EUROPEAN **PHARMACEUTICAL** REVIEW

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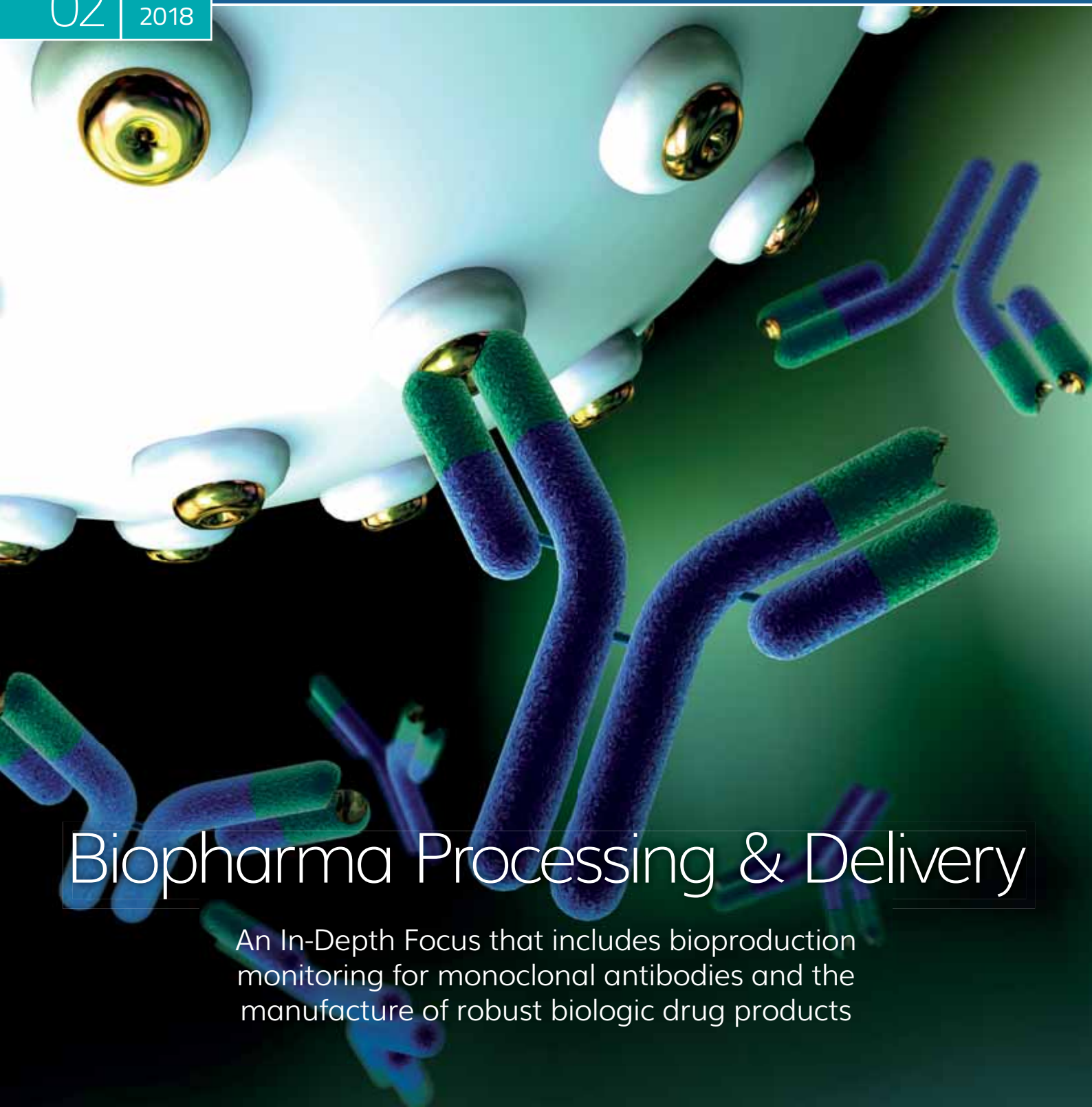

Biopharma Processing & Delivery

An In-Depth Focus that includes bioproduction monitoring for monoclonal antibodies and the manufacture of robust biologic drug products

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HOW ARE YOU ADDRESSING RISK MANAGEMENT AND DATA INTEGRITY?

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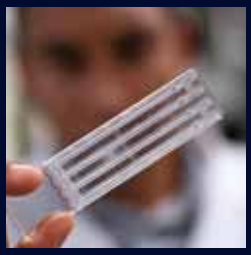
ENDOSAFE® ENDOTOXIN TESTING



Through reducing much of the time, accessories, and subjectivity associated with the traditional qualitative gel-clot test, Endosafe® LAL cartridge technology has long supported micro QC managers' efforts to produce rapid, accurate endotoxin results while adhering to rigorous data integrity standards. The need for the preparation of standard curves and long incubations has been eliminated, as our cartridges provide quantitative results in 15 minutes. The robust assay optimizes the use of LAL, requiring minimal preparation and no technical training, removing as much potential for human error as possible. The flexible Endosafe® testing platforms all generate the objective electronic data that has become the expectation in today's high volume testing environment. Calculations and analysis performed by the readers likewise remove user subjectivity, providing a clear picture of product quality that is essential to both mitigate risk and maintain cGMP compliance. **Discover how to remove subjectivity from your endotoxin testing at www.criver.com/cartridges**

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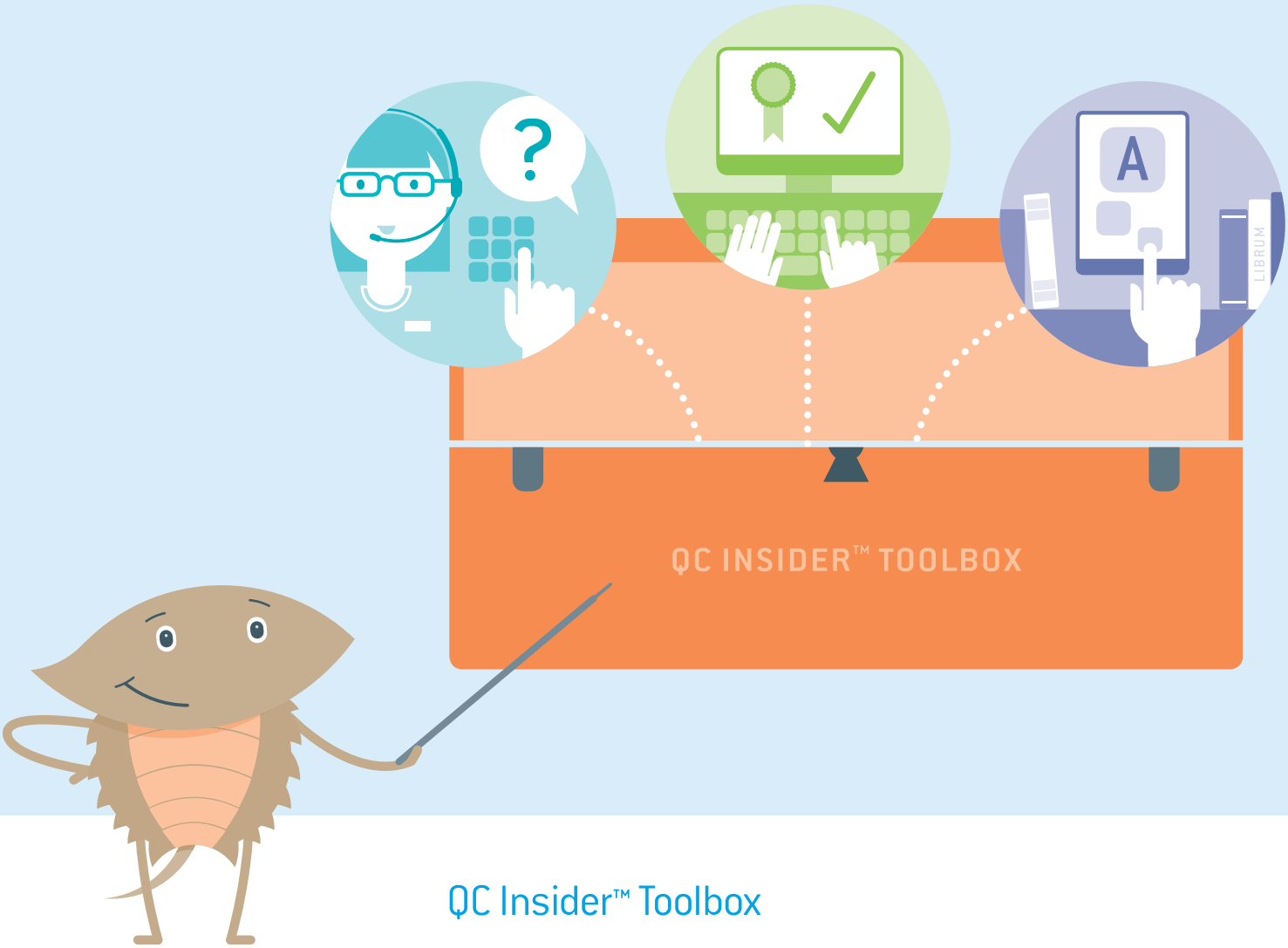

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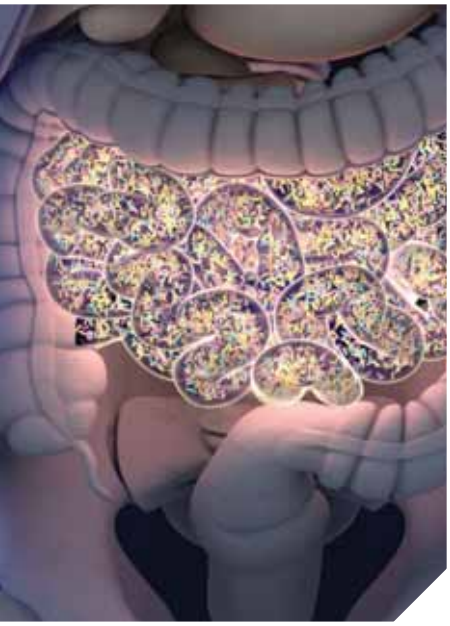

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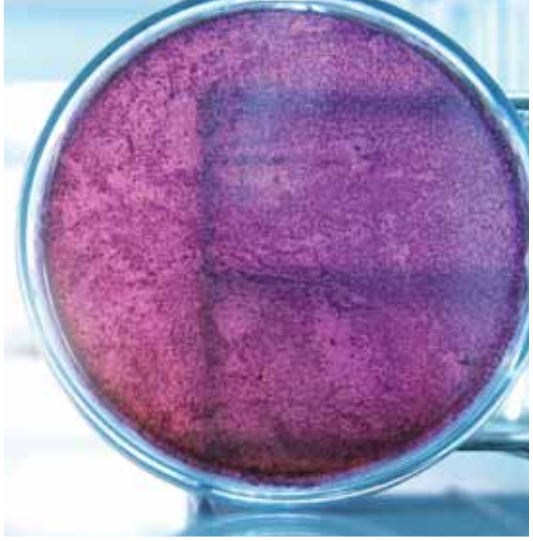

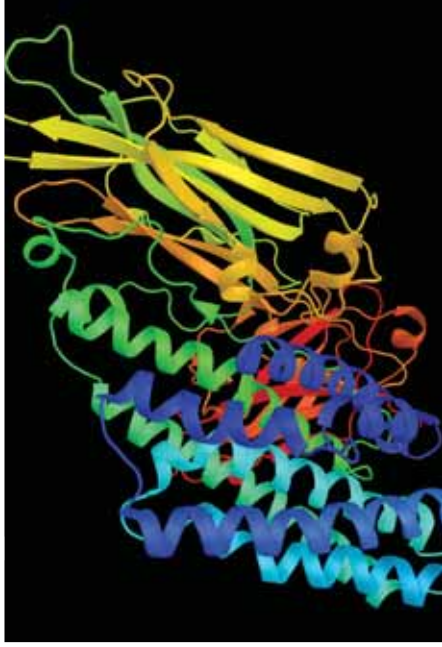

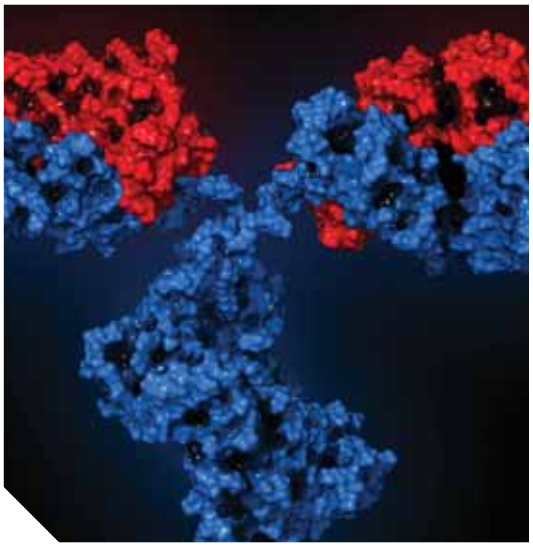

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Harnessing the power of data more effectively

Using data as efficiently as possible is a key theme of this edition of *European Pharmaceutical Review*.

MIKE STONES
EDITOR
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DATA may have become the new oil – the world's most valuable resource; aside from people – but are we using that data wisely? Few industries rely on such huge quantities of data as the pharmaceutical sector. So, it makes sense to ensure data planning, organisation and integrity are managed as efficiently as possible – both now and in the future.

In his Foreword, Dave P Elder considers both the opportunities and challenges that big data presents to the pharmaceutical sector. Read his views on this highly topical subject on page 9.

Continuing the theme of data management, we include a roundtable on track and trace technology featuring the views of five industry leaders on page 56.

In addition to data, this edition also contains detailed insight into three key areas, with an In-Depth Focus devoted to each topic. Those are: Biopharma Processing & Development, starting on page 17, Microbiology, starting on page 35, and Formulation, Development and Delivery, starting on page 47.

With the pharmaceutical sector's busy calendar of events now well underway, we bring you two show previews; starting on page 55 with Making Pharmaceuticals, which takes place between 24-25 April in Coventry. Next is the ACHEMA 2018 show preview, for the event which takes place between 11-15 June in Frankfurt.

We conclude with the latest in our Guide to ... Series. This focuses on the testing services offered by five leading providers. Our detailed guide to their testing services begins on page 75.

So, we hope you enjoy this data-rich edition of *European Pharmaceutical Review*. If you would like to join the debate about any of the topics raised, please contact us and share your views with other pharmaceutical industry professionals. You can also contribute to the discussion via Twitter, LinkedIn and Facebook. Please let us know what you think about the subjects under discussion.

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Big data: big opportunities or big challenges?

Dave P Elder

JPAG Member and David P Elder Consultancy

Every aspect of pharmaceutical research and development involves the generation of huge quantities of data (ie, big data), with the expectation that we can turn this information rapidly into useful knowledge, which in turn can be used to make 'data-driven' decisions to better understand and control processes. This derived knowledge can also be used to reduce costs, improve efficiencies, reduce development times and facilitate rapid post-approval changes.[1]

HOWEVER, despite the increased focus on quality in the pharmaceutical industry, there has been a significant increase in the number and severity of quality defects. Food and Drug Administration (FDA) has indicated that for the pharmaceutical industry to "continue to be successful, drug manufacturing must become agile, rapidly scalable, efficient, reliable – and less costly".[2] These challenges can only be met by making better use of data and knowledge, allowing us to reduce the enormous cost of poor quality and, in parallel, improve data integrity.

How will industry cope with big data?

FDA recently published its guidance on modernising the pharmaceutical manufacturing base.[3,4] FDA has also highlighted that modernising the manufacturing base needs to be supported by integrated strategies towards product and process understanding, underpinned by real-time monitoring of critical process data, which taken together should support better understanding, monitoring and process control.[5,6]

The regulatory strategy within the EU is similar.[7] However, all of these novel approaches generate significant volumes of data, ie, information rich, which necessitate enhanced data management solutions and enhanced data infrastructure to collect, process and analyse these data-rich streams.[8]

Therefore, the connectivity of equipment, people, processes, services and supply chains all contribute towards 'Pharma 4.0'.[9] Industry 4.0 technologies will enable manufacturers to have better visibility of ongoing operations, allowing them to be more responsive to information about changes in raw materials, inventory, assets, quality, waste, output and customer demands, highlighting improvement opportunities and ensuring that actions are taken, saving time, money and resource.

Data integrity

However, at the same time that both industry and regulators want to embrace and embed 'big data' into their decision-making process, there are parallel concerns and significant unease about data integrity in general.

FDA and European Medicines Agency (EMA) have issued draft guidance on the subject of data integrity,[10,11] due to the increasing numbers of cGMP violations involving data integrity during routine cGMP inspections.[12] Agencies are increasingly concerned about these trends because data integrity is a critically important element of industry's responsibility "to ensure the safety, efficacy, and quality of drugs, and of FDA's ability to protect the public health". These data integrity related cGMP violations have led to numerous regulatory actions, "including warning letters, import alerts, and consent decrees".

In parallel, EMA has developed a set of Q&As with guidance for stakeholders on actions that assure data integrity and also minimises data integrity risks at all stages of the data lifecycle in all pharmaceutical quality systems (PQS).[13]

Conclusion

The significant cost of a poor-quality culture is often underestimated by the pharmaceutical industry. For example, consider what would be the organisational outcome if the costs for non-conformity (NC) or out of specification (OOS) outcomes were presented in annual reports? Undoubtedly this would fuel an appetite for significant change. However, these quality challenges can only be addressed by making better use of data and knowledge to facilitate better and quicker decision making. Although there are ongoing concerns about data integrity, compliance could actually improve if better 'data-driven' decisions could be made and the human element is removed from the equation.

BIOGRAPHY

DAVE ELDER has nearly 40 years of service within the pharmaceutical industry at Sterling, Syntext and GlaxoSmithKline. He is now an independent GMC consultant. Dr Elder is a visiting professor at King's College, London, and is a member of the British Pharmacopoeia. He is a member of the Joint Pharmaceutical Analysis Group (JPAG) and a member of the Analytical Division Council of the Royal Society of Chemistry.

REFERENCES

To view references, please visit: **europeanpharmaceuticalreview.com/2-18-Elder**

ROUND UP

The editor's pick of the most interesting developments within the pharmaceutical industry

Diabetes drugs linked to inflammatory bowel disease

DIGESTIVE HEALTH

THE diabetes drugs known as dipeptidyl peptidase-4 (DDP-4) inhibitors have been linked to an increased risk of inflammatory bowel disease, the digestive condition that causes stomach pain and bloating.

While the Canadian researchers stressed that the absolute risk was low and that their findings need to be replicated, they said: "Physicians should be made aware of this possible association."

The researchers, led by Laurent Azoulay at McGill University, set out to assess whether the use of DPP-4 inhibitors is associated with inflammatory bowel disease in patients with type 2 diabetes.

They analysed data from the UK's Clinical Practice Research Database for 141,170 patients aged at least 18 years of age, who started antidiabetic drugs between 2007 and 2016.

Patients were initially treated with insulin and those with a history of inflammatory bowel disease or similar conditions were excluded. Factors such as age, weight (BMI), smoking status, alcohol-related disorders and complications of diabetes were considered.

Participants were monitored for an average of three and a half years, during which time 208 new cases of inflammatory bowel disease were recorded (an incidence rate of 37.7 per 100,000 person-years).

Overall, use of DPP-4 inhibitors was associated with a 75% increased risk of inflammatory bowel disease (53.4 cases per 100,000 person-years) compared with the use of other antidiabetic drugs (34.5 cases per 100,000 person-years).

This association increased with longer durations of DPP-4 inhibitor use, reaching a peak after three to four years and decreasing after more than four years of use.

Compound may help to tackle obesity

WEIGHT LOSS

A COMPOUND called semaglutide that mimics a naturally-occurring appetite-regulating hormone may help people who have obesity but not diabetes to lose weight, according to a new study.

Semaglutide has a chemical structure similar to the hormone glucagon-like peptide 1 (GLP-1), which regulates both insulin secretion and appetite. In December, the US Food and Drug Administration approved the semaglutide injection Ozempic as a once-weekly adjunct to diet and exercise to improve glycaemic control in adults with type 2 diabetes.

"This randomised study of weight loss induced with semaglutide in people with obesity but without diabetes has shown the highest weight reductions yet seen for any pharmaceutical intervention," said lead author Dr Patrick M. O'Neil, Director of the Weight Management Center and Professor of Psychiatry and Behavioral Sciences at the Medical University of South Carolina in Charleston.

The new study included 957 participants, 35% of whom were male. All participants had a body mass index (BMI) of at least 30 but did not have diabetes. They were randomly assigned to seven different groups. Five groups received different doses of semaglutide (between 0.05mg and 0.4mg) via injection once daily; a sixth group received a placebo, and a seventh group received 3mg of the diabetes drug Saxenda. All participants received monthly diet and exercise counselling.

After one year, all participants receiving semaglutide had lost significantly more weight than those receiving the placebo. The higher the dose participants received, the greater their average weight loss.

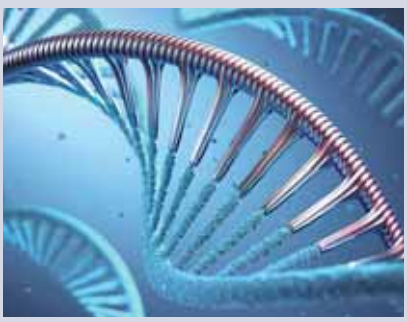
Participants who received 0.05mg of semaglutide daily lost an average of 6% of their body weight; the 0.1mg group lost an average of 8.6%; the 0.3mg group lost an average of 11.2%; and those receiving a daily dose of 0.4mg lost an average of 13.8%. Those receiving liraglutide lost an average of 7.8% of their body weight, while those in the placebo group lost only 2.3% on average.

New lung cancer hope from RNA-based therapy

RNA MOLECULES

A 40-50% success rate in curing lung tumours in mice has been achieved by turning down the activity of a specific RNA molecule.

Researchers led by Chandrasekhar Kanduri, Professor of Medical Biochemistry and Cell Biology, studied how tumour development is influenced by long non-coding RNA molecules. These molecules are produced from the part of the genome that previously classified as junk DNA but have been shown to regulate cell division, among other functions.



The researchers studied 16 cancer types comprising 6,419 solid tumours and 701 normal tissue samples which were used as controls. The aim of the research was to find long noncoding RNA molecules that are active during the phase of cell division in which the genetic material is copied.

Using an in-house developed technology and modern RNA sequencing, the researchers identified 570 long noncoding RNA molecules that are expressed differently depending on the type of cancer, as well as 633 new independent biomarkers that can be used to predict and treat 14 types of cancer. The results are expected to be important for cancer researchers in many parts of the world.

Nanorobots programmed to seek and destroy cancer tumours

NANOTECHNOLOGY

NANOROBOTS have been programmed for the first time to seek and destroy cancer tumours by cutting off their blood supply.

After adapting a mouse tumour model, human cancer cells were injected into a mouse to induce aggressive tumour growth, nanorobots were then deployed to kill the tumours.

Made from a flat, rectangular DNA origami sheet, 90 nanometres by 60 nanometres in size, each nanorobot has a key blood-clotting enzyme called thrombin attached to the surface.

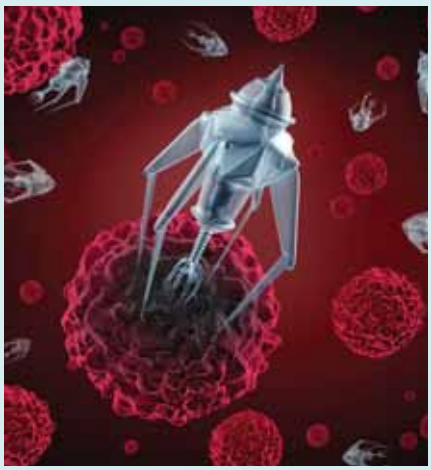
Hao Yan, Director of the ASU Biodesign Institute's Center for Molecular Design and Biomimetics and the Milton Glick Professor in the School of Molecular Sciences, said: "We have developed the first fully autonomous, DNA robotic system for a very precise drug design and targeted cancer therapy."

The nanorobots can be used for many types of cancer, since all solid tumour-feeding blood vessels are essentially the same, added Dr Yan.

Thrombin can block tumour blood flow by clotting the blood within the vessels that feed tumour growth, causing a kind of 'tumour mini-heart attack' and leading to tumour tissue death.

Programming nanorobots to attack only cancer cells entails equipping them with a special payload, called a DNA aptamer, on its surface. The DNA aptamer can specifically target a protein, called nucleolin, that is made in high amounts only on the surface of tumour endothelial cells – and not found on the surface of healthy cells.

Yuliang Zhao, also a Professor at NCNST and lead scientist of the international collaborative team, said: "The nanorobot proved to be safe and immunologically inert for use in normal mice



and also in Bama miniature pigs, showing no detectable changes in normal blood coagulation or cell morphology."

Data integrity in maintenance management systems

Glen Maxwell

ENGIE International FM

Data Integrity, in recent years, has become a key focus for regulators in industry governed by the requirements of Good Manufacturing Practice (GMP).

REGULATORY bodies expect data related to the production and release of product to be reliable, accurate, auditable, available and secure. The Food and Drug Administration (FDA) definition of Data Integrity is as follows: "*FDA expects data to be Attributable, Legible, Contemporaneously Recorded, Original or a True Copy, and Accurate (ALCOA). FDA expects that data be reliable and accurate, which means companies need to implement meaningful and effective strategies to manage their data integrity risks.*"

> ***"The need for reliable, accurate, traceable maintenance data is just as important as other systems in the production process and will receive a similar level of scrutiny during regulatory inspections"***

In recent years there have been an increasing number of GMP violations related to data integrity. In 2015 and 2016 around 80% of warning letters issued by the FDA contained data integrity issues.

In the MHRA GMP Inspection Deficiency Data Trend (Dosage Forms) 2016 examples of deficiencies related to integrity of data included the following:

- Data integrity assessments were focused on system compliance and failed to consider the

impact of business processes on the integrity of data, for example manual transfer of data between electronic systems
- The investigation relating to a data integrity failure, whereby fictitious utility monitoring data was recorded, lacked sufficient detail to demonstrate whether wilful intent was suspected
- Printouts of particle count data from HEPA filter testing were not transferred from thermal paper to non-volatile media to ensure the integrity of the record throughout the retention period.

Data Integrity requirements apply to all data affecting the quality of the production process and its final output. This includes information from Maintenance Management Systems.

Data on location and asset performance and maintenance status are critical to the production process, so it is important that the requirements of the data integrity standards are applied to the data in the Maintenance Management System.

Data integrity in maintenance services

The need for reliable, accurate, traceable maintenance data is just as important as other systems in the production process and will receive a similar level of scrutiny during regulatory inspections.

Data related to the performance of a location, system, asset or maintainable component is used to inform the validity of product release. Failure to ensure this data meets the data integrity requirements as applied to all other scientific data will lead to deficiency reports during regulatory inspections.

Maintenance management system

Data integrity in the Maintenance Management System starts with asset capture. This is the process of identifying locations, systems, assets and maintainable components that fall within the scope of the maintenance programme.

During this process assets are categorised according to their criticality, the overall maintenance and compliance regime is defined, and the scheduling of planned maintenance is implemented.

When asset capture is performed effectively, the maintenance management system can then be used to provide reliable data to report on the effectiveness of the maintenance programme, and to ensure out of specification assets are not involved in the production processes.

The Maintenance Management System is used to create work orders for both planned and reactive maintenance tasks, and to assign tasks to skilled operatives. Once assigned, data entered by the ›

ABOVE: There have been an increasing number of GMP violations related to data integrity

operative is used to track time of attendance, and to enter results of the maintenance activity.

Maintenance management models

Some maintenance management systems are purely paper-based, some are based on a combination of software applications, and some are hybrid systems where software and paper-based approaches are combined to manage maintenance workflow.

Computerised Maintenance Management System (CMMS) refers to software packages with connection to production and other assets, and to maintenance operatives via smart devices.

It is necessary to consider all maintenance management models when discussing the integrity of data associated with the maintenance of critical systems and assets.

Paper-Based systems

In paper-based systems, the application of Good Documentation Practice (GDP) can provide some assurance that data is acquired, recorded, and stored correctly.

Standard operating procedures and forms shown to be under document control, date and time stamps applied at the point the data is recorded, signature by the operative entering the data, and supervisor review and sign off can all be evidenced in an effective system.

However, a paper-based system can never eliminate the potential for data integrity deficit. Inconsistent recording can occur, such as differing formats of data entry between operatives, ineligible or spoiled records (environments are often harsh), transcription errors and loss of original copies. All these issues present significant risks to data integrity.

There is always the potential for the loss of an original record, and even the amendment of maintenance data after the event that may not be auditable.

Paper-based systems rely on the integrity of the people involved to a huge extent. There is also a reliance on over processing of data in its acquisition and review, and the data recorded is difficult to analyse effectively.

Paper and software hybrid systems

Some maintenance management systems, based on a collection of software tools and paper-based approaches, are merely used as a work order scheduling system. Jobs are scheduled and logged, work orders created, paper copies printed and made available for a qualified maintenance operative to pick up.

Maintenance details are then written onto the paper copy after the maintenance activity has been completed, along with date and time information. Ideally there should be a review by a supervisor.

As with purely paper-based systems, the risk of data entry error and data loss during this process is ever present. Entering the data from paper into the CMMS, typically done by the help desk or administration teams, adds another risk of error during transcription of the data. Often the people transcribing the details are not engineers and may not understand the terminology used or recognise the risks presented by incomplete data.

While this kind of hybrid maintenance management system does provide a repository for maintenance information, including jobs logged, assignment histories, and completion data, the same risk to data integrity exist.

Data entry errors, inaccurate results, unreliable data and over processing make it difficult to prove the status of locations, systems and assets at the time of production or an adverse event.

CMMS

CMMS are used to capture asset information and define maintenance and compliances regimes in a structured database.

Work orders are generated automatically for planned maintenance events, and reactive maintenance requests logged on request. Work orders generated are assigned electronically to skilled and qualified operatives using a smart device.

Once the work order is assigned, the CMMS can track the attendance of the operative and the time taken to complete the maintenance task. Asset tagging with readable codes, so the operator can 'check-in' at the asset, provides evidence of attendance at the very least.

BIOGRAPHY

GLEN MAXWELL is Quality and Compliance Manager for ENGIE International FM. He has over eight years' experience in multi-service facilities management (FM) contracts and over the past two years has been involved in compliance activities for FM contracts with international site portfolios. Previously, Glen spent 15 years working in scientific software, delivering enterprise laboratory information management systems and chromatography data systems to the pharmaceutical industry.

Information from the CMMS is used to ensure that the operative follows the maintenance regime associated with the asset in the CMMS.

The operative can then enter the results of the maintenance using a smart device. If necessary, an automated review step can be included in the workflow prior to the data being securely uploaded to the CMMS. So, while there is still a requirement to manually enter details of maintenance performed, this is uploaded to the CMMS without additional transcription steps or further processing of the data.

System performance data can also be acquired from asset monitoring systems and recorded against the asset in the CMMS, eliminating data entry and processing.

By using an integrated CMMS and eliminating the use of paper or other management software, the risk of data entry error is reduced. Accurate trend analysis can be performed, and maintenance strategies can be monitored and improved with a greater degree of confidence in the accuracy of the data.

As with any system, there can never be 100% assurance that data integrity is maintained, but the risk of integrity deficit is reduced. Any post event changes to the data are logged in the system audit trail, and there is little risk of losing the original data.

Maintenance Performance dashboards can be made available that show the status of premises and equipment and allow maintenance managers to control availability of locations and assets in the production process.

Augmented reality, connected operators and tagged and connected assets all offer the potential for a paradigm shift in maintenance strategies.

Conclusion

Data integrity presents a challenge in all maintenance management system models, but the aim is always to minimise the possibility of integrity deficit and eliminate over processing of data by systems and people.

The more data processing steps there are, the more opportunity there is for error, loss and even data manipulation – all of which puts the organisation at risk of failing to meet data integrity standards.

CMMS are a must for all modern facilities to reduce the risk of data integrity breaches, and to improve the effectiveness of the maintenance programme.

Paper-based and hybrid systems can no longer provide the level of data integrity required, even where GDP is effectively implemented and followed.

Integrated CMMS, with data capture from environmental and other monitoring systems, smart assets, and connected operators gives a greater assurance that maintenance data are accurate and meet integrity requirements.

EVENTS DIARY

Keeping you up to date with forthcoming events in the industry

april

23 BioProcess International European Summit

Date: 23–25 April 2018
Location: Amsterdam, The Netherlands

24 Making Pharmaceuticals

Date: 24–25 April 2018
Location: Coventry, UK

24 CPhI North America

Date: 24–26 April 2018
Location: Philadelphia, USA

june

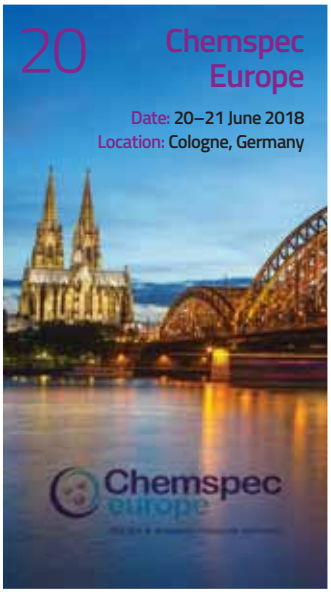
03 ASMS Conference on Mass Spectrometry and Allied Topics 2018

Date: 3–7 June 2018
Location: San Diego, USA

11 ACHEMA

Date: 11–15 June 2018
Location: Frankfurt, Germany

20 Chemspec Europe



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BIOPHARMA PROCESSING & DEVELOPMENT

Patients and caregivers expect drug products to be safe, efficacious, deliver the performance described on the label, perform consistently over the proposed shelf-life and be manufactured in a manner by which quality can be ensured. Atul Saluja, Mark Yang and Bernardo Perez-Ramirez, from Global Pharmaceutical Development Biologics, Sanofi, consider the manufacture of robust biologic drug products.

The development of a well-controlled, large-scale biopharmaceuticals production is dependent upon extensive characterisation of molecules and the associated process according to Guillaume Tremintin and Stuart Pengelley, from Bruker Daltonics.

Bioproduction facilities for recombinant monoclonal antibodies and antibody-drug conjugates are demanding greater method compatibility and ease of use. Kyle D'Silva, from Thermo Fisher Scientific, explains how bioproduction monitoring for monoclonal antibodies is becoming easier.

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Enabling the manufacture of robust biologic drug products

Atul Saluja, Mark Yang and Bernardo Perez-Ramirez

Global Pharmaceutical Development Biologics, Sanofi

Patients and caregivers expect that drug products are safe, efficacious, deliver the same performance as described on the label, perform consistently over the proposed shelf-life and are manufactured in a manner by which quality can be ensured. The quality, safety and efficacy of any product can only be ensured through a comprehensive understanding of the biology of the drug, its mechanism of action (efficacy) and dose-response relationship (safety).[1]

BIOGRAPHY

MARK YANG is a Director in Global Pharmaceutic Development Biologics in Sanofi, where he leads a team responsible for formulation and lyo process development, process scale up, and technology transfers. Before joining Sanofi, Dr Yang was Associate Director at Acceleron Pharma, where he built the formulation lab / team and oversaw CTM manufacturing in US and EU. Dr Yang also worked for many years in drug delivery and protein formulation in Baxter and Altus. He is the recipient of VP Award from Sanofi Genzyme, Outstanding Technology Achievement Award for High Concentration mAb Formulation from Baxter, and Fellows Award for Research Excellence from NIH. Besides numerous patents, he has published over two dozen peer-reviewed research papers.

SUBSEQUENTLY, the drug products need to be designed, developed, and manufactured to meet the desired quality attributes. The concept of the Quality by Design (QbD) approach for product manufacture has been implemented in the pharmaceutical industry and is described in detail in the industry-wide International Council for Harmonisation of Technical Requirements for Pharmaceuticals (ICH) Harmonised Tripartite guidelines.[2-5] The two key elements of the QbD approach that impact the overall development strategy are: i) the identification / understanding of the critical quality attributes (CQAs) of a molecule that could potentially affect the molecule's safety and efficacy and ii) the process design space generally defined as the range of process inputs that help ensure the output.

As regards the QbD approach in the biopharmaceutical industry, several guidelines are now available to guide the end-to-end development of robust drug products. ICH Q8R[2] defines QbD as a systematic approach to development that begins with well-defined objectives based on fundamental science, process understanding and quality risk management. ICH Q9,[3] ICH Q10[4] and ICH Q11[5] provide principles and tools to address quality risk management, pharmaceutical quality systems as well as the development and manufacture of drug substance and drug product.

Target product profile and desired product characteristics

Early steps in drug product design, including formulation selection and process development, require a clear definition of the target product profile (TPP), a quality target product profile (QTTP) and identification of the critical quality attributes (CQAs). The TPP is a summary of the desired product attributes or the final goal that will in turn ensure a drug product that is safe, efficacious, stable and convenient for use by patients and caregivers. The so-called quality TPP, or simply QTTP, provides a summary of the specific desired quality attributes that must be achieved to ensure quality for the product and fulfill the needs of the TPP. Most often the TPP and the associated QTPP evolve over the course of development. As additional clinical data becomes available, business needs and market landscape change, the TPP needs to be adjusted accordingly. Regardless of the stage of development ie, early to supply phase I / II or late to supply phase 3 and commercial batches, the manufacturing process must always be designed to fulfill the needs of the latest TPP and the associated product characteristics ie, its quality attributes.

Identification of preliminary critical quality attributes

A critical quality attribute is defined as a physical, chemical, biological, or microbiological property that should be between an appropriate limit, range, or distribution to ensure the desired product quality.[2] Essential to the identification of CQAs is an assessment of the extent to which variation in a given quality attribute impacts overall product quality (safety and efficacy). Stress studies provide a means to quickly identify risks to quality and are critical for efficient drug product development. Temperature and pH are common variables to stress proteins and help to identify CQAs. However, adequate stress conditions should be chosen to provide meaningful data, such that the stability trends are consistent across storage temperatures.

The identification of CQAs requires a concerted response analysis to numerous stress conditions that mimic processing steps. However, during early development when the manufacturing process has not been fully defined, some general processes such as freeze / thaw, extreme pH conditions

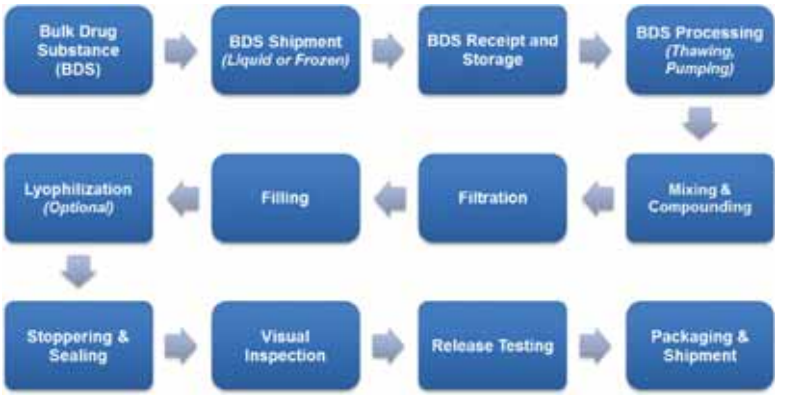
(viral inactivation), mechanical stress (pumping, including ultrafiltration) and holding times can be assumed to be critical to product quality. Quality attributes deemed critical at this early stage of development are often defined as preliminary or presumptive CQAs (pCQAs). Often the analysis of the experimental data is not simple and other parameters, such as the severity of a particular instability impacting potency and safety, need to be considered for the identification of pCQAs (or CQAs in general as development evolves).[6] The severity should also be linked to the probability of a given instability impacting product quality. Once CQAs are identified, it is essential to perform a risk analysis to formally (i) identify the failure modes for a given CQA, (ii) quantify the level of risk, and (iii) ultimately guide the drug product development strategy. Failure modes and effects analysis (FMEA) tools can be used to rank the importance of factors based on probability, severity and detectability.[7]

Process characterisation and design space

Manufacture of biologic drug products is usually accomplished through a set of material transfer and processing unit operations designed to convert the bulk drug substance (BDS) into drug product (***Figure 1***). Routinely, the BDS is stored in liquid or frozen form, shipped to the drug product manufacturing site, compounded and processed accordingly for filtration and filling and finally converted to either liquid or lyophilised drug product. Subsequently, the drug product undergoes visual inspection prior to final release testing, packaging and shipment.[8] It is imperative that at the end of the series of material transfer and processing operations, product quality, as defined by maintenance of CQAs within a predefined range or above and below a certain acceptance limit, be maintained from batch-to-batch. To ensure this result, it is critical to understand, through process development, characterisation and risk assessment exercises, the impact of input process parameters, raw material attributes and the associated variability on product quality.[9] A successful process characterisation exercise should first result in a clearly identified set of critical material attributes (CMAs) and critical process parameters (CPPs). Secondly, process characterisation should result in a process design space with well-defined boundaries for individual CPPs. A design space is defined as the multidimensional combination and interaction of input variables (eg, material attributes) and process parameters that have been demonstrated to provide assurance of quality.[2]

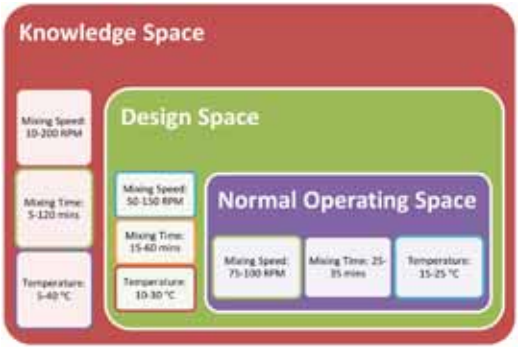
The need for quality to be built into the product is inherently related to its robustness ie, the ability of the product to withstand variations in material attributes and process parameters while still meeting the required quality attribute targets.[10] The concept of design space to manufacture a robust product is not intended to provide a discrete and sharp 'edge of failure' with attributes and input values outside of it resulting in a failed product with 100% certainty. Rather, it is a probabilistic definition with the likelihood of success being the highest if inputs can be maintained within the design space.[11] Thus, it is imperative, and certainly more informative, to characterise a given process with respect to the impact of inputs on product quality into distinct but somewhat diffuse 'zones'. These zones are customarily defined as (i) knowledge space – characterised by a broad range of putative CPP and CMA values beyond what will be routinely encountered during at-scale manufacture, (ii) design space – range of CPP and CMA values with highest probability of success for at-scale manufacture and (iii) normal operating space – range of CPP values most likely to be encountered and CMAs recommended for at-scale manufacture.[12] A representative example of design space and the associated zones outside and within it for a mixing unit operation impacting a drug product CQA (% aggregates for example) is provided in ***Figure 2***.

FIGURE 1



ABOVE: Representative biologic drug product manufacturing process

FIGURE 2



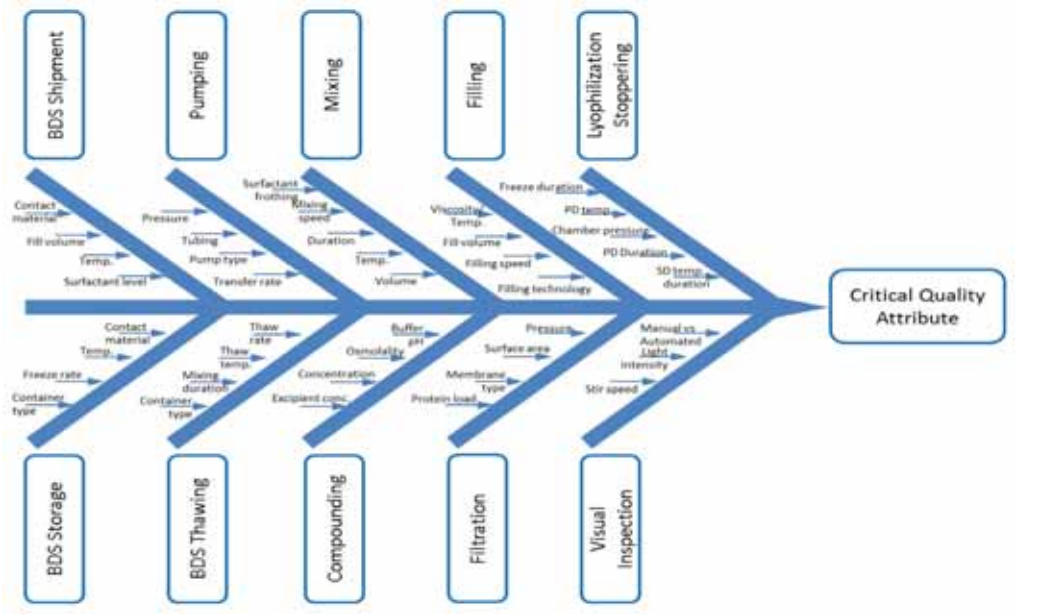
ABOVE: Concept of design space and operating ranges for a representative mixing unit operation

BIOGRAPHY

BERNARDO PEREZ-RAMIREZ is Senior Scientific Director of Global Pharmaceutical Development Biologics at Sanofi in Framingham-Massachusetts. Bernardo is responsible for development of drug products for therapeutic proteins including formulation, process development, technology evaluation and drug device integration. He is Adjunct professor at the Department of Biomedical Engineering at Tufts University-Boston. He received his PhD in Biochemistry from the University of Missouri and did post-doctoral research in physical biochemistry at Brandeis University. He began his biopharmaceutical career at Genetics Institute (now Pfizer) and joined Sanofi/ Genzyme in 2004.

RIGHT: Ishikawa diagram showing cause and effect relationship between representative input variables and critical quality attribute

FIGURE 3



BIOGRAPHY

ATUL SALUJA is Associate Director in Global Pharmaceutic Development Biologics at Sanofi where he is engaged with drug product development of early and late stage biologic assets. Before joining Sanofi, he worked at BMS, Amgen and KBI Biopharma in product development groups. Over the past decade, Atul has successfully delivered on various clinical development programmes, transferred products from lab to site enabling manufacture of validation batches, instituted lifecycle management process improvements, as well as workflow improvements through strategic initiatives spanning a broad development space. He actively engages with the scientific community through presentations, symposia, focus groups, and publications in peer-reviewed journals. Atul received his PhD in Pharmaceutics from University of Connecticut in 2006 (high concentration protein formulations) and has been with the industry since.

Design space can be developed for each discrete unit operation or for the overall manufacturing process. While the latter provides the most operational flexibility, the former is a relatively simpler approach since the interaction of diverse process parameters and material attributes from all the different unit operations can be kept to a minimum.[2] The Ishikawa (or cause and effect) diagram (***Figure 3***) shows one such approach to the development of design space for each bulk transfer or processing step impacting a given CQA. Material attributes and process inputs for each unit operation which can potentially impact the CQA are individually characterised during process characterisation studies and can be classified as either critical to product quality (CPP) or simply as a process parameter that needs to be monitored to assure that the unit operation is running in a controlled manner.[13] The process of developing cause and effect relationships and the parameter and inputs to consider during process characterisation studies, essentially starts from the basic QTPP which defines the fundamental quality attributes desired for the final product. The desired QTPP helps in defining the CQAs which, in turn, lead into an iterative process of risk assessment to select relevant inputs and process parameters to evaluate, process development and characterisation studies.

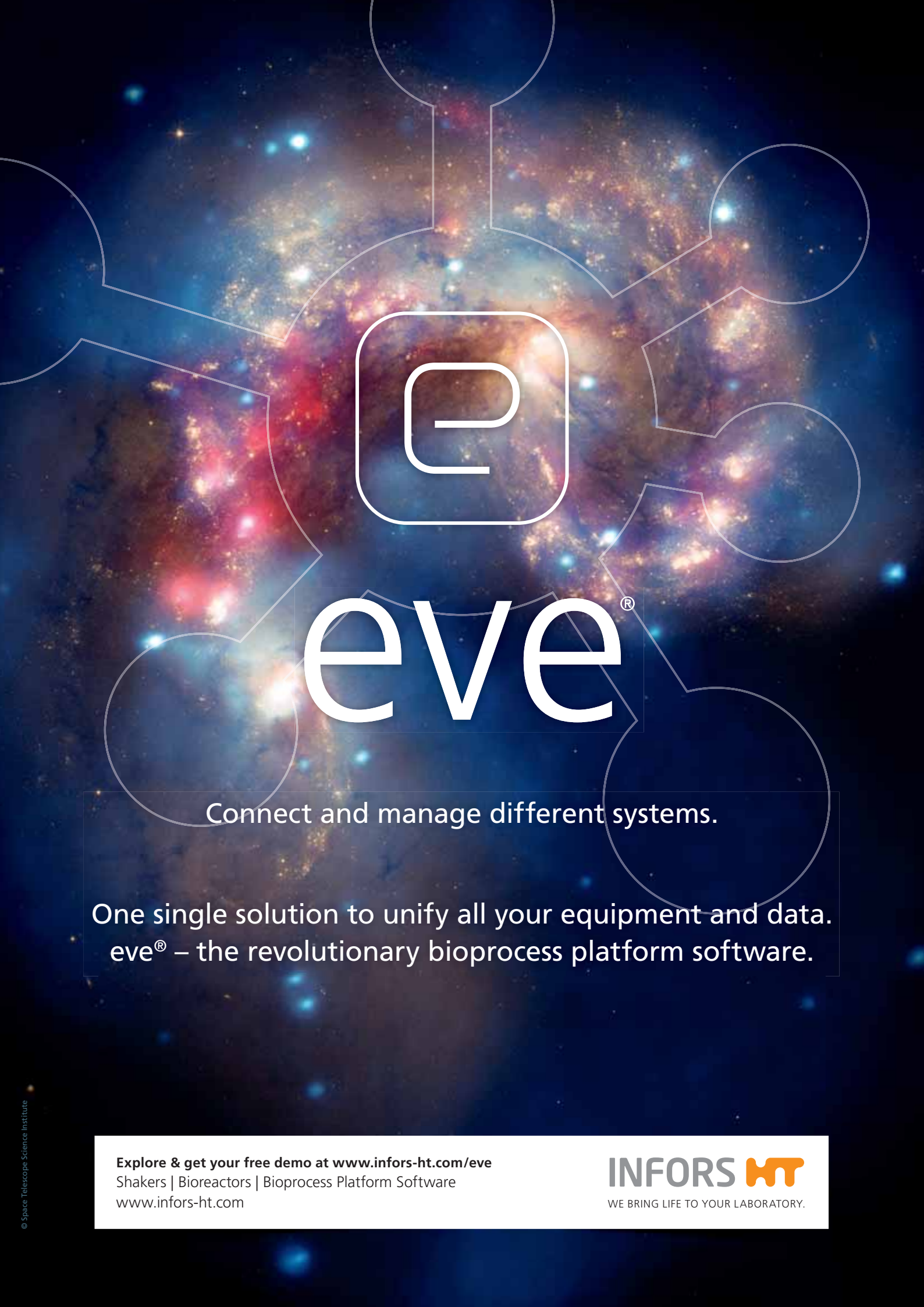
Once the CPPs and CQAs for a given product are defined, a process control strategy can be developed around those CPPs to ensure the result, ie, product quality, is met. Process development studies conducted at lab scale assist in the development of a broad knowledge space and in defining the potential criticality of a given process parameter or material input. Since these studies are conducted much earlier than scale-up runs and the material tends to be more heterogeneous and in limited quantity, scale-down lab models can be leveraged to minimise material consumption.

Process monitoring and continuous validation

The implementation of an effective manufacturing process at scale is not a discrete event that cannot change or evolve over time. In fact, process development studies, well-defined process control strategy, effective knowledge and technology transfer, and the process validation campaign all together only fulfil the first objective (ie, product realisation) of the three key pillars of the modern pharmaceutical quality system.[4] The other two objectives, the maintenance of state of control over the manufacturing process and continuous process improvement, are specifically aimed at ensuring consistent process performance throughout the product lifecycle all the way to product discontinuation. Per ASTM E2537-08,[14] continuous quality verification (CQV) is described as an approach to process validation where manufacturing process performance is continuously monitored, evaluated and adjusted as necessary and is consistent with a lifecycle approach to process validation. In essence, these objectives emphasise the need to not only establish a well-designed process at the commercial scale, based on extensive process characterisation but limited at-scale experience, but also to maintain, verify and constantly improve upon it.[15,16]

REFERENCES

To view references, visit: **europeanpharmaceuticalreview.com/2-18- Perez-Ramirez**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Automating biopharmaceutical development: from early stage to QC

Guillaume Tremintin and Stuart Pengelley

Bruker Daltonics

The development of a well-controlled, large-scale biopharmaceuticals production is dependent upon extensive characterisation of molecules and the associated process. Additionally, the number of biosimilar candidates is rising quickly as patents expire; this development pathway is placing a heightened emphasis on biophysical characterisation to prove similarity. This includes primary structure verification, detection of product-related impurities, and evaluation of process-related impurities.

LEFT: Mass spectrometry has evolved into the gold standard for biopharmaceutical characterisation and process development

BIOGRAPHY

STUART PENGELLEY is a Senior Scientist at Bruker Daltonics where he develops solutions and methodologies for biopharma applications.

THE complexity of a protein´s heterogeneity profile, compared with small molecules, demands a number of complementary techniques to achieve sufficient characterisation.

Traditional low-resolution methods, such as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), reveal a protein's purity but do not offer any information on the amino acid sequence, any post-translational modifications (PTMs) present or details of the secondary or tertiary structure. Due to its versatility and high mass resolving power, mass spectrometry (MS) has evolved into the gold standard for biopharmaceutical characterisation and process development. Until recently, only trained MS

specialists were able to interpret the data output from biopharmaceutical characterisation workflows, but with the incorporation of tailored, simple-to-use software, scientists across the development pipeline are able to gain insights from MS reports.

Analysing biopharmaceutical data

Current MS-based assays for protein drug development traditionally couple HPLC (high-pressure liquid chromatography) with an electrospray ionisation (ESI) mass spectrometer. Users initially submit a sample sequence with appropriate HPLC and MS methods to perform the measurements and the data generated must then be analysed to obtain protein structure information. Whereas these steps had previously been restricted to trained mass spectrometrists, biopharma industry researchers without formal MS training are now able to obtain and interpret this information using newly available software solutions.

These measurements can be carried out at the intact protein, domain (subunits) or peptide level using MS only or MS/MS for top-down and bottom-up analysis. Both high resolution ESI Quadrupole Time-of-Flight (QTOF) and Matrix-Assisted Laser Desorption/Ionisation (MALDI)-TOF data can be employed for these analyses.

Common biopharma workflows

One of the most widely-used assays is liquid chromatography-mass spectrometry (LC-MS) peptide mapping, where the peptides derived from an enzymatic digest of the protein are identified, to infer the protein sequence and its modifications. The peptide data is then processed and compared with the theoretical protein sequence to provide sequence coverage information.

Intact protein analysis

In contrast to bottom-up analysis for sequence validation of antibodies, where the protein is digested into peptides and analysed using LC/MS-MS, intact protein analysis looks at an intact antibody to establish the protein mass and some heterogeneity, such as the glycoforms profile. Intact mass workflows are rapid (approximately 10 minutes) as only a short gradient is needed when performed on ESI-QTOF MS systems. For more powerful characterisation, however, middle-down analysis is often used.

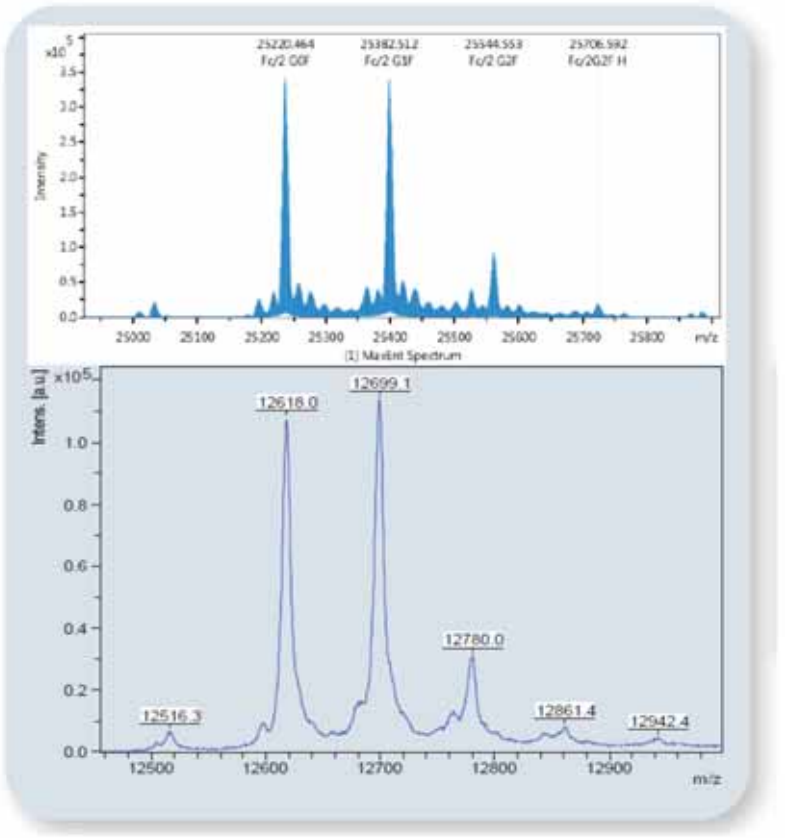
The domain approach

Middle-down experiments are used for the analysis of monoclonal antibody (mAb) subunits, for simple confirmation and relative quantitation of the modifications present on the mAb subunit. MAb subunit analysis involves the digest of the mAb heavy chain into two domains using the IdeS enzyme, followed by reduction. This produces three subunits (including the light chain) which are then separated with HPLC. When the MS measurement is performed with an ultrahigh resolution QTOF, the resulting measurement allows determination of the subunit isotopic pattern with high fidelity. This aspect allows an algorithm to generate the monoisotopic molecular weights, usually with a precision better than 1ppm. This enables the simple and accurate quantification of protein modifications, and even the detection of deamidation at the domain level.

Selecting clones based on their similarity to a target attribute profile is essential to choose optimal cell lines and growth conditions. ***Figure 1*** illustrates how such determinations can be made at the subunit level with highly informative spectra that require minimal samples preparation and data interpretation.

MAbs were IdeS digested (Genovis), diluted into matrix for Fc-glycan profiling using MALDI-MS, and major glycans relative intensities (G0F, G1F and G2F) were measured by direct profiling of the intact Fc-domain. Attributes such as the match of the glycan profile against a reference profile were reported in BioPharma Compass 3.0, providing multiple data points to

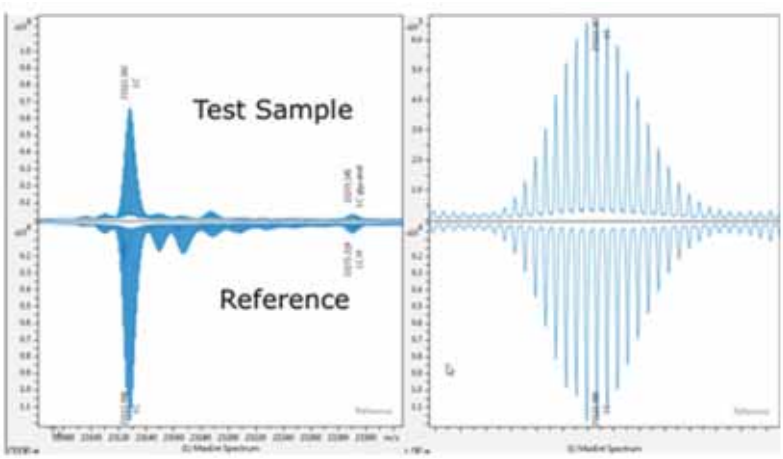
FIGURE 1



ABOVE: NISTmAb Fc glycoprofile from UHR-QTOF (top, deconvoluted) and from autofleX speed MALDI-TOF (bottom, 2+ charge state)

> *"Due to its versatility and high mass resolving power, mass spectrometry (MS) has evolved into the gold standard for biopharmaceutical characterisation and process development"*

FIGURE 2



ABOVE: Butterfly plot showing a subunit deconvoluted spectrum from a test sample and a reference

decide, for example, which clones to select for further rounds of screening.[1]

Subunit analysis enables the precise determination of the protein domains' molecular weight (providing evidence of the primary sequence correctness) and the associated heterogeneities, including glycosylation which is an essential attribute. This is a helpful tool to assess a molecule's liabilities before it progresses through the development pipeline, saving time and costs.

RIGHT: A third important workflow is top-down sequencing (TDS), which allows the direct access to amino acid residue level information directly from undigested proteins

Top-down protein sequencing

A third important workflow is top-down sequencing (TDS), which allows the direct access to amino acid residue level information directly from undigested proteins. Intact protein sequencing by top-down MS can provide N- and C-termini characterisation, which is important in ensuring that signal peptides are removed in early stage development. TDS detects and localises chemical modifications, PTMs and mutations, and is used to check the integrity of the sequence responsible for binding in later stages. MALDI is often used for TDS to proof read and check the complete amino acid sequence of the antibody and MALDI-TOF is a uniquely fast, easy and automated sequence confirmation approach with the added benefit of removing the complexities associated with HPLC. The obtained sequence information can be used particularly well when combined with accurate intact mass information from QTOF MS, allowing a rapid check to verify if the sequence is complete and/or if unexpected modifications are present.

Novel characterisation workflows

New software capabilities enable multi attribute screening workflows by MALDI, designed to speed up clone selection. The clone selection step in the development process allows R&D scientists to choose the best cell line match for potential use as a biotherapeutic, from 10s-100s of clones. To support this, glycan profiles of intact Fc-domains can be screened using MALDI-TOF MS, providing an insight to this essential attribute. In addition, it can provide antibody identities rapidly, based on differentiating abundant peptides in peptide mass fingerprints. Such screening streamlines the comparison of attributes at the intact protein level.

In addition, a new MALDI rapid identity workflow provides rapid confirmation of a sample's identity, which is useful during downstream processing and fill and finish operations. The complete analysis time, including lab work, data acquisition and processing, is 20 minutes, with each individual measurement taking in the order of five seconds allowing the analysis of hundreds of samples per hour.

Automation: accelerating time to results

Reducing time to sample and meeting data integrity requirements are primary goals for biopharma R&D scientists. Automating routine tasks such as data acquisition and data processing frees up valuable time, and automated procedures enhance results reproducibility. It is ideal to obtain a rapid match / no-match confirmation when comparing samples with a reference dataset, in an easy-to-interpret manner, so that a number of users can assess the results.

Systems often require manual data acquisition and processing. By removing the need for human decision-making, acquisition and processing is completed in a single automated step, reducing the time to result. New software includes a rapid green light/red flag system, allowing researchers to quickly establish a match or not, with the option for closer inspection of the chromatogram or MS spectra if necessary, for example using butterfly plots (***Figure 2***).

MS in biopharma QC

There is a growing interest to implement more specific assays for product release. MS is able to deliver highly specific measurements that can make some of the determinations required by regulatory authorities, for example under ICH Q6B. However, regulatory bodies require a high degree of data security and traceability of the experiment and associated reports, so it is important for labs to have tools to assist with the acquisition of data under 21CFR11 compliance.

The future of biopharma development

Increasingly innovative software solutions for biopharmaceutical characterisation have made it quicker and simpler to progress biotherapeutic drug candidates safely to market. However, some challenges remain. For example, the industry is currently lacking a feedback mechanism in the optimisation cycle of process control. Such a mechanism would ideally improve on a given process, on a constant loop, for example by adjusting to yield a target glycoprofile. The feedback loop would decide how much nutrient should go into the culture in the fermentation system, rather than a human, further reducing the chance of human error.

With regards to QC, there is some debate over whether older assays should be completely replaced by MS, because this technology provides unparalleled insights into the biopharmaceutical process. These aspects are being evaluated both by the industry and the regulatory agencies. But continued innovation in this highly specific technique suggests the rising usage of MS in regulated environments is likely to continue.

Biopharmaceutical scientists constantly strive for deeper insights into proteins and biomolecules, in shorter time frames. Recent technology improvements have enabled more complex workflows to become routine. The time taken to complete top-down analysis, intact antibody and subunit analysis with ESI-QTOF and MALDI-TOF can be reduced by implementing intuitive software which automates the acquisition, analysis and reporting of data. The subsequent data output enables automated sequence confirmation, visual data comparisons and key quality attributes for rapid, safe biopharmaceutical characterisation and development.

BIOGRAPHY

GUILLAUME TREMINTIN
is Market Manager BioPharma at Bruker Daltonics where he manages the development and commercialisation of Bruker mass spectrometry solutions for the biopharmaceutical industry.

REFERENCE

1. Resemann, A., Evers, W., Ju, Y., Tremintin, G. and Suckau, D. (2018) Rapid MALDI-MS Assays for mAb Development, Production Control and Release, CASSS AT Europe, P-148-W.

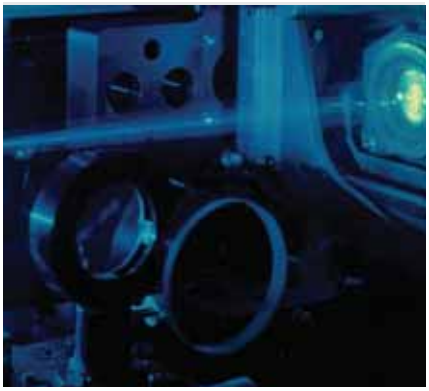
Raman spectroscopy enables Advanced Process Control in upstream bioprocessing

The Food and Drug Administration's (FDA's) Quality by Design (QbD) and Process Analytical Technology (PAT) initiatives brought a paradigm shift to using *in situ* PAT instead of grab sampling / off-line analysis.

PAT and QbD are strongly encouraged by regulatory agencies for their recognised benefits of increased scientific understanding, improved process efficiency, and consistent product quality. QbD and PAT initiatives support bioprocessing and emphasise the importance of real-time analysis in biopharmaceutical manufacturing. Implementation of PAT and QbD principles are driving innovation in the way biopharmaceutical companies operate.

Raman spectroscopy is an established PAT in small-molecule pharmaceuticals and bioprocessing, from the laboratory to manufacturing.[1] In bioprocessing, Raman spectroscopy provides an *in situ* and real-time analysis of multiple biochemical attributes without sample extraction from the bioreactor. In mammalian cell cultures, a single *in situ* Raman probe measures glucose, lactate, amino acids, and cell viability.[2,3] An *in situ* Raman measurement is representative of the process, that supports advanced process control (APC) strategies necessary to realise high-yield production and ensure consistent product quality. Raman-based feedback control allows for in-process corrections so that output variability is reduced, even with variable inputs. And, the high specificity of Raman spectroscopy enables cross-scale model transfer without significant model rework.[4,5] These features have been harnessed by industry leaders to realise yield improvements, ensure robust process understanding and apply APC to impact product critical quality attributes (CQAs).

A recent study by Berry et al. describes rapid generation of Raman-based feedback control of glucose during a CHO cell bioprocess to optimise the product's glycation CQA.[6] Significant glucose control was achieved after only two calibration steps and eight concurrent bioreactor runs. Raman-based feedback control supported a targeted concentration condition or a stepwise condition, demonstrating Raman as a robust method to integrate into a controller of an industrially relevant bioprocess. Other recent studies have demonstrated that Raman spectroscopy is compatible with a non-linear model predictive controller and that Raman-based feedback control of lactate in cell cultures increased yield up to 85% over the historical process.[7,8]



In bioprocessing, Raman spectroscopy provides an *in situ* and real-time analysis of multiple biochemical attributes without sample extraction from the bioreactor

The biopharmaceutical industry will continue to see developments in yield improvements, novel platforms, perfusion-based manufacturing, PAT for downstream processes, and original approaches to manufacturing facility design. Working closely with industry groups and leaders, we ensure that Raman spectroscopy meets not only the scientific application needs but also facility and engineering requirements. Raman spectroscopy has demonstrated value in biopharmaceutical manufacturing, from scientific understanding to process control. Our award-winning *in situ* monitoring solutions are trusted as a scalable PAT in bioprocessing. ■

Reference

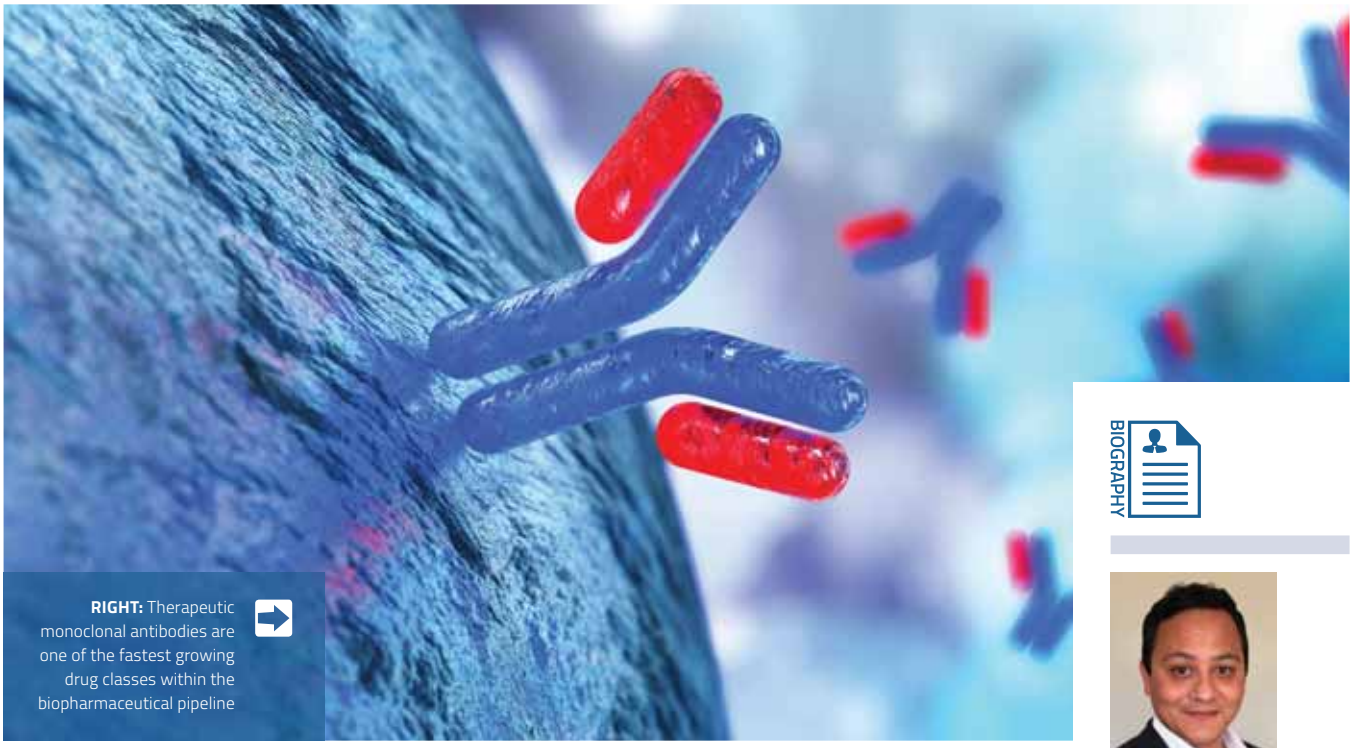
1. Esmonde-White KA, Cuellar M, Uerpmann C, Lenain B, Lewis IR (2016) Raman spectroscopy as a process analytical technology for pharmaceutical manufacturing and bioprocessing. Anal Bioanal Chem 409:637–649 . doi: 10.1007/s00216-016-9824-1
2. Abu-Absi NR, Kenty BM, Cuellar ME, Borys MC, Sakhamuri S, Strachan DJ, Hausladen MC, Li ZJ (2011) Real time monitoring of multiple parameters in mammalian cell culture bioreactors using an in-line Raman spectroscopy probe. Biotechnol Bioeng 108:1215–1221 . doi: 10.1002/bit.23023
3. Whelan J, Craven S, Glennon B (2012) In situ Raman spectroscopy for simultaneous monitoring of multiple process parameters in mammalian cell culture bioreactors. Biotechnol Prog 28:1355–1362 . doi: 10.1002/btpr.1590
4. Berry B, Moretto J, Matthews T, Smelko J, Wiltberger K (2015) Cross-scale predictive modeling of CHO cell culture growth and metabolites using Raman spectroscopy and multivariate analysis. Biotechnol Prog 31:566–577 . doi: 10.1002/btpr.2035
5. Mehdizadeh H, Lauri D, Karry KM, Moshgbar M, Procopio-Melino R, Drapeau D (2015) Generic Raman-based calibration models enabling real-time monitoring of cell culture bioreactors. Biotechnol Prog 31:1004–1013 . doi: 10.1002/btpr.2079
6. Berry BN, Dobrowsky TM, Timson RC, Kshirsagar R, Ryll T, Wiltberger K (2016) Quick generation of Raman spectroscopy based in-process glucose control to influence biopharmaceutical protein product quality during mammalian cell culture. Biotechnol Prog 32:224–234 . doi: 10.1002/btpr.2205
7. Craven S, Whelan J, Glennon B (2014) Glucose concentration control of a fed-batch mammalian cell bioprocess using a nonlinear model predictive controller. J Process Control 24:344–357 . doi: 10.1016/j.jprocont.2014.02.007
8. Matthews TE, Berry BN, Smelko J, Moretto J, Moore B, Wiltberger K (2016) Closed loop control of lactate concentration in mammalian cell culture by Raman spectroscopy leads to improved cell density, viability and biopharmaceutical protein production. Biotechnol Bioeng 113:2416–2424 . doi: 10.1002/bit.26018

Bioproduction monitoring for monoclonal antibodies just got easier

Kyle D'Silva

Thermo Fisher Scientific

Driven by the need for more robust and efficient analytical methods, bioproduction facilities for recombinant monoclonal antibodies and antibody-drug conjugates are demanding greater method compatibility and ease of use.



RIGHT: Therapeutic monoclonal antibodies are one of the fastest growing drug classes within the biopharmaceutical pipeline

THERAPEUTIC monoclonal antibodies (mAb) are one of the fastest growing drug classes within the biopharmaceutical pipeline – faster than gene/cell therapies and even vaccines.[1] mAb products move to market at a rate of three to five new products approved per year.[1] And, while their design and basic approach is not new, their market popularity can be attributed to their effectiveness. With precise targeting and low risk of immunogenicity, it is easy to see the potential that these drugs offer.

Biotherapeutics such as mAbs and antibody-drug conjugates (ADCs) are designed to treat a variety of diseases, from cancer and autoimmune diseases to less common indications.[2] Typically achieving higher approval rates than other drug types, total quantities of the products produced annually are increasing based on demand.[2] With this growth, comes bottlenecks in scaling, particularly for biotherapeutics that require rigorous quality control (QC) and quality assurance (QA) processes following stringent regulations.

Controlling variation

A biotherapeutic is an effective therapy given its target specificity and reduced risk for toxicity. However, biotherapeutics are also extremely complex to produce. Standard small molecule drugs, such as aspirin, are directly synthesised with set protocols, leaving little room for variation or

BIOGRAPHY

KYLE D'SILVA has a PhD in applied analytical chemistry from University of Leeds and over 15 years of experience working for several analytical instrument vendors in applications, product management and product marketing roles. Today, Kyle focuses on technologies and workflows using chromatography and mass spectrometry, for both pharmaceutical and biopharmaceutical applications, for Thermo Fisher Scientific.

"With this growth, comes bottlenecks in scaling, particularly for biotherapeutics that require rigorous quality control (QC) and quality assurance (QA) processes following stringent regulations"

efficacy issues. However, large molecule biologics, such as mAbs, are produced within a biological system using cell culture, and are prone to process related product variation.

Post-translational modifications (PTM), such as N- and C-terminal modifications, deamidation, oxidation, glycation and glycosylation can result in notable heterogeneity of mAb products including acidic and basic charge variants, leading to potential significant impacts on product activity.[3] Environmental sources are a likely cause of product variation, from cell stress to media changes that can modify a protein product.[3]

Experimental variables such as clone selection or process optimisation during the early stages of mAb development require monitoring and screening approaches for correct production set-up. Similarly, real-time analysis of mAb batches during manufacturing is also necessary to measure critical quality attributes (CQA) required to characterise a biotherapeutic and enable control of the final product.[3] Given the inherent complexity of molecules within a biological system, the need to control these protein variations within bioproduction calls for direct analytical procedures that ensure the original protein is manufactured for confirmed safety and efficacy of a drug.

Current methods come with big challenges

Methods for monitoring therapeutic protein quality traditionally rely on a variety of stand-alone chromatographic approaches, each with the capability to determine another variable of the product; such as glycosylation or charge variation. Many chromatographic approaches employ high-salt buffers and cannot typically be partnered with mass detection due to incompatibilities of mass spectrometry with such mobile phases. Moreover, cell culture media also contains components that make direct measurement a challenge for mass spectrometry. Consequently, some traditional analytical processes can take days to obtain final drug molecule measurements due to laborious multidimensional chromatography and sample preparation routines. Alternative rapid and sensitive methods that enable immediate CQA characterisation could improve both quality and productivity.[3]

Ion exchange liquid chromatography (IEX) is currently a preferred method for protein variant characterisation due to its ability to recognise charge variations between isoforms of a mAb drug molecule.[4] The ability to characterise and monitor charge variants is critical to ensure product stability, safety and reliable processing. When IEX identifies

product charge variants, deviations in peak area or the appearance of new peaks can then be characterised to evaluate whether any unwanted PTMs have occurred.[5] Since modifications can alter binding capabilities or cause conformational changes, characterisation is extremely important to mitigate any risk of aggregate formation or immunogenicity. IEX can be initially used to identify alterations in isoform charge variant patterns followed by fractionation of a peak of interest, and subsequent analysis by peptide mapping and / or intact mass analysis.[6] However, this multistep process is both time and labour consuming.

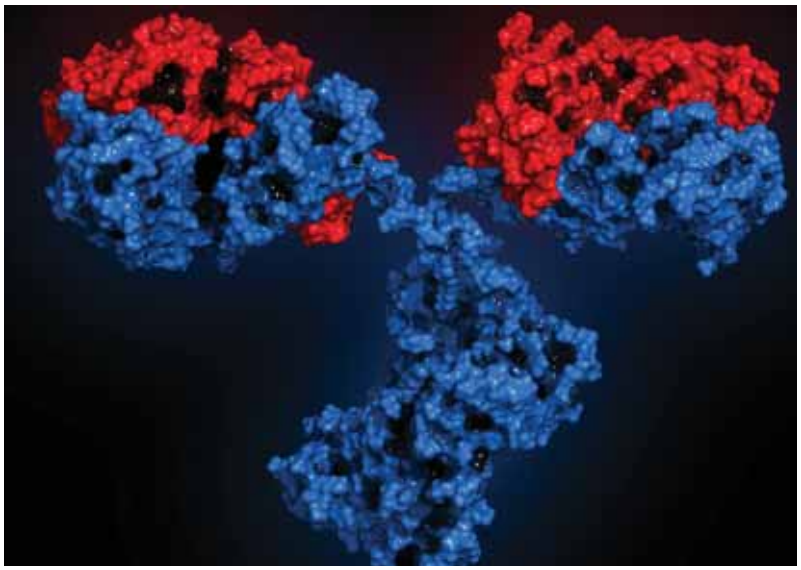
While a single IEX-MS workflow would be highly beneficial for variant analysis to confirm peak identity of potential isoforms, traditional IEX chromatography methods impede this association. Such methods are limited using salts and the presence of other components in the mobile phase that can reduce robustness of the MS instrument and decrease sensitivity.[3,4] Given these limitations, often several desalting or purification steps are employed in order to directly connect chromatography with MS analysis.[3] Not only have these supplemental steps increased time spent and added workflow complexity, but they were shown to result in low resolution or poor spectral quality.

Additionally, protein denaturation can occur during chromatography, which causes loss of tertiary structure, eliminating the potential for MS analysis to capture information about the specific charge state of the native protein.[3] Native state analysis of large molecules by MS allows investigation into changes in conformation that can affect function, providing insight into how a modified sequence may change a protein's structure and function.

Two new opportunities for charge variant mass analysis

Recent developments in capillary electrophoresis show promise in advancing charge-based separations in combination with mass spectrometry to rapidly improve workflow and laboratory efficiency in the characterisation of biopharmaceuticals. A capillary electrophoresis-electrospray ionisation-mass spectrometry (CE-ESI-MS) method applying microfluidics in a small chip-based separation module (908 Devices ZipChip System) can generate quality information through a single analysis step, completed in three minutes. The information generated from this three-minute, one step analysis, includes the determination of intact product variant mass and charge heterogeneity

"Not only have these supplemental steps increased time spent and added workflow complexity, but they were shown to result in low resolution or poor spectral quality"



ABOVE: Monoclonal antibodies

screening of mAb isoforms. The entire analytical process can take 1.5 hours or less including preparation, analysis, and data processing.[6] Consequently, the technique is now being deployed in biopharmaceutical development laboratories, including those at Biogen.[8]

Integrated microfluidics within the CE-ESI device channels control analyte adsorption to channel walls and ion migration induced by an applied potential while maintaining effective separation.[6,7] This benefits the system by focusing the targeted protein throughout the capillary for more effective separation. The combined strategy is successful in separating mAb variants with high resolution and identifying the mass difference between isoforms in addition to glycosylation patterns for each variant. Connecting CE-ESI with Orbitrap-based MS (Thermo Fisher Scientific) creates a rapid and comprehensive approach for the separation and identification of intact mAb charge variants. Abundant protein isoforms and glycosylation profiles can be screened directly from cell culture media without additional protein purification. Whilst minor variants may remain unseparated in the electropherogram, screening of main lysine variants are easily implemented using this approach.[8]

> *"Current approaches cannot comprehensively meet these required quality attributes without laborious procedures involving intermediate protein purification to reach high resolution mass analysis"*

While CE-ESI-MS offers a rapid alternative to the current multi-step protocols, recent advances in methodology coupling IEX to MS have also shown great promise.[4] While the ZipChip offers incredibly fast screening analysis, IEX-MS has been demonstrated by researchers at the National Institute for BioProcess Research and Training (NIBRT, Ireland) as a viable alternate approach based on a new method utilising pH gradient elution with volatile, low ionic strength buffers and high resolution accurate mass spectrometry (Thermo Scientific Q Exactive BioPharma system).[4]

NIBRT's approach too offers a simple strategy for mAb characterisation on the intact level without any need for sample preparation. However, NIBRT was also able to demonstrate that IEX-MS data generated from even minor variants was sufficient in quality to achieve component identification with high mass accuracy, allowing for the identification of critical quality attributes including accurate intact mass, lysine truncation, glycosylation, and deamidation from a single LC–MS injection.

Conclusions

Regulatory guidelines require the characterisation of the original protein sequence and any new PTMs present on mAbs to ensure that the final therapeutic protein product meets quality specifications. Current approaches cannot comprehensively meet these required quality attributes without laborious procedures involving intermediate protein purification to reach high resolution mass analysis.

Introducing new methodologies to improve upon strategic bottlenecks in bioproduction ensures quality of the final product. New developments in IEX-MS methods are beginning to show promise for hyphenation of charge variant and intact mass workflows. For those wanting speed and rapid screening, CE-ESI-MS offers proven, fast and efficient separation for screening of mAb products and their variants. It represents a simple workflow that brings a higher level of quality and confidence to bioproduction workflows for complete variant analysis.

REFERENCES

1. Ecker DM, Jones SD, Levine HL. The therapeutic monoclonal antibody market. mAbs Volume 7, 2015 – 1, 9-14. https://www.tandfonline.com/doi/full/10.4161/19420862.2015.989042
2. Medicines in Development Report – Biologics. http://phrma-docs.phrma.org/sites/default/files/pdf/biologics2013.pdf
3. Wang Y, Feng P, Sosic Z, Zang L. Monitoring Glycosylation Profile and Protein Titer in Cell Culture Samples Using ZipChip CE-MS. J Anal Bioanal Tech 2017, 8:2.
4. Füssl F, Cook K, Scheffler K, Farrell A, Mittermayr S, Bones J. Charge Variant Analysis of Monoclonal Antibodies Using Direct Coupled pH Gradient Cation Exchange Chromatography to High-Resolution Native Mass Spectrometry. Analytical Chemistry, 2018, Volume 90, Issue 7, 4669-4676.
5. Leblanc Y, Ramon C, Bihoreau N, Chevreux G. Charge variants characterization of a monoclonal antibody by ion exchange chromatography coupled on-line to native mass spectrometry: Case study after a long-term storage at +5 °C. Journal of Chromatography B. Volume 1048, 24 March 2017, Pages 130-139.
6. Redman EA, Batz NG, Mellors JS, Ramsey JM. Integrated Microfluidic Capillary Electrophoresis-Electrospray Ionization Devices with Online MS Detection for the Separation and Characterization of Intact Monoclonal Antibody Variants. Anal. Chem., 2015, 87 (4), pp 2264–2272.
7. Ghosal S. Effect of Analyte Adsorption on the Electroosmotic Flow in Microfluidic Channels. Anal. Chem., 2002, 74 (4), pp 771–775.
8. www.thermofisher.com/us/en/home/global/forms/industrial/discover-more-rapid-biotherapeutics-characterization-ce-esi-ms.html

Moving bioprocess data into the cloud and towards 'big data' – the first steps

Daniel Egger, Director Marketing & Software Development

Eric Abellan, Product Manager Software

Discussions regarding the storage, handling and exchange of data in the life sciences are often focused on genomics and proteomics. Data management is also an issue in other disciplines, eg, bioprocessing, which has its own requirements for storing, handling and understanding large amounts of data.

THIS is especially true for the integration of several key elements of bioprocessing, such as the planning of experimental / manufacturing campaigns, acquisition of local process data and the ability to relate this to data for quality control plus statistical process control.

The database methodologies used for Supervisory Control and Data Acquisition (SCADA) bioprocess data are a good solution for the traditional data types currently being stored, eg, sensor values, set points and parameters based on analytical data. However, not all bioprocess information fits neatly into the standard relational database model, since batch records should also include experimental details and specifics such as media, validation, recipes and soft-sensors, as well as microbial and cell culture characteristics. Combined data of several diverse types stored in one central location and accessible through a common interface is key to generating knowledge from data.

In general, the latest NoSQL (Not Only SQL) databases are better suited for handling 'big data', which tends to be less structured. Such databases enable rapid data processing and retrieval, and address record keeping involving evolving data requirements with an emphasis on scalability. Moreover, these methodologies are easing the path towards cloud-based storage solutions with unlimited data sharing already in widespread use.

With the possibility to manage 'big data' in the bioprocessing field, the topic of bioprocess hardware heterogeneity and diversity of new forms of bioprocess data has to be addressed. The ability for next-generation bioprocess platform software (such as eve from INFORS-HT) to allow the import of common offline file formats (such as Excel), Computer System Validation and movement of data to and from third-party software using a standardised approach, is vital. Also, for equipment data exchange, all Open Platform Communications standards (DA classic, XML DA, UA) provide a network-based solution covering a wide range of controllers, sensors, etc. As a modern data interface using web services, the REST API allows real-time data exchange to and from external data sources, irrespective of the origins of the third-party software, and stores data in one centralised database for subsequent storage, analysis and knowledge creation.

Combining all these elements allows software solutions that take the whole bioprocess and provides a user-friendly, understandable series of workflows to allow users to move seamlessly through project planning, experiment planning, recipe creation of batch processes (including the use of standard or customised soft sensors), plus integration of third-party devices. This functionality, combined with multi-user access via a web browser, is the key development of many modern bioprocess software packages. The actual bioprocess can then be navigated with monitoring, data acquisition, alarm handling, production of an audit trail and inclusion of off-line data, plus notes. Finally, the archived information can be viewed, analysed and made available for process optimisation and further improvement.

With the possibility of making bioprocess 'big data' available and searchable in a rapid way using modern software, eg, eve, an unlimited potential of aggregating diverse data into information and then knowledge is unleashed. New information technology lets us visualise bioprocess data in a holistic way and provides the tools necessary to navigate the data jungle. With the quantities and quality of raw data increasing exponentially, this is a key driver for new discoveries and better-optimised pharmaceuticals.

PAT's key role in pharmaceutical manufacturing 'cannot be overemphasised'

Adeyinka Temitope Aina

Pharmaceutical Manufacturing Technology Centre, Department of Chemical and Environmental Sciences, University of Limerick

The relative importance of the applications of Process Analytical Technology (PAT) in the manufacturing sphere, particularly in pharmaceutical manufacturing, cannot be overemphasised.

BIOGRAPHY

ADEYINKA TEMITOPE AINA was an innovation fellow at the Pharmaceutical Manufacturing Technology Centre, Ireland. He has extensive research experience in pharmaceutical and biomedical applications. He did his doctoral training at the Laboratory of Biophysics and Surface Analysis at the School of Pharmacy, University of Nottingham, UK.

HENCE the regulatory authorities have always supported and motivated manufacturers to improve on their efforts in securing product quality / efficacy, knowing full well the importance of these attributes in drug products.[1]

To further encourage manufacturers in the above regard, the US Food and Drug Administration (FDA) issued guidance documentation in 2004,[2] to serve as a developmental framework to foster novel perspectives in pharmaceutical development, manufacturing and product integrity.[3,4]

PAT is a multi-faceted, interdisciplinary approach that incorporates several scientific and engineering sub-themes. Those include:

Process chemistry development, process analytical chemistry and spectroscopic analysis, multivariate statistics, chemometrics, chemical engineering, process engineering design and control engineering,[4] as typified in ***Figure 1***.

A globally accepted definition of PAT, as it relates to pharmaceutical manufacturing, is: "A system for designing, analysing, and controlling manufacturing through timely measurements (ie, during processing) of critical quality and performance attributes of raw and in-process materials and processes with the goal of ensuring final product quality."[1,2,4]

In other words, quality cannot be tested into products; it should be built-in or should be by design.[1,4,5]

One area of pharmaceutical manufacturing that has seen extensive improvement and deployment of PAT is sensor development; especially using inline / at-line and online monitoring systems with a view to achieve process automation. This is particularly essential as pharmaceutical manufacturers aim to achieve optimal and timely delivery of products in order to reduce costs in a highly competitive market.

Miniature mass spectrometer technology

The Pulliam *et al* study[6] is a practical example of this. Using a miniature mass spectrometer technology (based on recent improvements in chemical sampling), they were able to simultaneously monitor multiple reactions, achieving high reproducibility with minor relative errors for major products.

Peles *et al*[7,8,9] deployed a novel light-emitting diode (LED) array-based light induced fluorescence (LIF) sensor to analytically monitor pharmaceutical cleaning verification. The LIF sensor facilitated a significant enhancement in the analysis time required for quantitative detection.

Using an ultra-performance liquid chromatography (UPLC) system, Waters PATROL UPLC, as an online PAT monitoring tool, Jang *et al*, [10] were able to simultaneously account for changes in concentration during crystallisation (due to solubility, dissolution and degradation), real time calibration of UV / Vis / Raman spectroscopy and, at the same time, monitor product purity.

Enhanced PAT-based process monitoring[11] has also seen wider usage, and this has contributed immensely to process understanding. One area of pharmaceutical manufacturing that has benefited from it is the optimisation and control of continuous crystallisation.[12-15]

Using an automated Intelligent Decision Support (IDS) system, Powell *et al*[16] studied the continuous crystallisation of paracetamol form I. Their experimental set up, incorporating an integrated PAT array (which included the use of Raman spectroscopy), allowed them to deduce critical information such as: crystal nucleation and growth as well as crystal morphology.

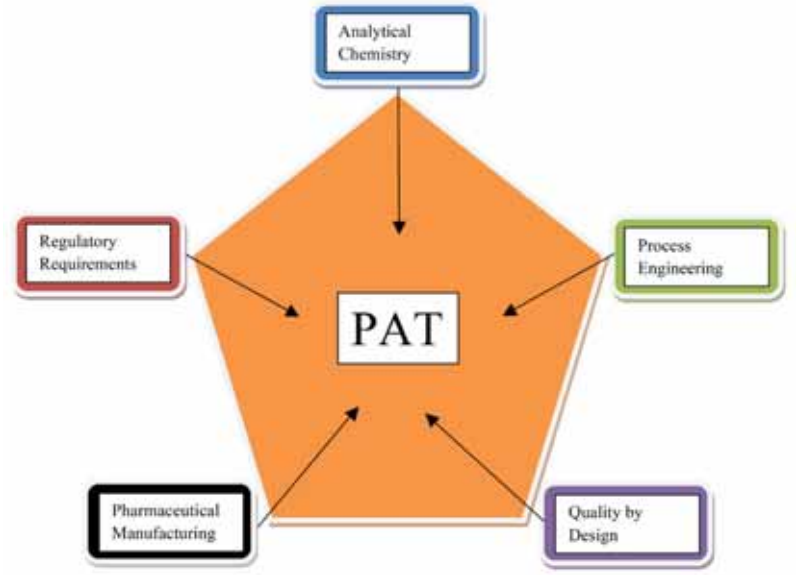
Simone *et al*[17] used a combination of UV / Vis spectroscopy, focused beam reflectance measurement (FBRM) and the CryPRINS software (Crystallisation Process Informatics System) as PAT tools, to monitor the crystallisation stage of vitamin B12 crude product extracted through fermentation. Their results showed a link between impurities and the nature of crystal growth of vitamin B12. Other authors have adopted a similar approach in their own studies.[18,19]

Results from the study carried out by Šahnić *et al* using an in-line Raman spectroscopic method to monitor the synthesis of Omeprazole showed that the model deployed was not only successful in a kilo-lab scale but could also be optimised as a quick response process analytical tool.[20]

Conclusions

In conclusion, the potentials inherent in the use of PAT, are supported by an industry market forecast that the process analytical technology market is expected to grow at a compound annual growth rate of between 13% and 13.5% over the forecast period 2017 – 2023.[21] Regrettably, more than a decade after the FDA issued the regulatory guidelines, the regular usage of PAT in an integrated manner to design, detect and control critical process parameters and performance characteristics through timely and proper measurements remains largely under-utilised across the pharmaceutical industry.[1]

FIGURE 1



ABOVE: Multidisciplinary attributes of PAT

> *"PAT is a multi-faceted, interdisciplinary approach that incorporates several scientific and engineering sub-themes"*

REFERENCES

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MICROBIOLOGY

Limulus amebocyte lysate users, compendial experts and regulators are still orienting themselves to the recombinant factor C (rFC) assay. Changes to compendial standards do not occur overnight and users willing to change must perform the alternative validation procedure USP <1225>, explains Kevin L Williams, from bioMerieux.

There have been many changes in pharmaceutical microbiology as we progress into the 21st century. Jeanne Moldenhauer, from Excellent Pharma Consulting, discusses some of the recent changes in areas of interest to microbiologists.

The environment in pyrogen and endotoxin testing is also changing significantly. The key developments are reviewed by Marsha Steed, from Concordia Valsource, Johannes Reich, from Microcoat, and Josh Eaton, from Parenteral Drug Association.

Specificity in the recombinant factor C test for endotoxin

Kevin L Williams

bioMerieux

Limulus amebocyte lysate (LAL) users, compendial experts and regulators are still orienting themselves to the recombinant factor C (rFC) assay. Changes to compendial standards do not occur overnight and, for now, users willing to change must perform the alternative validation procedure USP <1225>.

WHEN validating an alternative assay, the specificity of the assay for the impurity to be detected is a requirement. The test must be able to distinguish the impurity in the sample from other, non-related substances and impurities. With the recombinant version of the horseshoe crab biosensor, rFC, there are three levels of specificity provided compared with LAL testing. This paper seeks to highlight three different specificities of rFC that include (i) Enzymatic, (ii) Spectral, and (iii) Genetic level specificity.

Level 1. Enzymatic specificity

The molecular interaction of the factor C biosensor with endotoxin is an ancient enzymatic specificity developed in some invertebrates1 in which the zymogen protein interacts with and binds endotoxin in the region of factor C sushi peptides2. This binding then facilitates the breaking of a specific bond at the serine protease end (the opposite end) of the zymogen. The factor C molecule has now become '*activated*' through its interaction with endotoxin and, in this new form, is specific for the next protein in the serine protease cascade (factor B). In the case of the recombinant protein, the activation of the zymogen reacts with a small fluorophore peptide of a specificity that mimics factor C's interaction with factor B (***Figure 1***). This refers to enzymatic activation of factor C zymogen by endotoxin.

Derived from references 2 and 3: "During the lipopolysaccharide-mediated activation of factor C, its single-chain form is converted to a two-chain intermediate form with an 80-kDa heavy and a 43-kDa light chain, and the light chain is subsequently cleaved at a unique Phe-Ile linkage to form a 7.9-kDa A chain and a 34-kDa B chain held together with a disulfide bond(s). The resulting three-chain factor C shows an ability to activate factor B and to hydrolyse a synthetic tripeptide substrate, Boc-Val-Pro-Arg-NH-Np."[4]

It has been known for some time that LAL contains an alternative enzymatic pathway that can be activated by fungal and cellulosic breakdown byproducts, called the beta-glucan pathway. Non-endotoxin LAL reactive materials (LRM) in drug raw materials and products caused considerable concern upon the initial discovery of the additional pathway.[5,6] As ***Figure 2*** shows, beta-glucan acts upon the proclotting enzyme rather than factor C. Thus, LAL is not specific for endotoxin, whereas recombinant factor C assay is specific only for endotoxin. LAL users can use a beta-glucan blocking buffer to create an LAL test that is specific for endotoxin,[7] see ***Figure 2***.

Level 2. Spectral specificity

Specificity here explores the fluorescent spectrophotometric detection method used with recombinant factor C assays. Various non-specific colour changes can occur with absorbance-based test methods (colourimetric and turbidimetric), whereas the fluorescent method used by recombinant factor C employs a very specific fluorophore with very specific excitation and emission wavelengths. In fluorescent assays, the excitation of a sample using a specific wavelength (380nm) and a different specific wavelength for emission detection (445nm) provides another level of specificity in performing the assay. Thus, random interference in terms of colour change, light ingress or other chemical change related occurrences are invisible to the fluorescent test.

Absorbance tests have been used successfully for several decades in LAL chromogenic and turbidimetric assays. But for any given, specific sample one may need to troubleshoot why a consistent assay cannot be obtained. Geisler lists some of the factors that can affect absorbance assays.

BIOGRAPHY

KEVIN L WILLIAMS has worked in the pharmaceutical field for 35 years. The bulk of his career (30 years) was spent at Eli Lilly & Company, developing quality control tests for microbial and endotoxin detection. After Lilly he worked at Hospira, Lonza, and currently works for bioMerieux. He is the author of: Endotoxins 2nd Edition (Marcel Dekker, 2001) and Endotoxins 3rd Edition (Informa Healthcare, 2007). Most recently at BioMerieux he has been helping to set up a dedicated endotoxin test lab in the Chicago area.

- Selectivity: UV / Vis spectrophotometer does not discriminate between the sample of interest and contaminants that absorb at the same wavelength
- Stray light: the detectors used in spectrophotometers are broadband, meaning they respond to all the light that reaches them. If there are impurities in the sample that reflect light, an erroneous reading may be recorded. Stray light also causes a decrease in absorbance and reduces the linearity range of the instrument
- Sample conditions: absorption results can be influenced by temperature, pH, impurities and contaminants. All these factors can change the absorption properties of the sample, leading to inaccurate readings.

Geisler also outlines the general advantages of fluorescent methods.

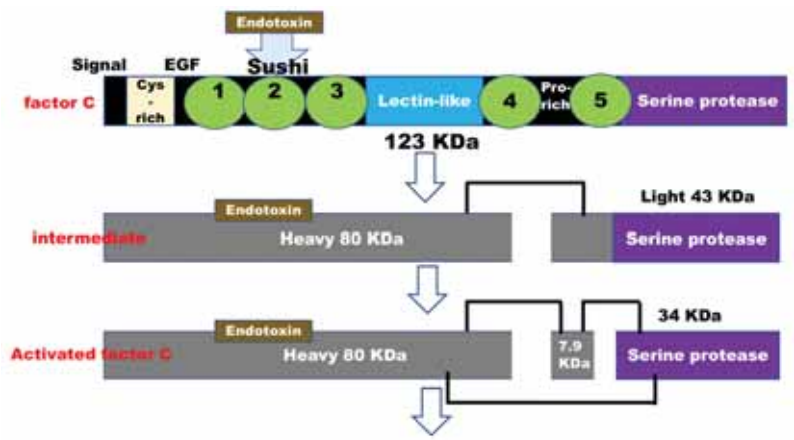
- Sensitivity: the sensitivity of fluorescence detection is approximately 1,000 times greater than absorption spectrophotometric methods
- Specificity: only molecules that fluoresce are detected by this method, resulting in greater specificity compared with UV / Vis absorption
- Wide concentration range: fluorimetry generally can detect more than three to six log orders of concentration without sample dilution or modification of the sample
- Accurate results: the sensitivity and specificity of fluorescence measurement leads to potentially more precise and accurate readings.

It is this fluorescent sensitivity and specificity that allows rFC to achieve a very sensitive level of detection without the need for the additional cascade proteins: 0.05 EU/mL for a 20-minute test, 0.005 EU/mL for a 60-minute test and 0.001 EU/mL using a 120-minute test.

Level 3. Genetic specificity

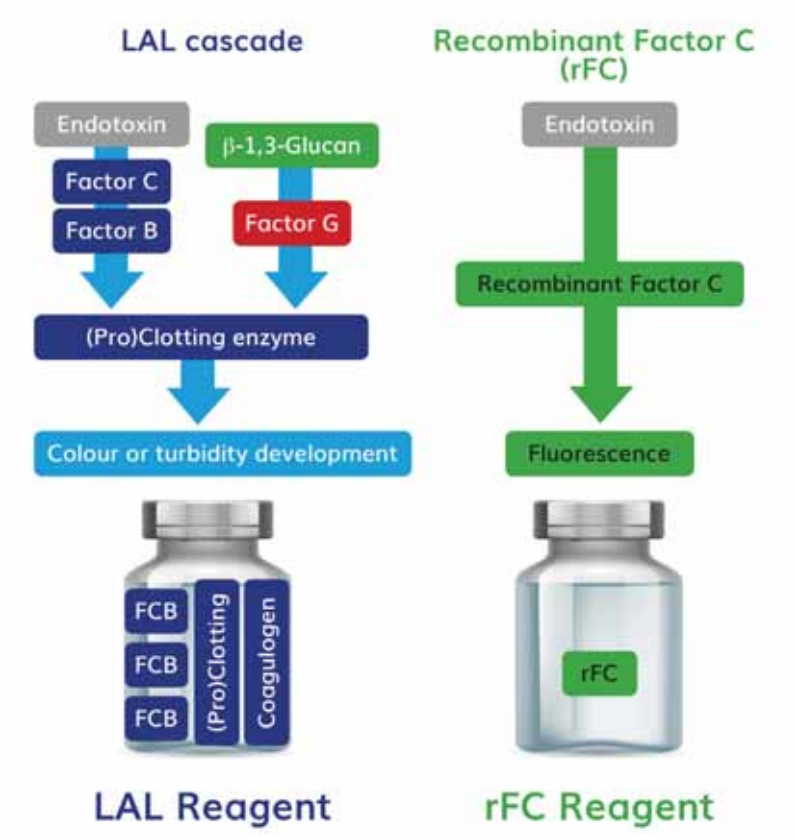
It is good to remember where the rFC protein has come from. See ***Figure 4***. The horseshoe crab factor C gene was originally cloned from *Carcinoscorpius rotundicauda* at the National University of Singapore by Jeak Ling Ding and Bow Ho.[9] DNA recombinant technology was developed in the 1970s and culminated with the cloning and expression of the insulin protein as the first recombinant drug (in 1982). The subsequent biotechnology revolution is a powerful story that has culminated in the cloning and expression of dozens of molecules that have drastically improved human health. These drugs include monoclonal antibodies used to treat cancer, infection, and autoimmune disease; cytokines and enzymes used to replace those genetically deficient in specific functions such as blood coagulation. The 'at will' expression of natural proteins via recombinant methods can be viewed as perhaps the third great

FIGURE 1



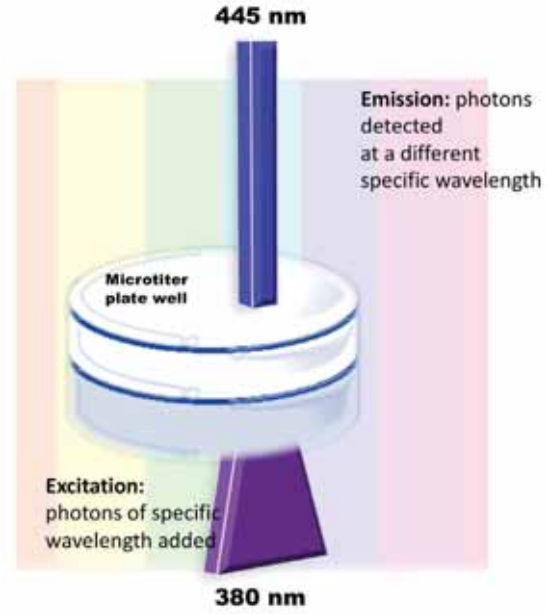
ABOVE: Activation of factor C activates factor B (LAL) or releases fluorophore (rFC)

FIGURE 2



ABOVE: The simplification of the protein test milieu provides the first level of specificity for endotoxin testing via rFC

FIGURE 3



RIGHT: The spectral specificity of fluorescence detection. Background light of the entire optical spectrum that is not 445nm is not collected. 445nm is the resonance of the fluorophore released by the enzymatic reaction

REFERENCES

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advancement in the prevention and treatment of disease; the first two being increasing sanitation (reducing water-borne illness) and vaccination. Vaccination has come to be improved by DNA recombinant methods, where the antigenic protein can be produced in a purified form thus removing potential risks associated with the use of attenuated organisms (microbial and viral).

Prior to the availability of recombinant methods some animal proteins, including bovine and porcine insulin, were harvested from cow and pig pancreatic glands beginning in the 1920s. The squeezing of proteins from a mass of organ tissue[10] and subsequently processing it for injection requires a great amount of raw materials. According to *Diabetes Forecast*, more than two tons of pig organs were needed to extract a mere eight ounces of purified animal insulin.[11] Biologic drugs today (except for a few vaccines) are produced via biotechnology. Given the anticipated upswing in the number of tests and companies performing LAL testing world-wide, the sustainability of endotoxin via LAL testing is an important concern for the pharmaceutical industry.

The development of animal-based proteins and the subsequent transition to recombinant sourced proteins to protect human health seems an under-appreciated topic. Consider briefly, the insulin story.

In 1921, a young surgeon named Frederick Banting and his assistant Charles Best figured out how to remove insulin from a dog's pancreas. Skeptical colleagues said the stuff looked like "thick brown muck," but little did they know this would lead to life and hope for millions of people with diabetes.

With this murky concoction, Banting and Best kept another dog with severe diabetes alive for 70 days – the dog died only when there was no more extract. With this success, the researchers went a step further. A more refined and pure form of insulin was developed, this time from the pancreases of cattle.

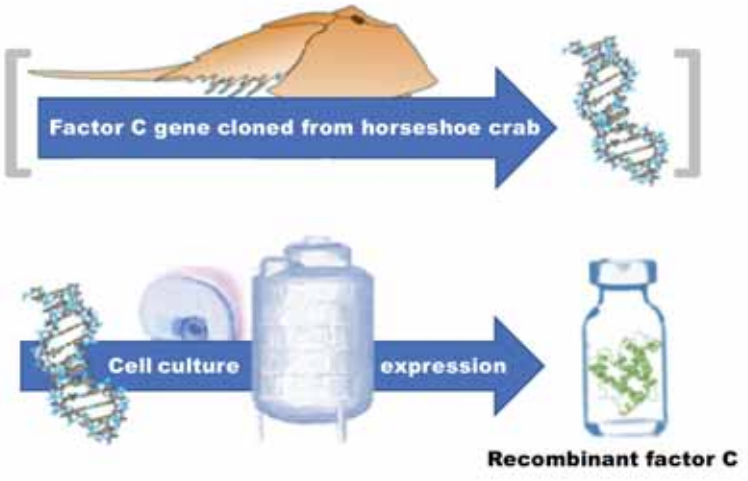
In January 1922, Leonard Thompson, a 14-year-old boy dying from diabetes in a Toronto hospital, became the first person to receive an injection of insulin. Within 24 hours, Leonard's dangerously high blood glucose levels dropped to near-normal levels.

The news about insulin spread around the world like wildfire. In 1923, Banting and Macleod received the Nobel Prize in Medicine.

Soon after, the medical firm Eli Lilly started large-scale production of insulin. It wasn't long before there was enough insulin to supply the entire North American continent. In the decades to follow, manufacturers developed a variety of slower-acting insulins, the first introduced by Novo Nordisk Pharmaceuticals, Inc., in 1936.[12]

Of course, the story didn't end there, as Eli Lilly developed a recombinant version of human insulin which began to be sold in 1982, thus kicking off the recombinant revolution. Lilly has also become one of the first big pharmaceutical companies to pursue testing with rFC in lieu of LAL.[13] The 'at will' expression of recombinant molecules allows the production of valuable proteins in unlimited amounts in an animal-free manner. The story of recombinant factor C, while not miraculous like the insulin story, provides the similar assurance of a sustainable supply.

FIGURE 4



ABOVE: Genetic specificity. Once the gene has been cloned, the desired protein can be produced without the animal from which it was derived, in this case the horseshoe crab biosensor protein factor C

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Jeanne Moldenhauer

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There have been many changes in pharmaceutical microbiology as we progressed into the 21st century. Some of these changes have been due to the advance of rapid microbiological methods and knowledge gained from the study of the human microbiome, while others are changes to conventional testing methods. This paper discusses some of the recent changes in areas of interest to microbiologists.

BIOGRAPHY

JEANNE MOLDENHAUER, Vice-President of Excellent Pharma Consulting, has more than 30 years' experience in the pharmaceutical industry. She chaired the Environmental Monitoring / Microbiology Interest Group of Parenteral Drug Association for more than 15 years, served on the Scientific Advisory Board of PDA for 20 years, founded the Rapid Microbiology User's Group, and is a member of American Society for Quality, and Regulatory Affairs Professionals Society. She is the author of many books and numerous publications, including book chapters and magazine articles.

The Human Microbiome Project (HMP)

The data obtained from this project has been providing many details about the relationship between humans and microorganisms. The data provides a description of the large number of microorganisms colonised in humans. Some of these organisms may be "opportunistic pathogens" that are able to cause human diseases. Basically, the relationships of these organisms can be commensal, symbiotic or pathogenic. A commensal relationship is one in which one of the organisms, either the human or the bacterium, benefits from the relationship, while symbiotic relationships benefit both the human and the bacterial species. Pathogenic relationships are those where the microorganism is known to cause a human infection.[1] Understanding these relationships more clearly will have a significant impact on much of the microbiological testing we perform for the pharmaceutical industry.

Another advancement in this field is the study of whether artificial intelligence can be used to study gut microbes in patients. This project involves both the Human Microbiome Project and artificial intelligence. This would allow for the microbes to also have artificial intelligence to evaluate the microorganism and its impact on different diseases, eg, predicting the success of surgery, curing obesity, and so forth.[2]

Microorganisms become the active ingredient in pharmaceuticals

There is a variety of new topical probiotic personal care products that has been introduced. Farris indicated that "the studies reviewed suggest that topical prebiotics, probiotics, and

bacterial cell lysates do provide demonstrable skin benefits." This has resulted in topical products that include live microorganisms. In some cases, the product may include more than 50,000 colony forming units of a microorganism as the active ingredient. The problem is how one passes some of the tests in the *United States Pharmacopeia* (USP), such as, for example, USP <61> for microbial enumeration testing, <62> the testing for specified microorganisms and <51> the test for antimicrobial preservative effectiveness. Some of the problems this causes include determining methods to preserve the formulation, while using a preservative with a narrow spectrum to maintain the viability and efficacy of the active ingredient.[3]

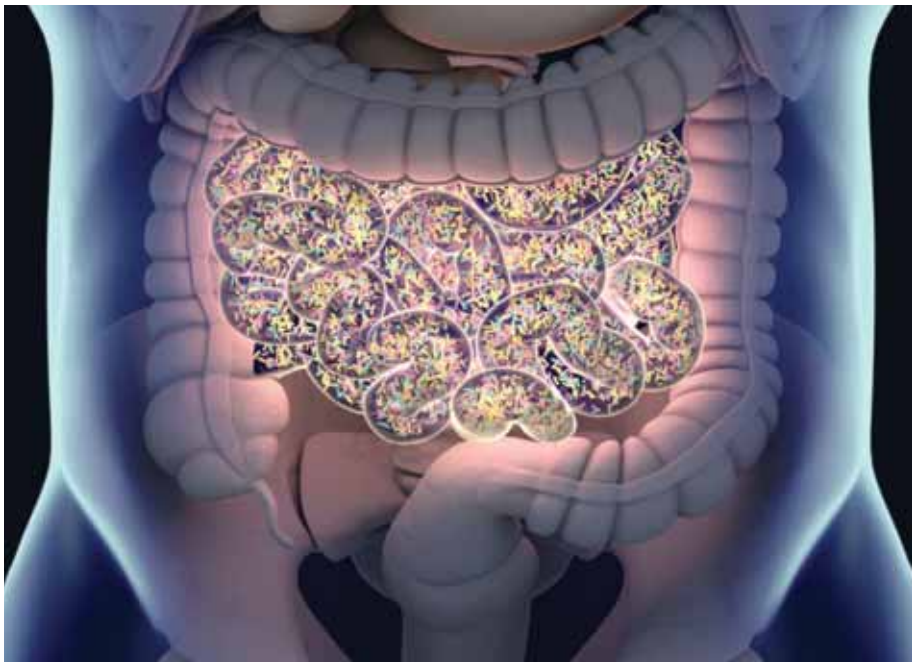
Even some bacteriophages are used in topical products – an active ingredient that is a virus that infects replicates within a bacterium.[3]

Faecal transplantation is a newer use of microbiology. Faecal transplantation (or bacteriotherapy) is the transfer of stool from a healthy donor into the gastrointestinal tract for treating recurrent C. difficile colitis or other diseases. Many of these diseases can be complications of antibiotic therapy.[4] There are companies that are working to isolate these "healthy microorganisms" and convert them into pharmaceutical active ingredients and products. This can produce many challenges for the microbiologist. Some of these challenges include:

- Healthy individuals are initially needed to provide the faecal material for transplant
- Isolation of the microorganisms that "make a difference" in treating the specific disease
- Finding a methodology to culture these organisms, maintaining their health benefits, while creating a situation where they can be routinely cultured
- Determining a methodology to evaluate efficacy, without giving it to patients
- Creating environmental monitoring procedures that monitor for contaminants but understands that the microorganisms are part of the product, and so forth.

Culture media

The Food and Drug Administration demonstrated an increased interest in *Burkholderia cepacia* Complex (BCC). This organism has caused issues for cystic fibrosis patients. In 2016, a group of "healthy" hospitalised patients got sick from BCC in a stool softener. This resulted in the FDA issuing new requirements for the testing of aqueous-based, non-sterile pharmaceuticals, including a test for absence of BCC.[5] Additionally, Metcalfe[6] presented that BCC could have unusual kinetics in pharmaceutical products.



ABOVE: Gut bacteria in the small intestine

Media manufacturers have started to market new microbiological media; either selective for BCC, or media that will recover BCC (but is not selective). These media can be useful but should be evaluated to ensure whether or not they are selective for BCC. In some cases, the existing types of media (eg, TSA, R2A, SDA) have also been shown to grow these organisms at the same rate.

The evaluation of rapid microbiological methods has led to the utilisation of other types of media than those traditionally used. One such example is the use of Schaedler blood agar with rapid sterility testing in the Milliflex rapid.[7]

Facilities used to manufacture antibiotics need to be able to neutralise the monitoring media utilised to ensure that if microorganisms are present they can be recovered. Different enzymes are needed depending upon the type of antibiotic. Additionally, sterility testing of antibiotics requires neutralisation to allow contaminants present to grow. Another advance in culture media relates to use of enzymes to inactivate various antibiotics. This is important to ensure. Traditionally, β-lactamases were readily available to add to culture media. Today, a variety of media are available with the enzymes already incorporated into the media, eg, specific betalactamases for use with penicillins, cephalosporins (first to fifth generation) and carbapenems.[8]

Alternative or rapid microbiological methods

While many rapid methods have been introduced in the past 20 or 30 years, the route to implementation has been slow, yet it does not diminish the importance of these methods. It appears that

"Avian influenza is a major epidemiological concern. A new biosensor has been developed to determine whether this virus is present in blood samples in about three minutes"

the implementation of these systems in water testing is moving forward. This allows for almost all water testing to be released in real time. While endotoxin testing is not necessarily real time, the handheld units can provide results in near-real time.

Identification systems

There have been many advances in the methods available for "rapid" identification testing. Today, both identification testing and strain typing can be performed using automated systems.

Pathogen detection

Sandle[9] provides an overview of several new methods for rapid pathogen detection. He indicates that rapid is probably not a good term to use, as it is subjective. It is probably better to refer to these methods as alternative. Diagnosing diseases sooner is a key concern of many clinical laboratories, as is the determination of the appropriate level of antimicrobial to prescribe. A prototype chip is available that uses two nanolitres of volume to determine whether any of several antimicrobials are effective against a microbe.

Other tests include C-reactive protein (CRP) blood tests to show if an infection is present. This test is based upon the correlation of inflammation in the body.[9]

Avian influenza is a major epidemiological concern. A new biosensor has been developed to determine whether this virus is present in blood samples in about three minutes. This method uses gold nanoparticles that allow the viral particles to be detected with a nanobiosensor.[9]

Quantitative polymerase chain reaction (qPCR) methods have also been developed to detect pathogens.[9] Other non-molecular assays, like immunoassays, have also been developed for the detection of pathogens.[10]

Antibiotic resistance / sensitivity

Professor Jürgen Popp, of the Leibniz-IPHT, discusses the use of Raman spectroscopy to provide a rapid result (under two hours) to determine whether a bacterial strain is resistant or sensitive to an antibiotic. Furthermore, one can obtain information on the concentration of antibiotic needed to constrain bacterial growth.[11]

Molecular assays have been developed for quick detection of antibiotic resistance. Chromogenic agars can be an inexpensive option in place of molecular assays.[10]

REFERENCES

To view references, visit:
europeanpharmaceuticalreview.com/2-18-Moldenhauer

Overcoming the challenges of data security in the microbiology lab

Many global organisations realise that regulatory agencies worldwide are increasingly focusing their efforts on data integrity in laboratories. This elevated scrutiny has led to a number of guidance documents being published on the subject.

DATA drives every decision in the lab, so ensuring it is accurate, relevant and reliable is critical in supporting confident decisions on product quality and safety. Newly-issued global guidance documents communicate increasing data integrity requirements, making many organisations aware of existing gaps and deficiencies in their data and reporting. Recently, data integrity lapses have been brought to the fore by regulatory agencies citing violations and inadequacies in findings from inspections, audits, and warning letters. Warning letters divert worker attention away from their daily activities towards corrective and preventive actions, which can cost significant time and money. These violations can also tarnish the company's reputation and provide competitors with an opportunity to increase their market share.

While reliably detecting contamination as soon as possible has clear benefits, many labs underestimate the impact it can have on both productivity and the length of their out-of-stock response procedures, which can include investigations and corrective actions and require valuable lab personnel. Technicians, supervisors, and managers must drop what they are doing and participate in planning, execution, and report writing. This means they are not necessarily contributing to the day-to-day activities that maintain a lab's average productivity / throughput.

It is often easy to overlook the time savings of rapid micro methods when retesting or corrective actions are necessary. However, since a rapid testing method can cut critical days from your reaction time and lead to confident investigation closeouts sooner, its value should be recognised as an essential tool for relieving pressure from production and manufacturing groups who are waiting to resume operation. Reducing test time means reducing response time.

An environmental monitoring programme requires accurate detection and identification of microorganisms. Many methods rely on visual reads and human interpretation of the result. Errors or misinterpretation of readouts can lead to inaccuracies, which jeopardises data integrity. Lab automation represents 'the' big step toward process validation, cGMP compliance, and other rigorous regulatory standards improvements, as it is an attribute for sophisticated microbial detection identification solutions and simplification of practices. Advantages of lab automation include: increased productivity with more samples processed per person; a shift from batch processing to continuous manufacturing; the ability to handle surge demands; assurance that the sample is processed correctly; reduction in technical and transcription errors, and improvement in traceability.

Automation also supports demonstration of real-time process monitoring, allowing organisations to take action to course correct and empower laboratories to control their process – not the other way around. This information is powerful in aiding investigations, unearthing root causes earlier, and utilising data or facts as support.

Finally, regulatory authorities expect organisations to track and trend data and take action based on unexpected or undesirable observations and log out-of-specification results. The old method of manual entry on a spreadsheet is no longer considered sufficient for recording data. Now, automated solutions allow laboratories to continuously, accurately, and securely monitor results through real-time tracking and trending and create custom alerts to address issues and simplify lab investigations. We can now identify trends before they become a bigger problem. From environmental monitoring to rapid methods and tracking and trending, as regulators continue to focus attention on accurate, secure data, it is clear that automation is the answer that will alleviate the pressures of investigations and their costly effect on your budget, uptime, supplies, and QC/QA services. ■

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The changing environment in pyrogen and endotoxin testing

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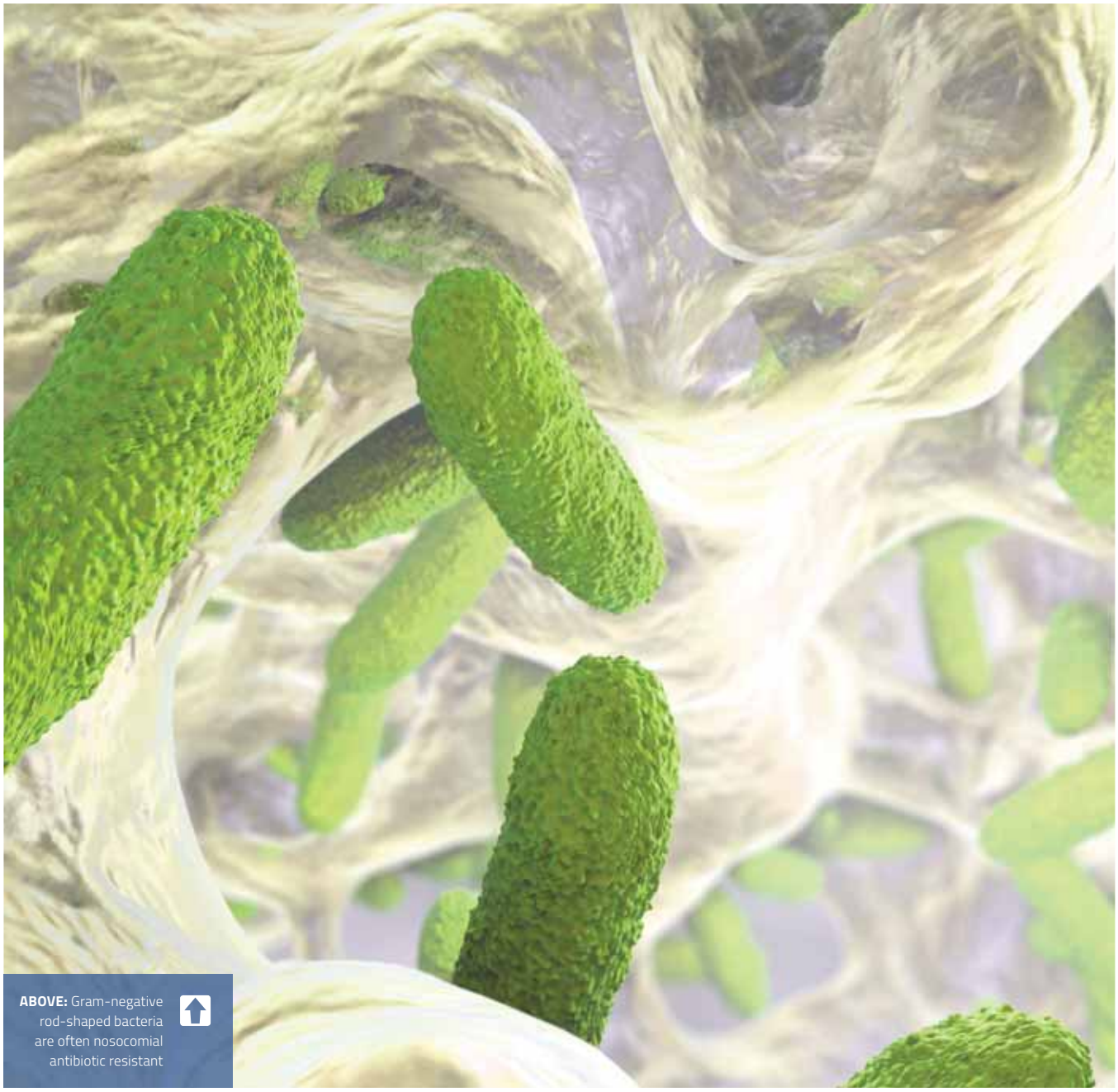
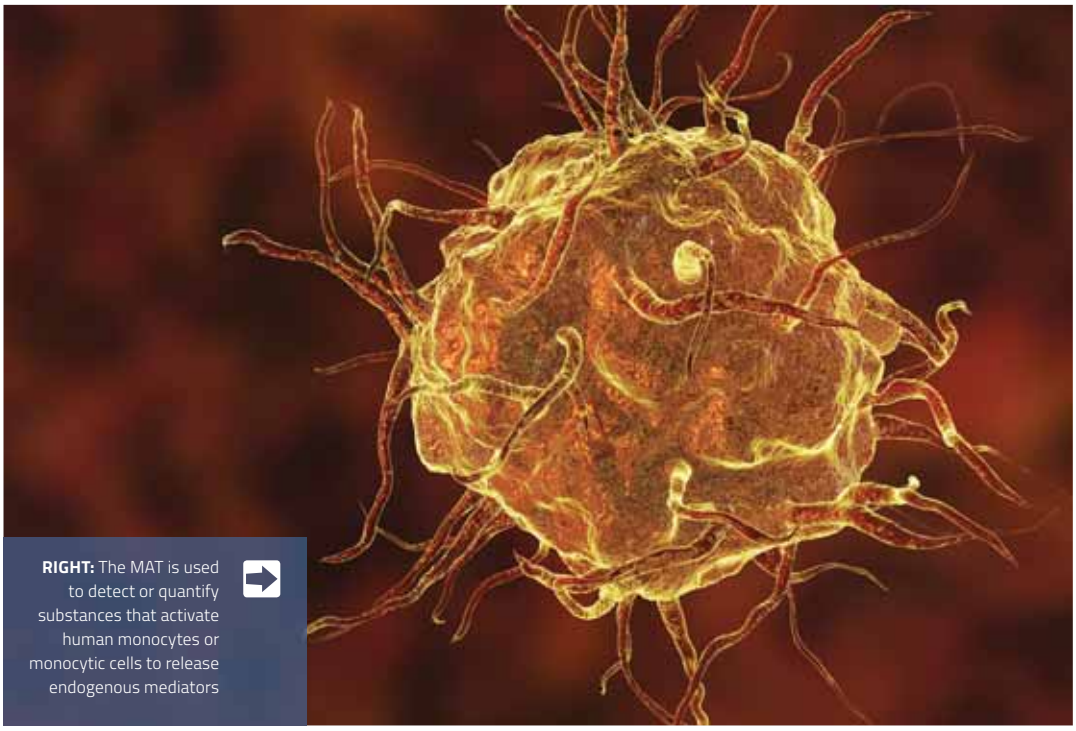
Pyrogen testing of drug products for parenteral administration is a mandatory task. Regulatory authorities require that each batch of drug product is pyrogen-free. Historically, the rabbit pyrogen test (RPT) was the required test but in most cases can be replaced by the endotoxin specific Limulus Amebocyte Lysate (LAL) test.



ABOVE: Gram-negative rod-shaped bacteria are often nosocomial antibiotic resistant



RIGHT: The MAT is used to detect or quantify substances that activate human monocytes or monocytic cells to release endogenous mediators

AS TECHNOLOGIES have advanced, new test methods like the recombinant Factor C Test (rFC) and Monocyte Activation Test (MAT) have been developed and made commercially available. However, the question as to when rFC and MAT versus LAL and the rabbit pyrogen test may be used can be confusing.

For instance, the MAT is used to detect or quantify substances that activate human monocytes or monocytic cells to release endogenous mediators. Therefore, bacterial endotoxins as well as non-endotoxin have been shown to stimulate the production of pro-inflammatory cytokines (eg, interleukins). These cytokines have a role in fever pathogenesis. Consequently, the MAT is intended to detect the presence of pyrogens in the test sample and include peptidoglycans, lipoteichoic acids, synthetic bacterial lipoproteins, and flagellin.

According to the regulations, to ensure quality control of parenteral drugs the suitability of the MAT must be demonstrated in a product-specific validation. In Europe, chapter 2.6.30 of the *European Pharmacopoeia* provides compendial guidance.

The MAT method

Moreover, MAT is thereby intended as replacement of the rabbit pyrogen test. In other countries, including the US, the test is classified as an alternative method and must follow the specific associated regulations. Benefits of the MAT method are that it is an *in-vitro* test method and allows for detection of a broad range of pyrogens in addition to endotoxins.[1]

An additional method in this field is the rFC test. This advanced test for bacterial endotoxins is used to quantify endotoxins from Gram-negative bacteria using a recombinant protein, Factor C (rFC), derived from the gene sequence of horseshoe crab and expressed in a cell culture manufacturing environment. The rFC test is currently classified as an alternative method and thus requires additional validation efforts compared with compendial methods. However, due to the benefits of recombinant tests, these are currently evaluated by several drug manufacturers and regulatory authorities.

Recently published articles have shown similar specificity for bacterial endotoxins as LAL.[2] Furthermore, rFC has been shown to minimise false positive results and improve assay specificity (eg, insensitive to beta glucan). As a consequence, the use of rFC is a robust method for the replacement of LAL and can be validated for the detection of bacterial endotoxins in a variety of pharmaceutical products.[3]

Pyrogenic substances beyond endotoxin

Taken together, alternative methods allow modernisation of the quality control environment of parenteral drug manufacture by eliminating animal-based tests. First, the application of the MAT may be an excellent method to replace the rabbit pyrogen test for detection of potential pyrogenic substances beyond endotoxin. Second, the rFC test is a sustainable method to

BIOGRAPHY

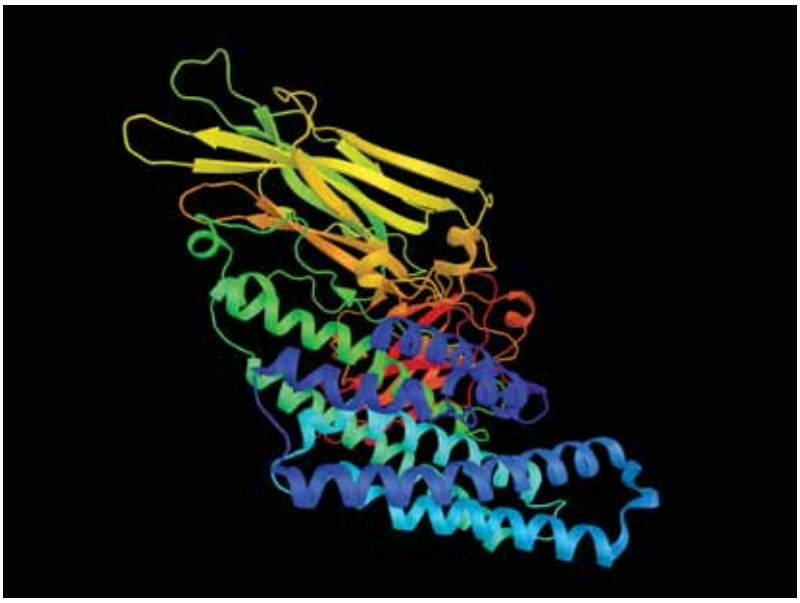
MARSHA STEED, Senior Consultant with Concordia Valsource, has over 20 years of experience in the pharmaceutical, biopharmaceutical, cell therapy / gene therapy and medical device industries with extensive experience in quality and microbiology. Her leadership experience includes global experience at manufacturing locations in North and South America, Europe and Asia. Marsha is actively involved in the PDA and is a member of the Scientific Advisory Board (SAB), the Education Advisory Board (EAB), the Annual Microbiology Meeting planning committee as well as numerous task forces.

BIOGRAPHY

JOHANNES REICH works in Endotoxin Testing with Microcoat. He has held previous positions as doktorand at Universität Regensburg, Germany. He studied at the Marcoule Institute for Separative Chemistry, France.

BIOGRAPHY

JOSH EATON is Senior Project Manager, Parenteral Drug Association (PDA). Josh works with PDA members to facilitate the production of technical reports and assists in the organisation and coordination of scientific and regulatory affairs activities and strategic goals.



ABOVE: Bacterial endotoxins as well as non-endotoxins have been shown to stimulate the production of pro-inflammatory cytokines

replace the LAL test for highly sensitive detection of bacterial endotoxin.

Thus, rFC and MAT are valuable methods and complement one another. Last, but not least, these methods support the European Directive (2010/63/EU) for the protection of animals used for scientific purposes.

In addition to the progress in alternative methods for endotoxin testing outlined above, a team of PDA member volunteers has worked for the past two years to develop a comprehensive overview of the phenomenon of low endotoxin recovery (LER). The purpose was to support the ongoing practice of endotoxin testing for product safety and patient well-being.

The technical report is now near to completion and aims to fulfill four main goals to aid the biopharmaceutical industry. Those are:

1. Describe the underlying mechanisms and contributing factors of LER
2. Summarise the potential clinical impact of the LER phenomenon
3. Present guidelines for developing LER hold-time study designs
4. Provide strategies for product-based mitigation of LER.

The authoring team included members from more than a dozen pharmaceutical companies, several service and supply company representatives, and a number of academic and regulatory agency contributors. More information on this technical report and endotoxin testing will be given at PDA's Pharmaceutical Microbiology Conference this coming October, taking place simultaneously in Berlin, Germany and Bethesda, USA. Check PDA's website for more details: www.pda.org.

REFERENCES

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FORMULATION, DEVELOPMENT & DELIVERY

Lead optimisation for discovery of small molecule drug candidates is well established, but less so for biological drug candidates. Gjalt Huisman, Vice President and Head of Biotherapeutics, Codexis, Inc., considers early biotherapeutic lead optimisation for more efficient drug discovery and development.

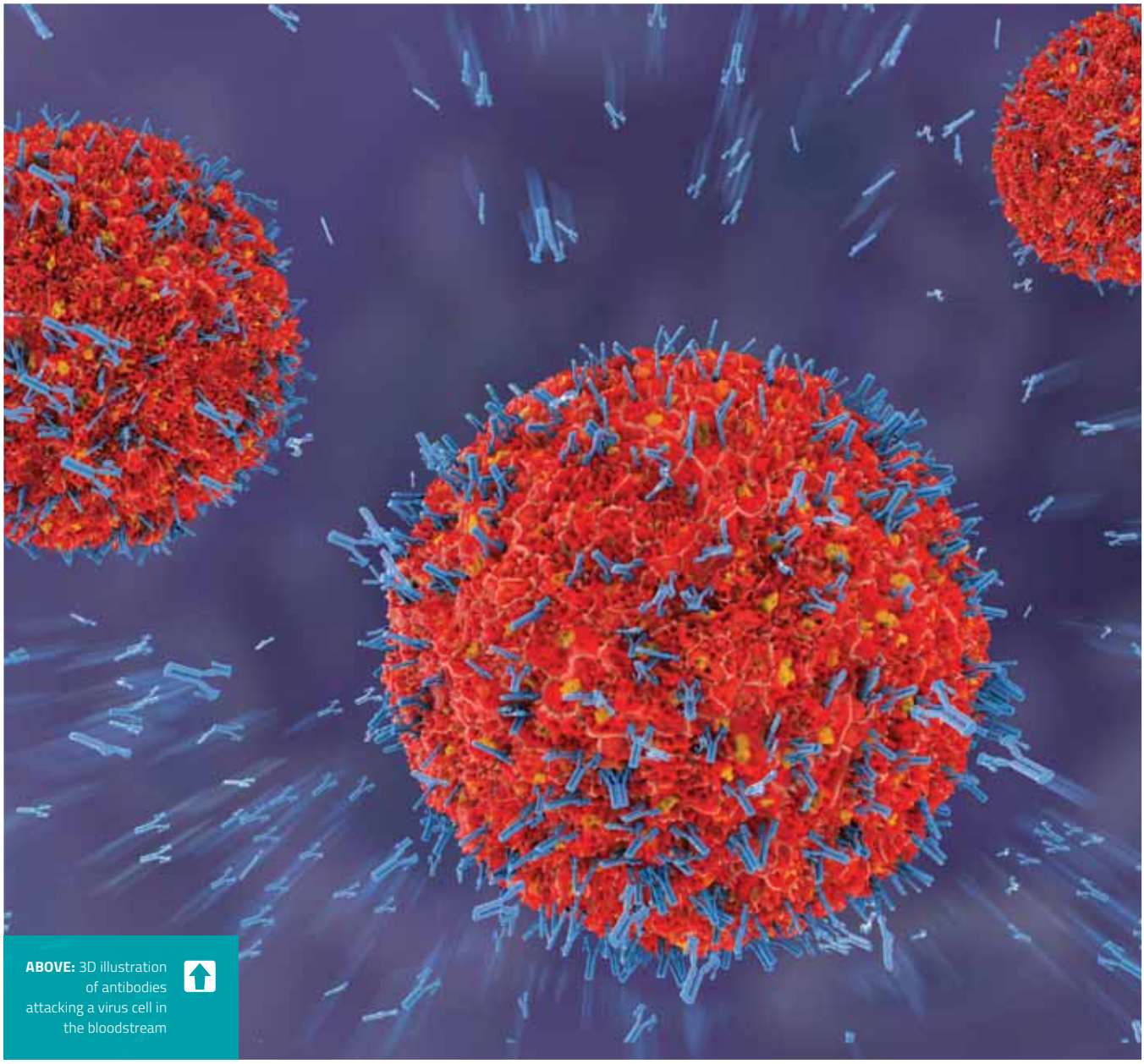
Turning a promising preclinical compound into a drug candidate that is fit for first-in-human trials is a complex, multi-stage process requiring expertise in formulation development and clinical trial management. The path to turning promising compounds into drug candidates is mapped by Torkel Gren, from Recipharm Pharmaceutical Development AB, and Anders Millerhovf, from CTC Clinical Trial Consultants AB.

Early biotherapeutic lead optimisation for more efficient drug discovery and development

Gjalt Huisman

Vice President and Head of Biotherapeutics, Codexis, Inc.

Lead optimisation for discovery of small molecule drug candidates is well established, but less so for biological drug candidates. While biologics are much easier to modify than non-natural small molecules, via the introduction of changes in their amino acid sequence through genetic engineering technologies, this approach is frequently met with trepidation.



ABOVE: 3D illustration of antibodies attacking a virus cell in the bloodstream

GENERALLY, the risk-benefit balance of small improvements resulting from a few mutations tilts towards potential safety concerns. However, large improvements in biologics function can drive significant benefits in efficacy and safety and are, thus, highly relevant to patients and should be extremely attractive to the pharmaceutical industry. Up until now, such improvements have been difficult to generate.

Lead optimisation in pharmaceutical discovery: small molecules versus biologics

Biological lead compounds, as soon as they have been identified as having desired therapeutic activity, generally undergo little further modification to improve potency and physical characteristics. In contrast, small molecule drug leads typically see substantial optimisation in order to optimise potency, bioavailability, and minimisation of off-target effects. For example, atorvastatin was the fifth HMG-CoA reductase inhibitor to enter the market nine years after lovastatin – and became the best-selling drug in the world in 2003. Statins all have a common 3,5-dihydroxyhexanoate (DHH) core, with remarkable chemical structure variation at the 6-position. Many variants of the DHH-core have been generated and tested, and, ultimately, eight statins reached the market. Ideally, such highly efficacious compounds would have been identified earlier.

Such a compound-rich historic timeline is common for small molecule drugs, but very rare for protein drugs, with the exception of antibodies. Antibody optimisation is routinely practiced, relying on the ability to sort out (select) antibodies with desired properties (such as: Affinity, specificity, developability and stability) via ultra high-throughput screening protocols. The key principle that supports this workflow is the fact that the antibody and the DNA encoding can be linked throughout the procedure using display (virus and yeast) technologies. In contrast, optimisation of non-antibody biological candidates is largely restricted to post-production modification (for example, by PEGylation or genetic introduction of additional sequences such as PASylation or XTEN technologies) to increase half-life and mask potential immunogenic epitopes. Otherwise, improvements are sought in formulation development to minimise stability liabilities or post-production aggregation.

Antibody engineering approaches have clearly been successful: In 2016, 27 of the top 200 drugs contained an antibody, either as stand-alone, fusion, or drug-conjugate (*MedAdNews* 2017), while small molecule drugs numbered 136. Other biologics in this list include protein drugs (n=20), vaccines (n=8), and peptide drugs (n=10) including seven insulins. While antibody sequences are typically optimised for function, other biopharmaceuticals, therapeutic enzymes, and other therapeutic proteins are not. Do such un-optimised biopharmaceuticals provide sufficient efficacy to patients and completely resolve the symptoms of disease? We suspect, by and large, they do not.

> *"Such a compound-rich historic timeline is common for small molecule drugs, but very rare for protein drugs, with the exception of antibodies"*

The reason biologics are not as effective as one would like is related to the process that generated these molecules: Darwinian evolution. Darwinian evolution provides a beautiful interplay between the internal and external environments of a living organism to arrive at a state where the organism can thrive. However, Darwinian evolution in man is relatively slow and, with the exception of the immune response, no mechanisms have evolved naturally to treat modern disease. Insulin is naturally secreted by the pancreas to regulate glucose levels; the molecule did not naturally evolve to be administered as an injectable to diabetes patients. Similarly, lysosomal enzymes mature intracellularly as they are transported from the endoplasmic reticulum, via the Golgi apparatus, to the lysosome; they did not naturally evolve to find the lysosomes in all relevant tissues from an infusion bag every other week for the treatment of inborn errors of metabolism such as Gaucher, Fabry, or Pompe Disease.

Novel technologies are required to provide biotherapeutic candidates that optimally meet the need of the patient. Directed evolution of proteins is a well-established engine to discover enzyme catalysts for small molecule drug manufacturing (see *Nature 2010* for a review). This body of work has established that enzymes can be modified at >15% of their primary sequence, resulting in many orders of magnitude performance improvement from a combination of increased activity, specificity (affinity), stability, and others. For small molecule manufacturing, lead optimisation is focused on variables related to pharmacokinetics and pharmacodynamics, such as bioavailability, half-life, activity, and selectivity. These characteristics are critically important for biologics as well, and directed evolution using relevant high-throughput assay technologies can provide greatly improved biological lead candidates.

Directed evolution comprises the accumulation of beneficial mutations in the protein of interest to generate a protein variant with the desired attributes for a specific application. As in natural

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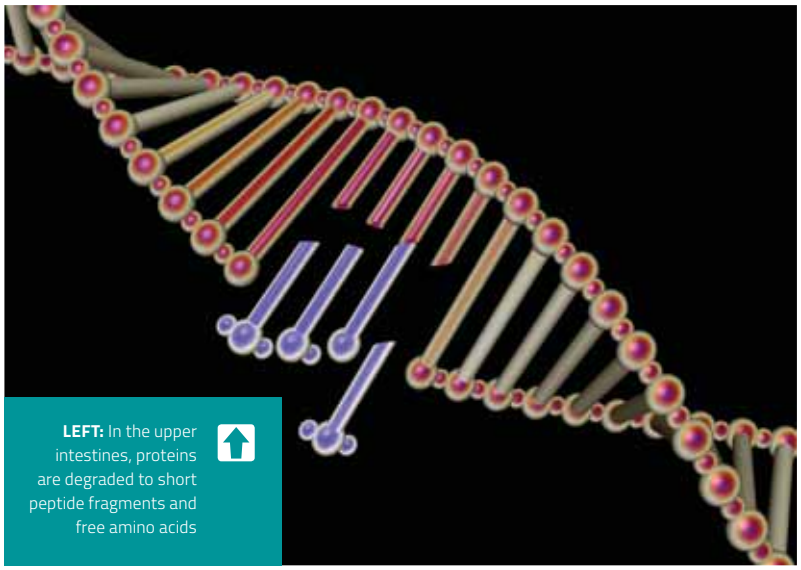

LEFT: In the upper intestines, proteins are degraded to short peptide fragments and free amino acids

BIOGRAPHY

GJALT HUISMAN is Vice President and Head of Biotherapeutics at Codexis, Inc., where he is responsible for the generation of novel biological drug candidates via the application of advanced directed evolution technologies. After receiving his PhD from the University of Groningen in the Netherlands, he was a post-doctoral fellow at Harvard Medical School before joining Metabolix Inc. He joined Maxygen, the parent company of Codexis, in 1998 and held various positions of a techno-commercial nature, initially focusing on the biocatalysis portfolio and then on the biotherapeutics initiative since 2013. In 2014, he was awarded the Biocat Industry Award in recognition of his outstanding achievements in biocatalysis.

evolution, directed evolution is a continuous, iterative process where different mutations accumulate combinatorially when screened for desired therapeutic function. In its most effective forms, directed evolution combines high-throughput (HTP) molecular biology, HTP screening, and HTP sequencing, supported by a laboratory information system and bioinformatics infrastructure to orchestrate and coordinate a highly efficient workflow. As a result, greatly improved proteins can be generated in short periods of time. This is illustrated in the example below.

Biologic lead optimisation for function in the upper intestines

The environment of the upper intestines, specifically the duodenum and jejunum, provides a harsh environment in which polymeric food components are efficiently degraded to monomers and oligomers by the action of hydrolytic enzymes. Proteins are degraded to short peptide fragments and free amino acids, polysaccharides to individual sugars, and fats to fatty acids and glycerol by the action of proteases, amylases, and lipases. While this digestive process has naturally evolved to be highly efficient, in some disease settings it would be desirable to augment it to remove toxic metabolites from within the confines of the GI-tract, thereby minimising exposure to the patient.

Almost 40 years ago, Hoskins *et al.* explored the use of an encapsulated enzyme to remove phenylalanine in the GI-tract for the treatment of hyperphenylalaninemia (also known as phenylketonuria or PKU). Several studies followed, in which the enzyme was protected from proteolysis in the duodenum and jejunum by various immobilisation, encapsulation and chemical modification (PEGylation) technologies, and rational protein engineering, ultimately demonstrating that the lack of a readily obtainable, GI-stable enzyme prevented the realisation of this approach. However, using our technologies and screening of more than 50,000 proteins, over eight rounds of directed evolution led to the identification of an enzyme that is sufficiently stable in the GI tract of dogs and monkeys to remove clinically relevant amounts of phenylalanine (*U.S. Patent* 9,611,468).

This GI-stable, phenylalanine degrading enzyme was obtained in about nine months using a combination of structure-guided library design, HTP molecular biology, HTP screening, and HTP sequencing. The rationale that was followed included site-saturation mutagenesis of the surface of the protein to identify new variants in which proteolytic sites had either been removed or made inaccessible for the GI proteases, trypsin and chymotrypsin. In addition, more stable variants were identified by subjecting libraries to heat treatment, ultimately culminating in an enzyme that is readily manufactured at large scale. The final enzyme that is slated to enter the clinic in 2018 contains >20 mutations. Exactly why this highly evolved enzyme is so protease stable is unknown and the total number of predicted trypsin and chymotrypsin cleavage sites barely changed. Clearly, and not unexpectedly, it is not just the presence of such sites at the surface that determines the enzyme's propensity to be proteolytically degraded, accessibility of such sites is a key component as well.

Future opportunities

Advanced directed evolution technologies hold tremendous promise to deliver new biologic lead candidates. Biologics are seldom a panacea for disease treatments and, moreover, are always difficult to manufacture due to their natural instability. Biological leads can now be readily optimised for increased efficacy and manufacturing. Increased stability of biologics – whether to serum, to the intracellular environment, to conditions in the lysosome, or to the GI-tract – ultimately has a significant impact on the efficacy of the drug.

Immunogenicity is generally a first concern when considering the potential consequences of protein sequence changes. However, let us not forget that immunogenicity is the result of a process in which proteins are degraded to smaller fragments, some of which have a high affinity to the major histocompatibility complex II (MHC-II) and are presented to the T-cells. The dynamics of this essentially chemical process are fully determined by the primary sequence of the protein and hence should be addressable with the right technology. Advanced directed evolution has arrived at the doorstep of making this happen.

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The path to clinic: turning promising compounds into drug candidates

Torkel Gren
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Anders Millerhovf
CTC Clinical Trial Consultants AB

Turning a promising preclinical compound into a drug candidate that is fit for first-in-human (FIH) trials is a complex, multi-stage process requiring expertise in formulation development and clinical trial management.

LEFT: The speed with which a promising library compound can be taken from lab to pharmacy will have a big impact on the cost of development and product revenue

FROM a cost management perspective, it is vital that early phase development and manufacture of materials is completed quickly and efficiently. In addition, while the focus is on getting to FIH trials as quickly as possible, developers also need to keep later-stage trials and commercialisation in mind.

An integrated, structured approach is critical to successfully navigating a pathway from the laboratory to the clinic.

> *"The speed with which a promising library compound can be taken from lab to the pharmacy will have a huge impact on overall development cost and, ultimately, the revenue the product will generate when commercialised"*

Formulation simplicity

Pharmaceutical companies rely on the ability to develop products that are effective and safe as quickly as possible.

The speed with which a promising library compound can be taken from lab to the pharmacy will have a huge impact on overall development cost and, ultimately, the revenue the product will generate when commercialised.

This time pressure dictates the first steps on the pathway to clinic. Most formulations used in FIH studies are oral suspensions, which can be made quickly. In addition, oral suspensions can be modified to allow trial staff to modify the dosages.

Another reason simple oral formulations are favoured for FIH trials is that they are cheaper to produce than solid dose formulations. Most compounds that show promise in labs do not go on to be developed into commercial products.[1] In short, over-engineering a phase I drug that fails is a costly waste of resources.

Therefore, in most cases oral solid suspensions or powder in bottles provide drug companies with an effective way of ensuring cost effective dosing in FIH trials.

However, for some new chemical entities (NCEs) the production of oral suspensions is not possible, for example if they are unstable in an aqueous environment. In such circumstances, capsules are used in FIH studies. In addition, large molecules such as proteins are often poorly absorbed from the gastrointestinal tract and are also metabolised. This type of NCE is often administered by intravenous injection or infusion.

Materials production

Supplies of active pharmaceutical ingredient (API) during early phase development are likely to be limited, which is another reason why simple formulations are favoured for FIH studies as they require less drug substance for development.

The majority of sponsors call on contract development and manufacturing organisations (CDMOs) to produce drug substance and drug product for early phase trials. The rationale for this is that CDMOs are highly experienced at developing production processes quickly. In addition, working with a partner that can coordinate all aspects of the process can help to manage the complexity of the FIH study while ensuring the necessary flexibility as needs change.

It also critical that the drug substance and drug product is manufactured in compliance with Good Manufacturing Practices (GMP). The set up and maintenance of a suitable quality system is a complex task and this is another reason why CDMOs are often used. For example, in the European Union (EU), batches of medicinal product must be certified by a Qualified Person (QP) before they can be released.

The purpose of the QP release is to guarantee that the product is safe to use. To ensure a swift release process, it is advantageous if all parts of the manufacturing process, including quality control (QC), packaging and labelling are performed by one supplier.

Commercialisation in mind

Simplicity may be key when developing formulations for FIH trials, but over-simplification should be avoided. A compound that is successful in a FIH study usually needs to be reformulated for later stage studies where factors like shelf life, rather than stability for the duration of the trial, become a factor.

As a result, there is a growing body of opinion that stability and bioavailability should be carefully considered during the development of formulations for FIH.

The rationale for this approach is that paying attention to stability and bioavailability characteristics at the earliest possible stage allows for better informed go / no-go development decisions, which let pharmaceutical companies make most effective use of their R&D budgets.

In addition, any useful stability and bioavailability data can help shape the development and production of formulations for later phase trials and potential commercialisation.

One of the most effective ways of approaching this is to work with a contractor that has experience in early as well as late stage development.

Starting with the end goal in mind will allow potential challenges to be addressed early. Understanding of the critical parameters that impact drug quality and help to design strong processes and control strategies will ensure the

BIOGRAPHY

TORKEL GREN is General Manager at Recipharm Pharmaceutical Development AB. Torkel holds degrees in Pharmacy and Business Administration as well a PhD in Pharmaceutics (Uppsala University). He has worked in the pharmaceutical industry since 1988 and has held a number of scientist and manager positions in Europe and US. He was lead formulator and co-inventor of Detrol OD / Detrusitol SR and is a member of the board of the Swedish Pharmaceutical Society. In 2017, Torkel led the launch of Recipharm Pathway to Clinic, designed to guide customers through the full phase one journey from formulation development to clinic. For more information, visit Recipharm Pathway to Clinic.

RIGHT: The pathway from the laboratory to the trial centre is perilous even for the most promising compound

BIOGRAPHY

ANDERS MILLERHOVF is CEO at CTC Clinical Trial Consultants AB. He holds a degree in Medical Biology (Linköping University). Anders has worked in the life science industry since 2002 and has been focused on Phase 0 – Phase 2a projects in various project management and director positions. He has been a partner at CTC since 2012. For more information on the clinical conduct of early phase clinical trials, please visit the website at CTC Clinical Trial Consultants AB.

manufacturing platform is optimised for late stage development.

Clinical trial planning

Another step on the path to a clinic is the planning of the clinical trial and it is vital that teams involved in early phase formulation development communicate with the teams who will design protocols and run the trials.

Trial planning should begin early and involve the sponsor and its partners. The main focus is the production of a trial synopsis that defines the project's objectives and endpoints. This document will be the blueprint for the study protocol.

Collaboration between the manufacturing and trial teams can accelerate the study start-up by allowing subject screening to start as soon as the project has been cleared by regulators.

New drug substances are usually tested in FIH studies in single ascending dose (SAD) cohorts and multiple ascending dose (MAD) cohorts. It is critical dosages can be adjusted during such studies to identify the safe limits for subsequent studies.

It is therefore vital that clinicians and staff involved with monitoring a candidate's safety, pharmacokinetic and pharmacodynamics properties communicate with formulation developers. This is particularly important if the compound in question is deemed to be high risk, eg, if it is a new class of compounds, has a new mechanism of action or has a steep dose-response relationship.[2]

Oversight

Early phase drug development requires drug substance manufacturing, formulation development, trial management, clinical implementation and bioanalysis expertise.

The multidisciplinary nature of such projects usually means that multiple parties are involved. Whether involving separate groups within a large organisation or third-party contractors, effective management requires a detailed plan and oversight.

In addition, it is also clear that effective collaboration between the different disciplines will increase the chance of success and speed up the pathway to clinic.

It is advisable to put together a detailed plan for the project that includes input from all disciplines involved. While details will be subject to change, open communication will help to mitigate any potential challenges.

In addition, cross disciplinary collaboration reduces delays between the different stages. For example, product development should be started when a quality API is ready, the clinical study should begin when the regulatory approvals are in place and the product is released and bioanalysis should commence when samples from the first subjects are ready and then run in parallel with the remaining part of the clinical study.

Conclusion

The pathway from the laboratory to the clinical trial centre is perilous even for the most promising compound. Most will not make the journey successfully and even for the few that do, success during late stage development is never guaranteed.

Pharmaceutical companies can increase the likelihood that their compounds stand a chance in clinical development by developing a detailed preparatory plan for the FIH study while also keeping late stage trials and commercialisation in mind. Putting together such plans can only be achieved if there is good communication between all parties involved in the research, from drug substance manufacturers to clinical research teams.

Marshalling such disparate resources is a challenge that requires an understanding of the role each party plays in the research, the time aspects of each stage and knowledge of applicable regulations.

In short, the pathway from lab to trial centre is best navigated when there is effective oversight of all the activities involved.

REFERENCES

1. http://www.fdareview.org/03_drug_development.php
2. https://www.sciencedirect.com/science/article/pii/S1359644616304342

Making Pharmaceuticals: Offers more than 35 hours of free-access content

One of the UK'S biggest free-to-attend pharmaceutical conferences, Making Pharmaceuticals, takes place between Tuesday 24 April and Wednesday 25 April. Here, we preview the event that offers more than 35 hours of free-access content dedicated to the pharmaceutical sector.

24-25 APRIL 2018

COVENTRY, UK

MAKING PHARMACEUTICALS brings together the key decision makers and innovators from across the pharmaceuticals sector. The two-day event offers the opportunity to meet, network and do business with hundreds of professionals looking to find solutions, obtain answers, and buy the products and services they need to fulfil their pharmaceutical development and manufacturing requirements.

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This year's event features areas of visitor interest including:

- Contract formulation, manufacture and filling
- Manufacturing and processing
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- Labelling
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- Waste management
- Good manufacturing practices and environmental hygiene
- Laboratory and analytical equipment
- Test equipment, analysis and testing services
- Regulatory environment

Conference delivers over 35 hours of free access content

Designed to bring together everyone involved in the development life cycle leading to successful commercial manufacture of pharmaceuticals, Making Pharmaceuticals provides an essential focus for the many different skills and range of expertise necessary to deliver consistent pharmaceutical products. The conference is a key feature of this year's event, which has been developed with the assistance of supporting organisations and institutions.

Visitors will benefit from more than 35 hours of free-access content delivered across five rooms in current sessions.

Topics under discussion include:

- Innovation in semi disposable manufacturing technology for ATEX environments
- The agility of continuous direct compression
- Serialisation and product security
- Manufacturing challenges: Dusty Dusty – and how DExToRR can help you not to explode
- Expanded design space in hot melt extrusion with high productivity hypromellose acetate succinate
- Phospholipids as business opportunities for pharmaceutical line extension products
- Global serialisation requirements: The pathway to successful serialisation implementation
- Unique silica microspheres for difficult to formulate actives and excipients
- Connecting the disconnected: Overcoming the disconnects within your labelling supply chain that risk compliance
- Disruptive innovation – a bitter sweet pill to swallow
- Improving biologics downstream operation through formulation innovation
- Development of open access pilot line for targeted drug delivery

The attendee numbers at last year's Making Pharmaceuticals grew by 45%, indicating that the event's conference content and exhibitor profile are becoming even more popular with industry professionals. All visitors who collect their visitor badges before 10 am will receive a voucher for a complimentary breakfast bap and a hot beverage.

Exhibitors forge new business partnerships

Exhibitors at this year's event will be able to forge new business partnerships, meet face-to-face with new and existing clients, and learn about the latest innovations and developments in the pharmaceutical industry while networking with other key industry professionals. Exhibitors also benefit from the platform to showcase their products, services and expertise to the market place, launch new products and services and focus on future investment and business strategies. ■

www.makingpharma.com

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Five expert views on Track and Trace

Welcome to *European Pharmaceutical Review's* roundtable devoted to track and trace technology. We have brought together five industry experts to focus on how the contribution of track and trace technology is shaping the pharmaceutical sector and explain what factors are driving that change.

What are the key elements of a successful track and trace system for the pharmaceutical sector?

ETTORE CUCCETTI: For the successful implementation of a pharmaceutical serialisation system, understanding of the packaging process, country-specific regulations, in-depth knowledge of process compliance and, most importantly, the right implementation partner are crucial. Implementation partners must possess skills such as project management expertise, service and maintenance capability, and financial stability. Also, as these projects are more of a software deployments scenario ranging from medium- to large-size scope, then system validation, security, handling and maintenance of large quantities of data is critical to the business.

Selection of lean architecture-based software solutions along with modular hardware design is a must, not only to drive changes in current packaging processes, but also to accommodate future complex additional – or amendments to – regulations in the process.

Pharmaceutical companies also need to consider minimal disruption to existing processes and its impact on productivity, to successfully reap the benefits from the serialisation system.

WILLIAM MINAEFF, SR: As a serialisation project progresses, there are many challenges that need to be faced and many sources of complexity. Serialisation failures most frequently come from not understanding the scope of a serialisation project. For example: not identifying all stakeholders and their requirements, regarding packaging, label change, supply chain, IT and enterprise resource planning.

There are five key elements of a successful track and trace serialisation solution for the pharmaceutical sector, which are:

- Configurability; meaning no specific development needed, with easy updates and upgrades management, allowing for virtually no chance of human error
- Scalability; meaning when the initial roll-out phase is achieved, you can smoothly deploy serialisation on additional lines without repeating the whole process
- Interoperability; meaning increased flexibility to choose the hardware that best fits your objectives, and uses any of your existing equipment when valuable for serialisation and minimises costs and delays in delivery
- Site level management capabilities; meaning the most secured and efficient approach to cope with change management. Having a centralised single point from where you can manage all your configuration and processes will help facilitate configuration management, data exchange, reporting, change management and validation support as well as IT governance. Site level management capability minimises risks and helps prepare you to meet future track and trace challenges.

DR STEFAN OEING: Beside powerful HW units, which can flexibly handle different packaging formats on all

levels in combination with serialisation and track and trace coding (aggregation), the overall SW-System itself will become increasingly important. On Level 2 (Line Management), the SW solution must be able to communicate in real time to all involved HW-units and provide an accurate picture of the commissioning process. This also covers any kind of manual rework necessary during commissioning. After completing batch production, balancing of data must be possible between all physical units (moved into stock), and all data sent to Level 3 software (site manager). Afterwards, post-lot processes must be accurately managed through pre-defined workflows, covering the generation of all regulated reports, the assembling of any shipment, and the reporting to any higher level SW (internally or externally, Level 4 and Level 5). Finally, the interaction with a (national) database will assure that the status of each code is up-to-date and available for all relevant participants of the track and trace process.

YANIK BEAULIEU: The key elements of a successful track and trace system are three-fold. First, the flexibility to interact with multiple third-party IT systems (ERP/MES, EPCIS server, government instances, etc). Second, the reliability and ease of use to minimally impact productivity. Third, adaptability through consistency: every packaging line is different, yet constancy between all lines / equipment is important for users.

JOE LIPARI: A successful track and trace system requires flexibility to meet the unique connectivity needs of your supply chain. All systems are not created equal, but all systems in pharma are validated. Having a track and trace system capable of interfacing with a myriad of systems requiring varying data and messaging standards is key to successfully integrating the supply chain.

What benefits do such systems deliver to industry and the consumers they serve?

ETTORE CUCCETTI: Implementation of a serialisation system will be beneficial across the pharmaceutical value chain right from the marketing authorisation holder (MAH) and contract manufacturing organisation (CMO), to the supply chain partner, pharmacies and end-consumer. The pharmaceutical companies will be benefited with advanced capabilities to improve the supply chain and data security between different stakeholders. A track and trace system helps to monitor and control the counterfeiting issue and ensures brand protection.

DR STEFAN OEING: Such systems fulfil legal requirements, which aim to protect the consumers against counterfeiting. But they can do much more; for example, monitor packaging systems as well as internal and external logistics processes. Based on this, processes can be optimised, and components with best vs. worst performance and/or quality can be identified. This can take place in different locations, eg, at the pharmaceutical manufacturer or along the legal supply chain. The overall picture can also provide valuable market information for different regions. Serialisation and track and trace codes can also be used to establish direct communication to the consumer, which opens the door for generating customer loyalty and gaining valuable customer insight.

YANIK BEAULIEU: The main goal is obviously to secure the supply chain against counterfeit product for the end user, but brand protection is also a significant benefit. Many other benefits could be named, such as minimising the impact of recalls, logistics and handling advantages during production / distribution. This leads to interesting avenues in the future. Those include stock shortage prevention, cold chain distribution control or the end user having access to more information about the product in their possession, including advertising and health recommendations.

JOE LIPARI: In a word, visibility. The holy grail of track and trace is having full transparency into where a product has been. The value this delivers to a consumer is the confidence that the product you or your loved ones are consuming is from a trusted source.

How has the importance of track and trace systems changed over the past 10 years?

ETTORE CUCCETTI: We have seen a major transformation in the perspective of customers over past decade – from meeting the mandates to utilising master data for betterment of production planning and supply chain management.

> *"The main goal is obviously to secure the supply chain against counterfeit product for the end user, but brand protection is also a significant benefit"*
> YANIK BEAULIEU

> *"The holy grail of track and trace is having full transparency into where a product has been"*
> JOE LIPARI

Today, the implementation is not only limited to printing on cartons, but also includes data management and data security.

The industry is now looking towards single source solution providers that help them through compliance adherence, creating significant value addition to business and consumer safety.

WILLIAM MINAEFF, SR: The pharma marketplace is under increasing pressures to balance shareholder values and rights with regulatory requirements, global market place competitiveness, consumer perception, and intellectual property protection as well as return on investment on research. Most research does not yield a marketable, profitable product yet there is an increasing viewpoint of big pharma as simply profit driven machines. These pressures exist, yet all responsible pharma companies I have ever been involved with have deep commitments to patient safety as their first and most important goal.

> *"Most research does not yield a marketable, profitable product yet there is an increasing viewpoint of big pharma as simply profit driven machines"*
>
> WILLIAM MINAEFF, SR

DR STEFAN OEING: Track and trace systems are very much related to computer capabilities, cloud computing, data security and integrity, and overall data availability. There has been and continues to be significant progress in all these areas. At the same time, industrially applicable track and trace systems for the pharmaceutical sector were perfected. For example, higher level software systems are increasingly working generically and are supplied with flexible workflow engines. Furthermore, the need to create standardised, generic interfaces to central systems such as country hubs, gateways, etc, has been identified. This will ease interoperability between all participants of a complex track and trace system significantly.

> *"Track and trace systems are very much related to computer capabilities, cloud computing, data security and integrity, and overall data availability"*
>
> DR STEFAN OEING

YANIK BEAULIEU: The main change has been the passage from a potential law into reality. Although many challenges still remain, we can see that traceability is a worldwide movement. It goes much further than simply applying a serial number to a container. Solution providers now offer a broad range of customisable and complete solutions from manufacturer to dispenser.

JOE LIPARI: I would argue that track and trace was just as important 10 years ago as it is today. We've all been tracking uniquely identified packages through our logistics provider of choice for quite some time now. The intensity of importance has certainly changed in pharma as global regulations have taken shape and placed a substantial interest on the ability to not only share data with trade partners but report to regulatory agencies as well.

What factors are driving those changes?

ETTORE CUCCETTI: There are few key actors that are driving this change. The foremost reason is the need for pharmaceutical industry to thwart the industry-crippling issue of counterfeiting of the products. Another reason is that track and trace industry is mainly driven by country-specific regulations across the globe. Add to these, technology advancements such as: the Internet of Things, machine learning, big data analytics and artificial intelligence – all of which are playing a key role in bringing about disruption at various levels across many industries.

WILLIAM MINAEFF, SR: I believe that as more and more information is made available to the marketplace, more transparency of the products' lifecycle will be exposed through increased implementation of integrated software systems. Across the enterprise, consumers will be better informed about what's involved in the world of pharma and hopefully that will help to shine a light on what goes into producing these products.

DR STEFAN OEING: The pharmaceutical sector is becoming ever more interconnected and internationalised. This applies both to production with contract manufacturing organisations and to data provision for all addressed markets. Increasingly, more countries are legislating for the serialisation and aggregation of pharmaceutical products, so that a holistic software solution becomes indispensable.

YANIK BEAULIEU: Many countries are having their laws / recommendations reinforced. The appearance of some national hubs contributes in expanding track and trace to the whole supply chain. That is allowing repackagers, distributors and dispensers to jump into the game. Meanwhile, the early adopters and corporate global deployment is helping to set the pace for organisations to reach serialisation

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JOE LIPARI: Within life sciences, compliance is the key driver. Federal regulations mandate track and trace requirements. Within other verticals, the desire for transparency into where goods are sourced and how they travel through the supply chain is a major driver for traceability. There is increased pressure to bring transparency to where food is sourced, how goods are manufactured, sustainability, and the need to combat diversion and dishonest supply chain actors.

"As a technology, blockchain is bringing a wave of changes across industries, especially in the finance industry"

ETTORE CUCCETTI

Will track and trace technology increasingly feature blockchain?

ETTORE CUCCETTI: The aim of the track and trace solution is to track the source or, in other words, the authenticity of the product throughout the supply chain up until the consumer. As a technology, blockchain is bringing a wave of changes across industries, especially in the finance industry. One can attribute its success to its capabilities to provide inherent transparency, security and reliability. The pharma industry will follow suit. When it comes to track and trace, we anticipate many companies will incorporate blockchain in their business processes in years to come.

WILLIAM MINAEFF, SR: The world is getting smaller, but the size of the data is increasing rapidly. The need to support multiple markets with geographic and regulatory requirements becomes a driver for an increase in software systems that reduce errors by ensuring the correct requirements are applied. This allows a packaging system operator or a warehouse material handler to focus on their respective roles and let the software manage the complexities of the modern global manufacturer and simplify the process for the operational staff. The impact is felt from the planning and ability to promise algorithms that support sales efforts across the marketspaces, to the most granular operational tasks needed to fulfil the production requirements and the multiple systems and sub-systems that exist between them.

DR STEFAN OEING: Blockchain will be the appropriate means to ensure data integrity and validity, especially for chained data. This is already being implemented within some track and trace systems, eg, to secure audit trail messages in databases. Increasingly, however, this technology will also be used in external communications. It typically manages a peer-to-peer network, collectively adhering to a protocol for validating new blocks. Once recorded, the data in any given block cannot be altered retroactively without the alteration of all subsequent blocks, which requires collusion of the network majority. In this respect, blockchains are secure by design. This makes blockchains suitable for the recording of events, medical records, identity management, transaction processing, documenting provenance, and much more.

YANIK BEAULIEU: It is a strong possibility. With the actual interest about blockchain and the evolution of technology, the secure aspect of data transaction between cloud platform can definitely benefit from the blockchain concept.

JOE LIPARI: There is a sharply polarised view within the healthcare industry with regard to blockchain. This truly hyper partisan view of a technology leaves most scratching their heads regarding what to believe. Blockchain, as a technology, has the potential to redefine how business is conducted. Let's face it, different industries are ripe for disruption including any industry involved in supply chain operations. While pharmaceutical and healthcare trading partners may not be exchanging bitcoins for drug products, development of an interoperable platform that will meet track and trace requirements could absolutely be met using blockchain technology. Systech are currently investing in multiple pilots utilising the technology and are optimistic that it can bring real value.

WHAT DO YOU THINK?

Do you share the views of our industry insiders? Or, are there points you think need to be added to our debate? Please email your comments to Mstones@russellpublishing.com to join the discussion about track and trace technology.

Meanwhile, watch out for our experts' comments on how track and trace will develop in the pharmaceutical sector over the next 10 years by visiting www.europeanpharmaceuticalreview.com

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European Pharmaceutical Review is pleased to announce the following show partners who will be exhibiting at ACHEMA 2018:

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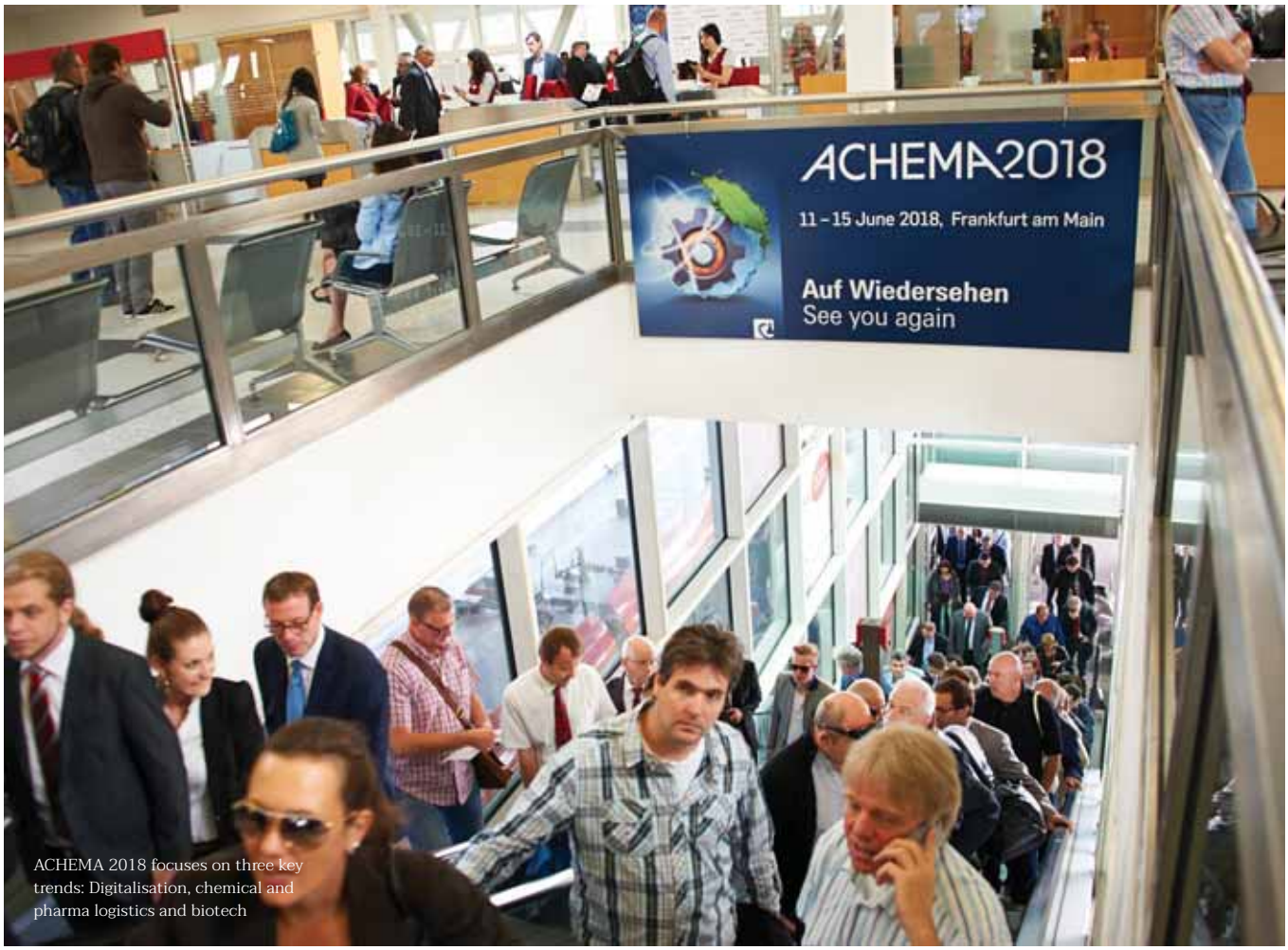
ACHEMA focal topics turn the spotlight on significant trends

What will the process industry look like in 2025? More flexible, more integrated and more biological, experts say. Three focal topics will bring 'Flexible Production', 'Chemical and pharma logistics', and 'Biotech for Chemistry' to the forefront at ACHEMA 2018.



ACHEMA 2018 focuses on three key trends: Digitalisation, chemical and pharma logistics and biotech

MEGATRENDS affect whole industries, from equipment to processes, to business models. Consequently, they cannot be covered within one exhibition group. ACHEMA answers this by defining three focal topics that draw attention to developments affecting all stakeholders in the process industry, from lab supplier to pump developer, to plant engineer and operator. Thus, aided by markings at the stand to dedicated topical magazines, visitors can get an overview on where the process industry is headed.

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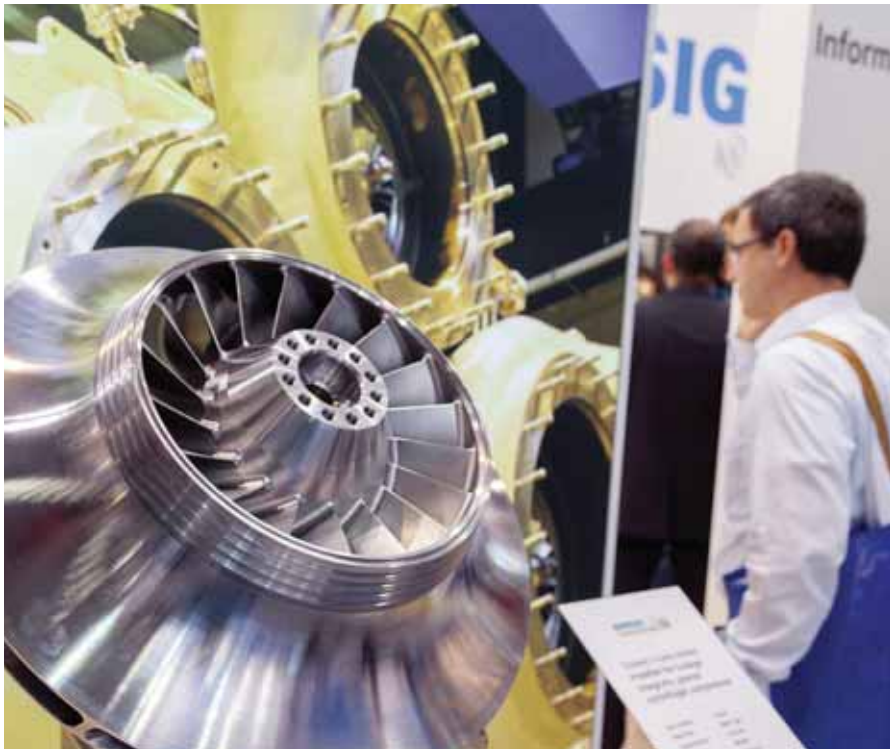

ACHEMA 2018's focal topic 'Flexible Production' will feature robust technologies that allow for variations of production volume, depending on factors such as energy supply

ACHEMA 2018 focusses on three trends: Digitisation, chemical and pharma logistics, and biotech. Digitisation has been a major driver of the process industry for some time – and it is no end in itself. "Future chemical production has to react more flexibly – to different raw materials, to a volatile energy supply, and to customer demands for more individualised products," said Dr Andreas Förster, Subject Matter Expert Chemistry at DECHEMA.

The focal topic, 'Flexible Production', at ACHEMA 2018 specifically addresses these aspects:

- Modular plants that can be assembled from 'plug and play' components according to the requirements of different processes, production volume or locations
- Robust technologies that allow for variations of production volume depending on, eg, energy supply
- Automated process control that uses real time measurements to optimise processes.

"Numerous exhibitors offer relevant products or services," said Dr Marlene Etschmann, responsible for communicating on the focal topics at DECHEMA Ausstellungs-GmbH. "The focal topics provide them with a platform to showcase their offerings across the whole exhibition."

Closely related to flexible production are chemical and pharma logistics. These used to be perceived as something happening outside the factory gate, but in times of integrated supply chains they have become a significant factor in production. In some areas, like personalised medicine, logistics even become part of the product: new therapies rely on samples being transported fast and reliably from the bedside to the lab. With track-and-trace technologies the location of the sample can be determined at any time – an important feature in quality control not only in the pharmaceutical, but also in the chemical industry. ACHEMA 2018 takes this into account: new solutions are not only presented in the growing exhibition group – pharma, packaging and storage technologies. In addition, the logistics hotspot in hall 1 offers plenty of opportunities for information and exchange.

The third focal topic, 'Biotech for Chemistry', showcases the integration of chemical and biotechnological methods. They are no longer strictly separated; pragmatically, the method of choice is the one promising the best results. Citric acid,

for example, has been produced since the 1920s by purely biotechnological means, but for acetic acid the chemical process is still more competitive. This leads to questions regarding the development of robust production strains as well as the selection of solvents at the interface between biotechnological and chemical reaction steps.

"More than ever, biotechnologists, chemists and engineers have to cooperate closely in these processes. Backward reasoning becomes even more important than it is already the case in the chemical industry," explained Dr Kathrin Rübberdt, Head of the biotechnology department at DECHEMA. ACHEMA is the forum that covers the whole development and value chain, and offers stakeholders the chance to explore exactly this type of cooperation.

Extensive information is provided for each of the focal topics in the run-up to ACHEMA and on site. Exhibitors offering corresponding technologies and solutions are easy to find following dedicated marks in the halls. The ACHEMA app and a dedicated magazine for each individual focal topic give a comprehensive overview and provide orientation.

This year's event features a series of special forums, including ones devoted to: Cyber security, and International powder and nanotechnology.

The Cyber security – why and how workshop takes place on Thursday, 14 June at the Congress Center Messe Frankfurt (CMF), Room Illusion 1. The forum examines the need to improve digital security in an age increasingly dominated by smartphones, internet, social networks, clouds, smart remote working and other factors. What will Industry 4.0 and the Internet of Things mean for the pharmaceutical sector? Find out at this workshop organised by ISA Italy Section.

The International Powder and Nanotechnology Forum takes place on Tuesday, 12 June and Wednesday, 13 June at the Congress Center Messe Frankfurt, Room Spektrum. This is the forum for the exchange of ideas between industry and academia and a platform for interdisciplinary cooperation in the field of powder technology. This fourth event at ACHEMA is organised with the joint support

Cyber security will take centre stage in one of ACHEMA 2018's special forums, as attention turns to improving digital security

ACHEMA 2018 will explore the future of the pharmaceutical industry through three key themes: 'Flexible Production', Chemical and pharma logistics' and Biotech for Chemistry'

of the Society of Chemical Engineers, Japan (SCEJ) and DECHEMA.

The forum on powder technology offers the opportunity to learn about the latest developments in the field of powder technology, in particular about innovations and technological trends in Japan.

The Forum will present cutting-edge technology, the key themes being materials, nanotechnology / biotechnology, crystallisation, pharmaceutical science and technology and also simulation and modeling. These themes reflect the strengths of the Japanese process industry, and process industries in general, from the chemical and pharmaceutical industry to the energy sector through to environmental technology. This forum is organised by the Committee of the International Powder and Nanotechnology Forum 2018.

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2018

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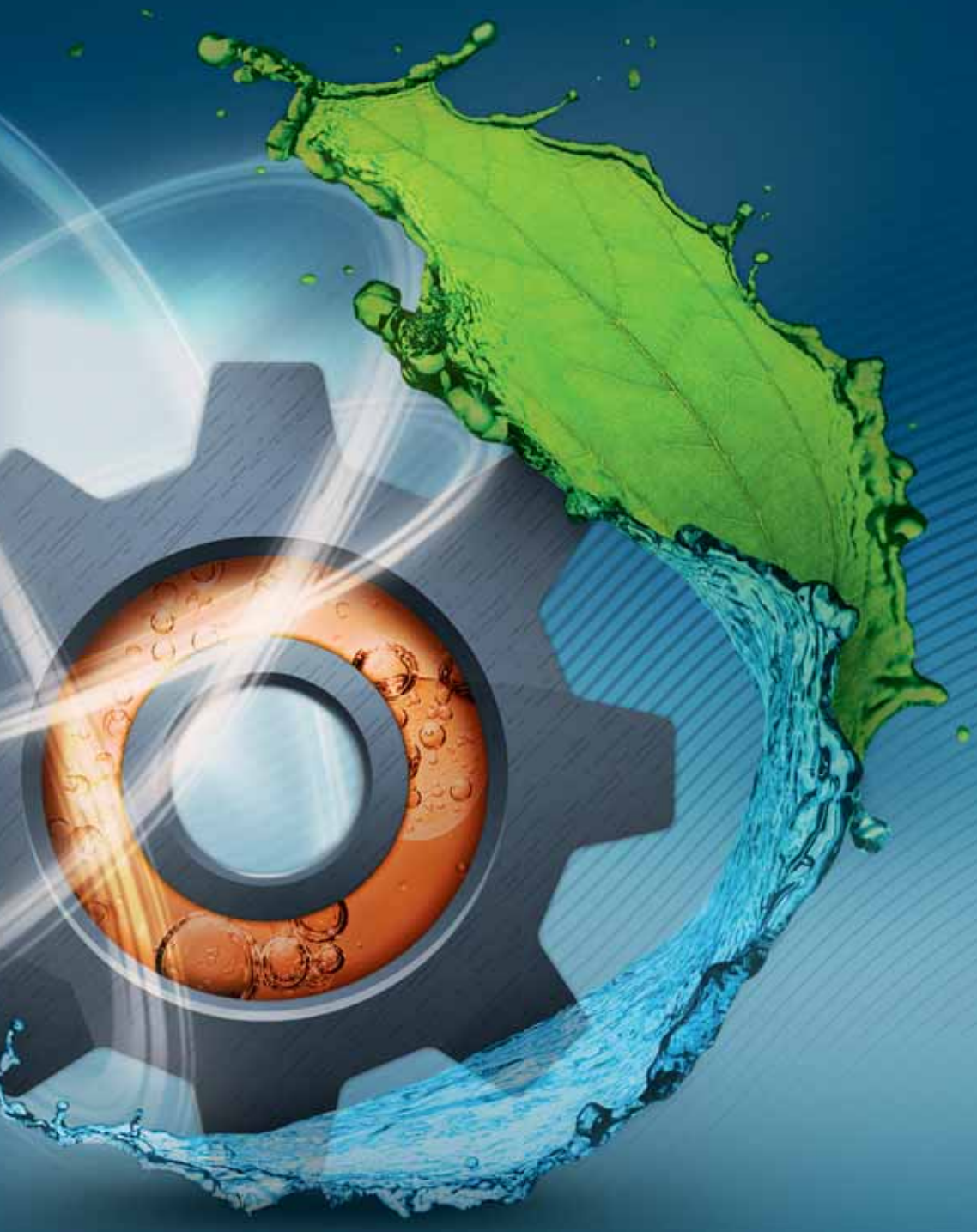

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Spatial light modulators as PAT sensors: Raman applications

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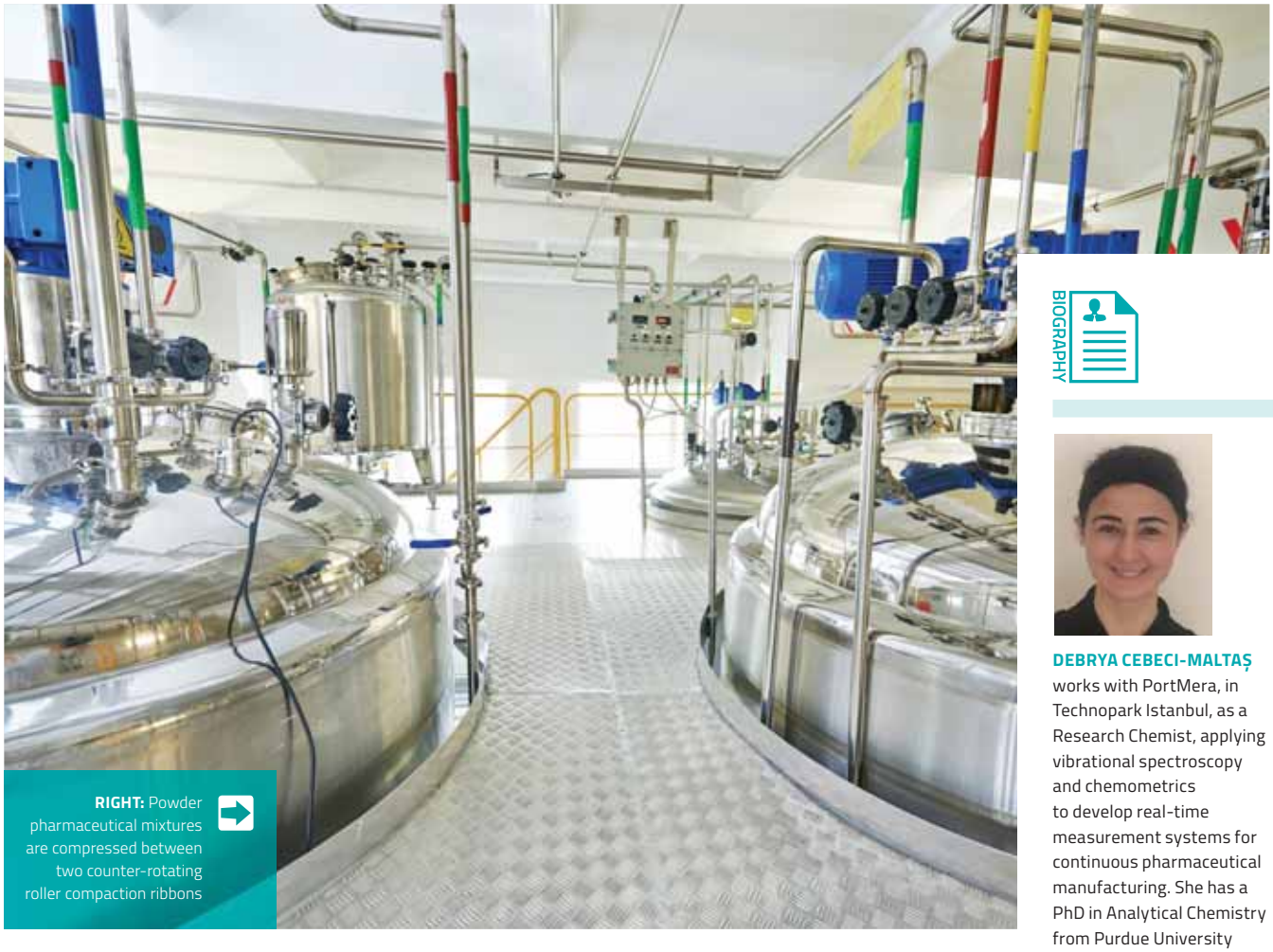
PortMera Research, Istanbul, Turkey

Rodolfo Pinal and Lynne S. Taylor
Department of Industrial and Physical Pharmacy, Purdue University, US

Dor Ben-Amotz
Department of Chemistry, Purdue University, US

A new way of applying Raman spectroscopy has been introduced in recent years to overcome the limitations of normal Raman.[1,2,3] These systems employ spatial light modulators (SLM) for advanced light controlling. Two different types of spatial light modulators are introduced in these studies: digital micromirror devices (DMD) and liquid crystal SLMs (LC-SLM).[1,2,4]



RIGHT: Powder pharmaceutical mixtures are compressed between two counter-rotating roller compaction ribbons

THE fundamental advantage of SLM-based Raman systems over traditional Raman instruments is that a single channel detector is used to collect all the light transmitted by SLMs, thus achieving a higher signal-to-noise ratio when the same light is distributed over the many cells of an array detector in traditional CCD-based Raman systems. This means that a shorter collection time would generate the same quality of signal from that of CCD based systems. Accordingly, the introduction of SLMs into the Raman systems speeds up the collection of Raman data, which makes it attractive as a PAT tool.

The LC-based SLMs consist of linear arrays of liquid crystal pixels (eg, 12,288 pixels), whose

BIOGRAPHY

DEBRYA CEBECI-MALTAŞ works with PortMera, in Technopark Istanbul, as a Research Chemist, applying vibrational spectroscopy and chemometrics to develop real-time measurement systems for continuous pharmaceutical manufacturing. She has a PhD in Analytical Chemistry from Purdue University and holds an MBA degree from Ball State University. Before joining PortMera, she worked at Food and Drug Administration as a postdoctoral fellow, generating spectroscopy methods for at-field counterfeit screening applications.

reflectivity is computer programmable and dictated by the filter functions specific for an application. DMD-SLMs, on the other hand, consist of hundreds of thousands (eg, 1920 x 1080) of highly reflective moving micromirrors controlled by underlying complementary metal-oxide semiconductor (CMOS) electronics. Both LC and DMD SLMs provide a variable transmittance (or reflectance) gray scale. Due to this feature they may readily be used to produce filter functions of any shape.

LC-SLMs use optical polarisation to produce phase or amplitude modulated variable filter functions, while DMDs can produce this functionality by controlling the number and duration of mirrors that are in the 'on' state during measurement. SLM-Raman systems defined in these references[1, 2, 4] work by directing the scattered Raman photons from the sample to the SLM. When photons reach the SLM, they will be modulated by the filters already loaded. This modulated light is then sent to the low noise, single channel detector, then measured and recorded by the system. SLM-Raman in compressive detection mode does not measure the whole spectrum, but rather measures the response of the spectrum coming from the sample to the filter functions (essentially recording the dot product of the spectrum vector and filter vector loaded on SLM). This response provides qualitative or quantitative information about the query components in the sample.

Due to its programmable nature, SLM-Raman instruments can function in different detection modalities utilising different filter functions. They function as compressive detection systems if it is operated on filter functions loaded on the SLM or as a general spectrometer scanning the wavelength range that the user determines without filters. Note that the speed advantage of SLM Raman over traditional CCD-based Raman systems is achieved only by using trained filter functions, which are tuned to optimally detect components under investigation.

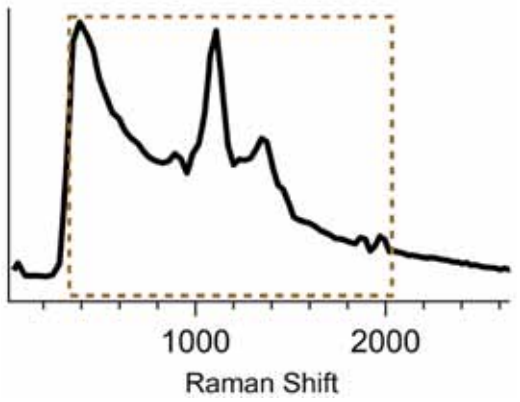
Density variation on roller compaction ribbon

Powder pharmaceutical mixtures are compressed between two counter-rotating rollers to form roller compaction ribbons. The ribbons are then milled into granules. Density gradients in ribbons determine the particle size distribution of granules, which eventually determines the tableting behaviour and final properties of the drug. Thus, determining the ribbon quality quickly is critical for PAT to reduce the delay time in manufacturing.

Here, LC-SLM Raman in imaging mode is employed to monitor the density variations in a ribbon. A univariate filter function is used for this study. Univariate technique correlates one independent variable, such as quantity, to a single dependent variable, such as peak area or intensity. Here, MCC hadamard spectrum is collected (***Figure 1***) and then scaled to a maximum value of one to set the liquid crystal pixels to the maximum SLM transmittance. For the filter function, the pixels corresponding to Raman shift values between 355-2020 cm^{-1} are set to 100% T (to maximise the reflectivity of the SLM), so that only those Raman lights are sent to the detector and recorded as a response. All other pixels are set to 0% transmittance by 'turning them off'. The response values obtained from this specific filter essentially gives the area between 355-2020 cm^{-1}.

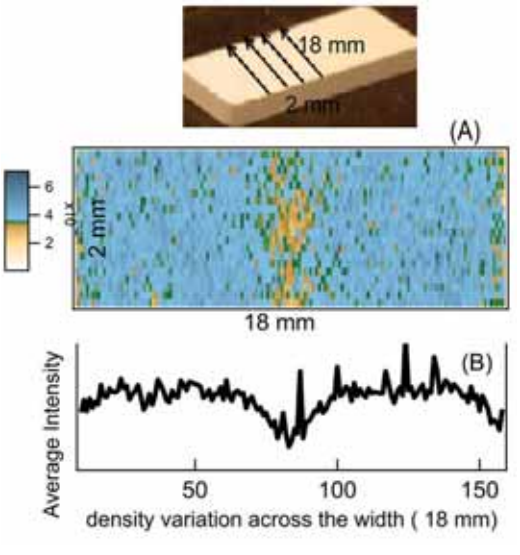
The Raman intensity map was collected with an LC-SLM Raman instrument equipped with a laser line at the 785nm and with 80mW power at the sample. A 20x (NA 0.40) NIR objective lens was used to focus the laser onto the sample and collect

FIGURE 1



LEFT: Hadamard Raman spectrum of pure MCC. Filter function is generated by setting pixels in the boxed area to 100% transmittance

FIGURE 2



LEFT: Density variation measurements across the width of MCC ribbon. Density gradients across the 20 lines of width are imaged in (A). Bottom figure (B) shows the average of all 20 lines across the width

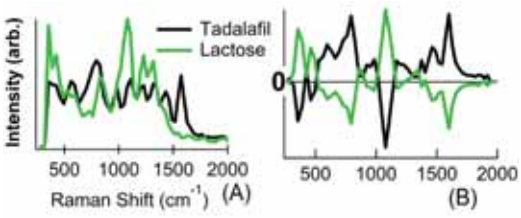
BIOGRAPHY

DOR BEN-AMOTZ obtained his PhD from UC Berkeley, followed by a postdoctoral fellowship with Dudley Herschbach at the Exxon Corporate Research Lab in Annandale, US. He has been a faculty member in the Department of Chemistry at Purdue University since 1989. His recent experimental and theoretical interests include hydration-shell spectroscopy, liquid theory, hyperspectral compressive imaging, and new ways of teaching physical chemistry.

LYNNE S. TAYLOR is a Professor at the Industrial and Physical Pharmacy department at Purdue University.

RODOLFO PINAL is Associate Professor of Industrial and Physical Pharmacy at Purdue University. Before his academic appointment, he worked for 13 years at Hoffmann-La Roche, in Nutley, US, where he was head of the solid-state characterisation laboratory. His research interests include the study of raw material functionality, aimed at the development of composites for applications to the design and manufacture of patient-centric, personalised medications.

FIGURE 3



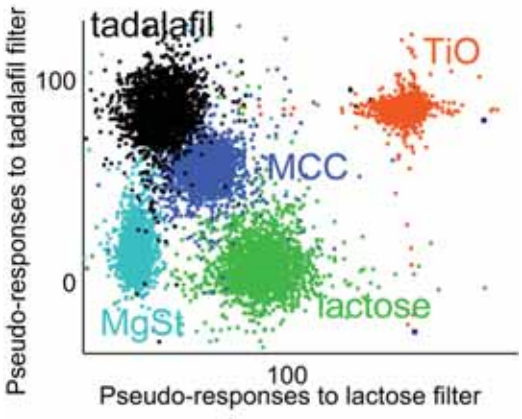
ABOVE: A is pure component spectra. B is PLS derived multivariate filter function for tadalafil and lactose

the scattered Raman photons. A density map with an area of 2 x 18mm2 (20 x 180 pixels) is formed by collecting a series of spectral responses from adjacent locations on the sample (***Figure 2A***). The surface of the ribbon is scanned with 100µm step size, both across the width and length. A total of 3,600 points on the ribbon is measured in about one minute using only one filter function, which would otherwise take about one hour with array-based Raman spectroscopy with a typical collection time of one second. ***Figure 2B*** shows the average density variation across the width of the ribbon for 20 lines measured. Both ***Figures A and B*** suggest that the density in the middle of the ribbon is lower than the sides for this specific MCC ribbon. The edges also look somewhat less dense.

Identity / verification sensor

Univariate filters may be valuable for their simplicity when the investigated material has a unique observable peak. However, isolating a unique peak belonging to the query chemical may not always be possible considering the number of ingredients typically present in a pharmaceutical formulation.

FIGURE 4



ABOVE: LC-SLM Raman responses of raw materials to the filters in Figure 3B. Each cluster represents ~1200 spectral responses measured on ~1200 points on the sample

Alternatively, pretraining using multivariate algorithms (PLS, PCA etc) may be used to determine filter functions. Although this approach requires more computational effort prior to data acquisition than the univariate approach, multivariate algorithms, such as PLS, have a better selectivity by maximising the variance between the components investigated. Thus these filter functions are often very effective in detecting even subtle spectral differences.

Rapid and effective techniques for verification / classification of raw materials for manufacturing are an essential part of the PAT programme. A successful application of LC-SLM Raman spectroscopy as an effective PAT sensor for identity / verification testing of raw materials has recently been reported.[3] For this study, the LC-SLM Raman instrument is used with PLS filters trained on only two components, to compress full-spectral data coming from each point on the sample onto a single channel avalanche photodiode (APD) detector. Note that measuring the light reflected by the filter on SLM is equivalent to obtaining the dot-product of the vectors corresponding to the spectrum from the sample and the filter function.

Multivariate PLS filter functions are generated on tadalafil and lactose spectra. The excipients investigated here are all common components used in pharmaceutical products. PLS-DA filters trained on only these two raw materials were able to discriminate three other common raw materials used in drugs. ***Figure 3*** shows the hadamard Raman spectra of the samples used for training. The responses of tadalafil and lactose, as well as microcrystalline cellulose (MCC), magnesium stearate (MgSt), and titanium (IV) oxide to these filter functions are then measured with the LC-SLM Raman system. The collection time for 1,200 points on the sample was only a minute, which would take 20 minutes with a typical collection time of one second per spectrum using array-based Raman systems. The filters used in this study are trained to identify only lactose and tadalafil. Thus the high discrimination of tadalafil and lactose is expected. However, the results showed that three other ingredients to the same filters can also be differentiated (***Figure 4***). The abscissa of ***Figure 4*** denotes the responses to the lactose filter while the ordinate represents the responses to the tadalafil filter. This implementation demonstrates the feasibility of training a LC-SLM Raman system using a relatively small library of materials and validating not only the materials in the library but also materials that are not included or trained.

This research was financially supported by a grant from the National Science Foundation (Grant IDBR 0754740), by NSF-Engineering Research Center for Structured Organic Particulate Systems (ERC-SOPS, EEC-0540855) and by a grant from the Lilly Endowment Inc. to the College of Pharmacy.

REFERENCES

To view references, visit: **europeanpharmaceuticalreview.com/2-18-Cebici**

The 'Guide to...' series offers readers a comparison between key products and companies within specific categories. Forthcoming topics include: BioProcessing Technology, Manufacturing, and Outsourcing.

TESTING

Welcome to *European Pharmaceutical Review's* **Guide to Testing.** In this edition, five leading testing service providers explain how they cater for the pharmaceutical industry's changing needs.

ASSOCIATES OF CAPE COD, INC., as the pioneer of the Limulus amebocyte lysate testing methodology, specialises in bacterial endotoxin and (1→3)-ß-D-glucan detection, using Food and Drug Association-licensed chromogenic, turbidimetric and gel-clot reagent technologies.

BUTTERWORTH LABORATORIES LTD is a UK-based contract laboratory providing analytical chemistry services to the global pharmaceutical industry in support of R&D, clinical development and licensed manufacture.

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Products:

Our reagents and instruments are used within the Pharmaceutical, Medical Device and Biotechnology industries for quality control and product release. We offer instrumentation for USP BET compliant endotoxin testing to our customers in over 80 countries worldwide.

Services:

ACC's CTS laboratory specializes in testing for endotoxin and glucan contamination and has the most extensive experience of any endotoxin and glucan testing laboratory in the world.

Our Beacon Diagnostics® laboratory is a CLIA-certified laboratory specializing in (1→3)-β-D-glucan analysis to support the diagnosis of Invasive Fungal Disease (IFD).

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Comprehensive consultation, validation, training, and support

As the pioneers of the Limulus amebocyte lysate (LAL) testing methodology, Associates of Cape Cod, Inc., (ACC), specialises in bacterial endotoxin and (1→3)-ß-D-glucan detection, using Food and Drug Association-licensed chromogenic, turbidimetric and gel-clot reagent technologies.

THROUGH comprehensive consultation, validation, training, and routine support, ACC provides customers in the pharmaceutical, medical device, biotechnology, compounding pharmacy and dialysis industries with a thorough endotoxin detection solution.

ACC's products quantify the presence of bacterial endotoxin as well as (1→3)-ß-D-glucans. In addition to LAL reagents, ACC also provides instrumentation, software and ancillary products to run the bacterial endotoxins test (BET) assay. This includes, but is not limited to, the Pyros Kinetix Flex incubating tube reader, Biotek ELx808 incubating plate reader, Pyroclear brand dilution tubes, reaction tubes and Pyroplates. ACC releases the Pyroclear brand products at <0.001 EU/mL and <1.56 pg/mL. For customers establishing a testing system, ACC offers on-site consultation, system validation and customisable technical training seminars. Lastly, ACC has a contract testing service (CTS) lab that provides product characterisation, validation, investigative and final product testing.

Associates of Cape Cod, Inc. is headquartered in East Falmouth, US, with additional offices located in Liverpool, Knowsley, UK and Mörfelden-Walldorf, Germany. Both the East Falmouth and Liverpool office boast Quality Control and CTS labs.

ACC is the only major provider of LAL reagents to exclusively focus on the endotoxin and (1→3)-ß-D-glucan testing space. The team at ACC is solely focused on providing the highest quality reagents with the absolute best technical support in industry. Additionally, Associates of Cape Cod, Inc. sells and supports their proprietary Pyros Kinetix Flex tube reader system – the only open-ended system on the market, which allows end users to continuously add samples as an assay is running. This test setup allows analysts to monitor and ensure the validity of the assay, and reduce the time and cost of re-tests. ACC's Pyrotell-T kinetic turbidimetric and Pyrochrome kinetic chromogenic reagents are the most sensitive in the world with detection limits down to 0.001EU/mL, and are both validated to be run with as little as 50µl per reaction. This makes ACC the most sensitive and cost-effective solution in the kinetic endotoxin detection market.

ACC is committed to the sustainability and longevity of the American Horseshoe crab species (Limulus Polyphemus), and continues to work closely with local authorities to ensure the impact on the local population is limited. ■

COMPANY DETAILS

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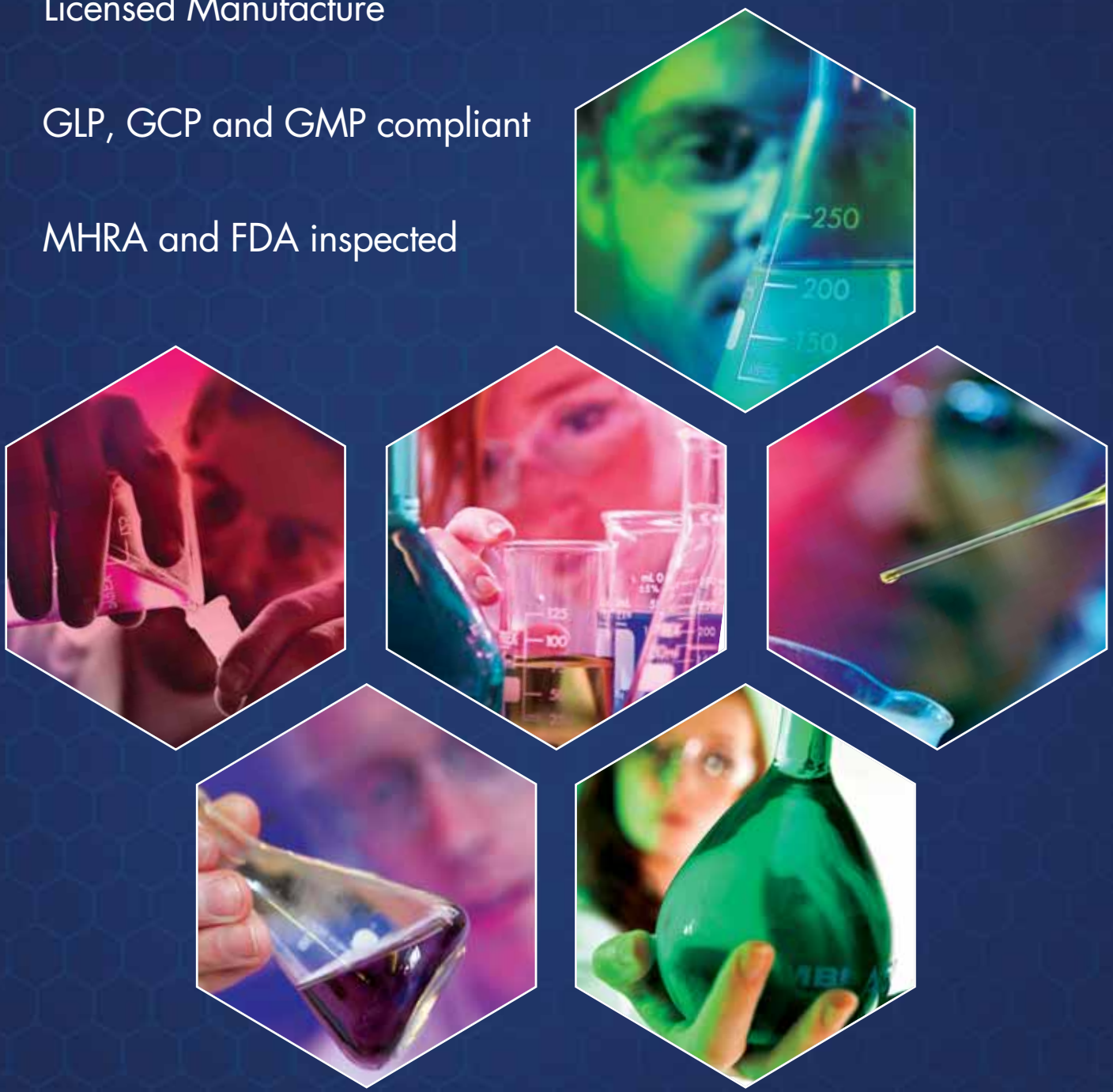

LEADERS IN PHARMACEUTICAL ANALYSIS

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Analytical chemistry services for the global pharmaceutical industry

Butterworth Laboratories Ltd is a UK-based contract laboratory providing analytical chemistry services to the global pharmaceutical industry in support of R&D, clinical development and licensed manufacture. We are GLP, GCP and GMP compliant, FDA and MHRA inspected.

WE HAVE extensive experience in the analysis of raw materials, finished products, devices and packaging in accordance with internationally published (*European Pharmacopoeia, British Pharmacopoeia, United States Pharmacopeia, Pharma Japan, Japanese Pharmaceutical Excipients*) client-supplied or in-house developed methods.

A wide range of state-of-the-art analytical instrumentation complements our classical competencies, enabling us to provide the most up-to-date and cost effective analytical services.

What are the main areas that you can test for?

We predominantly focus our analysis on confirming the identity and demonstrating the purity, safety and quality of materials using techniques such as: HPLC, GC, IC and ICP contractions all to cGMP standards. Classical wet chemistry, elemental impurity and residual solvents analysis are specialisms of ours.

What are the main challenges that the pharmaceutical sector faces in testing for QA/QC?

In terms of quality control, the age-old challenge is whether to retain in-house analytical capabilities as a manufacturer, or outsource to a dedicated contract analytical supplier, such as ourselves. This should really be driven by the assessment of core and non-core activities. Many companies choose to outsource raw material analysis and keep drug product testing in-house; the former often being complex and sporadic and the latter being consistent and time constrained.

What additional services do you provide?

As analytical chemists, we can use our equipment and expertise for many applications across the wider industry. We offer development and validation of methods for use in quality control analysis.

What additional benefits are there to working with you?

Our business is independent, so there are no shareholder dividends to be paid. This means we can re-invest our profits in two key ways. Those are: staff retention and capital investment in new equipment and laboratory facilities.

Retaining talent and expertise is critical to offering a first-class contract analytical service. It ensures that we can advise customers on the correct analytical approach and also quickly problem solve when required.

Investing in new and updated instrumentation ensures that we are offering the most up-to-date analytical methods to our customers.

The trend in the pharmaceutical industry is to globalise testing services. How can your company address the local needs of users?

We have an established reputation forged over nearly 44 years of operation. This provides our local customers with absolute confidence in the quality of service they will receive, allowing them to focus on their core business, safe in the knowledge that their analysis is in experienced hands.

However, globalisation has reduced barriers, and approximately 30% of our business is outside the UK, with many businesses choosing us over local suppliers. This demonstrates that we can offer as good a quality service to customers outside of the UK as within it. ■

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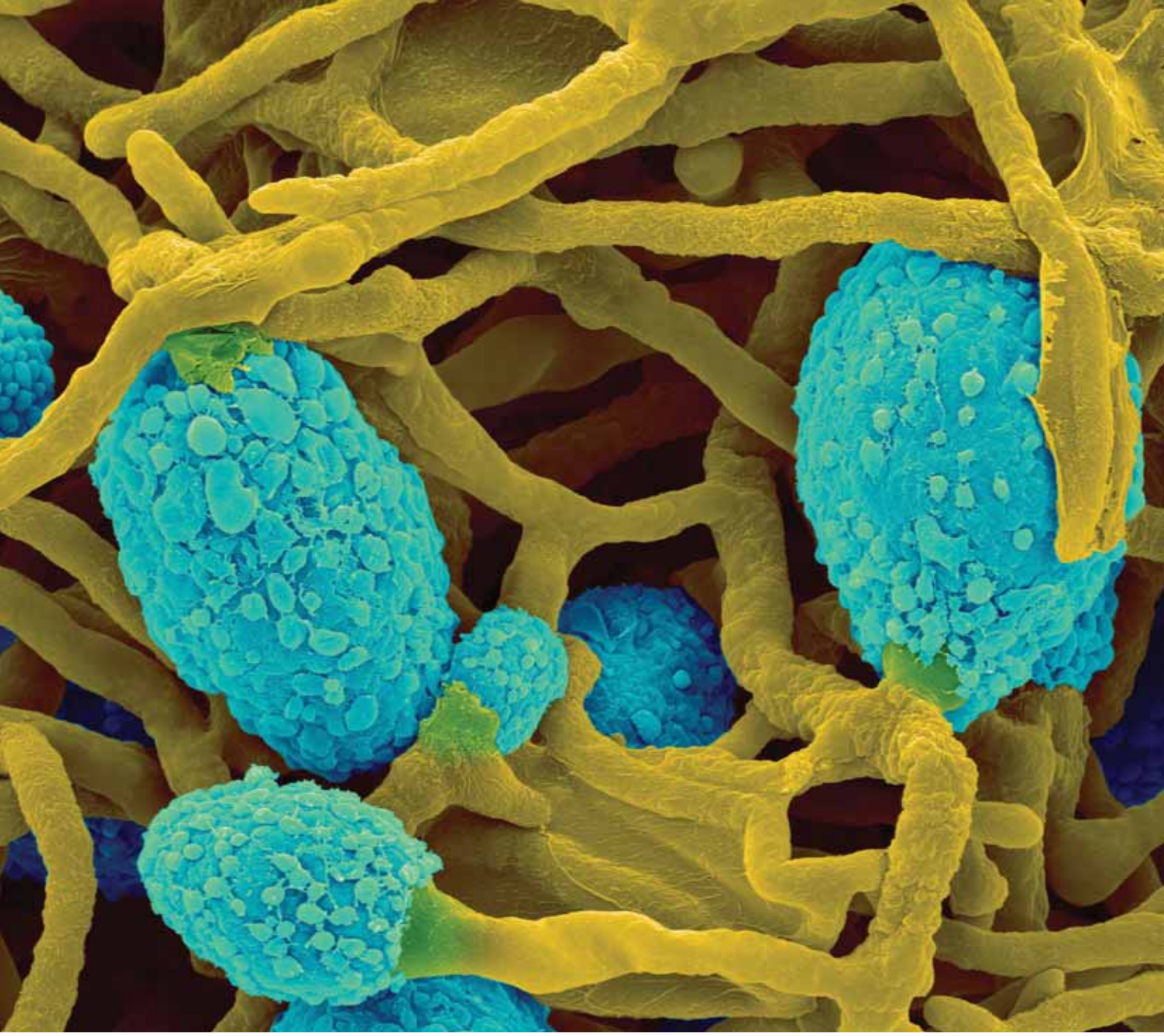

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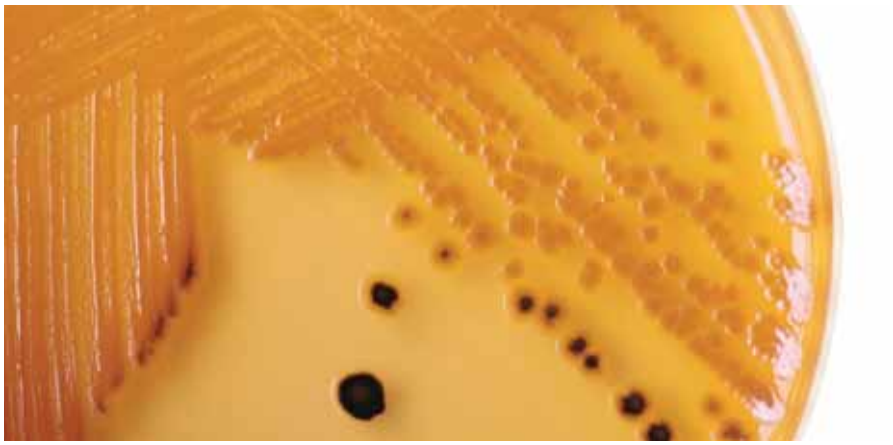
Data drives every decision in the lab to support confident decisions

Charles River Microbial Solutions believes data drives every decision in the lab, so ensuring it is accurate, relevant, and reliable is critical to support confident decisions on product quality and safety.

How confident can you be that your quality control (QC) lab's data is complete, secure, and accurate? Furthermore, process validation, cGMP compliance, and other rigorous regulatory standards are required attributes for sophisticated microbial identification solutions and simplification of practices.



Outsourcing to a compliant, data integrity-driven organisation, such as any of our Microbial Solutions laboratories, is thus the most cost efficient solution. It avoids tremendous investment in technology upgrades, and assures a streamlined micro QC process through innovative data management applications.

With its two centres of excellence in Lyon, France and Dublin, Ireland, Charles River's Microbial Solutions group plays an integral role in endotoxin testing, microbial detection, and identification in Europe. The facilities deliver the industry's most comprehensive portfolio of leading micro QC products and services, featuring Endosafe®, Accugenix®, and Celsis® solutions, enabling clients to run their manufacturing processes smoothly while implementing high quality environmental monitoring programmes.

The bacterial endotoxin test is a highly sensitive and exquisitely specific *in vitro* assay for medical devices, cellular therapies, and parenteral products, and the Charles River technical services laboratory can support customers performing these critical tests. Experienced specialists can help improve the compliance and efficiency of endotoxin testing programmes and achieve control, consistency, and precision in the laboratories they support. They can help identify which technology is right for your lab and assist with product validation programmes, regulatory compliance guidance, and troubleshooting technical issues.

A quarterly audit of limulus amebocyte lysate (LAL) testing aptitude, the Charles River Proficiency Test Program (PTP) is also available to verify the accuracy of professionals' LAL results by providing a confidential and external audit of lab analysts and to confidentially compare their results to those of other laboratories, in order to demonstrate competence and improve procedures and methods in accordance with *European Pharmacopoeia, British Pharmacopoeia, United States Pharmacopeia* and Japanese pharmaceutical regulations.

How do you strengthen objectivity in decision making?

Reliable and secure tracking and trending tools automatically aggregate ID metadata, improving your ability to apply objectivity to decision-making. Our ID services use the most relevant databases for Environmental Monitoring along with the most accurate commercially available technologies, DNA sequencing and Matrix Assisted Laser Desorption / Ionisation (MALDI-TOF). Outsourcing to our experts can help you decrease your cost per reportable ID and improve your operational efficiencies, all while reducing compliance risk. They offer several turnaround time options, including same-day processing, to meet any time requirements.

Protecting the integrity of your products requires a reliable partner at all critical junctures in the QC process. Charles River has the tools to provide you with accurate, relevant, and reliable data that can fuel confident in-house decisions on product quality and contamination control, in turn reducing hold times, eliminating days from production cycles, and streamlining your supply chains. ■

COMPANY DETAILS

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WEB: www.criver.com/microbialsolutions

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Designed to give clients laboratory services support

Eurofins' PSS Insourcing Solutions® (PSS) employs and manages full-time scientists, managers and support staff to perform a defined scope of work at a client's facility. Our project-based insourcing solutions are specifically designed to give clients laboratory services support with flexible timeframes and no worries about co-employment and other regulatory concerns, at lower costs than fixed headcounts.

What are the main services provided?

We infuse our more than 55-year track record of scientific and laboratory operations expertise, as well as HR best practices, to recruit, hire, train and manage highly qualified scientists to perform laboratory services at your site, using your quality systems and equipment. Our teams will help you set up your laboratory and validate equipment according to your SOPs and lean laboratory practices on a project basis. PSS employees are offered lean training.

Our PSS programme, including onsite management, can accommodate groups of any size, and we adjust the length of term to meet your needs.

What are the main areas that you can test for?

Our PSS Insourcing Solutions® provides services for clients who require lab services at their facility. We have proven success providing dedicated teams for a variety of technical disciplines that span the drug development pipeline, including:

- Analytical chemistry
- Microbiology
- Environmental monitoring
- Proteomics
- Biochemistry
- Quality assurance
- Sampling
- Stability
- Biopharmaceuticals
- Process development
- Biomedical engineering
- Method development / method validation
- Microscopy
- Bioinformatics
- Genomics
- Cell & molecular biology
- Immunochemistry
- Sample management
- Medical device testing
- Project management
- Bioassay characterisation.

What additional services do you provide?

Eurofins' PSS Insourcing Solutions® is the leading managed service provider that offers a comprehensive benefits package, as well as training, development and career advancement opportunities. Offering these additional benefits allows us to attract, retain and motivate high-calibre employees to serve you. We guarantee our model and indemnify clients from co-employment under our practices. Our on-site dedicated leaders manage our full-time employees and scope of services with measurable KPIs or service metrics to ensure we exceed your expectations.

How many testing locations do you operate?

With more than 1,600 employees worldwide, PSS provides services at more than 70 client sites in over 15 countries throughout North America, Europe, and Asia-Pacific and is part of Eurofins BioPharma Product Testing, operating 28 laboratory locations with over 1,200,000 ft^2 across 16 countries worldwide.

What are the other benefits to working with you?

PSS offers significant benefits, including:

- Provides insourcing services for a defined scope of work managed by our technical leaders
- Solves potential co-employment issues and challenges associated with staff augmentation
- Avoids turnover rate that traditional temporary staffing programmes face by offering job security, employee benefits, great place-to-work practices, and more long-term career opportunities
- Usually costs you less than your own full-time employees with a comprehensive benefits package, including training and overhead costs
- Gives you a detailed scope of the effectiveness of the insourcing programme through performance management metrics and lean cost saving initiatives
- Provides a resource for technical expertise and support through laboratory staff at global facilities.

Is there any other information you think would be helpful?

Our output per money spent will exceed that of other suppliers, through effective metric-driven management, lean project support / management, and cross utilisation of resources.

PSS is the only insourcing solution in the industry to have been recognised with 10 strategic partner awards for outstanding service in the past nine years. ■

COMPANY DETAILS

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eurofins | BioPharma Product Testing PSS Insourcing Solutions

Put Your Quality Control in Safe Hands

Over 50 Years of Expertise in Medical Device & Pharmaceutical Testing

Wickham Laboratories
Contract Analytical Services

mail@wickhamlabs.co.uk
www.wickhamlabs.co.uk

Wickham Laboratories Ltd: backed by five decades of global experience

Wickham Laboratories Ltd, backed by five decades of global experience in Good Manufacturing Practices (GMP) / Good Laboratory Practices (GLP) contract testing, is an established name in the fields of pharmaceutical and medical device contract testing, research and consultancy. We conduct business with clients worldwide and the combined expertise of our laboratory technicians and managers enables us to be fully conversant with global regulatory expectations.

WE ARE committed to providing a quality testing service, combining high levels of client satisfaction with the maintenance of appropriate accreditation. This, and the continual pursuit of delivering excellence, remain central to our business.

What are the main areas that you can test for?

Our core services are microbiology and toxicology testing for the medical device and pharmaceutical industries, including:

- Antimicrobial / preservative efficacy
- Bacterial endotoxin (LAL) and Monocyte Activation Test (MAT)
- Bioburden determination
- Biological indicator enumeration
- Cytotoxicity testing
- Environmental monitoring
- *In vitro* diagnostic assays such as ELISA, BCA and Western Blot
- Microbial identification, both traditional and rapid (MALDI-ToF)
- Microbial ingress
- Microbial limits including TAMC / TYMC and absence of specified pathogens
- Potency bioassays and abnormal toxicity on biological products
- Rabbit pyrogen (RPT)
- Stability storage and testing
- Sterility testing
- USP Class I-VI tests and ISO 10993 Series tests.

What additional services do you provide?

In addition to testing services, Wickham Laboratories offers global support and consultancy relevant to a wide range of medical device and pharmaceutical development and manufacturing concerns, including:

- Biocompatibility assessments
- Cleanroom qualification
- Process validation / identification of contamination sources
- Regulatory applications support
- Training on appropriate cleaning practices
- Validation of water systems.

Our experts have many combined years of experience in pharmaceutical and medical device testing and can assist in on-site visits or via telephone or email consultations. In collaboration with our preferred partners, we have helped many customers successfully achieve accreditations for products in various international markets.

Number of testing centres and locations

We have one main testing centre in our Hoeford Point location, which is a 4,000 metre facility segregated into independent microbiology and toxicology laboratories.

What additional benefits are there to working with you?

Wickham Laboratories has over 50 years of experience in testing services and a broad range of technical expertise in identifying and providing solutions for our clients. Our long-standing global experience in contract testing means there is rarely a problem that has not previously been encountered and resolved by our experts.

We operate in accordance with GMP and GLP as well as to International Organization for Standardization (ISO) and International Council for on Harmonisation (ICH) guidelines.

Other information you think would be helpful?

In addition to the discrete laboratories themselves, we also have dedicated facilities for sample booking and media preparation. Our sample receipt team utilises systems operating to FDA 21CFR part 11, which ensures safe handling and full traceability of samples, and most of the media used to test these samples is prepared and processed by our in-house media preparation department.

At Wickham Laboratories, we operate a secure site, with an onsite security team 24/7. We are also equipped with a team of in-house qualified service engineers, enabling a self-sufficient facility without major service disruptions.

We are routinely inspected by the Medicines and Healthcare products Regulatory Agency (MHRA) and Food and Drug Administration, and offer the opportunity for clients to conduct audits on a regular basis. ■

COMPANY DETAILS

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Panasonic biomedical has become PHCbi

We have not changed our innovative technology. The European sales and service organization stays the same. Our vision on sample security has not altered and we keep on designing the best biomedical equipment.

The new brand for Panasonic healthcare biomedical division will be PHCbi.

You can still rely on our undisputable quality and reliable products like:

VIP ECO Ultra Low Temperature Freezer

Low energy consumption due to Natural Hydrocarbon refrigerants and inverter compressors.

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www.phchd.com/eu/biomedical