Catalyst

Spring 2014

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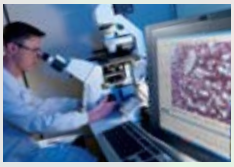

Cover story:
LGC is supporting the cell-based therapy industries by developing methods that underpin cell manufacturing quality. **p8**

This newsletter contains highlights of LGC's activities as a National Measurement Institute under the UK National Measurement System. In this role, LGC 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 our research and the importance of collaboration with innovative companies is recognised. We hope you find this newsletter useful and we welcome your feedback at nmshelp@lgcgroup.com.

Catalyst Editorial Team

LGC

National Measurement System

What's new?

Science with impact

Talk on nanoparticles: Dr Dorota Bartczak (Researcher, Inorganic Analysis) gave a well-received talk on the characterisation of inorganic nanoparticles in food at Queen's University, Belfast (QUB) in January 2014. Dorota described LGC's pioneering work in multidimensional approaches, based on the combination of asymmetric flow field flow fractionation (FFF) with a range of detection techniques, for the characterisation of metal(loid) oxide nanoparticles such as silica (SiO_2) added to food. Given the complexity of nano-objects and of the sample matrix in most foodstuffs, the use of a single measurement technique often results in ambiguous detection and characterisation. In this lecture, Dr Bartczak demonstrated that the use of multi-method approaches is essential to provide comprehensive information and reduced bias.

Training on Digital PCR: LGC scientists led training sessions focussing on analytical quality topics at two conferences in the USA. Dr Jim Huggett (Science Leader, Nucleic Acid Metrology) led a short course on designing digital PCR experiments at the Digital PCR Conference: Technologies and Tools for Precision Diagnostics, October 2013. Rebecca Sanders (Researcher, Molecular Biology) led a session on PCR Quality Control Considerations at the Molecular Medicine Tri-Con in February 2014. The session covered special considerations for dPCR and strategies for experimental design.

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Let's agree to agree - the importance of measurement standards and the role of National Measurement Institutes

Measurement is essential to our everyday lives. We all make daily use of measurements of physical quantities such as mass, length and time. However, the results from chemical and biological measurements are equally important, helping to ensure the safety and effectiveness of our medicines and treatments, and the nutritional content, quality and security of our food supply. Trade also relies on measurement – without reliable measurements it is not possible to establish fair trading between buyers and sellers.

Historically, measurement systems evolved differently across the world. Individual societies developed their own unique systems for measurements used in trade and commerce. However, with the expansion of international trade it became necessary to agree on a set of measurement units that are recognised throughout the world. The resulting system – the International System of Units (SI) – specifies a set of seven base units from which many other units of measurement can be derived.

We take it for granted that if we fill a car with petrol the volume will be delivered accurately, and that we can set our clocks to a reliable national time standard. But this doesn't occur by accident. Reliable measurements require suitable measurement standards to enable measurement results to be linked to appropriate references, such as the definition of an SI unit. Results that can be linked back to a suitable reference are said to be 'metrologically traceable'.

Fundamental to the development and maintenance of the measurement standards required for all types of measurement is the worldwide network of National Measurement Institutes (NMIs). These institutes are responsible for developing and maintaining national measurement standards for their country, and ensuring that the standards are traceable to internationally recognised references. Measurement standards give results meaning and enable the comparison of results, regardless of when or where the measurements were made. The NMIs, through the standards they produce, provide an infrastructure for global confidence in measurements.

In the UK, LGC is the designated NMI for chemical and bioanalytical measurements. Typically an NMI is responsible for:

- Dissemination of sound measurement science by providing the tools to assist organisations in achieving accurate measurement results;
- Research in metrology and the development of new and improved measurement methods and measurement standards;
- Providing advice to government to ensure sound policy and regulation;
- Maintaining a general overview of the national calibration/traceability hierarchy.

Some of the key activities in support of the above include:

- Development of high accuracy measurement procedures that can be used to assign values to measurement standards;
- Participation in comparisons at the highest international level to demonstrate and confirm measurement capabilities;
- Production and development of certified reference materials;
- Provision of calibration services;
- Dissemination of measurement best practice through protocols, standards and harmonised procedures.

Establishing measurement traceability and demonstrating comparability between National Measurement Institutes

The International Bureau of Weights and Measures (BIPM) operates under the supervision of the International Committee for Weights and Measures (CIPM). Its mandate is to provide the basis for a single, coherent system of measurements throughout the world, traceable to the SI. The CIPM has set up a number of Consultative Committees which bring together experts in particular fields of measurement. The relevant Consultative Committee for chemical measurements is the Consultative Committee for Amount of Substance: Metrology in Chemistry (CCQM). The National Measurement Institutes establish the equivalence of their measurements through 'Key Comparison' studies coordinated by the Consultative Committees. These studies involve the analysis of a well characterised substance by participating NMIs, to determine whether individual performances are comparable. This measurement equivalence is essential to enable the production of the reference materials required to maintain the international measurement infrastructure outlined above.

Scientists at LGC use a wide range of analytical skills to participate in CCQM studies

LGC, as a designated NMI, regularly leads and participates in these key comparison studies. Recently LGC has coordinated a key comparison study to measure a range of analytes, including potassium, calcium, iron, magnesium and selenomethionine, in a serum matrix (CCQM K107). LGC also participated in CCQM study K98 "Pb (lead) isotopes in bronze", to develop a method to determine isotope ratios of lead in bronze co-ordinated by the German Federal Institute for Materials Research and Testing (BAM), and CCQM Study K104 "Avermectin – Characterisation of Organic Substances for Chemical Purity" where HPLC, LC-MS and qNMR methods were optimised and used to identify impurities in the test material.

New projects supported by the National Measurement System

Traceability for mercury measurements: This project aims to establish the required metrological infrastructure for mercury measurements in environmental samples, needed for current and future legislation aimed at controlling mercury emissions and releases. Mercury in its many chemical forms is highly toxic to human, animal and plant health as a result of its ability to accumulate in terrestrial and aquatic bio-systems.

'Point-of-test' technology for rapid and accurate screening for trace levels of drugs and explosives: National security screening is a challenging area due to the ever expanding array of terrorist threats combined with the complexity and size of modern transport systems. The adoption of more sophisticated technology is key to combating these threats and maintaining public security. This project will be investigating the potential of transportable mass spectrometry as a new, more robust 'Point-of-test' technology for rapid and accurate screening for trace levels of drugs and explosives at airports and other borders.

Developing mass spectrometry-based methods for protein clinical diagnostics: Immunoassays are widely used in routine clinical laboratories; however, they are known to suffer from cross-reactivity due to antibodies recognising different antigens present in the same sample. In order to establish a higher level of accuracy and precision in routine clinical measurements, reference measurement procedures are required. An essential component of this activity is to understand and identify which protein(s) are being measured in immunoassays. This project aims to develop mass spectrometry-based methods to facilitate the definition of the measurand(s) in protein clinical diagnostics and to assist identification of the major sources of variability in routine analysis.

For further information on these projects please contact the NMS helpdesk: nmshelp@lgcgroup.com

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Microbial profiling workshop: On 1 May 2014 LGC hosted a training event focused on the use of Next Generation Sequencing (NGS) for the detection of human infectious disease. The event, "Pathogenic microbes sequencing workshop", was the second instalment of LGC's "Microbial Molecular Profiling" series, following on from the successful workshop organised in April 2013. The workshop was attended by post-doctoral scientists, facility managers and principal investigators with an interest in introducing the technology into their laboratory, and provided an overview of current NGS strategies and potential applications, as well as the challenges faced by those who are applying this technology to study infectious diseases.

New technical guide: LGC has published a "Good practice guide for the application of qPCR", aimed at individuals who are starting to use qPCR and realise that, while this method is easy to perform in the laboratory, numerous factors must be considered to ensure that the method will be applied correctly and to ensure the quality of the analytical data. See page 5 for further details.

New training courses: LGC is enhancing its long running programme of training courses in analytical quality with the addition of courses to help testing laboratories implement and maintain quality management systems as well as helping analysts take advantage of software packages to analyse data efficiently and effectively. The new courses "Understanding ISO/IEC 17025, requirements for analytical laboratories" and "Using Excel spreadsheets for data analysis in testing laboratories" will help customers meet regulatory demands and produce reliable data. Details of LGC's full analytical quality training programme are available at www.lgcgroup.com/training

Published papers

Recent papers co-authored by LGC scientists

- Huggett JF and Whale A, Digital PCR as a novel technology and its potential implication on molecular diagnostics, Clin. Chem., 2013, 59(12), 1691-1693
- Malinovskiy D, Dunn P and Goenaga-Infante H, Determination of absolute $^{13}C/^{12}C$ isotope amount ratio measurements by MC-ICPMS using calibration with synthetic isotope mixtures, J. Anal. At. Spectrom., 2013, 28, 1760-1771
- Sanders R et al, Evaluation of digital PCR for absolute RNA quantification, PLOS ONE, 2013, 8(9): e75296. Doi: 10.1371/journal.pone.0075296
- Pritchard C, O'Connor G, Ashcroft AE, The role of ion mobility spectrometry-mass spectrometry in the analysis of protein reference standards, Anal. Chem., 2013, 85(15), 7205-7212
- Nischwitz V et al, Speciation studies of vanadium in human liver (HepG2) cells after in vitro exposure to bis(maltolato) oxovanadium(IV) using HPLC online with elemental and molecular mass spectrometry, Metallomics, 2013, 5, 1685-1697
- Annesley TM et al, Standardization of LC-MS for therapeutic drug monitoring of tacrolimus, Clin. Chem., 2013, 59(11), 1630-1637
- Cryar A et al, Towards absolute quantification of allergenic proteins in food – lysozyme in wine as a model system for metrologically traceable mass spectrometry methods and certified reference materials, J AOAC Int., 2013, 96(6), 1350-1361
- Bustin SA et al, The need for transparency and good practices in the qPCR literature, Nat. Methods, 2013, 10, 1063-1067
- Kuselman I et al, House-of-security approach to measurement in analytical chemistry: quantification of human error using expert judgments, Accred. Qual. Assur., 2013, 18 (6), 459-467
- Thompson M and Ellison SLR, Towards an uncertainty paradigm of detection capability, Anal. Methods, 2013, 5, 5857-5861
- Taylor A et al, Atomic spectrometry update: Review of advances in the analysis of clinical and biological materials, foods and beverages, J. Anal. At. Spectrom., 2014, 29, 386-426

Standardising measurement in molecular biology

Promoting transparency and good practice in qPCR publications

LGC scientists, in collaboration with other leading molecular biologists, have evaluated the quality of scientific publications concerned with the measurement of RNA and DNA by qPCR.

PCR is a technique widely used in molecular biology to identify and quantify DNA. The technique can be very powerful in elucidating gene and transcriptome profiles for greater understanding of biological processes, and there is a widespread belief that it is easily mastered. To achieve reliable results with qPCR, however, requires fine-tuning of the measurement process with an understanding of all the factors that may influence the results. In order to encourage increased transparency in reported data, the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines were developed and published in 2009 [1]. These guidelines outline the essential and desirable experimental information that should be included in any peer reviewed publication reporting qPCR data, to allow critical assessment by the reader as well as the opportunity to reproduce experimental methods.

LGC's bioanalytical metrology experts have collaborated on a project to evaluate the quality of qPCR research published over the last five years. In total, over 1700 publications were assessed in two surveys that investigated how different journals faired when quality was assessed, and whether the MIQE guidelines were impacting on the scientific community by increasing the transparency and rigour of molecular research. This study, published in Nature Methods [2], has shown that the quality of reported qPCR data cannot be evaluated in a high percentage of publications owing to a lack of transparent reporting of technical and quality-control details. This deficiency makes it difficult to assess the biological or clinical relevance of the results. Reporting standards were found to be significantly improved in publications that cite the MIQE guidelines, however, not all papers citing the guidelines contained all the essential technical information. Overall, the findings from the surveys suggest that improvement is needed in both the uptake of the guidelines and the reporting standards to enable comparability and reproducibility of experimental methods.

LGC scientists have continued to collaborate on guidance aimed at improving the quality of molecular measurements and recently led the development of guidelines on the publication of quantitative digital PCR (dPCR) experiments [3].

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[1] Bustin SA et al, The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments, Clin. Chem., 2009, 55(4), 611-622

[2] Bustin SA et al, The need for transparency and good practices in the qPCR literature, Nat. Methods, 2013, 10, 1063-1067

[3] Huggett JF et al, The digital MIQE guidelines: Minimum information for publication of quantitative digital PCR experiments, Clin. Chem., 2013, 59(6), 892-902

LGC publishes a new good practice guide for the application of qPCR

Currently, quantitative real-time polymerase chain reaction (qPCR) is regarded as the 'gold standard' in the quantitative analysis of nucleic acids, be it DNA, RNA or micro-RNA molecules. The main reasons for its success are its high sensitivity, robustness, good reproducibility, broad dynamic quantification range, and very importantly, affordability. However, completing qPCR assays to a high standard of analytical quality can be challenging.

qPCR has a large number of applications in a wide range of areas, including healthcare and food safety. It is therefore of paramount importance that the results obtained are reliable in themselves and comparable across different laboratories.

LGC scientists, in collaboration with Sigma-Aldrich™, have produced a good practice guide on the application of qPCR. The guide is aimed at individuals who are starting to use qPCR and realise that, while this method is easy to perform in the laboratory, numerous factors must be considered to ensure that the method will be applied correctly.

The guide is freely available from the NMS Chemical and Bioanalytical website

Standardising RNA measurements

RNA molecules play a crucial role in gene expression and function, and therefore are useful biomarkers for the diagnosis and monitoring of both communicable (infectious) and non communicable diseases. Quantification of RNA, both for gene expression (e.g. messenger RNA) and viral (e.g. HIV) measurement, is widely employed in molecular biology and increasingly important in diagnostic fields. With current technologies RNA is measured indirectly, as it needs to be converted into complementary DNA before it can be amplified and quantified in a PCR reaction. This conversion step, Reverse Transcription (RT), can be both inefficient and imprecise contributing significantly to the uncertainty in measurement results, even when using validated commercial kits.

Digital PCR (dPCR) is increasingly recognised as an extremely precise and reproducible technique, offering the potential for accurate, robust and highly sensitive measurements. It provides absolute quantification, through the measurement of individual RNA or DNA molecules, rather than relative quantification offered by more conventional qPCR methods.

LGC scientists have completed a novel study which provides insight into the sources of variability in quantitative RNA analysis using dPCR by assigning comprehensive uncertainty values; as well as demonstrating how standardisation and protocol harmonisation reduces measurement variability.

This study investigated the causes of variability when measuring RNA. While there have been a number of studies that characterise dPCR capabilities for DNA quantification, there has been little work to investigate similar parameters using reverse transcription RT-dPCR for RNA analysis. While the variation observed between the different RT-dPCR kits was not unexpected, given the variability that is already known to be present in RT-qPCR experiments, it was the level of fundamental measurement biases within the data for each kit group that this study exposed, for the first time (Figure 1).

The study further supports the need for quality control materials and reference materials for measurement of RNA target molecules. It is also clear from the study's findings that there is an overwhelming need to standardise measurement procedures to ensure more robust, accurate and meaningful results. This is especially important for studies that involve comparisons between multiple laboratories.

Rebecca Sanders, Researcher in Molecular and Cell Biology at LGC, concludes "The wider issue we are concerned with at LGC is the variability and biases in RNA and DNA measurement. We are working to identify the points in the experimental process at which these errors may occur. Ultimately, we want confidence that what is measured is actually a true reflection of what is there, and that any differences observed due to natural biological variation can be differentiated from the technical error."

Read more

Sanders R, Mason DJ, Foy CA, Huggett JF, Evaluation of digital PCR for absolute RNA Quantification, PLOS ONE, 2013, 8(9): e75296. Doi:10.1371/journal.pone.0075296

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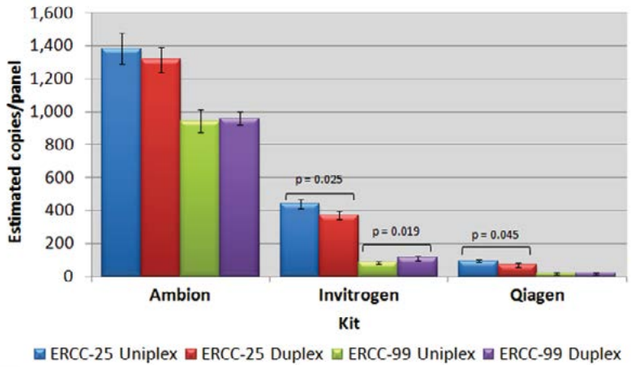

Figure 1: One-step kit comparison. Three different one-step RT-qPCR kits were compared in both uniplex and duplex formats, by dPCR. Two external targets, ERCC-25 and ERCC-99 were analysed. Error bars: 95% Confidence intervals. n=3 replicate panels. Equivalent UV estimates: ERCC-25 1185 copies/panel, 95% CI 17.34. ERCC-99 1185 copies/panel, 95% CI 26.19.

Reducing production time and cost - new ways to characterise organic reference materials

Most analytical measurements are the result of comparison between the sample of interest and a reference. In analytical chemistry, the reference will often be a 'pure substance reference material' – i.e. a compound of specified purity. The amount of analyte in a test sample is determined by comparing the value obtained for the sample (e.g. absorbance at a particular wavelength in spectroscopic methods) with the value obtained for the reference material, obtained under the same conditions. When the property value assigned to a reference material (for example %purity) has a stated uncertainty, is traceable to the SI, and has been characterised using primary methods, the material is classed as a 'certified reference material' (CRM).

This raises the question – how can a pure substance CRM be characterised if it is the first reference material for a particular compound? Such CRMs are generally not 100% pure, and in some cases contain up to 20 minor impurities. The traditional approach to assigning a purity value is to quantify every impurity present in the sample and to subtract the total impurities from 100%. It is a major undertaking to resolve, identify and accurately quantify each impurity while retaining SI traceability, resulting in a significant analytical burden.

Most detectors used in chromatographic systems (e.g. UV, mass spectrometry) do not have constant response from one analyte to another. Accurate quantitation of impurities is therefore impossible without reference materials of known purity for each of those impurities to allow for calibration of response factors. Rarely, if ever, are reference materials available for all the impurities present in a material and therefore a considerable uncertainty needs to be incorporated into the purity value assigned to the material to account for this.

While traditionally this has been the most effective approach to organic purity determination, the need to measure many separate analytes to infer the purity of the main component makes it a slow and expensive process, not conducive to producing reference materials quickly and cost-effectively.

The chromatography industry has strived for many years to generate a 'universal detector' that will give equal response to different analytes. Such equivalence in detector response would not only allow the impurities to be quantified directly, but also potentially enable direct assays against a certified reference material of an unrelated compound. While several technologies have shown promise, none have the range of applicability and performance required to solve the problem of quantifying multiple impurities.

Quantitative nuclear magnetic resonance technology (qNMR) is currently gaining traction as a source of direct assays for purity determination. The simple 1H qNMR assay has been used for many years for niche applications, particularly in high throughput areas but is now gathering momentum in the higher accuracy, metrology world due to its ability to provide measurement results that are directly traceable to the SI.

LGC has been successfully employing this approach in parallel to conventional analysis for several years to provide additional confidence in the purity of many of its small molecule organic CRMs and establish the credibility of the technique in its own right. The major challenge of qNMR technology, however, is the complexity of the NMR spectra for all but the simplest of molecules. LGC has been developing methodologies to minimise the uncertainty of measurement in qNMR assays to allow its exploitation over a much larger range of analytes.

Reference materials recently produced by LGC

New materials

ERM-FC030a	Phenyl salicylate certified for melting point
LGC7220	Horsemeat authenticity standard for meat species work
LGC7240	1 % Horsemeat in beef for authenticity testing of species present
LGC7241	10 % Horsemeat in beef for authenticity testing of species present
LGC7242	1 % Pork in beef for authenticity testing of species present
LGC7243	10 % Pork in beef for authenticity testing of species present
ERM-AC410a	40 % alcohol solution certified for density and alcohol content (50 mL unit size)

Replacement materials

LGC7221	Beef authenticity standard for meat species work
LGC7222	Pork authenticity standard for meat species work
ERM-AC406f	40 % alcohol solution certified for density and alcohol content
ERM-AC407d	70 % alcohol solution certified for density and alcohol content
ERM-AC409b	20 mg/100 mL ethanol solution certified for ethanol content

The need to quantify the purity of larger molecules, such as peptides, rules out 1H qNMR in all but a few cases so alternative NMR approaches that allow improved peak resolution are being employed. Techniques such as HSQC (heteronuclear single quantum coherence spectra) allow simplification of the NMR spectra and resolution of peaks by selective filtering of the NMR signals and dispersion in a second frequency domain. However, the direct application of these alternative NMR experiments has the potential to generate the type of analyte specific bias that the use of NMR is trying to avoid. Therefore, modified variants are being developed to minimise this bias and quantify any remaining uncertainty.

By careful matching of NMR signals from the analyte and qNMR reference standard so they possess similar coupling constants, multiplicities and correlation times, these biases can be reduced to less than 20%. The additional improvement required to deliver the accuracy required in most quantitative applications is being met by the calibration of the signal attenuation during the experiments (time zero HSQC). Such approaches have been shown to reduce the amino acid residue specific bias within each peptide from 20% to less than 1%.

Whilst the minimising of the uncertainty associated with such measurements is very important, it is the ability to assign a value to that uncertainty that makes these methods suitable for use in characterising reference materials. Such improved approaches to purity determination are showing great potential to speed up the development of the next generation of complex organic reference materials.

LGC's expertise in the application of qNMR is increasingly being recognised at the national and international level, which has led to collaborative initiatives with other National Measurement Institutes to help them improve their technical capabilities in this area. LGC has also been invited to be part of a working group tasked with delivering an IUPAC technical report on the SI (International System) Value Assignment of Purity of Organic Compounds.

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Underpinning quality in cell manufacturing

The development of advanced cell-based therapies as a tool to combat tissue damage and disease is revolutionising healthcare, and is a potential growth area for the high value manufacturing industry in the UK. Identified by the government as one of the eight great technologies, regenerative medicine is one of the areas where the UK has the potential to be a global leader. The research projects described in this feature can assist in unlocking the potential of new products, and help the UK cell-based therapies industry bring products to the market more quickly.

Quality control in cell manufacture – keeping cells under control

The cell therapy industry relies upon the production of large batches of cells which need to follow strict quality controls. However, the large-scale production of cells presents a range of technical challenges:

- Cells need to have a high proliferation potential in order to reach the cell densities required for therapeutic application. This is why immature stem cells, with high proliferation rates, are often used.
- Expansion from a relatively small cell population into an industrial batch can lead to the amplification of small initial biological differences, eventually leading to significant batch-to-batch variability and low potency in the cell product.
- Scaling up cell culture leads to high density cell cultures, which result in more stressful growth conditions for the cells, altering their proliferation, their differentiation, and ultimately their overall quality.
- There is currently no generic method to monitor and measure the quality of cells manufactured in such conditions because each clinical application requires a unique stem cell source and processing, and monitoring is often performed subjectively by experts in the field.

LGC has already developed and tested a generic approach to rapidly authenticate cell products by measuring the expression of cell membrane markers. This innovative method relied upon labelling generic membrane proteins (expressed on all cells, but in differing amounts depending on the tissue of origin) with fluorescently-conjugated antibodies. The fluorescence signals, proportional to the amount of markers in the cell membrane, were accurately quantified by high-throughput flow cytometry. The robustness of the measurements was enhanced by the introduction of calibration beads combined with a proprietary automated normalisation procedure, which further decreased the variability of the measurements.

LGC is now testing the application of this method for the monitoring of scaled-up cell culture for industrial and clinical applications. During the current project, "Phenotypic Profiling for Stem Cell Manufacture", the concept has been primarily tested on stem cell models. Stem cells were grown in a range of conditions replicating cell manufacture, and were induced to differentiate along the neuronal lineage. They were cultured in both conventional two-dimensional (2D) culture flasks and onto three-dimensional (3D) microcarriers in spinner flasks, to mimic actual industrial processes. In addition, chemically-induced stress was used to trigger and control cell apoptosis (programmed cell death), which typically occurs when cells are grown in high-density, and when nutrients are rapidly depleted from the medium. A range of 16 markers were screened to cover all aspects of cell quality, covering undifferentiated stem cell state to differentiated state, and stress related phenotypic changes. The combination of undifferentiated and differentiated stem cells, 2D and 3D culture systems, unstressed and stressed conditions, offered a suitable and comprehensive model for testing cell quality from large scale cultures.

The flow cytometry phenotypic profiling (Figure 1) allowed the identification of a subset of particularly stable markers which related directly to cell quality. The ability to measure such markers should guide and stimulate the development of quantitative release specifications for batches of cells grown industrially and for clinical applications.

LGC scientists will use the knowledge generated in this project to support cell therapy applications in a number of scenarios. Haematopoietic (blood-making) stem cells from cord blood will be tested, with the aim of defining release criteria to improve the outcome of cord blood transplants and helping to optimise national cord blood resources.

LGC will also apply its measurement capability to cells produced by a biotechnology company that has developed stem cell lines which can be turned into hepatocytes (liver cells) for in vitro drug testing, as an alternative to animal testing. Finally, LGC are discussing providing assistance to the Cell Therapy Catapult in validating cell-based assays which are currently used to give a competitive edge to the UK biotechnology and regenerative medicine industry.

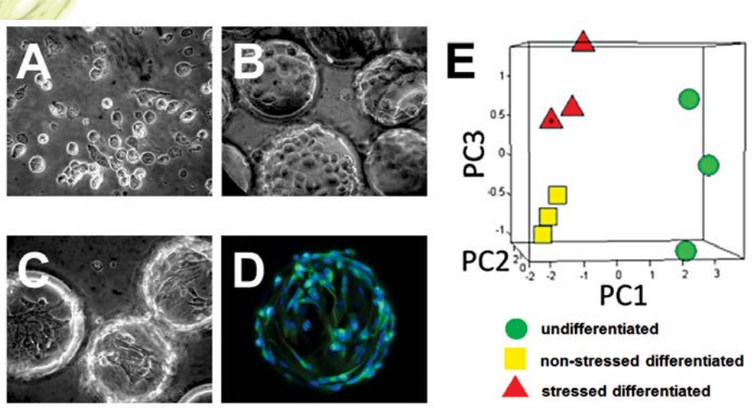

Figure 1. Neural stem cells were grown undifferentiated in 2D flasks (A) and at the surface of Cytodex 3 microcarriers in spinner flasks (B). They were expanded and induced to differentiate into neural cells (C), which was confirmed by a change of morphology and the expression of the mature neuronal marker βIII-tubulin (D, in green, with cell nuclei in blue). Chemical stress was also used to stimulate cellular stress and trigger apoptosis, mimicking stress when scale up production goes wrong. Principal component analysis of the phenotypic profiles (E) demonstrated that the test was discriminating between undifferentiated, differentiated, and stressed cells.

Getting to know single cells

Development of successful cell-based therapies requires precise knowledge about the quality of the cell lines used in the manufacturing processes. Current standard techniques for analysing large cell populations are hindered by the fact that subtle differences between cells, which together maintain the fine balance of intercellular dynamics in cell populations, are blurred into an average picture of the global population. This is therefore an artificial representation of the true, discrete nature of the underlying single cell components. Furthermore, when using standard analytical approaches, fundamental differences between rare cancer cells in a much larger background of healthy cells can be masked because the current approaches do not have the sensitivity required to pick up the smallest traces of target analytes.

Single cell analysis represents a major step forward in the field of cell biology, potentially overcoming some of the limitations associated with intrinsic variability in, and 'background noise' associated with, whole cell populations.

LGC scientists, using cutting edge technologies, have pioneered the first extensively characterised analytical workflow that can be used to reliably analyse gene expression patterns in undisturbed single cells with a high success rate.

This analytical method has been thoroughly optimised to alleviate some of the current drawbacks of single cell analysis. Such drawbacks include the extensive – potentially stressful – cell handling procedures required to isolate live individual cells prior to their analysis, such as the enzymatic detachment from culture dishes, circulation of the cells through microfluidic systems, and long incubation time once in suspension. Other significant downsides include the lack of reference analytes or cells, and most importantly a fundamental lack of knowledge of the inherent cell-to-cell variability at the discrete cell level.

In order to alleviate these shortcomings, LGC has partnered with a UK-based world leader in regenerative medicine specialising in the treatment of stroke patients. Stroke is the third largest cause of death and the single largest cause of adult disability in the developed world. This partnership provided LGC with access to the company's clinical cell line, a highly homogeneous and well characterised undifferentiated (immature) neural stem cell population, as well as a sister cell line, a not-so-potent clonal line isolated from the same donor tissue in the early days of the product development.

Because of their immature status, these cells could be biochemically forced into differentiation (maturation into more adult forms), thus exacerbating their initially small differences. This tuneable model proved ideal for testing a range of hypotheses relating to cell-to-cell homogeneity and biological variability, and subsequently to allow differentiation between biological variability and the biases introduced by the analytical process.

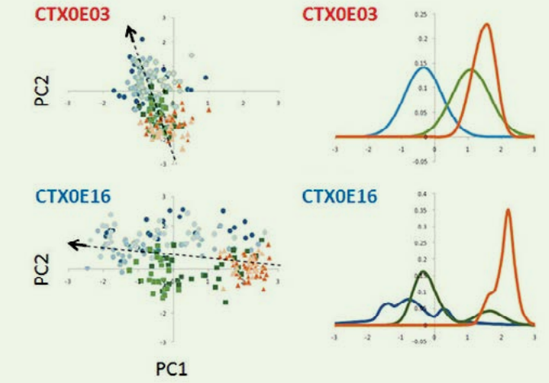

Figure 3. Principal component analysis of gene expression profiles (left) demonstrated differences between sister cell line CTX0E03 (clinical) and CTX0E16. Histograms (right) modelled along the differentiation axis (arrows) show increased heterogeneity of CTX0E16 compared to CTX0E03 upon neuronal differentiation.

Using these cells, the pitfalls of current single cell analysis protocols where minimized or removed altogether. A process to chemically 'freeze' the cells in undisturbed states was developed, which allowed for a highly successful, flexible and efficient cell isolation protocol using automated contact-free (contamination-free) laser micro-dissection over long periods of time. The same samples could also be stored and revisited for retrospective validation if necessary. Nanofluidic RT-qPCR (reverse transcription-quantitative real-time PCR) (Fluidigm® dynamic array) was integrated to provide high-throughput sensitive RNA measurements at the single cell level which, combined with stringent quality controls and optimised normalisation procedures, provided robust gene expression patterns characteristic of the cell lines studied (Figure 2).

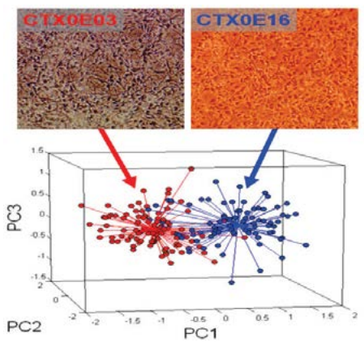

Figure 2. Top: Bright field microscope images of the two neural sister cell lines used in this study (CTX0E03 and CTX0E16). They have a very similar morphology. Bottom: Principal Component Analysis of the gene expression profiles measured on single cells reveals two distinct clusters corresponding to each cell line (one point represents one single cell). After normalisation, this clustering indicates that subtle differences are responsible for such distinct gene expression patterns.

The method also found increased heterogeneity between single cells in the non-effective cell line compared to its clinical counterpart (Figure 3), demonstrating the utility of this approach for quality control of stem cell derived cell products.

Building upon this work, LGC will look at integrating many of its analytical platforms to the multiparametric analysis of regenerative medicine products. Improvements in the ability to characterise single cells quickly and robustly will enable the detection, and consequently the reduction of, intrinsic cell variability and therefore allow better/safer cell-based therapies to be developed.

However, improvements in the analysis of single cells benefits not only cell-based therapy development, but impacts upon the wider pharmaceutical, diagnostic, forensic and environmental sectors; for example, for analysis of single circulating tumour cells (CTCs) for cancer monitoring.

Read more

Fox BC, Devonshire AS, Baradez MO, Marshall D, Foy CA, Comparison of RT-qPCR methods and platforms for single cell gene expression analysis, Anal. Biochem., 2012, 427(2), 178-86.

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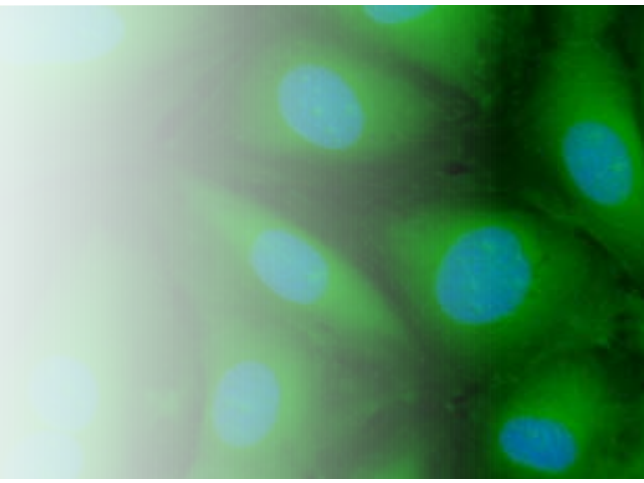
Measurement provides structure, removes chaos, reduces waste, ensures open and fair markets, supports precision where required and saves lives, money and time.



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