have been disseminated to key grass-roots stakeholder communities (e.g. MGED, ERCC, MAQC, CLGGS, EuroGentest) through active participation in the communities.

Impact

miRNA analysis is a novel and emerging field which has immense potential for studying gene expression, developing therapeutic interventions and discovering novel biomarkers associated with various disease conditions. This field promises to have a significant impact on healthcare, pharmaceutical and diagnostic industries, but there are currently no standardised methods or reference standards for miRNA analysis which could be used as internal QC controls for method development and cross-platform comparisons. Thus, developing a panel of standards and robust standardised methodologies will help solve the problem of inconsistencies related to experimental and data analysis procedures. This will have a huge impact on boosting confidence in the validity of data, especially for regulatory submissions for *in vitro* diagnostic products. The

work being proposed in this project is necessary to ensure that the wide spectrum of healthcare benefits promised by this field have the sound scientific backing needed to win regulatory and clinical acceptance.

Summary of Technical Work

- Select and evaluate miRNA extraction and analysis approaches.
- Identify critical points of variability and uncertainty to allow robust analysis of miRNA to be developed.
- Develop a panel of synthetic miRNA spike-in standards consisting of 2-5 target miRNAs.
- Test the panel by spiking-in to complex RNA for evaluating platform/assay performance as well as cross-platform comparison.
- Produce recommendations and guidance notes to establish a framework for standardisation of miRNA measurements.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW a minimum of 3 miRNA extraction and analysis approaches have been identified and evaluated.			
2	CEW the robustness and major sources of variability of the approaches from deliverable 1 have been determined.			
3	CEW a panel of miRNA standards has been developed. CEW the developed panel has been evaluated in a complex RNA background.			
4	CEW recommendations and guidance notes on robust standardised approaches for analysing miRNA drafted. CEW framework for standardisation of miRNA measurements has been disseminated to relevant analytical communities.			
			Total cost	

BA11					
Project Author/ Contact	**Author: Carole Foy** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Reference Standard Requirements for High Throughput Sequencing and Metagenomics

Project Objectives

- Identify critical points of technical uncertainty and error for novel, ultra-high throughput sequencing technologies for microbe detection in complex samples.
- Determine key performance criteria such as sensitivity, accuracy, robustness.
- Develop robust protocols for accurate measurement of microbial composition of complex samples.
- Identify reference standard requirements.

Background and Rationale

Next Generation Sequencing (NGS) is a disruptive technology that could significantly impact on the way that many analyses (e.g. identification, speciation, genetic testing) are performed. Our experience of how other disruptive technologies have been implemented (e.g. microarrays) is that inappropriate use and interpretation can be made through a lack of standardisation or appreciation of the required protocols and the associated errors and uncertainties. We aim to address this using a key application area (microbe/pathogen identification) as the exemplar.

Recent advances in genomics technologies such as NGS for ultra-high throughput sequencing is enabling the comprehensive study of genetic material recovered directly from clinical and environmental samples (metagenomics). Such approaches allow a rapid, in-depth analysis of entire communities of microbes, without the need for culturing, and offer major opportunities for stakeholders across many disciplines such as human and animal health, agriculture and energy, and to address current environmental concerns (draft final RSC chemical roadmap and NIST international workshop on 'Accelerating Innovation in 21st Century Biosciences: Identifying the Measurement Standards and Challenges). Study of the human microbiome (human-associated microorganisms) using NGS technologies has been added to the NIH 'Roadmap' for medical research (http://nihroadmap.nih.gov/hmp/) and a large European project (MetaHIT) has recently been funded to gain knowledge of the human gut metagenome. Such studies will lead to new tools in nutrition, drug discovery, and preventative medicine and may expand our understanding of complex diseases including obesity, cancer, and immune disorders. A recent Foresight Action Plan by the OSI entitled 'Infectious Diseases: Preparing for the Future' highlights the urgent need for novel diagnostic methods and advanced technologies to identify infective disease agents in a timely manner.

Molecular approaches for pathogen detection offer a rapid option for detection and identification, and can identify microbes not amenable to culturing. However, specific PCR-based approaches lack the ability to systematically survey the entire composition of a sample, especially at the trace component level. Microarray-based approaches suffer from specificity issues due to cross hybridisation of closely related sequences. Standard sequencing technologies suffer from cost, throughput and sensitivity issues. NGS approaches have the potential to address many of the limitations of current approaches. However, as with many of today's emerging (disruptive) technologies, the promise of the technology is already resulting in its rapid adoption before a framework of validation tools and standards are in place. This is particularly pertinent for NGS. Key stakeholders in the genetics, healthcare, environmental, safety and hygiene sectors have all realised the potential for the technology, but have raised concerns about the current uncertainty and quality of information it provides - where errors could have devastating consequences, e.g. mis-diagnosis - and have called for rigorous technical evaluation of NGS tools to highlight the strengths and potential pitfalls of the systems.

This project aims to supply the required rigour by evaluating reference standard requirements for high throughput sequencing NGS platforms and develop appropriate validation tools, standards and guidance documents in order to help realise the full potential of NGS technology.

The involvement of the NMS to address these issues is essential to ensure that appropriate standards and guidelines are developed that can be used across platforms and multiple disciplines. Previous collaborations with the microarray community to develop standards, guidelines and performance indicators for array-based measurements places the NMS in an ideal position to develop metrology for NGS technology.

Impact

Metagenomics is a new discipline which makes use of the explosion of powerful new genomics tools (such as NGS) to allow in-depth analysis of entire communities of, for example, microbes/pathogens. Application of these tools is expected to have widespread impact across human and animal health, agriculture and energy, and address current environmental concerns. However, the technology is rapidly advancing and is currently ahead of measurement requirement setting, without which data rigour and comparison will restrict the uptake and correct implementation of the technology. Rigorous evaluation and knowledge transfer of guidance on the key measurement issues associated with these technologies is required urgently – an identified priority during the recent NIST international workshop. This will lead to societal (and associated economic) benefits in terms of protecting the public interest and security, and improving quality of life through reduction of disease burden.

Summary of Technical Work

The potential of NGS to accurately identify and quantify multiple microbes will be assessed initially using the Roche FLX NGS system as a model platform. This will be achieved using reference strains of microbes mixed at known concentrations to allow an estimation of the sensitivity, specificity, accuracy and robustness of the approach and to identify potential errors and sources of uncertainty and variability in the process. Access to additional NGS platforms (such as the Illumina Genome Analyser and the ABI Solid system) will be sought and 'real world' samples tested to compare platform concordance and fitness for purpose. Guidance notes, standards requirements and validation protocols will be produced and disseminated across sectors through the proposed TSB Innovation Platform on Infectious Diseases and KTNs etc. Identification of a potential future reference standard will also be highlighted.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW performance characteristics of Roche FLX NGS system have been evaluated using mixed reference microbes.			
2	CEW evaluation extended to include at least 1, preferably 2, additional NGS platforms. CEW when standardised NGS method validation approaches have been developed.			
3	CEW 'real-world' samples such as food, environment and/or clinical samples have been evaluated to determine fitness for purpose of metagenomics approaches, identify standards requirements and assess applicability of validation approaches developed in deliverable 2.			
4	CEW standards requirements identified, guidance notes produced, protocols validated and potential reference standards identified.			
			Total cost	

BA12					
Project Author(s)/ Contact	**Authors: Damian Marshall/Maaike Schutte** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Predictive Toxicology

Project Objectives

To help UK industry develop better cell-based measurement techniques by understanding the parameters under which cell-based assays can be used robustly to:

- Develop measurement tools to assess the variability in cell-based systems and link phase I compound metabolism with the downstream conjugation and transport of test compounds.
- To assess the suitability of *in vitro* models to predict accurately compound fate within the liver.
- To provide guidelines for the use of improved measurement methods in predictive toxicology.

Background and Rationale

The European Federation of Pharmaceutical Industries and Associations (EFPIA) estimates that the probability of a drug reaching market following preclinical studies is around 1%, with the main causes of drug failure being lack of efficacy (25%), high toxicity (20%) and low safety (12%). The potential therefore to fail drugs at the preclinical stage using accurate and robust *in vitro* predictive toxicology screens offers the pharmaceutical sector the opportunity to make huge financial savings. The Tufts centre for the study of drug development has estimated that the full capitalised resource cost for new pharmaceutical drug development is in excess of $800m with ~75% of this development cost being attributed to failed drugs. This cost rises proportionately for biopharmaceuticals to $1.3bn. Leading pharmaceutical companies such as AstraZeneca estimate that the cost of development from target identification through to candidate drug which is heavily reliant on *in vitro* screens costs ~$335m. Clearly an increase in the reliability of *in vitro* screens would have a huge impact on the cost of the drug discovery process estimated by the FDA to be an average of $100m for a 10% improvement in the development process.

In vitro assays are currently the best alternative to costly animal testing for pharmaceutical and toxicological purposes. However, they present many disadvantages in terms of key performance characteristics and are often associated with high levels of variability. Liver models represent one of the main *in vitro* systems for drug toxicity assessment. Most of them use either cancer-derived cell lines or primary cells for *in vitro* screens. However, both systems have limitations. Cell lines have the advantage of being readily expandable but lack many of the key liver markers necessary to predict modes of toxicity, whilst primary cells have the advantage of being derived directly from the liver but can't be expanded and can suffer high batch-to-batch variability. These differences make the process of accurately measuring compound toxicity *in vitro* difficult, particularly between the different phases involved in the metabolism and transport of toxic compounds.

Previous work under the NMS CBM Programme (DD3) has shown the variability in liver-specific marker expression and phase I Cytochrome P450 activity between the different *in vitro* systems and how this can be improved using novel 3D cell culture technology. This work represented a major step forward towards improving both the reliability and comparability of compound toxicity screens in primary cell models. It was however concentrated on measuring phase I enzyme expression. Linking these measurements to compound fate via phase II metabolism and phase III transport represents a major metrology challenge but would allow a more complete understanding of compound toxicity within target organs such as the liver. Analytical technologies which can be used to study the formation of metabolites *in vitro* have already been developed within the NMS under previous programmes. This project will build on this success through method comparison applicable to the predictive toxicology field and an assessment of their application to emergent technologies aimed at improving the robustness of drug screens and lowering drug attrition rates.

Impact

This project will utilise the *in vitro* compound toxicity measurement capability already developed within the NMI under previous and current NMS programmes (projects CT1 and CT2, and DD3). These projects have had a marked impact on the development of novel cell culture models for predicting compound toxicity and contributed to significant venture capital investment in UK biotech, as well as helping UK companies demonstrate improved product performance. Dissemination of these outputs through conference meetings has lead to national and international interest in the developed measurement techniques and models.

The application of measurement techniques to *in vitro* liver models and to state-of-the-art emergent technologies is helping establish the credibility of the NMS within the international biometrology arena and meeting the needs of the pharmaceutical industry by enhancing the overall understanding of how metabolism affects the efficacy and possible toxicity of compounds. Knowledge transfer from this project will be achieved through established links with major UK pharmaceutical and consumer product companies and emergent technology providers, and to other interested parties through key international societies and conferences.

Summary of Technical Work

To investigate the interplay of phase II and phase III metabolism model compounds with known in-vivo modes of action will be selected. Three dimensional cultures of hepatocytes will be established and optimised to allow phase II metabolism of the test compound to be investigated using mass spectroscopy and gene expression analysis both in standard culture and 3D culture conditions. This will then be extended to examine the effect of phase III transport proteins on the absorption and excretion of the model compound in the different cell systems and culture systems.

Activities:

- Establish primary hepatocyte culture in a three dimensional culture environment using commercially available 3D scaffolds and/or establishing sandwich culture of primary hepatocytes.
- Establishment of measurement techniques to characterise in-vitro phase II compound modifications
- Establishment of measurement techniques to characterise in-vitro phase III compound transport properties
- Examination of the link between all three phases of in-vitro compound liver toxicity
- Provision of guidelines for the use of improved measurement methods in predictive toxicology.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW in house capability established for sandwich culture of primary hepatocytes and assessment of cell cytotoxic response to model toxic compounds			
2	CEW LC-MS methods to analyse drug metabolites formed during phase I and II metabolism have been evaluated			
3	CEW phase II and phase III response of liver cells to test compounds and correlation to phase I metabolism studies has been evaluated			
4	CEW fitness for purpose of stem cell models for phase I, II and III drug metabolism studies has been established			
5	CEW guidelines produced for cell model metabolism studies and wider knowledge transfer activities have been completed			
			Total cost	

BA13					
Project Author/ Contact	**Author: Milena Quaglia** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

QC Materials for Mass Spectrometry-based Proteomic Analysis

Project Objectives

The objective is to provide capabilities and methods for the assessment and production of system suitability standards for mass spectrometry (MS)-based proteomic facilities. The developed material will enable the comparison of data from different MS-based proteomic platforms and aid in method validation. The developed standards will consist of peptides from a selection of target digested proteins. The proteins will be selected from those used to create a matrix serum standard under an existing NMS Programme project (DD1). The developed material will support a proposed series of QC materials (from peptides, digested proteins and serum spike proteins) to facilitate measurement and method comparisons between proteomic facilities. It is proposed to link this to informatics-based pattern-matching approaches for the assessment of instrumental set-up.

Background and Rationale

Progress in bio-pharmaceutical production and the growth in protein-based diagnostics markers require an increasing number of tools to provide protein identification and quantification. A large number of studies have been performed in the proteomic area to assess and compare methods for protein identification and quantification. Many of these have highlighted the poor reproducibility and repeatability of proteomics experiments, with many laboratories failing to identify constituent proteins. A lack of standards to perform suitability tests and method validation has been highlighted. This has resulted in a number of proteomic-based standards being developed. Many of these standards are complex (to reflect the complexity of the samples being run) and the output from their analysis can be hard to assess for a routine user.

The development of a single source, simple, system suitability standard would help current practitioners:

- Assess optimum instrument set-up of these complex proteomic platforms.
- Quickly validate, compare and set-up new platforms.
- Improve measurement comparability between laboratories.

The past decade has seen a dramatic increase in the development and routine use of multiple protein-based methods (proteomics) to support bio-marker discovery, cell expression profiling and bio-pharmaceuticals. Major advances have occurred in protein identification and protein quantification. However, few tools have been implemented by industry-based proteomic facilities and the discovery rate of approved biomarkers is still low. This is mainly due to the lack of suitability tests to assure intra- and inter-laboratory reproducibility of proteomic chromatographic and mass spectrometry platforms, the lack of protein standards and to a poor validation of methods generally applied in the proteomic area.

Current NMS projects aim to provide validation tools for biomarker discovery. Characterisation of some of the proteins chosen for the delivery of this project, by various liquid chromatography mass spectrometry platforms, would provide reference samples for suitability tests and would represent a follow up of current projects.

Impact

The importance of simple protein standards for calibration or method development in proteomics is widely recognised and has been highlighted by the Human Proteome Organization (HUPO). In 2006, at a HUPO pre-meeting, the need for standards for benchmarking purposes characterised by known and constant protein composition was highlighted.

Currently the most mature standard, known as the Universal Proteomics Standard, is supplied by Sigma-Aldrich and is a mixture of human proteins that span a wide range of molecular weights and pI values. This standard enables researchers to better assess proteomic strategies and troubleshoot protocols. However its high complexity is the main drawback for suitability testing and the standardisation of multiple platforms. Recently Invitrogen Corporation, a provider of essential life science technologies for research, production and diagnosis, in collaboration with HUPO, announced the launch of the HUPO Gold Mass Spectrometry Protein Standard sampling programme. This was made available at the start of 2008 and is the first commercially available all-recombinant human protein standards. Although the HUPO Gold MS Protein Standard is expected to become an essential tool in data validation for mass spectrometry related analysis, its high complexity again limits its usefulness for system suitability testing.

Previous NMS Programme projects have developed the capability of assigning traceable values for protein quantification, identifying proteins by using various liquid chromatography mass spectrometry platforms and validating proteomic-based methods for relative protein quantification. In developing these capabilities extensive research and interaction with end-users has provided an insight into the impact greatly simplified standards would have in the sector. The production of a mixture of digested proteins for a system suitability tests would not only be of extreme benefit to the scientific community, but also consolidate the in-house capabilities of producing standards for proteomic based analyses.

Summary of Technical Work

At least two proteins, from the panel selected under the current DD1 project will be selected, tryptic digested and analysed using different liquid chromatography mass spectrometry platforms. Reproducibility studies will be performed and a list of identified stable, modified and not modified peptides will be provided.

Methods to assess stability and homogeneity will be developed to enable the production of a suitable QC material.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW a literature search has been performed to identify the main requirements for suitability tests of proteomic platforms.			
2	CEW tryptic digested proteins have been analysed using at least 4 liquid chromatography and mass spectrometry platforms (including ESI and MALDI systems). CEW a list of identified peptides has been derived, with observed modifications.			
3	CEW preliminary stability testing on a candidate material has been completed.			
4	CEW outputs disseminated.			
			Total cost	

BA14					
Project Author(s)/ Contact	**Authors: Damian Marshall/Carole Foy** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Development of a Robust Toxicogenomic-Based Measurement Capability to Support REACH Legislation

Project Objectives

To support UK chemical industry through the development of screening techniques to aid chemical registration under the EU REACH Directive:

- To provide evidence of the "fitness for purpose" of toxicogenomic-based approaches to accurately and robustly measure changes in biochemical pathways and biological processes involved in causing hepatotoxicity, using well characterised chemical compounds.
- To evaluate the "fitness for purpose" of human in-vitro cell models (HepG2, HepaRG and primary hepatocytes) for toxicogenomic assays by determining the inherent variability of the systems and comparison to in-vivo data.
- To provide guidelines for the use of appropriate cell models and toxicogenomics as a tool for improved prediction of hepatotoxicity in humans.

Background and Rationale

The EU REACH (Registration, Evaluation and Authorisation of Chemicals) Directive requires ~30,000 chemical substances to be registered in terms of their intrinsic hazard and intended use. The European Commission has estimated that this will cost industry £1.5-3.5bn over 11 years, although this could rise as high as £8.6bn. One of the major hurdles to assessing chemicals under REACH is that current methods for toxicity assessment largely rely on animal-based testing. As a consequence, in 'a worst-case scenario', ~12.8 million animals and >2000 years would be required for testing (British Union for the Abolition of Vivisection, BUAV). In agreement with UK Home Office advocation of reduced animal testing as part of their 3R's programme (reduction, refinement, replacement), BUAV have therefore published a comprehensive plan for the replacement of animal testing, involving a combination of *in vitro*, human *in vivo* and *in silico* approaches. The OECD SIDS (Screening Information Data Set) suggests a longer time-frame without the additional capacity for a high throughput platform approach, which forms an important component of this proposal.

The pattern of genes expressed in a cell is characteristic of its current state and virtually all differences in cell state or type are correlated with changes in mRNA levels. Identifying changes in gene expression patterns in response to toxins (toxicogenomics) offers the potential to understand the complex pathways of toxicity, identify specific 'molecular fingerprints' for particular toxins/drugs or classes of drugs and, ultimately, identify early molecular biomarkers of toxicity and pre-clinical and clinical biomarkers of toxicity, efficacy and exposure. However, a number of fundamental questions remain unanswered – the robustness of data generated from the toxicogenomic assays, the behaviour of *in vitro* models in relation to *in vivo* models, the comparability of response across different human in-vitro models (e.g. primary cells and cell lines) due to physiological and metabolic differences, and the validity of the molecular profiles as predictors of phenotypic toxic end points.

Deriving high quality, meaningful toxicogenomic information from *in vitro* systems can be difficult. Primary cell systems derived directly from the organ of interest have the advantage of expressing relevant markers, but can be hard to obtain and suffer from batch-to-batch variability. Commercial cell lines on the other hand have low variability, but are nearly always derived from cancer sources and lack complete biomarker expression profiles. Examination of the areas that impact measurement uncertainty in toxicogenomic data in response to a panel of well characterised model chemical compounds can be used as a basis for examination of cell model systems. Once understood, translating *in vitro* toxicogenomic data forward into a predictive human (*in vivo*) model to allow assessment of chemical hazard will be possible. This will facilitate wider uptake of the technology and allow its potential for routine incorporation into integrated testing strategies to be examined.

Impact

Understanding the impact of the variables which currently hinder the widespread application of toxicogenomic measurements to comply with REACH legislation, and the translation of this understanding to the development of high throughput screens for initial toxicity testing, will be of huge benefit to the chemical industry and related stakeholders. Provision of a robust toxicogenomic approach will improve adherence to REACH by introducing

associated efficiencies and reducing requirements for animal testing. Maximum use of industry associations and European initiatives and European Commission organisations will be made to ensure knowledge transfer from the project.

This project also builds on a NMS Joint Industry Project examining the robustness of array-based technologies to measure cytotoxic response of a human cancer cell line to a model compound, this project incorporates, and then builds upon, the outputs of the JIP which involves major UK consumer product company and instrument manufacturer, animal replacement charities and European Commission organisations.

Summary of Technical Work

This project will select a panel of well characterised toxins which fall under REACH testing legislation and which have well documented *in vivo* human response. Up to three different human *In vitro* models based on primary human hepatocytes and human derived liver cell lines (e.g. HepG2 and HepaRG) will be used to generate robust toxicogenomic datasets in response to multiple doses of the selected toxins across multiple time points. The specific molecular pathways identified by the toxicogenomics experiments will be correlated with documented phenotypic responses observed in vivo and the predictive value of the profiles generated investigated.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW a minimum of 3 and maximum of 10 model toxin doses/time points for investigation have been selected and relevant human cell models have been established in-house			
2	CEW standardised in-vitro protocols have been produced which minimise the inherent variability of each human liver cell model			
3	CEW toxicogenomics data from *in vitro* human models has been generated			
4	CEW data from deliverable 2 has been compared to *in vivo* human response			
5	CEW guidelines as to applicability of using toxicogenomics and *in vitro* models for toxicity testing for REACH compliance have been produced			
			Total cost	

BA15					
Project Author/ Contact	**Author: Damian Marshall** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Characterisation of Tissue Engineered Products (Joint EMRP JRP4 *"Regenerative Medicine"* Project)

Project Objectives

To support the UK tissue engineering community through the development of measurement tools to assess product quality by:

- Providing measurement solutions to facilitate compliance with European regulations to characterise/ measure all components present in final tissue engineered products including manufacturing impurities.
- Developing high throughput screening tools to measure cell 'quality'.
- Providing guidelines for the use of cell-based assays to characterise cells in tissue engineered products.

Background and Rationale

Tissue engineering is an emerging healthcare technology for regenerating, repairing or replacing damaged tissue and is at the centre of emerging approaches of regenerative medicine. As with any pioneering new technology regulation is critical for product quality, safety and development. Under new regulations published in 2007 the majority, if not all, tissue engineered products fall under the Advanced Therapy Medicinal Products (ATMP) Regulation which comes into force in December 2008. However, there remain differing opinions within the tissue engineering community as to the potential pharmaceutical action of the cellular material within related products, a problem increased by difficulties in measuring cell quality. Tissue engineering companies supported by their trade associations (Association of British Healthcare Industries [ABHI] and its European counter-part, Eucomed) have defined common priority issues that impact directly on monitoring product quality and that require innovative measurement solutions.

Ensuring 'cell quality' in terms of viability, phenotype, stress status and functional activity is difficult due to the influence the manufacture processes can have upon the cells. This can be particularly problematic for allogeneic or stem cell products which have lengthy manufacturing procedures. EMEA guidelines stipulate that the characterisation of cell-based medical products should encompass all components present in the finished product (CHMP/410869/06). This is again challenging for products which contain cells in combination with scaffolds, matrices or innovative devices. The ability to measure 'cell quality', impurities and the response of the cells to various manufacturing insults will be of huge benefit in generating better/safer tissue engineered products and will help drive future innovation by giving more control of the cellular component of the products.

A number of assays are available to measure cell behaviour *in vitro*. However, their suitability for tissue engineering applications remains to be validated fully. Viability can be measured through a range of techniques which mostly use colorimetric or fluorescent readouts; cell stress can be measured using ELISA; phenotypic characteristics can be assayed using immunocytochemistry or flow cytometry; and protein impurities can be measured using antibody-based techniques. All these methods have potential limitations when applied to tissue engineering. Assessing their suitability and generating standard protocols applicable to tissue engineered products will represent a major step forward for product characterisation. In addition the application of new techniques to measure product quality, impurities or carry over products using mass spectrometry will help in the development of high throughput screens which can be incorporated into product quality procedures.

Impact

The UK is currently one of Europe's leading countries (2000 companies, 85% of whom are SMEs with annual turnover <£5m, representing 11% market share) in tissue engineering R&D in a global market currently estimated to be attracting €4bn/year investment (IPTS technical report on tissue engineering). The provision of generic measurement-focussed solutions and standards which support product characterisation for safer human health applications will be of huge benefit to tissue engineering companies too small to develop their scientific infrastructure and thereby fully supports the timely intervention by the NMS in this emergent field. The associated drive for application of state of the art metrology to facilitate product development will also build on the integrated technology platforms available within the NMI to establish a leading edge measurement support

capability for take-up by industry.

Wide dissemination of the outputs of this project will be achieved through current links with leading UK tissue engineering companies, UK Networks in the field, current membership of standardisation committees (BSI RGM/1, ISO TC150/SC7) and the respective trade associations. This project will also complement international joint research, examining different measurement tools for tissue engineering, including surface characterisation (NPL, UK), coherent anti-stokes raman (INRIM, Italy) and flow cytometry (PTB, Germany), and will link with the established capabilities within the NMI for high accuracy trace measurement in biological systems. These international joint research collaborations will extend the expertise derived under the current NMS CBM Programme (HT1) and wider EMRP JRP4 – Regenerative Medicine projects.

Summary of Technical Work

This project will assess the suitability of different measurement techniques to characterise 'cell quality' in a range of tissue engineered product models. The ability of colorimetric, fluorescent and antibody-based techniques, adapted to measure cell characteristics, will be examined and guidelines for standard approaches produced. The potential for mass spectrometry-based high throughput assays to measure impurities and manufacture carry-over will also be examined. Knowledge transfer will specifically take the form of presentations/peer review and guidelines to measure 'cell quality' in tissue engineered products.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW characterised models to represent tissue engineered products are completed.			
2	CEW the reproducibility of colorimetric, fluorimetric and antibody - based assays to measure cell quality in tissue engineered models have been evaluated.			
3	CEW mass spectrometry-based techniques to measure impurities in TE products have been developed.			
4	CEW results presented at a conference/peer reviewed for publication, production of guidelines, and KT.			
			Total cost	

IS1					
Project Author(s)/ Contact	**Authors: Rebeca Santamaria-Fernandez/Milena Quaglia Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Certification of Reference Standards for Absolute Carbon Isotope Ratios Traceable to the SI

Project Objectives

To produce the first material certified for carbon isotope ratios traceable to the SI.

To develop a liquid chromatography (LC) method for the analysis of a compound of interest using mobile phases compatible with carbon isotope ratio measurements and to evaluate coupling LC to multicollector inductively coupled mass spectrometer (MC-ICP-MS).

To provide high accuracy carbon isotope ratio values for organic compounds of metrology interest to industry and forensic laboratories. This will include the evaluation of a compound/s of interest to produce a carbon isotopic CRM (certified for isotope ratios rather than relative differences in VPDB scale).

Background and Rationale

The most common technique to measure carbon isotopic abundance variations is gas source isotope ratio mass spectrometry (GS-IRMS) where carbon is measured as CO_2. CO_2 isotope ratio amounts are then measured monitoring molecular ion masses 44, 45 and 46. IRMS measurements are made against a reference gas and values are expressed as differences in ‰ in the VPDB scale. The $^{13}C/^{12}C$ information becomes available through measurement of ion-currents at m/z 44 and 45 and application of an ‘oxygen’ correction to the measured ion-current ratios.

SI traceable carbon isotope ratio amounts can be measured using a MC-ICP-MS. The advantage of the method over conventional GS-IRMS measurements is that carbon isotope amount ratios are measured as C^+ instead of CO_2^+ and ‘oxygen correction’ is not required. In addition, despite the recent efforts of instrument manufacturers, there has not, to date, been a successful commercial coupling of LC to an IRMS for measurement of high accuracy carbon isotope ratios in complex mixtures. The main constraints are related to the use of aqueous mobile phases thus limiting the chromatographic separation mechanism. This project will develop non-organic mobile phases for the analysis of a compound of interest and the coupling of LC to a MC-ICP-MS for the measurement of carbon isotope ratios evaluated.

Reference standards for carbon isotope ratio values are unavailable and existing materials are certified for $\delta^{13}C$ value relative to a reference standard that defines the VPDB scale. This project will provide, for the first time, absolute carbon isotope ratios traceable to the SI.

Impact

Research carried out under NMS project CBM I3 has established capabilities to measure traceable sulfur [1] and carbon [2] isotope ratios in pharmaceutical samples and proved the applicability of the method for counterfeit detection and forensic evidence [3,4]. This project will provide relevant reference standards with assigned carbon isotope ratios for the first time. The assignment of traceable carbon isotopic ratios to materials of interest will not only provide an effective tool for drug testing laboratories, forensic analysts (especially through the Forensic Isotope Ratio Mass Spectrometry network (FIRMS)) and researchers to assess their accuracy, but also help them gain traceability to the SI for these measurands. These stakeholders will benefit from the knowledge transfer of the developed strategy through publication of peer-reviewed scientific papers and presentations at scientific meetings.

1. R Santamaria-Fernandez and R Hearn, Rapid Communications in Mass Spectrometry, 2008.
2. R Santamaria-Fernandez, D Carter and R Hearn, Analytical Chemistry, 2008
3. R Santamaria-Fernandez, JC Wolff and R Hearn, Journal of Analytical Atomic Spectrometry, 2008.
4. R Santamaria-Fernandez, JC Wolff and R Hearn, in preparation, submitted to Science and Justice.

Summary of Technical Work

Selection & preliminary screening of carbon isotope ratio values for a key analyte such as benzocaine, polyethylene glycol, testosterone in urine etc.

Definition of best metrological approaches for the certification of carbon isotope ratio values.

Evaluation of non-organic mobile phases/chromatographic methods to analyse compounds of interest for carbon isotope ratio measurements. Optimisation of chromatographic conditions with chosen mobile phases for accurate measurement of carbon isotope ratios by MC-ICP-MS.

Collaboration with NMIA to provide carbon isotope ratio values on underivatised steroids (NMIA certification of $\delta^{13}C$ values in testosterone in urine). Collaboration with FIRMS and participation in FIRMS inter-laboratory exercises.

Certification of carbon isotope ratio values for the material of interest. Confirmation values from collaborators (including universities, forensic investigators and industry) for the same material.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW consultation with FIRMS members, anti-doping agencies (NADO, WADA etc.), forensic researchers, instrument manufacturers etc has been completed. When a literature review has identified potential candidate compound/s. CEW preliminary carbon isotope ratio values for the target analyte in candidate materials has been completed.			
2	CEW candidate material to be certified for carbon isotope ratios has been evaluated. CEW best approaches for certification of isotope ratio values by MC-ICP-MS including bulk analysis, laser ablation, etc have been identified.			
3	CEW coupling LC to the MC-ICP-MS has been evaluated. CEW a LC method for the analysis of the compound of interest using non-organic mobile phases compatible with carbon isotope ratio measurements has been developed. CEW the coupling of LC separations with MC-ICP-MS (including the effect on measurement uncertainty) has been assessed.			
4	CEW collaboration with NMIA to obtain confirmation values for underivatised steroids in serum candidate material has been established. CEW a FIRMS inter-laboratory exercise with candidate material has been completed.			
5	CEW carbon isotope ratio values have been certified for the chosen material.			
6	CEW project findings have been disseminated at appropriate conferences/meetings and through at least 2 peer-review publications.			
			Total cost	

IS2					
Project Author(s)/ Contact	**Authors: Heidi Goenaga-Infante/Zoe Hall** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Elemental Tagging/Labelling and HPLC-ICPMS for Environmental Applications

Project Objectives

To develop methodologies with improved detection capabilities for small organic molecules by ICP-MS *via* tagging or labelling strategies. This will be achieved by:

- Development of methodology based on the combination of chromatography with in parallel ICP-MS and ESI MS/MS to evaluate the potential of selected reagents for the derivatisation of organic molecules (with no hetero-element detectable by ICP-MS) and the characterisation of bioconjugates.
- Development of methodology based on the combination of a high resolution separation method with ICP-MS for the accurate quantitation of environmentally-relevant organic molecules (e.g. estrogens in natural waters) artificially labelled with a tag (such as P or S) by means of derivatisation.

Background and Rationale

ICP-MS has become one of the most versatile and sensitive tools in bioinorganic analytical chemistry for the determination of most of the elements present in the periodic table. Besides their importance for living systems, elements may also be used as natural tags in a wide range of biomolecules. This opens a unique possibility to detect and quantify these biomolecules with outstanding sensitivity and selectivity via their natural element tag (already detectable by ICP-MS) or via labelling the biomolecule with a tag by means of derivatisation. This project will exploit such benefits for the accurate quantification of organic molecules of relevance to environment (e.g. estrogens). It is important to note that the successful application of this approach is strongly dependent on the complementary application of ESI- or MALDI-based molecule-specific detection techniques, since knowledge of the tag stoichiometry is essential, especially for quantitative analysis.

Natural and synthetic estrogens are of environmental concern in surface waters, causing endocrine disruption in aquatic organisms at part per trillion levels. The future regulation of estrogens is likely to be set at a particularly low value for the synthetic estrogen ethynylestradiol (sub parts-per-trillion), which may be below some monitoring laboratories' limit of detection using traditional LC-ESI-MS/MS (which are the most widely used techniques in comparison with LC-fluorescence or immunochemical techniques due to increased selectivity). Necessary sample preparation methods, involving at least a 1000-fold concentration step, are laborious and time consuming. Derivatisation reactions such as dansylation (for labelling estrogens with ICP-ionisable elements such as sulphur and phosphorus) offer a promising approach for improving estrogen limits of detection with minimised efforts on sample preparation, thereby leading to an improved quality of analytical result (e.g. improved measurement uncertainty).

Impact

The development of analytical methods for small organic molecule quantification at ultra-trace levels based on elemental mass spectrometry and capable of providing reliable data to support law enforcement will be particularly beneficial to the water industry, environmental legislators, clinical experts and academics involved in the process of risk assessments relating to pollution control and restoration of aquatic ecosystems.

Summary of Technical Work

Labelling of biomolecules with a tag by means of derivatisation. Development of sample preparation/separation methodologies. Investigation of the label/biomolecule stoichiometry, labelling efficiency and selectivity (parameters which are necessary for further quantitative studies). Evaluation of the detection capabilities of ICP-MS on the labelled species compared to conventional methods. Characterisation of bioconjugates using complementary techniques such as MALDI-MS and ESI-MS.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW the feasibility for labelling biomolecules with a tag detectable by ICP/MS (e.g. estrogens with S/metal-reagents) has been assessed.			
2	CEW a hyphenated technique based on the combination of chromatography with elemental and molecular MS has evaluated the potential of selected reagents for biomolecule derivatisation and the characterisation of the bioconjugates.			
3	CEW the analytical capabilities of HPLC-ICP-MS, in comparison with conventional organic MS methods, have been assessed for the accurate quantification of labelled biomolecules at ultra-trace levels in real samples.			
4	CEW knowledge transfer achieved through presentation of results at an appropriate scientific meeting and at least 2 peer-reviewed publications in the scientific literature.			
			Total cost	

IS3					
Project Author/ Contact	**Author: Ruth Hearn** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

High Accuracy Quantification

Project Objectives

An underpinning project that will address the calibration fundamentals required for many of the new projects proposed for the CBM Programme. The aims are to provide high accuracy calibration strategies and the associated uncertainty protocols in cases where normal IDMS methodology is not applicable. In addition, a more efficient strategy for the amount content value assignment for CRMs and PT schemes will be developed.

The benefit of such work would be improved accuracy, traceability and value for money for a wide range of applications covered by the CBM Programme as well as innovative techniques accessible to the wider analytical community.

Background and Rationale

LGC has a strong background in high accuracy quantification using isotope dilution methodologies and has gained accreditation to ISO Guide 34 for the certification of reference materials using exact matching IDMS. However, the complexity of current metrology activities is such that new calibration approaches must be developed, validated and implemented.

The project will include strategies for dealing with situations where isotopically labelled species are not available such as species unspecific (post column) isotope dilution and standard addition techniques as well as the use of IDMS in challenging situations such as fast eluting peaks or reduced chromatographic separation.

A second aspect to the project is the improvement of our existing IDMS methodologies for greater accuracy and efficiency in all of our quantitative analysis and certification campaigns. This may include double injection techniques for the introduction of calibrations blends within the same chromatographic run as the sample and the use of continuous sample introduction for organic IDMS, via peak parking or using high resolution MS & MS/MS for added selectivity. Also, robust approaches to the use of quantitative GCxGCMS and fast GCMS for the certification of environmental contaminants will be investigated.

The effect on uncertainty will be a key component in all of these studies.

Impact

These fundamental studies will form the foundations for many of the other applied metrology projects within this programme.

The project will enable us to produce high calibre traceable measurements in the most efficient and cost effective manner with uncertainties that are fit-for-purpose. This will enable us to react to the more immediate needs of the analytical community for help in delivering higher quality data of economic, public and environmental benefit, as well as giving the community access to innovative techniques.

Summary of Technical Work

The technical work will span a wide area of high accuracy quantification measurements including inorganic, organic and speciation. It will include assessment of the fundamentals of improved isotope dilution techniques as well as other high accuracy quantitative methods and the evaluation of the impact on uncertainty.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW a post-column isotope dilution methodology has been validated by comparison with species specific measurements. CEW recommendations for future use of the method have been made to support metallomics and speciation studies.			
2	CEW standard addition protocols have been developed where isotopically labelled standards are not available. CEW recommendations for uncertainty calculations have been included.			

3	CEW a report detailing experimental studies on improvements to existing IDMS methodology (such as double injection techniques, peak parking, continuous IDMS, non-ideal isotope ratios and other alternative approaches) has been produced. CEW the report includes recommendations for selection of appropriate methods and the impact on uncertainty.			
4	CEW a practical assessment and report has been completed on the impact on uncertainty for challenging analyses (such as fast peaks (e.g. from CE) and reduced chromatographic separation (e.g. from SPME/headspace))			
5	CEW approaches developed for traceable quantitative GCxGCMS and FastGCMS. CEW the feasibility of combining techniques to improve robustness, traceability, accuracy and throughput of CRMs has been assessed.			
			Total cost	

IS4					
Project Author(s)/ Contact	**Authors: Heidi Goenaga-Infante/ John Entwisle** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Characterisation of a Methionine Certified Reference Material

Project Objectives

To characterise an existing animal feed reference material for its content of methionine in order to help UK field laboratories, industry and academia to validate their analytical methodologies for the quantitation of this S-aminoacid in a feed matrix. Such methods will be essential tools for quality control of new products, and their legislation and use.

Background and Rationale

In order to achieve valid and traceable measurements, UK analytical laboratories need fit-for-purpose standards and CRMs for key applications, e.g. where there are measurement problems, regulatory requirements, or a need for acceptance of UK data by trading partners. L-methionine (Met) is a S-containing essential amino acid and has been an important feed supplement in the UK for many years. Currently under Commission Directive 98/67/EU (http://ec.europa.eu/food/food/animalnutrition/labelling/marktlab02_en.pdf), DL-Met is permitted as a feed additive with a tolerance of +/-20% of the declared value.

The accurate determination of methionine in complex food/feed samples is challenging due to the instability of the amino acid, which can easily become oxidised during the analytical procedure and, in particular, during extraction. Extraction of Met from supplements of Se has been performed using methanesulfonic acid with derivatisation prior to analysis by GC-MS with species-specific IDMS using ^{13}C-enriched methionine. Using this methodology, a Se-yeast material (SELM-1) has been characterised for its MET content by NRC-CNRC, Canada and it is the only existing food supplement CRM certified for its Met content.

Novel methodology for the accurate quantification of Met in food supplements currently under derivation is based on the use of microwave energy (to accelerate acid hydrolysis of Met from its matrix) followed by accurate quantitation by HPLC-ICP-IDMS using ^{34}S-enriched methionine without need for Met derivatisation. However, its performance characteristics have not been assessed fully through comparison with the more traditional GC-IDMS analysis method in order to establish the best approach for extending the certification of an existing complex animal feed material, e.g. LGC 7173 RM (already certified for trace element content), for its Met content.

Impact

The global production of Met and Met analog compounds exceeded 600,000 metric tons in 2005 and has been growing by at least 5% p.a. Assuming an average price of $2.93/kg, this translates to annual sales of nearly $1.8 billion for Met compounds. Production of a more relevant CRM already available to the livestock industry and analytical communities will help underpin better optimisation of the nutritional quality and economics of feed through extension of its traceable reference values, thereby enhancing both animal and human welfare.

Summary of Technical Work

Comparison of species-specific double isotope dilution mass spectrometry (IDMS) with reversed phase HPLC-ICPMS using ^{34}S-enriched methionine (developed during a former NMS programme) with species-specific IDMS by GC-MS using ^{13}C-enriched methionine. The most suitable method will be used for homogeneity and stability testing, and certification measurements.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW the performance characteristics of GC-IDMS using ^{13}C-labels have been assessed for the measurement of methionine in a poultry feed sample (LGC 7173).			
2	CEW homogeneity and stability testing of the Met content of the poultry feed material has been determined using the most appropriate method.			

3	CEW knowledge transfer has been achieved through certification of LGC 7173 for its methionine content and the certification report submitted.			
			Total cost	

IS5					
Project Author(s)/ Contact	**Authors: Patrick Galler/ Heidi Goenaga-Infante/ Ruth Hearn Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Simultaneous Measurement of Sulfur With Other Metals for Bioclinical and Food Applications

Project Objectives

To study collision cell fundamentals for the simultaneous determination of elements such as Se, As and S, which coexist (often associated to the same biomolecule) in bioclinical and food/supplement samples in order to better remove spectral interferences, whilst reducing loss of low mass sensitivity, the biggest problem in most applications. In particular:

- Optimize simultaneous multi-element detection (e.g. lower background equivalent concentration, better LODs for S/Se or S/As) using collision reaction cell ICP-MS by investigating the effect of different collision gases and mixtures with and without kinetic energy discrimination (KED).
- Apply optimised conditions to the simultaneous element quantitation (e.g. S/Se or S/As associated with biomolecules) for environmental and biological samples.
- Report best practice guidelines for ICP collision cell mass spectrometric multi-elemental determinations.

Background and Rationale

Collision cells are employed routinely for removal of spectral interferences in ICP mass spectrometry. Depending on the nature of the collision gas, analyte-gas collisions will result in either redistribution of kinetic energy or formation of reaction products, enabling analyte/interference separation. The underlying mechanisms of collision and/or reaction cells are not yet fully understood. Typically experimental parameters are optimised for quantification of a single target analyte, resulting in reduced sensitivity of other elements. An example is the simultaneous determination of sulphur with other low/medium mass elements (e.g. As or Se). Spectral oxygen-based interferences on the detection of S isotopes can easily be removed using xenon (Xe) as the collision gas. However, the biggest problem with Xe mode is the loss of low/medium mass (e.g. Fe, Se, As, Zn) sensitivity. The problem may be addressed using a collision gas that improves efficiency of Xe reaction through collisional focusing, allowing lower Xe flow and hence reduced loss of low mass sensitivity. Alternative approaches may be the use of oxygen cell gas, thus detecting the oxides or the use of hydrogen mode, thus measuring SH and elements (e.g. Se), which work well in this mode. Furthermore, the effect of applying a difference in bias potentials between the quadrupole mass analyser and the octopole cell (KED) on removing cell-formed unwanted interfering ions may be investigated.

Impact

Knowing the effects and interactions of experimental parameters in ICP collision/reaction cells will enable the optimisation of measurement conditions suitable for more than a single element at once. Significant reduction of time and thereby cost associated with reduced numbers of runs for simultaneous quantification can be anticipated for future work, for example in metallomics and biological elemental speciation. Improved efficiencies will underpin current status as the UK's designated NMI for biochemical measurements and offer improved customer focus and service through the associated chemical calibration facility. Effective knowledge transfer of the developed strategies to relevant industrial sectors (through relevant industry groups and trade associations etc), healthcare & medical sector representatives and academia will be achieved through publication of peer reviewed scientific papers and presentations at scientific meetings, thereby widening the analytical communities' appreciation of better measurement performance when using more efficient ICP-MS/collision reaction cell technology.

Summary of Technical Work

- Thorough understanding of collision cell design of instrumentation within use
- Identification of relevant experimental parameters
- Systematic investigation of effect of experimental parameters on target analytes
- Investigation of the suitability of developed methods to analyse real samples.

Deliverables				
No.	**Deliverable**	**Start**	**End**	**Cost**
1	CEW methodology improved for the simultaneous quantification of target analyte groups (e.g. S/As, S/Se or S/Se/As) in real samples of relevance to nutrition, health or environment.			
2	CEW knowledge transfer of improved methodology achieved through conference presentation and peer reviewed scientific publication, and recommendations for multi-elemental determinations by ICP/collision cell MS published.			
			Total cost	

IS6					
Project Author(s)/ Contact	**Authors: Heidi Goenaga-Infante/ Emma Peachey** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Quantitative Speciation Methods for Characterisation of a Speciated Plant Reference Material

Project Objectives

This project will develop novel IDMS methodologies for the quantitation of hetero-atom compounds in different plants organs in metal-accumulating plants (for mass balance purposes) allowing the characterisation of a new speciated plant reference material in support of phytoremediation and biofortification technologies. In particular, the project will:

- Develop reference methodology (e.g. post-column IDMS with isotope pattern deconvolution *vs* species-specific IDMS and standard addition techniques) for the accurate simultaneous quantitation and identification of target heteroatom-containing biomolecules (e.g. Se or As-phytochelatins, methyl-Se species, Hg-methylHg-thiol bound compounds) in extracts of metal-accumulating plants (*Indian mustard, Allium plants* or *Arabidopsis Thaliana*) exposed to metal(loids).
- Investigate species instability/inter-conversion during the analytical procedure through use of isotopically-enriched compounds and isotope-ratio techniques.
- Characterise a new speciated plant matrix reference material.

Background and Rationale

Speciation/metallomic studies of metal-containing biomolecules in relation to phytoremediation and food research are of increasing interest to industry and academia for environmental monitoring. Although a number of metallomic approaches to study element speciation in plants have been reported, the accurate quantification of relevant element species in plant materials remains a challenge. This is mostly due to the lack of calibration standards and reference materials for validation of the speciation methods and in-solution instability of target compounds (e.g. plant phytochelatins). This project will extend the existing methodologies developed under the current CBM Programme to provide accurate IDMS quantification of multiple elemental species and the study of their interactions in, not only leaves, but other plant organs for mass balance purposes. Enrichment of elemental species with stable isotopes will enable study of species inter-conversion that may occur during storage, extraction and separation. In the case of As, which is monoisotopic, quantitation of its compounds (e.g. As-phytochelatins) will rely on standard addition protocols using standards of phytochelatins, which will be characterised with traceable inorganic As standards. The combination of these approaches will allow production of a new speciated plant reference material, which is urgently needed for the purpose of verification of accuracy of speciation methods and quality assurance needs.

Impact

The development and validation of isotope ratio (IDMS) methodologies and standard addition protocols for multiple elements/species in metal-accumulating will provide the basis for characterisation of a new speciated certified plant reference material to support the development of future regulation and the development of new and more efficacious bio-fortification and phytoremediation processes for health and environmental application. There is thus keen support from industry, academia, and environmental and governmental bodies (e.g. Southampton and Nottingham University, Forest Research, WRC, ABNA-Feed ingredients) for the work. Finally, knowledge transfer of the developed strategies to academia, industry and the healthcare sector will be achieved through publication of peer reviewed scientific papers and presentations at scientific meetings.

Phytoremediation has potential for safe, selective clean-up of environmental contaminants, with value added through the recovery of valuable and increasingly scarce trace metals. In bio-fortification, which uses conventional crop breeding to optimise levels of key nutrients, chemical speciation will help select varieties that maximise the bioavailability, and hence nutritional benefits, of trace elements.

Summary of Technical Work

Development of IDMS methodologies (post-column IDMS with the isotope pattern deconvolution approach [link with the proposed project on high accurate quantitation] for the simultaneous accurate quantitation and identification of different known and unknown elemental species of Se and Hg in plant organs. Development of standard addition methodologies with HPLC-ICP-MS for the quantitation of As species in plant extracts. Improvement of collision/reaction cell optimisation for the simultaneous detection of sulfur with other low/ medium mass elements (link to proposed project on collision reaction cells). Development and validation of methodology to study species inter-conversion during storage, extraction and separation, through use of isotopically-enriched compounds and isotope ratio techniques. Application of the validated methodology to characterisation of a new speciated plant reference material.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW reference methodology developed for the accurate simultaneous quantitation and identification of target heteroatom-containing biomolecules in plant extracts.			
2	CEW isotope ratio-based methodology developed to study species inter-conversion in plants.			
3	CEW reference methods/strategies applied to characterisation of a new speciated plant reference material.			
4	CEW knowledge transfer achieved through at least 1 conference presentation, publication of the methodologies derived in deliverables 1 and 2 in a peer reviewed scientific publication, and release of the CRM.			
			Total cost	

IS7					
Project Author/ Contact	**Author: Heidi Goenaga-Infante** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Speciation Methodologies for Quantifying DNA Adduct Formation with Metallo-drugs

Project Objectives

To improve the detection capabilities (selectivity and sensitivity) of LC-ICP-MS methods for the quantification of low picograms of P associated with biomolecules such as DNA-adduct formation with metallodrugs (e.g. *cis*-Pt or *oxali*-Pt). This will be achieved by:

- Development of methodology for preconcentration and quantitative extraction of metal/P-biomolecules (e.g. *cis*Pt or oxaliPt adducts with oligonucleotides) at low pg levels from DNA samples
- Development of methodology based on the coupling of capillary LC with interference reducing ICP-MS *via* ^{31}P- (and/or elemental isotope e.g ^{195}Pt) specific detection that will enable quantitation of P-containing biomolecules such as *cis*-Pt or oxaliplatin adducts with DNA nucleotides using calibration with synthetic (and well characterised) *metallo-drug* adducts with commercially available nucleotides.
- Evaluation of the feasibility of the developed methodology to detect/quantify the adducts of metallo-drugs (e.g. Pt drugs) formed *in vivo* in cancer studies.

Background and Rationale

The measurement of phosphorus in biologically relevant samples can provide useful information about the phosphorylation state of a protein or it can be used for the detection and quantification of RNA or DNA. ICP-MS represents the most sensitive spectrometric-based technique for the determination of phosphorus and with the new developments on collision / reaction cell technology, detection of P-containing biomolecules at parts-per-billion levels (e.g. in P-peptides, non-modified nucleotides and DNA adducts with styrene) can be achieved by coupling quadrupole-based ICP-MS with a high resolution separation technique. However, their accurate quantitation is still a challenge due to the naturally monoisotopic nature of P, making the use of IDMS quantitation approaches impossible.

The detection and quantification of DNA-adducts with metallo-drugs such as Pt-drugs (e.g *cis*Pt ot *oxali*Pt) formed *in vivo* in cancer models is of extraordinary present interest. Improving the efficiency of Pt-drugs by increasing drug selectivity and minimising side-effects requires the development of very sensitive methods since adduct formation with DNA is GG or AG -specific. The most sensitive method for detecting DNA-adduct formation (1 adduct in 10^{10} nucleotides) is based on ^{32}P-post labelling. However, it is a complex assay (involving radioactive P) and experimental conditions can vary depending on the nature of the adduct. There is therefore still a lack of specific robust methods to selectively detect and accurately quantify those adducts formed in vivo at low concentrations relevant to the clinic.

This project builds on current NMI capability for accurate quantitation of trace phosphorus using collision / reaction cell ICP-MS as developed under the current CBM Programme.

Impact

The formation of DNA-adducts with Pt drugs could be a pharmacokinetic parameter to optimise in cancer therapy. The development of novel methods for their traceable measurement will help improve drug efficacy and directly impact cancer research and treatment. Moreover, these advances in analytical methodology will enhance the UK NMS calibration capability and measurement science. The methodology developed will find applicability to the evaluation of the effect of other metallodrugs (e.g. Se or V) on cancer treatment. Knowledge transfer of the developed strategies to academics, industry and the healthcare sector will be achieved through publication of peer reviewed scientific papers and presentations at scientific meetings.

Summary of Technical Work

Development of novel extraction and preconcentration methods for P/metal-biomolecules at low picrogram levels from biological samples such as DNA. Use of pure traceable P inorganic standards for the characterisation of DNA nucleotide standards. Development of capillary LC/CE in combination with interference reducing (collision /reaction cell or double focusing magnetic sector) ICP-MS for detection/accurate quantitation of P-biomolecules such as DNA adducts with metalo-drugs (e.g. Pt drugs) via metal (e.g.Pt) and P detection. Enhancement of detection capabilities of ICPMS (lower blank signals and higher sensitivity for P) using novel sample introduction techniques. Synthesis and characterisation (using organic MS techniques) of metallo-drugs (e.g.*cis*Pt) adducts formed with commercially available nucleotides to be used for quantification.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW methodology for the extraction and preconcentration of P-biomolecules such as *cis*Pt or *oxali*Pt adducts with DNA has been established.			
2	CEW reference methodology based on the coupling of capillary LC/CE with ICP-MS for the accurate quantitation of DNA adducts with metallodrugs (e.g. Pt drugs) at pg levels via ^{31}P and elemental ion (e.g. ^{195}Pt) detection has been established.			
3	CEW the newly develop methods to detect/quantify adducts of metallo-drugs (e.g. Pt drugs) formed *in vivo* have been applied to cancer studies with animals exposed to specific drugs.			
4	CEW findings disseminated at appropriate conferences/meetings and through at least 2 peer review publications.			
			Total cost	

IS8					
Project Author/ Contact	**Author: Heidi Goenaga-Infante** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Traceable Measurement of Vanadium-containing Biomolecules

Project Objectives

To provide traceable measurement for the identification and quantitation of vanadium-containing biomolecules in biological materials (e.g. serum and tissues) to underpin UK industry and diabetes research into novel, safer and more efficient drugs through:

- Development and validation of novel quantitative extraction procedures for small metabolites and macromolecules of vanadium (e.g. V-transferrin) from biological fluids and tissues
- Development of 2D-chromatography (size exclusion followed by capillary reversed phase, capillary electrophoresis (CE) or fast protein liquid chromatography (LC) coupled to collision cell ICP-MS methodology for the detection/quantification of vanadium species in relevant models
- Development of enhanced detection capabilities for selective species identification using the parallel combination of high resolution separation with ICP-MS and organic MS (MALDI-MS or ESI QTOF-MS).

Background and Rationale

LGC has become a recognised world leader in elemental speciation through its developed metrology capability for phosphorus (P) and selenium (Se). Applying these developed principles to effect traceable measurement capability for vanadium compounds, for future application to clinical trial scenarios envisaged within a current Innovation R&D project, is timely. Vanadium drugs (e.g. the organic compound bis-maltolato oxovanadium-BMVO) have potential medicinal use in the treatment of diabetes mellitus - a disease that affects >2m people in the UK alone, at a cost of ~5% of NHS expenditure (1,2) – as, unlike insulin, vanadium compounds are low molecular weight species that are stable in gastric juice and can be administered orally. Binding of vanadium to transferrin *in vitro* in response to vanadium drugs (e.g. BMVO) has been reported. Despite numerous studies, the mechanism/s by which vanadium mediates its observed metabolic effects *in vivo* is still not completely understood owing to the difficulty in achieving highly sensitive/selective determination of this element using conventional ICP-MS detection (due to spectral interferences) and potential transformation of the target species during sample handling and species separation associated with organic MS strategies. Clarification of the biochemical forms and physiological functions of this element in higher organisms will be facilitated greatly by the provision of accurate measurement methods for traceable quantification and identification of ultra-trace levels of vanadium-containing biomolecules in biological materials.

1. Boden G, Chen X, Ruiz J, et al. *Metabolism*. 1996;45:1130-1135.
2. Halberstam M, Cohen N, Shlimovich P, et al. *Diabetes*. 1996;45:659-666.

Impact

The provision of methods for traceable quantification and identification of ultra-trace levels of vanadium-containing biomolecules will enable UK industry and researchers to develop more efficacious vanadium drugs/ supplements, potentially in the form of individualised treatment regimes. Moreover, the support for diabetes studies will help optimise vanadium 'insulin-mimics' for diabetes treatment. The project outputs will be transferred directly for use in relevant clinical trials to measure diabetes improvement in response to vanadium drugs planned within a current Innovation R&D project. Relevant user organisations are involved actively in the project and have a strong interest in the project outputs, but knowledge transfer of developed strategies to academics, industry and the healthcare sector will be achieved through publication of peer reviewed scientific papers and presentations at scientific meetings.

Summary of Technical Work

To develop and validate methodology based on the combination of novel extraction procedures and 2D separations coupled with ICP-MS and/or ESI- or MALDI-MS for the accurate determination and identification of target vanadium-containing biomolecules at low ppb levels in complex biological samples. Such methodology presents clear metrology challenges (difficulties for the interference-reducing detection of vanadium by ICP-MS, complexity of the sample, the low concentration and instability of target species, the limited amount of sample to perform total element and speciation analysis). These problems will be addressed by investigating the

capabilities of fast protein LC or CE as a second separation/fractionation mechanism coupled with collision/ reaction cell ICP-MS (with standard addition protocols). CE is well suited for the investigation of intact metal-containing proteins due to its high resolution capability, low sample requirement (nanolitre range) and the absence of packing susceptible to interaction with metals disturbing the complexation equilibria. Its interfacing to ICP-MS (which is very challenging) will be investigated for separation/detection of vanadium-containing transferring, albumin or ferritin isoforms. The potential of the parallel coupling of chromatography with ICP-MS and ES MS/MS will be investigated for the identification of vanadium compounds in samples generated from diabetic animals supplemented with vanadium drugs. Comparative studies of different ligands used to synthesise vanadium compounds with insulin-mimetic properties will be useful in the development of more effective antidiabetic and glucose-lowering drugs.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW a method for the extraction of a wide range of intact species of vanadium from biological cells/tissues has been established			
2	CEW a method based on the combination of 2D chromatography (size exclusion followed by reversed phase HPLC) with ICP-MS and MALDI/ESI-MS/MS for the identification and quantitation of ultra-trace vanadium compounds *via* vanadium off-line measurements, post-column IDMS or standard addition protocols has been established			
3	CEW a method based on the combination of a molecular weight fractionation step (e.g. by size-exclusion or using microfiltration membranes) followed by fast protein LC or CE with ICP-MS and MALDI/ESI-MS/MS for the measurement and identification of isoforms at ppb levels in cell/tissue extracts, incubated samples and cell lysates has been established			
4	CEW results have been disseminated at appropriate conferences/meetings and through at least 3 peer-review publications			
			Total cost	

OA1					
Project Author/ Contact	**Author: Steve Wood** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

International Metrology Measurements

Project Objectives

To ensure that the UK's chemical measurement system is linked internationally by:

- Developing and maintaining the infrastructure and competencies needed to fulfil UK obligations under the CIPM MRA, such that UK chemical standards are recognised globally
- Maintaining the UK's calibration and measurement capability (CMC) claims and underpinning the provision of calibration services to UK organisations.

Background and Rationale

This project maintains and develops capability to enable LGC perform its NMI role within the UK.

The CIPM Mutual Recognition Arrangement (MRA) requires the UK to participate in international comparisons of measurements (pilot and key comparisons), to develop and maintain quality systems and to demonstrate competence in calibration and measurement activities in chemical and biochemical measurement.

This in turn enables LGC through the UK Chemical Calibration Facility (UK CCF) to deliver high level chemical calibration services fir a wide range of different analytes and matrices to UK organisations to support trading activities and to enable compliance with legislation. The services provided by the UK CCF support proficiency testing schemes (FAPAS, WEQAS, UK NEQAS), product release, confirmatory measurements and QC material calibration.

LGC's obligations under the CIPM MRA require a significant commitment to infrastructure development (participation in comparisons, representation of chemical and biochemical metrology interests in CCQM and EUROMET), the development of personnel, quality systems and processes.

Impact

This project is principally underpinning, but the high accuracy calibration services and the assignment of reference values (e.g. through PT schemes and to reference materials) provide a direct and significant benefit to a wide range of UK stakeholders across all sectors. Successful participation in the studies currently under discussion for the 2009-2012 period would help guard against contamination in the food chain, deliver safer consumer products and more sustainable chemicals, underpin a diversified energy strategy and foster innovation for improved healthcare.

Summary of Technical Work

At this stage it is not possible to provide a definitive list of studies which will be undertaken. Participation in a specific study is influenced by a number of factors including the relevance to UK needs, fit with LGC capability, need to participate in key comparisons to support or maintain existing CMC claims; need to participate in studies in support of NMIs in developing economies. Some of these factors are outside LGC's control.

Studies currently under discussion in the CCQM bioanalysis, inorganic analysis and organic analysis working groups include: protein quantification by mass spectrometry (EMRP linked); RNA transcribe (key comparison), generic cell authenticity testing; brominated flame retardants in plastics; metal in plastics; malachite green in fish tissue (co-ordination); organic substance purity (3 key comparison studies, including aldrin and tetracycline) and bio fuels.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW UK CMC claims have been established and maintained through the participation in or organisation of at least 18 chemical and biochemical CCQM key comparisons or pilot studies and regional EURAMET comparisons over 3 years,			
2	CEW EURAMET TC-Q Annual Reports have been prepared and submitted; LGC CMC claims reviewed and submitted to the BIPM			

	and JCTLM databases; CMCs of other NMI's evaluated as required by EURAMET; and CRM data input to the COMAR database.			
3	CEW ISO/IEC 17025 accreditation for calibration and testing measurements to support measurement capability has been retained through successful audit performance (zero cost to NMS).			
4	CEW at least 4 calibration and reference measurement services to be delivered to UK organisations annually with direct costs paid for by customers have been delivered (zero cost to NMS).			
			Total cost	

OA2					
Project Author/ Contact	**Author: Nick Boley** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Methods and Materials to Support Clinical Laboratory Measurements

Project Objectives

To develop methods and associated Certified Reference Materials (CRMs) in support of the clinical measurement and laboratory medicine community. The methods and materials will meet the requirements of the In Vitro Diagnostic Medical Devices (IVD) Directive (EC IVDD, 98/79/EC), and comply with the requirements for listing on the database of higher-order reference materials of the Joint Committee for Traceability in Laboratory Medicine (JCTM).

To disseminate the knowledge developed to both laboratories and accreditation bodies via a series of presentations and publications with support from the Clinical Reference Material User Group (CRMUG).

Background and Rationale

The development of isotope dilution mass spectrometry methodology for a range of clinical analytes has been funded in previous NMS programmes, resulting in the availability of methods and CRMs for creatinine and electrolytes in serum, testosterone in serum and, under development in the 2006-2009 NMS Chem Bio programme, digoxin in serum, tacrolimus in blood and trace metals in serum. These materials have been developed in collaboration with organisations heavily involved in clinical laboratory medicine, who have prepared candidate materials and provided confirmatory measurement data, e.g. Cardiff Bioanalytical Sciences, WEQAS, TEQAS, and UKNEQAS.

The need for traceable high-quality CRMs remains strong and is reinforced by the demands of ISO 15189 (Medical Laboratories - Particular Requirements for Competence). For organic analytes, LC-MS is being increasingly used in hospital laboratories, although many still use immunoassay techniques, which are known to have limited traceability and problems with interferences.

Consultation with CRMUG has identified the key clinical analyte/matrix combinations as being of interest:

- Theophylline in serum: Theophylline is a bronchodilatory drug, and accurate monitoring of its concentration in serum is important in assessing patients and facilitating appropriate dosage.
- Cyclosporine in blood: Cyclosporine is an immunosuppressant drug used following organ transplantation, and accurate measurements are necessary to monitor and apply the appropriate patient dosage.
- Buprenorphine in urine or oral fluid: Buprenorphine is a very powerful opiate analgesic, used in the treatment of opiate addiction.
- Vitamin D in serum: Vitamin D deficiency is increasing in the UK, making monitoring and treatment more important. Vitamin D is produced in the body from the pro-hormones D1 and D2, and measurement of the active form is most important.
- Total metals in urine: The monitoring of metals in urine is important in assessing patients for nutrient deficiencies and for diagnosis of causes of toxicity.

Impact

The availability of further CRMs in the clinical and laboratory medicine sector will enable accurate and traceable measurements to be made for further key analytes. The results from such measurements will be used to determine appropriate treatments and drug dosages for patients, and will help reduce the quantity and therefore cost of drugs prescribed, ensure the comparability of measurements from different platforms and enable more consistent and comparable results to be obtained between different laboratories. There will also be benefits for other areas including workplace drug testing and forensic pathology.

Summary of Technical Work

The current and future needs of the laboratory medicine community will be confirmed in consultation with the CRMUG immediately prior to the start of the project to ensure the methods and materials to be developed continue to be a priority and there is no duplication with the activities of other NMIs. Traceable mass spectrometric reference methods will be developed and used to produce two CRMs and, where appropriate,

associated calibration standards.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW, in consultation with members of the Clinical RMUG, the key measurement and CRM needs of the clinical and laboratory medicine community have been confirmed, the CRMs to be produced selected and the key issues, risks and strategies evaluated.			
2	CEW reference methods have been developed and validated for two key analyte/matrix combinations and two Certified Reference Materials and, where appropriate, associated calibration standards are available for sale.			
3	CEW reference methods and production approaches have been accepted for publication in a peer reviewed journal.			
			Total cost	

OA3					
Project Author/ Contact	**Author: Nick Boley** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Methods and Materials to Support the EU Water Framework Directive

Project Objectives

To develop methodology for the production and certification of reference materials in support of the European Union's Water Framework Directive (WFD), and its daughter directives.

To produce two CRMs of the appropriate analyte/matrix to support UK laboratories implement the WFD.

Background and Rationale

The adoption of the European Union's Water Framework Directive (2000/60/EC) requires member states to establish water quality monitoring programmes for assessment and planning purposes. A comprehensive quality assurance programme is needed as part of this in order to reduce costs and associated with repeat measurements, and this is laid out in the QA/QC directive (q.v.). The need for high quality, traceable reference materials for use by laboratories has been referred to explicitly in the draft QA/QC directive, to be approved by the European Parliament by the end of 2008. These reference materials will significantly aid laboratories in demonstrating traceability and comparability across Europe. The directive requires laboratories to use appropriate certified reference materials (CRMs) from accredited producers.

An EU funded project (EAQC-WISE*, www.eaqc-wise.net) was established to suggest a quality control system, which would coordinate tailor-made proficiency testing activities, reference material production, research and training at the EU level in support of water and soil policies, with regular exchanges of good practice. This project has identified significant gaps in the provision of appropriate CRMs, and many of the "missing" materials present complex technical challenges to produce, such as the dissolution or dispersion of non-polar organic compounds. Examples of "missing" materials include organics in waters (e.g. current "priority substances". diuron, pentabromodiphenylether, atrazine, simazine, chloroalkanes and trichlorobenzenes) and metals and inorganic species (including Cr (VI) and TBT) in waters at concentrations relevant to the WFD limits.

The number of producers with the capability to overcome such challenges in Europe is low and it is unlikely that any significant funding will be available, at least in the short to medium term, from the European Commission for this work.

[* **E**uropean **A**nalytical **Q**uality **C**ontrol in support of the Water Framework Directive via the **W**ater **I**nformation **S**ystem for **E**urope]

Impact

The development of measurement capability and CRMs in support of the WFD will contribute significantly to the ability of monitoring laboratories in the UK, and the rest of the EU, to measure key pollutants in river basin waters in an accurate and traceable manner, and in a consistent manner to their peers. This will have a significant impact on the quality of measurements in this highly regulated area and, hence, the interpretation of data and subsequent planning/actions to control environmental pollution, protect water resources and maintain or improve their ecological status.

Summary of Technical Work

Key reference material needs, issues and risks will be ascertained (much of this data will be available through EAQC-WISE which is due to present its final report in December 2008, and the corresponding EU Expert Groups to be set up in early 2009) and, where appropriate, collaborations with other leading RM producers in the EU will be established to prevent any duplication of effort.

Two CRMs will be prepared, characterised and certified for specific analytes.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW key CRM needs, issues and risks in support of the Water Framework Directive have been evaluated and identified and production priorities and collaboration requirements have been agreed with other EU producers (e.g. IRMM, BAM).			

2	CEW a reference material certified for one or more of, for example, Diuron, Atrazine, Simazine Pentabromodiphenylether, and other pesticides in water is available for sale.			
3	CEW a reference material certified for one or more of, for example, Cr (VI), TBT, chloroalkanes and trichlorobenzenes in water is available for sale.			
4	CEW an article has been submitted for publication in a peer review journal			
			Total cost	

OA4					
Project Author Contact	**Author: Nick Boley** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Measurements and Materials to Support ROHS/WEEE Legislation and VOC Emission Control

Project Objectives

The project will build on the expertise being developed in NMSChemBio 2006-2009 project R7 for the determination of restricted substances such as Cadmium, Lead, Mercury, Chromium, polybrominated flame retardants (PBBs and PBDEs) and VOCs, and will provide:

- traceable reference values in support of the Restriction of Hazardous Substances (ROHS) Directive, designed to minimise the impact of Waste Electrical and Electronic Equipment (WEEE) on the environment.
- a reference material (RM) characterised for one or two volatile organic compounds (VOCs) in a building or vehicle product. (Note this is dependent on the results of a feasibility study currently underway in NMS CBM Programme project R7.

The feasibility of traceable measurement of Cr (VI) in a plastic material will be investigated and if practical, methodology for the determination of Cr (VI) in plastics will be developed and validated.

Background and Rationale

A review of the activity in the development of reference materials to support the ROHS/WEEE directives completed under the NMS CBM Programme project R7 concluded that National Metrology Institutes (NMIs) in the Far East and Europe have well established programmes for the production and certification of these materials.

To protect UK organisations impacted by the ROHS/WEEE legislation, methodology has been developed for the accurate measurement of restricted substances in WEEE. Capability to make these measurements has been confirmed by successful participation in the CCQM-P106 study for Cd, Cr, Hg and Pb in polypropylene.

Cr (VI) is a key analyte in the legislation. Thus far the measurement focus has concentrated on total chromium. Published methods for determination of Cr (VI) in water matrices using Dionex columns containing both anionic and cationic groups which retain the Cr (VI) and Cr (III) are available. However the extraction of Cr (VI) from matrix materials presents a significant challenge and it is proposed to carry out a feasibility study

The feasibility of producing a reference material for VOCs in building products is also under investigation the NMS CBM Programme project R7, specifically issues affecting stability, homogeneity and shelf life. The outputs from this work will be used to develop a reference material representative of construction and/or vehicle products to support laboratories through validation of emissions testing procedures and for quality assurance in routine analysis.

Impact

The development and dissemination of traceable measurement methods for ROHS analytes will enable UK manufacturers and suppliers of electrical and electronic goods to demonstrate compliance with legislation that aims to protect human health and the environment.

Methods for Cr (VI), PBBs and PBDEs will, in particular, assist significantly in improving measurements in this technically complex and challenging area. The development of a traceable method for Cr(VI) determination will have a wider applicability to other sectors, e.g. environmental.

VOC emissions from building and vehicle materials impact on indoor air quality and potentially expose the total UK population to harmful substances. The development of a reference material for VOCs in building and/or vehicle products will provide, for the first time, a measure of traceability for laboratories in the these sectors when carrying out measurements of toxic VOCs in the built environment, helping to ensure exposure does not exceed regulatory limits.

Summary of Technical Work

Reference values for Pb, Cd, Hg, Cr, PBBs and PBDEs at, or near, the legislative level in plastics will be provided to UK organisation and the European Reference Material (ERM) co-operation partners to assist compliance with legislation and the production of certified reference materials. To demonstrate capability, the NMI will participate in relevant international inter-comparison studies for total metals and brominated flame retardants - the funding for these studies is provided for in the proposed International Metrology Measurements project.

A traceable measurement method for the determination of Cr (VI) in plastic materials will be developed and validated and Cr (VI) stability in these materials will be evaluated.

A certified reference material for one or two VOCs in a building or vehicle material will be prepared, subject to the outcome of a feasibility study project under the NMS CBM Programme.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW reference values in support of the ROHS directive to UK organisations for establishing compliance with the legislation and/or the ERM cooperation to support reference material production have been provided.			
2a	CEW the feasibility of extraction and measurement of Cr (VI) species in a plastic material has been assessed.			
2b	CEW an appropriate methodology for the determination of Cr (VI) species in plastics has been developed and validated and the stability of Cr (VI) in a candidate reference material evaluated. (Dependent on the outcome of 2a).			
3	CEW a building/vehicle CRM containing one or two VOCs has been produced and is available for sale. (Note: deliverable dependent on outcome of project R7 in current NMS CBM Programme).			
4	CEW knowledge transfer dissemination and project management completed.			
			Total cost	

OA5					
Project Author/ Contact	**Author: Milena Quaglia** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Evaluation of Matrix Suppression Effects using Rapid High Resolution Separation Systems and On-Line Sample Clean Up Strategies on Quantitative MS Detection

Project Objectives

To assess the effects on accurate quantification of LCMS(MS) technologies by:

- better understanding the influence of high resolution chromatographic stationary phase morphology on matrix suppression.
- investigating the importance of on-line sample cleanup strategies for the reduction of matrix suppression and improve accurate quantification.

Background and Rationale

The past decade has seen the evolution of tandem mass spectrometry (QqQ) as the preferred choice for trace level quantitative analysis for a number of application sectors. The initial capital expenditure is often offset by the sample throughput capabilities of such technologies. Initially the selectivity of the tandem MS experiment was used to justify the reduced chromatographic resolution. However, this loss of resolution had an impact on the accuracy of quantitative measurements due to the presence of other compounds in the source at the time the analyte was present (competing reactions or matrix suppression). This resulted in the increased use of isotopically-labelled internal standards (often at great expense) or excessively long chromatographic run times to combat these effects. In order to address this issue, whilst keeping sample throughput high, instrument manufacturers investigated the use of multiplexed chromatographic systems and, more recently, rapid high resolution chromatography. These rapid high resolution systems are now standard in most laboratories.

Ion suppression is a complex phenomenon which occurs in liquid chromatography mass spectrometry (LCMS) and adversely affects sensitivity, accuracy and precision of the analytical method. Over the past decade several studies have been performed to limit ion suppression generated by using conventional reverse phase LCMS systems. However this phenomenon can neither be predicted nor avoided. The recent launch on the market of a broad range of novel stationary phases (e.g. 1.7-2µm shell particle, hydrophilic interaction LC stationary phases) introduced additional chromatographic parameters which influence matrix suppression. The evaluation of the matrix effects generated by the use of these new column technologies has become critical due to their increased use in drug discovery and diagnosis, where high speed and high resolution are required.

Chromatographic separation and sample preparation have been considered key factors in the reduction of matrix effects. However, investigations into the influence of the stationary phase morphology (particle size and porosity) on matrix suppression effects have never been carried out. These studies are important to understand the chromatographic component of this phenomenon.

Preliminary experiments (Quaglia et al, PBA 2008) have shown a potential reduction of matrix suppression due to the minimized eddy and longitudinal diffusion generated using sub-2µ particulate packed-bed columns. However further investigations should be performed to better understand and predict this phenomenon.

Impact

Today's laboratories are under pressure to increase both routine and research sample throughput. Speed has become a premium value driver for all separation strategies, especially LC where the 'ultra fast' share of the current total LC market now exceeds 10%, with an annual growth rate of at least 20% expected in the future.

Challenges to early adoption of sub-2µ particulate columns for standardized protocols, particularly pharmaceutical methods based on United States Pharmacopeia (USP) monographs have already arisen. However, lack of knowledge of the influence of chromatographic parameters induced by using ultra-fast LC on matrix suppression could adversely affect sensitivity, accuracy and precision of analytical methods.

Matrix suppression effect studies will support the scientific community with investigations into the impact of novel analytical strategies on sensitivity, accuracy and precision of these approaches. A thorough scientific evaluation into the effects of rapid high resolution chromatography on accuracy, robustness of the published strategies (simple on line clean up technologies) for the reduction of adverse effects would be timely.

Summary of Technical Work

Selection of a range of columns packed with different stationary phase morphologies (e.g. particle size range, 1.7-10µm). Selection of a group of analytes and methods to be used for the evaluation of Van Deemter, Knox plots and matrix suppression effect studies. Evaluation of the chromatographic performance of the columns by ultra-fast LC UV detection and measurement of the influence of particle morphology on matrix suppression by LCMS. Comparison of matrix suppression effects obtained by using diverse on-line sample clean-up strategies, such as restricted access materials and turbulent flow chromatography.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW chromatographic performance of columns characterised by different particle size (range 1.7-10µm) and/or porosity has been evaluated.			
2	CEW matrix suppression effects obtained by analysing small molecules (e.g sudan dye, creatinine, malachite green, estrogens) with different particle morphology chromatographic columns have been measured. CEW these results have been compared with those from the chromatographic performance evaluation.			
3	CEW performance of on-line sample clean-up strategies, such as turbulent flow chromatography and restricted access materials, have been compared.			
4	CEW findings disseminated at appropriate conferences/ meetings and through at least 1 peer-review publication.			
			Total cost	

OA6					
Project Author Contact	**Author: Steve Wood** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Enthalpy of Fusion Reference Measurements and Materials - Feasibility Study

Project Objectives

To secure the long term availability of certified reference materials for the calibration of calorimeters.

The feasibility of establishing a UK adiabatic calorimetry capability for the calibration of the enthalpy of fusion of pure substance materials and production of enthalpy of fusion CRMs to a specification agreed with key industrial/pharmaceutical stakeholders will be completed. The study will identify supply routes, produce a design specification and detailed drawings with full costings, and schedule for production of an adiabatic calorimeter capable of achieving the required uncertainty of measurement for determination of enthalpy of fusion.

Background and Rationale

Differential scanning calorimetry (DSC) is one of the most widely used thermal analysis techniques for the characterization of solids. It enables thermal events such as melting, recrystallisation, decomposition, and glass transitions events to be monitored as a function of temperature giving valuable insight into solid form characteristics, mechanisms of polymorphic transformations, and solid-state degradation pathways.

For traceable measurements, DSC and other calorimetry instruments are calibrated using high purity materials, certified for enthalpy of fusion. Traditionally there have been very few producers of such materials (a search of the COMAR database of Certified Reference Materials, shows only the UK and the Chinese National Metrology Institute (NIM China) as having available materials. NIST also have a small number of certified enthalpy of fusion standards, but are withdrawing from this area as their technical expertise is lost through retirement. Such materials are generally very stable and current stocks were produced several years ago. However LGC stocks will not last indefinitely and provision is needed to develop the capability to replace these important materials when they sell out, in three to five years time.

The UK's range of enthalpy of fusion CRMs were characterised by an acknowledged world expert based at the University of Oslo, Norway, who has now retired. Full details of the approach used in the characterisation are available, but no working equipment is available.

Impact

Certified enthalpy of fusion standards are used by a wide variety of industries (including the major and generic pharmaceutical industry, the rubber industry and general scientific establishments) to calibrate thermal measurement equipment, including differential scanning calorimetry (DSC).

This feasibility study is a necessary first step to sustaining traceable DSC (and other forms of calorimetry), which is widely used in the pharmaceutical industry in product development, manufacture and product release.

The UK pharmaceutical market is highly fragmented: only one company has a ten per cent share, most of the rest are far smaller. Of the major medicines sold in the UK, around half were developed in British laboratories. In 2007, sales of prescription medicines to the NHS were £10.3 billion and pharmaceutical industry exports in were £14.6 billion, creating a trade surplus of £4.3 billion (source ABPI).

Summary of Technical Work

LGC, in collaboration with NPL, other technical experts, equipment suppliers and key customers/users, review enthalpy of fusion work previously carried out at University of Oslo; develop a specification for an adiabatic calorimeter capable of measuring enthalpy of fusion; identify suppliers capable of designing and building a calorimeter to the required design; prepare a validation plan for the system once built.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW a project working group comprising key stakeholders (technical experts, potential suppliers and customers) has been established.			

2	CEW a specification for enthalpy of fusion CRMs (e.g. spheres or discs, range of materials, uncertainty requirements) has been agreed.			
3	CEW a design specification and detailed drawings with full costings and schedule for the production of an adiabatic calorimeter capable of achieving the required uncertainty of measurement for determination of enthalpy of fusion is available and approved by stakeholders.			
4	CEW a full validation plan, with uncertainty budget, is available.			
5	CEW design specification and validation plan has been disseminated to key stakeholders (e.g. via the Thermal Methods Group of the Royal Society of Chemistry).			
			Total cost	

OA7					
Project Author/ Contact	**Author: Gill Holcombe** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Improved Certified Reference Materials for Food Analysis

Project Objectives

To investigate and develop appropriate packaging options to enable the production of 'wet' meat and fish reference materials containing analytes appropriate to the relevant legislation with the necessary product stability.

To develop a traceable measurement method for hydroxyproline in food based products and to produce corresponding certified reference materials and calibration standards.

Background and Rationale

It is important that analytical laboratories in the food sector have available certified reference materials, which closely match their routine samples in both analyte levels and matrix type. This was recognised in the widely cited, nine-sectored food triangle of The Association of Analytical Communities (AOAC) International, published in 1993, in which foods are positioned according to their fat, protein and carbohydrate content. The premise is that foods, and therefore reference materials, in a particular sector are representative of other foods within the same sector.

There is a paucity of materials in the area of analysis of meat and fish products even thought these are a very important area of nutritional and contaminant interest. Most currently available fish reference materials are freeze dried and are characterised for organic contaminants, with a smaller number characterised for elements. Two recently released 'wet' fish materials from NIST, characterised for organic analytes, proximates and a few elements are sold as frozen samples and must be kept at -80 °C during transport, storage and during sampling, making them expensive to distribute and difficult to work with (Most routine laboratories do not have -80 °C storage).

Improved production and packaging methods are required to provide true-to-life materials which can be transported at minimum cost, but still adequately represent materials under test. Any new materials must be sufficiently homogeneous and stable to ensure fit-for-purpose uncertainties (comprising homogeneity, stability and characterisation contributions) of the certified values. Previous wet fish reference materials (LGC 7101 mackerel paste and LGC 7160 crab paste), sealed in cans, had to be withdrawn from sale due to failure of the packaging. The only meat based material sold by NIST is canned.

Appropriate food reference materials are required to ensure food products in the UK meet the relevant legislative requirements, e.g. the levels of fat and (indirectly) hydroxyproline (an amino acid used an indicator of meat quality) in meat are regulated [Statutory Instrument 2003 No. 2075 (The Meat Products (England) Regulations 2003), EU Directive/13/EC for definitions of meat}.

Traceable measurement methods are not available for the main constituents of food materials and for some constituents traceability is not applicable, e.g. fat and dietary fibre because they are defined by the measurement procedure. However, hydroxyproline is a defined entity and traceability is possible. Two meat based reference materials characterised for hydroxyproline content are available (ERM-BB501a; 0.33 ± 0.03 g/100g and SMRD 2000-6; 0.133 ± 0.016 g/100g), although neither material is certified for hydroxyproline. In addition, it is known that SMRD 2000-6 will sell out within a few years, leaving a gap in the AOAC food triangle.

Certain metallic contaminants can cause a risk to public health either at low or high levels and consequently are regulated within the EU [Regulation (EC) 1881/2006 of December 2006 setting maximum levels for certain contaminants in foodstuffs, *Official Journal* **L 364**, 20/12/2006, 0005-0024].Traceable methods have been developed under previous CBM Programmes for Pb, Cd, Sn and Hg, and the procedures will be applied to the new fish and meat materials.

Impact

The CRMs will assist the UK analytical community by providing relevant materials for validation and ongoing performance checks of analytical methods to ensure food products in the UK meet the appropriate legislative requirements. This will, in turn, help to protect UK consumers from low quality and contaminated food products, improve the provision of information to consumers and dieticians by more accurate food labelling, assisting educated diet choices and underpin better nutrition, and consequently health, of the population as a whole.

Summary of Technical Work

Production and packaging options for 'wet' food products e.g. addition of stabilisers, cryogrinding, metallised pouches will be evaluated and compared against frozen and freeze dried product for stability. A traceable measurement method will be developed for hydroxyproline in food products and the production of appropriate calibration standards prepared and certified. One meat reference material in appropriate packaging will be prepared and certified for hydroxyproline and selected elements of contaminant interest, with additional characterisation through inter-laboratory studies. One fish reference material in appropriate packaging will be prepared and certified for selected elements of contaminant interest, with additional characterisation through inter-laboratory studies.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW investigation of packaging options for wet food matrices has been completed.			
2	CEW traceable measurement method/s has been developed for hydroxyproline in food products.			
3	CEW appropriate calibration standards have been produced and characterised.			
4	CEW a meat reference material for proximates, elements and hydroxyproline has been produced and characterised.			
5	CEW a fish reference material for proximates and elements has been produced and characterised.			
6	CEW an article on the production and characterisation methodology has been submitted to peer review publication.			
			Total cost	

OA8					
Project Author/ Contact	**Author: Steve Ellison** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Improved Characterisation and Estimation Methods for Proficiency Testing and Reference Material Certification

Project Objectives

- Implement and compare improved robust estimators for inter-laboratory studies, including improved methods for bimodal data and robust methods for handling data with uncertainties.
- Develop and assess robust methods for maximum likelihood estimation for data including non-detects or other censored values as well as extreme outliers.
- Review existing RM stability and homogeneity designs with a view to assessing their statistical power and make recommendations for improvement.
- Examine and develop methods for stability testing of reference materials for qualitative characteristics and for handling degradation in homogeneity.

Background and Rationale

Accreditation and QA for chemical and biological measurements rely heavily on inter-laboratory study, particularly proficiency tests, and on certified reference materials for validation of existing and novel measurement procedures. Most current interlaboratory studies (including PT and some RM production studies) use robust estimation methods originating in the 1970's and promoted in analytical chemistry in the mid 1980's. These methods have proven effective, but most either have comparatively poor statistical efficiency (and therefore large uncertainties) or have comparatively weaker resistance to outlying values. Further, there are essentially no authoritative treatments of the uncertainty associated with these estimators in chemical measurement applications. Only one method intended specifically to address robust estimation for multimodal chemical measurement data has been published, and there are no accepted methods for handling non-detects or censored values (e.g. "less than…" reports) in PT schemes other than recommendations for their removal – a practice which causes positive bias in consensus assigned values.

More recent statistical developments in the statistical community have, however, included the development of robust estimators with simultaneous high efficiency and outlier resistance, and several methods now exist for treating multimodal data, including (for example) kernel density estimation and maximum likelihood estimation. Some of these methods explicitly allow for reported uncertainties reported results. There is therefore scope to improve practice in interlaboratory studies in chemical and biological measurement by implementing, comparing and disseminating improved estimation methods applied directly to chemical and biological measurement problems.

Similarly, recent developments in statistical theory and methods have potential to improve practice in characterisation of reference materials. Homogeneity tests currently have low power and there is potential to improve power and reduce costs by combining stability and homogeneity testing into a single experiment, if appropriate designs can be developed. Developments in stability design and analysis are also needed; in particular, improved modelling methods for semiquantitative and qualitative data have potential applications in stability assessment. Current stability assessment methods rely on detection of approximately monotonic change in reference value with approximately constant variance. Real materials, however, often show other patterns of change, including increased probability of catastrophic failure or changes in homogeneity rather than mean value. Statistical methods based on survival analysis have the potential to provide a basis for assessing the significance of these types of change, and are additionally applicable to qualitative reference materials (such as microbial culture collections).

Impact

Demonstration of improved methods for estimation of consensus values in proficiency testing will provide reliable reference values in situations where current methods are biased, improving the early detection of measurement problems as well as increasing confidence in scoring methods.

Improving stability and homogeneity designs will facilitate development of reference materials in all chemical and biological measurement sectors, with consequent improvements in measurement quality.

Summary of Technical Work

The technical programme will:

- Assess the performance of new estimation methods for PT and make recommendations for their use.
- Develop new methods for handling censored and multimodal data in proficiency tests.
- Examine methods for stability testing for reference materials, including qualitative materials that accommodate changes in homogeneity or changes in probability of failure.
- Develop a methodology for combining stability testing and homogeneity testing to improve the reliability of both.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW a study of new robust estimators and estimators for multimodal data has been published, with authoritative recommendations for their use.			
2	CEW a study of methods for incorporating the treatment of censored (“less than” and semiquantitative) data in proficiency testing has been published.			
3	CEW recommendations published for combined stability and homogeneity tests			
4	CEW a study has been completed on the feasibility of applying survival analysis and related techniques to reference material stability testing.			
			Total cost	

OA9					
Project Author/ Contact	**Author: Zoe Hall** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

International Harmonisation of Reference Measurements for Malachite Green

Project Objectives

This project will harmonise global measurements for a priority food contaminant. In an effort to realise this aim, the following will be investigated:

- Production of a suitable material, with an appropriate homogeneity and stability, for use in an international inter-comparison.
- Co-ordination of, and participation, in a CCQM inter-comparison.
- Production of a certified reference material (CRM) for malachite green (MG) in fish.

Background and Rationale

Malachite green (MG) has been used in the fish farm industry as a parasiticide and antifungal agent. However discovery of the wide range of toxicological effects (including potential carcinogenesis) caused by MG, and its major metabolite leucomalachite green (LMG), has resulted in its declined use. The commercial use of MG has been banned in the EU since 2002 with the minimum required performance limit (MRPL) for laboratories carrying out surveillance for these compounds being 2µg kg^{-1} for the sum of MG and LMG.

Previously, a method has been developed to accurately measure MG in salmon by exact matching isotope dilution mass spectrometry (IDMS). This method has been applied successfully to determine the total MG content in salmon tissue as part of a pilot CCQM inter-comparison. During this exercise it was noted that a major limitation was the homogeneity of a 'real' food-like material (i.e. not lyophilised), particularly with respect to LMG. Improvements in material homogeneity, stability and transportation will be of key importance for the development of a successful CRM.

The determination of MG in salmon tissue is a complex analysis involving the extraction of trace levels of potentially unstable analytes from a solid matrix. Success in a follow-up CCQM exercise would be indicative of a laboratories' ability to measure µg kg^{-1} levels of medium to high polarity residues in fish tissue. It would also demonstrate a high level of analytical capability in extraction from a solid matrix and the ability to measure inter-converting and unstable analytes.

Improvements to the method used to assign the value to the CRM should be focussed on increasing the extraction efficiency of MG without significant losses; current in-house methodology employs a 16h extraction time. Analyte instability precludes the use of harsh conditions such as high temperatures and extreme pH; advantages may be gained from enzymatic and/or microwave digestion or the use of radical scavengers which can act as stabilisers.

Impact

The UK currently has in excess of 1200 fish farms producing some 180K tonnes of salmon and trout. This generates exports >£400M p.a. for Scotland alone, while the UK's total fish exports are worth >£1Bn p.a. The confirmed presence of MG in Canadian fish (from an unknown source) bound for the US resulted in the suspension of all operations on the contaminated farm. With aquaculture trade increasing and the development of a truly global market, consistency in the testing of banned substances will be essential in the adjudication of trade disputes. The availability of a CRM for malachite green in fish will underpin measurements made by regulatory laboratories by acting as a quality control to validate testing laboratories' methodology and thereby allowing increased confidence in data and enforcement of any infringements. The analysis of MG in fish tissue is known to be challenging, primarily due to issues surrounding extraction and analyte stability. From a metrological point of view, the successful completion of a CCQM study would demonstrate a high level of analytical capability in participating laboratories.

Summary of Technical Work

The technical work first requires the procurement of a suitable matrix material with naturally occurring MG and its metabolites. Due to difficulties in dosing the fish, this will require blending with blank fish, to obtain a residue

concentration at/or close to the MRPL. This mixing process requires the candidate material to be homogenised carefully ensuring limited degradation and inter-conversion of the MG species. Stability studies, which will include stability whilst in transit and shipping of the material, will also be crucial.

The previous developed method will be refined for use with this specific material. Small changes in lipid and water content were seen to effect extraction conditions in these previous studies. Having access to the same blank fish that was used to produce the CRM will enable spiking experiments to be performed to fully validate the equilibration of the labelled material, which is essential for the high accuracy IDMS procedure used.

Finally the material will be used in a CCQM inter-comparison which has already been approved by the Organic Analysis Working Group of CCQM. The developed method will be used for the UK's participation in such a study.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW a suitable material has been procured and prepared; this includes investigation to improve homogeneity (e.g. freeze-shattering under liquid nitrogen or blending with liquid CO_2) and transportation.			
2	CEW extraction methodology that limits the inter-conversion of species within the selected sample/matrix has been developed.			
3	CEW homogeneity and stability testing, and value assignment of the candidate CRM have been completed.			
4	CEW knowledge transfer activities completed and CCQM study co-ordinated and reported (see CCQM project).			
			Total cost	

OA10					
Project Author/ Contact	**Author: Chris Hopley** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Feasibility Study for Traceable Methods to Assign Polyfluorinated Compound and Perfluorinated Persistent Organic Pollutant CRMs

Project Objectives

To assess the issues associated with the measurement of polyfluorinated compounds in environmental samples and investigate the requirements of industry for perfluorinated persistent organic pollutant certified reference materials.

Background and Rationale

Perflourinated compounds, salts of which are used widely as surfactants, are exceptionally stable in the environment and, as a result, their historical use means that their fate and exposure to humans is of increasing concern. Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), in particular, have been classified as persistent organic pollutants (POPs). PFOS was detected in the bodies of almost all people tested for it in a study by the U.S. Government. Other studies have found that animals exposed to PFOS, in levels comparable to those commonly seen in humans, had altered immune functionality or were even toxic.

EU regulations on PFOS come into effect in 2008, setting a limit of 0.005 %/m of PFOS in substances and preparations. Currently, analysis is performed using LC-MS/MS. However, a number of such compounds, including PFHxA, PFHpA, PFOA, PFNA, PFDeA, PFDoA, PFBSH, PFHxSH, PFOS and PFOSA, are posing measurement challenges. Good chromatography is required coupled with highly specific mass spectrometry detection (trace level presence in complex matrices, e.g. waste water and food), the stability of the compounds often causes issues (instrumental carryover effects) and high blank levels are seen (fluorinated polymers often used in the manufacture of analytical instrumentation).

The provision of traceable methods for analysis of these compounds in environmental/industrial samples is therefore a high priority. Through consultation with industry and testing laboratories, the feasibility for production of relevant certified reference materials (CRMs) is proposed.

Impact

Polyfluorinated compounds are being recognised as a new persistent organic pollutant (POP) due to their exceptional stability in the environment. They have been found worldwide at low levels, and measurement tools are needed to support monitoring and control measures. The production of perfluorinated CRMs in consultation with industry and testing laboratories would be timely; PFOS being the priority in terms of controlling known risks to human health and enforcing related regulation. The new materials would be instrumental in ascertaining the competence of routine testing laboratories, providing confidence in analytical measurements used to support legislation and enabling innovative approaches to improve the analysis of these compounds.

Summary of Technical Work

The PNECs for freshwater and marine water are 25 µg/l and 2.5 µg/l respectively and represent the minimum detection limits that must be achieved for the measurement of PFOS.

Initial work would focus on the assessment of background level contamination issues and ‘standard practice’ instrumentation for measurement of PFOS in aqueous samples. The feasibility for use of isotope dilution mass spectrometry (IDMS) will be established; PFOS is mass deficient due to its fluorine content but very stable and MS fragmentation is difficult. Sample preparation options such as online sampling or sample concentration will be investigated. Recommendations for a PFOS RM will be made.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW instrumentation (LCMS, LC/LCMS, GCMS) assessed which enables measurement of PFOS in aqueous samples at detection limit, without interference. CEW use of TOF for IDMS assessed.			

2	CEW background levels of PFOS contamination in laboratory detected and levels required for a RM determined.			
3	CEW possible sample preparation options assessed for a potential PFOS RM (waste water/spiked water/concentrated sample).			
4	CEW knowledge transfer achieved through publication of project findings in a suitable scientific literature format available to related stakeholders.			
			Total cost	

OA11					
Project Author/ Contact	**Author: Thierry Le Goff** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Improved Traceability of Pure Certified Reference Materials (CRMs)

Project Objectives

The main objectives of this project are:

- Production of certified ultra pure standards by semi-preparative and their subsequent use to characterise larger batches of the same measurand (solids and solutions of key analytes).
- Extend the number of traditional analytical techniques to determine organic purity of a compound e.g. the development of method to assess purity by thermogravimetric analysis - mass spectrometry (TGA-MS). The potential of TGA-MS to determine moisture, volatiles (residual solvents) and inorganic residue will be investigated and the use of new detectors for HPLC especially FID (Cambridge Scientific Instrument).
- Comparison studies for key purity parameters i.e.
    - Bilateral study of specific moisture determination by oven coulometric Karl Fischer and ambient coulometric Karl Fisher (NMIA) using existing pure CRMs.
    - Comparison study of inorganic residue determination by ICP-MS, ICP-OES, ashing and TGA using existing CRMs.
    - Influence of parameters such as moisture, inorganic content, residual solvents and pans on DSC measurements.

Background and Rationale

Purity of an organic substance can be defined as the mass fraction of the substance under investigation in a given sample. This cannot be determined directly and traceability of the purity value of an organic substance is complex. Traditionally, organic purity values are determined using analytical techniques such as high performance liquid chromatography (HPLC), gas chromatography (GC) or differential scanning calorimetry (DSC) and subsequent correction for the key purity parameters of water content, inorganic residue and residual solvents. However, HPLC, GC and DSC are secondary methods and are not directly traceable to SI units unless a pure CRM of the measurand is used. These CRMs are, in particular, prone to problems including:

- Non-detectable organic impurities (UV)
- Co-elution (HPLC and GC)
- On column degradation (GC and HPLC)
- Impurity response factors (UV, MS, ELSD and FID) relative to main compound significantly different from 1
- Thermal instability or non-volatility (DSC and GC) of the compound of interest
- Impurities not forming a solution in the melt of the main compound (DSC)

Due to these limitations the mean of the purity values determined by the traditional analytical methods is used to calculate the organic purity. However, some compounds are only amenable to analysis by one technique increasing the complexity of the purity assessment [Le Goff and Wood, ABC, 2008a] and the uncertainty of the determined purity value, a drawback for high accuracy measurements.

Earlier this year, the Organic Analysis Working Group (OAWG) of CCQM stressed the importance of the purity assessment of all calibrants used for high accuracy measurements to ensure traceability of results. A semi-preparative HPLC approach based upon the recovery of the pure fraction of the measurand (mass) from an injected sample (mass) was developed successfully for the determination of the purity of organic compounds in the range from 95-99 % m/m [Le Goff and Wood, ABC, 2008b] and is directly traceable to SI units ensuring traceability. There were additional indications that the pure substance could also be used as a calibrant to determine the purity of an unknown sample with an acceptable uncertainty (U = 0.7 % m/m k = 2). The proposed project will develop this preliminary observation to produce ultra pure substances by semi-preparative HPLC with full purity assessments and demonstrate their use as calibrants to characterise larger batches of the same measurand, thereby improving traceability of the produced standards.

Impact

This project delivers, for the first time, the CCQM OAWG traceability requirements for full purity assessment of pure substances/calibrants by developing new and existing capabilities within the calibration laboratory of the UK NMI for chemical and biochemical analysis. Then improved capabilities will be demonstrated by applying them to the high accuracy measurement of novel, difficult to produce, pure CRMs, e.g. amino acids and steroids.

This project will upgrade core UK NMI capability (and, through CCQM, extend and strengthen the global metrological resource for organic chemical analysis, with wide ranging impacts on food chain, healthcare and environmental protection.

Summary of Technical Work

- Semi-preparative HPLC purification (method development), purity assessment of ultra pure standards (organic purity, moisture, inorganic content and residual solvents) and direct assay by HPLC using purified standards.
- Specification, evaluation, development and validation of TGA-MS instrumentation.
- Feasibility study on the use of HPLC-FID (prototype) for purity assessment.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW ultra pure substances produced by semi-preparative HPLC have been used to certify larger batches of the same measurand (solids and solutions).			
2	CEW a method to assess purity by TGA-MS has been developed and TGA-MS suitability assessed for determination of moisture, volatiles (residual solvents) and inorganic residues.			
3	CEW the applicability of new detectors for HPLC (FID) has been demonstrated.			
4	CEW the influence of parameters such as moisture, inorganic content and residual solvents on DSC measurements have been assessed.			
5	CEW a comparison study of inorganic residue determination by ICP-MS, ICP-OES, ashing and TGA has been completed.			
6	CEW a bilateral study of specific moisture determination by oven coulometric Karl Fischer and ambient coulometric Karl Fisher (NMIA) has been completed.			
7	CEW knowledge transfer to OAWG CCQM has been completed.			
			Total cost	

OA12					
Project Author/ Contact	**Author: Gill Holcombe** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Reference Material Certification and Support

Project Objectives

To develop and validate improved procedures for stability assessment, stability uncertainty evaluation and on-going monitoring of reference materials.

To provide advice, support and an update service to customers on the use of NMS CBM reference materials in accord with the requirements of ISO Guide 34.

Background and Rationale

ISO Guide 34 (General requirements for the competency of reference material producers) requires reference material producers to provide a post distribution service to the customers and users of their materials. This includes on-going monitoring of the stability of reference materials and the provision of guidance and technical services.

Stability testing, the evaluation of the uncertainty of stability measurements and their contribution to the total uncertainty of a certified reference material are critical activities in the certification and on-going monitoring of the majority of reference materials. The exceptions are those which, by their nature, are stable such as metals and metal alloys certified for elemental content. The publication of the 3rd edition of ISO Guide 35 (Reference materials, general and statistical principles for certification) in 2007 and the increase in the number of reference material producers accredited to ISO Guide 34, has given greater significance to stability testing and the associated uncertainty of such measurements. One widespread approach to stability testing and uncertainty determination is the so called 'isochronous' method. Here a test scheme is established with samples stored at a number of different conditions for different lengths of time. Once samples have been exposed to the test conditions for the appropriate period they are moved to a sufficiently low temperature such that any degradation is slowed to a negligible rate. Typically the temperature used to 'freeze' any degradation is –80 °C and a number of samples are stored at this temperature to act as reference points against which the test samples are compared. When all storage conditions and times have been completed all samples (including a reference sample) are removed from storage and analysed at the same time, i.e. isochronously.

However this is not an approach which can be applied to all materials, e.g. some clinical materials will be damaged if stored below 0 °C. In addition the effect of changing the phase of many materials is not known.

A systematic study of the stabilities of a variety of materials using a number of stability test regimes is needed to provide information to develop practical stability testing protocols and uncertainty assessment methodologies for all reference material producers.

Impact

Stability testing and uncertainty assessment impact all reference material producers and particularly those accredited to the requirements of ISO Guide 34. It is anticipated that the outputs from this project will be disseminated to the reference material producer and user communities through a series of presentations and publications, and become accepted by the ISO committee for reference materials (REMCO) for inclusion in any further revision of ISO Guide 35.

Summary of Technical Work

A number of stability test schemes will be established for a range of NMS CBM reference materials which are not suitable for monitoring using the conventional isochronous approach. Materials to be tested will include clinical materials (e.g. testosterone in serum and digoxin in serum), forensic alcohol and reference spirits, kava kava, platinum group metals in autocatalyst and biological reference materials such as glycans, using appropriate, fit-for-purpose measurement methods with low uncertainties. Work will be carried out in collaboration with UKAS, members of the NMS CBM UK Reference Materials Working Group, particularly those accredited to ISO Guide 34, and European Reference Material co-operation partners (BAM and IRMM).

Deliverables				
No.	**Deliverable**	**Start**	**End**	**Cost**
1	CEW improved procedures for stability assessment, stability uncertainty evaluation and on-going monitoring of reference materials have been developed, validated and disseminated.			
2	CEW advice, support and technical services has been provided to customers and users of NMS CBM reference materials.			
			Total cost	

OA13					
Project Author/ Contact	**Author: Chris Hopley** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Traceable Values for Dioxin-like PCBs

Project Objectives

To develop the capability for the traceable analysis of Dioxin-like Polychlorinated Bi-Phenyls (PCBs).

To develop and validate methods capable of independently measuring the 12 dioxin-like PCBs in a variety of matrices traceable to the SI.

Background and Rationale

PCBs are known persistent organic pollutants and have been analysed for many years. However most work has been based on standard PCB markers (Arcolors/Clophen mixes or the Dutch seven), selected to indicate the presence of PCBs in general with a distribution of retention times across a standard gas chromatogram.

Recent studies have shown that a certain number of specific congeners (12), known as Dioxin-like PCBs, have high toxic equivalency factors of an order of magnitude more than most PCBs. Dioxin-like PCBs are found in soils, sediments and fatty food products and exhibit similar toxicity to Dioxins due to the unique structure of the 12 PCB congeners. The ingestion of these 12 PCBs from the environment account for at least 1/3 of the daily intake to humans of toxic Dioxins/Furans/Dioxin-like PCBs and there is evidence of causal developmental effects in the growing foetus. New approaches are required to measure the lower (than traditional) concentrations of these congeners with low uncertainties, both in terms of maximising sensitivity and optimising separation to enable interference free measurements of the individual congeners.

3 of the 12 Dioxin-like PCBs have been certified under previous NMS Programmes and are available as ERM materials. Methods and procedures have been developed over a number of years to enable these measurements to be carried out, e.g. dealing with dirty matrices (sewage sludge), addressing separation issues (custom capillary columns/multi-dimensional separation) and optimising measurement/detection (comparison of sector/bench-top quad/TOF). However the current methods would have to be further refined to cope with the low levels and alternative congeners of the Dioxin-like PCBs, to attain low level traceable measurements.

The measurement of Dioxins has been a priority for a number of years. However PCBs were only measured as a total indication of PCB contamination and Dioxin-like PCBs are not measured as a matter of course, as they are generally at lower concentrations than other indicator PCBs routinely monitored. However, recently the EU revised the limits and action levels incorporating Dioxin-like PCBs with the aim of removing the most contaminated foods from the market and identifying possible problem areas, thereby reducing dietary exposure.

With improvements in separation and sample preparation the development of accurate traceable measurements for these compounds is a priority, to better understand the exposure routes and contamination of the Dioxin-like PCBs in environmental materials and food and feed products.

Impact

The development of a measurement capability, built on previous NMS work, and the availability of reference materials with traceable certified values for the Dioxin-like PCBs will help monitoring & enforcement of the recently introduced (2006) limits for total dioxins and dioxin-like PCBs in any food or feed marketed in the EU and help environmental monitoring certification schemes (such as MCERTS) establish realistic limits for these materials in soils and other matrices.

Summary of Technical Work

Appropriate quantities of two or three additional pure dioxin-like PCBs will be procured and characterised for purity using well developed in-house methodologies. CRMs will be produced and made available for sale where the quantities of material available permit.

A range of analytical approaches for the certification of the Dioxin-like PCBs in matrix materials will be investigated, e.g. extraction of the analytes using normal phase HPLC clean-up methodology using hypercarb columns to separate the Dioxin-like PCBs and various GC-MS strategies for quantitation (multi-D GC, accurate mass and MS/MS). The most promising approaches will be developed and validated for the traceable quantitation of the dioxin-like PCBs in soil and/or sewage sludge matrices.

Deliverables				
No.	**Deliverable**	**Start**	**End**	**Cost**
1	CEW additional pure dioxin-like PCBs have been procured and characterised and one or two made available for sale, where quantities permit.			
2	CEW separation and isolation/clean-up methodologies for dioxin like PCBs have been developed and validated			
3	CEW the impact of advanced GC MS strategies (Multi-D GC, accurate mass and MS/MS) for the analysis of Dioxin-like PCBs have been investigated.			
4	CEW traceable values for priority Dioxin-like PCBs have been assigned to PT scheme provider.			
5	CEW an article has been submitted for publication in a peer reviewed journal.			
			Total cost	

S1 Surface and Nanoanalysis of Micro and Nanoparticles for Innovation and Environment Health and Safety

Contact michael.adeogun@npl.co.uk

Project Objectives

This major multi-technique project will develop the key metrology for the chemical analysis of micro- and nanoparticles:

- To develop a practical mounting method for micro- and nanoparticles, from 10 nm diameter to PM10, suitable for use in a range of techniques including AES, XPS, SIMS and AFM.
- To develop the essential underpinning metrology and provide a practical procedure for analysts for the chemical characterisation of micro and nanoparticles using surface-analysis techniques
- To evaluate the measurement capability of the high-resolution imaging techniques for the chemical characterisation of nanoparticles, such as TEM+EELS, 3D Atom Probe and NanoPIXE, and the metrology needs.

Background and Rationale

Nanoparticles are front-runner nanotechnologies key to high innovation products such as biodiagnostics, drug delivery, medical imaging (contrast agents), cosmetics and sunscreens through to catalysts. Certainly, for many of these applications, it is clear that the surface chemistry of the particles is a critical parameter for innovation. For instance, for manufactured nanoparticles, characterisation of surface chemistry in terms of coating layers and contamination, are essential. The nanotechnology field is growing strongly and it is estimated that by 2015 products incorporating nanotechnology will contribute $1000 billion to the global economy [EPSRC Nanotechnology Strategy Group, 2006] of which nanoparticles will have a major share. Concomitantly with this economic importance, there is increasing concern over the potential health risks of nanomaterials. The international toxicology community is making progress in identifying which parameters of nanoparticles have the strongest effect on mammalian cell toxicity. In general, three key parameters have been identified that can give reliable predications of toxicity; particle size distribution (also aspect ratio), specific particle surface area and surface chemistry/charge. The characterisation of the surface and bulk chemistry of nanoparticles is therefore an underpinning requirement for innovation and determining the environment, health and safety aspects. Recent key reports—by the Defra-funded REFNANO project, the Nanotechnologies Research Coordination Group (NRCG) and the OECD (Organisation for Economic Cooperation and Development) Working Party on Manufactured Nanomaterials—have highlighted the need for the characterisation of nanoparticle surface chemistry. In particular, the OECD, through the PROSPEcT project, has tasked UK researchers, including NPL, with the characterisation of zinc oxide and cerium oxide nanoparticles. Consultation with industry has also shown that there is a strong need for nanoparticle characterisation on surfaces, including their dispersion and surface chemistry. The need for nanometrology of chemical properties is also reinforced in a recent statement by the UK Government [Statement by the UK Government about Nanotechnologies, March 2008].

This multi-technique project is proposed to develop the essential underpinning metrology for the chemical characterisation of nanoparticle to PM10 particles that are relevant to further innovation and toxicology studies. However, to meet these needs is currently a major challenge and no robust methodologies exist for valid measurements. To ensure the effective use of surface chemical techniques for nanoparticle characterisation, it is necessary to develop new approaches and standardised methods for sample preparation and analysis, and improve our understanding of the fundamentals that differentiate nanoparticle analysis from bulk analysis. For example, SIMS is capable of providing the sensitivity and nanometre depth resolution to give rich chemical information and even compositional depth profiles of nanoparticles. This has been anecdotally demonstrated, but a systematic study is needed to produce a reliable and validated procedure for analysts. Finally, it is essential to adapt a multi-technique approach in the characterisation of nanoparticles to achieve a complete understanding of their structure and chemistry. High resolution imaging techniques, such as STEM+EELS, 3D Atom Probe and NanoPIXE, have been demonstrated but there is a requirement to review the state of the art and metrology requirements of these techniques: For example the significance of beam damage.

Impact

Strong regulatory drivers, increased calls for standardisation by ISO TC201 and ISO TC229 for the measurement and characterisation of the surface chemistry of nanoparticles, and the need for reference measurements of nanoparticle surface chemistry for toxicology studies will play a key role in determining the future use of nanomaterials in various applications, and their economical and quality of life impacts. To meet these needs, this project will develop underpinning nanometrology, characterisation and standardisation for nanoparticles. The metrology will also be extended to industrially relevant particles up to PM10, which are of interest to the environmental and toxicological communities.

This project's development of the underpinning metrology and characterisation of nanoparticles will certainly be of importance to the development of nanotechnology standards and regulations, in general. Further, a better understanding of engineered nanoparticles' surfaces, including their dispersion and surface chemistry together with compositional depth profiles of multi-layered nanoparticles, will be highly important in the development of innovative high added value products in healthcare, energy, personal care, and transportation applications. This will have high impact across the gamut of industries and major companies involved in using nanomaterials—including AstraZeneca, GSK, Johnson & Johnson, Unilever, Proctor & Gamble, 3M, Abbott, Inverness Medical, Kodak, GE Healthcare, SkyePharma, Oxonica, L'Oreal, PowderJect and Johnson Matthey—and therefore, the UK economy. Therefore, this work will improve the knowledge base of nanomaterials, thereby reducing health risks and facilitating the safe introduction of new and improved products to the market place.

Summary of Technical Work

In this project, a method and procedure will be developed for the mounting of micro and nanoparticles for analysis by SIMS, XPS and AFM, as well as FIB sectioning methods for preparing samples for STEM+EELS. It is currently very difficult to immobilise particles onto surfaces without introducing contamination. Improperly mounted particles can cause severe problems in analysis and damage to instrument, for example, if they are not sufficiently bound and are subsequently attracted into the analyser.

A fundamental study will be carried out to evaluate the sputtering yield of model nanoparticles for both atomic and cluster primary beams. The sputtering yield is the principal fundamental parameter in SIMS analysis, but this is expected to be significantly different for nanoparticles compared to bulk material because the dimension of nanoparticles is similar to the size of the SIMS collision cascade (excited volume of material) and the larger available surface area for emission. Currently, there are no reliable data available to analysts to relate the sputtering yield of nanoparticles to that of the bulk material.

A method and procedure will be developed for the practical SIMS compositional depth profiling of inorganic nanoparticles, using model materials with different core and surface chemistries. This work will utilise the reference data for sputtering yields, allowing depth resolution, detection limits and practical guidance to be given to analysts. In addition, we will establish capability in high-resolution nanoparticle imaging techniques. These methods will be used to study nanoparticles of key interest to environmental health and safety community (such as ZnO and CeO_2) and nanoparticles for diagnostics and contrast agents.

Deliverables

No.	Deliverable
1	Development of methods to mount sub-micron particles and nanoparticles for AES, XPS, SIMS and AFM analysis.
2	Sputtering yields of nanoparticles compared with bulk values – development of theory and experimental data.
3	Development of methods and procedures for the compositional depth profiles of inorganic nanoparticles.
4	Evaluation of high-resolution imaging techniques for the chemical characterisation of nanoparticles.
5	Knowledge transfer including provision of expert advice to UK industry and academia, publications, presentations at meetings, the production of reference data on the website and key input into BSI CII/60 and ISO TC201 and TC 229.

S2 Multivariate methods for identification, classification and quantification in spectroscopy and imaging

Contact michael.adeogun@npl.co.uk

Project Objectives

- To develop a method for the predictive correlation of surface chemical spectra with surface properties, and validate with an industrially relevant problem.
- To establish rapid, robust analysis of surface spectra and images, using multivariate and informatics methods, for the identification of sample chemistry, and the classification and discrimination of chemically similar materials.
- To disseminate results and provide simple guidance to analysts on the application of multivariate methods, and to encourage the uptake of these methods in industry.

Background and Rationale

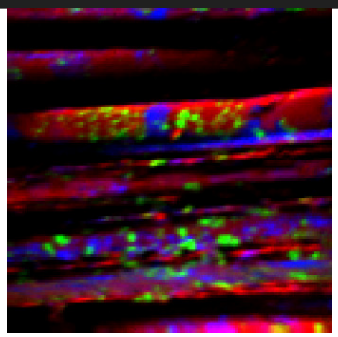


Fig 1. Multivariate analysis of hair fibres – rapid localisation of conditioning agents

- Molecular surfaces are key to many high added value and innovative products, for example the complex formulations used in personal care products shown distributed on hair fibres in Fig 1 [the UK personal care market is worth over £10 billion]. Consequently, there is strong demand for analytical methods that can identify and quantify organics at surfaces and relate this to product performance. The InsightFaraday report "Technology Roadmap for High Throughput Technologies" identifies the benefits of rapid data handling and interpretation to R&D sectors involving complex multi-component product formulations, including personal care products, cosmetics, household products, coatings and inks.

- In many emerging technologies, the correct design of surface chemistry is critical to the functionality of the material, such as surface wettability and biological response. Secondary Ion Mass Spectrometry (SIMS) is a powerful and versatile surface analytical technique, capable of generating spectra, images and depth profiles containing rich molecular information. It is a powerful technique for the identification and quantification of complex molecules at surfaces and interfaces. In particular, with modern cluster ion beam sources, the technique enables molecular imaging at spatial resolutions down to 200 nm with 3D profiling. This results in very large hyperspectral data sets with millions of data points that can only effectively be analysed using multivariate methods.

- The increased power and throughout of modern SIMS instruments now means that the bottle-neck is now the analysis time rather than the acquisition time. Together with the increasing chemical complexity of the real-life samples, this necessitates the implementation of new data-analysis methodologies, which are capable of exploiting the wealth of information obtained from SIMS in a robust and speedy manner.

- Consultation with industry experts has highlighted the urgent need for reliable multivariate analysis methods and guidance on statistically valid data analysis. This project was the highest-ranking area in the industry consultation (SAMS) workshop [SAMS Workshop, NPL Report October 2008].

- Work in the previous NMS programme has established an international track record for NPL in this important multivariate analysis area. An NPL guide on multivariate analysis has been downloaded over 5000 times and a recent published review of the area was cited 10 times within the first year of publication.

- This project has very strong opportunities for collaborative work with industry driven by their needs to understand complex systems. Collaborations to use multivariate analysis are already in place with Cambridge Display Technology (CDT) on molecular surface patterning and Unilever on the analysis of fibres.

Impact

This project will develop and validate novel data handling, processing and multivariate analyses to industrially relevant spectra and hyperspectral images in order to enhance the application of such methods to cope with complex 'real-world' data and provide statistically robust insight into the key surface chemistries. Direct impact to industries include understanding and controlling surface chemical patterning in the organic electronic and ink-jet printing industries, mapping the molecular distribution in diagnostic arrays, understanding the bioactivity of devices in terms of the surface chemistry and biological effect – for example, how changing the surface polymer composition can affect anti-fouling properties and characterising the distribution of molecules on fibres such as hair and cotton from complex personal care formulations.

This project will also impact strongly on UK-based analytical services laboratories (including CSMA Ltd., Intertek MSG, Evans Analytical Group, Molecular Profiles Ltd., Loughborough Surface Analysis Ltd.) by facilitating the analysis of increasing complex samples from customers and faster analysis that provides higher confidence and statistical support for data interpretation. These companies provide essential measurements to UK high-innovation companies. The reduction in analytical turnaround time will lead to improved cost effectiveness to industry customers, enabling the wider uptake of surface chemical analysis using SIMS to aid product innovation, process optimisation and failure analysis.

This project has strong potential for collaboration; co-funding is already available through the Technology Programme SCOPE project (Surface Conditioning of Plastic Electronics) with CDT, University of Liverpool and University of Cambridge to correlate surface chemical spectra with wettability for plastic electronics materials using multivariate methods. Pharmaceutical companies and the University of Nottingham are keen to collaborate with NPL for the analysis of high throughput screening methods for the identification of novel polymeric materials that mitigate the growth of biofilms. We will maximise impact through co-organising, with Prof B Tyler (USA), the 59th IUVSTA workshop on "Surface Chemical Analysis – Improving Data Interpretation by Multivariate and Informatics Techniques". This will have major international impact, bringing together experts, industry analysts and chemometricians to coordinate scientific debate about the latest advances and disseminate best practice for the use of multivariate analysis in surface chemical analysis. We have a strong track record of impact in this area through tutorials, papers, book chapters and we will increase this through inward secondments from industry.

Summary of Technical Work

In this work, a clear framework will be established for industry analysts on the robust application of quantitative and predictive multivariate methods such as partial least squares (PLS). Recommendations will be given for robust training and calibration of the model, using appropriate cross-validation and validation procedures, and effective data scaling. We will also use statistical expertise from the NMS Software Support for Metrology programme. The applicability of models to predicting new data, and the associated predictive errors will be studied and clarified. In addition, methods and procedures will be developed for effective comparison and discrimination of multiple spectra using multivariate analysis, principal component analysis (PCA) and discriminant function analysis (DFA). Additionally, resolution of depth profiles using MCR, taking into account detector linearity and effect of normalisation, will be defined. Finally, quantitative analysis strategies will be developed for the rapid analysis of high-resolution, hyperspectral SIMS images, including the use of multivariate analysis to identify regions of interest and extract maximum chemical information, and methods to reduce the effects of topography during data processing to obtain more accurate and quantitative results. For all methods, clear guidance on the most suitable methodology and data scaling will be provided and incorporated into the "Practical Chemometrics Guide" available on the NPL website.

Deliverables

No.	Deliverable
1	Development of a methodology for the quantification and correlation of surface chemical spectra with surface properties.
2	Validation of methodology on an industrially relevant problem.
3	Development of a method for rapid comparison and discrimination of SIMS spectra.
4	Development of quantitative analysis strategies for complex hyperspectral SIMS images.
5	Knowledge transfer including provision of expert advice to UK industry and academia, publications, presentation at meetings, key input into BSI CII/60 and ISO TC201, development of Practical Chemometrics Guide on the website and co-chairing 59th IUVSTA Workshop on Multivariate and Informatics Techniques.

S3 G-Tip Innovation and Cluster Ion Metrology

Contact michael.adeogun@npl.co.uk

Project Objectives

- To develop the G-Tip innovation to maximise uptake and impact for analysts and to broaden the instrument base that the G-Tip is available to.
- To develop G-SIMS & SMILES for pharmaceuticals and biologically relevant materials. Allowing convenient and specific identification of molecules through molecular structure.
- Develop a data and model to characterise the fragmentation of organic molecules by cluster Ion beams

Background and Rationale

Innovation is key to the "race to the top" for UK competitiveness in the global economy [The Race to the Top, A Review of Government's Science and Innovation Policies "Sainsbury Review", 2007]. The Department of Innovation Universities and Skills (DIUS) are committed to developing the UK as the "Innovation Nation". *The UK can only prosper in a globalised economy through innovation and high-added value businesses* [DIUS Innovation Nation 2008].

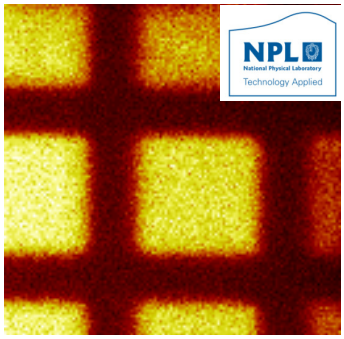


Fig 1. Imaging G-SIMS using G-Tip technology

G-SIMS (gentle-SIMS) is one example of NMS award winning innovation. G-SIMS is a powerful method that considerably simplifies complex static secondary ion mass spectrometry (SSIMS) analysis of organics at surfaces. However, a barrier to the wider uptake of G-SIMS into the community is the requirement for two ion beams producing suitably different fragmentation conditions and the need for their spatial registration (spatial alignment) at the surface, which is especially important for heterogeneous samples. Recently, a novel bismuth-manganese emitter, known as the G-Tip, has been developed in the current programme that is used with the popular liquid metal ion sources. This is a very powerful new development that makes G-SIMS technology available to a much larger user community allowing the NMS outputs to be exploited with increased impact (the first two SSIMS instruments have just been shipped with the G-Tip technology included). (The G-Tip is part of the NPL Technology Applied scheme.)

There is now a strong requirement for the analysis of organic surfaces, including the identification and localisation of molecules, for which G-SIMS with G-Tip technology is excellent. The UK's Advanced and High-Value Manufacturing industry accounts for approximately 15% of GDP, 50% of exports and employs over 3 million people [TSB – Driving Innovation High Value Manufacturing, 2008]. UK industry is particularly strong in high-added value devices that are based on organic materials such as polymers, multi-component formulations as well as molecularly functionalised surfaces. These include medical devices with a global sector size of (£120B), diagnostics (£23B), Personal care (£40B), Cosmetics and Pharmaceuticals (£53B). Consequently there is strong demand for analytical methods that can identify and quantify organics at surfaces and relate this to product performance. For the identification of complex biomolecules, mass spectra are often insufficient and molecular structure is needed, as the mass alone cannot give unique identification. SIMS contains latent information about molecular structure within the spectra from the fragmentation of molecular ions. G-SIMS related Fragmentation Pathway Mapping (G-SIMS-FPM) and SMILES have been shown to make a powerful method to easily access this information and evaluate the molecular structure of complex molecules at surfaces. So far, G-SIMS-FPM and SMILES have been evaluated for a range of small organics using both positive and negative spectra. This has shown to be very powerful but the method needs extending to important industrial materials, such as pharmaceuticals and other molecules relevant to biotechnology.

Impact

For many medical devices and technologies—including drug delivery systems, diagnostic arrays, coronary stents, catheters, artificial implants, wound healing and tissue engineering—surface molecular chemistry is critical. The attachment or adsorption of biomolecules on engineered surfaces plays a crucial role in determining the performance of biomedical devices and assays. For these devices the relationship between the surface concentration, orientation, structure and activity of immobilised biomolecules is key. Similar requirements are to be found in other high innovation sectors such as personal-care products (fibres, biofilms and cosmetics) and organic electronics (OLEDS, displays, photovoltaics, RFID). There is an urgent requirement to develop methods to characterise molecules at surfaces. Addressing the lack of properly validated and traceable metrology in this area is becoming increasingly important as the number of novel and innovative diagnostic and therapeutic devices grows. Therefore, the results from this project will impact innovation and measurement capability in wide range of industries and address the needs of various companies, including Unilever, Smith & Nephew, Johnson & Johnson, AstraZeneca, GSK, CDT, PLL, Pfizer, Abbot, Inverness Medical, Shell, BP, BAE, Pilkington, Syngenta, 3M and Proctor and Gamble. This will be achieved through high impact scientific publications, inward and outward secondments as well as strategically aligned co-funded projects that directly support industry through, for example, the Technology Programme.

Summary of Technical Work

In this project we will take the G-Tip innovation forward. The G-Tip also gives automatic alignment of the two primary ions and consequently gives excellent G-SIMS imaging and spectroscopy. At present G-SIMS spectra are acquired sequentially: the Bi^+ spectrum first, followed by the Mn^+ spectrum. Taking the G-Tip innovation to the next level for ease of use for analysts would involve a new configuration to enable real-time G-SIMS using pulse-by-pulse modes and scan-by-scan modes. This would then allow real-time acquisition of G-SIMS images and would be even more convenient for analysts. The effectiveness of this new innovation will be evaluated on a range of different chemically patterned reference materials. So far, the G-Tip is available on the ION-TOF instrument.

It has often been observed that cluster primary ion beams increase the amount of fragmentation peaks as well as enhancing the molecular ion signal. This is clear if G-SIMS analysis is conducted using a cluster primary ion and an atomic primary ion such as Bi_3 and Bi. This effect may be owing to the thermal spike properties of the cluster primary ion causing heavy fragmentation around the ion track with the molecular ions being emitted at a later time and further away as the spike cools. This leads to two important features. We will use the fragmentation cascades observed for G-SIMS using cluster and atomic primary ions to further understand the fragmentation and processes involved in the thermal spike process and secondly evaluate if it may be possible to use cluster ion beams for G-SIMS in some conditions. A recent analysis has shown that the fragment cascades, which are generally parallel lines, contain additional structure such as strong dips for polycyclic aromatic ions. The fragmentation cascades will be studied in more detail using molecular modelling (*ab initio*) calculations to provide a more fundamental understanding and to see if further structural information may be extracted.

In this project, G-SIMS-FPM and SMILES will be developed to provide an automated system for interpreting molecular structure from mass spectra. This will need to additionally incorporate general rules of mass spectrometry that are relevant to G-SIMS in the automated SMILES system. This method will then be applied to pharmaceuticals as well as other biotechnology relevant molecules.

Deliverables

No.	Deliverable
1	Develop G-Tip innovation to maximise uptake and impact for analysts
2	Develop G-SIMS & SMILES for pharmaceuticals and biologically relevant materials
3	Develop a data and model to characterise the fragmentation of organic molecules by cluster Ion beams
4	KT including provision of expert advice to UK industry and academia, 4 publications, presentations at meetings, the production of reference data on the website and key input into BSI CII/60 and ISO TC 201.

S4 Quantitative Imaging XPS

Contact michael.adeogun@npl.co.uk

Project Objectives

The aim of this project is to enable the quantitative chemical analysis of micrometre-scale features on surfaces using instrumental capabilities already widely available to analysts in the UK. We will do this by:

- Investigating methods for translating XPS images into quantitative chemical maps.
- Establishing simplified or automated algorithms to enable reliable quantitative XPS imaging.
- Providing a good practice guide on X-ray beam damage of organic materials.

Background and Rationale

Within the contexts of an aging society and an added value, knowledge intensive manufacturing base, UK industry has to maintain a high innovation rate to increase its global competitiveness. Areas highlighted for growth include MEMS devices, microfluidics and microarrays, sensors and diagnostics and organic electronics. These areas impact upon many major UK industries, including the medical, pharmaceutical, chemical and aerospace sectors. Within the medical sensors and diagnostics area, point of care diagnostics and theranostics offer enhanced life expectancy and a high quality of life into old age. Many of the advances rely on microfluidic technologies; in such devices, surface functionality includes anti-fouling coatings and areas on the device with specific binding activity. Organic electronics relies on the printing of functional electrically active material in precise locations. This, in turn, often requires the chemical patterning of the substrate onto which the organic layer is deposited. In these application areas, local surface properties are extremely important and usually related to the local chemical composition on the micrometre scale. These precisely engineered, functional organic coatings are not amenable to the traditional tools of microanalysis, which damage organic materials too quickly for meaningful analyses to be made.

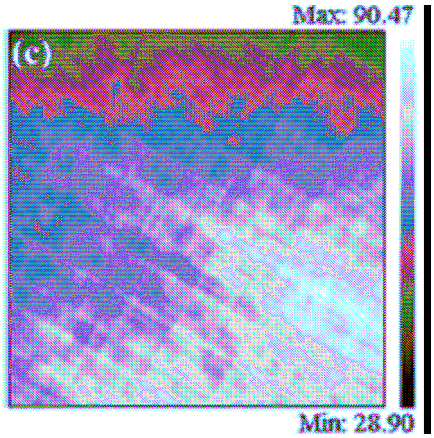


Figure 1: Intensity across an image of a homogenous sample shows significant inhomogeneity. (Walton

There is a need for spatially resolved, quantitative surface analysis within the emerging areas of biotechnology, organic electronics and microfluidics as well as in the traditional areas of microelectronics and materials science. XPS imaging meets this need and, although it has a lower spatial resolution than techniques such as SIMS and AES, it has the respective advantages of a straightforward route to quantification and applicability to a wider range of materials, including organics. However, significant work is required to enable meaningful information to be extracted from XPS images. Figure 1 demonstrates the lateral inhomogeneity in intensity due to issues such as local detector response, electron dispersion and X-ray illumination. Peak positions and widths are also affected. Such issues need to be described and compensated for. This topic was highly ranked in a consultation with the relevant industries and this ranking reflects a current need for guidance on the use and application of imaging XPS.

Impact

Within this work, we will determine the reliability of the different methods used to quantify XPS images, and, if appropriate, develop new methods. Guidance will be produced on the use of optimal experimental parameters and acquisition times to achieve requisite levels of precision in the measurement of composition. In addition, the effect of beam damage on delicate organic samples will be reviewed and linked to the work to establish the feasibility of using XPS imaging for such materials. This work will underpin the employment of XPS imaging in the future. We will work to ensure that it is relevant to the widest possible range of commercial instruments and that the protocols used for quantitative imaging are easy to implement and accessible. The UK is the market leader in sales of imaging XPS instruments and therefore the project directly impacts the UK economy.

With established guidelines and protocols, coupled with practical algorithms that can be easily implemented, users of XPS instrumentation will be able to perform imaging with greater confidence. By providing this confidence, NPL will enable analytical service providers and academic institutions to measure surface compositions of heterogeneous surfaces with spatial resolutions of a few micrometres within specified levels of precision. Peer-reviewed publications and a web-based guide will show impact. Use of the protocols developed in this project by instrument manufacturers, including KRATOS and Thermo-Fisher within the UK, and producers of XPS analysis software, such as CASA, will also demonstrate impact.

Summary of Technical Work

Instruments capable of providing spatially resolved maps of monoenergetic photoemission intensity have been available for many years, and this feature is available on a number of modern instruments. This capability, whilst it is attractive to a large number of users, is not widely utilised. Use of this capability is limited by the difficulty in translating XPS images into a quantitative measure of local concentration. Simple methods involving the subtraction of ‘background’ images from ‘peak’ images appear unreliable. Recent work by Walton and Fairley (e.g. SIA 38, 1230, 2006) has indicated that this goal is feasible if a careful characterisation of the spectrometer is carried out. At this stage, it is unclear to what extent such procedures can be translated to other instruments and samples, or if they can be simplified or automated to enable a wider uptake of imaging XPS. Another major issue is the long exposure of the sample to radiation during imaging, multivariate techniques may be used to minimise this exposure, but imaging may still be inappropriate for delicate organic samples.

We will: 1) Characterise the imaging mode of the NPL photoelectron spectrometer, 2) determine the instrumental response as a function of position within the image, 3) establish the effects of pass energy, illumination, magnification and electron kinetic energy, 4) investigate the constancy of instrumental response over time and on different samples, 5) investigate different algorithms for quantitative XPS imaging, 6) determine the X-ray exposure required to obtain requisite levels of precision for different samples and algorithms, 7) investigate the applicability of different algorithms to alternative imaging modes, 8) collaborate with instrument manufacturers and users, which would be helpful for this aspect of the work, 9) publish a web-based guide on damage of organic samples during XPS will be collated from available literature.

Deliverables

No.	Deliverable
1	Protocol for the characterisation of imaging XPS spectrometers.
2	Publication of reliable algorithms for quantitative imaging XPS
3	Publication of a web based good practice guide on minimising and accounting for X-ray beam damage of organic materials
4	Knowledge transfer including provision of expert advice to UK industry and academia, publications, presentations at meetings, the production of reference data on the website and key input into BSI CII/60 and ISO TC201

S5 Organic Depth Profiling in SIMS and XPS

Contact michael.adeogun@npl.co.uk

Project Objectives

- Develop a reference material and procedures for organic depth profiling and establish a crucial reference framework for reproducibility and repeatability.
- Investigate the use of XPS as a quantitative analytical technique for organic depth profiling using cluster ion beams.
- Develop standard quantitative measures and fundamental metrology of organic depth profiling to give clear guidance to industrial users.
- Evaluate the potential of novel massive cluster ion beams, such as Ar_{2000}.

Background and Rationale

Innovative, functional materials can lead to improvements in the quality of life, lower manufacturing costs and reduced environmental impact. For example, organic electronics offer the promise of cheap mass production of circuitry and displays integrated into everyday objects. In the near future, medical devices will be able to resist bacterial colonisation and reduce the currently high rates of infections (up to 20% for some devices) and the associated suffering and cost caused by reinterventions. Advanced drug delivery can effectively target disease, both reducing side effects and improving patient compliance. The composition and structure of organic films are important for many novel, high added value products, such as those highlighted above. Such technologies are encompassed within the TSB key technology areas of 'high value manufacturing' and 'advanced materials' and are also identified as UK priority technology areas in Council for Science and Technology Report "Strategic Decision Making For Technology Policy" (2007). Innovative advances crucially rely upon achieving the correct spatial and in-depth distribution of components.

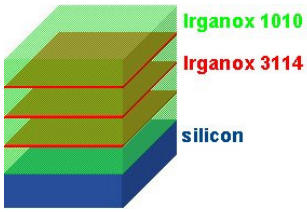


Fig 1. Innovative NPL organic delta layer

With increasing complexity in organic-film formulation—for example, the creation of multilayered, spatially patterned films or films with specified concentration gradients—problems associated with misalignment, intermixing, diffusion, phase separation, migration and contamination of components become difficult to diagnose without an effective measurement tool. It is therefore important that appropriate, validated techniques are developed to identify and localise chemical components in organic films, as well as to obtain quantitative measurements of their concentration. The realisation that cluster ion beams may be used to sputter away layers of organic materials without causing high levels of damage to the underlying material has given rise to the field of organic depth profiling. This provides a step-change in the capability of obtaining 3D molecular information that no other techniques can provide.

Organic depth profiling is highly promising for the investigation of compositions in organic coatings, with depth resolutions on the scale of ~10 nm and the ability to be combined with imaging spectroscopies to allow high resolution, 3D visualisation of the distribution of organic and bio-materials. However, many challenges and unresolved issues are currently preventing its routine uptake in industry, including a lack of knowledge on the sputtering, damage and roughening mechanisms. There is an urgent need for guidance on applicability to different systems and validated methods for profile quantification, and a need for a clear reference material and framework for intercomparison. In addition, while organic depth profiling with cluster ion beams is becoming commercially available to XPS instruments, studies are so far limited to phenomenological investigations. XPS possesses the advantages of direct quantification and a lower sensitivity to subtle forms of chemical damage during the transient period, and the use of XPS organic depth profiling is expected to grow strongly in industry. Finally, massive gas cluster ions have been recently demonstrated to radically suppress fragmentation of biomolecules compared to other cluster ions, allowing apparently damage-free etching through microns of organic materials. This is especially relevant to organic semiconductor materials that are difficult to profile with existing methods. It is therefore vital that massive cluster ions are evaluated and the basic metrology developed rapidly, in order to address the enormous potential of this emerging technology. Following the success of the IUVSTA workshop led by NPL in this important area and recent pioneering research, NPL is recognised as an International Lead in this key area. This project is essential to take these important developments forward to maximise the benefits to industry.

Impact

This project has a large potential impact on leading UK high technology sectors such as advanced materials (advanced multilayer functional coatings e.g. Becker Industrial Coatings Ltd.), pharmaceuticals (drug delivery systems), medical devices (drug coated implants and coronary stents) and organic electronics (3D molecular assemblies, e.g. Plastic Logic Ltd., Cambridge Display Technology Ltd.). 3D molecular characterisation using organic depth profiling is a key enabler in the development of these innovative, high added value products. Within the emerging area of organic depth profiling, there is a pressing need for assessing the repeatability and comparability between different laboratories as well as guidance on quantitative, correct and meaningful interpretation of data. NPL is ideally placed to provide reference materials and procedures based on its international reputation in the area acquired over the past few years. Clear reference materials, procedures and quantification of key profile parameters will provide the foundation for possible future development of an ISO standard or guide on quantitative organic depth profiling. These reference materials will be made available to the broader community, already 50 labs worldwide have expressed an interest and their uptake would be an excellent demonstration of the project's impact. The quantification of depth profiles will allow direct comparisons between different experimental arrangements and laboratories, and facilitate the mapping of material parameters leading to clearer data on the appropriate analysis conditions for each material. By addressing the issues of organic depth profiling with XPS analysis, we will impact directly on a number of industries and commercial analytical laboratories that have started to use this approach, but are working without references and standard procedures. An XPS manufacturer has offered a cluster ion source to NPL to assist with this work. Finally, by adopting a forward looking approach and exploring the promising new technique of massive cluster ion sputtering, for which a secondment of Joanna Lee to Kyoto University will demonstrate impact, we will maximise the potential for this new technology to radically expand analytical capability in the UK and enable the UK to stay at the forefront of international competition.

Summary of Technical Work

In this project, we will build upon the world leading capability of NPL in organic depth profiling to establish reference materials and protocols for organic depth profiles using SIMS. The innovative NPL organic delta-layer (see Fig 1) reference sample will be developed and made available to analysts, and the results of an interlaboratory study using these reference materials will be evaluated to establish the reproducibility of organic depth profiling and provide guidance to analysts for optimising experimental parameters. We will develop methods for quantitative depth profiling of organic thin films, investigating matrix effects on the quantitative determination of analyte concentrations. We will develop quantitative measurements for key parameters in organic depth profiling, such as depth resolution, roughness, damage and variations in sputter yield. Using the expertise gained with SIMS, we will determine the suitability of XPS as a complementary analytical tool for organic depth profiling, using an appropriate reference material. We will assess the chemical sensitivity, quantification, sensitivity to damage and depth resolution and investigate any special issues that arise. Potential collaborations and co-funding include in-kind contribution from XPS instrument manufacturers for minor equipment and cluster ion sources. We will develop existing links with the pharmaceutical, personal care and organic electronics sectors to ensure relevance to the anticipated beneficiaries of the technique. We will investigate the potential of massive argon clusters, such as Ar_{2000}, as a sputtering source using appropriate standard samples and compare with theory. We will characterise the damage and fragmentations, evaluate the depth resolution achievable and obtain fundamental sputtering yield data. Results will be compared to data from Bi_3 and C_{60}, providing a basic understanding of massive cluster sputtering.

Deliverables

No.	Deliverable
1	Development of a reference material and procedure for organic depth profiling using SIMS and XPS.
2	Development of a method for quantitative organic depth profiling using XPS and cluster ion beams.
3	Quantification of depth resolution, damage, variation in sputtering yield, roughness and matrix effects in organic depth profiles.
4	Evaluation of sputtering using massive cluster ion beams Ar_{2000} – establishment of theory and experimental data.
5	KT including provision of expert advice to UK industry and academia, publications, presentations at meetings, the production of reference data on the website and key input into BSI CII/60 and ISO TC 201.

S6	Analysis of molecular layers - Ultrathin layers on polymers and Molecular Orientation
Contact	michael.adeogun@npl.co.uk

Project Objectives

- To develop reliable and valid measurements of organic nanolayers on organic substrates using atomic and cluster ion beams.
- To develop a multi-technique method, using SIMS and polarised optical methods, to identify the orientation of molecules relevant to pharmaceuticals, diagnostics and personal care.
- To establish a fundamental knowledge base to understand the enhanced molecular emission using cluster ion beams and provide a framework to guide analysts for the optimum choice of primary ion species and energy for molecular nanolayers and thick films.

Background and Rationale

Functionalised organic surfaces and molecular engineered surfaces are key to the gamut of high-innovation and high-added value industries. Consultation with UK industry [SAMS workshop, NPL report October 2008] ranked this project amongst the highest needs. Additionally, the evidence base for measurement needs is strongly supported in key published reports. For example:

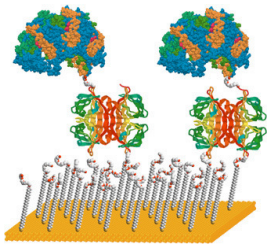


Fig 1. Biomolecularly engineered diagnostics

- The UK biotechnology industry is second only to the USA. The bioscience healthcare sector (excluding major pharmaceutical companies) covers over 1,100 diagnostic, device, service and supply companies, employing 100,000 people and generating revenues of £11 billion. (BBSRC Technology Strategy 2007). Here, a major measurement issue is the activity of biomolecules on the surface and key to this is molecular orientation.
- An important factor driving the UK biomaterial sector is interfacial control (Biomaterials Foresight), here surface chemistry such as molecular orientation allow biomaterial properties to adapt to a changing *in vivo* environment. Surface chemistry can be designed with anti-microbial, anti-thrombogenic, analgesic and cell differentiating capabilities. This is critical for medical devices, which have a global market of £120 billion, and is one of the key priority technology areas highlighted by the CST for Government investment (Strategic decision making for technology policy).
- The UK is an innovation hotbed and has a world leading academic base in the rapidly growing emerging technology of Plastic Electronics. The UK's Council for Science and Technology has highlighted plastic electronics as one of six top-priority areas for government support [CST Strategic Decision Making (2007)]. A major issue is the characterisation of nanolayer organic films, interfacial regions and ink-jet printing.
- The interaction of biological molecules with surfaces and analysis of their surface concentration, orientation and structure, is of major importance in nanobiotechnology and nanomedicine (DIUS ChemBio strategy 2008 & EU FP7 Nanomedicine Technology Platform, November 2006). It is essential for the development of these nanotechnologies to have nanometrology in place (Statement by the UK Government about nanotechnologies, March 2008).
- Locating and manipulating a molecule on a surface is one of the first steps towards bottom up approach of manufacturing at the nanoscale; for devices such as protein microarrays, microfluidic channels, or biosensors. Knowledge of the orientation of a molecule in 3-dimensions is also critically important since orientation determines the chemical accessibility of the molecule and biological activity.

Surface analysis techniques, such as secondary ion mass spectrometry, have shown amazing potential to probe material nanolayers, providing rich information about the structure of the material. Potentially, this technique could also provide information about the orientation of molecules (Green *et al*, SIA 2008) and quantity of doping and mixing in multilayer systems (Shard *et al*, J.Phys.Chem.B 2008). NPL is world leading in developing these areas, and has published 6 papers in the last year in this field, putting NPL in a strong position to develop these abilities further to enable reliable measurements of organic quantification, molecular orientation, and depth resolution that are critical for the wide range of industries outlined above.

Impact

For many medical devices and technologies—including drug delivery systems, diagnostic arrays, coronary stents, catheters, artificial implants, wound healing and tissue engineering—the surface molecular chemistry is critical. The attachment or adsorption of biomolecules on engineered surfaces plays a crucial role in determining the performance of biomedical devices and assays. For these devices, the relationship between the surface concentration, orientation, structure and activity of immobilised biomolecules are key. Similar requirements are to be found in other high innovation sectors such as personal-care products (fibres, biofilms and cosmetics) and organic electronics (OLEDS, displays, photovoltaics, RFID). Specific examples where these issues are critical include: 3M using thin polymer layers to develop novel organic LCDs; Unilever developing novel dirt repellent textile coatings; CDT developing thin multilayer systems for organic electronics, where careful quantification of the multilayer mixing is required; anti-microbial surface chemistry developed by a range of companies, including Wells plastics Ltd. in the UK, which depend on orientation of molecules; and self-assembled monolayer surfaces for the characterisation of biomolecules such as those used by Prof. Ryan at Oxford University.

Addressing the lack of properly validated and traceable metrology in this area is becoming increasingly important as the number of novel and innovative diagnostic and therapeutic devices grows. Results from this project will impact innovation and measurement capability in a wide range of industries and companies active in drug delivery, functionalised surfaces, characterisation of organic electronics and biological assays and microfluidic devices. This will be achieved through high impact scientific publications, inward and outward secondments as well as strategically aligned co-funded projects that directly support industry through, for example, the Technology Programme.

Summary of Technical Work

In the present programme we have achieved significant advances in characterising the ion yield for molecules on an inorganic substrate for different cluster primary ions. These systems are relevant to silicon-based technologies, for example. However, for molecules on an organic substrate the stopping power in the substrate is lower and the spectra are consequently very different. In this work, we will conduct a careful study of the effect of substrate on secondary ion yields of thin layers of molecules on organic materials, such as the blooming of additives on polymer surfaces. Results in the literature have shown that SSIMS is able to distinguish between proteins on different surfaces and between different protein orientations on surfaces. However, the data is unclear and not systematically evaluated. Controversy exists because it is known that the primary ion causes significant fragmentation of proteins but apparently is surface sensitive to the top 2 nm. This issue needs to be resolved before it can be used as a reliable method in industry. In this project, we use a reference set of biomolecules with controlled orientation to develop data and a model. Additionally, we will use a single molecule fluorescence microscopy based technique, varying the polarisation of the excitation laser in all the three directions, to identify the orientation of the dipoles of fluorophores in the molecules. These complementary measurements will be cross-validated to ensure a robust analysis.

A major recent development by NPL has shown that the molecular ion yield is proportional to the square of the total sputter yield. This has allowed, for the first time, experimental data for Irganox 1010 for a wide range of atomic and cluster ion beams to be plotted on a straight line. This allows analysts to easily compare different ion beams and energies and allow predictions for new sources such as larger fullerenes. However, this revolutionary approach has been controversial and further work is essential to validate it for molecules of different sizes and substrates as well as positive and negative secondary ions.

Deliverables

No.	Deliverable
1	Develop method to characterise nanolayers to thick layers of organics on organic substrates
2	Identify the sampling depth of SIMS for biomolecules and the ability to interpret molecular orientation of biomolecules at surfaces
3	Develop method to identify the 3D orientation of molecules at surfaces using optical methods.
4	Develop theory and data to characterise the positive and negative molecular ion yield for different materials and primary ion species.
5	KT including provision of expert advice to UK industry and academia, 3 publications, presentations at meetings, the production of reference data on the website and key input into BSI CII/60 and ISO TC 201.

S7 Characterisation of AFM-Probe-Chemistry (CAPC)

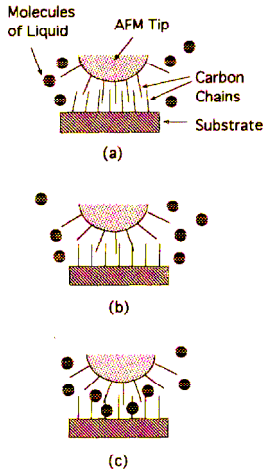
Contact michael.adeogun@npl.co.uk

Project Objectives

- To understand the underpinning science of tip-sample surface interaction for functionalised probes (of size 20 nm to 200 nm diameter) and to demonstrate the chemical-specificity in chemical force microscopy.
- To engage with research and development in industry and academia, proactively disseminate outputs and support innovation in the UK industries by using AFM chemical measurement.
- To develop a comprehensive protocol for the use of AFM with a chemically functionalised tip, including tip preparation and tip characterisation (tip-geometry and tip-chemistry).
- To provide a base for making AFM chemical measurements more repeatable and quantifiable.

Background and Rationale

Atomic Force Microscopy (AFM) is a useful surface analysis technique that finds use in a large variety of applications such as pharmaceuticals, health and personal care, nanotechnology and biotechnology. In particular, AFM is a key technique for emerging nanotechnology and nano-biotechnology applications. However, two major obstacles experienced by industrial AFM users are affecting its greater uptake by industry: (a) users' lack of confidence about the state of the tip (both shape and chemistry) as well as the sample surface during scanning, and (b) interpretation of AFM images. In reality, an image of a surface is influenced by both tip-shape and tip-chemistry. Erosion and deformation of the tip-end during a measurement are also common problems. It should be noted that a vast majority of characterisation of SPM tips relates to global tip shape (tip shank) instead of the very end of the tip (tip apex) and neglects the tip-chemistry[1]. In addition:



Different type of interactions of functionalised tip and surface-(a) entanglement of molecules (b) repulsion of molecules (c) entrapment of liquid molecules

- A number of R&D laboratories, which do not have specialist scientists conducting AFM measurements, are unable to utilise the full potential of AFM. For this reason, in the consultation of relevant industrial R&D stakeholders on various topics related to surface analysis, the investigation of tip-sample interaction scored very highly.
- Tip sample interaction was highlighted in a recent ISO TC/201/SC 9 meeting on scanning probe microscopy, which called for standards for probe characterisation (chemistry and geometry).
- Lord Sainsbury assigned scanning probe microscopy as one of NPL's priority technical areas[2].

NPL already has a strong knowledge base in the field through work in past and current programs and collaborations with pioneering scientists. For example:

- Working on characterisation of tip-shape (the end of the tip) in the current ChemBio programme (2006-2009) and ready to investigate tip-sample interaction as the next step.
- Simon Attwood, a NPL sponsored PhD student working with Prof. Mark Welland, (U of Cambridge) on chemical force microscopy, has developed the necessary expertise to handle the complexity of the problems.

It is a mammoth task to develop a complete understanding of tip-sample interaction and perform quantitative AFM measurements. Therefore, this project proposes to address the first two project objectives listed above and prepare the groundwork to begin the third project objective.

---

[1] A. Yacoot and L. Koenders, J. Phys. D: Appl. Phys. 41 (2008) 103001

[2] The Race to the Top: A review of government's science and innovation policies, Lord Sainsbury of Turville, October 2007

Impact

- This project will provide maps showing the parameter space for different mechanisms, which will help industrial research on a variety of fields related to AFM measurement. This will be done through a review of the work previously undertaken on tip functionalisation, tip-characterisation and tip-sample interaction.
- The work on chemical speciation will impact a number of industry sectors, including pharmaceuticals, biosensors, nanomedicine and organic electronics, enabling users to differentiate between various chemical species using chemical force microscopy or friction force microscopy.
- This project will develop underpinning metrology and contribute strongly to the development of international standards in SPM measurements in ISO TC/201/SC9 and especially in WG5 on characterisation of probes of which BSI holds the convenorship.
- The project will also increase the national and international recognition of NPL's activities in the area of scanning probe microscopy with at least 3 peer reviewed journal publications and presentations at international and national conferences. It will also foster greater collaboration between academia and industry (NPL) through NPL-EPSRC postdoctoral funding scheme, should our application be successful.
- As part of the KT project, a workshop involving SPM manufacturers, such as Asylum Research, JPK Instruments and Veeco, will be run to specifically disseminate the outcomes and best practice.

Overall, in industries where chemical specificity is extremely important in product development and innovation, this project will provide AFM users with greater confidence in AFM results, thus increasing the uptake of instrumentation and ultimately leading to cheaper nanoscale analysis. Given that, internationally, the UK is one of the key players in supplying nanoscale characterisation tools and the global market for nanotools is predicted to grow from ~$2 billion in 2008 to ~$8 billion in 2013 (according to data from BCC Research), this project could strengthen the UK's position in this market area.

Summary of Technical Work

This project will initially review the existing work in following areas; tip-functionalisation, tip-geometry and tip-sample interaction in the context of the needs of UK industries. This will highlight areas that need further understanding of the influence of tip geometry, role of non-uniform functionalisation of tips and AFM operating parameters in tip-sample interaction. Maps showing the parameter space for each process will be generated. Due to the complex nature of the problem, the project will focus on measurements with spherical tips as a function of radius (20 to 200 nm), functionalised with thiol-terminated organic molecules of different chain lengths or amino acids in contact with functionalised contact-printed surfaces. The coverage and orientation of the functional group will be characterised by SIMS and XPS. The tip-chemistry and tip-geometry will be characterised in-situ using functionalised one-dimensional nanostructures, such as carbon nanotubes. The project aligns well to the Technology Programme (TP) and the FP7 themes of nanotechnology, advanced materials, bioscience and electronics, and the Strategic Priority Theme 6 and 7 of the ChemBio Metrology Programme Strategy.

Deliverables

No.	Deliverable
1	Generate a map showing different mechanisms/process of interaction between the tip and the sample surface
2	Prepare calibration sample using one dimensional nanostructure e.g. carbon nanotubes
3	Develop a protocol for characterising tip-shape as well as tip chemistry using the functionalised one dimensional nanostructure
4	Demonstrate chemical specificity using the functionalised tips
5	Organise workshop on AFM for industrial users, involving SPM manufacturers and universities, publish 3 peer reviewed paper

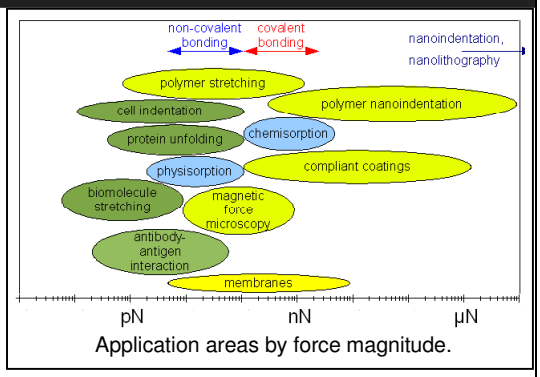
S8 Novel AFM Modes for Soft-surface Imaging (NAMSI)

Contact michael.adeogun@npl.co.uk

Project Objectives

- To develop underpinning metrology for the nanoscale characterisation of soft samples using Atomic Force Microscopy (AFM), and to support measurements used in the healthcare, pharmaceutical, polymer, organic electronics, advanced materials and food technology industries.
- To build a greater understanding of multi-frequency AFM techniques, and the qualitative and quantitative information that these techniques can yield.
- To determine and quantify the factors that limit the spatial resolution of force-distance and force-volume AFM measurements.

Background and Rationale



Application areas by force magnitude.

In various market sectors, a requirement exists for the detailed surface and nanoanalysis of "soft samples", such as biological tissues, biocompatible materials and hydrogels. Also, there have been specific calls for the development of scanning probe methods for characterisation of cells and cell components (*European Technology Platform on Nanomedicine*, 2006). This need is becoming ever more prominent across a greater breadth of sectors concerned with measurements on soft polymers and blends, gel phases, weakly-supported membranes and compliant films and coatings. Such sectors include those of advanced materials, plastic electronics, cosmetics and food technologies, where applications are typically concerned with the development of high value-added products—all areas that a recent UK-government statement on nanotechnologies highlighted because of their potentially huge benefits to the UK economy, as enabling technologies in high value-added manufacturing, and to society at large, and which also identified the need for the development of nanometrology to underpin progress in nanotechnologies Statement by the UK government about nanotechnologies, 2008). In addition, Lord Sainsbury assigned scanning probe microscopy as one of NPL's priority technical areas (The Race to the Top: A review of government's science and innovation policies, Lord Sainsbury of Turville, October 2007).

However, although AFM is used increasingly by industry to measure various characteristics of hard samples, such as semiconductor and mineral surfaces, AFM techniques can easily damage soft materials. Consultation with industry has highlighted strongly the need for guidelines concerning the use of a number of emerging, novel AFM modes—such as amplitude-, frequency- and phase-modulation modes—which exert very low forces only on the sample and so can potentially be used in the analysis of soft samples. These modes can also offer information about the sample beyond topography but, at present, their contrast mechanisms are not well understood: there is a demand for better understanding in this area, with a view towards quantification. Quantitative, nanoscale maps of mechanical characteristics can be achieved through force-volume measurements, although here there is a need for guidelines concerning the limits of resolution of this technique, and the influence each force-distance measurement has on those that follow it, due to sample damage.

NPL is well placed to carry out this work in collaboration with well-known experts on low-force and high-resolution imaging. We are building on a strategic collaboration with AFM manufacturer, Asylum Research, who will contribute in kind to realise this project. We will also engage with biotechnology and pharmaceutical companies and disseminate the outputs from this project.

Impact

This project will provide a competitive advantage to a number of industry sectors important to both the UK and European economy by opening up AFM to new markets and applications, especially in high-added value areas such as nanomedicine, nanobiotechnology and organic electronics. For example:

- Businesses concerned with smart materials and functional coatings will benefit from the development of fundamental understanding and a metrological framework for the quantitative measurement of nanoscale adhesion and elasticity.
- The biotechnology and nanobiotechnology sectors, and the developing fields of nanomedicine and nanotoxicology, will benefit from the publication of guidelines comparing methods for the measurement of soft, delicate samples at high resolution; we will work with NPL's Biotechnology group (Dr. Anna Hills) and Biomaterials group (Dr. Paul Tomlins) to disseminate the knowledge to relevant industries.
- The uptake of dynamic AFM modes by industry will be encouraged through the provision of recommendations for the use of these novel modes, potentially stimulating growth in the instrumentation sector, which has long been an area of strength for the UK. Internationally, the UK is one of the key players in supplying nanoscale characterisation tools and the global market for nanotools is predicted to grow from ~$2 billion in 2008 to ~$8 billion in 2013 (according to data from BCC Research).
- NPL will gain both national and international recognition of its activities in the area of scanning probe microscopy, through exposure in peer-reviewed journals and at conferences.
- NPL's reputation as a world-leading NMI in surface and nano-analysis and its status as a leader in the metrology of AFM techniques will be developed through contributions to future standards in ISO/TC201.

Summary of Technical Work

Dynamic AFM modes, using both conventional and novel probes, will be evaluated for imaging soft samples, with comparison to images obtained using SICM. Levels of sample damage and the presence of image artefacts will be determined as a function of imaging parameters, such as *feedback gains* and *free-oscillation:set-point ratio*. The theory underlying complementary contrast mechanisms in these dynamic imaging modes and new, multi-frequency modes will be reviewed and developed. This will be achieved by comparison of information derived from these contrast mechanisms to quasi-static force-distance measurements, and the production of reference samples.

The resolution of force-volume measurements, and the effects of individual force-distance measurements on the sample, will be investigated both theoretically and experimentally. Finite element modelling will be used to investigate theoretical limits of force-volume resolution. Experimental work will concern the limit of resolution as a function of measurement parameters such as applied maximum load and probe displacement, using reference samples in which there are sharp boundaries between regions with different elastic moduli. The results will be used to produce a guide for UK analysts concerning the limitations, key factors and optimisation of force-distance and force-volume AFM measurements.

Deliverables

No.	Deliverable
1	A report and/or publications and conference contribution providing guidelines and recommendations for imaging of soft samples with AFM.
2	One or more publications and conference contributions concerned with the physics underpinning dynamic and multi-frequency AFM modes.
3	Publications concerning the spatial resolution of force-distance and force-volume AFM modes.
4	Knowledge Transfer

S9	Measurement of Inter-Particulate Interaction (MIPI)
Contact	michael.adeogun@npl.co.uk

Project Objectives

- Develop methods for measuring the interaction between functionalised nanoparticles based on (a) stochastic study of dynamic interaction using light scattering/microscopy (b) interaction force measurement using Photon Force Microscopy.
- Engage with those industries working with nanoparticles for biodiagnostics and nanomedicine and demonstrate the capability of the technique by performing measurements on specific systems relevant for those industries. We will proactively disseminate the knowledge generated in this project to industries and academia.
- Develop a fundamental understanding of interparticulate interactions, such as the different types of long and short range interaction forces between various particulate systems, which will be beneficial for a wide variety of application areas where micron and nanometre-size particles are used, or occur in the environment.

Background and Rationale

Nanoparticles often display improved or novel properties, compared to their bulk counterparts, because of their nanosize and very high surface area to volume ratios. Nanomaterials, such as silver nanoparticles, are increasingly finding use in myriad products, including cosmetics, home appliances, clothing, healthcare, pharmaceuticals and specialty chemicals. How well we manage the risk of using nanoparticles depends on how well we understand and measure their properties. The risks and benefits of using nanoparticles are being weighed with utmost care by regulators and manufacturers: Various nanoscale measurement techniques are being used by researchers to understand the impact of nanomaterials on the public health and the environment. Today, there is very little understanding of the behaviours of these nanoparticles when they are released in air, water or when they enter into biological organisms. Long and short-range forces, along with chemical affinity, determine the nature of interaction between particles. For example:

- In biotech products and devices, the interaction between two particulates, or biomolecules and a particulate, can determine the functionality of a system. For example, in nanoparticle-based biodiagnostic devices, the interaction between the functionalised nanoparticles and the biomolecules determines the sensitivity of the device; in nanomedicine, the interaction between the target molecule/surface and the nanoparticulate determines the efficacy of a drug.
- For waterborne particles, often there is a dynamic equilibrium between particles. In colloidal systems, such as clay or sunscreens, the interaction between particles is extremely complex. A clearer understanding of such systems can benefit a wide range of industries including food, pharmaceuticals and cosmetics through improved product formulations.
- The degree of aggregation of particles in air, especially when their sizes are at the nanometre scale, depends on the chemical and physical interaction between them.
- Certain nanoparticles are toxic due to their nature of interaction with biological cells and organs. If we can measure the interaction, we will be able to prevent or promote certain events resulting from inter-particulate interactions.

There is significant interest from various funding bodies in measuring interaction between nanoparticles to understand the toxicological effects of nanoparticles. For example, several future calls from FP7 relate to the better understanding the interactions of nanoparticles with biological systems and the environment. Today, no suitable method exists for measuring the interactions between nanoparticles that is traceable to SI. For the technology examples listed above, Scanning Probe Microscopy (SPM) is already in use for nanoscale measurements on 2-D surfaces. The emergence of novel SPM tools, such as Photon Force Microscopy (PFM), now allows in-vitro measurement of interaction forces as well as mapping the topography in 3-D. In chemical and biological systems, rate constants are often used to define a dynamic equilibrium between particles; however, this value is not directly correlated to the absolute force of interaction. In this work, we propose to use PFM to measure the force of interaction between two functionalised nanoparticles in water and use light scattering experiments to determine the dynamic equilibrium constant.

Impact

- This project will bring direct benefit to various industries and emerging application areas, such as biodiagnostics, nanomedicine and drug delivery research, where interactions of nanoparticles can play a very crucial role in determining the performance of product formulations, systems and devices—ultimately leading to better, lower cost products and strengthening the UK's economy.
- From a regulatory and legislative standpoint, this project will have immense impact on our understanding the interaction between nanoparticles (both natural and man-made) and biological systems such as proteins, cell surfaces and membranes. These measurements will provide insight into modelling the toxicological effects of the nanoparticles, thereby helping industry and regulators determine the future safe use of nanomaterials in various applications, improve people's quality of life and allay the concerns of public health and consumer groups.
- This project focuses on developing a method of measuring interaction between two nanoparticles in water using two techniques. However, this work can be extended to a variety of liquid mediums, leading to a knowledge base that will be useful in areas such as studying the interaction between particulates in food, aggregation of nanoparticles in the workplace and the behaviour of nanoparticles in healthcare products.
- The scientific impact of this project will be achieved through a minimum of three peer reviewed scientific papers and two conference presentations. We will also disseminate this knowledge proactively to bring the benefits to the UK industry.

Summary of Technical Work

Novel SPM-based methods, such as Photon Force Microscopy (PFM), will be used to measure the interaction forces between two species A and B. A stochastic method (e.g. fluorescent/light scattering technique) will be used for experimental work to estimate the dynamic equilibrium between them (schematic shown in figure 1). New thermodynamic parameter, equivalent of Gibb's energy of reaction (ΔGr), will be calculated from stochastic measurements and correlated to the SI unit of force, which will be directly measured using a calibrated PFM in the force range of few tens of pico-newton.

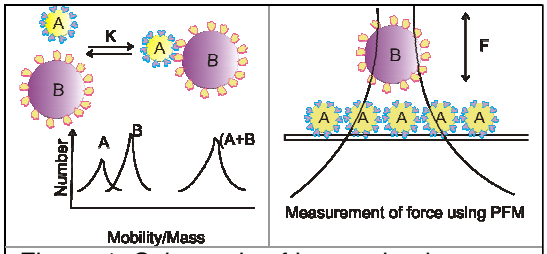


Figure-1: Schematic of interaction between two nanoparticles and measurement using stochastic process and direct force measurement

Metal nanoparticles (gold or silver) functionalised with antigen and antibody will be used as a model particle-system in water-based medium. The laser trap in PFM will be calibrated through experimental measurements using either the Brownian motion of the particle in the trap or lamellar flow through a microfluidic device. Multiphysics modelling will be performed using COMSOL to validate the experimental calibration of the trap. Light scattering experiments will be performed at the laboratory at Nanosight Ltd, UK. Measurements will be performed on particulate systems relevant for biodiagnostics and/or nanomedicine industries.

Deliverables

No.	Deliverable
1	Preparation of suitable engineered nanoparticles for interaction measurement
2	Calibration and modelling of the laser trap in Photon Force Microscope
3	Measurement of interactions of functionalised nanoparticles using light scattering and PFM experiment on model system
4	Demonstration of measurement of interactions on particulate systems relevant for biodiagnostics/nanomedicine industries
5	Engage with industries, publish 3 peer reviewed paper and two conference presentations

S10a Ambient and Imaging Mass Spectrometry

Contact michael.adeogun@npl.co.uk

Project Objectives

- To build on a successful collaboration with LGC to develop the metrological framework for DESI.
- To improve DESI repeatability and constancy to better than 10%, to build confidence in the technique for measurements in healthcare, security and pharmaceuticals.
- To provide a user guide to direct analysts through the range and effectiveness of DESI.
- To establish the capability of DESI for a range of measurements including imaging, depth sensing, reaction monitoring and low detection limits.

Background and Rationale

For biological studies, there is a strong demand for analytical techniques that can operate in ambient conditions and *in vivo*. For example, across a wide range of biochemical areas it is increasingly important to use label-free imaging for *in vivo* analysis—a research priority highlighted in "Nanomedicine-European Technology platform 2006". Further, this need has been identified as a major metrology requirement in a recent EU report on needs for standardisation in Research and Development in nanotechnologies [Nanostrand 2008, NMP4-CT-2006-033167]. The DIUS ChemBio strategy for 2008 also states that a key aim is to "support innovation and competitiveness through the development of reliable leading-edge measurement". One of the major drivers is "chemical analysis in ambient conditions". Finally, consultation with industry showed a strong demand for the development of metrology in ambient mass spectrometry. At the consultation workshop [SAMS V, NPL Report October 2008] this project scored very highly. Therefore, it is clear that ambient mass spectrometry techniques, like Desorption Electrospray Surface Ionisation (DESI) and related methods, are strategically important. Figure 1 shows how different analytical techniques contribute to the biological measurement requirements that span from quantification and identification through to structure, function and activity as well as the operating environment from *in vacuo* through to *in vivo*.

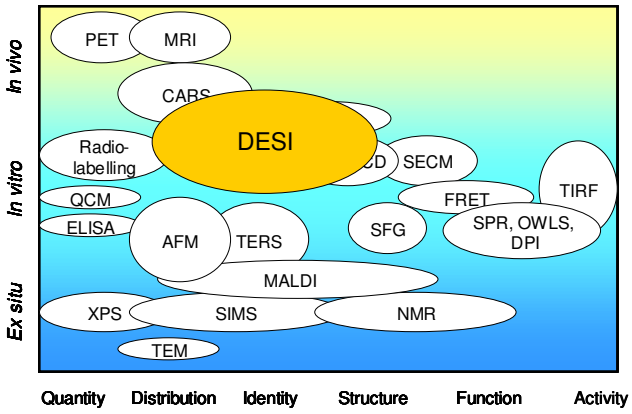


Fig 1. Nanobiotechnology – Map of techniques [EU Nanostrand, 2008]

DESI is a powerful new mass spectrometry technique that can identify pharmaceuticals, explosives, proteins and a range of biological materials [Science 306 (2004) 471]; is capable of molecular image analysis for a range of samples including tissues [Science 311 (2006) 1566], with optimum spatial resolution of the order of 100 µm; is capable of femtomole sensitivity [Anal. Chem. 77 (2005) 6755]; provides high throughput analysis [Anal. Chem. 80 (2008) 6131] and real time analysis for reaction monitoring [Anal. Chem. 79 (2007) 5064]. In addition, DESI can be combined with mini-MS for a handheld ambient molecular analysis device [Anal. Chem. 80 (2008) 4026].

Interest in ambient mass spectrometry is demonstrated by the fact that over 1000 papers have been published in this area since 2004. However, many users report issues in its robustness and reliability. Typically repeatability is 50% and, for industrial use, this needs to be regularly < 10 %, and results need to be comparable between instruments. A strong collaboration between NPL and LGC has made significant progress in improving the reliability of DESI, investigating the effects of certain parameters and furthering our understanding of the DESI process by exploring the DESI/surface interaction and efficiency of the process. This project aims to take the technique forward and fulfil its potential for ambient imaging, in field, *in vitro* or *in vivo*, which requires underpinning metrological research to enhance the usability, robustness and reliability of the technique.

Impact

There is a major requirement for analytical techniques with high chemical specificity and sensitivity that can operate in ambient conditions and are portable. In many high innovation sectors—such as pharmaceuticals and health and personal care—for many devices and products, the surface chemistry is critical but the samples are not compatible with vacuum-based surface chemical analytical techniques. Molecular examination of complex surfaces, *in situ*, *in vitro* or *in vivo*, to give rapid chemical or biological analysis as well as image analysis, would be very useful in a wide range of application areas. There are many strong examples of the potential of DESI and complementary techniques in areas such as high throughput analysis of pharmaceuticals, detection of explosives, forensic analysis, environmental monitoring, counterfeit detection, imaging of cancer tumours or monitoring of catalytic reactions. However, the development and implementation of consistent definitions, measurement methods and reference materials are necessary to ensure comparable results from day-to-day and laboratory-to-laboratory operations.

This underpinning knowledge base project—in collaboration with LGC—will develop the foundations for DESI to provide reliable and valid measurements that enables industry to make full use of its potential and capabilities across myriad application sectors. This is essential to enable ambient MS to be developed beyond an exploratory research tool and into use in commercial analytical or testing laboratories, production lines or in field analysis. Instrument manufacturers ABI and Shimazdu have already shown interest in the progress of the DESI research at NPL and LGC and are keen to collaborate and develop this underpinning metrology further to ensure consistency across MS platforms.

Summary of Technical Work

This knowledge base project will build on, and grow, the successful collaboration with LGC in the current ChemBio programme that has made significant progress in developing the basic metrology for reliable analysis using DESI. Here, we aim to establish a simple procedure to set optimal DESI conditions; this will lead to improvements in the robustness and comparability of the technique. Then, from this base we will develop a method and procedure for the improvement of DESI repeatability and constancy to better than 10%. This work will develop in collaboration with instrument manufacturers such as ABI, Waters, Thermo and Shimadzu to ensure consistency across MS platforms. It is essential for industry to have a clear understanding of the regimes of effectiveness when using DESI – which materials it works well for and those for which it does not work. In this work, we will develop further our understanding of the DESI technique, investigating the effect of wettability, solvent composition and analyte chemistry and mass on the surface interaction and efficiency. Using this information we will map out what materials may be analysed effectively using DESI and provide clear guidance and validated protocols to help analysts optimise DESI for different classes of material. A metrological framework, including reference materials and validated procedures, will be developed to support and optimise the technique for a range of different capabilities. Firstly we will develop a protocol to evaluate sensitivity and efficiency of DESI for measuring limits of detection by understanding the erosion of material (analogous to sputtering yields) and efficiency of the DESI process and test this on a range of 10 materials. Secondly, a procedure for the characterisation and optimisation of DESI spatial resolution will be extended from our present work, including a reference material to easily measure spatial resolution. We will also explore the possibilities to improve limits of sensitivity and spatial resolution such as sniffers, grids, enclosures or other instrumental improvements. Thirdly, we will demonstrate the potential of DESI for additional capabilities and information such as depth sensitivity, using a multilayer system to measure depth resolution and real time analysis, exploring using DESI for reaction monitoring.

Deliverables

No.	Deliverable
1	Develop methods to improve the repeatability of DESI to better than 10%.
2	Identify the classes of materials and substrates for which DESI is effective and provide a guide to analysts.
3	Evaluate the detection sensitivity of DESI and measure the rate of material removal, depth resolution and useful lateral resolution.
4	KT including provision of expert advice to UK industry and academia, 3 publications, presentations at meetings, the production of reference data on the website.

S10b	Metrology for Innovative Ambient Mass Spectrometries and MALDI
Contact	michael.adeogun@npl.co.uk

Project Objectives

- To develop reliable and valid analysis using new ambient mass spectrometries (EESI and PADI).
- To provide a user guide detailing the capability of EESI and PADI for analysis of biological, pharmaceutical and personal care products.
- To survey the issues limiting the use of MALDI as a quantitative technique for healthcare and biotechnology imaging and identify where metrology is most needed and how best to couple NPL and LGC expertise to have significant impact.

Background and Rationale

The UK biotechnology industry comprises over 480 companies, employing 26,000 people, generating revenues of £4 billion and investing £1.8 billion in R&D. (BBSRC Technology Strategy 2007). Bioanalysis, in particular molecular imaging, is of particular interest to the biotechnology industry in enabling future innovation. For example, for nanomedicine, research priorities are toward the development of appropriate technologies for *in vivo* imaging, including the development of label-free detection; in point of care diagnostics, according to a MoD future technologies study (June 2007), "Molecular imaging is an area from which future high impact technological developments are likely to arise, a possibility being that of diagnosis at the point of examination" Further, the NanoRoadMap project identified molecular imaging as one of 11 topics that are key to nanotechnology for the health and medical sector. The DIUS ChemBio strategy for 2008 also notes that "underpinning metrology in new important technologies, such as matrix-assisted laser desorption/ionization (MALDI) imaging and desorption electrospray ionization (DESI), offers significant potential to provide 'information-rich' images for biomedical research and materials science."

The growing field of imaging mass spectrometry techniques, such as MALDI and emerging fields in ambient analysis such as Plasma Assisted Desorption Ionisation (PADI) and Extractive Electrospray Ionisation (EESI), have great potential in molecular analysis in field, *in vitro* or *in vivo* environments:

- PADI is a technique developed in the UK that uses cold helium plasma to desorb and ionise materials at surfaces under ambient conditions. Initial work shows its potential for direct analysis of pharmaceuticals, without high voltages or solvents.
- EESI has only been established in the last year and works on a similar basis to DESI, however it allows much gentler probing of a material, without using chemical solutions or high voltages. This gives it the potential for analysing *in vivo* with very low risk, a major advantage if it is to be used in diagnostics.
- MALDI is complementary to ambient MS, it is information rich, with detection of whole protein and sequencing of primary structure through peptide digest and MS/MS, with spatial resolution of < 50 µm. MALDI is a widespread technique, with over 7000 papers published in the last 5 years, and a growing area of interest is MALDI imaging, which is very useful for tissue mapping. However, much of the underpinning metrology has yet to be developed especially for biotechnology applications and repeatability of the technique is rarely better then 15%. This is not robust enough for measurements incorporated into health diagnostics. Consultation with UK industry and MALDI instrument manufacturers (the UK has an excellent MALDI export market) has led to very strong support for NPL and LGC developing the metrological approach in this area.

Molecular analysis of complex surfaces—*in situ*, *in vitro* or *in vivo*—that provides rapid chemical or biological analysis and image analysis is critical for various applications. There are many good examples of the potential of ambient mass spectrometry in high throughput analysis of pharmaceuticals, detection of explosives, imaging of cancer tumours or monitoring of catalytic reactions. However, the development of consistent definitions, measurement methods and reference materials are necessary to ensure repeatable measurements and equivalence between laboratories and to compare the rapidly growing range of techniques.

Impact

As part of this underpinning knowledge base project, in collaboration with LGC, we will develop the measurement infrastructure for reliable comparisons between several powerful new atmospheric techniques, PADI and EESI, extending the recent successful collaboration with LGC on DESI. EESI is particularly powerful for *in vivo* studies; PADI shows great potential for analysing materials more strongly bound to the surface that cannot be accessed by DESI. Potential collaboration with universities, regional agencies and EPSRC are already in discussion.

Consultation with UK industry has shown that there is a need for metrology to support MALDI. In particular, Shimazdu has expressed strong support for this work. The UK has a strong MALDI instrumentation sector, and the work in the NMS would have a high impact across a broad user base. Many of the issues in MALDI arise from surface problems—such as matrix deposition, coverage and the laser matrix interaction—therefore, surface analysis is critical to understanding and addressing these issues. In collaboration with LGC, who have some history of work in MALDI and excellent mass spectrometry expertise, we can start to address many of the metrological and quantitative issues surrounding MALDI. Collaboration with instrument manufacturers will ensure a consistent development across MALDI platforms.

Examples of industries and applications where ambient mass spectrometry and imaging mass spectrometries can have major impact include pharmaceutical and biomedical analysis (tumour analysis and medical device analysis, biological analysis for research and development or drug delivery testing), forensic analysis, explosive detection and battlefield analysis, environmental monitoring, testing of counterfeits in drugs and food, production line testing for consistency or faults and point of care diagnostics. Therefore, the impact of this work will be in the greater support and take up of the techniques for these potential areas.

Summary of Technical Work

In this work, we will begin the development of basic understanding of the EESI and PADI using the successful methodology and team that has led to rapid progress in DESI in the current ChemBio programme. This will include developing controlled instruments to allow a systematic study of the effects of key instrumental parameters and using surface analysis to explore the way material is desorbed from a surface and transferred to the mass spectrometer. The production of a user's guide will map out the complementarity of EESI, PADI and DESI for different applications, including analytical mass range, detection efficiency and the ability to desorb more strongly bound molecules (PADI).

MALDI is an extremely powerful mass spectrometry technique used widely in the biotechnology industry. However, repeatability is poor and much of this is thought to relate to the surface chemistry of the matrix and analyte as well as coupling of the laser with the matrix. Preliminary studies at NPL and LGC using AFM are beginning to show the extent of these issues. In this proposed work, we will survey the issues in MALDI that hinder it from being a repeatable, quantitative technique and identify where research from NPL and LGC may best make a significant impact.

Deliverables

No.	Deliverable
1	Establish controlled instrument for EESI and PADI
2	Evaluate the complementarity and molecular sensitivities of DESI, PADI and EESI.
3	Survey issues for repeatability and reproducibility in MALDI, especially relating to biotechnology and pharmaceuticals.
4	KT including provision of expert advice to UK industry and academia, 2 publications, presentations at meetings, the production of reference data on the website and begin development of standardisation for MALDI in ISO.

S10c					
Project Author/ Contact	**Author: Peter Stokes** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Assessment of the Quantitative Attributes of Imaging Mass Spectrometry

Project Objectives

To assess the application of quantitative approaches to the reproducibility and repeatability of imaging MS platforms and thereby support the development of new mass spectrometry (MS)-based imaging technologies. This requires:

- Determination of the quantitative potential of selected MS-based imaging technologies.
- Development of suitable calibration and measurement strategies for improving the accuracy and precision of quantitative imaging MS.
- Comparison of quantitative imaging strategies with more traditional forms of MS quantification.

Background and Rationale

Surface chemical analytical techniques such as LA-ICP-MS, ToF-SIMS, MALDI-ToF and DESI are extremely powerful and capable of providing high-sensitivity, qualitative and quantitative information on the distribution of a wide variety of molecular and/or elemental species within a sample. Imaging technologies are considered a priority area of research in the field of clinical (biomarker discovery), pharmaceutical (targeted drug detection), environmental (chemical fate) and material sciences.

Assessing new imaging MS technologies has been a joint area of activity at NPL and LGC under the current CBM Programme. Previously, activity has focussed on the assessment of different technologies for the production of high resolution spatial data. However, the current key area, still to be addressed for such technologies, is the robustness of the quantitative measurements being made. Imaging MS is inherently reliant on the capability of the mass spectrometer to distinguish the levels of specific compounds present at different locations (and hence build up an image). The lack of in-depth knowledge of the quantitative attributes of such approaches is limiting and impacts on the quality of the spatial images. The quantitative potential of imaging MS techniques has not been fully addressed and this project will address the essential requirements, to further develop quantitative MS imaging and assess its associated measurement uncertainty. The determination of limits of detection, repeatability and reproducibility are all essential to enable scientists working in different disciplines to assess the applicability of the technology for their imaging needs.

Using our expertise in quantitative mass spectrometry, different calibration strategies for the production of repeatable quantitative measurements across a surface will be assessed. The application of quantitative approaches to the standardisation of the each spectrum will improve the reliability and quality of the data, and increase confidence in the technology and key scientific processes reliant on MS imaging.

Impact

Development of the measurement infrastructure for emerging imaging mass spectrometry-based techniques, by evaluating what is required to achieve reliable, traceable quantitative measurements, will have a large impact in the medicinal and clinical diagnosis sectors. As new techniques and advances in technology enable greater resolution and specificity, the utility of such devices to the biotechnology and pharmaceutical sectors is becoming more apparent. These sectors are the biggest single contributors to the R&D investment within the UK (£7.6bn). The use of imaging technologies within them has already started to play a significant role in their understanding of process *in vivo*. Further advance in metrology to support such innovation is required to realise the true impact of imaging MS by expediting its development into reliable analytical tools in the future. Wider impacts range from improved product characterisation to enhancing public security and detecting crime.

Summary of Technical Work

During the current CBM Programme, UK NMIs have developed a successful relationship that has made significant progress in developing the basic underpinning metrology relating to the use of DESI and improved its usability considerably. This project aims to further develop applications in this field by extending such collaborative study to include the use of emerging ion sources, considering all aspects of quantitative approaches for mass spectrometry-based imaging technologies.

A review of the current quantitative method requirements and assessment of up-to-date models for quantitative imaging will precede the bulk of the work examining the effect of sample preparation techniques (principally cryo-sectioning, matrix application, digestion etc) and standardisation approaches (choice and incorporation of suitable internal standards versus external standardisation approaches).This will be delivered alongside the developmental work looking at emerging ambient MS techniques. The various fundamental aspects, such as repeatability and reproducibility, will be assessed and improved upon, where possible, in close collaboration with other NMI colleagues and other leading groups in the field to gain a full understanding and further develop the quantitative potential of these techniques.

The knowledge gained by the successful completion of this project will broaden the scope of the metrology base provided by the NMIs and lead to the development (with industry and academia) of a best practice guide for the production of reproducible mass spectrometry-based images that can be used to compliment more traditional measurements.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW industry consultation to assess the necessary quantitative attributes required for the successful application of imaging MS technologies has been completed.			
2	CEW the effects of sample preparation and presentation on imaging analysis have been investigated.			
3	CEW standardisation and calibration approaches have been investigated for the reduction of error and measurement uncertainty for quantitative imaging.			
4	CEW a best practice guide for the production of reproducible mass spectrometry-based images has been produced in association with industry.			
			Total cost	

TA1 Traceability for miniaturised electrochemical devices

Contact michael.adeogun@npl.co.uk

Project Objectives

- To provide underpinning traceability for existing ion selective electrodes (ISEs) and develop novel miniaturised ISEs and electroanalytical techniques for use in in-situ applications.
- To provide traceability and validation protocols for these novel techniques, especially at ultra trace concentration levels.
- To develop flexible calibration and data handling methods to maximise the accuracy of these measurements.
- To disseminate the outputs of this work via peer-reviewed publications and relevant learned society committees.

Background and Rationale

There continues to be a requirement for the measurement of a number of trace level species in environmental and biological environments, where electrochemical techniques provide the cheapest, most robust, and most appropriate method of analysis. In particular, a strong driver is the increasing drive toward miniaturisation of electrochemical devices so that measurements can be made easily in on-line or in-situ environments. Strong regulatory drivers for such measurements come from the EU water framework directive; EU air quality directives (especially for impinger-sampled field measurements); EU and IFCC requirements for blood gas and electrolyte concentration measurements, which are critical to health and biological balance; and WMO requirements for accurate salinity measurements for climate change assessment. This is also an area identified of high importance by the recent Chemistry Innovation KTN roadmap. The NRCG report on nanoparticles has identified the requirement to measure the leachable fraction of manufactured nanoparticles—another area where in-situ electrochemical techniques are particularly suitable.

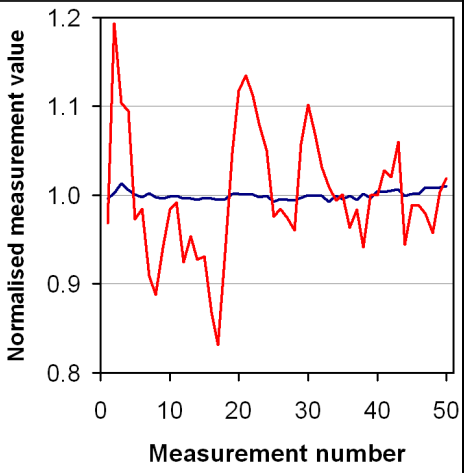


Figure. Fifty repeat electrochemical measurements of cadmium ions in a high ionic strength environment, with (blue) and without (red) internal standardisation

Moreover, requirements are emerging for the in-situ ion measurement within fuel cells and dye-sensitised solar cells, to examine parameters such as performance, degradation rate, and leaching from catalysts and electrodes. These measurements require highly sensitive methods and miniaturised devices, with novel solutions for how these devices might be made to work in environments where electric fields will interfere with traditional measurements. Micro- and nanostructured materials will play a large part in delivering these requirements, since this is essential to ensure that these miniaturised electrodes have a maximised contact area with the analyte solution for measurements. However, as devices become smaller and analytical matrices more challenging there is a need to ensure the accuracy and traceability of results—a requirement of the IFCC and the IVD directives—especially at ultra-low concentrations. These devices often lack the theoretical understanding and practical validation of more traditional macro-electrochemical devices, and often require new calibration and standardisation strategies (see Figure) in order to ensure the accuracy of the results.

This project aligns well with the results of the consultation process with the stakeholder community in the electroanalytical chemistry sub-section, which highlighted traceability for miniaturised environmental sensors, new ion selective electrodes (ISEs) for in-situ environments and new analytes, and flexible calibration strategies for on-line measurements as the most important areas where there was a requirement for measurement science input. The work proposed in the project also aligns well with DIUS Technology Programme priority areas of Smart, Bioactive and Nanostructured Materials for Health and Low Carbon Energy Technologies, with respect to the application of these electrodes to fuel cells and dye-sensitised solar cells. The work delivers the CBKB strategy in the environmental technologies and particles sub-themes, specifically in technology areas associated with ionic content, and the leachable content of particles. Co-funding, via a Defra LINK Programme project is already in place.

Impact

The outcomes of this work will provide underpinning traceability for existing ISEs and develop novel devices and measurement strategies for electroanalytical determinations of a variety of ionic species in complex environmental and biological environments. The dissemination of this work impacts widely across each industrial sector and will enable accuracy and traceability of these measurements to be ensured. Moreover, the production of methods for flexible calibration strategies for on-line and in-situ measurements should decrease the uncertainty of electroanalytical ion measurements, by providing tailored and responsive strategies to calibrate at the most appropriate concentration levels and at the optimum time period. The application of miniaturised devices for the measurements of ionic species, in particular metals, in fuel cell and dye-sensitised solar cell environments will be of special interest to these rapidly developing areas. In addition, this work has synergy with other NMS projects (in the biological themes of CBKB, and in the Materials Programme) and will therefore provide additional capability and impact in these areas.

The work will be disseminated via high impact peer-reviewed publications, and the project will also allow NPL to provide representation and dissemination on appropriate committees (in particular the SCI electrochemical technology group, and the RSC electroanalytical group). It is also the intention to disseminate the work via the CCQM Electrochemical Analysis Working Group, and encourage this group to look towards international comparisons of these secondary measurement methods. Impact will be assessed by the uptake of the new methods and devices in the user community and by the citations received by the peer review publications produced in the coming few years.

Summary of Technical Work

Initially, the project will develop novel electrodes and techniques for the determination of routine species, such as chloride, but on a miniaturised scale. Techniques such as polymer-coated microwires and micropatterned surfaces will be investigated for these devices. The project will then aim to produce ion-selective electrodes for novel ISE species such as heavy metals, using ion selective coatings for the microwires, or the micropatterned surfaces developed in the first stage. Ideally, these would then be small enough to be used in in-situ environments, such as fuel cells, and even in microfludic devices, for very low volume analysis. The application of these electrodes for measuring the leachable content of manufactured nanoparticles in biologically relevant fluids in very small volumes will be demonstrated. The project will then assess acceptable performance criteria for these electrodes, such as stability, selectivity and detection limits. Moreover, protocols for calibrating the response of these electrodes against standard solutions with traceable concentrations, and determining the uncertainty of their measured response, will be delivered. This is especially important at low concentration levels where the contribution of interfering species can be very large. The final stage of the project will be to identify and design flexible and responsive techniques for calibrating these electrodes so that uncertainty can be minimised, particularly when responding to changes in sample composition in on-line and in-situ environments. This will encompass the dynamic tailoring of calibration ranges and frequencies to obtain high accuracy measurements, with a minimum of detector downtime.

The outputs of this work will be disseminated via peer-reviewed publications and attendance at relevant learned society committees (RSC Electroanalytical and SCI Electrochemical Technology Groups). The project will continue to build on existing external collaborations at University College London associated with electroanalytical chemistry and fuel cells, and internal collaboration at NPL with the fuel cell group. Moreover, we will use internal NPL expertise to assist with any micropatterning required for the construction of the miniaturised electrodes. Co-funding is already in place from the Defra LINK Programme Project “PROSPEcT” where there is synergy related to NPL’s deliverable, which is in part aimed at measuring the leachable content and redox potential of engineered nanoparticles in environmental and biological matrices.

Deliverables

No.	Deliverable
1	Provide underpinning traceability for existing ISEs and develop novel miniaturised ISEs for use in in-situ environmental applications
2	Provide traceability, validation protocols and exemplar measurements for these novel electrodes
3	Develop flexible calibration and data handling methods to maximise the accuracy of these measurements
4	Disseminate the outputs of this work via at least three peer-reviewed publications and relevant learned society committees

TA2 Traceable and validated automatic measurements of mercury vapour in ambient air

Contact michael.adeogun@npl.co.uk

Project Objectives

- To develop a validated, traceable method for the automatic measurement of mercury vapour in ambient air in collaboration with industrial partners.
- To address and overcome the scientific challenges required to improve the accuracy of the automatic method to a fit-for purpose level.
- To undertake an equivalence trial to compare the performance of the new automatic method with the existing manual method in order to support new European legislation.
- To disseminate the results of the project via articles in leading peer-reviewed analytical science journals and to support the UK's interests by playing a key role on the relevant European standardisation committee.

Background and Rationale

Measurement of ambient air quality is an essential requirement of modern society due to its role in ensuring the health and improving the quality of life of the general public. These measurements also enable the emissions from industrial sources to be monitored and compliance with European target values to be assessed, as well as informing government policy development and assessing the effectiveness of abatement strategies. Mercury is a particularly toxic and persistent pollutant, and its potential for bioaccumulation means that it is particularly insidious and therefore essential to monitor and manage. Mercury vapour is emitted from a large variety of sources across the UK (see Figure) including coal-burning power plants, crematoria, and the chlor-alkali industry. Another source of great current interest is energy-efficient light bulbs, which release mercury when broken or inappropriately disposed of.

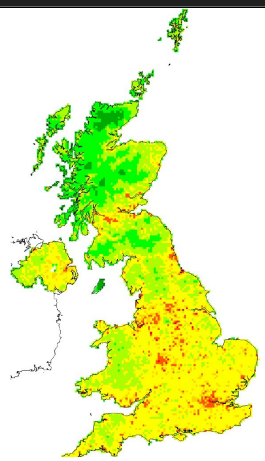


Figure: **Map of UK mercury vapour emissions (red highest, green lowest)**

The concentration of mercury vapour in ambient air at an extensive number of sites across the UK is measured by Defra as part of its UK Heavy Metals Monitoring Network. For these measurements, ambient air is sampled for a period of one to four weeks onto an adsorption tube, which is then sent to the laboratory for analysis. NPL has previously undertaken research to underpin and provide SI-traceability to these measurements and is world-leading in the field of mercury vapour analysis. For example, a recent paper, "Establishing SI traceability for measurements of mercury vapour" (*The Analyst*, 2008, 133, 946-953), won the 2008 Cooperation on International Traceability in Analytical Chemistry Award for the Most Important Paper on Metrology in Chemistry.

A CEN working group (TC264/WG25) is currently developing a standard method for the automatic measurement of mercury vapour (*i.e.* sampling and analysis *in situ*). This improves cost-efficiency and also has the benefit of providing data in shorter time periods (thus enabling identification of any 'spikes' in concentration caused by release episodes). It is expected that this automatic method will be adopted into mandatory European legislation during the three-year period of this project, meaning that work proposed here is both urgent and essential in order to position the UK to meet the challenges this legislation will bring. Full assessment of the validity and accuracy of this method is essential and requires a detailed study of a number of physical and chemical characteristics of the system, such as the dilution behaviour of the calibration system and assessment of the adsorption/desorption characteristics of the gold-coated silica sorbent material. This proposed project is aligned with the CBKB strategy in the Environmental Technologies and Particles areas, specifically addressing the trace analysis measurement requirements. It also aligns well with Defra's Air Quality Strategy for England, Scotland, Wales and Northern Ireland. The consultation exercise recently undertaken with key industrial, academic and government stakeholders identified the following two proposals as having a very high requirement for measurement science: "Development of accurate and traceable methods for automatic mercury measurements in ambient air" and "Demonstration of the equivalence between manual and automatic mercury vapour measurement". This proposed project squarely addresses these issues.

Impact

This traceable and validated automatic method for the measurement of mercury vapour in ambient air developed by this project (in collaboration with UK SMEs) will strengthen the knowledge base in this area, thus impacting upon UK government activity and policy through introduction into the Defra UK Heavy Metals Monitoring Network. This will ensure compliance with forthcoming European legislation and enable the Network to be operated more efficiently whilst also producing more accurate data. It will therefore also impact upon the quality of life of the UK public by ensuring that these measurements can be achieved with much greater confidence.

The completion of the equivalence trial will allow government to make strategic decisions on how to most efficiently organise its existing air quality networks in the future, whilst still ensuring that it meeting its legislative obligations. The data from these networks will also impact upon policy decisions by enabling assessment of compliance through activities such as emissions modelling. Accurate ambient concentration data will also help inform health studies relating to mercury exposure.

Impact on the scientific community will be achieved by the publication of at least three papers in leading peer-reviewed journals. This impact will be assessed through the number of citations these publications receive in future years, and the impact factor of the journals in which they are published. The project will also impact on the UK's standing in European air monitoring by enabling a leading role to be played in the development of standard methods by CEN working group TC264/WG25.

Summary of Technical Work

The initial deliverable of the project will focus on the development of a new, traceable automated method for the analysis of mercury vapour in ambient air. This is a highly challenging goal due to the ultra-trace levels of the analyte in ambient air (typically 1-10 $ng.m^{-3}$). These concentration levels are the reason for the lengthy sampling period (one to four weeks) currently employed for manual measurements across the UK; development of an automatic method with much shorter sampling times (thus enabling the detection of 'spikes' in concentration) will require the use of novel calibration and drift-correction methods.

As part of this work, the accuracy of the method will be optimised through a detailed study of the key factors that contribute to the overall uncertainty, such as: the dilution characteristics of the calibration system, the adsorption/adsorption characteristics of the gold-coated silica sorbent material, the calibration method employed (including the application of a novel drift correction procedure), design of the sampling system, and instrumental repeatability and reproducibility.

In the third technical deliverable of the project, an equivalence trial will be undertaken in order to compare the performance of the new automatic method with the existing manual method. The trial will consist of two stages, one during the winter and one during the summer, and will compare the results from multiple automatic and manual instruments.

The project will support the UK's interests on CEN working group TC264/WG25 and will lead to the publication of papers in leading peer-reviewed analytical science journals. (This follows on from work on the current manual method for mercury vapour analysis, which has seen two publications in the Royal Society of Chemistry's high-impact journal *The Analyst* (*e.g.* vol. 133, 2008, pp. 946-953 and pp. 1611-1618)). A presentation of the work will also be given at a leading environmental conference.

Co-funding of this project will be achieved by funding from CEN (which is already guaranteed).

Deliverables

No.	Deliverable
1	Development of a validated, traceable method for the automatic measurement of mercury vapour in ambient air (in collaboration with industrial partners).
2	Development of a full uncertainty budget for the automatic method, and technical solutions to improve its accuracy.
3	Completion of an equivalence trial to compare the performance of the new automatic method with the existing manual method
4	Dissemination of project outcomes by the publication of at least three peer reviewed publications, presentation at a leading conference, and representation at CEN WG TC264/WG25

TA3 Validated analytical strategies for emerging ambient pollutants

Contact michael.adeogun@npl.co.uk

Project Objectives

- To review the requirements for the measurement of emerging pollutants and the sampling, preparation and analysis techniques in current use
- To validate sampling and sample preparation methodologies for highest priority pollutants
- To demonstrate traceable measurements of these pollutants with full uncertainty statements in real environments
- Dissemination of project outcomes via peer reviewed publications and attendance at relevant standardisation committees

Background and Rationale

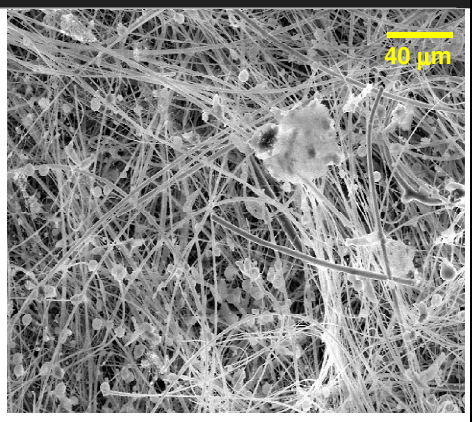


Figure. SEM image of a cellulose ester filter (the long fibres) with particulate matter collected on it.

Air pollution continues to be an issue of great concern to the scientific and medical communities, as well as to members of the general public, the media, and environmental pressure groups. Several requirements drive the need for air quality measurements, including: measuring the exposure of the general population to a variety of toxic compounds; assessing compliance with legislative limits or similar target values; informing policy development and assessing the effectiveness of abatement strategies. In addition, there is a need to provide air quality information for the general public and to inform other scientific endeavours (for example, climate change research), and to provide an infrastructure that can readily respond to new and rapidly changing requirements, such as the specification of new pollutants requiring measurement, or assessment of episodes, such as local, regional or trans-boundary pollution events. The determination of the total concentrations of specific pollutants in ambient air is of great importance within this framework. The general public and the environment can be exposed to several classes of hazardous compounds that occur naturally or are released by domestic or industrial processes. These measurements are challenging since the overall concentration of these pollutants is often very low. In particular, there is a need to respond to recent developments in legislation and policy aimed at UK air quality:

- The new EU air quality Directive 2008/50/EC, requires the measurement of a series of anions and cations in particulate matter (PM), which can be present in large concentrations in ambient particulates (see Figure). T
- The recent report by the Defra Expert Panel on Air Quality Standards recommends the measurement of selected metals and metalloids (such as Be, Cr, Ni and As), which are regarded particularly dangerous to human health and which are a priority for monitoring, some of which are not currently covered by national air quality networks. These pollutants are also identified as priorities by the EU NORMAN network of reference laboratories monitoring emerging pollutants. For these emerging pollutants no standard methods exist and validation work is required, especially in the sampling and sample preparation areas, to underpin the accuracy and traceability of these measurements, prior to these being routinely made as part of air quality assessment. Moreover for some of these pollutants there is little or no data concerning their concentration in UK ambient air.

This proposed project aligns well with the CBKB strategy in the Environmental Technologies and Particles areas, in particular with the measurement requirement identified in trace analysis, and the technology gaps identified in the chemical analysis of particle area. The consultation exercise in the environmental analytical chemistry sub-section highlighted measurements of emerging pollutants as the most important topic with regard to need for measurement science input, with very strong regulatory and quality of life drivers, amongst the relevant stakeholders. The work also aligns well with Defra's recent Air Quality Strategy for England, Scotland, Wales and Northern Ireland (2007).

Impact

This project will deliver accurate and validated methods for the measurements of regulated and emerging pollutants that will be used for environmental and air quality assessment. Moreover strategies will be provided for the sampling and measurement of emerging pollutants that will enable these measurements to be incorporated with confidence into existing air quality networks and environmental assessment infrastructure in the near future. The assessment of measurement uncertainty provided by the project will also enable those making similar measurements to gauge the accuracy of their own measurements, and enable assessment of compliance with data quality objectives for maximum allowable uncertainty specified in regulation. The measurements produced on real samples will also provide a valuable contribution to the UK data-set of ambient pollutants, especially in areas where very little data exists.

The project will also allow NPL to continue to drive forward this work within the context of the BSI shadow committee for CEN and ISO air quality matters – EH/2/3, and lead the UK's input at relevant air quality committees, by hosting and continuing to provide the secretariat for EH/2/3; and to allow the best interests of the UK to be represented on standardisation committees relevant to this project. Impact will be assessed primarily by the extent of NPL's input into the standards development within Europe, and the uptake of the validated procedures by the user community – in particular, other air quality reference laboratories and national air quality networks. Impact will also be assessed by the number of citations received by the peer-reviewed publications produced.

Summary of Technical Work

The project will focus mainly on sampling, extraction and sample preparation strategies, since high precision in the final analytical step is not usually the limiting factor for these measurements. The first step will be to review existing literature, especially any standard methods, for the measurement of the pollutants to be addressed by the project – initially the anions and cations identified in the new EU air quality directive, and the metals and metalloids identified in Defra's recent EPAQS consultation.

The most promising technique(s) will be examined further with a view to providing full method validation. In particular, the effect on the final measurement result on variables such as sampling flow rate, sampling media and sampling time will be investigated with respect to their effect on sampling efficiency and sample degradation. The performance of filter and resin-based sampling against liquid impinger sampling will be assessed.

The technical work will also address the extraction and sample preparation procedures for these pollutants, and determine the most efficient method to extract the maximum quantity of target analyte from the particulate matter. Final analyses for anions and cations will take place using ion chromatography, and for the metals and metalloids using ICP-MS, delivered using techniques validated during previous CBKB projects (or possibly polarography where appropriate). From these studies a full uncertainty budget will be formulated, and the method demonstrated on real ambient samples.

The project will support UK attendance at the proposed CEN committee aimed at standardising the measurement of anions and cations in particulate matter to ensure that the best practice established via this proposed project is disseminated for the benefit of the UK in Europe, and any standard method is consistent with research under this project. We would aim to collaborate with EU air quality reference laboratories (via AQUILA) interested in performing similar measurements.

Deliverables

No.	Deliverable
1	To review the requirements for the measurement of emerging pollutants and the sampling, preparation and analysis techniques in current use
2	To validate sampling, extraction, and sample preparation methodologies for relevant pollutants
3	To demonstrate traceable measurements of these pollutants with full uncertainty statements in real environments
4	Dissemination of project outcomes via at least three peer reviewed publications, attendance at relevant standardisation committees, co-ordination of BSI EH/2/3

TA4 Accurate compositional studies of size fractionated particulate matter to inform health studies

Contact michael.adeogun@npl.co.uk

Project Objectives

- To review existing work on the size fraction dependency of particulate matter composition and identify the key measurands and size fraction divisions where data is currently missing.
- To sample and provide validated measurements of relevant measurands in different particulate matter size fractions.
- To carry out field measurements using these methods in strategic locations.
- To disseminate the results via peer-review publications and attendance at relevant conferences.

Background and Rationale

The size fractionation of ambient particulate matter (PM) is of great interest to the environmental and toxicological communities. Whilst PM_{10} (particles with an aerodynamic radius of 10 microns or less) has historically been assumed to be the inhalable size fraction of PM, it is now clear that the actual size of the particulate matter determines how far into the respiratory system it can penetrate (see Figure) and this consequently determines, which biological structures the PM will end up interacting with. This has now been recognised in Europe and the new EU air quality directive will require Member States not only to measure PM_{10} and $PM_{2.5}$ mass, but also to analyse a sub-set of $PM_{2.5}$ samples for chemical composition—in particular, the most abundant water soluble species.

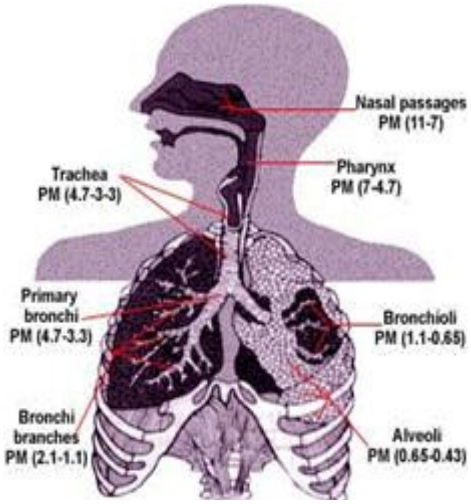


Figure. The extent of penetration into the human respiratory system of the PM_X size fraction.

More importantly, relatively little work has been done to determine and compare the chemical composition of different size fractions of PM. It is thought that composition will vary according to size fraction since, because of their differing sources, different compounds are present at different levels in each size fraction—in particular, those formed as primary or secondary particles. The corollary of this is that a more detailed understanding of the way that PM composition changes with size fraction will help in our understanding of the sources and origins of ambient pollutant in PM, and also add valuable knowledge in working towards mass closure studies for ambient PM.

No standard methods exist for the sampling, sample preparation and analysis of the chemical composition in size fractions below PM_{10} so existing methods would need to be assessed and validated to ensure that they were still suitable for use in this context.

In terms of health drivers, understanding the composition of various size fractions, and which parts of these will be soluble in biological fluids, allows a more detailed knowledge of exactly which chemicals assault different parts of the reparatory system, allowing more focussed toxicological and epidemiological efforts in this area. This work supports the requirements of the UK to provide chemical composition data to Europe for both PM_{10} and $PM_{2.5}$, and has strong regulatory and quality of life drivers. Research into the size fraction dependency of PM was identified as the most important topic in the consultation of relevant stakeholders in the environmental analytical chemistry sub-section, with data analysis methods to enable source apportionment also scoring very highly. The proposed work in this project aligns well with the environmental technologies and particles sub-themes of the CBKB strategies, in particular in the technology areas of chemical analysis of particles, chemometric techniques, and leachable content of particles.

Impact

The project will provide a thorough review of the work previously undertaken in the area and identify where there are gaps in the understanding of the composition of PM with changing size fraction. It will deliver valuable data and validated methodologies in support of the requirement for the UK to provide chemical composition measurements of the $PM_{2.5}$ and PM_{10} fraction of particulate matter. In addition, the data produced from field measurements will provide a useful addition to the data sets in this area that will be of great interest to other environmental scientists, and will provide toxicologists and epidemiologists with a new focus for research into the absorption of potentially harmful chemical particles via the lung.

The project will also examine ways of using the changing composition profile of PM with size fraction to provide more accurate methods for source apportionment of pollutants in PM, by the use of chemometric techniques. This will assist environmental policy makers in developing legislation and assessing abatement strategies.

The results of the project will be disseminated via high impact peer review publications, by attendance at relevant international conferences, and by engagement directly with relevant stakeholders in the toxicology and epidemiology communities and at the European Union Joint Research Centres (JRCs). Impact will be assessed by the extent of the take-up of the outputs of this project in the user communities, especially the EU JRCs, and by interaction with, and dissemination to, key toxicological and epidemiological researchers such as Bob Maynard (HPA), Ken Donaldson (U. Edinburgh) and Ian Mudway (Kings College London). Impact will also be assessed by the citations received by the peer-reviewed publication produced.

Summary of Technical Work

The project will initially involve a thorough review of the existing work in this area. This is expected to highlight gaps in the literature where there is little of no understanding of the composition of particular size fractions, with respect to particular chemical compounds.

Trial samples will be taken using cascade impactors to separate out the various sizes of particulate matter. The size fractions of interest will be analysed for the compounds of interest using either newly developed and validated methods, or existing standard methods for PM_{10} (which will need revalidation for the different size fractions being examined). In particular, the components of interest are expected to comprise metals (measured by stripping voltammetry or ICP-MS), anions and water-soluble metals (measured by ion selective electrodes or ion chromatography) and organic/elemental carbon (measured using existing capability from other CBKB projects).

A campaign of field measurements will then be undertaken to validate these measurements in the field and provide a data set of particulate composition against size fraction at different sites (industrial, rural, urban, roadside) and under different metrological conditions. From this data set, correlations between the compositions of different PM size fraction will be used to attempt to propose methods for source apportionment.

Deliverables

No.	Deliverable
1	Review existing work on the size fraction dependency of particulate matter composition and identify the key measurands and size fractions divisions where data is currently missing
2	Sample and provide validated measurements of relevant measurands in different particulate matter size fractions and
3	Carry out field measurements in strategic locations using the method developed
4	Disseminate the outputs of the project via at least three peer-review publications and attendance at relevant conferences

TA5 Quantitative SERS using optimised substrates and novel platforms

Contact michael.adeogun@npl.co.uk

Project Objectives

- To produce a robust definition and protocol for the determination of enhancement factors from SERS substrates.
- To develop novel structures using functionalised metal nanoparticles which optimise efficacy for trace sensing applications.
- To demonstrate the application of these novel substrates for high enhancement, reproducible SERS.
- To proactively disseminate outputs to the SERS community and encourage consensus on enhancement factor calculation.

Background and Rationale

Surface enhanced Raman spectroscopy (SERS) is beginning to realise its potential as an ultra-trace analysis technique for forensic, homeland security, quality of life and environmental applications, and it is already used in some specialist and research-based applications. NPL has been at the forefront of providing best practice solutions and guidance for these applications over the last few years. However, in most of these cases, SERS is used as a qualitative detection method, to determine the presence or absence of a target measurand. Quantitative measurements using SERS will require the calibration of each new substrate used for measurement. This is because the extent of measurement irreproducibility between substrates, even manufactured using the same procedures, means that calibrations cannot be transferred. It is clear now that to achieve trace analytical measurements optimised substrates are required that achieve a balance between reproducibility and enhancement such that they are viable for ultra-trace analysis, and can also be labelled with an enhancement factor ex-situ, based on the performance of other substrates fabricated using the same procedures. Functionalised metal nanoparticles, structured around a surface or template, have previously shown the most promise for achieving these goals.

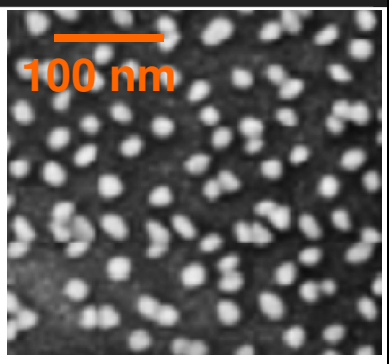


Figure. Electron microscope image of a NPL nano-structured SERS substrate created using silver nanoparticles tethered to a poly-lysine coated glass surface.

From the consultation of relevant stakeholders in the SERS and related technologies sub-section, the areas that scored most highly in both importance and requirement for measurement science were: the development of substrates aimed at better reproducibility and providing traceability for the quantification of enhancement factors. Moreover, there remains a pressing need for validated and traceable measurements in many industry sectors, where quality of life and UK competitiveness drivers are very strong. There is a need, therefore, to develop new SERS substrates (using both theoretical modelling and experimental procedures) which will achieve the enhancement and reproducibility required to deliver quantitative SERS, and also to develop robust theory and traceable methods for determining the enhancement factors delivered by the substrates. Further, the final stage is to use these substrates to address analytes on a practical analysis platform (possibly in a microfluidic device). This project aims to address the first two of these goals, and begin working towards the third, which would enable the application of SERS to a wider variety of analytical chemistry problems.

NPL is well placed to deliver these solutions because of established links and on-going collaborations with major groups at PTB and several UK universities such as Imperial (de Mello, Cohen), Strathclyde (Smith, Graham) and Manchester (Blanch). We also have a track record of highly cited publications in this area—11 in the last 4 years (see Figure)—proactive dissemination via high profile conferences, for example, organising the recent RSC Faraday Discussions meeting on SERS, and widespread take-up of our best-practice methods for practical qualitative SERS. The proposed project also aligns well with the aims of the particles and environmental technologies sub-themes of the CBKB strategy in the areas of trace chemical analysis of particles, functionalised substrates for SERS and calibrationless SERS.

Impact

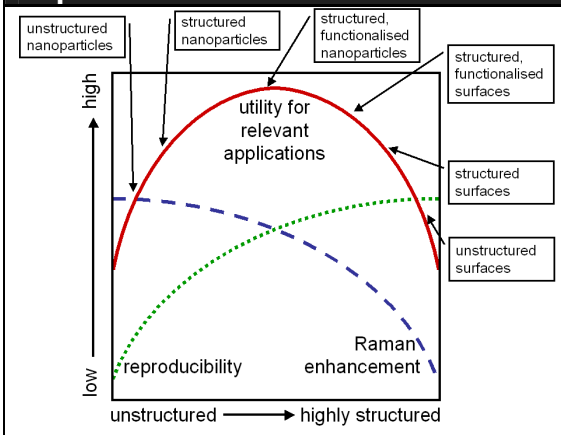


Figure. How different types of SERS substrate rate in achieving the balance between reproducibility and Raman enhancement to optimise utility for relevant applications

A major impact of the project will be to provide a validated method for quantifying enhancement factor with a robust physical basis. This would unify the many methods currently used in the literature such that enhancement and reproducibility properties claimed henceforth for new substrates could be rigorously compared with confidence. Through widespread and proactive dissemination of this method we would aim to work towards a consensus in the SERS community on the use of the method for definition, calculation and dissemination of enhancement factors—this will be a good measure of the impact of this project. The project will also deliver new substrates for quantitative SERS, exhibiting the optimum balance between enhancement and reproducibility to make these suitable for relevant industrial applications (see Figure) and will define the state-of-the-art in this area.

The move towards quantitative SERS by the pre-calibration of reproducible substrates will allow the use of SERS in more demanding applications where concentration level of analyte, rather than just presence or absence, is important. This final stage of the project, to demonstrate the application of the substrate and enhancement calculation techniques on a test system, will give a practical demonstration of these new techniques, encouraging increased take-up within the SERS community. This work will provide underpinning traceability and validation for the quantitative SERS applications in the clinical chemistry area, being developed jointly by NPL and PTB. Impact will also be assessed by the citations received by the peer-reviewed publication produced.

Summary of Technical Work

The work will initially involve an iterative loop of modelling, design and experimental fabrication of substrates in order to work towards the optimum balance of enhancement and reproducibility. It is likely that the initial substrates will use functionalised metal nanoparticles tethered to surfaces, or three-dimensional structures such as membranes, using bi-functionalised linker molecules (such as dithiols). Raman enhancement will be tested to determine three characteristics: 1) the average enhancement of the substrates, 2) the reproducibility between several substrates and, 3) the average repeatability across an individual substrate. Alongside this experimentation we will refine and validate techniques to determine the enhancement factor. This will involve assessing several different physical measures of enhancement, and producing a definition and measurement protocol that will produce traceable enhancement factors, which will be comparable across all substrate types. The techniques and substrates developed will finally be demonstrated in a test system with the aim to demonstrate efficacy and throughput of the analytical protocol developed.

We will also aim to work synergistically with other projects at NPL dealing with tip-enhanced Raman in order to investigate the physical characteristics of individual 'hot-spots' on these new substrates to better understand why and where the very large enhancements on these and similar substrates arise. We will continue our successful collaborations in SERS with PTB (Germany) for the fabrication of novel substrates, and with Imperial College and University College London, for Raman measurements and in order to work towards rigorous determination of the enhancement factor.

Deliverables

No.	Deliverable
1	Review of existing techniques for enhancement factor determination
2	Demonstration of the enhancement, reproducibility and repeatability of functionalised nanoparticle substrates
3	Application of the substrates and techniques developed to a test system
4	Pro-active dissemination of the outputs of the project through at least three peer-reviewed papers, and presentations at relevant international conferences

TA6 Measurements of ultra low permeability barriers for flexible organic electronics

Contact michael.adeogun@npl.co.uk

Project Objectives

- To review the performance of existing methods for measuring water vapour transmission rates (WVTR) through ultra low permeability barrier films for applications in plastic electronics.
- To determine the feasibility of extending NPL's trace water vapour analysis facility to measure WVTR.

Background and Rationale

The inorganic semiconductor industry has advanced rapidly in the past decades. However, due to fabrication cost rising steeply with size, inorganic semiconductor technology is unable to meet the increasing technological demands for large area, low-cost, flexible optoelectronic devices. Organic semiconductors offer a promising solution. Scientific and technological research into these materials has been strongly driven by the fact that they possess the processing and performance advantages for low cost, large area applications with many of the electronic and optical properties of inorganic semiconductors.

In their undoped state, organic semiconductors are usually medium to wide band gap semiconductors with easily tunable optical properties. Consequently they are of scientific and commercial interest owing to their applications in optoelectronic devices such as organic light emitting diodes (OLEDs), thin film transistors and solar cells that are of major importance in the technology drive to secure future energy supply and increase renewable energy. The UK has a world leading academic base in the rapidly growing emerging technology of plastic electronics the worldwide market for which is estimated to grow to £15B by 2015 [DIUS Technology Programme]. The UK's Council for Science and Technology has highlighted plastic electronics as one of six top-priority areas for government support [CST Strategic Decision Making (2007)].

A major obstacle for the introduction of flexible organic electronics into the commercial market is the limited lifetime of devices due primarily to their degradation in the presence of moisture and oxygen. Degradation is primarily due to the formation of non-emissive regions, or dark spot defects in the device. Thermal diffusion of oxygen causes the oxidation of both the metal at the electrode interface and the light-emitting material (for example). Water degradation acts by an electrochemical process, causing oxidation and possible delamination of the electrode. Impermeable barriers are used to minimise the exposure of the devices to the moisture and oxygen in the atmosphere. Barriers usually consist of polymeric substrates with multilayered barrier coatings comprising alternating organic/inorganic layers. A water vapour transmission rate (WVTR) value of 1×10^{-6} g/m^2/day has become the unofficial standard for the OLED industry to achieve a device lifetime of >10,000 hours. This value was originally estimated by calculating the amount of oxygen and water needed to degrade the reactive cathode. An impediment to the development of these barrier materials is the lack of an accurate and traceable method to measure the WVTR of novel structures as they are developed. At present the standard technique used in a variety of industries to measure the WVTR through flexible barrier materials is the "MOCON" test (ASTM F1249). In this method, a dry chamber is separated from a wet chamber of known temperature and humidity by the barrier material to be tested. The dry chamber and the wet chamber make up a diffusion cell in which the test film is sealed. Water vapour diffusing through the film mixes with the dry gas and the concentration of water vapour is measured. However, the limit of detection for the MOCON test is 5×10^{-4} g/m^2/day and therefore it does not have the required sensitivity to measure state of the art ultra barrier layers used in encapsulating devices such as OLEDs. Moreover, the measurement is extremely time consuming. A typical measurement can take a week or more at which point the user often learns that a given substrate lies below the detection limit.

Impact

Advances in barrier quality have challenged the sensitivity limits of available water vapour permeation measurement methods. In order for products such as OLEDs and solar cells that depend on these ultra low permeation barrier materials to be viable, there is a requirement for a method to provide accurate and reliable measurements of WVTR at these low permeation rates. The outcomes of this project will be a review of the performance of existing methods for measuring WVTR of ultra low permeation barriers and to explore the development of an alternative method to provide accuracy, traceability and validation for these measurements. By addressing these issues, we will impact directly on industry involved in the development of ultra low permeation barrier materials for applications in plastic electronics working without references and standard procedures (e.g. The Centre for Process and Innovation Ltd.). As a result, this work will have a significant impact on the plastic electronics industry (e.g. Plastic Logic Ltd., Cambridge Display Technology Ltd., G24 Innovations) as the development of suitable barrier materials is an essential stage in the delivery of flexible, lightweight and cheap electronic products to consumers such as disposable or wraparound displays, cheap identification tags, low cost solar cells and chemical and handheld medical diagnostic devices.

This work aligns with the aims of the technology strategy board to develop technologies needed for the products and services of the future for which plastic electronics is a priority area. This project has a large potential impact on a leading UK high technology sector and works to ensure that the UK is out front as a global leader in the development of new technologies to drive economic growth.

Summary of Technical Work

The technical work will be based on NPL's unique trace water facility traceable to mass that is currently used to address the metrology needs of the specialty gas and microelectronics industries. This has a world leading capability for generating an adjustable level of trace water (between 2 – 2000 nmol/mol with an uncertainty of 3%) by using continuous accurate measurements of mass loss from a permeation device coupled with a dilution system based on an array of critical flow orifices. The trace water vapour facility has been used in a recent Euromet comparison with NIST, NMIJ and PTB to make measurements on a travelling standard to ensure international standardisation of trace water.

Initially this project will review the performance of existing methods for measuring WVTR of ultra low permeation barriers. Methods such as the "MOCON" test (ASTM F1249) and an indirect measurement technique referred to as the "calcium test" will be reviewed. The project will then assess the feasibility of extending NPL's trace water vapour facility as a traceable alternative method for measuring WVTR of ultra low permeation barriers with a target WVTR of 5×10^{-4} g/m^2/day.

Deliverables

No.	Deliverable
1	Review the performance of existing methods for measuring WVTR for applications in plastic electronics and determine the capability of NPL's trace water vapour facility for addressing these measurement issues

NB1					
Project Author/ Contact	**Author: Damian Marshall** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Nanotoxicology - Dosimetry

Project Objectives

- To support UK industry in the development of safer nanomaterials, and products containing engineered nanoparticles (ENPs) by developing analytical tools which can be used to improve nanotoxicity dose measurements
- To develop analytical methods to accurately measure ENP characterisation/concentration/distribution in biological cell culture media, on the cell surface and within the cell
- To develop combinatorial approaches to measure the influence of media and ENP characteristics on cell dose measurements *in vitro*
- To develop a testing strategy that can be used to accurately assess ENP dose-response characteristics.

Background and Rationale

Engineered nanoparticles (ENPs) are already used as key components in over 400 commercially available consumer products and are driving innovation in a diverse range of key industrial sectors that directly impact on human health and well being, such as biomedical image contrast agents, drug delivery agents and biosensors. Despite this widespread use, their unique size characteristics have raised concerns over their potential toxicity and ability to adversely affect human health. The UK nanotechnologies research coordination group (NRCG) chaired by Defra has produced several key research reports relating to the potential harm posed by ENPs. The most recent of these reports in 2007 [characterising the potential risk posed by ENPs] highlights a need to understand characterisation, inter/intracellular transport and localisation of ENPs and their cellular toxicity, as well as recommending special attention be given to methodology and dosimetry issues. The need to develop measurement solutions to predict safety is also highlighted internationally in reports by the OECD [working party on manufactured nanomaterials 2008], the Environmental Protection Agency [EPA – nanotechnology white paper 2007] and the FDA [nanotechnology taskforce report 2007].

Understanding the potential harmful and/or toxic effects of ENPs is complex. Current methods rely on the well developed *in vitro* and *in vivo* testing processes developed for assessing chemical, pharmaceuticals and consumer products. Whilst comparisons can be drawn from these testing regimes, ENPs have unique properties which make measuring their potential toxic effects more problematic. As a consequence it is difficult to predict the human health effects of nanomaterials based on known risks for macrosized particles with the same chemical composition. One of the major problems is the measurement of dose of material to which the cells are actually exposed. One of the fundamental principles of pharmacology is that chemical activity (response) is proportional to the concentration of the affecter molecule at the site of action. Unlike soluble chemicals, ENPs are rarely in a homogeneous form and can aggregate and diffuse according to differences in their density, size and surface chemistry, all of which can change over time in solution. Consequently, there is a need to develop robust dose metrics that give a complete understanding of how ENP and media characteristics affect both *in vitro* cellular dose and corresponding toxicity profiles.

Measuring *in vitro* dose of nanoparticles both at the cell surface and within cells presents unique analytical challenges that are hard to address with a single technology platform. 'Gold standard' imaging methods such as scanning/transmission electron microscopy (S/TEM) and confocal microscopy can measure ENP localisation with varying levels of resolution, but can lack sensitivity. Analytical mass spectrometry methods such as ICP-MS, LC-MS and stable isotope tracing have high sensitivity but lack information on particle localisation, agglomeration status or particle size. A combinatorial approach would precipitate a more complete understanding of the factors which influence nanoparticle dosimetry and allow for improved *in vitro* dose-response assessments.

This project will utilise the international reputation of the NMS in high accuracy mass spectrometry, trace detection of molecules and advanced imaging to develop a combinatorial approach to measure nanoparticles dose response in an in-vitro cell system. The relationship between the nanoparticle dose in the biological media and the dose that is actually delivered to the cells will be quantitatively analysed using mass spectrometry and compared to information on particle size, agglomeration state and cell internalisation obtained using SEM and confocal microscopy techniques and cytotoxicity using standard assay techniques.

Impact

This project will impact the across the nanotechnology sector by proving analytical tools which can be incorporated into testing regimes to measure human health risks posed by ENPs. Assessing the potential hazard/toxicity effect of nanoparticles is limiting both the regulatory processes and public acceptance of nanomaterial safety following a number of recent safety scares. The potential for nanotechnology to improve human health and aid diagnostic and medical procedures is huge. However, this potential can only be realised if the nanoparticles can be shown to be safe and non-hazardous to human health, particularly those proposed for use in nanomedicine applications and consumer products.

Maintaining close links with nanomaterials producers and safety testing organisations, as well as interested groups and regulatory authorities, will ensure extensive transfer of the outputs of this project to key stakeholders within the nanomaterials community. Conference presentations and peer review publications will be used to complement this knowledge transfer to wider interested parties.

Summary of Technical Work

This project will adopt a combinatorial approach to measure the cellular exposure of ENPs in biological media and relate this to cytotoxicity in order to develop methods for accurate dose response analysis. Cell and nanoparticle imaging will be performed using EM, confocal imaging and novel 3D modelling tools to measure cell exposure, nanoparticle agglomeration state and intercellular nanoparticle localisation. This will be used to support mass spectrometry based approach to measure nanoparticles in biological media using ICP-MS, LCMS and/or stable isotope tracing techniques.

Activities

- Establish capability to image ENPs *in vitro* both at the cell surface and within cells using SEM and confocal microscopy
- Development of flow injection and microwave assisted digestion techniques to allow microlitre volumes sample analysis
- Establish capability to use analytical mass spectrometry to characterise and measure ENP concentration/ distribution in biological cell culture media at trace detection levels
- Develop combinatorial methods to assess *in vitro* dose response of cell systems to ENPs
- Production of guidance document for performing accurate nanoparticle dose responses *in vitro*.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW relevant nanoparticles with know toxicity have been chosen and appropriate cell model has been established in house			
2	CEW imaging techniques to measure nanoparticle localisation, internalisation and agglomeration have been investigated			
3	CEW and assessment of ICP-MS, LC-MS and/or stable isotope tracking to measure nanoparticle concentration at trace diction level within biological media before and after exposure to cells has been completed			
4	CEW the use of imaging and mass spectrometry to generate nanoparticle toxicity dose response profiles has been demonstrated			
5	CEW results presented at a conference/peer reviewed for publication, production of guidelines, and KT			
			Total cost	

NB2					
Project Author(s)/ Contact	**Authors: Damian Marshall/Steve Wood** **Contact: julian.braybrook@lgc.co.uk**	**Co-funding target**		**Total Cost**	

Nanotoxicology – Assay Standardisation

Project Objectives

To support the UK nanotechnology community through the development of standardised in-vitro methods which can be used to:

- Aid the development of engineered nanoparticle (ENP) hazard testing procedures by identifying areas of measurement uncertainty in commonly used cytotoxicity assays.
- Assess the suitability of 'classic' basal cytotoxicity and stress response assays to measure ENP toxicity.
- Assess the suitability of standardised methods to be transferred for high throughput screening regimes.
- Produce best practice guidelines for ENP cytotoxicity testing.

Background and Rationale

More than $1.1 trillion worth of products incorporated the use of nanotechnologies in 2007 with this figure set to rise to >$4.0 trillion by 2015 as emergent new materials are incorporated in novel medical and consumer applications [Nanomaterials State of the Market Q3 2008]. The innovation drive behind the develop of new nanotechnologies is dramatically increasing human exposure and raising concerns that harmless bulk substances might turn out to be toxic or carcinogenic in certain fibrous or nanoparticle (NP) forms. At present there is a lack of understanding of the nature and origin of the risks involved in the manufacture and use of engineered nanomaterials. The development of testing strategies to measure the hazard potential of nanoparticles is urgently needed and represents one of the biggest challenges to both material producers and regulatory authorities. Current OECD guidelines are not appropriate to generate reliable data to directly measure NP toxicity. The development of standardised, appropriate methodologies which would allow cross-study and interlaboratory comparisons of NP toxicity would be of huge benefit to the nanomaterial community. This would allow targeted screening of NPs and allow researchers and manufacturers to develop safer product which can exploit the unique characteristics of nanotechnologies.

Many studies have been published which demonstrate the potential cytotoxicity of NPs using *in vitro* cell models. Despite these reports there are still no standardised or validated methods established for nanotoxicity testing. This has led to the publication of conflicting and confusing data and is hindering the development of NP risk assessment strategies. Of the most commonly used measures of NP cytotoxicity developed for chemical and pharmaceutical testing, only one of them, the neutral red uptake (NRU) assay, is actually validated for testing chemicals under the REACH directive. Most of these 'classic' cytotoxicity and stress response assays rely on colorimetric or fluorescent outputs which may be impacted by NPs through absorbance of assay reagent, scattering light or quenching of fluorescent signals. The suitability of such assays needs to be evaluated and standardised methods and protocols developed.

Developing standardised methods for *in vitro* NP cytotoxicity testing is complicated further by the properties of the culture system. Few studies have been performed to measure the impact of these changes. Despite these potential shortcomings, *in vitro* systems still represent the best methods for high throughput analysis of nanoparticle cytotoxicity and understanding the limitations will allow better method development. This has been recognised in a number of international reports by the UK nanotechnologies research coordination group, NRCG, and recently at an European Union workshop [research projects on the safety of nanomaterials: reviewing the knowledge gaps 2008] which recognised specific knowledge gaps in the field of nanotoxicology and called for urgent action to be taken to:

- *Develop validated test methods for nanoparticle toxicity testing*
- *Develop rapid, user friendly, in vitro toxicity screens*
- *Develop guidelines for nanoparticle toxicity testing.*

The NMS is ideally suited to address the issues relating to the development of standardised protocols for NP toxicity testing through its core expertise in cell culture standardisation developed under the MFB 2004-2007 programme (CT1) and CBM 2007-2010 programme (LS3), as well as its role in improving toxicity testing measurements, CBM 2007-2010 programme (DD3). The project will add value to other NMS projects including

the P5 Bio-nanoparticle interaction project as well as internationally through a proposed FP7 project NAPIRA.

Impact

There is an urgent need for the development of appropriate standardised assays to test ENP cytotoxicity and to aid both manufacturers and regulators in assessing their safety. This project will have significant impact in this area by testing the fitness for purpose of 'classic' cytotoxicity assays, identifying the biggest sources of measurement uncertainty and developing assay protocols that can be used to increase both assay robustness and data reproducibility. Ensuring the reproducibility of these standardised assays will be key to their wider take up with the nanotechnology community. To ensure this happens this project will form the basis for an inter-laboratory data comparison exercise, providing the UK NRCG with a small subset of assays available for toxicity testing and subsequent validation.

Interaction will be maintained with key stakeholders in the nanomaterials community to ensure wide dissemination of the outputs of this project. These includes links with key ENP manufacturers, UK health and safety organisations, *in vitro* automated high throughput groups and interested UK organisations. Conference presentations and peer review publications will be used to complement this knowledge transfer to wider interested parties. This project will also complement international research collaborations through a proposed FP7 project NAPIRA.

Summary of Technical Work

This project will prepare and test up to three ENP's contributed by industrial partners and distributed to collaborators for testing. Preliminary analysis will examine the suitability of classic cytotoxicity assays to predict the toxic effect of the ENP's. Examination of the subsequent cytotoxicity data generated in each test site will allow areas of measurement uncertainty to be assessed and standardised protocols to be developed. These protocols will then be used for inter-laboratory data comparisons and to examine their potential for incorporation into automated HTP screens.

Activities

- Prepare, assess for homogeneity and stability, store and distribute to NAPIRA project partners 3000-4000 units of 3 bulk ENP materials according to the principles of ISO Guide 34 (General requirements for the competence of reference material producers).
- Develop a comprehensive list of commonly applied *in vitro* techniques and cell lines used to measure cytotoxicity and stress reactions in response to NPs.
- Decision on suitable panel assays and cell lines which can be developed into standardised methodologies and have potential for high throughput screening.
- Examination of variability of sources of measurement uncertainty in selected models.
- Development of standardised method/s for cytotoxicity measurements, confirmed by interlaboratory trial.

Deliverables

No.	Deliverable	Start	End	Cost
1	CEW when 3 candidate ENP reference materials have been prepared, stored and distributed to collaborative partners for testing			
2	CEW when a suitable panel of cell lines and assays which can be used to test NP toxicity have been selected			
3	CEW when major causes of measurement uncertainty in NP toxicity measurements have been determined			
4	CEW when standard protocols for NP toxicity testing which can be applied to automated HTP screens have been developed			
5	CEW results presented at a conference/peer reviewed for publication, production of standard protocols, and KT			
			Total cost	