EUROPEAN **PHARMACEUTICAL** REVIEW

ISSUE 02 | APR 2019

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Anticipating delays to market

It's not only Brexit that experiences hold ups; if you haven't prepared for the unexpected, you risk delaying your product to market.

NIKKI WITHERS
EDITOR
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THE COMPLEXITY of developing a combination product extends beyond the realms of its formulation; the product-package compatibility is key for product stability and performance requirements of the overall delivery system. In this issue's cover feature, James Mellman, Device Manager at Novartis, explains how he is constantly straddling two worlds – the formulation world and the delivery system world – to ensure successful development of a combination product. "If you don't have a compatible system with your primary packaging and formulation, you will not have a product that can be delivered," he says. To read the full story, including an unexpected case study example, turn to page 38.

Moving the focus to Europe, where Brexit delays continue, legal expert Paul Ranson touches upon possible impacts outside the UK/EU, with a look at the Association of South Eastern Nations (ASEAN) region. "It has been argued that the effect of the UK's exit from the EU might be to bolster ASEAN's desirability, centrality and its influence on potential trade and strategic partners," he says.

In our Microbiology In-Depth Focus series, concerns over the proposed revisions to the EU Good Manufacturing Practice Annex 1 are raised by pharmaceutical microbiologist Tony Cundell (page 60), while a team at GlaxoSmithKline have penned key considerations for microbiological environmental monitoring technology selection and evaluation (page 50).

Dave Elder continues his series on mutagenic impurities on page 7 by discussing the realities of the EMA's guidance for manufacturers regarding the processes to prevent nitrosamine impurities. He told me recently that the scenario continues to change...so I'm sure this won't be his final instalment.

Finally, five leading testing companies are showcasing their services in our Guide To series starting on page 71. We hear how ACC, Charles River, Eurofins, Nelson Labs and Wickham Labs stand out from the crowd with the services they offer.

Finally, we are 'revamping' our content offering going forward; from the next issue of *EPR* we will have In Depth Focus articles on Formulation, Development & Delivery, QA/QC and Analytical Techniques, Manufacturing, Packaging and Logistics, and Downstream Bioprocessing and Bioproduction in every issue. If you would like to contribute research, then please get in touch.

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Mutagenic impurities: a done deal? (part 3)

Dave P Elder

JPAG Member and David P Elder Consultancy

In the third part of this series on mutagenic impurities, Dave Elder discusses the realities of the EMA's guidance for manufacturers regarding the processes to prevent nitrosamine impurities.

IN THE previous *EPR* article focusing on the sartan contamination issue[1] it was highlighted that, based on evolving information, "The EU's emerging position appears to be a pragmatic combination of avoidance and an acceptable control strategy, ie, ppb limits on API specifications".

However, EMA subsequently issued further guidance at the beginning of February[2] showing that their stance has hardened, indicating that companies manufacturing sartans must avoid this issue completely in the future.

Manufacturers must "review their manufacturing processes so that they do not produce nitrosamine impurities". Companies will have a "transition period to make any necessary changes, during which strict temporary limits on levels of these impurities will apply". EMA further stated that "After this period, companies will have to demonstrate that their sartan products have no quantifiable levels of these impurities before they can be used in the EU".

Unfortunately, the downside of an avoidance strategy linked with a confirmation strategy is that demonstrating absolute absence (ie, 0 ppb) is an impossible undertaking. There is ample precedence for trying to mandate total absence of cancer-causing agents, without being able to achieve the desired result.

The most famous example is the 1958 US Delaney Clause. It states that no human or animal carcinogen, "shall be deliberately added to, or found as a contaminant in food".[3] However, enforcement by the FDA proved to be extremely problematic. Indeed, there was significant concern that an unwillingness to accept even the most infinitesimal risks could "force off the market many substances utilised in agriculture and food processing that are widely regarded as safe when used as intended".[4]

> *"Interestingly, there are many within the EMA's own safety working party (SWP) that viewed the "risk avoidance" strategy finally adopted by EMA with concern"*

Currently, the nitrosamine methodologies have ppb limits based on acceptable intakes (AI) for NDMA (Candesartan: 3000 ppb; Irbesartan: 320 ppb; Losartan: 620 ppb; Olmesartan: 2400 ppb; Valsartan: 300 ppb) and NDEA (Candesartan: 820 ppb; Irbesartan: 88 ppb; Losartan: 177 ppb; Olmesartan: 663 ppb; Valsartan: 82 ppb). EMA has indicated that after the transition period a limit of <30 ppb for both nitrosamines would be applicable. However, this confirmatory limit is difficult to justify, as it isn't based on a specific daily dose of any specified sartan, or on the specific nitrosamine impurity (ie, NDMA or NDEA), or on the AI limits for those specific nitrosamine impurities; it appears to be an arbitrary figure.

Interestingly, there are many within the EMA's own safety working party (SWP) that viewed the "risk avoidance" strategy finally adopted by EMA with concern. The so-called "risk minimisation" group[5] within SWP felt that the established AI strategy defined in ICH M7(R1)[6] employs a conservative risk assessment approach, which is considered suitable if levels are kept below these thresholds. The "risk minimisation" group felt that there were no compelling reasons to deviate from the accepted "risk/benefit balance regulatory practices for handling mutagenic impurities". This is based on the fact that there are no factual data to demonstrate that "NDMA and NDEA are fundamentally different from other mutagenic carcinogens, which are covered by the TTC framework in ICH M7(R1), besides being more potent". As such, the higher potency is handled by "defining compound-specific thresholds based on carcinogenicity data and by linear extrapolation". Thus, there is no requirement for a "no threshold" approach.

Ironically, we have reached the "same" position as earlier, with a combination of avoidance and an acceptable control strategy – ie, ppb limits on API specifications – although in the former case the limits were scientifically justifiable, safety-based limits (ie, AI-derived limits) whereas in the latter case the limits (ie, <30 ppb) are not.

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 the Analytical Division Council of the Royal Society of Chemistry.

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ROUND UP

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

Big pharma accused of no action on antibiotic threat

BIG PHARMA

THE PHARMACEUTICAL industry should match its words with action on researching new antibiotics to address the threat posed by drug-resistant superbugs, a former UK government adviser has said.

In a strongly-worded attack on major drug companies, Jim O'Neill, who headed a British government global review of antimicrobial resistance in 2016, said the industry had produced "endless talk and no action", and was engaged in little more than "spewing out nonsense" about the problem.

"If the pharma companies delivered one tenth of the commitment in their words, we might be getting somewhere," O'Neill told reporters at a briefing in London.

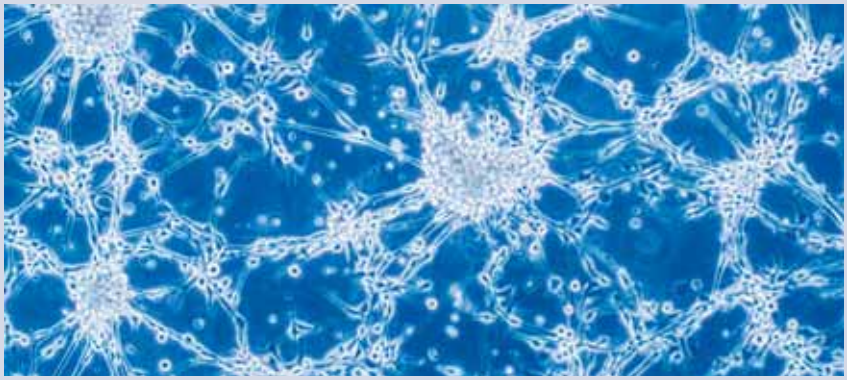
O'Neill, also formerly chief economist of Goldman Sachs, said his frustration at the lack of commitment by drug companies had reached a point where he now believes the best solution might be to create a government-funded 'utility' type drug company, which would not be beholden to shareholders.

O'Neill's 18-month-long review that concluded in 2016, found that the threat of antimicrobial resistance (AMR) could kill an extra 10 million people a year by 2050 and cost up to $100 trillion if nothing is done to slow or halt it.

Global health experts agree the world urgently needs new medicines to keep ahead of the superbugs, but pharmaceutical firms are reluctant to invest in developing drugs that would not be sold in large volumes because of the need to preserve them.

O'Neill has proposed a 'pay or play' solution to the problem, in which drug companies would be subject to a surcharge if they decide not to invest in research and development to bring successful new antimicrobial medicines to market.

For those firms who do decide to 'play', he suggests a reward of between $1 billion and $1.5 billion should be paid for any successful new antibiotic drug developed.



New glioblastoma cancer vaccine shows promise in phase 1b clinical trial

VACCINES

RESULTS from a phase 1b clinical trial of a new experimental glioblastoma vaccine developed by Jefferson and Imvax, show the treatment was tolerated well by patients, slowed tumour recurrence and prolonged patient survival.

The research was presented at an oral session of the American Association for Cancer Research (AACR) annual meeting on 31 March 2019 in Atlanta, Georgia.

Researchers treated 33 patients with newly diagnosed glioblastoma multiforme with the novel cancer vaccine (IGV-001) in a prospective phase 1b clinical study and compared outcomes to a historical comparator group of 35 patients treated with the same standard of care at the same institution. The results showed patients treated with the vaccine had improved progression-free survival and overall survival compared to the control group treated with standard of care alone.

"The response we see in some patients is very encouraging," says Dr David Andrews, Professor of Neurosurgery at the Vickie & Jack Farber Institute for Neuroscience – Jefferson Health and co-founder, Chief Medical Officer and interim Chief Executive Officer of Imvax. "We look forward to initiating a phase II trial later this year to confirm these phase 1b results."

FDA sends four warning letters for cGMP violations

GMP

THE US Food and Drug Administration (FDA) has posted warning letters to four companies who produce homeopathic drug products for significant violations of current good manufacturing practice (cGMP) regulations.

These included a letter to King Bio Inc. of Asheville, North Carolina. The FDA previously warned the public about the agency's serious concerns with the quality of drug products produced by King Bio.

"In late 2017, the FDA proposed a comprehensive, risk-based enforcement approach to drug products labelled as homeopathic and marketed without the required FDA approval. While the agency continues to examine this approach, the homeopathic industry has continued to grow, and we need to continue to address, consistent with our current enforcement policies, situations where products labelled as homeopathic are being marketed for serious diseases and/or conditions where the products haven't been shown to offer clinical benefits," said FDA Commissioner Scott Gottlieb.

The FDA also posted warning letters to additional companies for products labelled as homeopathic due to various quality and misbranding violations.

Drug linked to 33 deaths in Northern Ireland is reclassified

DRUG CLASSIFICATION

A PRESCRIPTION drug that has been linked to dozens of deaths in Northern Ireland is now being treated as a class C drug.

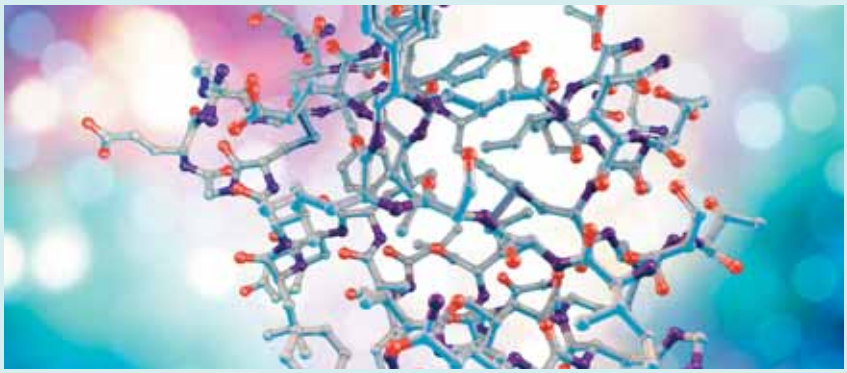
Pregabalin, also known as Lyrica, or 'buds', is used to treat nerve pain, epilepsy and anxiety.

The Northern Ireland Statistics and Research Agency has revealed a four-fold increase in deaths where pregabalin was listed on the death certificate from eight in 2016, to 33 in 2017.

New legislation comes into effect this week classifying pregabalin as a class C controlled drug under the Misuse of Drugs Act.

This means it is now illegal to possess pregabalin without a prescription, with the maximum penalty for unlawful possession of the drug being two years in prison. Selling or supplying pregabalin can now carry a prison sentence of 14 years.

Northern Ireland currently has the highest prescription rate for pregabalin within the UK, with a growing illicit market for the drug there.



Needle-free 'dominates' injectable drug delivery market

DRUG DELIVERY

NEEDLE-FREE injectable drug delivery devices are dominating the injectable drug delivery market, with the prefilled needle-free injector segment expected to take the lead due to its ease of application, a report has found.

Disposable injectors are the most commonly available type of injectors since they are user friendly and do not require special skills for administration.

An increase in prevalence of chronic diseases such as diabetes, cancer and rheumatoid arthritis along with other factors such as need for patient-controlled drug release and increased risk of needle stick injuries are the prominent factors expected to drive the growth of the injectable drug delivery market.

The report, titled 'Global Injectable Drug Delivery Market: Global Industry Analysis 2013-2017 and Opportunity Assessment, 2018-2028', suggests that technological advancements have resulted in better product offerings.

However, some factors such as strict regulatory framework and premium pricing of products are expected to hinder the growth of the injectable drug delivery market.

In terms of value, the global injectable drug delivery market is expected to expand at 10.9 percent CAGR over the period of 2018 through to 2028.

North America remains a dominant regional market (nearly 32 percent market share) and is expected to maintain its dominance due to the presence of a large number of manufacturers, mostly operating from the US.

Western Europe is expected to be the second largest market in injectable drug delivery, with Germany, the UK and France occupying major shares.

News continues overleaf...

Second potential male birth control pill passes human safety tests

CLINICAL TRIAL

AN EXPERIMENTAL male oral contraceptive – 11-beta-methyl-19-nortestosterone dodecylcarbonate, or 11-beta-MNTDC – has passed human safety tests. The pill is a modified testosterone that has the combined actions of a male hormone (androgen) and progesterone, said the study's co-senior investigator, Christina Wang, Associate Director, Clinical and Translational Science Institute at Los Angeles Biomed Research Institute (LA BioMed), Torrance, California.

"Our results suggest that this pill, which combines two hormonal activities in one, will decrease sperm production while preserving libido," Wang said.

The study took place in 40 healthy men at LA BioMed and the University of Washington in Seattle, Washington. Among men receiving 11-beta-MNTDC, the average circulating testosterone level dropped as low as in androgen deficiency, but the participants reportedly did not experience any severe side effects. Furthermore, no participant stopped taking the drug because of side effects, and all passed safety tests.

Effects due to low testosterone were minimal, according to co-senior investigator, Stephanie Page, Professor of Medicine at the University of Washington School of Medicine, because "11-beta-MNTDC mimics testosterone through the rest of the body but is not concentrated enough in the testes to support sperm production."

"Safe, reversible hormonal male contraception should be available in about 10 years," Wang predicted.

Three further Losartan batches recalled

RECALLS

AS A precautionary measure to protect public health, the Medicines and Healthcare products Regulatory Agency (MHRA) has recalled three batches of Losartan tablets due to contamination with the nitrosamine N-nitroso-N-methylamino butyric acid (NMBA).

The recall is taking place as part of the continued investigation into potential nitrosamine contamination of sartan-containing medicines, a class of medicine to treat blood pressure, heart attacks and heart failures.

Currently, there is no evidence that nitrosamine impurities can cause harm and patients are being advised to continue taking their medication.

The investigation into possible contamination of sartan medicines began in 2018, after the nitrosamine N-nitrosodimethylamine (NDMA) was identified in valsartan manufactured at a facility based in China.

In 2018, the MHRA recalled batches of valsartan-containing tablets to pharmacy level in July and November due to possible NDMA and N-nitrosodiethylamine (NDEA) contamination.

In January and February 2019, the MHRA recalled batches of irbesartan containing tablets after testing revealed possible contamination with NDEA.

The MHRA continues to monitor the situation in the UK and is comprehensively investigating the issue alongside the European Medicines Agency (EMA) and the European Directorate for the Quality of Medicines (EDQM).

Continuous manufacturing method used to make generic lomustine

CONTINUOUS MANUFACTURING

RESEARCHERS at Purdue University have developed an innovative and cost-effective continuous manufacturing method to make generic lomustine.

The team synthesised lomustine – an important agent for treatment of brain tumours and Hodgkin's lymphoma – using continuous flow methodology, and it is thought the approach could be applied to other products.

"Desorption electrospray ionisation mass spectrometry (DESI-MS) was used to quickly explore a large number of reaction conditions for one of the reaction steps and guide the efficient translation of optimised conditions for continuous lomustine production," write the authors in *Organic Process Research and Development*.

"Using only four inexpensive commercially available starting materials and a total residence time of nine minutes, lomustine was prepared via a linear sequence of two chemical reactions performed separately in two telescoped flow reactors."

The Food and Drug Administration (FDA) wants the pharmaceutical industry to get away from making drugs using the traditional batch method and switch to continuous manufacturing.

In February, the FDA put out a statement saying the continuous process allows manufacturers to more easily scale operations to meet demand and should help reduce drug shortages. The statement also said continuous manufacturing can provide a more robust, lower-cost and diverse supply of drug products.

Professor David Thompson, from Purdue's Department of Chemistry and a member of the Purdue University Center for Cancer Research, began working on applying his continuous manufacturing process for lomustine after reading an article written by Dr Henry Friedman, a neuro-oncologist from Duke University, about how the cost of lomustine had risen dramatically.

His aim was to make lomustine quickly and cheaply and within six months Thompson's team developed a method to make lomustine at a rate equivalent to one dose every two hours using continuous manufacture. His group is now developing methods to scale up the production rate.

"All of this is happening in a space that is the size of a small desk. A very small footprint," Thompson said.

Are you giving regulatory intelligence the platform it deserves?

Jürgen Hönig
Senior Director, Application Services, Head of Regulatory Affairs (Frankfurt), PharmaLex

Rob Williams
Senior Director, Regulatory Informatics and Digital Technologies, PharmaLex

Regulatory intelligence (RI) is becoming one of the most valuable strategic tools for goal-oriented pharma. In this article, Jürgen Hönig and Rob Williams discuss the key elements of developing a successful knowledge-management plan.

THE REGULATORY landscape is evolving at such a rate that, from the start of a project to its completion, compliance may frequently change – with new policies created and regulations adapted.

Knowledge in pharma management

From drug development through approval to marketing, having knowledge of the landscape is vital to efficiently navigate an ever-changing and increasingly challenging regulatory path. As the guidelines that govern the industry constantly adapt and change, this intelligence cannot be gathered as a one off; but instead must be continuously updated to maintain its value.

Each year, regulatory bodies issue hundreds of new guidelines and regulations globally. Staying abreast of every relevant update and

> *There's nothing more frustrating than knowing you have the information and experience required but are unable to use it in times of need*

maintaining compliance standards for each market is a considerable undertaking, but one that is of vital importance given its potential impact on everything from pre-clinical research to new drug approvals and the ongoing availability of existing products. The power of this knowledge is demonstrated during complex decision-making processes, especially development strategy and, importantly, chemistry, manufacturing and controls (CMC) maintenance. Harnessing and applying this knowledge enables re-evaluation of decisions and, once the impact of the decision has been reviewed, strategic intelligence to be garnered for future CMC maintenance decisions.

Competitive and strategic advantage

Despite its importance, RI can be highly resource intensive and therefore not considered a worthwhile investment or use of internal resources. Not only does intelligence gathering consume valuable time, but so too does its ongoing maintenance, which is essential to ensure that knowledge is up to date; and this is what poses a significant challenge for modern, goal-oriented pharma management teams.

It can be hard to justify the cost of maintaining a high level of knowledge when it is not fulfilling an immediate need. However, RI is of high strategic value to goal-oriented businesses in the most crucial times of company need and when changes occur in the wider environment. RI provides the information needed to anticipate change and influence policy. It can mean the difference between improving a product's design early in development or being faced with a series of challenges further down the line; potentially resulting in further months of costly time and investment for re-approvals, all of which could have been avoided if the ever-changing compliance requirements of regulatory bodies were efficiently monitored. Opting to outsource RI decreases the cost of investment for companies and frees up internal resource, often resulting in efficiency gains. In short, RI can provide an essential competitive and strategic advantage to pharma management, no matter the investment.

Knowledge management needs a powerful infrastructure

An efficient infrastructure to support the ongoing maintenance, analysis and management required is essential in any RI plan. This framework should provide immediate access to the most relevant, up-to-date information, making the infrastructure that supports the intelligence equally important to the gathering itself. This challenge can easily be remedied if outsourced; however, if this level of management is taken away you risk losing the ability to respond to varying needs of teams across the company. This can result in intelligence not being retrieved and the initial investment potentially wasted – there's nothing more frustrating than

BIOGRAPHY

ROB WILLIAMS is Senior Director, Regulatory Informatics and Digital Technologies, at PharmaLex. He has two decades of experience in the development of enterprise web-based solutions for global organisations. At PharmaLex he is responsible for driving the use of innovative technologies to improve regulatory processes.

REGULATORY INTELLIGENCE HAS FOUR KEY RESPONSIBILITIES:

- Gather, analyse and circulate relevant knowledge of the regulatory process
- Provide teams with bespoke advice based on information gathered
- Help with training and overarching education of internal teams
- Develop market insights to better guide future strategic decisions

> *An effective RI strategy allows for the development or management of a medicine to be taken from a reactive to a proactive process*

knowing you have the information and experience required but are unable to use it in times of need.

Modern infrastructure systems are increasingly able to rely on digital resources such as artificial intelligence (AI) and machine learning (ML) to simplify some of the biggest challenges. AI can make meeting basic regulatory obligations easier by providing advance notifications of events, updates or approvals; undertaking tasks that would ordinarily consume hours of human resource such as data extraction or entry; creating connections between siloed systems; and conducting advanced analytics that could shape future decision making. The constant flow of information within the regulatory field means that AI is proving itself particularly valuable in allowing the regulatory professional to focus their time on the more specialised tasks.

Converting knowledge to business value

To realise the added value from knowledge, pharma companies must apply insights to business operations. These need to be made available in a manageable, digestible format and then shared, used and implemented quickly for it to be a differentiator. Each team will need to access their relevant regulatory insights and knowledge resources to give them the appropriate tools to make fully informed decisions and anticipate change. This might sound obvious but is almost impossible to achieve without sufficient management and expertise.

The power of regulatory information is best demonstrated when it is applied to the value chain, which brings into account the complete list of requirements needed to deliver an effective, compliant and safe medicine that meets the evolving standards of regulatory authorities. The value chain will only be as strong as its weakest link, so to reach a successful conclusion each element needs to be managed, monitored and analysed meticulously. If RI is harnessed and applied at each stage, the links become stronger and the success of a medicine can become more assured.

Knowledge requires not only expertise, but experts

Information is power, but too much information does not lead to power if it isn't harnessed, analysed and applied properly. This requires experience. Experts within any field have more than enough knowledge required, including the

BIOGRAPHY

DR JÜRGEN HÖNIG is Senior Director, Application Services, Head of Regulatory Affairs at PharmaLex. He obtained his pharmacist training at University Heidelberg and has over 20 years' experience in regulatory strategy, regulatory operations, project management and business process optimisation. He joined PharmaLex in 1999 after several years in hospital pharmacy and academic research, mainly focused on phytochemistry and biochemical/pharmacological models for proof of concept.

BENEFITS AND CHALLENGES OF RI ACTIVITIES

LIKELY BENEFITS:

- Reduced time-to-market
- Improved data-driven decisions
- Fewer clinical studies
- Improved accuracy of submissions
- Improved policies
- Anticipating regulatory changes
- Tracking competitor activity

POTENTIAL CHALLENGES:

- Gathering and maintaining information is resource intensive
- Continuously needing to stay up-to-date
- Experience of the RI professional is highly impactful
- Managing the volume of information is time consuming
- Ability to analyse the information

> *The value chain will only be as strong as its weakest link, so to reach a successful conclusion each element needs to be managed, monitored and analysed meticulously*

experience of its implementation, the technical understanding of the field and the necessary skills, together with the ability to describe the facts in a way that gives vital context to the intelligence. Indeed, it is the expert's personal and strategic assessment that provides the greatest added value. Their personalities, specialisms, knowledge and instinct, combined with their manner of preparing relevant information, are all influential to the outcomes of the knowledge resource. Whether based internally or outsourced to an external provider, experts should not be underestimated or undervalued when establishing the importance placed on RI.

When faced with the sheer volume of information that regulatory professionals handle, requirements change from knowledge gathering to knowledge filtering. RI tools should be designed as cognitive tools, which are both useful and necessary for finding the appropriate information and providing a digital background library. This tool should be designed to evolve learning by telling, doing, and through discussion and reflection.

Move with the times

RI is not new, but the way information is being gathered and used has changed. The speed at which the regulatory environment is growing in complexity means compliance is now a very different process to that of the past. A wealth of information is readily available, can be obtained from many sources, and the resultant knowledge can be widely applied, touching on a broad range of fields and expertise. As the regulatory landscape evolves and grows in complexity, it is important that pharma companies of all sizes have access to regulatory professionals that are equipped to adapt and react, so that their regulatory knowledge can lead to intelligent regulatory affairs.

An effective RI strategy allows for the development or management of a medicine to be taken from a reactive to a proactive process; it is the tool that provides companies with the ability to pre-empt issues that may be faced, identify solutions before they arise, or eliminate them entirely. The efficiencies that can be gained from accurate and accessible RI can positively impact timelines to product launch – any platform with this level of influence should be held in the highest esteem.

Applying quality risk management to develop an isolator filling line

The Italian Biochemical Institute (IBI), Comecer and Particle Measuring Systems collaborated to construct an isolator filling line, and this webinar provided practical insights for Quality by Design principles and risk management techniques. Here are the answers to several pressing questions raised during the live stream.

How is the monitoring and classification sampling point defined?

The classification and monitoring sampling points are defined by two standards: ISO 14644-1 and ISO 14644-2. ISO 14644-1 requires the definition of the classification sampling point to be based on a table of values created using hypergeometric distribution. For monitoring, ISO 14644-2 requires a formal evaluation of a risk document to identify all areas/locations which may represent risk for the product and environmental cleanliness, and therefore require constant control.

Why is the location B1 (isolator is module B) not considered a critical control point?

The location B1 of this project is not considered critical because bags are gamma irradiated and are introduced to the isolator in secondary packaging. This packaging is decontaminated in the previous module A and transferred or stored in the B1 area, still inside the packaging to prevent any potential contamination.

Can I have more information about the FMEA and HACCP methodology?

There are several guidelines you can read to better understand the different risk assessment methodologies. The most important are ICH Q9, the FDA guideline, and PIC/S guidelines about risk assessment.

Is the product exclusive to IBI or can it be purchased?

Absolutely, subsequent to submission/approval, we are open to offer this innovation to anyone who is interested.

How was the VHP (H_2O_2) cycle designed? Did you just evaluate the microbial log reduction?

To validate the hydrogen peroxide cycle, a minimum of 3-log contamination reduction must be demonstrated. However, while developing a similar nonstandard application, it is extremely important to also evaluate the coverage of VHP fumigation among the whole isolator area.

Do you have a recommended method to evaluate the coverage of VHP?

As an example, in an early development stage we used coloured media, then we tested with chemical indicators before finalising our validation with bio indicators.

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BIOPHARMA PROCESSING & DEVELOPMENT

Biopharma developers and manufacturers need to know their molecules and understand their manufacturing processes better than ever before. In this article, Mike Wilson, Product Manager at Waters, discusses the benefits of mass analysis in biopharma and the current challenges of adopting new technologies.

An important step in the research and development of biopharmaceuticals is to identify molecules with favourable physical and chemical stability profiles. Yongchao Su and Wei Xu from Merck & Co and Bruce Yu from the University of Maryland School of Pharmacy discuss how solution, solid-state and benchtop nuclear magnetic resonance (NMR) methods can provide advanced biophysical characterisations of biological products.

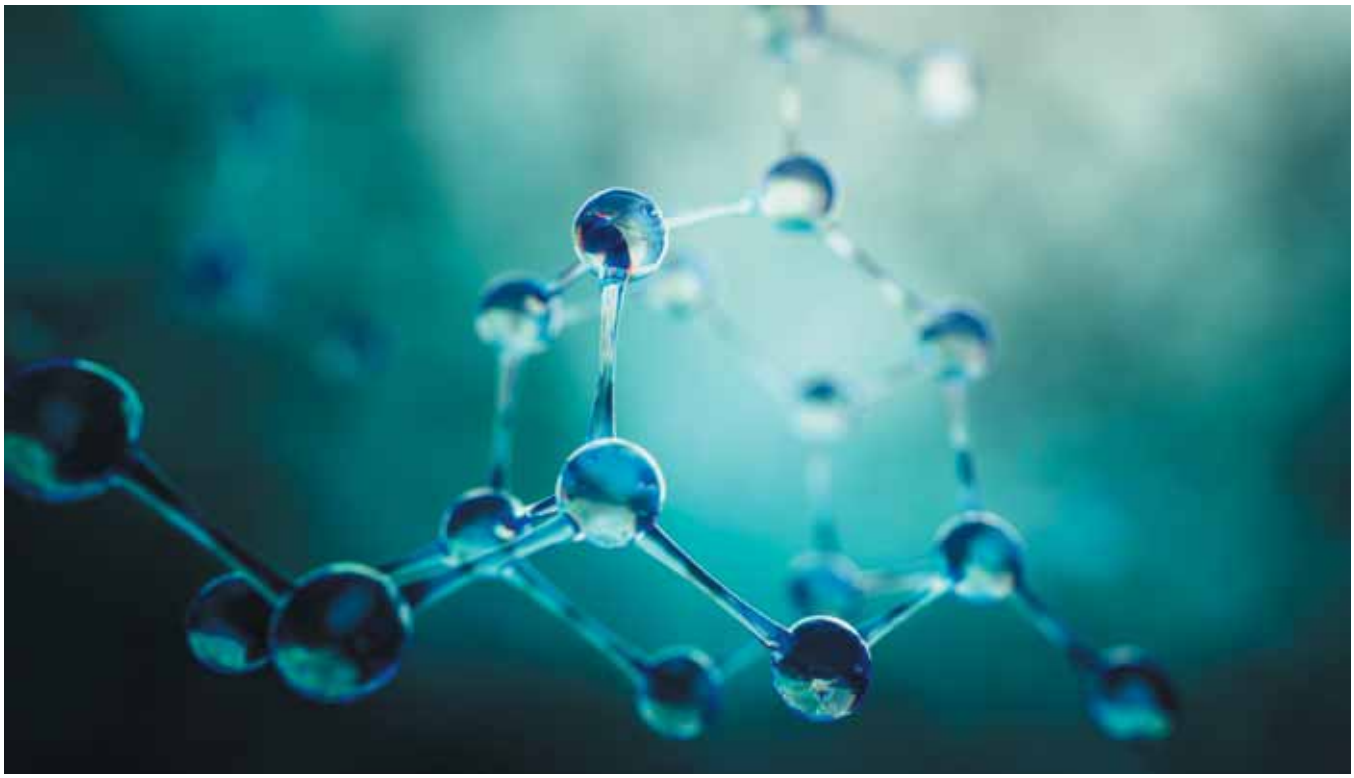
There are many business challenges to developing a biologics drug, one of which is effective data management. In this article, Unjulie Bhanot, IDBS, focuses on efficient data management policies and systems, and how they could improve biologics product development processes.

Routine mass analysis enables greater productivity in biopharma development

Mike Wilson

Product Manager, Benchtop TOF MS, Waters

Biopharma developers and manufacturers need to know their molecules and understand their manufacturing processes better than ever before. In this article, Mike Wilson discusses the benefits of mass analysis in biopharma and the current challenges of adopting new technologies.



IN THE biopharmaceutical industry, the cost of failure – or not failing fast enough – can be high. Recently, a large biopharma company reportedly paid a termination fee of around $155 million to their partner after halting commercialisation of a biosimilar. Three years after initiating their commercialisation project, the company cited the cost of manufacturing the drug as too high and its projected selling price too low to justify bringing it to market.[1]

Here, in a nutshell, is the challenging reality for biopharma today. Not only must companies grapple with increasingly complex large molecules and regulatory requirements that are becoming more stringent, they must also find ways to develop, manufacture and analyse these molecules in a more efficient and cost-effective manner.

Regulatory bodies around the world are continuing to update their guidance for the industry with more exacting requirements for analyte monitoring in development and QC.[2] Both the US FDA and the European Committee for Medicinal Products for Human Use have recently rejected biologic license applications (BLAs) due

"Better testing makes for better understanding and for the biopharma industry that will mean making better techniques more accessible"

to insufficient data concerning overall quality, impurities and stability.[3,4]

Knowledge and understanding

The message is clear: biopharma developers and manufacturers need to know their molecules and understand their manufacturing processes better than ever before. Yet the volume of attribute testing for large molecules is already high – roughly five to 10 times that used for small molecules.[5] Adding more testing to this load does not in itself result in better analysis and understanding, and it certainly won't help these companies to be more productive. Instead, better testing makes for better understanding, and for the biopharma industry that will mean making better techniques more accessible. Gold standard techniques, such as liquid chromatography-mass spectrometry (LC-MS) analysis, must become routine.

LC-MS analysis offers the ability to directly measure multiple molecular properties/attributes simultaneously, often with greater sensitivity and selectivity than can be achieved by more traditional assay techniques. Highly trained MS specialists, who have spent years learning the relevant instruments and techniques, carry out upstream "characterisation" work for biologics through which a quality target product profile (QTPP) for each drug candidate is established.

QTPPs typically include a full array of molecular attributes that should ideally be monitored throughout ongoing development work, including analytical method development and optimisation, clone selection, process development, formulation development and stability testing; in keeping with Quality by Design (QbD) principles.

Through extensive development testing, a subset of these attributes is then defined as critical quality attributes (CQAs). If altered, these attributes could potentially impact the purity, safety and/or efficacy of the final biotherapeutic drug product. Broader access to LC-MS analysis for monitoring of product attributes throughout late-stage development, manufacturing and even QC would not only enable better, more precise data to be captured, but it could also be carried out more efficiently.

Future promise

LC-MS's great promise for routine monitoring begs the question: just when will it become a true bedrock analytical tool for scientists and technicians throughout biopharma?

As development and therapeutic use of biologics grows, expanding access to high-powered LC-MS analysis – in other words, making LC-MS monitoring routine – will increase in importance.

As that importance grows, biopharmaceutical laboratories must increasingly explore how to use techniques such as multi-attribute monitoring (MAM) and LC-MS assays to monitor the CQAs of biologics during drug development. MAM assays offer the promise of detecting and measuring multiple CQAs simultaneously, which can streamline analytical workflows that have previously taken place in parallel, time-consuming processes.

BIOGRAPHY

MIKE WILSON achieved his PhD from the University of York, UK, where he developed innovative MS approaches for investigating the structure and fate of photosynthetic pigments in living organisms and historic environments. He then worked in the pharmaceutical industry as a characterisation specialist, where he developed his interest in the application of MS for creative problem solving, as well as coaching others in this approach. Mike has held roles within Service and Product Development at Waters, prior to his current role in Product Marketing.

"The biggest challenges to wide adoption lie in overcoming the high costs and complexity of operating and maintaining these advanced tools in regulated laboratory environments"

Two MAM approaches for biotherapeutic analysis are commonly implemented today:

1. Analysis of monoclonal antibody (mAb) subunit mass
2. Analysis of peptides from a protein digest – a peptide-mapping workflow.

Subunit mass MAM assays can be limited in their ability to monitor small mass changes, such as deamidations, and they are unable to generate site-specific information when multiple modifications are located on the same subunit fragment. In these situations, peptide-based analyses are viewed as complementary workflows.

High-resolution MS and liquid chromatography quadrupole time-of-flight (LC-QTOF) MS peptide mapping methods are indispensable for in-depth protein characterisation during biotherapeutics development, whereas optical detection is widely used for peptide mapping identity tests during QC product release.

Recently, LC-UV-MS peptide mapping methods have been developed for product identity release assays, taking advantage of the selectivity and specificity offered by MS detection.

The key benefit of a peptide mapping MAM approach is that it offers site-specific direct assessment of product quality attributes, and this has received significant interest in recent years. The following are further examples of its application:

- Amgen has been pioneering the deployment of a peptide mapping multi-attribute methodology to streamline biotherapeutics development and QC testing. In a 2015 publication, Amgen researchers described their efforts to monitor multiple product attributes for characterisation and process development using high-resolution MS analysis and shared their vision of a multi-attribute methodology workflow that can greatly reduce the number of assays needed during process development and quality control.[6]
- Roche Diagnostics published details of a peptide MAM approach for high-throughput sample preparation along with LC-MS analysis for quantification of deamidation, isomerisation, oxidation and glycosylation.[7]

In both studies, comparable results were obtained using a peptide mapping MAM method as with conventional optical detection assays. However, peptide mapping MAM using the highly complex research-grade mass spectrometers and informatics tools demonstrated in these studies could be quite challenging to deploy by regulated late development and QC laboratories.

In a recent internal survey of biopharmaceutical scientists, nearly 70 percent of respondents indicated that they expected to see a significant increase of MS utilisation in regulated development and QC in the future. However, they believe the biggest challenges to wide adoption lie in overcoming the high costs and complexity of operating and maintaining these advanced tools in regulated laboratory environments, and ensuring consistent, robust performance – as well as addressing data integrity and compliance.

Many biopharmaceutical companies are working on developing analytical platform solutions that support routine biopharmaceutical analyses and that can transcend the traditional silos of the discovery, development and manufacturing/QC pipeline. As such, fit-for-purpose deployment, ease-of-use and compliance are all factors that should be considered early on when adopting MAM methods.

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Emerging NMR techniques as advanced tools for characterising biological parenteral products

Yongchao Su and Wei Xu
Merck & Co

Bruce Yu
University of Maryland School of Pharmacy, Baltimore, United States

An important step in the research and development of biopharmaceuticals is to identify molecules with favourable physical and chemical stability profiles. Yongchao Su, Wei Xu and Bruce Yu discuss how solution, solid-state and benchtop nuclear magnetic resonance (NMR) methods can provide advanced biophysical characterisations of biological products.

BIOGRAPHY

YONGCHAO SU, PhD is an Associate Principal Scientist in Pharmaceutical Sciences at Merck & Co, Inc., US. He received postdoctoral training at Massachusetts Institute of Technology in 2011-2014 after his PhD research at Iowa State University in 2011. He works as the analytical lead on physicochemical characterisations of solid dosages and biophysical and biochemical characterisation of biologics. His research focuses on developing and utilising NMR techniques for mechanistic investigations of membrane permeability in drug delivery and structural characterisations of small and large molecule formulations.

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LARGE MOLECULES can exhibit complex structural behaviours due to their flexible molecular properties. An important step in the research and development of biopharmaceuticals is to identify molecules with favourable physical and chemical stability profiles, as these attributes are critical to the efficacy and safety of a drug product.[1] In parenteral formulations, the conformational flexibility of proteins can result in different intra- and intermolecular contacts and sometimes trigger physical aggregation and chemical degradation. Thermal and mechanical stress encountered during the manufacturing processes, transport and storage of the finished products often introduce additional stability risk. Biophysical characterisation plays an important role in the development and risk assessment of protein formulations. A wide array of analytical methods is necessary to probe the different aspects of the formulation, ranging from the molecular conformation to the higher order structure and the aggregated species. High-resolution characterisation tools are often required to determine structure, probe dynamics and study interaction in biological products. Particularly, NMR methods have emerged as a powerful set of techniques for biophysical characterisation (***Figure 1***).

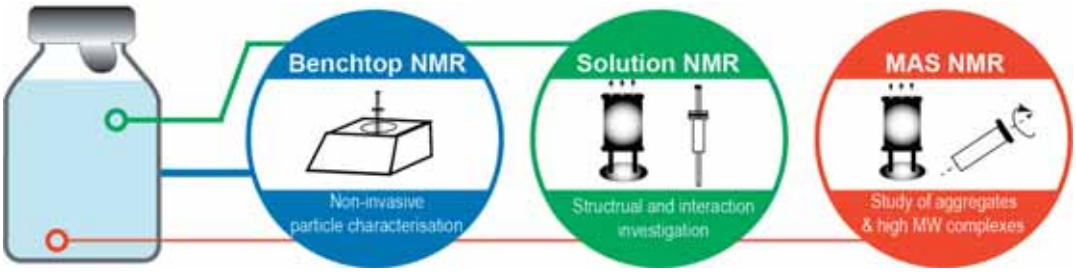
High-field solution NMR as a versatile tool for in-depth biophysical characterisations

From a microscopic point of view, physical and chemical stability is governed by the structure of the molecular species in a biologics formulation. NMR characterises structures from Å to µm size and molecular dynamics from picoseconds to seconds, covering almost a full range of molecular behaviours in biologics products.[2] Compared to conventional tools, NMR is a versatile technique for investigating chemical and molecular structure, dynamics, and interaction at a high resolution and sensitivity.

In recent years, solution NMR has been explored by academia, biopharmaceutical industry and regulatory agencies to analyse biologics products. Successful examples include characterisations of physiochemical properties of biologics and mechanistic investigation of stabilising and destabilising interactions in parenteral products. For example, monoclonal antibodies (mAbs) represent the largest category of protein therapeutics on the market. One-dimensional (1D) NMR has been used extensively to identify and quantify the stability of mAbs.[3-5] It has facilitated investigation of structural and hydrodynamic profiles, the effects of excipients on self-association, and conformational change associated with aggregation in mAb formulations. Two-dimensional (2D) NMR methods enable examination of higher-order structure (HOS); describe the secondary, tertiary and quaternary structures; and probe the critical molecular properties of mAbs.[6-8] Fingerprint spectra can provide a sensitive spectroscopic identification and comparison on quality attributes of the drug substance over the time course of formulation

> *"Compared to conventional tools, NMR is a versatile technique for investigating chemical and molecular structure, dynamics, and interaction at a high resolution and sensitivity"*

FIGURE 1



LEFT: Advanced NMR techniques for characterising biologics formulation

development. Protein-excipient interactions have been studied to probe the mechanism of formulation instability at the molecular level[9] and screen stabilising excipients.[10] A significant number of NMR studies have focused on protein aggregation. For example, the particle size distribution is a critical quality attribute of drug products. Diffusion ordered spectroscopy (DOSY) NMR measures diffusion coefficients, which can derive the hydrodynamic radius for quantifying size distribution.[11] DOSY-NMR can also simultaneously report the particle size of the drug substance and excipients and identify the protein-excipient complex in the formulation. Moreover, extensive efforts are made toward the mechanistic investigation of protein aggregation in studies of amyloid diseases.[12] The characterisations of prion protein oligomer, misfolding and effects of temperature, pH, metal ion and mechanical agitation in these studies offer opportunities for in-depth biophysical characterisations of large molecule formulations.

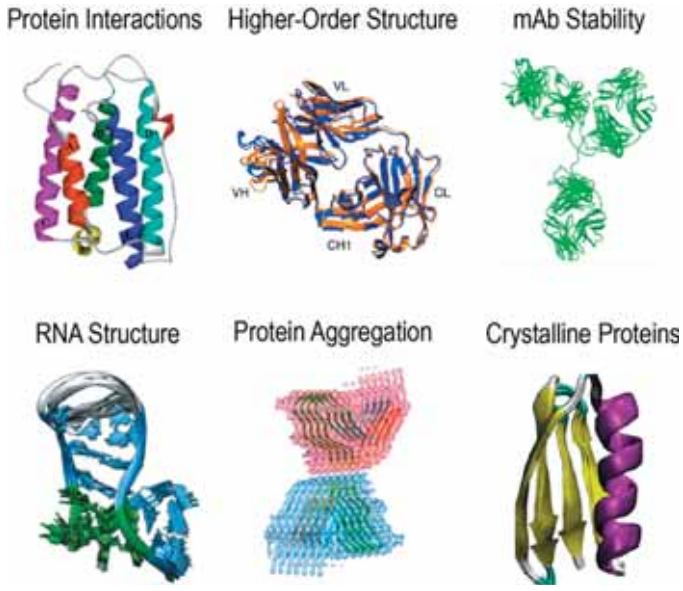
Solid-state NMR investigation of large biotherapeutic complexes and insoluble aggregates

In recent years, solid-state NMR (ssNMR) has been utilised to characterise a wide range of pharmaceutical materials including amorphous and crystalline dry powder, soft tissue specimen, fully-hydrated lipid systems, gel-like samples and insoluble protein complexes. In ssNMR experiments, samples are rotated at an angle (so-called magic angle spinning, MAS) with respect to the external magnetic field to average chemical shift anisotropies and dipolar interactions for narrower linewidth. As a cutting-edge technique, ultrafast MAS can spin up to 150kHz to generate ^{1}H ssNMR spectra with significantly enhanced sensitivity and resolution. Another revolutionary technique, dynamic nuclear polarisation (DNP), can boost the ssNMR sensitivity by transferring high polarisation from electrons. Proton-detected techniques and DNP-enhanced ssNMR have opened new avenues in biopharmaceutical applications.[13] Many large protein complexes and insoluble aggregates experience slower molecular dynamics and thus fall into the motional regime for MAS NMR to study. Existing applications include large protein assemblies, RNA structure, fibrils and crystalline proteins (***Figure 2***). Taking protein aggregations, for example, insoluble particles represent a significant risk for biological formulation developments. Glucagon or glucagon-like peptides exhibit a strong tendency of fibrilisation and consequently physical instability. MAS NMR has been demonstrated as a powerful tool for structure determination of disease-related amyloid fibrils.[14,15] This has immediate applicability for investigating insoluble aggregation of biological drugs. The goal is to solve the structure of undesired aggregation and identify the mechanistic cause of fibrillation. This approach can guide the rational design of the molecular motifs to improve the inherent stability of the development candidates. Besides insoluble aggregates, multidimensional

BIOGRAPHY

WEI XU, PhD is a Director of Preformulation at Merck & Co, Inc., US. She received her PhD in Medicinal Chemistry from Purdue University. She has over 20 years of drug development experience spanning from discovery support to late stage product development. She has led multiple cross-functional teams to advance new therapeutic molecules and pursue product value enhancement opportunities. Her research interests include materials and biophysical characterisation, predictive dissolution and novel formulation technologies.

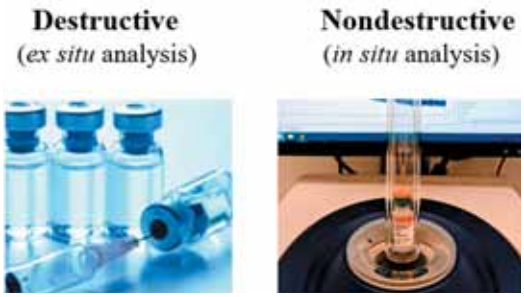
FIGURE 2



ABOVE: Solution and solid-state NMR characterisations of biopharmaceutical interesting molecular behaviours including protein-protein and protein-excipient interactions, RNA structure, higher-order structure (HOS), mAb stability, protein aggregation and crystalline proteins

RIGHT: Benchtop NMR as a nondestructive tool for drug product characterisation

FIGURE 3



BIOGRAPHY

BRUCE YU, PhD is a Professor of Pharmaceutical Sciences and Director of the Bio- and Nano-Technology Center at the University of Maryland School of Pharmacy. Dr Yu has conducted research on proteins, imaging agents and biomaterials. His current focus is on nondestructive analytics for biologics and complex drug products, including biomanufacturing. He received the 2004 Kimmel Scholar Award and the 2005 US Presidential Early Career Awards for Scientists and Engineers. He received a PhD in molecular biophysics from Johns Hopkins University.

MAS NMR is a primary tool for studying large protein complexes[16] and can be employed for characterising protein-excipient and protein-protein assemblies in the formulation. Moreover, MAS NMR has also been used to determine RNA structures[17] and shows great potential for investigating RNA-excipient complexes. In addition to the structural events in the sterile formulation, biological crystals present a new category of large molecular drugs. Bulk crystallisation provides one strategy for purification and requires solid-state structural analysis. Ultrafast MAS applications of micro- or nanocrystalline proteins have demonstrated successful examples of characterising large molecule drugs in the crystalline form.[18]

> *"Benchtop NMR presents a nondestructive biophysical tool for accessible and quantitative analysis, particularly at the drug product level"*

Time-domain benchtop NMR for probing biologics products

While high-field NMR techniques, whether solution or solid state, are apt to provide in-depth biophysical characterisations, they have limitations – such as expensive equipment with a large footprint, sophisticated and time-consuming operations, and high skillset requirements. Benchtop NMR presents a nondestructive biophysical tool for accessible and quantitative analysis, particularly at the drug product level. In the solid state, benchtop NMR has been used to analyse content uniformity of tablets[19] and powder weight in vials.[20] In the solution state, the water proton NMR signal has been used to detect mAb aggregates,[21,22] insulin[23] and aluminium adjuvant[24] filling levels. As illustrated in ***Figure 3***, benchtop NMR also exhibits great potential for quantitative analysis of aluminium-adjuvanted vaccines. Sedimentation of heterogeneous micron-sized particles in vaccines poses challenges to manufacturing. Benchtop $1H_2O$ NMR can potentially serve as an in-line tool to monitor aluminium adjuvant suspensions without need of optical transparency or physical contact.

Conclusion

Solution, solid-state and benchtop NMR methods provide advanced biophysical characterisations of biological products for quantitative assessment, structural characterisation and mechanistic investigation.

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Creating a modern landscape

Unjulie Bhanot

Solutions Consultant, IDBS

There are many business challenges to developing a biologics drug, one of which is effective data management. In this article, Unjulie Bhanot focuses on efficient data management policies and systems, and how they could improve biologics product development processes.

BIOGRAPHY

UNJULIE BHANOT is a UK-based Solutions Consultant at IDBS and has worked in the biologics R&D informatics space for over five years. Unjulie holds a BSc in Biochemistry and an MSc in Immunology, both from Imperial College London. Since joining IDBS in 2016, Unjulie has been responsible for designing and deploying informatics solutions for biologics-based organisations within Europe. In 2017, she took on a leading role in the development of the IDBS bioprocess solution. Prior to joining IDBS, Unjulie worked as an R&D scientist at both Lonza Biologics and UCB, and later went on to manage the deployment of the IDBS E-WorkBook Platform within the analytical services department at Lonza Biologics in the UK.

BIOLOGICS therapies, in the form of monoclonal antibodies and recombinant proteins, have proven success in the medical market with six out of the global top 10 treatments taking this form in 2017.[1] However, the development journey of a biologics drug to its final destination in the market can take up to 12 years;[2] a journey that always comes with its own scientific, operational and technological challenges.

Biopharma organisations are also under pressure to reassess their development strategies; factors such as the rise in scientific advances and changing therapeutic requirements are amplifying this pressure. Such organisations must ensure they benefit from the latest scientific and process innovations to shorten their time to market and release relevant and targeted molecules – all while adhering to the growing list of regulatory and compliance requirements and government policy reforms. Players in the space must also compete with the shift of drug development to growing markets, such as India and China, and their ability to support lower value markets.

Consequently, these large biopharma organisations are looking to partner with technology firms to leverage their talent to support the digital therapeutic market. Examples include the partnering of Pfizer and IBM to deliver technology that performs real-time monitoring of the symptoms of Parkinson's,[3] UCB and MC10 creating sensors that monitor key parameters to develop therapies for neurological disorders,[4] as well as Biogen and Verily (Google's life sciences arm) using sensors and software to study the biological and environmental factors contributing to multiple sclerosis.[3]

In-vitro companion devices are also being implemented in combination with drug development, to gauge which patient cohort will most likely benefit from the therapeutic biological product in development and identify those patients most likely to be at increased risk of non-beneficial side effects.[5] By using these technologies in tandem, organisations can target the right therapeutic areas and make decisions early on about whether to develop a molecule for a specific disease.

Tackling the business challenges

Owing to this combination of factors, organisations rely on four key areas to help support the development of a drug and overcome the business's challenges:

- Science and technology
- Resources and project planning
- Outsourcing and collaboration
- Business-driven compliance policies and procedures.

In the lab, these tools are tied together by the thread of data and information – such as performing scientific data analysis to understand the impact of a given instrument; using information from personnel training to plan experiments; or managing sample transfers based on release data.

Furthermore, organisations require both top-level and detailed views of their data to make informed decisions about the correct biological formation and purity of the drug, its efficacy and potency, and the impact of supplementary conduits and processes. Organisations also need an overall view of the development strategy and its success.

For data to become consumable information, context is critical. The ability to piece together that context, determine what data should be used before extracting relevant data, and compile/aggregate the pertinent data is critical. This requires efficient data management policies and systems.

Figure 1 depicts daily activities that scientists may encounter, in which data is either created, utilised, manipulated or managed, with most emphasis given to the scientist's key role of executing the science.

Current data management in labs

Inept management of any of these tasks has consequences for the business – be they only

small oversights that are immediately remediable. Whether the gap takes five minutes to close or a few hours, it's important to acknowledge that the impact extends to the overall business and could cause incremental damage.

Imagine a scenario where an instrument fails its calibration, but this data point is not recorded. Many people then use the instrument and their experiments fail; the mistake only being caught several experiments down the line and perhaps only linked to the instrument after much work has been reviewed. In this scenario, time has been lost, rework has been triggered and may also have exhausted reagent stock, project timelines are delayed and it could cause a compliance failure where experimental results have been used in GMP.

In an everyday scenario, the combination of these activities generates lots of data that is acquired or recorded, processed and analysed, stored and then disseminated. To follow the path of this data, and understand the relationship between the data points, scientists must assimilate process data with parameter data, together with experimental results.

While evidencing the science performed, scientists may also be expected to duplicate the same metadata across different systems.

FIGURE 1

LEFT: Activities a scientist may encounter in which data is either created, utilised, manipulated or managed

Additionally, with the deployment of instrumentation of the modern-day lab, such as high-throughput systems (HTS) or process analytical technology (PAT) tools, scientists are required to be proficient in drawing information from the reams of

"Players in the space must also compete with the shift of drug development to growing markets, such as India and China"

data these systems can yield.[6] To achieve this, they must master the skills needed to decide which sets of data are most relevant, provide the most insight, and should be moved forward for analysis and report compilation. Many R&D organisations are making the investment to ensure they either hire or train personnel to be confident analysing large volumes of data.[7]

Take a reasonably common example in screening analysis: each well in a 96-well plate may generate an image of 2MB (~200MB per plate), extend this across five time points and suddenly the volume of data jumps to 1GB. Now extrapolate this across 100 plates, across five time points, and 100GB of data from a single experiment can become difficult to manage. The scientist may also need to determine whether to analyse all wells across all timepoints across all plates.

There may also be some numerical calculations associated with these wells (absorbance values, concentrations, etc), what's their format? Can this information be extracted and consumed? Most importantly, are we able to reconcile the image with its corresponding numerical data, with the context of knowing what was in the given well on the defined plate? We can start to see how unmanageable the volume of data can be; and this does not even address the storage challenge.

Designing an effective data management strategy

An ideal solution need not be a complicated one; however, it should be purposeful, and its role and position should be fully defined. The strategy designed should serve both the scientists and the overall organisation.

It is here where seamlessly integrated systems hold most value – by creating and understanding the full laboratory and organisation landscape of all the moving parts, dependencies, human interventions and, most importantly, data collection and handoff points. Software can be strategically utilised to facilitate smoother data transaction, thus reducing the burden on the scientist while maintaining data integrity.

Systems that permit data to be recorded vicariously as part of performing a procedure and do not need manual duplication, will be the least burdensome on scientists. This will allow them to focus on their core work and for organisations to make an impact on both their returns and main business goals. Equally, systems that can communicate with one another without requiring human mediation and can streamline data transfers, will enhance reliability of the data in question.

When creating the 'landscape of the lab', organisations should map out the journey of the data. What purpose does it serve, what question is it trying to answer, who needs to consume the data, and, inevitably, is the quality of the data sufficient to validate the journey of the biologic through its development? Businesses may wish to consolidate business metrics with the reporting of scientific outcomes; for example, what was the duration of a particular stage of work, and was there an impact on its success or failure corresponding to the resources available?

Ultimately, organisations aim to deliver novel, high-quality therapeutics to patients faster and more cost effectively. Therefore, it is critical to understand the collective workforce (personnel and instrumentation) that contributes to this pursuit.

Considering our assessment of a day in the life of a scientist (***Figure 1***), it is clear to see the impact an integrated platform that allows data to be recorded in a consistent, structured manner and connects different factions of the workflow could have. Put simply, organisations would be better supported in the current competitive R&D landscape with their endeavour to bring their biologic to patients faster, with a system that could accurately calculate the length of time an experiment will take when scheduling work, or could track and store sample metadata in tasks managing sample testing.

What about a system that could manage the compliant use of instruments as well as their output? Or one that could ensure that the activities performed to support compliance could be an automatic outcome of users entering data in their experiments?

Even with these few examples, it is easy to see how the deployment of an enterprise-ready platform, specifically designed to support the biologics data workflow, could form the core of an effective data management strategy, empower businesses to make better decisions with improved product and process insight, shrink reporting timelines and expedite seamless data dissemination.

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Potential impact of Brexit outside the UK/EU

Paul Ranson

Consultant at global law firm, Morgan Lewis

There is hardly a shortage of views, forecasts and estimates of the impact of Brexit – hard, soft or otherwise – on the UK and EU generally, and on life sciences in particular; but less thought has been given to potential ramifications further afield, whether for developing or mature countries. In this article we touch upon some of the possible impacts, whether at geopolitical or life sciences-industry levels, with a focus on the Association of South Eastern Nations (ASEAN) region.

SIMON JOHNSON, former chief economist of the IMF, in a January 2019 article headlined "Brexit Does Not Matter", concluded that whilst Brexit may have an impact on British growth, it will not cause significant disruption to regional, let alone global, trade. In his view, the global economy's current uncertainty is due to a far greater extent to the political climate in the US.

However, beyond the level of global trade, some commentators take a broader view. In the aftermath of the UK referendum, Indonesia's trade minister at the time, Thomas Lembong, stated that he considered Brexit to be, not only a 'wake-up call' for the EU but also for ASEAN (Malaysia, Indonesia, Thailand, Philippines, Singapore, Brunei, Vietnam, Laos, Cambodia and Myanmar). He said that "Transnational unions cannot be allowed to become a project of the elites... We are failing to bring along our own people on the benefits of globalisation and international trade, even of international finance." He concluded

> *"Currently, the UK's withdrawal from the EU will deprive it of the benefits of existing Free Trade Agreements between the EU and non-European countries"*

that ASEAN could take lessons from Brexit on identifying and addressing the challenges of regionalism in the face of national priorities.

Conversely, some have looked at Brexit and concluded that any damage it – and the developments in other countries such as Italy, Greece and Hungary – does to the EU in reversing the 'ever closer union' logic, will benefit other regions such as ASEAN. It has been argued that the effect of the UK's exit from the EU might be to bolster ASEAN's desirability, centrality and its influence on potential trade and strategic partners.

Currently, the UK's withdrawal from the EU will deprive it of the benefits of existing Free Trade Agreements between the EU and non-European countries. For instance, the EU and Singapore have just negotiated and (in October 2018) signed a Free Trade Agreement intended to improve trade for goods such as pharmaceuticals. It is also negotiating deals with Vietnam, Indonesia and Malaysia. These would need to be renegotiated by the UK in the event of its departure from the EU.

Europe's international life sciences support

The European Commission, through the European Medicines Agency (EMA), is highly regarded internationally and has assumed a global role in seeking to assist less well-resourced regulatory agencies. For instance:

- Under Article 127 of Directive 2001/83/EC (on medicinal products for human use), the EMA issues certificates of medicinal products, based on World Health Organization recommendations, on behalf of the European Commission scheme to any country outside the EU. Such certificates confirm the marketing authorisation status of products and the good manufacturing practice (GMP) compliance status of the manufacturing site(s).
- The Agency also certifies products under Article 58 of Regulation (EC) No. 726/2004 (on the centralised procedure) by way of a scientific opinion, for the evaluation of certain medicinal products intended exclusively for markets outside the European Community. Such opinions are drawn up by the Committee for Medicinal Products for Human Use (CHMP), after consultation with the WHO, following a review of the quality, safety and efficacy data, analogous to the review undertaken via the centralised procedure.
- On a broader level, the EMA also plays a key role in harmonisation of worldwide regulatory standards. It is one of the three agencies that make up the International Conference on Harmonisation.
- The EMA and many other EU national medicines regulatory authorities are involved in the Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme (PIC/S). This is an international cooperation between pharmaceutical inspection authorities in the field of GMP. The PIC/S develops international GMP standards and quality systems of inspectorates in human and veterinary medicines and assesses national inspectorates and facilitates cooperation between national regulatory authorities. In practical terms, this means that if a country joins PIC/S they will recognise GMP inspections and assessments carried out by other PIC/S member countries, without the need for another inspection.

Several ASEAN countries are among the 72 countries that have authorised medicines evaluated through the Article 58 process. Indonesia, Malaysia, Singapore and Thailand also belong to PIC/S.

Questions have arisen in the light of the well-publicised teething problems of the EMA's move from London to Amsterdam and the likely loss to the EMA of the resources of the UK Medicines and Healthcare products Regulatory Agency (MHRA). The MHRA is reported to play a bigger role than any other national agency, carrying out some 30 percent of EMA assessments, vigilance and licensing work. However, whilst the EMA acknowledges that its role at an international level, such as on the harmonisation of global medicine regulation, will be temporarily scaled back to a more reactive level, it has stated that it

BIOGRAPHY

PAUL RANSON is a consultant at Morgan Lewis who focuses on the regulatory and commercial needs of the pharmaceutical, biotechnology and medical devices sectors. Paul's regulatory experience covers both marketing authorisation-related matters and market access, pricing and reimbursement issues. His commercial work is concentrated on transactions with a high degree of industry specificity including collaborations and outsourcing transactions.

will continue to process product-related requests and supply-chain integrity and procedures under Article 58. The suspension and scaling back of work is expected to last until 30 June 2019, but a decision will be taken this month as to when a full programme of work can resume.

Some effects for third countries of the UK's departure

The position of the European Commission is that any marketing authorisation or other licence, which is a legal requirement under EU laws held by a UK-based entity, will require a transfer to one based in the EU27. Similarly, any UK holder of a regulatory role such as a responsible person for manufacturing or pharmacovigilance purposes, or a responsible person for distribution purposes, must be replaced by an entity based in the remaining states. Additionally, the EU Pharmacovigilance System Master File (PSMF) must reside within an EU Member State.

> *"Some argue that Brexit substantially weakens the UK's position in global trade"*

Moreover, the current system, whereby the batch release of a product imported into the EU may take place in any of the EU28, will no longer apply within the UK. It will therefore make little sense for an importer of drug product into the EU to batch release in the UK. Instead, a separate batch release would be necessary for the EU27, and then again for the UK.

With medical devices, under the Medical Device Directives (eg, 93/42/EC) and the new Medical Device Regulations ((EU) 2017/745 and 746), a manufacturer not based in the EU is required to have an authorised representative located inside the EU. In addition, Notified Bodies, charged by the Commission with awarding CE-marking to approved devices, is dependent on EU location. Accordingly, those manufacturers using UK-based Notified Bodies will have to transition to Notified Bodies based within the EU27.

The free flow of medicinal and medical products between the UK and the EU27 would also end and third-country importers would need to deal with different, albeit possibly mirrored, requirements for the UK and the rest of the current EU.

As to the acceptability of UK medicines and devices for import; that would be a matter for local regulatory authorities, although it is worth noting that the MHRA is commonly accepted under the Article 58 procedure as an appropriate reference authority.

The future for third-country trade with the UK

The UK is the third largest biopharmaceutical cluster outside the East and West Coast of the United States. Its leading universities have excellent intellectual capital for third countries seeking to develop medicinal products and medical devices, making it unlikely that the research base will wither any time soon.

However, from a trading perspective, Brexit fundamentally alters Britain's relations with trading partners outside Europe. There are contrasting positions regarding the impact of Brexit on trade between the UK and the rest of the world, which might be said to encapsulate the 'Remainer' and 'Brexiteer' perspectives.

Some argue that Brexit substantially weakens the UK's position in global trade. The country would cease to enjoy the collective bargaining power of the EU and would stand alone in trade disputes before the World Trade Organization, due to the fact that, at present, withdrawal from the EU necessitates the UK's withdrawal from the European Union's common external tariff regime.

Others consider that Brexit strengthens the international trading position of the UK, allowing it to enter bilateral trade agreements with the rest of the world, rather than having to accept trade deals negotiated by Brussels. In particular, this camp would point to deals with China and India, stalled by the agricultural and other protectionist lobbies.

Only time will tell who has correctly interpreted the future but at the time of writing, the UK Government and Parliament continue to wrangle as to whether to pursue a soft Brexit (continuing to be closely tied to the EU) or a 'clean break' (leaving without any deal with the EU). The coming hours, days, weeks, months or even years may see a resolution.

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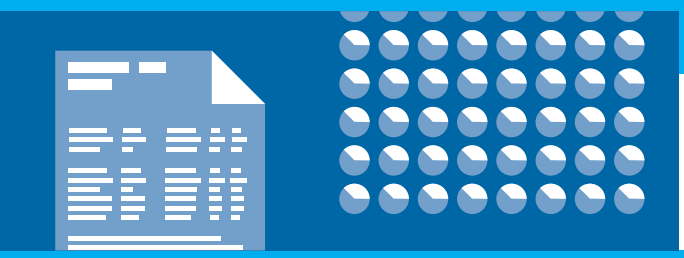

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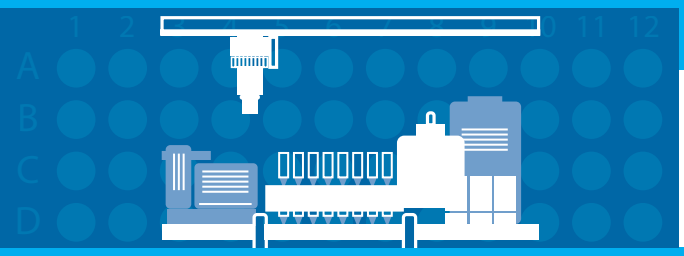

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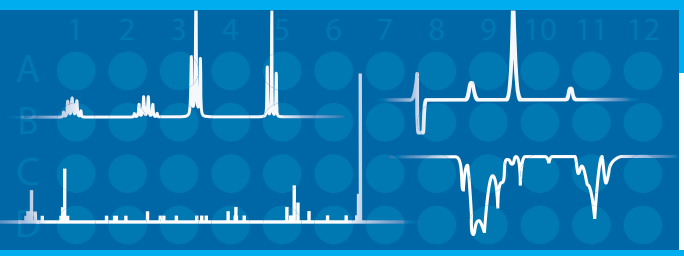

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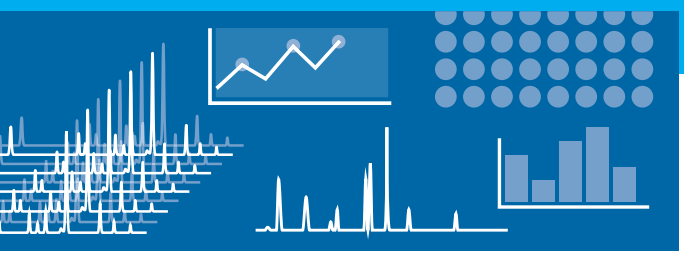

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DESIGNED to address the challenges still faced by scientists in high-throughput synthesis, Katalyst D2D helps ensure data integrity in high-throughput experimentation for reaction optimisation, process development, catalyst screening and scale-up. Katalyst's digital environment eliminates the manual transcription currently necessary between systems, as well as the painstaking process of manually gathering and interpreting all the associated analytical data. This transfer of tedious but necessary administrative tasks to a chemically-aware software application gives scientists more time for reaction development. Katalyst also automatically assembles live analytical and chemical information for entire studies in a single interface, providing scientists with results that are conveniently connected to each experiment for fast and effective decision-making.

Katalyst D2D supports the entire workflow of high-throughput experiments from Design to Decide, in a single user interface.

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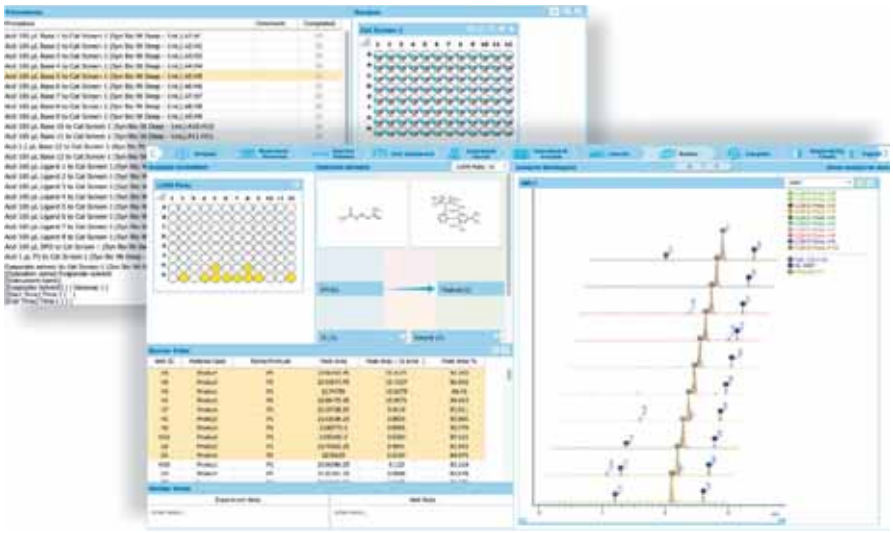
Plan – physical planning of experiments is made easy through integration with internal and third-party inventory systems, and access to the reaction vessels and hardware is available. The software enables the user to specify the physical arrangement of experiments, physical layout of materials to be dispensed, amounts, operations, procedures and parameters. The final sampling and analysis of experiments is also planned from the outset.



Execute – integration with lab automation hardware and lab execution systems means you no longer need to transcribe information from one system to another. The capability to create instruction lists for steps that require manual effort not only provides a timesaving but also further ensures data integrity. Analyses during, or at the conclusion of, a reaction may also be executed regardless of technique (eg, LC/UV/MS, NMR, IR) and instrument vendor.

Analyse – analysis of parallel experiments (often the bottleneck of high-throughput experimentation) may be expedited through automation or carried out manually. Analytical results are automatically associated with each individual well in an array to maintain data integrity and enable efficient decision-making.

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By streamlining high-throughput workflows, organisations are able to optimise the efficiency of laboratories, the analysis of array-based chemistry, and the return on investment in high-throughput robotic equipment.

Katalyst D2D is the application of choice for efficient, collaborative experimentation in array-based and parallel chemistry, from Design to Decide. ■

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Annex 1 revisions: Let's talk TOC and conductivity requirements for pharmaceutical water systems

In this webinar, Daniel Kellner-Steinmetz and Lukas Swanson from SUEZ address the requirements for continuous monitoring of water for injection (WFI) systems and how this can be achieved with total organic carbon (TOC) and conductivity. It also covers cold WFI production according to European Pharmacopoeia and how it applies to water systems as drafted in Annex 1. Here, the team answer some pressing questions that were asked during the live webinar session.

Are Sievers instruments compliant with CFR 21.11 and data integrity requirements?

Yes, Sievers instruments designed for pharmaceutical applications are fully compliant with 21 CFR Part 11 and FDA data integrity guidance. Sievers DataGuard software facilitates compliance with 21 CFR Part 11 and electronic records control requirements. It provides administratively controlled, multi-level and multi-user access, and complete audit trail functionality.

What potential changes to WFI monitoring may come into effect with the Annex 1 revisions on pharmaceutical water?

The Annex 1 draft revision mentions that WFI systems should include continuous monitoring systems such as TOC and conductivity. This explicit expectation is new.

Do you see, or would you recommend grab sampling along with RTRT?

While grab sampling is not required if the process and instrument are fully validated, many companies will still take grab samples. Grab samples can be analysed to verify POU or the online instrument and can be a good practice to ensure process control.

Can your online TOC analysers be integrated into a PLC? How can you view/export the data?

Yes, Sievers 500 RL On-Line TOC Analyzers can be integrated into a PLC. Additionally, the data can be transmitted via 4-20 mA output into any SCADA or data management system.

How many instruments do I need on my water loop for RTRT?

This depends. It can vary from one to several online analysers based on the level of acceptable risk. Online TOC instrumentation should be considered as part of the control strategy and placed at various positions within the water system as determined through quality risk management. As part of the RTRT assessment, companies should identify the number and location of higher risk POUs.

What instruments are available for continuous TOC monitoring, real-time testing and conductivity testing for pharmaceutical water systems?

The Sievers 500 RL TOC Analyzer can provide continuous, online quality assurance for pharmaceutical real-time testing and can be provided with the critical protocols required for RTT process validation. In alignment with industry guidance ASTM E2656, analytical instrument validation is required for real-time release testing. This can be achieved only with an analyser, such as the Sievers 500 RL On-Line TOC Analyzer. The Sievers 500 RL is also an optimal choice if the intended use is focused only on process control and process understanding to perform corrective actions in real time and ensure quality. The 500 RL also measures USP <645> conductivity in addition to USP <643> TOC, which provides added assurance that the purified water and WFI used in production meets quality requirements.

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The increasing complexity of formulations and active biological products raises new challenges for pre-filled syringe development. James Mellman, Device Manager at Novartis, discusses the challenges of selecting the right primary packaging for injectable formulations and how he has learnt to expect the unexpected.

Vaccinations have begun in a phase I human clinical trial testing a freeze-dried, temperature-stable formulation of an experimental tuberculosis vaccine candidate. In this article we hear from Dr Daniel Hoft and Christopher Fox about the formulation process and what this means for vaccine development going forward.

Expecting the unexpected

James Mellman

Device Manager, Novartis

The increasing complexity of formulations and active biological products raises new challenges for pre-filled syringe development. James Mellman, Device Manager at Novartis, speaks to Nikki Withers about the challenges of selecting the right primary packaging for injectable formulations and how he has learnt to expect the unexpected.

BIOGRAPHY

JAMES MELLMAN is a device manager for Novartis in Basel, Switzerland. His main responsibilities include developing primary packaging systems for combination products and supporting new technologies. He has worked on a multitude of injection device platforms including pre-filled syringes, needle safety devices, auto injectors and patch injectors. He has also helped to build and implement a renovated CCIT strategy for the Novartis Group. Before Novartis, James worked for Pfizer in their Devices Centre of Excellence in Cambridge, UK and Danone in their Advanced Medical Nutrition Division in Amsterdam, NL. He received his PhD from the University of Florida in Materials Science and Engineering in 2007 specialising in biomaterials.

DEVELOPING a successful combination product – a medicinal product combined with a delivery system – requires not only an understanding of the design parameters of the system, but also compatibility between the system and the formulation. "It is like a marriage between the container system and the drug product," explains Mellman. "If it is stable and works well, it will have a long, fruitful commercial life."

Mellman has worked on a multitude of injection device platforms including pre-filled syringes, needle safety devices, auto injectors and patch injectors. His main responsibilities at Novartis include developing primary packaging systems for combination products. "If you don't understand the delivery system in combination with the drug product in advance, you risk a delay to market," he explains, before providing a real-life example of a time when his team experienced an unanticipated incompatible system.

> *Product–package compatibility is key for product stability and the performance requirements of the overall delivery system*

"We had a product, a monoclonal antibody, and the commercial requirement was to put it into a disposable autoinjector containing a pre-filled syringe for home-use," he says. "The issue we faced was that the injection time was out of specification during stability testing (***Figure 1***).

"Everything was great for the first year, but as the product entered the second year, injection times increased, which is something we hadn't anticipated. When we examined this further, we realised that the silicone was doing a disappearing act on us. It was migrating away from the wall of the syringe over time (***Figure 2***). This was something we had never seen before, so it was quite striking."

The team soon realised that these changes were long term, leading to an unacceptable injection time. "This meant we had to go back to the drawing board," says Mellman. "We had to figure out what to do because the product was going to be delayed to market. It emphasised that we needed to find a more suitable primary packaging for this drug product."

Reflecting on this example, Mellman stresses that understanding the drug–container compatibility as an input to the delivery system is critical. "If you don't have a compatible system with your primary packaging and formulation, you will not have a product that can be delivered."

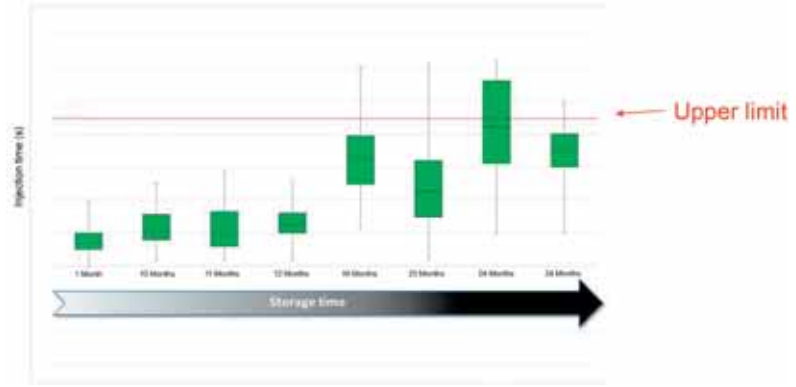
Key considerations for development

Mellman explains that the primary packaging acts as the containment system for the formulation of a combination product and is essential for maintaining the quality attributes of the drug product over its shelf life. Key considerations for successful combination product development include product-package compatibility, device design, human factors, and manufacturing quality (***Figure 3***). "Product–package compatibility is key for product stability and the performance requirements of the overall delivery system," he explains, emphasising that this is the first step to success. Sub-visible and visible particles must also be monitored, as well as contaminants from the primary packaging.

When discerning a suitable device design, he advises that it is of utmost importance to deliver the full dose at any time during the shelf life to match the label claim. Manufacturing quality must also be controlled, which includes the supply chain as well as their own fill and finish and assembly processes. Finally, ease of use and comfort for the user must be ensured. "There are a multitude of challenges that exist," he says.

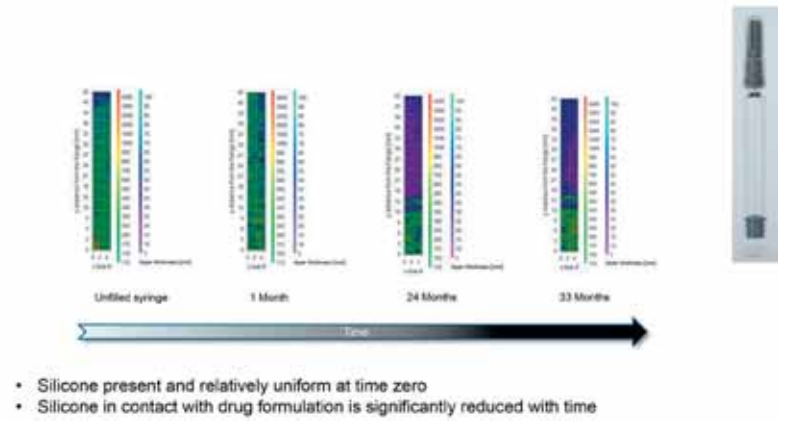
Added to this are the regulations associated with delivery devices, and these differ slightly between the US and the European markets. In the US, drug products combined with a device are called combination products and are governed by the FDA combination product legislation 21 CFR Part 4. In the EU they are known as either medical devices or medicinal products and are currently regulated by the Medical Device Directive (MDD), which will change to the Medical Device Regulation (MDR) in 2020 and be more similar to US regulations. "There are inherent differences between the two market's regulations, such as only one agency reviews the product in the US, ie, the FDA, and notified bodies will be used in Europe to review the device part of a single integral product, while EMEA will review the medicinal part. In any case, we work to be compliant in any market our product is marketed," says Mellman.

FIGURE 1



ABOVE: Case study; injection time increasing over time

FIGURE 2



BELOW: Case study; silicone thickness measurements show a decrease with storage time

FIGURE 3

ABOVE: Key considerations for successful combination product development

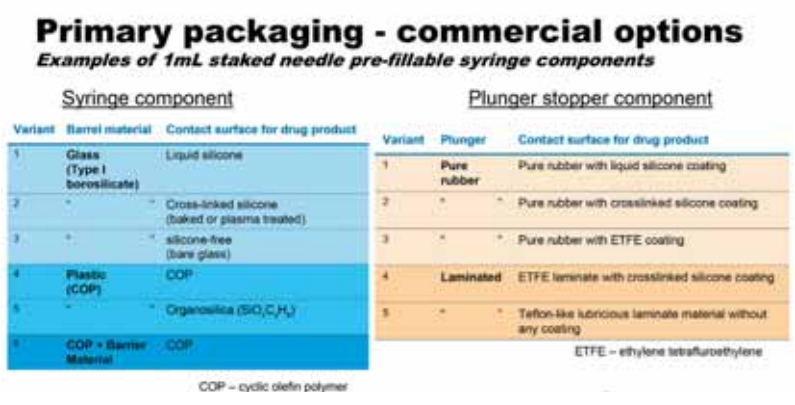
Formulation challenges

The increasing complexity of formulations and active biological products is proving to be a challenge with respect to developing primary packaging for injectables. Historically, pharmaceutical packaging was developed for small molecules, and these molecules do not behave the same as biologics or larger complex molecules. "There is a lot of added complexity around what we need to deliver now," says Mellman. "Some of the things that worked in the past are being tried today but cannot be applied to biologics."

Indeed, drug products are increasingly being delivered with monoclonal antibodies, and there are therapeutic peptides and proteins, antibody drug conjugates (ADCs), CAR T-cell therapy, protein multimers and adeno-associated viruses, which all present their own formulation and delivery challenges. "The formulators have to get it right, but we also have our own challenges in the delivery system," says Mellman. "These formulations are not necessarily going to fit into a 1ml pre-filled syringe. For example, adeno-associated viruses are frozen in a vial until use and may only need to be delivered in a 20 or 30µl capacity to a deep tissue, which requires a different delivery system than, for example, an autoinjector that injects into subcutaneous tissue."

BELOW: Examples of 1mL staked needle pre-fillable syringe components

FIGURE 4



> *"Working in combination products, I need to straddle both worlds – the formulation world and the delivery system world"*

Other formulation challenges include excipient effects, drug product concentration, and sensitivity to primary packaging contact surfaces over time. "Excipients can interact with silicone or other types of primary packaging, and it is not always understood what the contribution of small molecules or excipient effects will be on contact surfaces over time," says Mellman. "Some molecules may interact with silicone on the glass barrel and make it more soluble in water-based solutions, so the silicone layer can erode over time. Even polysorbate may break down over time and offer less protection to the protein. A number of potential interactions are possible."

To address this, companies may need to move towards state-of-the-art containment systems and develop their knowledge. One way to do this is by screening different packaging systems to see what does and does not work. "If we rely on things that have worked in the past and not judiciously screen, we may end up with failure in product development, which sets us back some time," says Mellman.

Ideally, he would like to use technology to develop relevant models to predict some of these complex reactions between formulas and the containment system, rather than studying one drug product or one containment system at a time. "In a sense it seems simple, but in reality, the process doesn't involve a single group of people. Working in combination products, I need to straddle both worlds – the formulation world and the delivery system world, in which the container lies between."

Current commercial options for primary packaging can be seen in ***Figure 4***. "These are what we can readily buy off the shelf," explains Mellman. "These are the tools in my toolbox. The challenge with new formulations is to work out which will work – and if they don't work for a formulation, why that is."

Mellman concludes that anticipating product–packaging interactions will be key to moving forward. "Expect the unexpected," he says. "If we start to understand the complexities of formulations and their interactions with delivery systems, we'll have a win-win situation, with reduced regulatory risks and a faster time to patients."

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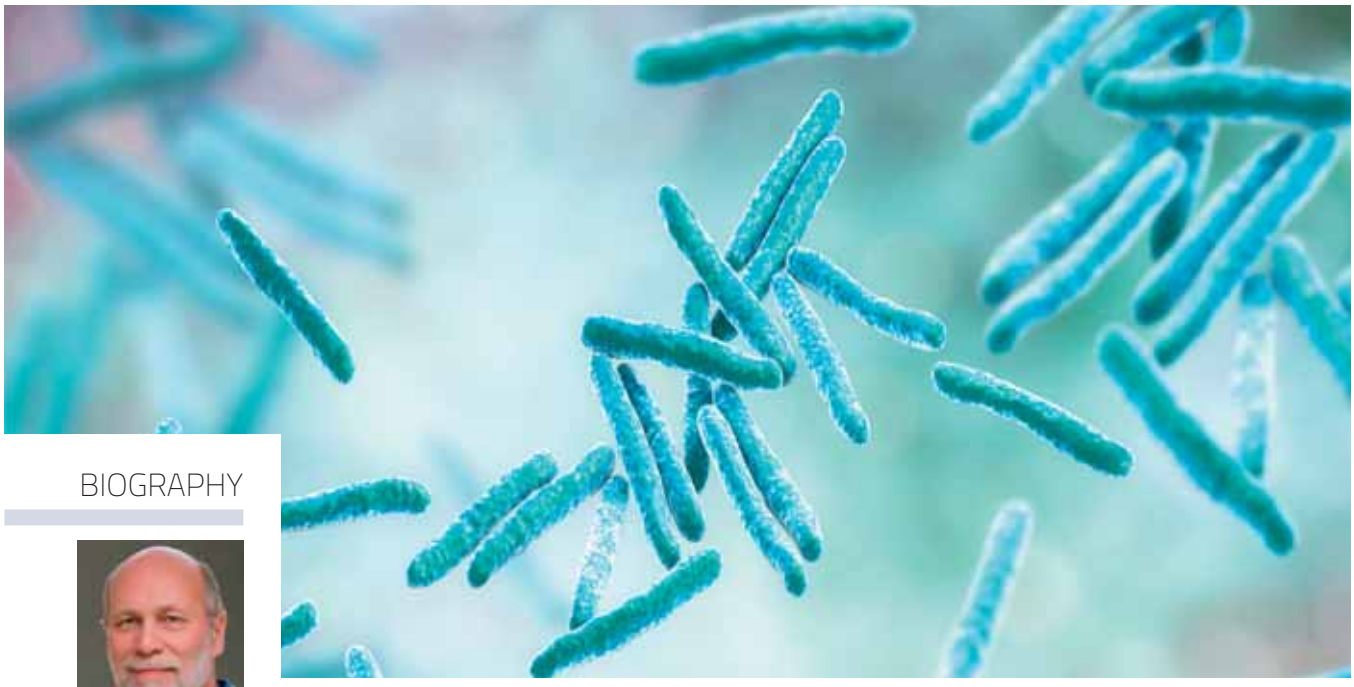
Tuberculosis: fighting the disease with a single vial

Dr Daniel Hoft
Saint Louis University School of Medicine

Christopher Fox
Infectious Disease Research Institute

Vaccinations have begun in a phase I human clinical trial testing a freeze-dried, temperature-stable formulation of an experimental tuberculosis vaccine candidate. Nikki Withers speaks to Dr Daniel Hoft and Christopher Fox about the formulation process and what this means for vaccine development going forward.



BIOGRAPHY

DR DANIEL HOFT treats patients for infectious diseases. His areas of expertise include tuberculosis, parasitology and vaccination. He is particularly interested in research into vaccine development. When not teaching or treating patients, Daniel collaborates with a clinical research site in Johannesburg, South Africa, and serves as a grant reviewer for the National Institutes of Health (NIH). He is also an investigator for the Aeras Global TB Vaccine Foundation and for the Gates Foundation.

TUBERCULOSIS (TB) is one of the leading infectious causes of death worldwide, infecting around one third of the world's population, says Dr Daniel Hoft, director of the Division of Infectious Diseases, Allergy and Immunology at the Saint Louis University School of Medicine, who is involved in developing a novel, freeze-dried vaccine to help fight the disease. "We are testing a state-of-the-art vaccine, which targets multiple vulnerable points in the TB bacterium's lifecycle," he says. "The vaccine does not require a cold chain, which is critically important."

Currently, the only Food and Drug Administration-approved vaccine for TB is Bacillus Calmette-Guérin (BCG). It is commonly given to babies in TB-endemic regions to protect children against meningitis and disseminated disease; however, the vaccine does not adequately prevent TB disease in adolescents and adults. Developing a highly effective TB vaccine would, therefore, be a crucial tool in ending the pandemic, according to the research team. However, many vaccines require a temperature-controlled system during transport, which can be costly and logistically challenging. "The worst problems with tuberculosis are in the developing world where it is difficult to maintain a cold chain," explains Dr Hoft.

Critical quality attributes

Christopher Fox, Vice President of Formulations at the Infectious Disease Research Institute and principal investigator of the project, explains they were interested in developing approaches to simplify administration and increase ease of delivery to resource-poor areas. "To our knowledge, no one had shown that a modern vaccine

containing a complex adjuvant formulation could be freeze-dried and still maintain its critical quality attributes," he says.

Compared to vaccines that require specific temperature-controlled environments, freeze-dried, or lyophilised, powder vaccines can be distributed at a lower cost to remote, low-resource settings. The powder formulations are mixed with sterile water for administering with a needle and syringe. "Freeze-dried vaccines may have significantly increased stability, enabling them to be exposed to temperatures outside of the cold chain and still retain potency," explains Fox. "Moreover, in the case of vaccines containing adjuvants, it enables a single-vial presentation rather than separate vials that are mixed prior to immunisation (one containing adjuvant and one containing vaccine antigen)."

Single-vial candidate

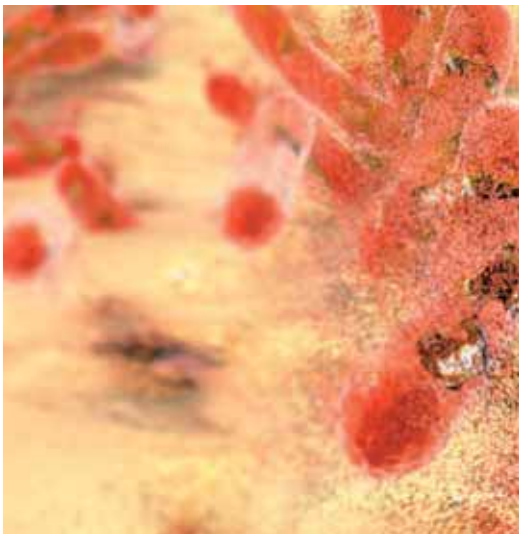
The experimental vaccine, ID93, is a recombinant vaccine candidate made from four proteins of *Mycobacterium tuberculosis*. The investigators are examining if a powder formulation combining ID93 and the adjuvant GLA-SE – an immune response-stimulating protein – in a single vial reconstituted with sterile water is as effective at inducing an immune response in participants as a previously tested two-vial combination of powdered ID93 and liquid GLA-SE.

"The two-vial presentation contains the antigen (powder) and adjuvant formulation (liquid) in separate vials, which requires the antigen to first be reconstituted with water and then mixed with the adjuvant formulation prior to administration," says Fox. The focus of the present trial is to ascertain whether the adjuvant could be present in the same vial as the vaccine in a form that is stable and does not require a cold chain, making it more effective in downstream implementation of vaccination.

DISEASE SCOPE AND AETIOLOGY

In 2017, there were more than 10 million cases of active TB worldwide, resulting in 1.6 million deaths.[1] This makes TB the number one cause of death from an infectious disease in the world. More than 95 percent of deaths occur in developing countries, and more than 50 percent in India, China, Indonesia, Pakistan and the Philippines.[1]

The main cause of TB is *Mycobacterium tuberculosis* infection, which is transmitted through infectious aerosol droplets 0.5–5.0µm in diameter. People with active TB exhibit symptoms such as chronic cough, fever, weight loss and night sweats. Around ninety percent of those infected have asymptomatic, latent TB infections. However, around 10 percent will reactivate, meaning the organism wakes up and causes active disease.



"A single-vial powder presentation contains both the vaccine antigen as well as the adjuvant formulation together in a single vial, which need only be reconstituted with water prior to administration," says Fox. "We designed a composition that optimised stability of the critical vaccine components as well as lyophilisation performance. The excipient composition enables the adjuvant formulation and the vaccine antigen to retain important physicochemical characteristics (particle diameter, etc) in the absence of water. The lyophilisation process was optimised to ensure complete drying and formation of an elegant cake structure."

To test the effectiveness of the single-vial vaccine, the researchers have enrolled 48 TB-naïve participants to receive two vaccinations 56 days apart. Of these, 24 people were vaccinated with the single-vial formulation of ID93 and GLA-SE, and the remaining participants received the previously tested two-vial presentation of powdered ID93 and liquid GLA-SE. The researchers will be monitoring any reactions to the vaccine and blood samples will be examined to determine if participants have generated an immune response.

"We think the approach we have demonstrated with this vaccine can serve as a platform that is adaptable to other vaccine candidates where thermostability and simplification of administration could have significant advantages in ensuring that these products are able to be delivered where they are needed most," concludes Fox.

FURTHER INFORMATION

The vaccine is being developed by scientists at the Infectious Disease Research Institute (IDRI) in Seattle, and the National Institute of Allergy and Infectious Diseases (NIAID) is supporting the current trial.

BIOGRAPHY

CHRISTOPHER FOX has played a role in developing, characterising and manufacturing cGMP vaccine adjuvant formulations for clinical evaluation of vaccine candidates against a variety of infectious diseases. He also leads IDRI's efforts to supply adjuvant and formulation expertise to global health researchers through the Global Health Vaccine Accelerator Platform funded by the Gates Foundation. Technology transfer to developing countries has also been a major focus of Chris's work. From 2010-2014, he led a BARDA-funded effort to transfer adjuvant manufacturing technology to the Cantacuzino Institute in Bucharest, Romania. He has also participated in projects in Brazil and India, where a vaccine formulation centre has been constructed to manufacture adjuvant formulations for clinical trials to evaluate malaria, leishmania and tuberculosis vaccines.

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Essentials in QbD, process and analytical controls

Thomas A. Little

President/CEO Thomas A. Little Consulting, BioAssay Sciences

The control strategy is a major component of any drug filing and must be carefully crafted. In this paper, Thomas A. Little details the process and analytical method elements of a well-defined control strategy and discusses how to identify and control the influence of critical process parameters.

BIOGRAPHY

DR THOMAS A. LITTLE is President/CEO of Bioassay Sciences and Thomas A. Little Consulting. He has extensive experience in developing and deploying statistical methods and QbD programmes aligned to heath authorities, CMC and business requirements. TLC is a strategic partner of SAS/JMP. Tom is a former professor from San Jose State University and has extensive experience in applying statistical and analytical methods to biologics, pharmaceuticals and medical device products development and manufacturing. He has published numerous articles and has presented papers on many aspects of data and analytics for systematic product development and QbD.

AN ESSENTIAL component of Quality by Design (QbD) is the control strategy associated with raw materials, intermediates, product release, processes, analytical chemistry and materials. The control strategy is a major component of any drug filing and must be carefully crafted. It should be based on scientific principles: a clear mechanistic understanding of what influences product performance, product knowledge, process understanding and quality risk management.[1] The following are the controls typically defined in the control strategy for any filing or submission:

Raw material control

- Vendor testing and certification within defined limits
- Internal raw material qualification and usage factors for tested materials
- Critical material attribute identification during characterisation
- Material/reagent stability and expiry.

Product controls

- Intermediates testing and specification limits[2]
- Drug substance and drug product release testing and specification limits.

Process controls

- In-process controls during manufacturing
- Closed loop controls with a clearly defined design space for adjustment[3]
- Control of identified critical process parameters (CPPs) with defined targets and operational limits (NOR and PAR ranges) from characterisation studies
- Control of critical-to-productivity parameters (CtP) with defined targets and limits
- In-process monitoring during manufacturing.

Analytical method control

- Reagent qualification and stability/expiry
- Equipment control and calibration
- Analyst certification and training
- Standards and systems suitability
- Standards control and expiry.

ICH Q8 Pharmaceutical Development[2] states in 2.5: a control strategy can include, but is not limited to, the following:

- Control of input material attributes (eg, drug substance, excipients, primary packaging materials) based on an understanding of their impact on processability or product quality

- Product specification(s)
- Controls for unit operations that have an impact on downstream processing or product quality (eg, the impact of drying on degradation, or particle size distribution of the granulate on dissolution)
- In-process or real-time release testing in lieu of end-product testing. A monitoring programme (eg, measurement and control of CQAs during processing)
- A monitoring programme (eg, full product testing at regular intervals) for verifying multivariate prediction models.

The primary focus of this paper is to detail the process and analytical method elements of a well-defined control strategy.

Process controls

In-process controls (IPC) during manufacturing should be used to control critical elements of the process. By measuring at key steps in the process, IPCs can be used to: 1) terminate the batch and/or move a batch to the next operation; 2) adjust/change downstream operations (feedforward control); 3) adjust upstream operations for the next batch (feedback control) and/or; 4) adjust the process in situ (during processing).

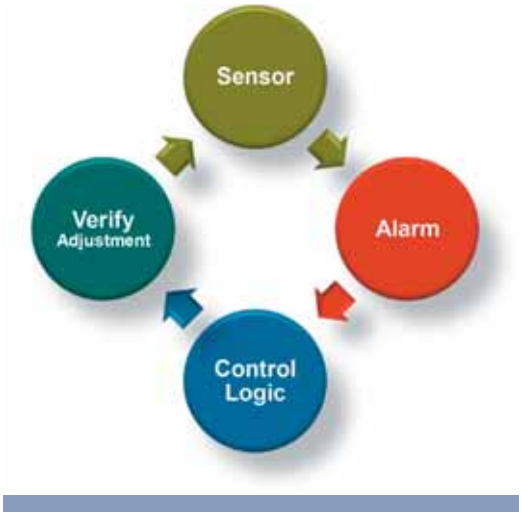
Closed-loop controls, as shown in ***Figure 1***, with a clearly defined design space for adjustment are very desirable. Closed-loop controls have four elements: 1) sensor; 2) alarms; 3) control logic; and 4) verification of the control action. Closed-loop controls may be XX controls or YX controls.

XX are factor/factor controls to make sure the factors are on target and well controlled. Proportional–integral–derivative (PID) loops on a thermal jacket would be an example of an XX temperature control. YX controls are response/factor controls to make sure the product is performing well by adjusting critical factors to influence outcomes on the response. Growth rates of a cell expansion (Y) may be controlled by adjusting media pH run-to-run or temperature in situ. Another example of YX control is loading of a purification column based on turbidity or titre.

Closed-loop sensors

The sensor is used to detect the changes in process performance and any deviations from clearly defined product and process targets. The sensor has three elements: 1) a sampling plan of what data is collected, sampling location and sample size; 2) probe or analytical measurement method; and 3) SPC control chart to detect deviation from target.

FIGURE 1



LEFT: Closed-loop control

Closed-loop alarms

Alarms detect the deviation from target and alert the operator/system of the need to adjust. Typically, three alarms are used: 1) one point beyond 3 sigma; 2) two out of three points beyond 2 sigma; and 3) nine in a row above or below the centre line or target. Never define an alarm that does not have a clear action associated with it.

Closed-loop control logic

The defined control logic indicates how adjustments are made. Control logic is typically derived from the results of designed experiments or a retrospective study. A typical adjustment algorithm is as follows:

New setting = Risk*((deviation from target)/slope coefficient of the X factor))+current setting

Risk = 0.5 if one point beyond 3 sigma
Risk = 0.75 if 2/3 points beyond 2 sigma
Risk = 1 if nine in a row, above or below centre line or target

All adjustments must be within a well-defined design space. The design space specifies the maximum amount of adjustment allowed. All closed-loop controls and adjustment design spaces are proposed by the applicant and approved by the health authority. If adjustments needed to control the process are outside of the design space further investigation or corrective action is warranted prior to any corrections.

Closed-loop verification

The next sampling point indicates whether the corrections or adjustments made are effective and the process is back in control and on target.

> *"CPPs may be found in formulation, media, upstream and downstream unit operations, and drug-product processing"*

FIGURE 2

Scaled Estimates

Continuous factors centered by mean, scaled by range/2

Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	92.124296	0.745461	123.58	<.0001 *
Protein Load (g/L)	-4.752041	0.600452	-7.91	<.0001 *
Buffer Molarity (mM)	-8.947703	0.575128	-15.56	<.0001 *
(Protein Load (g/L)-19.9321)*(Protein Load (g/L)-19.9321)	-2.107	1.145099	-1.84	0.0793
(Protein Load (g/L)-19.9321)*(Buffer Molarity (mM)-137)	-5.231745	0.62338	-8.39	<.0001 *
(Buffer Molarity (mM)-137)*(Buffer Molarity (mM)-137)	-3.151341	1.08283	-2.91	0.0081 *

ABOVE: Scaled estimates from a designed experiment

BELOW: Simulation of NOR/PAR ranges

Critical process parameters (CPPs), critical material attributes (CMAs) and critical to productivity (CtP)

Quality risk management and risk assessments may be used to identify potential factors that may impact CQAs. Risk assessment alone is not sufficient to detect and control CPPs. CPPs can reliably be assessed based on measurement of how factors directly influence the response. CPP identification is the direct result of designed experiments and measurement. When discussing CPPs, CMAs and CtPs will follow the same logic.

CPPs may be found in formulation, media, upstream and downstream unit operations, and drug-product processing. Due to the large number of unit operations and media complexity, it is easy to overlook processing parameters and materials that influence drug-substance and drug-product variation and CQAs. Failure to identify critical parameters will result in unexplainable variation during batch processing and lot acceptance.

The key steps to CPP selection and their application to process control is as follows:

- Identify CQAs for drug product and substance. Include critical responses for productivity (percent viability or titre)
- Define all materials, unit operations and equipment
- Define all product and process specification limits and acceptance criteria (include in-process controls and release testing)
- Qualify/validate all analytical methods and probes
- Complete high-level quality risk management and identify unit operations with risk to CQAs and productivity measures
- For unit operations with risk, complete a low-level factor/response selection for all factors, interactions, quadratics, and materials
- Generate design of experiments (DoEs) for unit operations or materials with risk
- Explore the design space for all key factors identified during the risk assessment using DoE or other multifactor methods
- Determine the factor effect size and scaled estimates
- Determine the percent of tolerance, percent of margin, or percent of mean, and identify all CPPs/CMAs
- Generally, more than 20 percent of tolerance, the factor is identified as CPP.

FIGURE 3

Identification of CPPs based on the scale estimate from DoEs

DoE and multifactor experiments help to isolate the influence of every factor and interaction on the critical responses associated with the drug substance or product. Analysis of the DoE will generate the scaled estimates (one half the change in Y relative to the change in X) also known as the half effect. ***Figure 2*** shows the scaled estimates and direct measure of how factors influence key responses. Figures were created using SAS/JMP®.

Multiplying the scaled estimate by two for all main effects and two-factor interactions, and by one for all quadratics, determines the full effect of each factor and/or model term. Dividing the full effect by the tolerance, margin or mean measures the factor or model term as a percent of tolerance, percent of design margin, or percent of the mean. The reason that ±20 percent of tolerance is considered critical, is it will likely cause shifts in the CQA response and probably result in out-of-specification (OOS) results; thus it makes the factor or material attribute critical.

FIGURE 4

Term	Scaled Estimate	Multiplier	Full Effect	CPP	Lower DOE Limit	Upper DOE Limit	Operational Range
Protein Load (g/L)	-4.752040782	2	-9.504081565	-23.76020391	15.1	26.3	11.2
Buffer Molarity (mM)	-8.947703454	2	-17.89540691	-44.73851727	128	146	18
(Protein Load (g/L)-19.9321)*(Protein Load (g/L)-19.9321)	-2.106999834	1	-2.106999834	-5.267499584	.	.	.
(Protein Load (g/L)-19.9321)*(Buffer Molarity (mM)-137)	-5.23174488	2	-10.46348976	-26.1587244	.	.	.
(Buffer Molarity (mM)-137)*(Buffer Molarity (mM)-137)	-3.151340873	1	-3.151340873	-7.878352182	.	.	.

Term	Scaled Estimate	Multiplier	Full Effect	CPP	PAR Lower Limit	PAR Upper Limit	Operational Range
Protein Load (g/L)	-1.106640839	2	-2.213281678	-5.533204195	13.9	20.1	6.2
Buffer Molarity (mM)	-3.090917193	2	-6.181834387	-15.45458597	131.3	141.3	10
(Protein Load (g/L)-19.9321)*(Protein Load (g/L)-19.9321)	-0.655953162	1	-0.655953162	-1.639882906	.	.	.
(Protein Load (g/L)-19.9321)*(Buffer Molarity (mM)-137)	-1.631299465	2	-3.262598929	-8.156497323	.	.	.
(Buffer Molarity (mM)-137)*(Buffer Molarity (mM)-137)	-0.984150064	1	-0.984150064	-2.460375159	.	.	.

LEFT: Before and after NOR/PAR limits and CPPs

CPPs are a function of the DoE range and their associated influence on CQAs. Increasing or decreasing the experimental range will influence CPP identification. During characterisation we want wide limits and don't mind identification of many CPPs; however, when filing a drug with the health authorities we want to demonstrate that our control of the parameters makes them not critical.

Control of CPPs/CtPs/CMAs

ICH Q11[5] discusses the need for a control strategy. After the identification of CPPs is complete, the next step is to control the influence of the factor so that it does not adversely cause uncontrolled variation in the process. The following are strategies that may be used to control CPPs:

1. Closed-loop control on the factor (PAT/ statistical process control with adjustments within the defined design space)
2. Modify equipment and associated control loops to a tighter range
3. Institute raw material qualification on incoming materials
4. Improve vendor material control and certificate of analysis
5. Change a material usage factor based on concentration or potency to control the influence of raw material variation in the process
6. Establish clear design targets with NOR and PAR limits that restrict the operational range of CPPs.

NOR and PAR range control

Normal operating ranges and proven acceptable ranges (NOR/PAR) are determined based on evaluating normal variation from the process (typically ±3 standard deviations for NOR and ±4.5 standard deviations for PAR) and its transmitted variation from a DoE simulation (***Figure 4***). Simulation from a design experiment is the preferred method of evaluation. Capability assessment is accomplished by measuring capability in PPM.

Edge of failure analysis is then used to evaluate and/or set the NOR/PAR limit. Setting a NOR or PAR limit will cause restriction in the factor range, and the characterised transmitted influence will be reduced.

Limiting the factors to the NOR and PAR range will reduce the influence of the factors, so they are now more controlled.

When reporting CPPs to health authorities, it is important to report those factors from any characterisation DoEs that are having a strong influence (>20 percent) on any CQA or productivity measure. It is, however, equally important to show how those CPPs will be systematically controlled. Establishing NORs and PARs that influence the factors and demonstrating how those limits reduce their influence so they are no longer critical is an effective way to control CPPs.

Analytical and test methods

Control of the analytical and test method[6] is equally important to process control. Failure to control the measurements will result in OOS and stability failures.

Analytical control systems should include the following:

- Reagent qualification and stability/expiry
- Equipment control and calibration
- Analyst certification and training
- Standards and/or positive/negative controls
- Systems suitability and closed-loop control of the method.

Summary and conclusions

Control effectiveness must be demonstrated during validation so that the variation caused to the drug substance or drug product is minimised and predictable. A discussion of the control strategy, identification and control of CPPs and the validation and control of analytical methods must be included in regulatory filings and is a key demonstration of product knowledge and process understanding.

> *"Establishing NORs and PARs that influence the factors and demonstrating how those limits reduce their influence so they are no longer critical is an effective way to control CPPs"*

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29 MAY 2019

15:00 BST

KEYNOTE SPEAKER:

DANIEL KELLNER-STEINMETZ
EMEA Application Lead
UPW/CV, SUEZ

Daniel Kellner-Steinmetz is the EMEA Applications Specialist for SUEZ, focusing on Life Science UPW and Cleaning Validation applications. He has eight years of experience in the quality, manufacturing and training areas of the pharmaceutical industry. Most recently he served as the Global Product Quality Lead for several commercial products at Shire (formerly Baxter) in Vienna. Daniel holds a Bachelor of Science in Biomedical Engineering.

TOC analysis is a widely-adopted method for detecting residual product or cleaning agents on manufacturing equipment. Compared to specific methods such as HPLC, TOC can provide better process understanding and efficiency for cleaning validation (CV), verification and continuous monitoring.

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MICROBIOLOGY

Modernisation of microbiological test methods involves far more than just the science. Paul Newby, Alice Laures and Lisa Wysocki from GlaxoSmithKline discuss criteria they have developed to assist in the selection process during consideration of new microbiological environmental monitoring technologies.

Appropriate standards for impurity tests are an important part of analytical testing. In this paper, Kevin Williams from bioMerieux outlines various requirements of standards for endotoxin, as stated by United States Pharmacopeia (USP), and elaborates on the definition of endotoxin as distinct from other cellular constituents.

Pharmaceutical microbiologist and consultant Tony Cundell discusses the proposed revisions to the EU Good Manufacturing Practice Annex 1, and expresses his concerns in terms of current industry practice and future innovation in sterile product manufacturing.

It's not just about the science

Paul J Newby, Alice Laures and Lisa Wysocki

GlaxoSmithKline

Modernisation of microbiological test methods involves far more than just the science. As new systems and technologies become available it becomes increasingly important that potential users can identify not only the technical requirements, but also the business case. Here, Paul Newby, Alice Laures and Lisa Wysocki from GlaxoSmithKline discuss criteria they have developed to assist in the selection process during consideration of new microbiological environmental monitoring technologies.

BIOGRAPHY

PAUL NEWBY has worked in the field of pharmaceutical microbiology, in both research and manufacture roles, for the past 30 years. His current role is Microbiology Modernisation Lead in the Future Analytical and Control Technology group within GSK Research and Development. Paul is a former member of the BP expert committee on microbiology and was involved in reviewing the initial EP chapter on Rapid Methods (5.1.4). He was also part of the PDA technical working party charged with updating Technical Report 33 'Evaluation, Validation and Implementation of New Microbiological Testing Methods'. He has recently become a Fellow of the Royal Society of Biology.

THERE is a real and growing need in pharmaceutical microbiology for the introduction of new analytical methods that can address the requirements of today's fast-paced industry. Many of the conventional growth-based microbiological techniques for product testing and environmental monitoring currently used in the pharmaceutical sector date from the nineteenth century.[1] Technology-driven solutions to drug development and manufacture are beginning to take shape. Initiatives such as the FDA's Process Analytical Technology, the publication of USP chapter 1223,[2] European Pharmacopoeia chapter 5.1.6,[3] and guidance documents such as the PDA Technical Report 33,[4] have helped to define the technical and validation requirements necessary for new microbiological tests. Growing areas such as cell-based therapies, biopharmaceutical products and new continuous processes will all benefit from real-time or near real-time analytical data and adapted analytical evaluation.

Environmental monitoring (EM) is an important aspect of pharmaceutical manufacture facility control.[5] It consists of air, surface, water and possibly operator monitoring, depending on facility needs. Wider facility monitoring will also include microbiological water analysis – for both water system monitoring and water as raw material (***Figure 1***).[6]

Microbiological EM is a very labour-intensive process, typically involving multiple samples that need to be tracked, incubated and eventually read by operators. Current microbiological methods for EM are growth based and results are not real-time – they can take several days. These methods include the use of settle plates, active air monitoring, swab and contact

plates. Real-time data for EM analysis is the desired state for new methods as it enables timely interventions, troubleshooting and remedial actions in manufacturing environments.[4,7,8,9]

Many regulatory agencies are keen to actively support pharmaceutical innovation and modernisation. These agencies recognise that the adoption of innovative approaches may present challenges, yet they support modernisation and improved quality. The FDA has an Emerging Technology Team (ETT), to which companies can submit questions and proposals regarding the use of specific emerging technologies. The Medicines and Healthcare Products Regulatory Agency (MHRA) has launched an 'Innovation Office' to help organisations who are developing innovative medicines and approaches. Pharma companies are now also working together in the pre-competitive space to support modernisation of microbiological test methods. The Microbiology Modernisation Cross-industry Consortium (MMCC), a cross industry group, has recently been formed between several companies including AstraZeneca, Merck, GSK, Pfizer, Johnson & Johnson, Jansen, Catapult and Sanofi to help build the case for new microbiological technology uptake.

Building the case for environmental monitoring

Technology selection is not a simple task due to the complexity of many test platforms and sometimes the lack of familiarity of the vendor with the prospective purchaser's requirements. To help with assessment and developments, several key requirements will be outlined for the following:

- Technical criteria
- Data integrity and archiving
- Business criteria.

They are also intended to be used during the seeking phase of a new technology to form the basis of an evaluation protocol, as well as assisting with an evaluation go/no-go decision (***Figure 2***).

1) Technical criteria

These technical criteria have been developed with integrated incubator and automated colony counting systems in mind, but are equally applicable to other rapid microbiological test systems.

Performance equivalence of the system must be equivalent to or better than the compendial methodology in terms of accuracy, precision, specificity, limit of detection, limit of quantitation, linearity, range and rates of false positive and negative results.[2] In all cases, these parameters should demonstrate equivalence equal to or better than the conventional test method in terms of enumeration and rates of false negatives and positive test results.

In terms of colony counting, a plate reader must have the ability to accurately read different media surfaces (ie, rough/smooth/indented). It must also accurately enumerate and record the number of colonies present on plates. The recording and retaining of an image of any growth present on plates and the ability to record numerical results for both positive and nil growth plates are all important requirements. It is essential that the system can distinguish between viable and non-viable microorganisms and artefacts.

The system should be capable of interfacing with site batch release systems to record additional details such as the media type, media batch number, and sample information.

If the system includes an incubator, it must be able to incubate plates at an accuracy of +/-0.5°C.

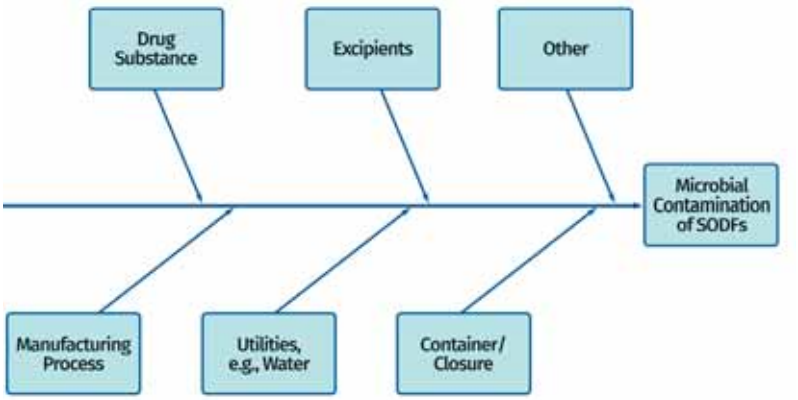
2) Data integrity and archiving

This section details data integrity and archiving acceptance criteria.[10,11] Samples must be trackable via a unique sample identifier for the duration of the analysis and comply with FDA guidance.[12] Individual sample data must be retrievable, and unique sample identifiers should include time stamp, duration, incubation status and sample set.

Individual login security must be in operation, which can attribute all actions within the application to specific individuals. If users of the system have a dual role, they must have a distinct user ID for each role including vendor and/or engineer access.

The system must be able to capture and archive all data generated in accordance with data retention requirements and ensure that stored data or methods cannot be altered without changes being captured in a secure, time-stamped and attributable audit trail. It must have an audit trail that records both application access and

FIGURE 1

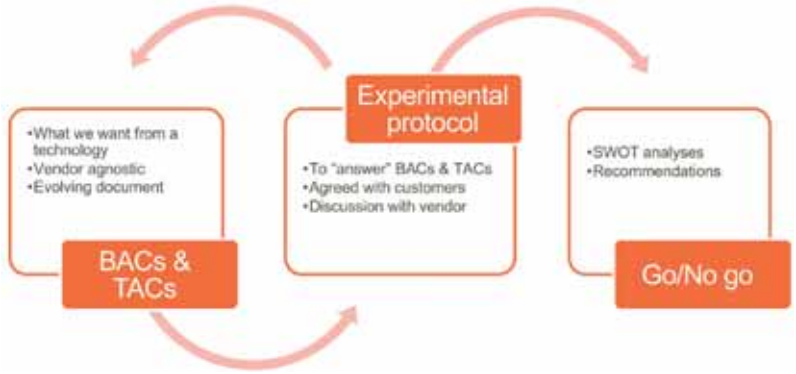


ABOVE: Fishbone diagram identifying potential sources of microbial contamination for a packaged solid oral dosage form (SODF)

BIOGRAPHY

LISA WYSOCKI is a Microbiology Technical Lead and Associate Fellow at GlaxoSmithKline in the CMC Analytical Group. She provides microbiology support for Pharmaceutical Product Development and Supply and leads an effort on new technology development and implementation for rapid microbiology methods. She has previous experience in molecular biology, microbial genetics, mammalian cell culture and biological sample management.

FIGURE 2



ABOVE: Process flow for use of BACs and TACs in technology selection and evaluation

actions performed by the administrator and all users of the system. Any data files, audit trails and method files must be protected from deletion within the system software and be protected from deletion/modification outside of the application – ie, Windows. Windows' version compatibility must also be considered.

The system must include capability for data backup and recovery to a remote (server-based) location. It must also be able to attribute distinct permissions to at least two user groups (ie, user and administrator) and assign users of the system to the relevant user group.

The programme name, current version number, original author, date created and modification history must be recorded within the source code.

3) Business criteria

New technologies often involve initial high purchase and operating costs. Business benefits and return on investment can be obscured with simple direct comparison of like-for-like with conventional methods. However, a broader evaluation that considers productivity gains, automation potential and higher throughput can present a different picture. Efficiencies in the context of site demand and sample throughput must be considered in terms of system capacity, which must be defined and able to meet specific site demands.

Time to result is important; therefore, the supplier should work with the customer to provide evidence of net time savings (eg, start-up time saving, total number of manufacturing and testing days, documentation and approval, sample preparation/movement). Evidence that the methodology is equivalent or better than the compendial methodology in terms of simplicity (ie, number of consumables, procedure) is useful. Additionally, the supplier can help the business case by providing evidence of error and investigation cost savings and any FTE savings.

The supplier must provide evidence of return on investment in terms of cost per analysis and cost per year in comparison to conventional tests over a three-year period, including capital, facilities and servicing costs. Scalable pricing is an advantage. All this information helps build a clear understanding of the upfront investment needs in terms of capital and facility requirements.

Conclusions

Automation and integrated data integrity functionality are increasingly important in microbiological EM processes. Patient safety and product quality considerations require a move towards real-time or near real-time EM analysis. The stage is set for the implementation of a whole new generation of test systems in pharmaceutical microbiology, including EM.

BIOGRAPHY

ALICE LAURES works for GlaxoSmithKline R&D in the UK Medicines Research Centre where she leads the Future Analytical and Control Technologies group – a multi-disciplinary team of technical experts in mass spectrometry, novel imaging technologies and rapid microbiological techniques. Alice is a cofounder of the Microbiology Modernisation Cross-Industry Consortium (MMCC), set up in 2018 to create a collaborative forum for pharmaceutical companies interested in developing the use of novel microbiological technologies. Alice also has experience in pharmaceutical analysis and spectroscopy.

ACKNOWLEDGEMENTS

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FURTHER INFORMATION

For more information about the Microbiology Modernisation Cross-industry Consortium (MMCC) please contact the authors.

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Endotoxin definition and standardisation

Kevin Williams

Senior Scientist at bioMérieux

Appropriate standards for impurity tests are an important part of analytical testing. In this paper, Kevin Williams outlines various requirements of standards for endotoxin, as stated by United States Pharmacopeia (USP), and elaborates on the definition of endotoxin as distinct from other cellular constituents.

BIOGRAPHY

KEVIN WILLIAMS worked for Eli Lilly for 30 years, developing QC tests for endotoxin detection, process control and control strategies of raw materials/excipients and container/closures. He wrote and edited the 2nd and 3rd editions of the book, Endotoxins. He currently works for Hyglos-bioMérieux.

Introduction

Endotoxin, or lipopolysaccharide (LPS), is a constituent of the outer leaflet (OL) of the outer membrane (OM) of gram-negative bacteria. It is a unique molecule used as a marker of prokaryotic invasion by metazoan immune systems and occurs as a singular constituent among multiple gram-negative bacteria OM constituents, including phospholipids and surface proteins.

LPS is purified away from other impurities as per USP reference standard quality requirements. A highly purified material is used as a standard endotoxin (RSE/CSE), which has served historically to define endotoxin analytically. In industry, there is some desire to prepare a non-purified or "natural standard" for various purposes and to call these preparations "natural endotoxin". This paper outlines some requirements of standards, as stated by United States Pharmacopeia (USP),[1] and elaborates the definition of endotoxin as distinct from other cellular constituents.

Standardisation

An excerpt from USP 40 General Requirements <11>, states:

> "Reference Standards provided by the United States Pharmacopeial Convention (USP Reference Standards, or RS) are **highly characterised** specimens reflective of specified drugs and foods (drug substances, biologics, excipients, dietary supplements, food ingredients, impurities, degradation products, reagents and performance verification standards). When approved as suitable for use as comparison standards for documentary tests or assays (ie, as a monograph component) in the USP or National Formulary (NF), USP RS also assume official status and legal recognition in the United States."

"Endotoxin is not a random occurrence in nature; it is the product of a bacterial manufacturing process"

The "impurity reference standards" section of USP <11> states: "Impurity Reference Standards may be presented as **purified single-component materials or as mixtures of more than one impurity**." For industry to maintain the current "endotoxin" standard (for BET <85>), rather than a "gram-negative cell wall" standard, it must be purified and highly characterised, otherwise it will be a "mixed impurity" standard and should be labelled as such.

Definition of endotoxin

Given the brief nature of this article, only a rudimentary sketch of the definition of LPS can be provided (***Figure 1***). The topics will be confined to items deemed relevant to endotoxin standardisation:

1. LPS biosynthesis
2. Reciprocal specificity of the host response
3. Asymmetry in the gram-negative bacteria OM
4. Contrasting constituents.

1. LPS biosynthesis

Endotoxin is not a random occurrence in nature; it is the product of a bacterial manufacturing process that involves nine separate enzymatic events, followed by export to the gram-negative bacteria cell surface. Unique sugars in LPS include the core sugar, KDO (3 deoxy-α-D-manno-octulosonic acid), and unique arrangements of sugars in the O-antigen moiety, which are used to distinguish bacterial strains when characterising various gram-negative bacteria foodborne illness outbreaks (serotyping).

The unique LPS structure and associated sugar residues and arrangement are outlined in ***Figure 2***. The enzymatic cascade used to "build" LPS, as encoded in the bacterial genome, is not shown but is extensively detailed by Wang and Quinn and supports that LPS is a specific functional unit.[2]

2. Reciprocal specificity of the host response

Metazoan systems have already defined endotoxin through their very specific responses. However, not all metazoan responses are the same. For decades, Limulus-based tests have been an effective analytical surrogate for supporting drug testing. The specificity of the mammalian response is extreme. A single molecule of LPS is selected (purified away) from an aggregate by lipopolysaccharide binding protein (LBP) or potentially as a monomer where serum albumin has been found to serve in lieu of LBP.[4] Once isolated, the LPS molecule is transferred to MD-2, which sits in TLR4, and brings about a transmembrane signalling event. This is about as "purified" as a molecule can get. TLR4 not only takes the measure of the molecule but also signals a response based upon the underlying variance in sub-molecular moieties.

To assert that LPS is "not purified" in nature seems a matter of semantics; it begins as a singular molecule in its biosynthesis and ends as a singular molecule in the mammalian receptor. For mammalian detection purposes, it is as purified as it could be by current analytical methods. See an overview of the (canonical) mammalian detection cascade in ***Figure 3***.[6]

3. Asymmetry in the gram-negative bacteria OM

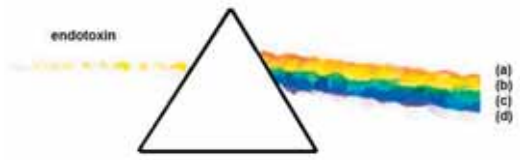
Gram-negative bacteria work, energetically speaking, to maintain the asymmetry associated with the OM. This maintains an efficient barrier against the formation of hydrophobic "patches" that might allow indiscriminate passage of unwanted substances (ie, antibiotics). This is one reason for the difficulty associated with treating gram-negative bacteria infections. If we look at a common textbook diagram of the gram-negative bacteria surface, it is clear where the "asymmetry" occurs (***Figure 4***).[6]

The asymmetry occurs as the OM OL consists of LPS and the inner leaflet consists of phospholipids (PL).[7] If sufficient PLs aggregate in the OL, the cell has specialised methods to reestablish asymmetry; including "floating" in new LPS molecules.[8] The longstanding definition of endotoxin as a singular molecular entity includes its unique ability to form this asymmetrical barrier. Thus, the proportionality of PLs and LPS as separate OM constituents (***Figure 3***) is an important property.

4. Contrasting constituents

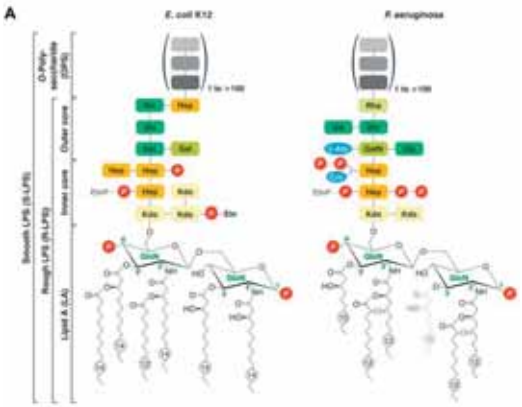
It is sometimes easier to define something by describing what it is not, rather than what it is. Endotoxin does not include other membrane-associated molecules. It is a quality or analytical marker for half a dozen bacterial residues that cannot otherwise be detected, and thus a critical quality attribute in pharma

FIGURE 1



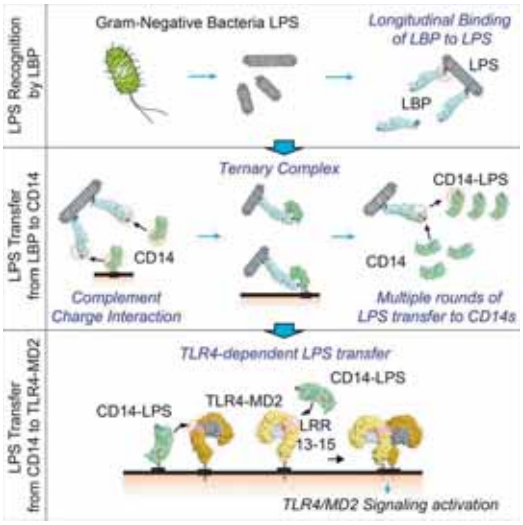
LEFT: Definition of endotoxin as broken into relevant constituent pieces

FIGURE 2



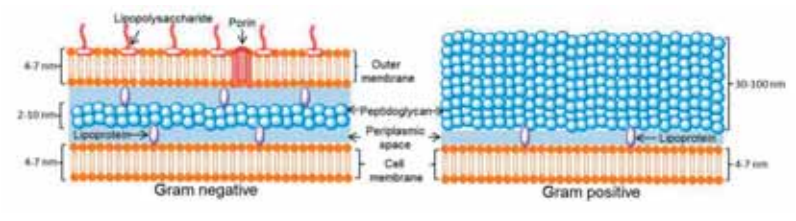
LEFT: LPS unique structural definition[3]

FIGURE 3



LEFT: LPS of gram-negative bacteria activates host innate immune responses. Ryu *et al.* investigated dynamic intermediates in the LBP-CD14-mediated LPS transfer to TLR4-MD-2 down to single-molecule resolution[5]

FIGURE 4



BELOW: Cell wall structure for gram-negative and positive bacteria[6]

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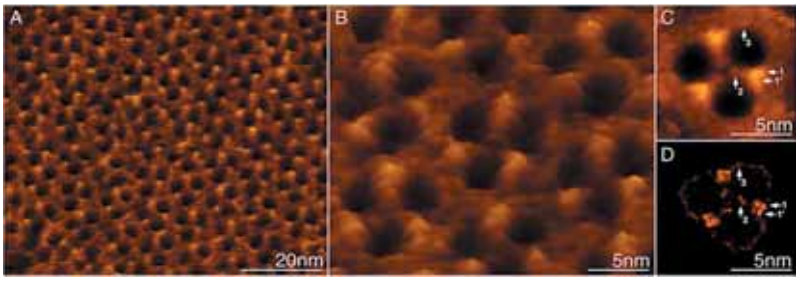
BELOW: Atomic model of the native supramolecular assembly of porins in the OM[9]

FIGURE 5



LEFT: High-resolution analysis of the periplasmic surface of the *R. denitrificans* OM. **A)** Membrane region densely packed with porin trimers. **B)** At high-resolution substructure is visible on individual trimers.[9]

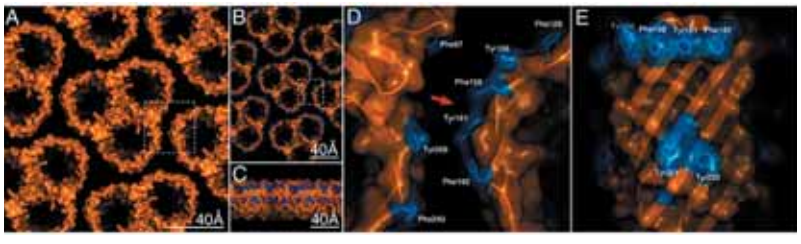
manufacturing. One OM constituent type that shares the gram-negative surface with LPS is a "porin". Porins are protein structures, typically present as trimers, that regulate the passage of water, solutes and nutrients in and out of the cell.

The concept of the gram-negative bacteria OM has evolved recently, as atomic force microscopy (AFM) studies of living bacteria identify holes dominating the gram-negative bacteria surface (***Figure 5***).[9] These woven protein strands form hollow "tubes" that extend from the surface down through the OM and into the cytoplasm (***Figure 6D*** and ***E***). LPS alerts manufacturers to the presence of gram-negative bacteria artifacts via Limulus-based tests; however, porins themselves cannot currently be detected.

Using atomic force microscopy (AFM), Oestreicher *et al*.[10] studied two prototypical gram-negative bacteria structures from *E. coli* and *Rhodobacter sphaeroides*, stating: "Our research clearly demonstrated that both of these types of cells have an outer surface that is covered in a network of nanometer-sized holes similar to *M. magneticum*." Like LPS, porins are separate, enduring structures that are immune reactive and difficult to destroy. "Porins are extremely sturdy proteins that can resist denaturation in the presence of 5M guanidium hydrochloride or two percent SDS at 70°C."[11] Porins should be of interest given that they are immunogenic in nature.[12,13]

While it doesn't change the role of LPS as a potent contaminant, it makes it clear that LPS does not represent the entire gram-negative surface, but rather the OM consists of several discrete substances.

FIGURE 6



Summary

The historical and continued use of purified LPS as a standardised impurity (RSE/CSE) is supported by a detailed definition of endotoxin:

(a) endotoxin is a unique structure with a specialised assembly (nine enzymatic steps)
(b) the specificity of the metazoan response to LPS (via TLR4) is separate from other responses (such as to porins that activate TLR2 or flagella that activates TLR5)
(c) it includes endotoxin's role in creating and maintaining the asymmetry of the OM in proportion to PLs
(d) its existence as a singular structure separate from and adjacent to other OM structures provides a contrast in terms of structure, function and immune reaction (TLR activation).

Efforts should be made to develop new tests to detect significant non-LPS membrane constituents. This forms a critical distinction. Rather than devising a "natural endotoxin" comprised of unpurified "cell wall" constituents of which only endotoxin can be detected, the individual constituents (endotoxin, phospholipids, porins, flagellin, etc) should have their own assays and standards as a means of broadening microbiological contamination control capabilities. This would provide necessary redundancy, whereas today the entire weight of detecting subcellular gram-negative bacterial artifacts falls on endotoxin.

Appropriate standards for impurity tests are an important part of analytical testing. As per USP <11>, an impurity mixture labelled as a "natural endotoxin" is not endotoxin, but a mixture that may contain endotoxins, proteins, phospholipids, nucleic acids and porins. Today's Limulus-based tests are specific for endotoxin, not for cell wall or cytoplasmic constituents that are not endotoxin; this specificity is inherent in both Limulus Factor C and mammalian TLR4 detection architecture. The definition of endotoxin is a cross-discipline definition and not one that can be casually re-defined.

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ENDOZYME II GO recombinant Factor C assay: fast, easy and consistent endotoxin testing

Authors: Gregory Devulder and Petra Schneider

Background

The vast majority of endotoxin tests for pharmaceuticals and medical devices, as well as their in-process intermediates and raw materials, are microplate-based. They require tedious reconstitution and dilution steps for preparation of Control Standard Endotoxin (CSE) dilutions and Positive Product Controls (PPCs). These time-consuming manual handling steps can result in substantial variability and a significant number of invalid results that demand repeat testing. To address these issues, as well as to reduce the time for microplate preparation, we have developed a ready-to-use microplate – the GOPLATE – embedding required CSE amounts in dried format. Thus, the conventional standard dilution has become completely obsolete; in turn, the GOPLATE significantly lowers the risk for human error and cost-intensive test repetition. The GOPLATE is included in a complete test kit, ENDOZYME II GO. The new microplate is the first of its kind, ready-to-use and enables high reduction in handling time as well as consistent standard curve and PPC accuracy.

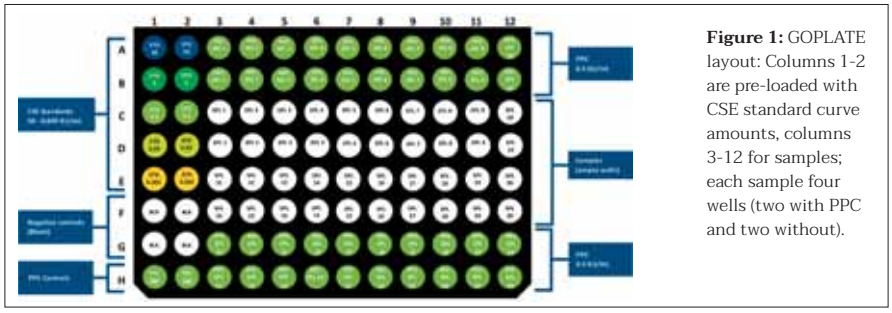

Figure 1: GOPLATE layout: Columns 1-2 are pre-loaded with CSE standard curve amounts, columns 3-12 for samples; each sample four wells (two with PPC and two without).

Recombinant horseshoe crab Factor C

Recombinant horseshoe crab Factor C (rFC) is an exact synthetic copy of the endotoxin-sensitive enzyme naturally harboured by the blood of horseshoe crabs. Compared with Limulus amebocyte lysate (LAL) methods, endotoxin detection assays based on rFC offer specificity, flexibility, lot-to-lot consistency and, importantly, a sustainable, animal-saving and secure source. rFC tests are available as fluorescence end-point assays in 96-well microplate format and are validated in the same way as conventional methods according to pharmacopoeia bacterial endotoxin testing chapters. The US FDA recently approved product release using rFC for Eli Lilly[1] and defines the requirements for rFC in Guidance for Industry Pyrogen and Endotoxins Testing.[2] The European Pharmacopoeia includes rFC as an alternative method in Ph. Eur. Chapter 5.1.10 and published the world's first draft general chapter for rFC, Ph. Eur. 2.6.32 in December 2018.[3] The Japanese Pharmaceutical and Medical Device Agency has so far published two collaborative studies demonstrating equivalence between rFC and LAL assays.[4,5]

Specifications and workflow

ENDOZYME II GO is the evolution of ENDOZYME II, using the same reagents in a more efficient way. The key component, GOPLATE, is pre-loaded with CSE for the standard curve 0.005–50 EU/mL and PPCs 0.5 EU/mL, all in duplicate replicates (***Figure 1***). The fast workflow of ENDOZYME II GO consists of three easy steps: 1) Addition of endotoxin-free water in standard curve and blank wells, and samples to the dedicated wells, 2) Preparation and addition of the assay reagent, 3) Running the assay in a fluorescence reader for 20-60 minutes depending on the desired sensitivity (0.05–0.005 EU/mL). Due to the streamlined workflow, the handling time for preparing a full GOPLATE is reduced by more than 50 percent (***Figure 2***).

Inclusivity

Endotoxin from 14 different strains of gram-negative bacteria and Reference Standard Endotoxin (RSE) were tested using the ENDOZYME II GO and two LAL tests in parallel. Samples were measured in serial dilutions in single replicates (n=44). Endotoxin values [EU/mL] were calculated from the respective standard curve and plotted as a logarithm. All three tests detected all the strains (100 percent

Table 1: False-positive signals and recovery of spiked endotoxin (PPC=0.5 EU/mL). Samples were tested in duplicates (n=2).

Conc. zymosan [mg/mL]	**ENDOZYME II GO**		**LAL 1**		**LAL 2**	
	Mean EU/mL	PPC recovery [%]	Mean EU/mL	PPC recovery [%]	Mean EU/mL	PPC recovery [%]
0.01	<0.005	98	6.6	2250	2.8	484
0.001	<0.005	96	0.27	479	0.43	286

Table 2: Coefficient of variation (CV) of back-calculated CSE standard curve concentrations (EU/mL) determined in different endotoxin assays. Each standard (0.005–5 EU/mL) was measured in four-fold determination (n=4).

Nominal conc. [EU/mL]	**ENDOZYME II GO** CV(EU/mL) [%]	**LAL 1** CV(EU/mL) [%]	**LAL 2** CV(EU/mL) [%]
5	1.6	3.0	6.8
0.5	3.0	4.1	5.6
0.05	2.1	3.7	3.2
0.005	6.2	7.1	19.3

Figure 2: Comparison of manual preparation time between ENDOZYME II GO manual kinetic chromogenic LAL; full 96-well plate with undiluted samples in duplicates. Exact preparation times depend on sample types, need for dilution, equipment and operator.

inclusivity). The rFC test correlated well to both LAL tests also in terms of the obtained EU/mL value, with correlation coefficients of 0.948 (94.8 percent) and 0.935 (93.5 percent), which was slightly higher than the correlation between the LAL tests, 0.933 (93.3 percent).

Exclusivity

The endotoxin detection of ENDOZYME II GO occurs with endotoxin-specific rFC. As expected, zymosan, a 1,3-β-glucan, only produced a false-positive signal with the tested LAL tests, but not with the rFC assay (***Table 1***).

Precision

Precision was assessed based on the coefficient of variation (CV) calculated on the back-calculated concentrations of the standard curves. The rFC test showed a higher intra-assay standard precision compared to the LAL tests due to accurately pre-loaded CSE (***Table 2***).

Interference testing

For evaluation of interferences, different samples such as common excipients, proteins, organic solvent and culture medium were tested in duplicates without and with PPCs of 0.5 EU/mL. A PPC recovery of 50–200 percent indicated a valid result. All three methods were comparable regarding tolerated concentrations of substances for valid PPCs. The rFC test yielded a higher rate of valid recoveries (***Table 3***).

Third-party evaluation

ENDOZYME II GO has been evaluated and validated by several users. In one study by Marine Marius (Sanofi Pasteur) in 2018, ENDOZYME II GO was compared with two LAL tests. Marius concluded that ENDOZYME II GO is an easy and suitable method for pharmaceutical waters and high-throughput testing, it is possible to automate, with very low variability between assays, it has best reproducibility recovery for CSE/RSE and the lowest rate of invalid results.[6]

Table 3: Percentage of PPC recovery (0.5 EU/mL) in different samples in ENDOZYME II GO compared to two LAL assays. Samples and spiked samples were tested in duplicate replicates (n=2). Red font colour highlights invalid PPC recovery.

Category	Substance	Conc./ dilution	PPC recovery in ENDOZYME II GO [%]	PPC recovery in LAL1 [%]	PPC recovery in LAL2 [%]
Common excipients	Sodium citrate	1 mM	112	161	92
		0.1 mM	106	161	118
	Dextrose	5%	80	134	92
		0.5%	95	165	108
	NaCl	500 mM	58	57	1
		50 mM	92	135	84
	Polysorbate 20	0.02%	77	61	50
		0.002%	101	69	43
	PBS	1x	82	96	36
		1:10	100	142	110
Proteins	MAB-33	0.1 mg/mL	117	603	46
		0.01 mg/mL	108	190	92
	HSA	1 mg/mL	94	332	138
		0.1 mg/mL	111	188	76
Organic solvent	Ethanol	1%	99	135	94
		0.1%	114	174	104
Culture medium	IMDM	100%	35	214	55
		10%	91	262	130

Conclusion

The ENDOZYME II GO rFC endotoxin detection assay has been shown to perform equivalent or better than the tested LAL tests, with higher rates of valid results, accuracy, precision and no false-positive results from β-glucans. From a workflow point of view, ENDOZYME II GO requires less than half the handling time of conventional microplate-based endotoxin tests and is a useful method for both manual and high-throughput automated testing. ■

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Petra Schneider is Project Manager in the Endotoxin Research & Development team of Hyglos – a bioMérieux company – and holds a MSc from the University of Applied Sciences Fulda, Germany.

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Concerns around Annex 1

Dr Tony Cundell

Microbiological Consulting, LLC

This article expresses the opinions of a pharmaceutical microbiologist on the proposed revisions to the EU Good Manufacturing Practice Annex 1 in terms of current industry practice and future innovation in sterile product manufacturing.

> *"Aseptic processing environments vary widely in terms of their risk to microbial product contamination from laminar flow hoods to isolator systems"*

AS A MICROBIOLOGICAL consultant working in the pharmaceutical industry, my reaction to the revisions to EU Guidelines to Good Manufacturing Practice – Medicinal Products for Human and Veterinary Use Annex 1. Manufacture of Sterile Medicinal Products was one of disquiet. Although we expect regulatory science to evolve, there is always a tension between industry best practice and the positions taken by regulators. Much of the Annex's content is, in my opinion, prescriptive, unsupported by technical literature, will not promote a broader understanding of sterile drug manufacturing and may discourage risk analysis.

My major concerns fall into the following four areas:

- Requirements in Annex 1 becoming the de facto rules that govern sterile product manufacturing globally when the revised Annex contains requirements that do not represent industry best practice and conflict with other regulatory guidance
- Setting microbial requirements for air, surfaces and personnel by air cleanliness classifications of aseptic process environments when they cannot be engineering standards and when

these environments vary greatly in design, operation and potential risk of product contamination
- Failure to recognise the poor analytical capabilities of current microbial monitoring methods
- Continuing to support the equivalency requirement for new microbiological technologies that are not dependent of microbial growth and the colony-forming unit.

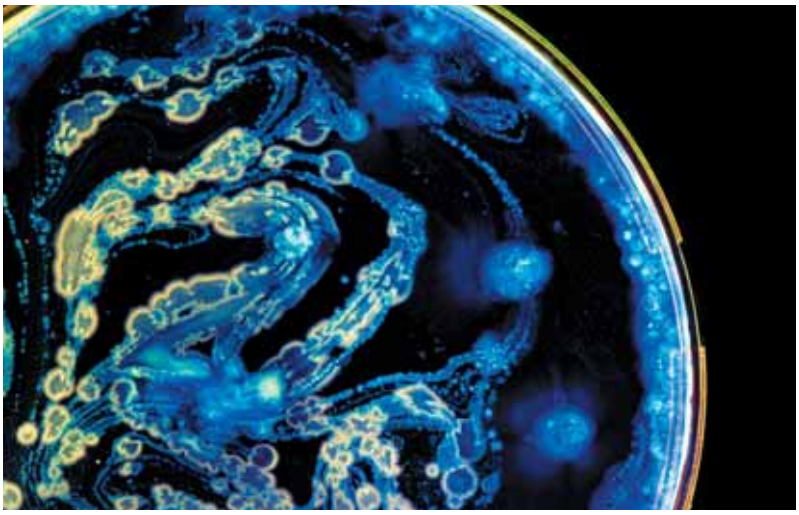
Other US viewpoints on the Annex 1 revision from experts in the area of aseptic processing and engineering were published last year.[1] ***Table 1*** describes the regulatory framework for GMPs in the US and EU.

The EU GMPs were first published in 1989, reconstructed in October 2005 and updated in December 2010. I believe that Annex 1 dates back to September 2003 and has been revised to align the cleanroom classification table and provide guidance on media simulations, bioburden monitoring and the capping of vials. The current extensive revision published in December 2017 and subject to considerable comment, will be official when finalised. The guidelines are unlikely to be revised again before the passage of a decade. With a "how to" and not a "what to do" approach, the pharmaceutical industry is bound to these mandatory requirements, which will have a dead hand on any advances in manufacturing and monitoring technologies.

The guide is presented in three parts and supplemented by a series of annexes, of which Annex 1. Manufacture of Sterile Medicinal Products is the topic of this article.

The EU Guideline is divided into:

- Part I: covering GMP principles for the manufacture of medicinal products
- Part II: covering GMP for active substances used as starting materials



- Part III: containing GMP-related documents, which clarify regulatory expectations.

These parts of the EU GMPs are high-level requirements comparable to those found in 21 CFR 211 Current Good Manufacturing Practice for Finished Pharmaceuticals.

Differences in sterile product current good manufacturing practice

The 2004 FDA Guidance for Industry Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice is not enforceable but recommends how a sterile drug manufacturer can comply with the cited sections of the GMP regulations and allows other approaches, as justified. In contrast, Annex 1 regulations must be complied with if the manufacturer wants to market their products in the EU. The revision contains many requirements that are not industry practice and are not scientifically justified. I recommend reviewing the recently published 2017 PDA Aseptic Processing Survey to benchmark industry practice.[2]

The revision was undertaken by a joint EMA and PIC/S working group that represents a broad base of multi-national pharmaceutical manufacturing

TABLE 1 **Statutory and regulatory framework for current good manufacturing practice in the areas of sterile product manufacture**

Framework	US Model	EU Model
Statutes	Laws passed by Congress and signed by the President, ie, Food, Drug and Cosmetic Act	Directives of the European Parliament under the Treaty establishing the European Community
Regulations	Written by the FDA and approved by the Executive Branch, ie, 21 CFR Part 211 Current Good Manufacturing Practice for Finished Pharmaceuticals (interpretative of Statutes)	EudraLex Volume 4 EU Guidelines to Good Manufacturing Practice – Medical Products for Human and Veterinary Use and Annex 1 Manufacture of Sterile Medical Products (Mandatory for products sold in the EU)
Guidance	FDA interpretation of the regulations written and approved within the FDA, ie, 2004 Guidance for Industry Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice (Advice non-binding on FDA or drug manufacturer)	

BIOGRAPHY

DR TONY CUNDELL has a PhD in Microbiology from the Lincoln University, New Zealand. He consults in the areas of microbial risk assessment, regulatory affairs and microbiological testing. Prior to November 2013 he worked for Merck Research Laboratories in Summit, New Jersey as the Senior Principal Scientist in early phase drug development. Earlier in his career, Tony worked at a director level in Quality Control and Product Development organisations at the New York Blood Center, Lederle Laboratories, Wyeth Pharmaceuticals and Schering-Plough. He is a member of the 2015-2020 U.S.P. Microbiology Committee of Experts where he takes a leadership role in the area of modern microbiology methods.

experience. The Annex 1 revision may make mutual recognition of GMP compliance inspections between the EMA and the FDA more difficult as there are unresolved differences in how to achieve GMP compliance between the two regulatory agencies. Both agencies will retain and exercise the option to conduct separate investigations for cause and pre-approval inspections, where the inspectional differences will be most clearly manifested with one agency taking regulatory action while the other may see no compliance issues. If manufacturing sites outside the EU cannot comply with these changes, this will increase drug shortages in the EU.

> *"Microorganisms are never uniformly distributed in the environment so reliable sampling is always an issue, especially at microbial densities"*

Setting microbial requirements by air cleanliness classifications

Setting air cleanliness standards for the engineering design and operation of an aseptic process environment based on particulate concentrations, air velocities, air changes, space pressurisation, temperature and relative humidity makes sense; whereas setting microbial monitoring standards does not, when often they are area use and people related. Aseptic processing environments vary widely in terms of their risk to microbial product contamination from laminar flow hoods to isolator systems. The ability to exclude people from aseptic processing has a hierarchy of risk – laminar flow hood > biological safety cabinets > restricted access barrier systems > open isolator systems > closed isolator systems with gloves > gloveless isolator systems – that is not fully recognised in Annex 1.

Analytical capabilities of current microbial monitoring methods

It is my experience that colleagues in the pharmaceutical industry who are not microbiologists expect too much of microbial monitoring methods, giving more creditability to the plate counts than is justified. These issues are more fundamental than the incubation conditions of environmental monitoring plates and media fills highlighted by the PDA review of the EU GMP Annex 1. Our methods are unreliable for the following reasons:

- Microorganisms are never uniformly distributed in the environment so reliable sampling is always an issue, especially at microbial densities
- Most microorganisms will not grow on standard microbiological growth media. The term "the great plate count anomaly" was coined by Staley and Konopka to describe the difference in orders of magnitude between the numbers of cells from natural environments that form colonies on agar media and the numbers countable by microscopic examination[3]
- As microorganisms in manufacturing facilities are frequently associated with shed skin cells, water droplets or dust particles, a colony-forming unit is most likely not derived from a single microbial cell
- Microbial levels are set below the limit of quantification where the accuracy and precision of the microbial counts are poor.

Equivalency requirement of new microbiological technologies

The USP general informational chapter <1223> Validation of Alternative Microbiological Methods contains a discussion of the limitations of the colony-forming unit and why microbial detection and enumeration methods that employ different signals to the colony-forming unit may not be equivalent to traditional methods. The signals may include laser-induced fluorescent particles, ATP bioluminescence, enzymic activity, headspace analysis and genomic weights. This was addressed in <1223> by broadening the validation options used to qualify an alternative microbiological method.

Four options are available to establish the equivalence of a candidate alternative analytical method: acceptable procedures (ie, merely meeting a minimum performance or acceptance requirement without a need to demonstrate equivalence to the compendial method); performance equivalence to the compendial method; results equivalence to the compendial method; and decision equivalence to the compendial method.

For example, light-induced fluorescent viable particle monitoring of air and pharmaceutical-grade water can provide continuous in-process monitoring data that is not equivalent to daily or weekly monitoring using colony-forming units obtained from plate counts but may be used to make superior decisions related to a loss of microbial control or adverse trends during sterile product manufacturing. As results are not equivalent, different alert/action levels and trending rules would need to be established, resulting in an improved level of environmental and operational control.[4,5,6] The positions taken in the revision to Annex 1 prevent the industry from taking advantage of this new technology.

Furthermore, real-time analysis of the in-process microbiological air quality is consistent with the principles of ICH Q8 Quality Risk Management (QRM), ICH Q9 Quality by Design (QbD) and Process Analytical Technology (PAT).

Conclusions

The working group needs to re-visit the proposed version and make additional changes.

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Advancing bacterial endotoxin testing with recombinant Factor C

Emerging recombinant Factor C (rFC) methods have many advantages to offer pharmaceutical quality control, including improved specificity, robustness, efficiency and alignment with 3R principles. First, global manufacturers have validated rFC and pharmacopoeias worldwide are on the move. Here, Karolina Heed provides an industry and regulatory update.

Why is there a need for alternatives to the Limulus-based reagents, and what are they?

For decades, the bacterial endotoxin test has relied on the blood cells of horseshoe crabs, which are currently assessed as vulnerable in North America and endangered in Asia by the IUCN Red List of Threatened Species. The Limulus amebocyte lysate (LAL) test is one of the few remaining tests still dependent on an animal source. Now it is, both ethically and practically, increasingly difficult to meet present and growing demand for reagents. That is where recombinant DNA technology comes into the picture; it has been well established for safe, affordable and sufficient production of synthetic reagents to replace animal sources. The molecular mechanism of the natural LAL reaction was first described in the 1980s. Central to this mechanism is the zymogen Factor C that functions as a natural biosensor responding to endotoxin. This essential role of Factor C was the reason that Ding *et al.* chose to express rFC as an alternative to LAL. Since then, further efficient and controlled rFC production processes have been established.

What actions are regulatory authorities currently taking?

Recent regulatory progress in the field of rFC endotoxin testing follows a decade of development efforts to establish high-performing methods. In September 2018, the US FDA for the first time approved a drug, Emgality from Eli Lilly (galcanezumab-gnlm), using rFC test for product release. To this end, rFC tests from two different suppliers had been extensively evaluated and compared to the LAL test, and validated according to USP chapters <1225> and <85> to replace LAL. A couple of months later, in December 2018, the European Pharmacopoeia published a world's-first draft of a general chapter for rFC; 2.6.32. *Test for bacterial endotoxins using recombinant factor C*. In February 2019, the Chinese Pharmacopoeia followed suit and published a draft chapter including the recombinant alternative to LAL. In the coming months, the US Pharmacopoeia will organise a workshop that includes alternative methods to LAL.

Who supplies rFC and how do the tests work?

Currently, two global suppliers, Lonza and bioMérieux, offer end-point fluorescence rFC tests for different applications ranging from high-throughput testing of water and in-process samples to product release and solutions for even the most challenging sample matrices. In rFC endotoxin tests, following activation by endotoxin present in a sample, rFC cleaves a substrate, which in turn gives a quantifiable fluorescence signal. All available rFC assays follow conventional bacterial endotoxin testing (BET) methodology in terms of acceptance criteria, and thereby provide absolute comparability of results. Method standardisation is essential, as companies replace LAL with rFC and perform the required method validation, eg, according to compendia chapters.

What are the advantages of rFC?

In addition to eliminating the animal source, rFC tests, such as bioMérieux's ENDONEXT assay range, also provide faster and easier workflows and more robust results. Lot-to-lot consistency, the exclusion of cross-reactivity with ß-glucan, and state-of-the-art sensitivity down to 0.001 EU/mL, are additional test advantages. Another important gain with rFC is the lowered rate of invalid results. In a recent evaluation study on water for pharmaceutical use, presented by Marine Marius from Sanofi Pasteur at the PharmaLab Congress in November 2018, the performance of the rFC test ENDOZYME II GO was compared with two LAL tests, including kinetic chromogenic and cartridge-based. Marius concluded that the rFC test was a suitable method for pharmaceutical waters and high-throughput testing. It showed very low variability between assays, best reproducibility and recovery of Control Standard Endotoxin (CSE) and Reference Standard Endotoxin (RSE), as well as the lowest rate of invalid results compared to the two LAL methods. All available data shows that rFC is a valid alternative to reagents harvested from animals in terms of purity, consistency and sustainability. ■

Karolina Heed, Global Solution Manager Endotoxins at bioMérieux

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30 APRIL-2 MAY 2019

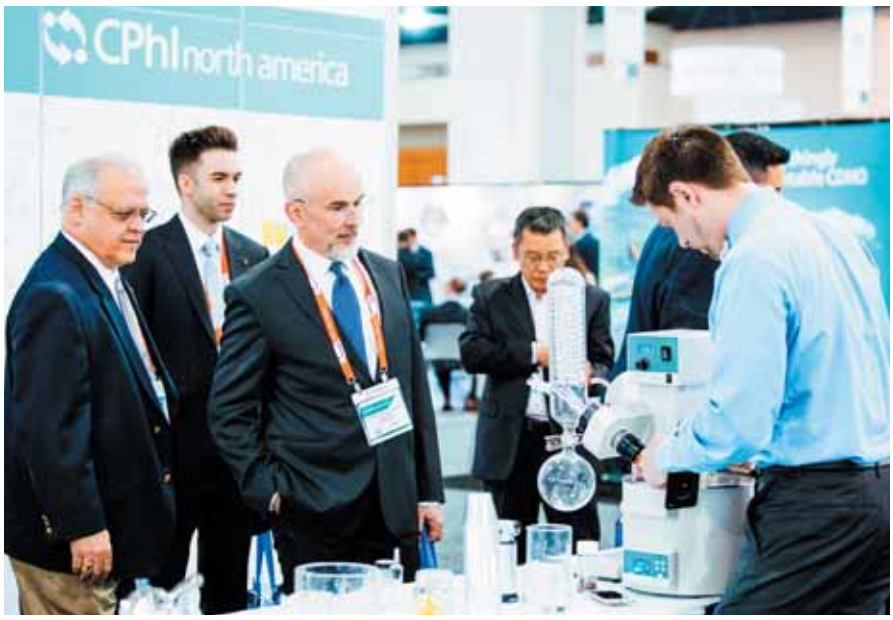
CHICAGO, US

For nearly 30 years, CPhI has organised premier, worldwide pharmaceutical events. Several annual gatherings comprise the global portfolio, but CPhI North America has become the critical link in a global chain connecting motivated buyers with industry-leading suppliers. In its third year, the event takes place at McCormick Place, Lakeside Center in Chicago, IL, from 30 April-2 May 2019.

CPhI NORTH AMERICA 2019 will feature seven strategic zones, that include the following:

- **NEW! bioLIVE:** The bioprocessing zone will unite the small and large molecule markets from the biopharma processing and manufacturing sectors.
- **CPhI:** The manufacturing ingredients zone will showcase APIs and their excipients in addition to their intersection with sustainability.
- **FDF:** The finished drug products zone will feature leading small and large molecule CDMO/CMOs for both excipients/formulations and finished dosage formulations.
- **iCSE:** The drug development zone will highlight CROs specialising in pre-clinical and clinical drug development research and analytical and lab services.
- **InformEx:** The specialty chemicals zone will showcase innovative molecule developments.
- **InnoPack:** The packaging zone will present pharmaceutical packaging highlighting sustainable, user-friendly and cost-effective solutions.
- **P-MEC:** The machinery zone will present innovations within pharmaceutical equipment, technology and machinery.

The conference programme features insights from more than 50 speakers that were selected to address topics of innovation in drug development and manufacturing.



Not to be missed is our keynote address by former Arizona Senator Jeff Flake. The former Republican Senator will give his opinions on pharma manufacturing, healthcare innovation and the direction of healthcare as he sees it under our current structure of divided government, as well as scenarios beyond 2020.

The show will also feature more than 50 hours of education on drug development, drug manufacturing and bioprocessing on the show floor theatres.

- **Insight Briefings Theatre** features thought-provoking seminars that take you inside the trends and technologies that are shaping the future of pharma.
- **Exhibitor Showcase Theatre** provides deep dives and customised presentations on the show's most sought-after solutions.
- **The BPI Theatre** in the bioLIVE zone will feature a diverse schedule developed to address hot button topics across biopharmaceutical research, development and manufacturing.

Returning this year is the **Women in Leadership Forum**, which will bring together female executives from across global pharma and chemical networks with the goal of uniting them to share experiences and trade knowledge.

Additionally, C&EN Media Group is bringing its next **Science Marketing Event**, open to all qualifying pharma marketing professionals. Join them to hear from event and media specialists, to learn best practices for achieving a comprehensive event marketing strategy.

While aiming to provide an insightful and educational experience for attendees, we are also focused on giving back to the community. We take part in many initiatives to make this possible, including:

- **Carbon mitigation:** we are aiming to reduce the carbon impact of our events by 11.4 percent by 2020 in an effort to reduce our contribution to climate change and its effects.
- **Waste management:** we plan to either reuse or recycle everything from our show both to reduce the amount of resources we use and the waste we create.

CPhI is proud to partner with International Medical Corps as our charity partner. International Medical Corps provides emergency relief to those struck by disaster – no matter where they are and what the conditions – working with them to recover, rebuild and gain the skills and tools required for self-reliance.

We are excited to bring CPhI North America to Chicago. With an expansive list of industry thought leaders in attendance, an abundance of networking opportunities, and the goal to foster innovation in our industry, we hope that the event and programme will be a truly valuable experience.

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Making Pharmaceuticals

30 APRIL-1 MAY 2019

COVENTRY, UK

Pharma heads to the Midlands

Making Pharmaceuticals returns to the Ricoh Arena in Coventry – one of the more recent jewels in the crown of this historic city – on 30 April-1 May. For two days, being sent to Coventry takes on an entirely new meaning as the city becomes the centre of the pharmaceutical world for the UK sector.

SINCE ITS launch in 2014, Making Pharmaceuticals has become the most important annual event in the pharmaceutical calendar and strives to achieve the following:

- An event that answers questions
- Offers attendees essential information to aid in pharmaceutical development
- Provide intellectual and material solutions to the major issues that the industry faces in delivering pharmaceutical products in an increasingly demanding sector.

Now in its sixth year, the event continues to galvanise and focus the efforts of the pharmaceutical industry on key challenges; ensuring pharmaceutical excellence is forefront in the development of new medicines, the navigation of new stringent regulations and the delivery of pharmaceutical products to the end user.

Unique in its approach to offering a pharmaceutical event to the pharma community, the two-day, comprehensive, technical conference at Making Pharmaceuticals is free to all attendees. It runs alongside the largest exhibition in the UK, where leading companies will be showcasing technologies and machinery, and offering their expertise.

Within the exhibition, companies will be offering services such as full product development, clinical trial manufacture, data integrity, serialisation software and hardware, regulatory advice, processing

machinery, pharmaceutical actives and excipients, packaging, water technologies, laboratory analysis, labelling, analytical equipment and a host of other products and services, all geared toward improving pharmaceutical product development.

The conference programme is technically comprehensive, with sessions from leading professionals and educational organisations, as well as presentations from key pharmaceutical experts and representatives from companies within the exhibition who are at the cutting edge of the pharmaceutical product lifecycle.

Five conference streams will run concurrently throughout the two-day conference, offering content that will help stimulate innovative ideas and develop products in an increasingly demanding and regulated industry.

Contributors to the 2019 programme include many of the leading professional associations that work to advance the UK pharmaceutical industry.

Register today at makingpharma.com where you will also be able to view the full list of exhibitors and the conference programme, or contact **clintonsturdey@stepex.com** for more information.

makingpharma.com

THE PHARMACEUTICAL EXCELLENCE AWARDS

The inaugural Pharmaceutical Excellence Awards will be taking place again this year at Making Pharmaceuticals. Celebrating the very best that the industry has to offer across four awards categories, the presentation ceremony takes place on the evening of 30 April at the Making Pharmaceuticals Dinner (RICOH Arena, Coventry). The four categories that the 2019 awards cover are:

- **Community Partnership of the Year**
- **Sustainable Achievement**
- **Innovation in Manufacturing**
- **Innovation in Distribution**

The shortlisted entrants for each of the awards are:

- **Community Partnership of the Year:** Ubichem Pharmaceutical and Clarity Compliance
- **Sustainable Achievement:** Veolia Water Technologies, Brenntag UK Ltd and iDi Pac Limited
- **Innovation in Manufacturing:** kg-pharma GmbH & Co.KG, iDi Pac Limited, Glatt Protech Ltd., Crystec Ltd. and SSPC, Bernal Institute, University of Limerick
- **Innovation in Distribution:** Hydropac UK Ltd. and Brenntag UK Ltd.

The night promises to be filled with laughter as the celebrity guest speaker is non-other than Shaun Williamson, famous for playing the legendary Barry in EastEnders, and more recently for appearances with Ricky Gervais in Extras and on Al Murrays' Pub Landlord.

Shaun is an ambassador for the British Wireless for the Blind Fund, which supplies specially adapted audio equipment to those that cannot afford it, and this is the officially supported charity for Making Pharmaceuticals 2019.

If you want to be a part of this glamorous night of dinner and awards for the pharma sector, tickets are available on the Making Pharmaceuticals website, at just £95+VAT per person. Go to: www.makingpharma.com/dinnerbooking/

Making Pharmaceuticals takes place at the RICOH Arena, Coventry from 30 April to 1 May, it is entirely free to attend the exhibition and conference, details can be found on the website: www.makingpharma.com

Chemspec europe

The fine & speciality chemicals exhibition

26-27 JUNE 2019

BASEL, SWITZERLAND

Transforming challenges into opportunities

Chemspec Europe 2019 explores novel chemical solutions to tackle new demands in politics and society.

AMIDST the current investment boom, UN climate talks and Brexit, the fine and speciality chemicals industry eagerly awaits their annual get-together at Chemspec Europe 2019 to discuss the latest market trends, technical innovations, business opportunities and regulatory issues in this rapidly-changing market.

Taking place on 26 and 27 June 2019 at Messe Basel in Switzerland – one of Europe's major hubs for the fine and speciality chemicals industry – Chemspec Europe 2019 unites an impressive array of experts, scientists, managers and thought leaders. The event provides a powerful industry platform to explore bespoke solutions, new approaches and innovative substances as well as discuss the latest market trends, technical innovations, business opportunities and regulatory issues in this rapidly-changing market. The event features a full spectrum of fine and speciality chemicals for various applications and industries, including pharmaceuticals.

The 2018 exhibition hosted 358 exhibitors and over 6,000 attendees from all over the world. The exhibitor list for the coming event reveals yet another strong line up, with the majority originating from European countries, as well as the US and Asia.

Operating in a highly-competitive market with complex regulations and environmental guidelines, the fine and speciality chemicals industry is currently experiencing a multitude of challenges from various sources whilst benefitting from high levels of investment, especially in green and bio-based technologies. "Many producers are busier than ever, but companies will have to manage change and innovate at unprecedented rates in terms of sustainability and operational excellence," says Liljana Goszdziewski, Exhibition Director of Chemspec Europe, on behalf of the organisers, Mack Brooks Exhibitions. "With the right mindset, the current challenges can be turned into opportunities for innovation and business growth."

Goszdziewski continues: "The upcoming Chemspec Europe unites an impressive array of experts, scientists, managers and thought leaders, all contributing to a powerful and well-known industry platform. Purchasers and agents have the chance to explore the latest technical advancements and to source specific ingredients or custom-made solutions in direct exchange with suppliers. The accompanying workshops and conferences offer plenty of additional opportunities to exchange industry know-how and form international relations with peers and business partners."

Extensive two-day conference programme alongside the exhibition

An outstanding two-day seminar programme provides further knowledge and insights into major industry developments as well as key strategies adopted by industry leaders to succeed and grow. All visitors and exhibitors of Chemspec Europe 2019 are invited to attend free of charge. The full conference programme and further updates will be published online.

Dedicated on-site areas

For the first time, visitors of Chemspec Europe can look forward to a new NanoTECH Pavilion, which exclusively highlights companies and organisations from the nanotechnology industry. The event features the following dedicated on-site areas: NanoTECH Pavilion, UK Pavilion, ORGANICA Feinchemie GmbH, and SOCMA Pavilion (Society of Chemical Manufacturers and Affiliates).

Chemspec Europe 2019 takes place on 26-27 June 2019 in Hall 1.0 of Messe Basel in Switzerland. Entrance tickets and the official show preview are now available. Visitors can sign up for the free newsletter to receive updates and notifications prior to the show. ■

chemspeceurope.com

HPLC 2019

16-20 JUNE 2019

MILAN, ITALY

The 48th international symposium on high-performance liquid phase separations and related techniques – HPLC2019 – is the largest, most recognised international appointment on high-performance liquid phase separations. The 2019 edition will take place in Milan, Italy from 16 to 20 June.

Scientific programme, main topics and speakers

HPLC2019 affords an insight into worldwide research that relates to the fundamental and practical aspects of separation science, with a focus on new and highly-relevant emerging trends. Key topics will include hyphenated techniques – most importantly liquid chromatography coupled to mass spectrometry (HPLC-MS), design and characterisation of stationary phases, micro- and nano-fluidics, supercritical fluid chromatography (SFC), capillary electrophoresis (CE) and their applications in proteomics, metabolomics, food analysis, characterisation of biopharmaceuticals and biosimilars.

There will be over 70 keynote speakers involved in the scientific sessions during the five-day conference. In addition, 16 short courses are scheduled on Sunday 16 June, where leading academic and industrial scientists will cover both the fundamentals as well as real-world application examples. All courses emphasise application and will provide valuable technical knowledge to implement techniques and solutions to improve job productivity and solve today's separation problems, as well as understand tomorrow's technology.

Call for abstracts and poster presentations

Poster displays will be arranged to allow intensive and comprehensive review and discussion between authors and delegates. The submission deadline for posters is 29 April, and these can be made through the HPLC2019 website. Don't miss the Best Poster Award – 10 awards will be delivered during the closing plenary session with winners receiving a certificate and a €300 cash prize.

Authors of both oral and poster presentations are invited to submit manuscripts based on their presentation(s) at the HPLC2019 Milan meeting for possible publication in the *Journal of Chromatography A* or *Journal of Chromatography B*, with the intention of publishing in a joint special issue dedicated to this symposium. For further information, visit our website.

Special events, awards and travel grants

Two new initiatives have been announced for HPLC this year: Separation Science Slam and HPLC Tube.

- **Separation Science Slam** is a competition for young scientists to present their research related to liquid chromatography workflow (before, during or after)
- **HPLC Tube** is a new scientific video contest, which requires no paper or abstract, but a video linked to the question: "How is your chromatography making a difference in the world?" *Deadline: 30 April.*

In addition to the Best Poster Award, six other awards will characterise the HPLC2019 symposium:

- Csaba Horváth Young Scientist Award
- Uwe D. Neue Award in Separation Science
- JFK Huber Lecture Award
- Georges Guiochon Faculty Fellowship
- Chromatographic Society Jubilee Medal
- Chromatographic Society Martin Medal.

To encourage the participation of young scientists, some of the most important scientific societies provide travel grants to participate at HPLC2019.

Social events

HPLC2019 Milan will maintain a conference tradition of organising an exciting meeting conducive to business, scientific and social exchange.

In addition to the scientific programme, the following social events are planned:

- The Opening Ceremony on Sunday 16 June at Milan Conservatorio "Giuseppe Verdi" – the largest music academy in Italy founded by Royal Napoleonic Decree in 1807. To celebrate the 500th anniversary since Leonardo's death, the opening ceremony will include a lecture by Professor Martin Kemp (Oxford University) on Leonardo with accompanying music.
- The Gala Dinner will take place on Wednesday 19 June at the "Central Courtyard" of Università degli Studi di Milano – a wonderful garden surrounded by neoclassical columns in the heart of Milan and a perfect Italian location to host guests from all over the world.
- Farewell drinks on Thursday 20 June at the Università Milano-Bicocca. This is an opportunity to celebrate and mark the end of the symposium, accompanied by a delicious cocktail.

Discover further details about HPLC 2019 on the official website.

hplc2019-milan.org

EVENTS DIARY

Keeping you up to date with forthcoming events in the industry

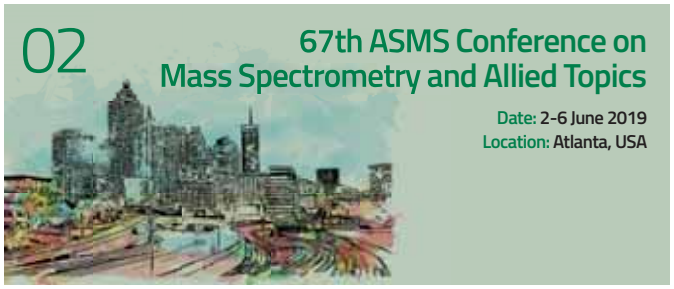
april

30 CPhI North America 2019
Date: 30 April-2 May 2019
Location: Chicago, USA

may

21 Labvolution 2019
Date: 21-23 May 2019
Location: Hanover, Germany

june



16 HPLC 2019 Milan
Date: 16-20 June 2019
Location: Milan, Italy

25 4th PDA Europe Annual Meeting
Date: 25-26 June 2019
Location: Amsterdam, The Netherlands

26 Chemspec Europe 2019
Date: 26-27 June 2019
Location: Basel, Switzerland

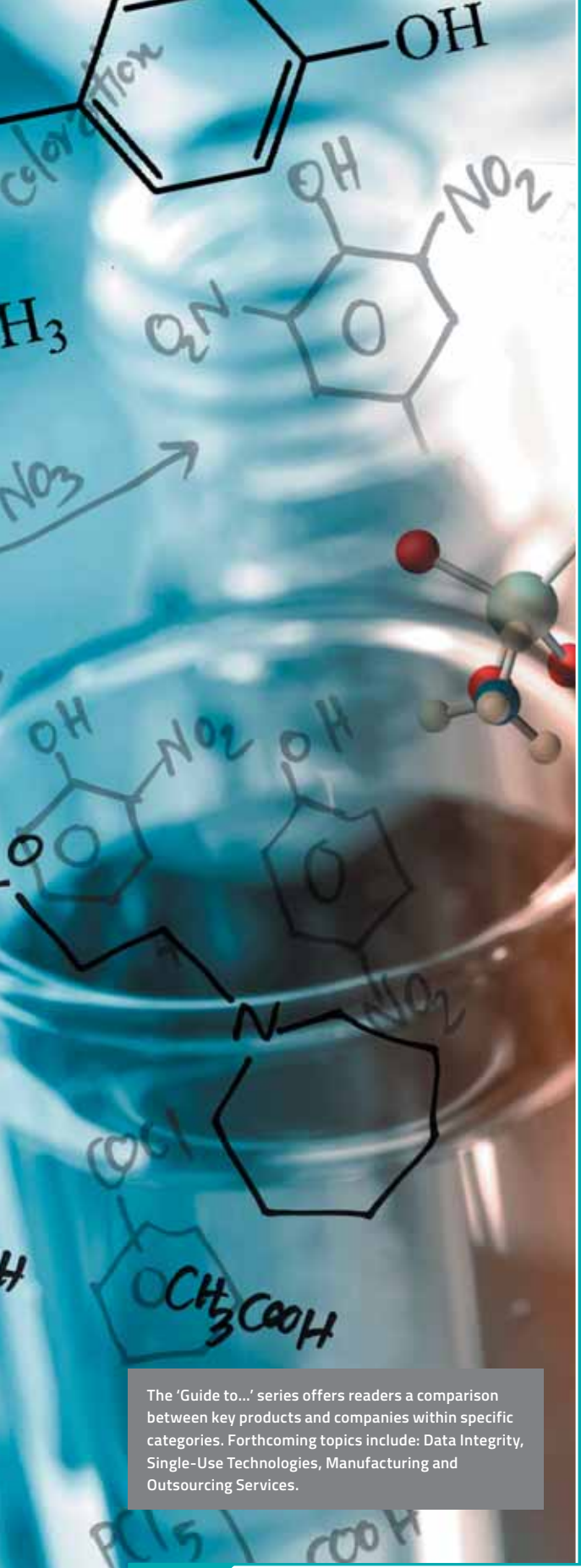
july

22 BPSA's 9th Annual Summit
Date: 22-24 July 2019
Location: Washington DC, USA

september

18 ISPE Europe Biotechnology Conference
Date: 25-26 September 2019
Location: Brussels, Belgium

For more information about other industry events, go to:
europeanpharmaceuticalreview.com/events



TESTING

Welcome to *European Pharmaceutical Review's* **Guide to Testing.** In this edition, five companies showcase their services and highlight how they stand out from the crowd.

ACC – Specialising in chromogenic and turbidimetric reagent technologies, Associates of Cape Cod, Inc. (ACC) has been a leader in endotoxin detection products and services for nearly 45 years. ACC pioneered LAL testing methodology and was the first FDA-licensed company to manufacture LAL reagents; ACC has grown to be an internationally recognised leader in endotoxin detection.

Charles River – Our microbial solutions portfolio of Endosafe endotoxin testing, Celsis rapid microbial detection, and Accugenix microbial identification and strain typing products and services facilitate confident and objective decision making, ensuring the integrity of the microbial data and minimising the risk to patients.

Eurofins – Eurofins BioPharma Product Testing offers complete CMC testing services for the bio/pharmaceutical industry, including all starting materials, process intermediates, drug substance, drug product, packaging and manufacturing support, through our broad technical expertise in biochemistry, molecular and cell biology, virology, chemistry and microbiology.

Nelson Labs – At Nelson Labs, we have a long history of partnering with pharmaceutical and biopharmaceutical companies. We perform a variety of testing to support your internal quality processes including: sterility (USP 71) testing in a cleanroom for isolator environment, particulates testing (USP 787, 788) using a variety of methods, bacterial endotoxin, filter validations, container closure integrity and USP compendia testing.

Wickham Labs – Wickham Laboratories Ltd, backed by five decades of global experience in GMP/GLP-regulated laboratory services, is an established name in the fields of pharmaceutical and medical device contract testing, research and consultancy.

The 'Guide to...' series offers readers a comparison between key products and companies within specific categories. Forthcoming topics include: Data Integrity, Single-Use Technologies, Manufacturing and Outsourcing Services.

ACC combines robust reagents, analysis and technical service expertise, to provide you with diverse solutions for endotoxin and glucan testing.

Associates of Cape Cod Int'l., Inc.
Your Endotoxin & Glucan Experts

CELEBRATING OUR 45 YEAR ANNIVERSARY

www.acciuk.co.uk • (+44) 151.547.7444

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 FDA-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.

ACC is headquartered in East Falmouth, US, with additional offices located in Liverpool, Knowsley, UK and Mörfelden-Walldorf, Germany. Both the East Falmouth and Liverpool offices boast Quality Control and CTS labs.

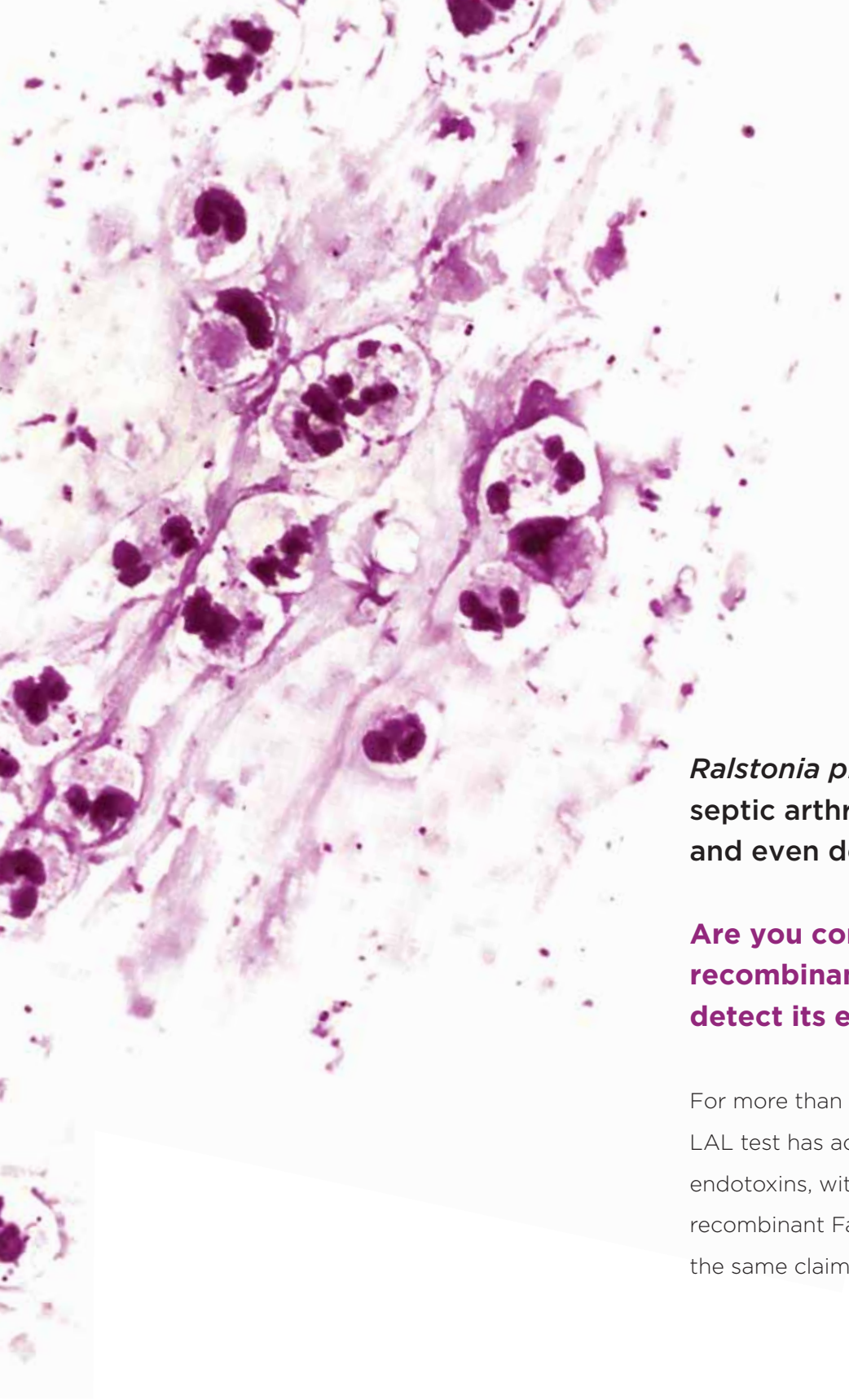
ACC is the only major provider of LAL reagents to exclusively focus on the endotoxin and (1→3)-ß-D-glucan testing space. The team at ACC is solely focused on providing the highest quality reagents with the absolute best technical support in industry. Additionally, ACC sells and supports their proprietary 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 0.001 EU/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 on 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

NAME: Associates of Cape Cod, Int'l., Inc.
HEADQUARTERS: East Falmouth, US
EMAIL: info@acciuk.co.uk
WEB: www.acciuk.co.uk

ASSOCIATES OF CAPE COD INCORPORATED
Specialists in Endotoxin and Glucan Detection



Ralstonia pickettii **can cause septic arthritis, osteomyelitis, and even death.**

Are you confident the current recombinant methods can detect its endotoxin?

For more than 40 years, the compendial LAL test has accurately detected bacterial endotoxins, without a single failure. Current recombinant Factor C products can't make the same claim.

Discover what's at risk at
www.criver.com/whatsatrisk

charles river

Charles River microbial solutions

Those who work in QC know that the job is more than a box to be checked, and that it can't be done effectively without confidence in the reported results. Tight timelines, regulatory demands, and stringent data integrity standards can make it hard to focus on what's really at stake in the QC process: the safety of the products and the lives of the patients.

What are the main services you provide?

Our microbial solutions portfolio of Endosafe endotoxin testing, Celsis rapid microbial detection, and Accugenix microbial identification and strain typing products and services facilitate confident and objective decision making, ensuring the integrity of the microbial data and minimising the risk to patients. Like our customers, patient safety is at the core of what we do.

What additional solutions do you provide?

Endosafe endotoxin detection

As cGMP and FDA-approved and licensed therapy manufacturers, the organisation our customers partner with for their solutions must also be held to those same standards. Our portfolio of FDA-licensed LAL products for rapid and traditional bacterial endotoxin testing solutions reduces retest rates, decreases variability, and improves turnaround times, enabling prompt, confident decisions about product safety.

Accugenix microbial identification and strain typing

Consistent and diligent EM practices are some of the best strategies to achieve operational improvements that eliminate risk to patient health. Our proprietary DNA sequencing and MALDI-TOF organism libraries are continuously optimised to maximise the accuracy of species-level identifications. We identify over 100,000 environmental isolates every year, enabling us to expand our organism libraries based on real samples frequently recovered from QC labs around the world, and to create the most relevant database for the pharmaceutical and medical device industries.

Celsis rapid microbial detection

When it comes to critical assays such as final product sterility, confidently finding nothing ultimately means everything. Celsis rapid microbial detection determines a product's sterility by providing a definitive yes or no answer to the most critical of decisions. Through reagent-catalysed amplified ATP bioluminescence rapid detection, our technologies can detect even the lowest levels of microbial contamination a week faster than the traditional method, unlocking new efficiencies to the QC workflow and a new level of confidence in the safety of our customers' products.

What makes your company stand out in the field?

At Charles River, we support developers and manufacturers from discovery through product release to deliver therapeutics to the patients who need them most.

As quality issues persist as a driving force behind product shortages, recalls and FDA warning letters, it is imperative that we identify ways to improve the quality control process. For more than 30 years, the Charles River microbial solutions team has continued to cultivate a portfolio of leading-edge technologies and services to keep you ahead of the curve. Our solutions are designed to streamline workflows, ensure the integrity of test data, and allow job completion with the confidence of total quality control. ■

COMPANY DETAILS

NAME: Charles River Microbial Solutions
CONTACT: Mélancolie Spedito-Jovial
EMAIL: askcharlesriver@crl.com
WEB: www.criver.com/whatsatrisk

charles river | microbial solutions

Largest scope of global services.

Sharpest focus on data integrity.

eurofins | BioPharma Product Testing

www.eurofins.com/biopharma

From Starting Materials through Finished Product Testing, Eurofins BioPharma Product Testing's 34 facilities in 17 countries deliver the world's most comprehensive scope of harmonized GMP testing services and seamless regulatory acceptance.

As we have grown to become the world's largest network of GMP product testing labs, we continue to uphold our founding promise of personal service and impeccable quality.

When the world awaits your product, choose the lab that provides complete capabilities and rigorous quality systems you can trust.

Comprehensive GMP Testing Services

Method Development & Validation • Release Testing • Stability Testing & Storage
Cell Banking Services • Virology Services • Facility & Process Validation
Chemistry • Biochemistry • Molecular & Cell Biology • Microbiology
Raw Materials Testing • Primary & Secondary Package Testing

Flexible Service Models

Fee For Service (FFS)
Full-Time-Equivalent (FTE)
Professional Scientific Services® (PSS)

Global Facilities

Australia	Denmark	India	Japan	Spain	UK
Belgium	France	Ireland	Netherlands	Sweden	US
Canada	Germany	Italy	New Zealand	Switzerland	



Eurofins BioPharma Product Testing

What are the main areas that you can test for?

Eurofins BioPharma Product Testing offers complete CMC testing services for the bio/pharmaceutical industry, including all starting materials, process intermediates, drug substance, drug product, packaging and manufacturing support, through our broad technical expertise in biochemistry, molecular and cell biology, virology, chemistry and microbiology.

Our breadth of services include:

- Method establishment (development, validation, transfer)
- Release testing
- Stability testing and storage
- Characterisation
- Residuals and impurities testing
- Raw materials testing
- Extractables and leachables testing
- Container and package testing
- Shipping studies
- Viral clearance and viral safety testing
- Bioassay and potency testing
- Cell banking services
- Critical reagents/reference standards management
- Disinfectant efficacy/cleaning validation studies
- Environmental monitoring
- Facility and process validation
- Organism identification
- Clinical trial material support
- Formulation development/testing
- Custom synthesis and radiolabelling.

What additional services/ solutions do you provide?

Our fundamental philosophy is to help clients efficiently allocate their research and manufacturing expenditures by strategically engaging them to meet their unique outsourcing needs. We offer clients the flexibility to manage testing programmes more efficiently through the choice of three unique service models. In addition to the most commonly used method in the industry, Fee-for-Service, we offer additional options, such as FTE and PSS insourcing programmes to allow choice of the best, most cost-effective service solution for your project goals at any of our global facilities.

Our FTE programme provides you with dedicated, full-time employees to work on your projects within our GMP facilities. Managed by us, your dedicated FTE employees will use our infrastructure, equipment and consumables to meet your project testing needs. Our team leaders manage your projects, direct the priorities of the team and can even integrate our operations with client systems/SOPs.

Our PSS Insourcing Solution programme places full-time scientists and technical support personnel, managed by us, directly at your facility to provide a non-permanent, long-term and cost-effective way to meet your staffing needs, while maintaining the same services, expertise and cGMP compliance available at our facilities.

What makes your company stand out in the field?

With a global capacity of more than 150,000 square metres and more than 34 facilities located in Australia, Belgium, Canada, Denmark, France, Germany, Ireland, Italy, India, Japan, Netherlands, New Zealand, Spain, Sweden, Switzerland, UK and the US, our network of GMP laboratories and vast experience allow us to support projects of any size from conception to market. Further, we have teams of scientists placed at more than 70 client facilities worldwide through our PSS Insourcing Solution.

Our local presence ensures personal service backed by a unique global breadth of harmonised capabilities that support all functional areas of bio/pharmaceutical drug development and manufacturing, including method development, microbiology, process validation and quality control.

We continue to expand our facilities, enhance our capabilities and service models, and continually invest in information technology (IT) to support our steadfast commitment to data quality and data integrity.

From the development of our secure, online data access portal LabAccess.com in 2007 to our recent efforts to develop and deploy our global Laboratory Management System (LIMS) and Electronic Laboratory Notebook (ELN) platforms, we continually develop solutions that further enhance data quality, data integrity and data accessibility for our clients.

We also continue to aggressively make investments to enhance and expand our market-leading biologics capabilities across our global network of Eurofins BioPharma Product Testing laboratories, as well as continuing to drive geographic expansion. All of this is focused on serving our clients with the world's largest and most comprehensive group of harmonised GMP laboratories. ■

COMPANY DETAILS

NAME: Eurofins BioPharma Product Testing
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WEB: www.Eurofins.com/BPT

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Safeguarding Global Heath™

- with every test we complete.

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BACTERIAL ENDOTOXIN
STERILITY TESTING (USP/AAMI)
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COMPENDIAL TESTING
REAL TIME &
ACCELERATED STABILITY

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Nelson Labs: supporting internal quality processes

At Nelson Labs, we have a long history of partnering with pharmaceutical and biopharmaceutical companies. We perform a variety of testing to support your internal quality processes including: sterility (USP 71) testing in a cleanroom for isolator environment, particulates testing (USP 787, 788) using a variety of methods, bacterial endotoxin, filter validations, container closure integrity and USP compendia testing. We have the expertise to support this testing whether the product is terminally sterilised with gamma, e-beam or ethylene oxide.

ADDITIONAL analytical services specialised for the pharma and biopharma industry include: stability testing, extractables and leachables, impurities and heavy metals. Quality control of APIs, raw materials, intermediates, finished products, impurities and degradation products.

We can help to support your facility with environmental and water system testing and validate cleaning processes inside the clean room. Together with our sister company, Sterigenics, we can help you evaluate alternatives to aseptic processing with a variety of terminal sterilisation methods.

What are the main areas that you can test for?

From development to delivery, we help get your products to market. Test services include:

- Extractables and leachables
- Biocompatibility and toxicology
- Sterilisation validations
- Sterility assurance
- Packaging solutions
- Facility and process validation.

Number of testing services/locations

With the addition of Nelson Labs Europe, we are the leading global extractables and leachables lab testing platform. Our scientists, technicians and service specialists diligently perform more than 700 rigorous tests in 13 global laboratory locations. With decades of expertise, we stand behind the quality of our results and the strength of our customer partnerships.

What makes your company stand out in the field?

Every year, hundreds of pharmaceutical and biopharmaceutical companies make Nelson Labs their testing laboratory of choice. For them, the decision is easy. Nelson Labs is a clear leader in the microbiology and analytical testing industry, offering more than 700 laboratory tests and employing more than 850 scientists and staff in state-of-the-art facilities. We are known for exceptional quality and rigorous testing standards, but it's our focus on the bigger picture that sets us apart. We look beyond test results and partner with you to achieve your long-term business goals — mitigating risk, being first to market and succeeding with your customers.

Why choose us?

Companies choose Nelson Laboratories for our:

- Thought leadership and approachable experts. We give you direct access to industry authorities who understand your business and add value every step of the way.
- Customer-centric culture. We take the time to understand your vision. Your goals become our goals.
- Real-time project management tools and a dedicated client portal. We provide proactive information, keeping you informed and in control.
- Metric-driven testing processes. We are our own toughest customer, holding ourselves to goals for things that matter most to you; like quality, turn-around time and testing accuracy.
- Expertise and support in global compliance. We act as a trusted advisor, helping you navigate the ever-changing compliance landscape.

See how we can help you mitigate your risk, be first to market and succeed with your customers. ■

COMPANY DETAILS

NAME: Nelson Labs
EMAIL: sales@nelsonlabs.com
WEB: www.nelsonlabs.com

Nelson Labs®
A Sotera Health company

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 GMP/GLP-regulated laboratory services, is an established name in the fields of pharmaceutical and medical device contract testing, research and consultancy.

DURING this time, we have been involved in testing for a wide range of industries. Each of these experiences has provided us with a unique skillset and strong scientific background, which we now apply to our specialised microbiology and toxicology services.

What are the main areas that you test for?

We supply microbiology and toxicology testing services for medical device and pharmaceutical industries, including:

- Absence of specified pathogens
- Antimicrobial/preservative efficacy
- Bacterial endotoxin (BET) and monocyte activation test (MAT)
- Bioburden determination
- Biological indicator enumeration
- Cytotoxicity
- In vitro diagnostic assays such as ELISA, BCA and Western blot
- Rapid microbial identification via MALDI-ToF
- Microbial ingress
- Microbial limits including TAMC/TYMC
- Potency bioassays and abnormal toxicity on biological products
- Rabbit pyrogen (RPT)
- Stability storage and testing
- Sterility
- USP plastics class I-VI tests.

What additional services/ solutions do you provide?

As well as laboratory testing, we offer global support and consultancy services relevant to a wide range of pharmaceutical and medical device development and manufacturing concerns, such as:

- Assay development
- Biological safety assessments
- Environmental monitoring assessments
- Process validation/identification of contamination sources in manufacturing
- Training on appropriate cleaning practices
- Validation of water systems.

Number of testing services/locations?

We have one main testing centre at our Hoeford Point location, with approximately 4,000 square metres (42,000 square feet) of space segregated into independent laboratory areas, which enables us to provide a wide range of testing services.

What makes your company stand out in the field?

Decades of global experience in testing services means we have developed strong technical expertise, which we can draw upon when identifying challenges and providing solutions for a broad range of products and testing scenarios. This long-standing global experience in contract testing enables us to assess our clients' bespoke requirements and advise on the best path forward for their projects.

We believe that supporting our clients at all stages of the testing lifecycle is invaluable for building strong, long-term relationships; communication has always been a key priority for us. We focus on responsiveness and flexibility, and ensure technicians are accessible to guarantee requirements are clearly communicated and products are tested to the satisfaction of both regulators and clients. In addition, our business managers in both toxicology and microbiology have regularly supported clients in understanding the full range of testing required for their regulatory submissions.

Other information that would be helpful?

In addition to the discrete laboratories themselves, we 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. The majority 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 a 24/7 onsite security team. We are also equipped with a team of in-house qualified service engineers, enabling a self-sufficient facility without major service disruptions. ■

COMPANY DETAILS

NAME: Wickham Laboratories
EMAIL: mail@wickhamlabs.co.uk
WEB: www.wickhamlabs.co.uk

Wickham Laboratories
Contract Analytical Services

Catalent®

DEVELOPMENT

your molecule has so much potential. our passion for development will help unlock it.

As the #1 global leader in drug development, Catalent's comprehensive capabilities, robust scientific approach, and broadest selection of enabling technologies can help you overcome the toughest development challenges. Our formulation experts have experience with thousands of new molecules to customize your project, doing it right the first time based on science and technology fit.

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BIOAVAILABILITY ENHANCEMENT
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EXPANDED OPTIFORM® SOLUTION SUITE

One accelerated, flexible, and data-driven solution combines all analytics, services and materials your molecule needs from candidate selection into Phase 1.

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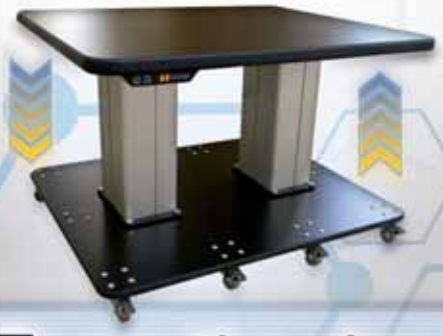
Mass Spectrometry benches & LC elevating tables



Contributes to
Lab Safety & Productivity

Increase
LC/MS Performance

Ease *the day-to-day use*

of your **LC/GC/MS** *system*

Movable

-75%
noise reduction
performance

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contact@ionbench.com