



Setting standards
in analytical science

National
Measurement
System



Catalyst

Newsletter published by LGC, the UK's designated National Measurement Institute for chemical and bioanalytical measurement.

Winter 2010

This newsletter contains news and highlights of LGC's activities under the UK National Measurement System Chemical and Biological Metrology Programme. The programme supports industry by improving the accuracy and reliability of chemical and bioanalytical measurements that are fundamental to the UK's international competitiveness. This is achieved through the application of leading-edge science and the development of improved measurement procedures to underpin some of the most challenging and important measurements made in the UK. The measurement needs of industry drive the direction of the programme and the importance of collaboration with innovative companies is recognised. We hope you find this newsletter useful and we welcome your feedback at nmshelp@lgc.co.uk.

Catalyst Editorial Team

Real-time cell measurements for nanoparticle safety testing

It is increasingly important to understand the potential risk that nanoparticles pose to human health. In response to this worldwide need, LGC is developing new *in vitro* testing regimes which can overcome the potentially misleading results obtained from traditional toxicity assays.

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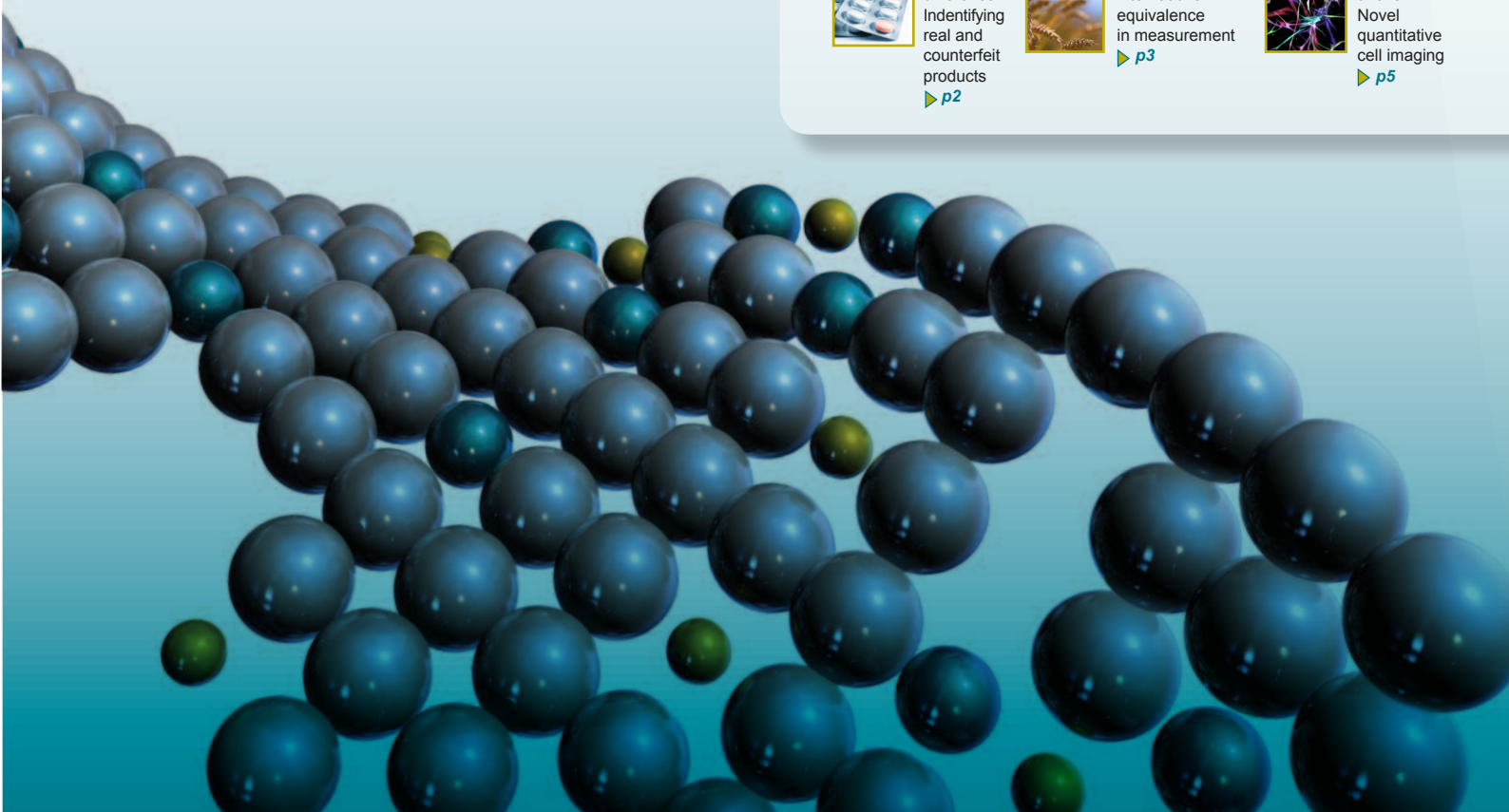
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A round up of LGC's recent activities under the Chemical and Biological Metrology programme

Knowledge transfer for impact

LGC's leading-edge science and the development of improved measurement procedures, underpin some of the most challenging and important measurements made in the UK. These case studies demonstrate how LGC is applying science for a safer world.

Success in molecular measurement

LGC organised a successful workshop on 'Accuracy in Molecular Measurement'. Following high quality presentations from speakers including Professor Stephen Bustin from Barts and the London School of Medicine and Dentistry, Queen Mary, Dr Stefaan Derveaux from Ghent University and Dr Tania Nolan from Sigma Aldrich, the day culminated in a lively discussion about current and new techniques, best practice and achieving accurate molecular measurements.

Establishing international bio-measurements

LGC, in collaboration with INRIM and NIST, is organising one of the first international comparison studies to ensure comparability in measurements to accurately determine cell number when adhered to a solid substrate.

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Supporting innovation in industry

LGC scientists, through collaboration with Reinnervate, a spin-out from Durham University, are growing cells in a novel three dimensional (3D) cell culture technology to imitate the natural structure of the liver and improve *in vitro* screening of potentially toxic compounds.

LGC activities in Eurachem network

For 21 years, LGC has been actively involved in Eurachem; a network of organisations from across Europe working, in cooperation, to provide a focus for analytical chemistry and quality related issues.

Collaboration for performance enhancement

LGC's collaboration with the National Measurement Institute Australia has helped to improve the accuracy of anti-doping programmes in sports.

Real-time cell measurements for nanoparticle safety testing



Nanomaterials are now incorporated into more than 800 commercial products accounting for over £104 billion in sales worldwide. These products impact on every aspect of human life – from paints that stop corrosion, to stain resistant fabrics and nanotechnology-driven catalysts for fuel cells. They are even incorporated into sun creams and antibacterial socks. The increased use of nanotechnologies is driven by the unique mechanical, thermal and catalytic properties that materials develop when structured at the nanoscale. These unique properties, while beneficial for technological innovation, could also make nanomaterials toxic to biological tissues, raising concern they could pose a risk to human health. However, while methodologies have been developed to test chemicals for toxicity, there are currently no standardised methods to measure the toxic effects of nanoparticles.

Standardisation has become a major issue for nano-toxicologists due to the inconsistent behaviour displayed by nanoparticles in traditional *in vitro* screening models, and by the variability in experimental methods employed by different laboratories. While high throughput *in vitro* screening regimes can offer rapid analysis of nanomaterial interactions at the cellular level, nanomaterials can potentially interact with soluble assay reagents, providing misleading results. To overcome this, LGC is validating a label free, real-time, cell electronic sensing system (xCELLigence) to measure changes in cell number following nanoparticle exposure. This technique enables continual analysis of the cells using electrical impedance measurements and provides quantitative information about the mechanisms and

levels of toxicity which can be missed when using traditional assays. This approach further develops standardised methods that can be used to improve the accuracy of *in vitro* data and enable a more realistic prediction of *in vivo* nanotoxicology.

“LGC's real-time nanoparticle toxicity measurements really demonstrate the versatile capabilities of xCELLigence and its potential for the provision of safety assurance in an emerging field.”

Chris Bullion, Technical Representative for Roche Diagnostics Limited, developers of xCELLigence

LGC's work on nanotoxicology was presented at the 2nd NanoImpactNet conference in March 2010 and at Nanotoxicology 2010 in June 2010.

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Spot the difference: Identifying real and counterfeit products

The use of stable isotope ratio mass spectrometry to provide data for forensic investigations has been evaluated by LGC. To be able to use isotopic data as evidence, methods must be validated, reference samples characterised and the uncertainty in results evaluated. LGC has developed methodologies for the high precision measurement of carbon, sulfur, lead and calcium isotope ratios using multicollector inductively coupled mass spectrometry (MC-ICP-MS) in samples of forensic interest such as counterfeit drugs and their packaging.

In collaboration with a leading pharmaceutical company, LGC scientists are using this high accuracy technique to help distinguish counterfeits from genuine drugs and packaging. Subtle variations in isotope ratios can indicate differences in the origin or production of a material. The technique, which consists of a laser ablation system coupled to the MC-ICP-MS, is being used to measure the isotope ratios of calcium and lead present in the ink used on pharmaceutical packaging to discriminate between genuine and counterfeit

products. The same technique is also being used to provide high accuracy sulfur isotope ratio measurements to identify counterfeit pharmaceutical drugs.

Due to the potential impact on health, and the cost to the pharmaceutical industry, counterfeit medicines need to be identified and closely monitored. Current widely used approaches for detecting counterfeits range from simple colorimetric methods, to more advanced techniques such as near-infrared spectroscopy (NIR) and liquid chromatography-mass spectrometry (LC-MS). However, counterfeits are becoming increasingly sophisticated – many contain the correct active ingredient at the right concentration, and their elemental composition is often very similar, if not identical to that of the genuine product. It may therefore be difficult to identify these materials using traditional techniques. LGC has evaluated approaches which combine isotopic data, from isotope ratio mass spectrometry (IRMS) and MC-ICP-MS, with trace metal profiling from ICP-MS to assist with the detection of counterfeit

drugs. The approach can also be used to aid identification of counterfeits from a common source by grouping them on the basis of differences in their elemental and isotopic composition. The research carried out at LGC has demonstrated the potential of the measurement of isotope ratios and elemental composition to be a powerful tool for pharmaceutical counterfeit detection.

LGC's work on the detection of counterfeit packaging is described in the paper 'Application of laser ablation multicollector inductively coupled plasma mass spectrometry for the measurement of calcium and lead isotope ratios in packaging for discriminatory purposes'

R. Santamaria-Fernandez and J.-C. Wolff, *Rapid Commun. Mass Spectrom.*, 2010, 24, 1993-1999.



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LGC facilitates international equivalence in measurement

In order to ensure the international traceability, comparability and acceptance of analytical results, there is a need across all measurement sectors for suitable reference materials to support both instrument calibration and method validation. LGC has a long history of reference material production and certification, and the approach used is now accredited to ISO Guide 34 (General requirements for the competence of reference materials producers). As the UK's

designated National Measurement Institute (NMI) for chemical and bioanalytical measurement, LGC regularly participates in and organises comparison studies to demonstrate measurement capabilities and benchmark against international peers. The CCQM (Consultative Committee for Amount of Substance) of the International Bureau of Weights and Measures (BIPM) coordinates these studies, the results of which are used to establish the equivalence of the measurements

undertaken by the NMIs. This measurement equivalence is essential to enable the production of the reference materials which underpin the measurements required for global trade, to facilitate innovation and demonstrate regulatory compliance.

The following articles describe two key areas – food analysis and clinical measurements – for which LGC is developing reference materials.

Supporting the food industry through traceable measurements



LGC scientists are using their expertise in speciation measurements in order to develop reference materials and reference methods for the accurate quantitation of total selenium and selenium species in selenium enriched foods and nutritional supplements.

Working in collaboration with the National Research Council Canada (NRCC), LGC has co-ordinated a CCQM key comparison study (CCQM-K60) and a parallel pilot study (CCQM P86.1) to accurately determine the mass fraction of selenomethionine (SeMet) and total selenium (at low mg kg^{-1} levels) in selenised wheat flour.

Selenium (Se) is an essential trace element that occurs as several chemical species and SeMet is a common natural food source of selenium. Wheat is one of the most important sources of selenium for humans but the European diet is reportedly lacking in this important element. The production of Se-enriched wheat flour offers an effective bio-fortified food for increased selenium intake. However, in order to protect public safety, reliable product characterisation is essential. Products with higher selenium levels than the maximum tolerable daily intake, or containing selenium in its toxic inorganic form, put the public at risk. Additionally, early studies have indicated that some bio-fortified products are inconsistent in their makeup relative to label indications. It is therefore essential that reliable measurement procedures are available to characterise products. Certified reference materials have a key role to play in the development and validation of methods.

Participation and successful performance in the key comparison study demonstrates LGC's capability to measure selenium and SeMet at low concentrations, in a complex matrix with low levels of uncertainty. LGC is using this measurement capability to develop certified reference materials for selenium in wheat flour and yeast dietary supplement tablets. These reference materials will enable manufacturers of

bio-fortified foods and food supplements to accurately characterise their products for correct levels of selenium.

In order to assist in the characterisation of matrix reference materials, LGC has also recently released an isotopically enriched SeMet reference material (selenomethionine enriched with ^{76}Se) providing an assessed value for the abundance of ^{76}Se . This is needed for the accurate quantification of SeMet by species specific isotope dilution mass spectrometry (IDMS).

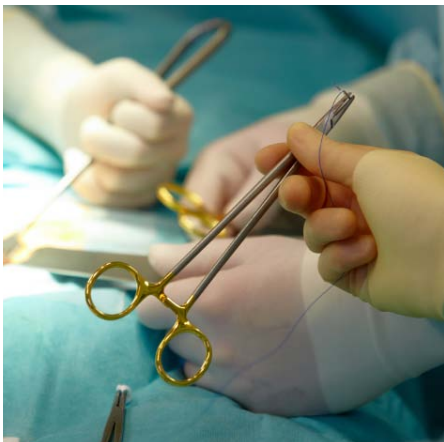
The results from the key comparison study on the determination of selenium species in wheat flour are described in the paper 'Key comparison CCQM-K60: Total selenium and selenomethionine in selenised wheat flour'

(H. Goenaga-Infante and M. Sargent, *Metrologia*, 2010, 47, 08012).

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Supporting healthcare through traceable measurements



The European Directive on *In Vitro* Diagnostic Medical Devices (98/79/EC) requires instrument manufacturers to demonstrate the traceability of values assigned to calibrators to higher level reference materials and/or reference measurement procedures. It is therefore essential that certified reference materials with traceable quantity values and low measurement uncertainties are produced to assist manufacturers in satisfying the objectives of the directive. Additionally, end users making clinical measurements need to ensure the validity of their measurements by routinely employing reference materials during method validation and ongoing quality control.

To meet these needs, LGC has produced a number of certified reference materials aimed at the clinical sector, including immunosuppressant drugs for post-surgery treatment and steroids for diagnosis and treatment of disease:

- Creatinine and electrolytes (Li, K, Ca, Mg & Na), at a range of concentrations, in frozen human serum (ERM DA250-253)
- Testosterone in frozen human serum (6 & 0.3 ng g⁻¹) (ERM DA345 & 346)
- Pure theophylline (ERM AC803)
- Digoxin in frozen human serum (in production, 0.8 & 2 ng g⁻¹) (ERM DA200 & 201)
- Pure digoxin (ERM AC200)

Work is also underway to produce certified reference materials for tacrolimus and sirolimus in frozen human whole blood.

Through successful participation in a number of related CCQM studies, LGC has demonstrated high accuracy measurement capabilities to support the healthcare community. The results from these studies are reported in the following papers:

Final report on CCQM-K69 key comparison: Testosterone glucuronide in human urine
Fong-Ha Liu et al, *Metrologia*, 2010, 47, 08018

Final report on CCQM-K63.a,b: Non-peptide hormones in serum: cortisol and progesterone
S. S.-C. Tai, and D. L. Diewer, *Metrologia*, 2010, 47, 08017

An international comparison of mass fraction purity assignment of theophylline: CCQM Pilot Study CCQM-P20.e (Theophylline)
S. Westwood et al, *Metrologia*, 2009, 46, 08019

CCQM-K12: The determination of creatinine in serum
M. J. Welch et al, *Metrologia*, 2003, 40, 08005

LGC's work on the production of reference materials containing creatinine and electrolytes is described in the paper 'Production and certification of four frozen human serum certified reference materials containing creatinine and electrolytes'

(R. P. Barbagallo et al, *Ann. Clin. Biochem.*, 2008, 45, 160-166).

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A selection of certified reference materials produced with support from the Chemical and Biological Metrology programme.

These matrix reference materials are suitable for the development, validation and quality control of analytical methods:

Chocolate certified for nitrogen, sugars, fat and butterfat content

Processed meat certified for proximates, chloride, nitrate and hydroxyproline content

Potato powder certified for iodine content

Tomato paste certified for cadmium, lead and tin content

Lager, brandy and wine certified for alcohol by volume

Orange juice solutions certified for degrees Brix and Refractive Index

Plant root powder certified for kavain content

Poultry feed certified for proximates and content of a range of elements

Hard and soft drinking waters certified for metals content

River water certified for content of trace elements

Sewage sludge certified for extractable metals content

Automobile catalyst certified for palladium and platinum content

Petrol and diesel certified for sulfur content

Reference materials for the calibration of measurement procedures:

Aqueous solutions certified for ethanol content

Benzoic acid and diphenylacetic acid melting point standards

Kavain certified for purity

Malachite green oxalate and leucomalachite green certified for purity

Nicotine certified for purity

NEW Selenomethionine enriched with ⁷⁶Se with assessed value for the abundance of ⁷⁶Se

Solvent yellow 124 certified for purity

Reference materials are commercially available through LGC Standards, www.lgcstandards.com.



Current publications describing LGC's work carried out under the Chemical and Biological Metrology programme

Validation of reference gene stability for APAP hepatotoxicity studies in different *in vitro* systems and identification of novel potential toxicity biomarkers
B. C. Fox et al, *Toxicol. In Vitro*, 2010, 24, 1962-1970

Achieving metrological traceability in chemical and bioanalytical measurement
V. Barwick and S. Wood, *J. Anal. At. Spectrom.*, 2010, 25, 785-799

Precise and traceable carbon isotope ratio measurements by multicollector ICP-MS: what next?
R. Santamaria-Fernandez, *Anal. Bioanal. Chem.*, 2010, 397(3), 973-978

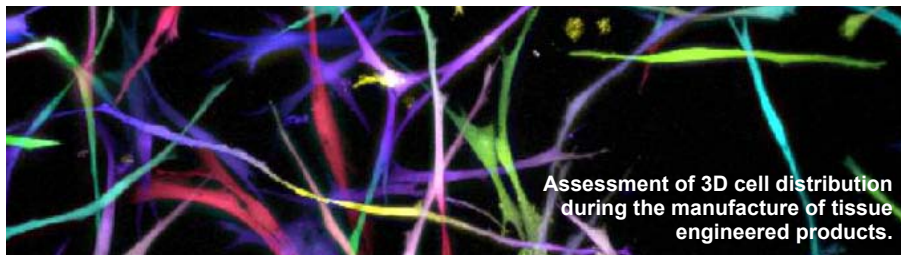
Evaluation of different analytical strategies for the quantification of sulfur-containing biomolecules by HPLC-ICP-MS: Application to the characterisation of ³⁴S-labelled yeast
J. Giner Martínez-Sierra et al, *J. Anal. At. Spectrom.*, 2010, 25, 989-997

The effect of electrospray solvent composition on desorption electrospray ionisation (DESI) efficiency and spatial resolution
F. Green et al, *Analyst*, 2010, 135, 731-737

Digital polymerase chain reaction; new diagnostic opportunities
J. Huggett and D. Scott, *European Pharmaceutical Review, Industry Focus* 10, pp 7-9

Isotopic characterisation of in-house purified progesterone for ¹³C/¹²C isotope ratios by multicollector ICP-MS
R. Santamaria-Fernandez and T. Le Goff, *J. Anal. At. Spectrom.*, 2010, 25, 378-383

Life through a lens: Novel quantitative cell imaging



LGC scientists, in collaboration with leading tissue engineering companies, have developed a rapid quantitative imaging approach to measure the quality of cells in tissue engineered products in order to improve manufacturing, storage and transportation processes.

Tissue engineering is an emerging healthcare technology which brings together biology, engineering and materials science to manufacture functional tissue replacement which can be used in clinical applications. As with any pioneering new technology, regulation is critical for product quality, safety and development. However the novel aspects that make the future of tissue engineering so promising also make complying with regulation more challenging.

Manufacturing processes can affect cells by imparting stresses which they would not normally be exposed to in their natural corporeal environment. This may trigger stress responses in the cells which affect cell behaviour and can, in extreme cases, lead to apoptosis (cell death) thus affecting cell viability and the overall quality of the final product. Determining product quality is a challenge that requires measurement of multiple biological characteristics, so the development and validation of assays are critical for establishing an accurate measure of cell quality.

Fluorescent probe technologies are now routinely used in cell biology to study important cellular events but are usually applied to cells growing in monolayer or in suspension. LGC has validated the use of this technology in combination with laser scanning confocal microscopy (LSCM) to enable multiplexed analysis of cells within 3D tissue engineered products.

Different fluorescent probes can be used to measure biological processes such as active metabolism (indicating cell

viability), DNA intercalation (indicating damaged or dead cells) and the production of reactive oxygen species which is linked to cell stress. In combination with LGC's custom designed image processing platform, measurements can be made both in 3D and in real-time.

Using this approach, LGC has demonstrated how cell viability and cell stress change, not only in response to manufacturing processes but also during storage and transportation. This information can be used to streamline processes, minimising the stress placed upon the cells, and ultimately improve the quality of tissue engineered products. This research contributes to defining metrics and analytical routes to support robust quality control in direct response to the needs of the UK tissue engineering industry.

“This methodology fills a technology gap that is holding back tissue engineering since quality cannot otherwise be confirmed without destroying the product.”

Dr Christopher Bravery, Director of Consulting on Advanced Biologicals

An oral presentation entitled 'The use of stress markers to inform on tissue engineered product quality' was delivered at the 2009 World Conference on Regenerative Medicine.

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Standardising biomarker measurements

Biomarkers are molecules such as proteins, DNA or RNA, which are measured to assist with the diagnosis of disease, monitoring of disease progress, identifying suitable patient treatments and evaluating the efficacy of the treatment. Biomarkers also have a crucial role to play in the development of new drug treatments and are therefore essential for enabling the effective diagnosis and treatment of diseases. However, the rate of approval for biomarker based tests is low with one of the key problems being a lack of robust validation strategies.

The development of a wide range of technologies and platforms for measuring biomarkers makes the evaluation and standardisation of data difficult due to differences in experimental protocols, and in methods of data processing and interpretation.

Much of the work in the area of biomarker validation is in response to the US Food and Drug Administration (FDA) 'Critical Path Initiative' published in 2004. This highlighted the fact that biomarker studies are currently held back by a lack of measurement standards to demonstrate measurement comparability across different platforms. Measurement standards are vital for improving the quality and reliability of diagnostic assays and generic reference materials are needed to enable validation and performance assessment of assays across multiple platforms.

Gene expression profiling is an important approach for detecting biomarkers. In order to accelerate the approval of novel diagnostic biomarkers, and address the issues highlighted by the FDA, it is vital that results from the different platforms used for measuring expression profiles are standardised. To achieve this goal requires suitable reference standards. LGC, in collaboration with NIST (US National Institute of Standards and Technology) and the ERCC (External RNA Controls Consortium), has prepared an RNA reference material from one of the ERCC standards. This RNA reference material has been used

to validate LGC's measurement capabilities and as a preliminary step to producing a commercially available reference material. LGC and NIST have also co-ordinated a CCQM pilot study (P103 Measurement of multiplexed biomarker panel of RNA transcripts) to assess the ability of participating organisations to accurately quantify a single RNA transcript using approaches such as qRT-PCR (quantitative reverse transcriptase PCR) and RT-digital PCR (reverse transcriptase digital PCR). Eleven laboratories participated in the study and overall, good concordance of measurement results was achieved. A report is currently being prepared and the next stage of the pilot study is being planned to include more challenging measurements of multiple transcripts.

In addition to the work on gene expression biomarkers, LGC is also characterising protein-based biomarkers. A panel of generic platform-independent reference standards has been developed and these are currently being characterised and quantified using high accuracy isotope dilution mass spectrometry (IDMS) measurement procedures. The standards are also

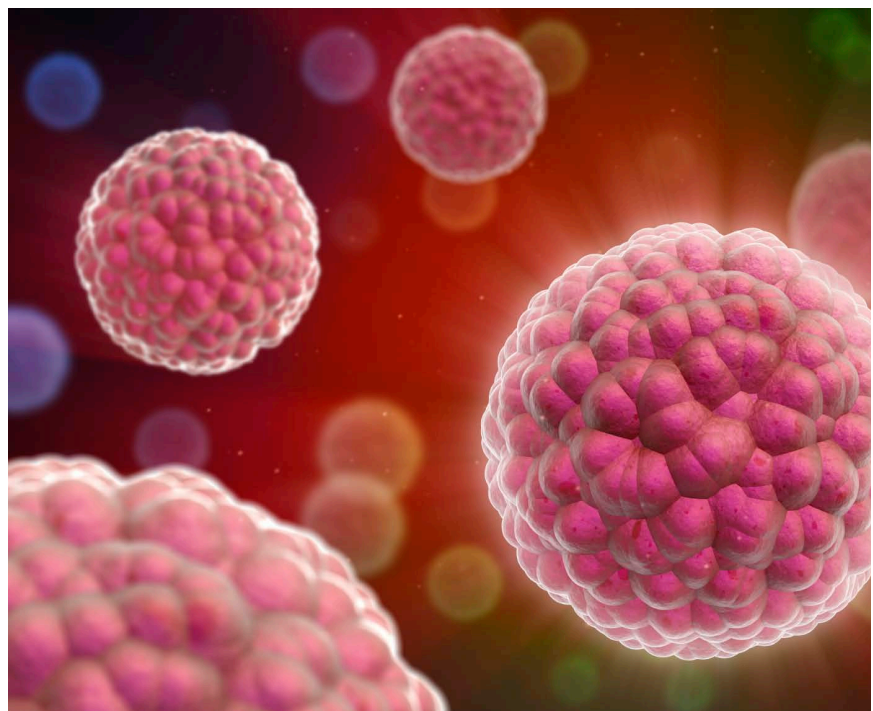
being distributed for evaluation by end users to assess the utility of the materials and obtain feedback on their applicability for a range of real world applications.

LGC's work on biomarkers was presented at the EDQM International Symposium on Pharmaceutical Reference Standards and at the GTCBio 3rd Annual Rediscovering Biomarkers Conference: Shifting the Drug Development Paradigm.

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