MICROBIOLOGY

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

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

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

Specificity in the recombinant factor C test for endotoxin

Kevin L Williams

bioMerieux

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

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

BIOGRAPHY

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

Level 1. Enzymatic specificity

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

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

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

Level 2. Spectral specificity

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

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

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

Geisler also outlines the general advantages of fluorescent methods.

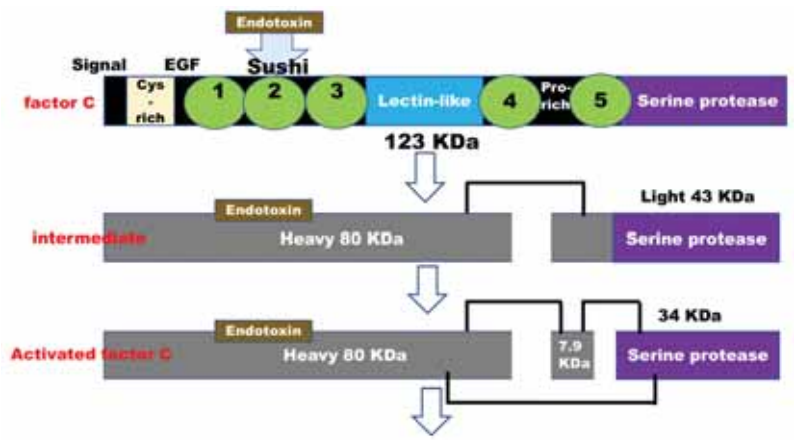
- Sensitivity: the sensitivity of fluorescence detection is approximately 1,000 times greater than absorption spectrophotometric methods
- Specificity: only molecules that fluoresce are detected by this method, resulting in greater specificity compared with UV / Vis absorption
- Wide concentration range: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

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

Level 3. Genetic specificity

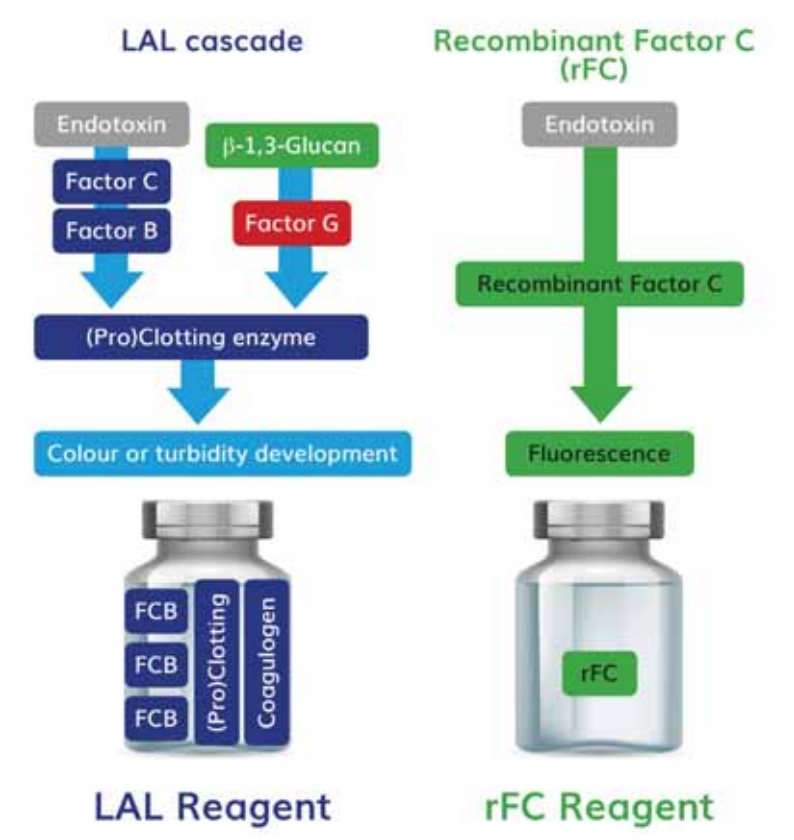
It is good to remember where the rFC protein has come from. See ***Figure 4***. The horseshoe crab factor C gene was originally cloned from *Carcinoscorpius rotundicauda* at the National University of Singapore by Jeak Ling Ding and Bow Ho.[9] DNA recombinant technology was developed in the 1970s and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

FIGURE 1



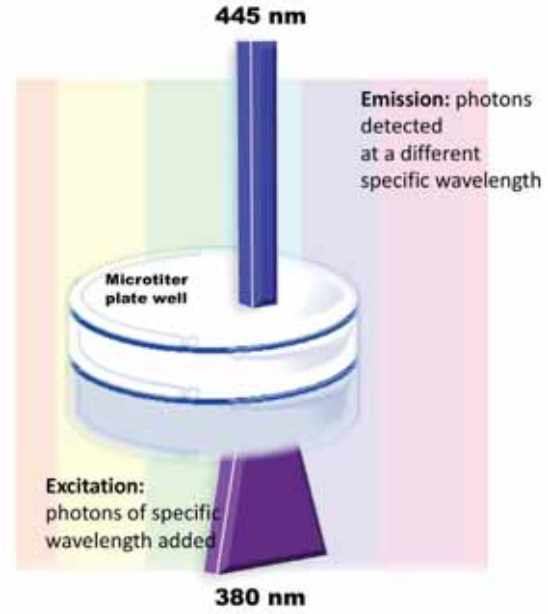
ABOVE: Activation of factor C activates factor B (LAL) or releases fluorophore (rFC)

FIGURE 2



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

FIGURE 3



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

REFERENCES

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

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

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

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

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

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

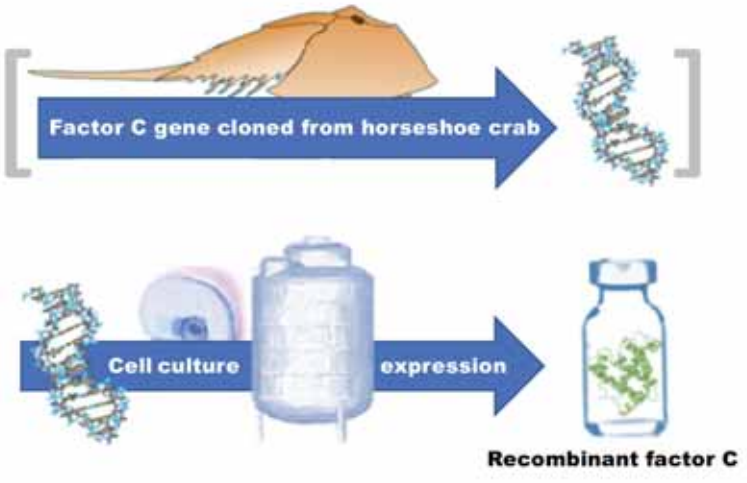
In January 1922, Leonard Thompson, a 14-year-old boy dying from diabetes in a Toronto hospital, became the first person to receive an injection of insulin. Within 24 hours, Leonard's dangerously high blood glucose levels dropped to near-normal levels.

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

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

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

FIGURE 4



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

BETTER FOR YOUR LAB.
BETTER FOR THE PLANET.

ENDONEXT™ endotoxin detection assays from bioMérieux are ushering in a new era of smarter, more sustainable pharmaceutical quality control.

Based on Recombinant Horseshoe Crab Factor C (rFC), ENDONEXT™ technology not only eliminates the need to harvest horseshoe crab blood—it makes your entire lab more efficient. With 100% endotoxin specificity, lot-to-lot consistency, and more streamlined workflows, ENDONEXT™ provides reliable results everywhere from in-process controls to final product testing on the most challenging matrices.

biomerieux-industry.com/endotoxin

BIOMÉRIEUX

The latest trends in pharmaceutical microbiology

Jeanne Moldenhauer

Excellent Pharma Consulting

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

BIOGRAPHY

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

The Human Microbiome Project (HMP)

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

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

Microorganisms become the active ingredient in pharmaceuticals

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

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

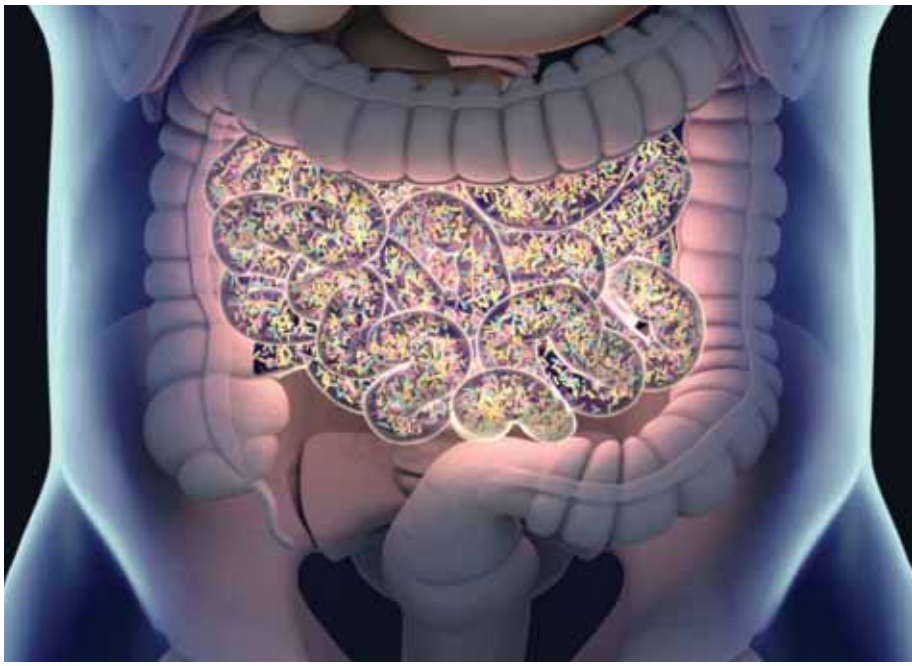
Even some bacteriophages are used in topical products – an active ingredient that is a virus that infects replicates within a bacterium.[3]

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

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

Culture media

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



ABOVE: Gut bacteria in the small intestine

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

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

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

Alternative or rapid microbiological methods

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

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

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

Identification systems

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

Pathogen detection

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

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

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

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

Antibiotic resistance / sensitivity

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

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

REFERENCES

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

Overcoming the challenges of data security in the microbiology lab

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

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

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

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

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

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

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

Mélancolie Spedito-Jovial, Europe Business and Marketing Manager, Charles River

charles river | microbial solutions

www.criver.com/microbialsolutions

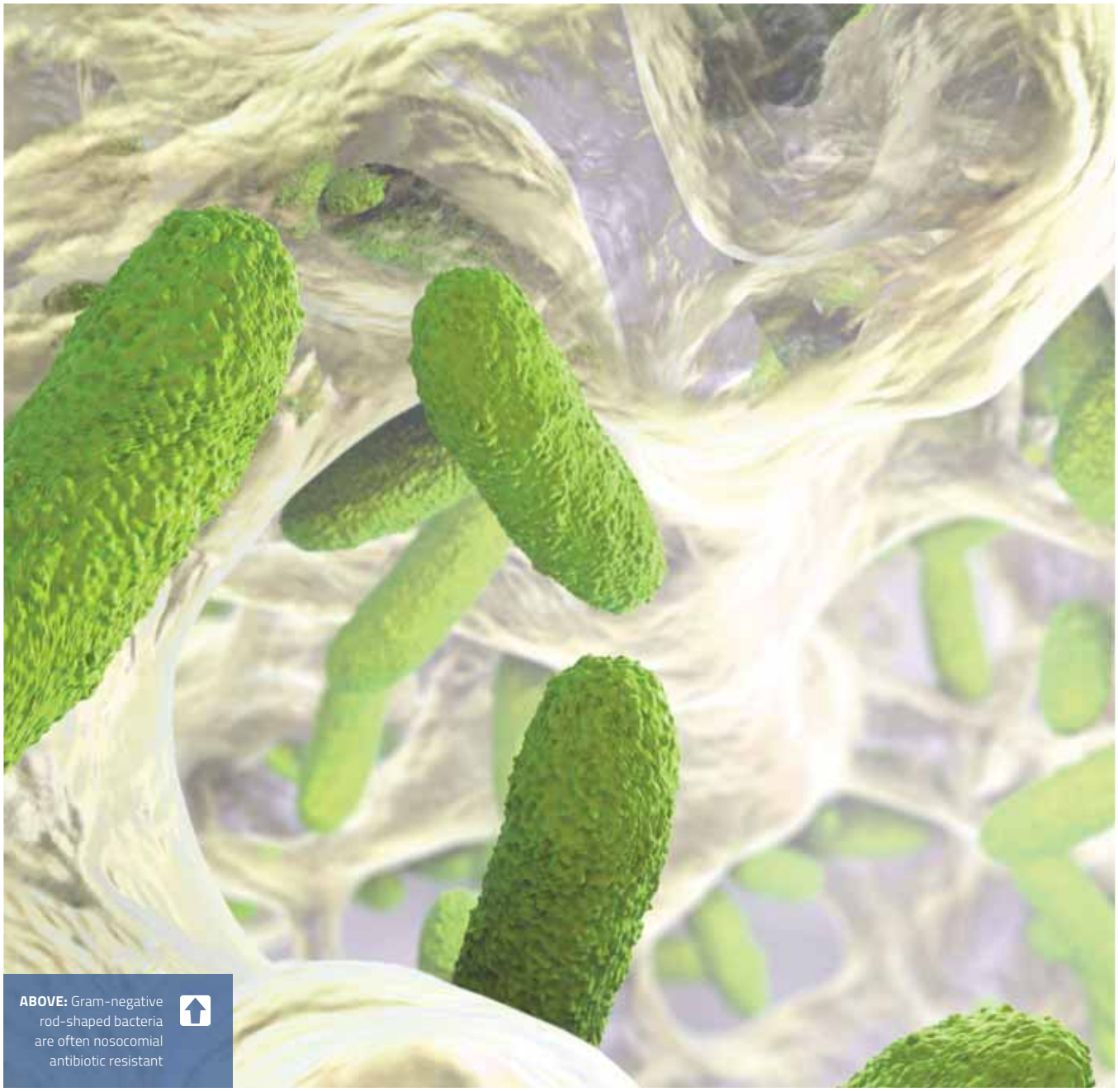
The changing environment in pyrogen and endotoxin testing

Marsha Steed
Senior Consultant,
Concordia Valsource

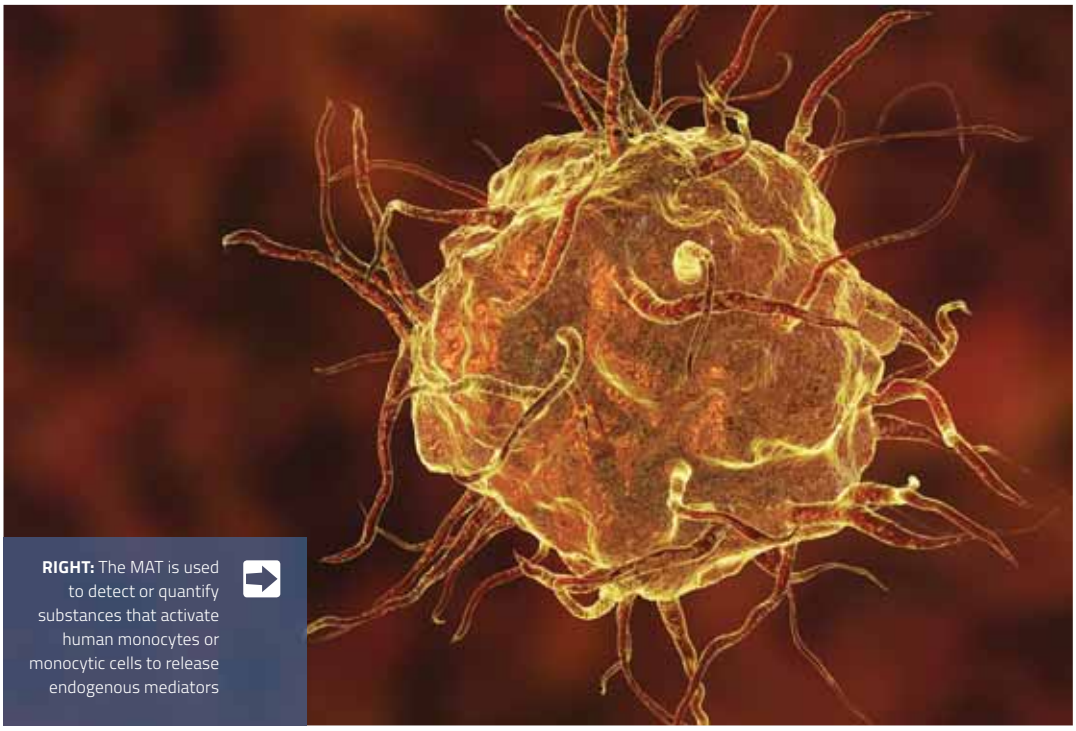
Johannes Reich
Endotoxin Testing,
Microcoat

Josh Eaton
Senior Project Manager,
Parenteral Drug Association (PDA)

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



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



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

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

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

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

The MAT method

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

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

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

Pyrogenic substances beyond endotoxin

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

BIOGRAPHY

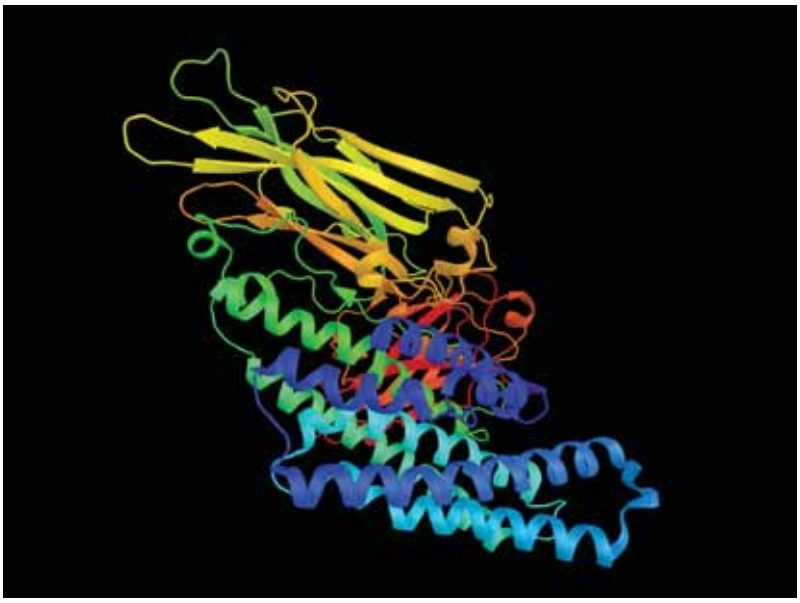
MARSHA STEED, Senior Consultant with Concordia Valsource, has over 20 years of experience in the pharmaceutical, biopharmaceutical, cell therapy / gene therapy and medical device industries with extensive experience in quality and microbiology. Her leadership experience includes global experience at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

BIOGRAPHY

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

BIOGRAPHY

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



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

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

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

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

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

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

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

REFERENCES

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

CONTEC®

Contec is a leading manufacturer of contamination control products for critical cleaning and disinfection in cleanroom manufacturing environments worldwide.

Contec's range of sterile alcohols and disinfectants is specifically designed for life science cleanroom use. A choice of 70% IPA or denatured ethanol is available with guaranteed low levels of endotoxins. Contec ProChlor is one of the fastest acting sporicides available with a log 6 kill against spores in 1 min. Contec HydroPure leaves no residue so is ideal for product contact areas. New products will be added to the cleanroom disinfectant range this year. All current and new products will be supported through the Biocidal Products Regulation registration process.

For more information please visit **www.contecinc.com**