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COVER FEATURES

IN THIS ISSUE »

It's Easy To See

BET Sustainability

LAL Reagent Comparison Table	Conventional LAL Reagent	**ACC's PyroSmart NextGen® (rCR) Reagent**	First Generation Competitor (rFC) Reagent
Sustainable Reagent (animal free)	**No**	✓ **Horseshoe Crab Blood Free**	✓ **Horseshoe Crab Blood Free**
Kinetic Assay	Kinetic	✓ Kinetic	✗ **No.** Endpoint only
Assay Setup	Single step reconstitution	✓ Single step reconstitution	✗ **No.** rFC requires three reagents in a 1:4:5 ratio and a 10 min. pre-incubation step
Same Standard Plate Reader	Incubating plate or tube reader at 405 nm	✓ **Yes.** Incubating plate or tube reader at 405 nm	✗ **No.** Fluorescent reader required
Derived From *Limulus* Amebocyte Lysate (LAL)	LAL	✓ **Yes.** rCR is recombinant LAL	✗ **No.** Based on *Carcinoscorpius* or *Tachypleus* Amebocyte Lysate (CAL/TAL)
Multi-step Cascade Pathway	Yes	✓ **Yes**	✗ **No**
Endotoxin Specific	No	✓ Endotoxin Specific	✓ Endotoxin Specific

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Message from the Editor

Transitions

Have you heard the term: liminal space?

I first learned of the term while watching the excellent AppleTV series Severance.

Without giving too much away, the series is about a team of office workers whose memories have been surgically divided between their work and personal lives, as the series progresses, and you learn more about each character you find out why each has chosen to have the procedure done. The office building that they work in is both familiar and eerie at the same time – which – as I found out is intentional, and a perfect example of liminal spaces.

So, what exactly is a liminal space?

I found a Forbes Health article that does a good job defining the term:

> *Derived from the Latin word "limen" which means "threshold," liminal space is a concept that may sound unfamiliar, but it's something you've likely experienced in your daily life.*
>
> *"Liminal space can best be described as going through a change or going from place to place—from one thing to the next," says transitions expert Melissa Cohen, a licensed clinical social worker in New York and founder of the mental wellness website A Redefined You. "It's the space between what is and what will happen next," she says. In other words, liminal space refers to the actual space or time in which you shift from one phase to another.*
>
> *What's interesting about this concept is not only does everyone experience it at some point in their lives, but some people also tolerate it better than others, experts say. And psychologically, it can have positive or negative ramifications. But there are ways to learn to thrive in liminality*

Less than two miles from my house is a small strip mall which, unfortunately, suffered during the pandemic. Almost all of the stores have closed – including the movie theater, diner, pet shop, and a bunch of others. The Macy's that was the "anchor" store also closed – and then was hastily converted into a "Vaccine Mega Site".

I think the site has been closed for at least a year – maybe more. But as you can see from the photo the sign still hangs on the building – faded – and a reminder of the worst of times during the pandemic.

Looking at this banner the other day – it gave me that "liminal" feeling. The feeling of transition, from one stage of life to another.

Are we "done" with COVID-19? I don't think so. This fall will see an uptick in cases, and a new vaccine. But, like the flu vaccine the yearly COVID-19 vaccines will become a fact of life.

We will all transition through our own liminal spaces – and learn to thrive.

Also – go watch Severance. It's so good.

Mike Auerbach
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« Editorial Advisory Board

CNPerspectives

CompareNetworks

American Pharmaceutical Review is one of several outstanding publications available from CompareNetworks, Inc. Here is a look at the insightful content our readers may enjoy from four of our sister resources: *Pharmaceutical Outsourcing*, Biocompare, Labcompare, and *Tablets & Capsules*.

How to De-Risk Clinical Trials in Today's Complex Environment

Pharmaceutical Outsourcing™

As the biopharmaceutical industry continues to face extraordinary challenges, it is becoming increasingly important for companies to identify strategic ways to balance managing financial risks with testing drugs that have the greatest potential for health impact. With this in mind, we need to better understand why the clinical trials landscape is becoming increasingly volatile, and what effect that may have in the future.

bit.ly/43FgpgY

A Guide to Endothelial Cell Markers

Biocompare

Endothelial cells line the interior surface of the circulatory system, forming a barrier that regulates the transport of fluids, substances, and cells to and from various tissues. This system includes blood vessels, which transport nutrients and oxygen to tissues throughout the body, and lymphatic vessels, which drain interstitial fluid; both serve as conduits for immune cell trafficking. Although most validated endothelial cell markers label blood vessel endothelial cells and lymphatic endothelial cells indiscriminately, some label lymphatic cells exclusively. Emerging datasets generated by next-generation sequencing have uncovered novel endothelial markers, enabling the identification of new endothelial subsets. However, whether these transcriptional markers are suitable for protein detection methods remains to be seen.

bit.ly/3Dt8BEM

Targeting the Undruggable: Methods to Enhance Targeted Protein Degrader Discovery, Optimization

labcompare

Abnormal protein expression or activity has been linked to the development of many devastating conditions, including cancer, autoimmune diseases, and neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's. These conditions often involve the misfolding, aggregation, or accumulation of proteins, leading to cellular dysfunction and disease pathology. What if these disease-causing proteins could be selectively eliminated to potentially cure the condition? This is the premise behind targeted protein degradation (TPD), an emerging drug modality that offers the potential to probe biological pathways and target proteins that have previously been considered "undruggable."

bit.ly/44Fcrq2

Re: 'Sourcing': Qualifying Excipient Vendors Can Strengthen Supply Chain

Tablets&Capsules

Qualifying multiple, interchangeable sources of excipients used in the manufacture of a drug product could reduce the potential impact of future global supply chain disruptions. However, careful consideration must be taken to ensure that unintended consequences do not result from limited evaluation of alternative sources. There are other good business reasons that qualification of multiple excipient sources should be considered during the drug product lifecycle—from formulation development, through commercial manufacturing.

bit.ly/44F7kGk

Social Media Connections

U.S. FDA
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@FDACBER is hosting their next virtual town hall Aug. 30 to answer questions related to nonclinical assessment of cell therapy and gene therapy products. Registrants can submit questions in advance of the event. Register now: https://bit.ly/3O0ds65

PhRMA
@PhRMA

Just released: The latest Patient Experience Survey reveals the difficulties patients experience with accessing and affording health care, including practices that put up barriers between patients and their medicines and jeopardize access to innovation: https://onphr.ma/3Q7gFTa

U.S. Pharmacopeia
@USPharmacopeia

USP has officially launched the Continuous Manufacturing Knowledge Center (CMKC), an online tool accessible anywhere at any time, allowing unlimited opportunities to research & collaborate. Sign up for free now to become a #CMKC Community member. https://cmkc.usp.org

World Health Organization (WHO)
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Health and care workers play an important role in helping people understand and use #SelfCare approaches and tools – whether to self-test for pregnancy, COVID-19 or HIV, manage their fertility or self-monitor chronic health conditions https://bit.ly/3OqQMfv

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New public consultation open! Comment now on a draft reflection paper on artificial intelligence & machine learning in the development, use and regulation of medicines for people and animals.

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Data published in @NEJM on our Group B streptococcus (GBS) maternal vaccine candidate suggest that maternal vaccination may help protect infants against invasive GBS. Read more: https://pfizer.com/news/press-release/press-release-detail/pfizer-announces-new-england-journal-medicine-publication

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Advanced Nanopharmacotherapies for Ocular Diseases

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Abstract

Vision impairment and blindness are critical problems worldwide. Major causes of vision loss include age-related macular degeneration (AMD), diabetes, retinitis pigmentosa (RP), and glaucoma. In order to improve treatment outcomes, multimodal imaging using photoacoustic microscopy (PAM), optical coherence tomography (OCT), fundus photography, and fluorescence imaging-guided treatment approaches can be utilized. This review explores novel nanopharmacotherapy treatments for a variety of ocular diseases. In the first section, the use of silicon nanoneedle embedded contact lenses for treatment of ocular angiogenesis including corneal neovascularization (CNV) is investigated. Next, combination chemotherapy and photothermal therapy with gold nanoparticles as drug delivery agents for the treatment of ocular tumors is discussed. Limitations and future applications are addressed.

Introduction

Ocular diseases cause hundreds of millions of people to experience vision loss and blindness. In 2020, more than 596 million people worldwide had been diagnosed with vision impairment, and its incidence is expected to increase to 895 million by 2050.[1] The development and progression of several ocular diseases such as AMD, RP, and glaucoma are still not fully understood, although there is evidence demonstrating that retinal pigment epithelial (RPE) or photoreceptor degeneration, ocular inflammation, development of angiogenesis, or accumulation of metabolic waste can be hallmarks of these diseases.[2] To date, many treatment methods have been investigated for these diseases.[3] To remove corneal angiogenesis, therapies include anti-vascular endothelial growth factor (VEGF) topical therapy[2] and conventional laser photocoagulation,[4] but they both carry suboptimal efficacy and significant side effects.

To visualize the effectiveness of these treatments, multimodal imaging can be used. Optical coherence tomography (OCT) is a non-invasive technique that produces cross sectional images of the retina. Retinal damage such as RPE death and neovascularization can be readily visualized with OCT.[5,6,7,8] Photoacoustic imaging is non-invasive and uses non-ionizing laser pulses. Chromophores like hemoglobin, melanin, and lipids absorb the laser light, generating acoustic waves.[9] Using various contrast agents, photoacoustic microscopy (PAM) imaging can provide high resolution and high depth images of the retinal vasculature as well as molecular or cellular imaging with contrast agents. PAM imaging has a high spatial and temporal resolution that allows for the differentiation between choroidal and retinal vessels.[10-16] Contrast agents such as nanoparticles, nanorods, and nanostars have all been used to facilitate molecular and cellular imaging as well as provide improved visualization of the retinal and choroidal vasculature.[9,17-19] This review will focus on gold nanoparticles as a potential contrast agent due to its versatility as a drug carrier, thermal agent for photothermal treatments, and biocompatibility.

Silicon Nanoneedle Embedded Contact Lens for Long Term Drug Delivery

Current routes of drug delivery for ocular targets rely heavily on eye drops or frequent intravitreal injections. The former has a low bioavailability due to the many ocular barriers surrounding the eye. Only around 5% of the drug will reach the desired target, making eye drops cost inefficient due to the large amount of drug lost during administration.[20] The latter, while more efficient in delivering drugs, is off-putting for many patients scared of needles, is invasive, and carries significant risks including risk of infection that can lead to blindness.

Recently, nanomedicine has been explored as a novel method to improve the capability of drug delivery to the target.[21] Nanomedicine can be utilized for various applications in biomedicine such as treatment, diagnosis, opening the blood brain barrier, and

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stimulation of neural tissues.[21-23] Nanomedicine covers nanoparticles, nanoliposomes, nanocapsules, nanocages, nanohydrogels, nanomicelles, nanodendrimers, nanomaterials, nanobiosensors, and nanophotosensitizer probes.[9,17,24,25] Today, nanomaterials with different sizes, shapes and materials are being widely investigated for enhancing the current paths of drug delivery, improving the overall bioavailability of drugs, decreasing side effects and toxicities, and improving treatment efficiency.

Nanoneedles can be used for delivery of drugs and other agents into the nucleus or cytoplasm in living cells. Nanoneedles can effectively transfect siRNA in corneal endothelium cells.[26] Esfandyarpour *et al.* used a nanoneedle biosensor as an electronic probe to measure electrical response of nucleic acids and proteins.[27] Maurini *et al.* have used porous silicon nanoneedles to develop a nanoinjection approach for RNA interference therapy targeting the human corneal endothelium to effectively reduce levels of p16 protein and promote cell proliferation.[28] In addition, their studies demonstrated that nanoinjection targeting the endothelial layer of explanted human corneas preserves cellular structure and did not induce any significant apoptosis to the human corneal endothelium. To be effectively applied in different applications, the surface of nanoneedles needs to be modified by conjugation with other drugs and materials.

Park *et al.* have introduced silicon nanoneedles (Si NNs) integrated in a tear-soluble contact lens that can be effective, painless, and easy to administer to treat corneal angiogenesis. The Si NNs are first transferred to a polymethyl meth-acrylate (PMMA) film and then pressed into a contact lens shaped mold to finally transfer the Si NNs to the tear soluble lens. Park *et al.* reported a yield of >98% for the entire transfer process of the Si NNs. The final product had a lens thickness of 37-43 µm and a needle base diameter of 900 nm.[24] A great benefit is that these Si NNs can be fabricated with different sizes and shapes. The Si NNs can safely penetrate ocular barriers without scarring the cornea as well as allow long term sustained ocular delivery of drugs.[24] Microneedles can also be used for drug delivery but are larger, which can lead to more discomfort and damage.[29-32]

Dissolution Kinetics

Park *et al.* reported quick dissolution of the water-soluble contact lens within a minute of application with multi-month-long sustained delivery of drugs through the Si NN (Figure 1A,C). It was also reported that no corneal punctures were found due to the NN, and the NN were inserted stably into the cornea without any washing away (Figure 1B,D). The drug release kinetics of the Si NNs could also be increased through deposition of a passivation layer such as Al_2O_3. Figure 1E-H show the drug delivery process. Si NNs are able to degrade via hydrolysis reaction in the presence of tears. It was reported that the degradation of the Si NNs increases linearly with time from 3.5 nm/day to 16.6 nm/day. However, this reaction could be significantly slowed with a passivation layer to 0.05 nm/day. Once the passivation layer completely dissolved, the rate returned to the original degradation rate.

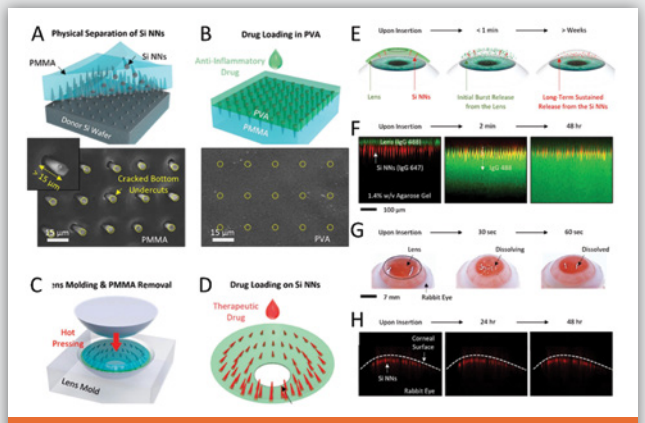

Figure 1. Schematic silicon nanoneedles fabrication and conjugation with anti-inflammatory drug: (A) the transfer of the Si NNs to a PMMA film. (B) the encapsulation of a water-soluble film with anti-inflammatory drug. (C) the hot-pressing of the resulting structure into a lens-shape mold. (D) the loading of therapeutic drugs to the surface of the Si NNs. (E) Time-lapse schematic illustrations of the biphasic drug delivery process. (F) Time-lapse confocal fluorescence microscopy images for the biphasic release of IgG 488 (green) and 647 (red) from the tear-soluble contact lens and the Si NNs, respectively. (G) Time-lapse photographs of the enucleated rabbit eye with the tear-soluble contact lens while being dissolved. (H) Time-lapse cross-sectional confocal fluorescence microscopy images of the enucleated rabbit eye with the Si NNs embedded into the cornea. Adapted with permission from Ref.[24]

In Vivo Evaluation

Park *et al.*'s Si NNs were evaluated in a rabbit choroidal neovascularization (CNV) model (Figure 2). In order to monitor treatment efficacy, the Si NNs were used to release ocular drugs like bevacizumab to treat CNV.[27] New Zealand white rabbits were used in this study. Rabbit eyes have been heavily used as the most translatable animal model for ocular diseases due to its many similarities to human eyes.[33] Some of these similarities include corneal thickness, axial length, curvature, and even CNV pathology.[34] Rabbit CNV development was monitored over 28 days with OCT, color fundus, and red-free imaging. Two experimental groups were tested: short Si NNs (10 µm) with 1.5 µg bevacizumab and long Si NNs (60 µm) with 14 µg bevacizumab. Three controls groups were also tested: no treatment, subconjunctival bevacizumab injections without Si NNs, and non-dosed Si NNs. Both experimental groups proved to be highly effective in treating CNV without significant differences. Reduction of CNV was observed through the first 9 days with complete resolution of CNV within the 28 days. This contrasts with the no treatment group which showed no decrease in CNV throughout the 28 days. The subconjunctival injection group only showed incomplete reduction of CNV. Most importantly, it was noted that the minimum dosage of bevacizumab required for CNV treatment (1.5 µg) was significantly lower in the experimental groups than traditional treatment methods such as subconjunctival injections or topical ophthalmic drops.[35-37]

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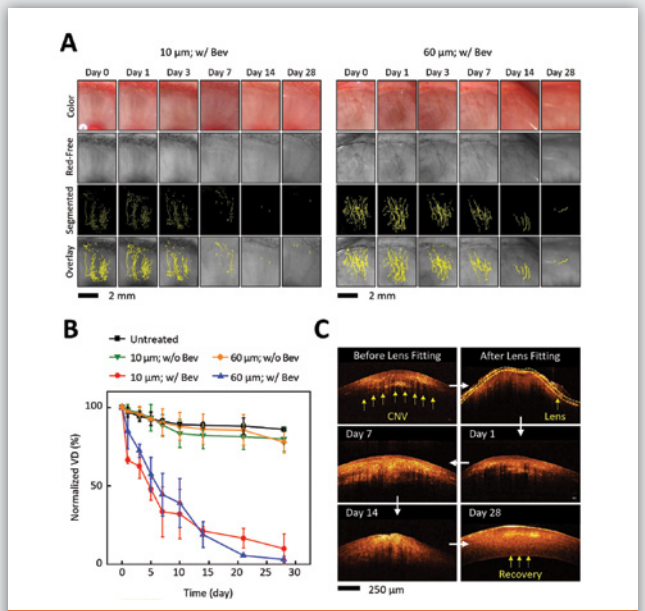

Figure 2. *In vivo* CNV treatment with Si NNs in a rabbit model. (A) Color fundus photography, red-free, segmented, and overlay images of CNV pre (day 0) and post treatment at different time points: days 1, 3, 7, 14, and 28 (i.e., on-therapy) using the 10 μm-long (left panel) and 60 μm-long (right panel) Si NNs. (B) A panel of VD analysis to quantify the dynamic change of CNV from day 0 to 28. (C) 2D OCT images of the rabbit eye under therapy using the 60 μm-long Si NNs at day 0 (i.e., right before and after the lens fitting) and days 1, 7, 14, and 28 (i.e., on-therapy). Adapted with permission from Ref.[24]

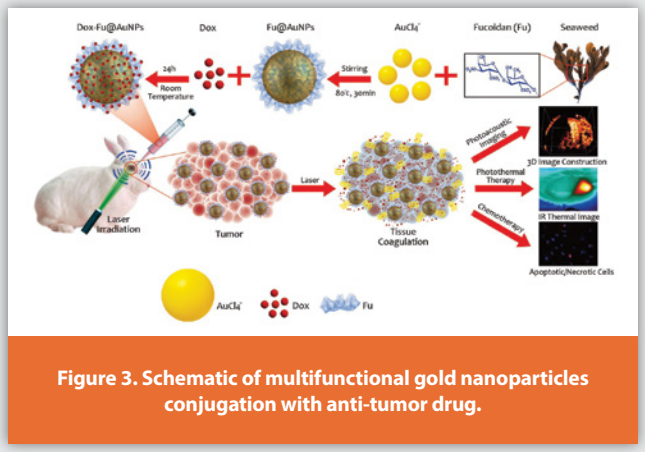

Figure 3. Schematic of multifunctional gold nanoparticles conjugation with anti-tumor drug.

Combination of Chemotherapy and Photothermal Therapy for Treatment of Ocular Tumors

Common types of ocular tumors include metastases from other malignant cancers, choroidal melanomas, retinoblastoma, medulloepithelioma, retinal astrocytic hamartomas, neuroblastoma, leukemia, amd infantile hemangioma.[38,39] The diagnosis of ocular tumors is usually associated with very invasive methods such as histological analysis, lumbar puncture, and bone marrow biopsies. These methods are commonly performed in patients in clinics with high risk of extraocular diseases.

Current treatments for choroidal melanomas have significant limitations, including high recurrence rates and infection.[40] Using gold nanoparticles as a photothermal sink for tumor treatment seems highly promising and doubles as a contrast agent that allows for precise 3D visualization of the tumor's position in the eye. Gold nanoparticles (NPs) readily absorb light, and the resulting heat can irreversibly damage tumors (Figure 3). Furthermore, drugs such as Doxorubicin (Dox) can also be administered using functionalized gold NPs. Dox has known chemotherapeutic effects such as inhibiting nucleic acid synthesis. Fucoidan (Fu) has also been recently investigated as a cancer medication given its anti-tumor and anti-inflammation properties.[41,42] In addition, Fu is able to reduce the cytotoxic effects of heavy metal nanoparticles.[43,44]

Kim *et al.* reported using Fu coated gold NPs dosed with Dox to successfully kill choroidal tumors without recurrence two weeks after treatment administration. Fu was a great candidate to conjugate Dox with gold NPs as both are positively charged with Fu being negatively charged. Upon irradiation with a 0.11 W/cm^2 laser, 100, 200, and 300 μg/ml gold NPs reached a max temperature of 48.6 ± 3.0, 56.8 ± 1.6, and 64.6 ± 1.7°C respectively. It was previously reported that temperatures in the range of 55–70°C can induce irreversible heat damage to tumors.[45]

In vitro testing revealed the max cell viability achieved in the absence of irradiation using Dox-Fu gold NPs was 38% with a concentration of 300 μg/ml. With a 2-minute laser irradiation, cell viability reached a peak low of 12% for Fu-Dox gold NPs at a concentration of 200 μg/ml. Interestingly, irradiated Fu gold NPs without Dox resulted in almost no cell death. The researchers attributed this to the fact that Dox-less Fu gold NPs only reached a temperature of 41 °C. However, the cytotoxic effects of the gold NPs cannot be fully attributed to Dox as the photothermal effect in cytotoxicity of tumor cells was significant. As discussed earlier, in the absence of laser irradiation, Dox-Fu gold NPs only result in a cell viability of 38% versus the 12% viability with laser treatment.

Similar results were observed *in vivo* (Figure 4A-D). Tumor size and progression was monitored in VX2 tumor-bearing rabbits for two weeks. The authors reported observing an increase in tumor size for rabbits injected with just Dox-Fu gold NPs without laser irradiation. The group that received both Dox-Fu gold NPs with laser treatment saw a decrease in tumor size up until day nine when the tumor began to disappear.

Imaging guidance can help to confirm the diagnosis and determine staging. Imaging modalities used to evaluate the ocular tumors include orbital ultrasonography (US) or ultrasound biomicroscopy, computed tomography (CT), magnetic resonance imaging (MRI), and photoacoustic imaging (PAI).[46,47,48]

Kim *et al.* used photoacoustic (PA) imaging to visualize eye tumors (Figure 4E-F). To enhance the PA signals, the authors used gold NPs.

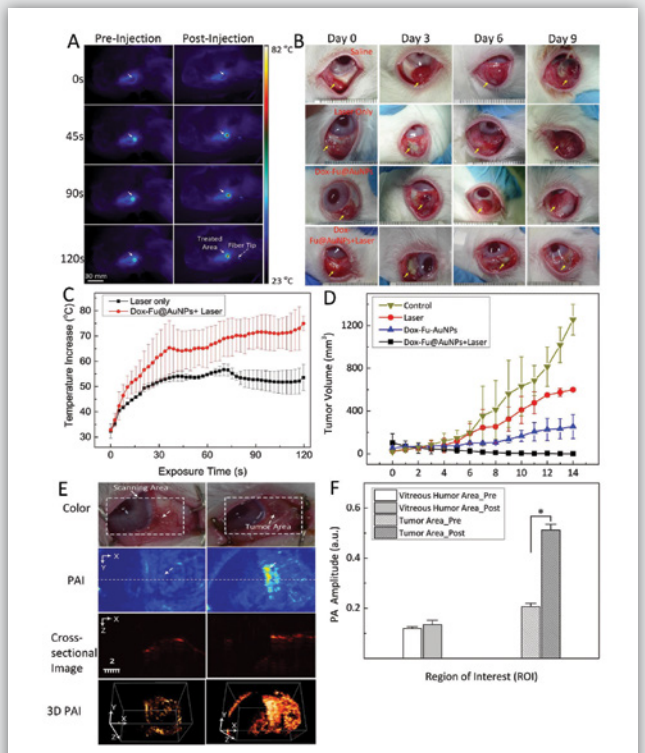

Figure 4. Photoacoustic imaging (PAI) and chemo-photothermal effects of Dox-Fu@AuNPs in rabbit eye tumor model. (A) *In vivo* infrared thermographic maps of eye tumors before and after injection with Dox-Fu@AuNPs followed by laser illumination at different times from 0s to 120 s. (B) Color photographs of the rabbit eye tumors treated with saline as control, laser only, Dox-Fu@AuNPs only, and Dox-Fu@AuNPs with laser irradiation. (C) The graph indicates temporal development of temperature from the irradiated area in the tumor during the laser irradiation (laser only vs. Dox-Fu@AuNPs with laser irradiation: N = 20). (D) Graph of tumor volume acquired before and after treatment over a period of 14 days. (E) Photoacoustic imaging of rabbit eye tumor before and after Dox-Fu@AuNPs injection. (F) Quantitative photoacoustic signals obtained from different positions in the rabbit eye tumor before and after injection of Dox-Fu@AuNPs. Adapted with permission from Ref.[46]

A big benefit of using gold NPs as agent of drug delivery is its ability to also act as a contrast agent for visualization of the tumor. After an injection of 100 µl of 200 µg/ml of Dox-Fu gold NPs, the contrast of the tumor was 2.6 times higher than the surrounding tissue. A 3D visualization of the tumor volume can also be constructed by combining a series of B-scan cross sectional images, allowing us to clearly see the borders of the tumor.

Limitations and Future Clinical Applications

Pharmacotherapies for ocular diseases have shown significant promise in recent years. However, there remain several challenges that need further investigation, including lack of effective treatments for some diseases, difficulty in developing drugs that can penetrate the eye's barriers, limited understanding of the underlying mechanisms of some diseases, and the high cost of treatments, which can make them inaccessible to some patients. In order to translate to clinical applications, there are several factors that need to be addressed, such as development of more effective and specific treatments for specific ocular diseases, investigation of new drug delivery methods to improve treatment efficacy and reduce side effects, and continued investment in research to improve our understanding of ocular diseases and identify new targets for treatment. Recently, the gene therapy voretigene neparvovec-rzyl (Luxturna) from Spark Therapeutics has been launched as a novel treatment technique to treat patients with Leber congenital amaurosis but carries a very significant treatment cost for both eyes of more than $850,000. It would be beneficial to reduce treatment cost to increase the use of gene therapy and regenerative medicine.

Conclusions

In conclusion, advanced nanopharmacotherapies represent a promising strategy for the treatment of ocular diseases. These advanced techniques have been utilized to develop new and more effective therapeutics to deliver drugs to the eye. However, significant challenges still remain, including the need to overcome the barriers that prevent drugs from reaching their intended targets in the eye, and the need to improve our understanding of the underlying mechanisms of ocular diseases. Despite these challenges, Despite these challenges, results to date provide evidence that advances in ocular therapeutics advances in ocular therapeutics have the potential to revolutionize the treatment of ocular diseases and improve patient outcomes. Further research and investment in this area is therefore critical to ensure that the benefits of these advances are realized and that patients have access to the most effective treatments for their conditions.

Funding

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References

1. Burton, M. J. *et al.* The Lancet global health Commission on global eye health: vision beyond 2020. The Lancet Global Health 9, e489-e551 (2021).

2. Solomon, S. D., Lindsley, K., Vedula, S. S., Krzystolik, M. G. & Hawkins, B. S. Anti-vascular endothelial growth factor for neovascular age-related macular degeneration. Cochrane Database of Systematic Reviews (2014).
3. Mitchell, P., Liew, G., Gopinath, B. & Wong, T. Y. Age-related macular degeneration. The Lancet 392, 1147-1159 (2018).
4. Matsumoto, H. *et al.* Quadrant laser photocoagulation trial to ameliorate choroidal congestion in central serous chorioretinopathy. Jpn. J. Ophthalmol., 1-8 (2023).
5. Yeung, L., Lima, V. C., Garcia, P., Landa, G. & Rosen, R. B. Correlation between spectral domain optical coherence tomography findings and fluorescein angiography patterns in diabetic macular edema. Ophthalmology 116, 1158-1167 (2009).
6. Vaz-Pereira, S. *et al.* Optical coherence tomography features of active and inactive retinal neovascularization in proliferative diabetic retinopathy. Retina 36, 1132-1142 (2016).
7. Ishibazawa, A. *et al.* Characteristics of retinal neovascularization in proliferative diabetic retinopathy imaged by optical coherence tomography angiography. Invest. Ophthalmol. Vis. Sci. 57, 6247-6255 (2016).
8. Kuehlewein, L. *et al.* Optical coherence tomography angiography of type 1 neovascularization in age-related macular degeneration. Am. J. Ophthalmol. 160, 739-748. e732 (2015).
9. Nguyen, V.-P. *et al.* Gold nanorod enhanced photoacoustic microscopy and optical coherence tomography of choroidal neovascularization. ACS Applied Materials & Interfaces 13, 40214-40228 (2021).
10. Nguyen, V. P. *et al.* Contrast agent enhanced multimodal photoacoustic microscopy and optical coherence tomography for imaging of rabbit choroidal and retinal vessels in vivo. Sci. Rep. 9, 1-17 (2019).
11. Tian, C., Zhang, W., Nguyen, V. P., Wang, X. & Paulus, Y. M. Novel photoacoustic microscopy and optical coherence tomography dual-modality chorioretinal imaging in living rabbit eyes. JoVE (Journal of Visualized Experiments), e57135 (2018).
12. Tian, C., Zhang, W., Mordovanakis, A., Wang, X. & Paulus, Y. M. Noninvasive chorioretinal imaging in living rabbits using integrated photoacoustic microscopy and optical coherence tomography. Opt. Express 25, 15947-15955 (2017).
13. Zhang, W. *et al.* High-resolution, in vivo multimodal photoacoustic microscopy, optical coherence tomography, and fluorescence microscopy imaging of rabbit retinal neovascularization. Light: Science & Applications 7, 103 (2018).
14. Nguyen, V. P., Li, Y., Zhang, W., Wang, X. & Paulus, Y. M. High-resolution multimodal photoacoustic microscopy and optical coherence tomography image-guided laser induced branch retinal vein occlusion in living rabbits. Sci. Rep. 9, 1-14 (2019).
15. Nguyen, V. P., Li, Y., Zhang, W., Wang, X. & Paulus, Y. M. Multi-wavelength, en-face photoacoustic microscopy and optical coherence tomography imaging for early and selective detection of laser induced retinal vein occlusion. Biomedical optics express 9, 5915-5938 (2018).
16. Nguyen, V. P. *et al.* In vivo 3D imaging of retinal neovascularization using multimodal photoacoustic microscopy and optical coherence tomography imaging. Journal of imaging 4, 150 (2018).
17. Nguyen, V. P. *et al.* Chain-like gold nanoparticle clusters for multimodal photoacoustic microscopy and optical coherence tomography enhanced molecular imaging. Nature Communications 12, 34 (2021).
18. Nguyen, V.-P. *et al.* Plasmonic gold nanostar-enhanced multimodal photoacoustic microscopy and optical coherence tomography molecular imaging to evaluate choroidal neovascularization. ACS sensors 5, 3070-3081 (2020).
19. Chen, J. *et al.* Thin layer-protected gold nanoparticles for targeted multimodal imaging with photoacoustic and CT. Pharmaceuticals 14, 1075 (2021).
20. Zhang, K., Zhang, L. & Weinreb, R. N. Ophthalmic drug discovery: novel targets and mechanisms for retinal diseases and glaucoma. Nature reviews Drug discovery 11, 541-559 (2012).
21. Reimondez-Troitiño, S., Csaba, N., Alonso, M. & De La Fuente, M. Nanotherapies for the treatment of ocular diseases. Eur. J. Pharm. Biopharm. 95, 279-293 (2015).
22. Chow, E. K.-H. & Ho, D. Cancer nanomedicine: from drug delivery to imaging. Sci. Transl. Med. 5, 216rv214-216rv214 (2013).
23. Weber, J., Beard, P. C. & Bohndiek, S. E. Contrast agents for molecular photoacoustic imaging. Nature methods 13, 639-650 (2016).
24. Park, W. *et al.* Biodegradable silicon nanoneedles for ocular drug delivery. Science Advances 8, eabn1772 (2022).
25. Sauvage, F. *et al.* Laser-induced nanobubbles safely ablate vitreous opacities in vivo. Nature nanotechnology 17, 552-559 (2022).
26. Zahir-Jouzdani, F., Mottaghitalab, F., Dinarvand, M. & Atyabi, F. siRNA delivery for treatment of degenerative diseases, new hopes and challenges. Journal of Drug Delivery Science and Technology 45, 428-441 (2018).
27. Esfandyarpour, R. *et al.* Label-free electronic probing of nucleic acids and proteins at the nanoscale using the nanoneedle biosensor. Biomicrofluidics 7, 044114 (2013).
28. Maurizi, E. *et al.* Nanoneedles Induce Targeted siRNA Silencing of p16 in the Human Corneal Endothelium. Advanced Science 9, 2203257 (2022).
29. Wu, Y. *et al.* Nanosuspension-loaded dissolving bilayer microneedles for hydrophobic drug delivery to the posterior segment of the eye. Biomaterials Advances 137, 212767 (2022).
30. Yadav, K. S., Rajpurohit, R. & Sharma, S. Glaucoma: current treatment and impact of advanced drug delivery systems. Life Sci. 221, 362-376 (2019).
31. Khandan, O., Kahook, M. Y. & Rao, M. P. Fenestrated microneedles for ocular drug delivery. Sensors and Actuators B: Chemical 223, 15-23 (2016).
32. Kim, Y.-C., Park, J.-H. & Prausnitz, M. R. Microneedles for drug and vaccine delivery. Advanced drug delivery reviews 64, 1547-1568 (2012).
33. Del Amo, E. M. & Urtti, A. Rabbit as an animal model for intravitreal pharmacokinetics: Clinical predictability and quality of the published data. Exp. Eye Res. 137, 111-124 (2015).
34. Qin, Y. *et al.* Photo-mediated ultrasound therapy for the treatment of corneal neovascularization in rabbit eyes. Translational Vision Science & Technology 9, 16-16 (2020).
35. Ucgul, R. K., Celebi, S., Yilmaz, N. S., Bukan, N. & Ucgul, A. Y. Intrastromal versus subconjunctival anti-VEGF agents for treatment of corneal neovascularization: a rabbit study. Eye 35, 3123-3130 (2021).
36. Bakri, S. J., Snyder, M. R., Reid, J. M., Pulido, J. S. & Singh, R. J. Pharmacokinetics of intravitreal bevacizumab (Avastin). Ophthalmology 114, 855-859 (2007).
37. Kim, Y. C., Edelhauser, H. F. & Prausnitz, M. R. Targeted delivery of antiglaucoma drugs to the supraciliary space using microneedles. Invest. Ophthalmol. Vis. Sci. 55, 7387-7397 (2014).
38. Rao, A. A., Naheedy, J. H., Chen, J. Y.-Y., Robbins, S. L. & Ramkumar, H. L. A clinical update and radiologic review of pediatric orbital and ocular tumors. J. Oncol. 2013 (2013).
39. Triozzi, P. L., Eng, C. & Singh, A. D. Targeted therapy for uveal melanoma. Cancer Treat. Rev. 34, 247-258 (2008).
40. Nie, S., Xing, Y., Kim, G. J. & Simons, J. W. Nanotechnology applications in cancer. Annu. Rev. Biomed. Eng. 9, 257-288 (2007).
41. Atashrazm, F., Lowenthal, R. M., Woods, G. M., Holloway, A. F. & Dickinson, J. L. Fucoidan and cancer: A multifunctional molecule with anti-tumor potential. Mar. Drugs 13, 2327-2346 (2015).
42. Li, B., Lu, F., Wei, X. & Zhao, R. Fucoidan: structure and bioactivity. Molecules 13, 1671-1695 (2008).
43. Lu, K.-Y. *et al.* Development of a new type of multifunctional fucoidan-based nanoparticles for anticancer drug delivery. Carbohydrate polymers 165, 410-420 (2017).
44. Tengdelius, M. *et al.* Synthesis and anticancer properties of fucoidan-mimetic glycopolymer coated gold nanoparticles. Chemical Communications 51, 8532-8535 (2015).
45. Thomsen, S. Pathologic analysis of photothermal and photomechanical effects of laser–tissue interactions. Photochem. Photobiol. 53, 825-835 (1991).
46. Kim, H. *et al.* Doxorubicin-fucoidan-gold nanoparticles composite for dual-chemo-photothermal treatment on eye tumors. Oncotarget 8, 113719 (2017).
47. de Graaf, P. *et al.* Guidelines for imaging retinoblastoma: imaging principles and MRI standardization. Pediatr. Radiol. 42, 2-14 (2012).
48. Aerts, I. *et al.* Retinoblastoma. Orphanet J. Rare Dis. 1, 1-11 (2006).

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Process Validation and Sterility Assurance

Relations and Requirements

Robert Dream

Managing Director
HDR Company LLC

The purpose of the production and process control subsystem (including sterilization process controls) is to manufacture products that meet specifications. Developing processes that are adequate to produce drug products that meet specifications, validating (or fully verifying the results of) those processes, and monitoring and controlling the processes are all steps that help assure the result will meet specifications. For sterilization processes, the primary product specification is the desired Sterility Assurance Level (SAL). Other specifications may include sterilant residues and endotoxin levels, Figure 1.[7]

EU Point of Consideration

When parametric release of sterility is proposed, the Guideline on Real Tme Release Testing, EMA/CHMP/QWP/811210/2009-Rev1 (human products only,[4] the Guideline on Parametric Release, EMEA/CVMP/QWP/339588/2005 (veterinary products only)[5] and the text of Ph. Eur. Chapter 5.1.1[6] should be taken into account. The bioburden control criteria should be specified prior to all sterilization processes.

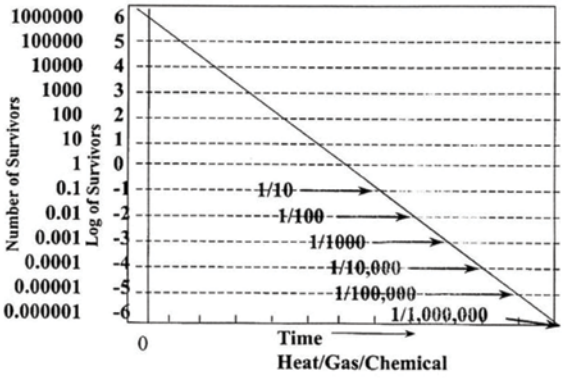

Figure 1. Sterility Assurance Levels for heat, gas, or chemicals.

High bioburden acceptance criteria should not be justified by the capacity of the sterilization process or any bioburden reducing step before sterilization. The levels of bacterial endotoxins in the finished product can be impacted by the bioburden and bacterial endotoxins in the components (i.e., active substance, excipients and containers), and by microbiological contaminants introduced during manufacture. To ensure an acceptable level of bacterial endotoxins in the finished product, the level of microbiological contaminants of the components should be minimal. Acceptance criteria for bioburden and, where relevant, bacterial endotoxins in components and bulk solutions should be specified. All filters used in the manufacture of the finished product that come in contact with the finished product, or with any component (substance or intermediate product) incorporated in the finished product should be described and the information stated should be provided in the quality dossier. The information should be in line with the requirements stated in EudraLex GMP Annex 1.[2] For ATMPs, the Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products should be followed.[3] If a secondary container (e.g., secondary pouch for infusion bags or blisters intended to keep the outside of the container sterile) is used to provide a specific protection to the medicinal product, the packaging process should be described, including a risk assessment, since it may affect the sterility of the finished product; for example, trapping moisture between the primary and secondary containers. Information should be provided as to when the packaging step is performed (before or after sterilization) and any aseptic techniques employed. The proposed processes should be justified from a microbiological perspective. If the use of a secondary container means additional sterilization of the finished product is performed, this should be justified with regard to sterility assurance and any potential impact on finished product quality.[1]

US FDA Point of Consideration

Sterilization Process Controls-Inspectional Objectives

Inspectional Objectives are to confirm that the sterilization process was validated by reviewing the validation study. Review the specific

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procedure(s) for the sterilization process selected and the methods for controlling and monitoring the process. Verify that the process is controlled and monitored.

The purpose of the production and process control subsystem (including sterilization process controls) is to manufacture drug products that meet specifications (Figure 2), inspectional process controls are to[7,13]:

1. Confirm that the sterilization process was validated by reviewing the validation study.
2. Review the specific procedure(s) for the sterilization process selected and the methods for controlling and monitoring the process. Verify that the process is controlled and monitored.
3. If review of the Drug Product History Records (including process control and monitoring records, acceptance activity records, etc.) reveals that the sterilization process is outside the firm's tolerance for operating or performance parameters:
    a. Determine whether the nonconformances were handled appropriately; and
    b. Review the equipment adjustment, calibration and maintenance
4. If the sterilization process is software controlled, confirm that the software was validated.
5. Verify that personnel have been appropriately qualified and trained to implement the sterilization process.

Relationship Between the Stages of Process Validation and Sterility Assurance

As part of stage 1 of the lifecycle of process validation is process design, that is the activity of defining the commercial manufacturing process that will be reflected in planned master production and control records. The goal of this stage is to design a process suitable for routine commercial manufacturing that can consistently deliver a product that meets its quality attributes.[9]

During stage one the process design establishes a Strategy for Process Control, US FDA expects controls to include both examination of material quality and equipment monitoring. Special attention to controlling the process through operational limits and in-process monitoring is essential in two possible scenarios:

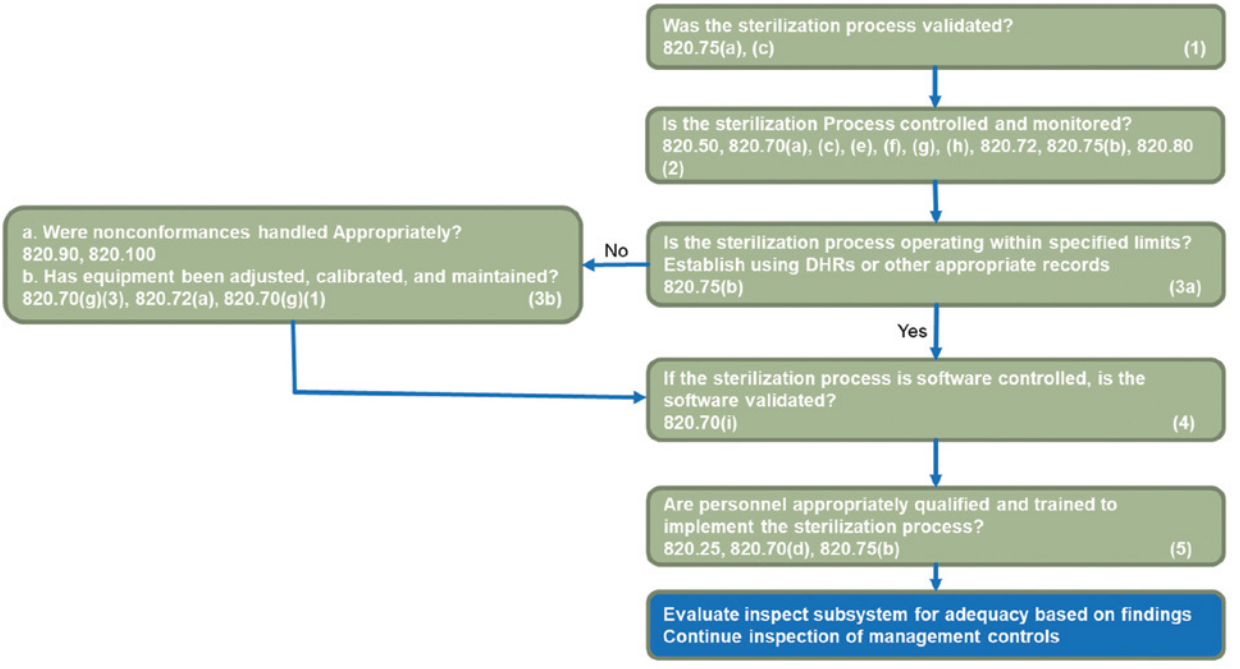

Figure 2. Sterilization Process Controls Decision Flow Chart (US-FDA)[7,22]

1. When the product attribute is not readily measurable due to limitations of sampling or detectability (e.g., viral clearance or microbial contamination), example:

Probability of Detection of Viruses at Low Concentrations: At low virus concentrations (e.g., in the range of 10 to 1,000 infectious particles per liter), it is evident that a sample of a few milliliters may or may not contain infectious particles. The probability, p, that this sample does not contain infectious viruses is:

$$p = ((V-v)/V)^n$$

when V (liter) is the overall volume of the material to be tested, v (liter) is the volume of the sample and n is the absolute number of infectious particles statistically distributed in V.

If V >> v, this equation can be approximated by the Poisson distribution:

$$p = e^{-cv}$$

when c is the concentration of infectious particles per liter. Or,

$$c = \ln (p/-v)$$

As an example, if a sample volume of 1 ml is tested, the probabilities p at virus concentrations ranging from 10 to 1,000 infectious particles per liter are:

c	10	100	1000
v (Liter)	0.001	0.001	0.001
p	0.99	0.90	0.37

This indicates that for a concentration of 1,000 viruses per liter, in 37% of sampling, 1 ml will not contain a virus particle.

If only a portion of a sample is tested for viruses and the test is negative, the amount of virus which would have to be present in the total sample to achieve a positive result should be calculated and this value taken into account when calculating a reduction factor. Confidence limits at 95% are desirable. However, in some instances, this may not be practical due to material limitations.[14]

2. When intermediates and products cannot be highly characterized and well-defined quality attributes cannot be identified.

As part stage 2 of the process validation lifecycle, A written protocol that specifies the manufacturing conditions, controls, testing, and expected outcomes is essential for this stage of process validation. It is recommended that the protocol discuss the following elements:

- The manufacturing conditions, including operating parameters, processing limits, and component (raw material) inputs.
- The data to be collected and when and how it will be evaluated.
- Tests to be performed (in-process, release, characterization) and acceptance criteria for each significant processing step.
- The sampling plan, including sampling points, number of samples, and the frequency of sampling for each unit operation and attribute. The number of samples should be adequate to provide sufficient statistical confidence of quality both within a batch and between batches. The confidence level selected can be based on risk analysis as it relates to the particular attribute under examination. Sampling during this stage should be more extensive than is typical during routine production.
- Criteria and process performance indicators that allow for a science- and risk-based decision about the ability of the process to consistently produce quality products. The criteria should include:
  - » A description of the statistical methods to be used in analyzing all collected data (e.g., statistical metrics defining both intra-batch and inter-batch variability).
  - » Provision for addressing deviations from expected conditions and handling of nonconforming data. Data should not be excluded from further consideration in terms of PPQ without a documented, science-based justification.[15]
- Design of facilities and the qualification of utilities and equipment, personnel training and qualification, and verification of material sources (components and container/closures), if not previously accomplished.
- Status of the validation of analytical methods used in measuring the process, in process materials, and the product.
- Review and approval of the protocol by appropriate departments and the quality unit.

These controls are established in the master production and control records (see § 211.186(a) and (b)(9)).[8]

The sterility assurance is incorporated into the design during Stage 1 and built into the design and is demonstrated through PPQ protocol execution, Stage 2. Sterility assurance a perquisite to support the execution of PQ and PV to establish product continued process verification lifecycle, stage 3, Figure 3.

Sterility assurance cannot be seen as an entity independent from the process/product qualification and validation. Sterility assurance is generally a prerequisite to starting the PPQ process, however the process should be designed not only taking into consideration the drug substance/drug product, but also the combination of sterility assurance and the drug product quality.

Process Validation is Well Defined by Regulations

Generally, early process design experiments do not need to be performed under the cGMP conditions required for drugs intended for commercial distribution that are manufactured during Stage 2 (process qualification) and Stage 3 (continued process verification). They

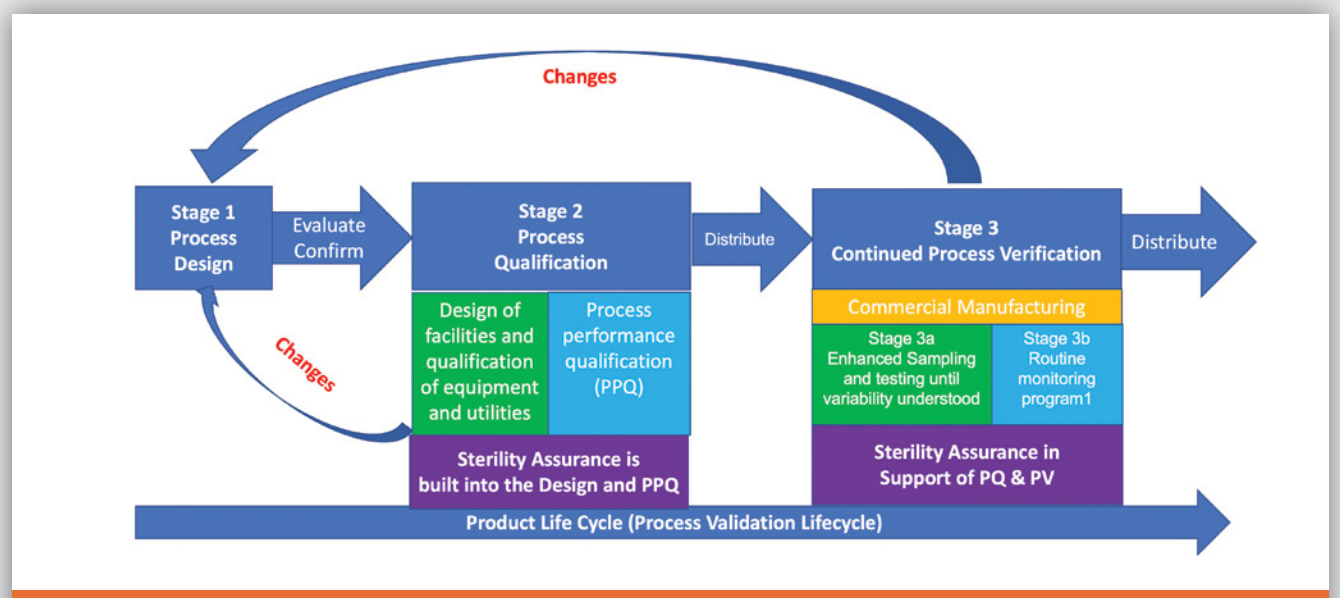

Figure 3. Process Validation Lifecycle and Sterility Assurance Relations.

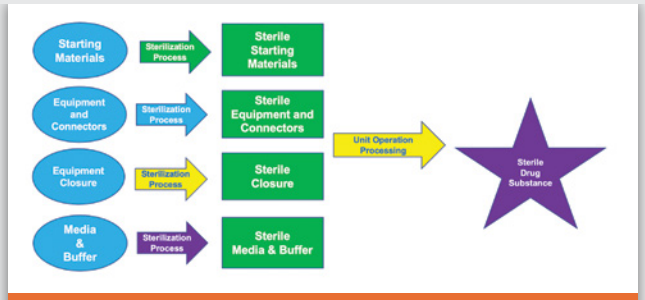

Figure 4. Drug substance processing can use multiple sterilization processes each optimized for the individual component.

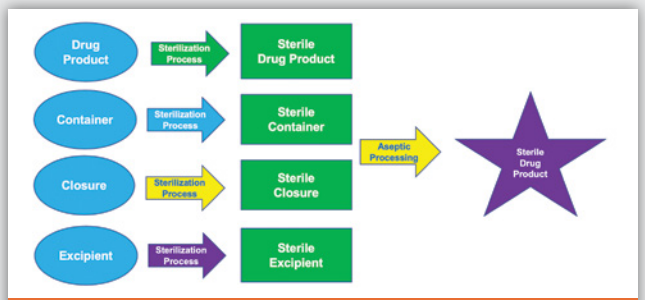

Figure 5. Drug product processing can use multiple sterilization processes each optimized for the individual component

should, however, be conducted in accordance with sound scientific methods and principles, including good documentation practices. This recommendation is consistent with ICH Q10 Pharmaceutical Quality System. Decisions and justification of the controls should be sufficiently documented and internally reviewed to verify and preserve their value for use or adaptation later in the lifecycle of the process and product.[9]

For parenteral drug product the essential element of aseptic processing as defined in Figure 6.

1. It means ensuring a product is sterile, with
    a. Very low endotoxins
    b. Very low foreign particles

Figure 6. Essential element of aseptic processing.

c. The entire manufacturing process shall be designed to fit the characteristic of the product:
    i. Excipients
    ii. Drug Substance
    iii. Container
    iv. Platform
    v. Administration route
2. The process can be divided in steps (Figure 4 and 5), and for each step shall have as a minimum control of bioburden, endotoxins and particles
3. At some point in time the main product components.
    a. Formulation
    b. Equipment in contact with product
    c. Container closure
    d. Environment where product is exposed

Aseptic processes are designed to minimize exposure of sterile articles to the potential contamination hazards of the manufacturing operation. Limiting the duration of exposure of sterile product elements, providing the highest possible environmental control, optimizing process flow, and designing equipment to prevent entrainment of lower quality air into the Class 100 (ISO 5) (and or grade A) clean area are essential to achieving high assurance of sterility.

Science and Risk Based Approaches

These approaches should be used to obtain information needed/required to make decisions related to the evaluation, design, qualification, validation, operation, and monitoring sterile product manufacturing processes. Risk and science-based approaches should be used to develop and implement control strategies and acceptance criteria designed to assure the establishment and maintenance of manufacturing conditions which affect the sterility of products. Sterile drug product manufacturing processes and testing requirements should have a basis in and relevance to risk to product quality and patient safety. Risk management and assessment methods should be developed to not only identify risk but allow for the improvement of processes and control strategies.

The effectiveness of certain traditional testing and monitoring methods as control strategies should be reevaluated. As technology has been introduced and knowledge acquired, the usefulness and value of testing procedures have changed. Testing and monitoring should be designed, performed, and its results evaluated based on scientific value, risk to product quality and patient safety, and usefulness to the determination of process control. Where testing and monitoring approaches and methods no longer meet the needs or are not optimal, their replacement or modification should be considered. The use of outdated testing and monitoring methods have the potential to add risk, provide false sense of control, be ineffective, and deploy resources in a manner which may not be efficient or optimal. Thus, detracting from the development and use of more effective testing and monitoring approaches.

Precautions Against Microbial Contamination

The test for sterility is carried out under aseptic conditions. In order to achieve such conditions, the test environment has to be adapted to the way in which the sterility test is performed. The precautions taken to avoid contamination are such that they do not affect any micro-organisms which are to be revealed in the test. The working conditions in which the tests are performed are monitored regularly by appropriate sampling of the working area and by carrying out appropriate controls.[11]

What Should Sterility Assurance Level Demonstrate?

A sterility assurance level of 10^{-6} or better (Figure 1) should be demonstrated for a sterilization process. Refer to the FDA guidance entitled "Guideline for the Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products".[12] Various industry governing bodies, such as the Association for the Advancement of Medical Instrumentation (AAMI), provide a range of guidance documents, recommendations, and standards for sterility assurance monitoring. The American National Standards Institute (ANSI) is the United States national standard body and all US standards and recommended practices with national recognition must be accepted by ANSI. Professional Associations like the International Association for Healthcare Central Service Material Management (IAHCSSM) provide education on best practices utilizing ANSI/AAMI standards and guidelines.

- **ANSI/AAMI ST79:** This standard is the comprehensive guide for healthcare **steam sterilization**. This guide covers a range of 15 sections including Personnel, Material Management, Sterilization, and Process Monitoring and Improvement. Sterility assurance monitoring for steam sterilization is covered within Section 13, the Process Monitoring and Improvement section.[16]
- **ANSI/AAMI ST58:** This standard is the most recognized guideline for **vaporized hydrogen peroxide sterilization** in healthcare facilities. This standard includes many aspects of the VHP process including packaging and record keeping. It also provides recommendations for an assurance monitoring program under Section 9, Quality Control.[17]

Absolute Sensitivity Threshold

Absolute sensitivity threshold is a key metric that represents the theoretical minimum amount of light required to observe any

meaningful signal. It is the number of photons required in order to acquire a signal that is equivalent to the noise observed by the sensor. The term "absolute threshold" is also used in experimental research to refer to the smallest level of stimulus that can be detected, usually defined as at least half the time.

Also, absolute sensitivity threshold is the number of photons needed to get a signal equivalent to the noise observed by the sensor. This is an important metric because it represents the theoretical minimum amount of light needed to observe any meaningful signal at all.

EMVA1288[18] is a standard that defines what aspects of camera performance to measure, how to measure them and how to present the results in a unified method. This illustration will help understand the various aspects of imaging performance of an imaging sensor. It will outline the basic concepts that are important to understand when considering how an image sensor converts light into a digital image and ultimately defines the performance of the sensor. Figure 7 presents a single pixel and highlights these concepts.

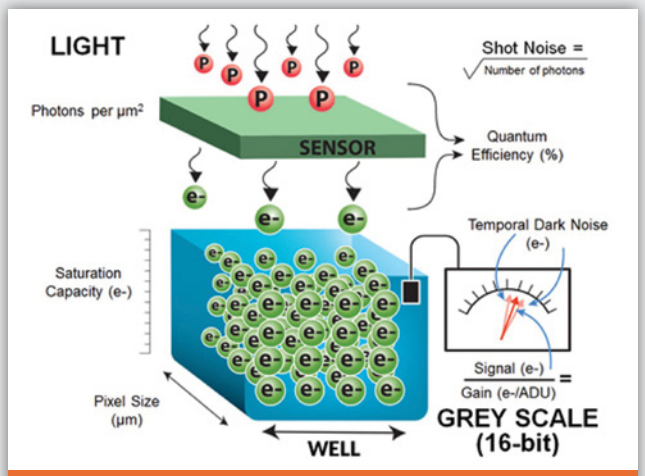

Figure 7. How an image sensor convert light into a digital image.[21]

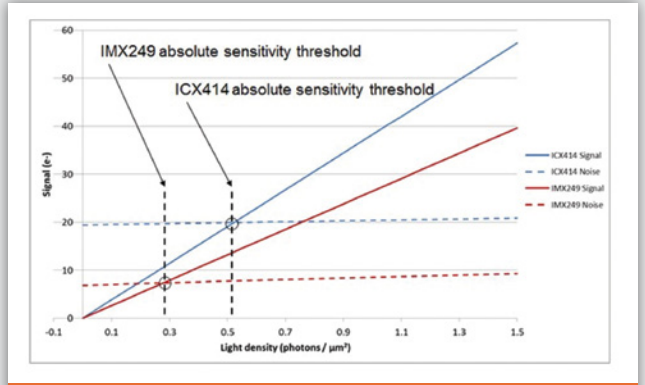

Figure 8. Mako G-234 with Sony IMX249 runs 41.0 frames per second at 2.4 MP resolution.[19,20]

The graph in Figure 8, shows the relationship between the signal and the noise of a camera. When the noise reaches the noise floor, we have reached absolute sensitivity threshold. IMX249 will reach absolute sensitivity threshold at a lower light density and thus perform better in lower light applications.

Mako is an attractively designed GigE (Gigabit Ethernet) Vision-compliant camera in a compact rugged industrial housing. Many models include advanced functionalities such as Precision Time Protocol (PTP), Trigger over Ethernet (ToE) Action Commands, and Power over Ethernet (PoE).

Aseptic Process Simulation (APS)

Aseptic process simulation (APS) is a study that simulates the aseptic filling process by using growth media instead of the actual product. It is required by regulators[13] to demonstrate the sterility confidence and the aseptic capability of the process. It involves qualified or validated elements such as HVAC systems, cleanroom environment, material transfer, equipment, sterilization processes, and sterilizing filtration. It also requires operator training, skills, supervision, quality assurance, and microbiological monitoring. It is performed as closely to the actual production procedure as possible.

It consists of a minimum of three initial media simulations and repeat media simulations at six-monthly intervals.

Key Words:

- Aseptic process simulation
- Media fills
- Aseptic process qualification
- Aseptic process validation
- Risk assessment

Sterility Assurance in Aseptic Processing

Sterility assurance in aseptic processing requires contributing elements—such as;

- Heating, Ventilation, and Air Conditioning (HVAC) system
- Clean-room environment
- Material transfer
- Equipment
- Manufacturing process steps
- Sterilization processes
- Sterilizing filtration

to be qualified and validated as applicable and for personnel to be trained and qualified.

References

1. Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container; 6 March 2019 EMA/CHMP/CVMP/QWP/850374/2015. Sterilisation guideline - adopted by CxMP - 10.12.2018 (europa.eu)
2. The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use, Annex 1, Manufacture of Sterile Medicinal Products; 20220825_gmp-an1_en_0.pdf (europa.eu).
3. EudraLex The Rules Governing Medicinal Products in the European Union Volume 4 Good Manufacturing Practice; Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products; EUROPEAN COMMISSION, 22 November 2017. 2017_11_22_ guidelines_gmp_for_atmps_0.pdf (europa.eu)
4. Guideline on Real Time Release Testing (formerly Guideline on Parametric Release), Final; 29 March 2012 EMA/CHMP/QWP/811210/2009-Rev1, 1 October 2012. Real Time Release Testing guideline (europa.eu).
5. COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE (CVMP), London, 16 October 2006 EMEA/CVMP/QWP/339588/2005. Microsoft Word - Guideline on Parametric Release (europa.eu).
6. EUROPEAN PHARMACOPOEIA 5.0 5.1.1. Methods of preparation of sterile products; 01/2005:50101. 5.1.1. Methods of preparation of sterile products.pdf (uspbpep.com).
7. Sterilization Process Controls, Production and process controls, subsystem sterilization process controls; US FDA, Content current as of 03/28/2023. sterilization process controls-inspectional objectives - Search (bing.com).
8. § 211.186 Master production and control records. eCFR :: 21 CFR 211.186 -- Master production and control records.
9. Guidance for Industry, Process Validation: General Principles and Practices, January 2011, Current Good Manufacturing Practices (CGMP), Revision 1. Process Validation: General Principles and Practices (fda.gov).
10. Guidance for Industry Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice, September 2004 Pharmaceutical CGMPs. U.S. Department of Health and Human Services Food and Drug Administration; Guidance for Industry (fda.gov).
11. ICH Topic Q4B Annex 8 Sterility Test General Chapter, Step 3, ANNEX 6 TO NOTE FOR EVALUATION AND RECOMMENDATION OF PHARMACOPOEIAL TEXTS FOR USE IN THE ICH REGIONS ON STERILITY TEST GENERAL CHAPTER (EMEA/CHMP/ICH/645592/2008). December 2008 (europa.eu)
12. Guidance for Industry for the Submission Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products, November 1994. US-FDA, For the Submission of Documentation for Sterilization Process (fda.gov).
13. Guidance for Industry Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice, September 2004, Pharmaceutical CGMPs, U.S. Department of Health and Human Services Food and Drug Administration; Guidance for Industry (fda.gov).
14. INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE ICH HARMONISED GUIDELINE VIRAL SAFETY EVALUATION OF BIOTECHNOLOGY PRODUCTS DERIVED FROM CELL LINES OF HUMAN OR ANIMAL ORIGIN, Q5A(R2) Draft version Endorsed on 29 September 2022. https://database.ich.org/sites/default/files/ICH_Q5A%28R2%29_Step2_draft_ Guideline_2022_0826.pdf
15. FDA's Guidance for Industry, Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production, available at http://www.fda.gov/downloads/Drugs/ GuidanceComplianceRegulatoryInformation/Guidances/ucm070287.pdf
16. ANSI/AAMI ST79 – Comprehensive Guide to Steam Sterilization and Sterility Assurance in Health Care Facilities.
17. ANSI/AAMI ST58:2013/(R)2018, guideline for vaporized hydrogen peroxide sterilization in healthcare facilities.
18. EMVA Standard 1288 Standard for Characterization of Image Sensors and Cameras Release 3.1 December 30, 2016, Issued by European Machine Vision Association www.emva.org
19. Mako G-234 | 2.35 MP Sony IMX249 CMOS sensor – Allied Vision; https://www.alliedvision.com/en/camera-selector/detail/mako/G-234/
20. Absolute Sensitivity threshold, IMX249 graph, absolute sensitivity - Bing images.
21. SATURATION CAPACITY (also known as Full Well Depth), Teledyne FLIR, MVA 1288 Overview: Imaging Performance | Teledyne FLIR.
22. 21 CFR Part 820 (up to date as of 7/06/2023) Quality System Regulation, 21 CFR Part 820 (up to date as of 7-06-2023).pdf.

Managing A Cleanroom Cleaning Team

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Abstract

We often take for granted the human aspect of meeting objectives for compliance. While the patient is our ultimate customer, developing a program of contamination control is complex and is significantly dependent on proper human behavior to achieve successful outcomes. The interdependencies of a robust process, quality of input materials, controlled facility and equipment, and operators following validated procedures are the parts of a puzzle that must fit well to ensure successful implementation of a contamination control strategy.

Designing a sterile product process can, and does, move in the direction of removing human operators wherever possible. Robotics and containment provide methods to reduce human intervention. Yet, pharmaceutical cleanrooms commonly do not operate without human presence, although with varying degrees of activity. One of the routine human activities that support contamination control of a cleanroom is cleaning and disinfection. There are equipment and tools that can be sterilized prior to manufacturing use, but there are also many surfaces and areas that require routine oversight and disinfection to maintain control over microbiological cross-contamination.

This article will discuss the best practice approach for supervising and training cleanroom cleaning operators and managing the cleaning activities.

Introduction

The human aspect of meeting objectives for compliance is often taken for granted. While the patient is our ultimate customer, developing a program of contamination control is complex and significantly dependent on the correct human behavior to achieve successful outcomes. The interdependencies of a robust process, quality of input materials, controlled facility and equipment, and operators following validated procedures are each a piece of a puzzle that must fit well to ensure successful implementation of a contamination control strategy (Figure 1).

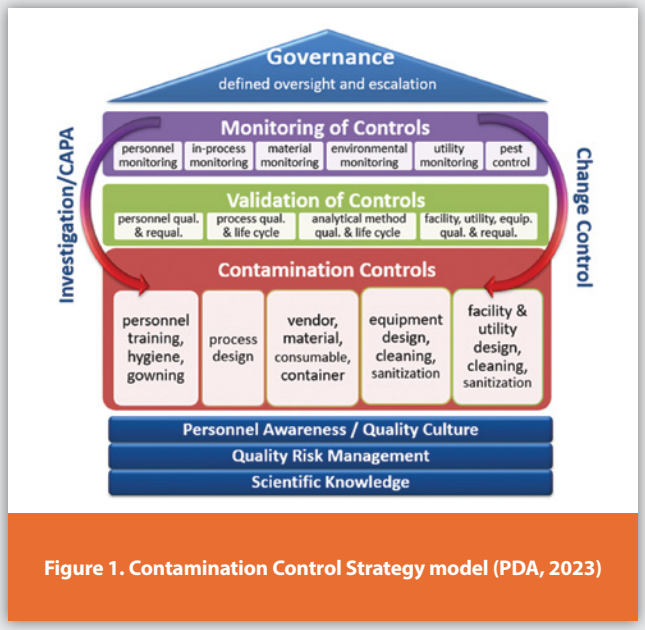

Figure 1. Contamination Control Strategy model (PDA, 2023)

Designing a sterile product process can, and does, aim to remove human operators wherever possible. Robotics and containment provide means to reduce human intervention. Yet, pharmaceutical cleanrooms commonly do not operate without human presence, although with varying degrees of activity. One of the routine human activities that support contamination control of a cleanroom is cleaning and disinfection. There are equipment and tools that can be sterilized prior to manufacturing use, but there are also many surfaces and areas that require routine oversight and disinfection to maintain control over microbiological contamination.

The cleaning and disinfection activities are performed by individuals who may have this activity as a single, focused role or as one in a list of many other roles. There can be one or more individuals assigned to perform this role. The individuals assigned to cleaning and disinfection can be either full-time or part-time employees of the cleanroom owner, or they could be third-party contractors responsible for one or more

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cleanroom facilities. Regardless of the employer, the individual(s) who performs cleaning and disinfection should be given sufficient training, adequate tools, respect by their supervision, and they should show good behavior and integrity for the purpose and facility in which they work. A consistent and successful cleanroom cleaning staff is aware of the purpose and importance of their responsibilities. Their management should have high quality oversight.

This article will discuss the best practice approach and tips for supervising and training cleanroom cleaning operators and managing the cleaning activities.

Training

Learning foundation

A cleanroom cleaning operations team can consist of a range of demographics, cultures and personalities, with diversity just as significant as in the foundations of a pharmaceutical workplace.

Many 'GMP cleaners' (GMP: good manufacturing practices) start their careers as non-GMP cleaners (such as with office and general facility cleaning), giving them a foundation with which they can build and can learn about GMP manufacturing and the cleanroom contamination controls, including cleaning and disinfection. Training to develop the cleaner's contamination control thinking as well as reinforce best practices for cleaning and disinfection will require some time investment. Training should be given in appropriately timed sessions and should cover the basics of sterile manufacturing, sterility assurance, microbiology, safe handling of cleaning and disinfection agents, preparation and disposal of disinfectants, and appropriate application methods.[2] Training is considered an initial introduction of ideas and concepts, whereas learning is an ongoing investment in the expansion and retention of information. When planning weekly or monthly staff team meetings, it is a good practice to establish time in the agenda for learning.

Provide a format for the training content, based on the communication style(s) of the team (know your audience). If you have more junior members of the team looking for development opportunities, perhaps look to engage them to create and deliver the training packages. Not only will this expand the cleaning team's understanding of your operation and the importance of their role in it, but it will also help to foster wider team engagement and community.

The foundation of a good cleaning team is providing and instilling a sense of ownership and accountability. The most important aspect is how their work impacts the process.

Along with the role ownership objective, they should be trained on the manufacturing operation of a cleanroom and how critical cleanroom operations are. The use of real-life examples and simplicity is helpful. For example, relate the cleanroom activities to the resulting end product which is critical and potentially dangerous to the patient. With this perspective, the cleaning team can understand the significance of their activities. In other words, control and patient safety starts with how the cleanroom is controlled and maintained, including their cleaning actions in the cleanroom. The flipside of this could also be useful for learning: if you don't have a clean facility to work in, you don't have a place to manufacture medicine, and all involved in a manufacturing process are 'cogs in the wheel.' If one of those cogs break down, then we don't have an aseptic operation. Everyone has an equally important role to perform.

The training needs to ensure that at the end the cleaning operators are as independent as possible, because they're on their own. They need to be able to make appropriate decisions, follow procedures and know how that is going to impact the processes down the line from their cleaning. So, the real key training for the cleaning operators is how their work fits into the quality of the process.

Another good trait of a cleaning operator is knowing when to ask for help, being able to speak up and let someone know potential issues. A sense of ownership should be instilled during the training so that the cleaning operator feels empowered to speak up and communicate. This is important for a cleanroom environment. That's the way to mitigate. You mitigate early and you mitigate as soon as you know. With a team, it needs to be collaborative.

Key Knowledge and Skills

These are some of the key areas of knowledge and skills to express in a holistic training program:

- Aseptic gowning and gloving procedures
- Microbiology
- Cleanroom controls
- Decontamination agent applications
- Safe handling and storage of cleaning agents and equipment

The cleaning team should understand what the cleanroom is and the ancillary rooms that they will be entering and cleaning, and at a high level, the manufacturing function performed in each of these rooms.

A high-level explanation should include the engineering controls, (e.g., HEPA filters, air pressure and air flow), that are built in place which support the cleanroom such as equipment, instrumentation and design aspects for maintaining control for high quality air and contamination prevention.

It is good to mainly focus on the people aspects, as humans are the most common source of contamination in a cleanroom. Subsequently, what do we do about that? This is where training objectives get into the gowning behaviors and why it's so important for the cleaning operators to understand and be skilled in proper aseptic gowning and cleanroom behaviors, just as the aseptic operators are expected.

Aseptic Gloving

Gloving training should develop a person's understanding and experience with microorganisms. Start with proper sterile gloving

procedures, and fundamentally show individuals where and how microorganisms can be transferred.

For example, some trainers use a plating exercise using the steps below:

1. Each operator's (and trainer's) fingers and wrists are sampled microbiologically using plated media, prior to putting on gloves or sanitizing hands.
2. They are then instructed to put on the sterile gloves 'like any other glove' (e.g., garden glove) they have donned before.
3. Another microbiological sampling of the operator's gloves is then performed.
4. The trainer then puts on their own sterile gloves in an aseptic manner, explaining how and why this procedure is followed, and then has them sampled.

The explanation is a good transition into aseptic glove training. After a couple of days of plate incubation, the team is brought together to see the microbiological results of non-sanitized hands, poor glove handling, compared to the lower bioburden results from proper aseptic glove handling.

The glove training will also stress the importance of skilled aseptic technique and the consequences of good and poor technique, which also leads to what surfaces a cleaning operator should and should not contact with their gloved hands in the cleanroom they are cleaning.

In simple terms, a sterile or decontaminated surface can remain as such until it has been contacted by a non-sterile entity, such as human gloved hands. Every operator should be aware of what they touch in a cleanroom. Some people have personalities that enhance clean surface awareness, and some do not. It is important for a trainer to influence improved clean surface awareness in initial and ongoing training.

Aseptic Gown Training and Qualification

Gowning training and qualification of cleaning personnel should align with aseptic operator training and qualification. Each individual should understand how to follow the procedure and a trainer should be able to express improvement recommendations at any time. Both a technical assessment and a microbiological assessment should be complementary for qualification and each requalification. Increased frequency of technical assessments (how to gown properly) may provide for development of a timelier improvement plan before problems lead to contamination issues. The use of in-operation cameras could complement or replace periodic technical in-person assessment.

For camera use, they should not be used in training to show bad examples. They should only be used for investigations or in training to show good examples, which is a better culture to instill. However sometimes it is helpful to watch yourself, as it can be used for improvements in training for proper aseptic gowning.

The capabilities and experience of a good trainer are critical for this training. Knowing individual's patterns during gowning exercises are important and the ability to constructively express improvement recommendations will enhance the integrity of the individuals for cleanroom cleaning activities.

Cleaning and Disinfection in the Cleanroom

The application of agents onto surfaces by cleanroom cleaners is critical. Just think about the cleanroom space that has all white walls. The design of a cleanroom looks like one continuous space. There are no markers when you're looking at the walls to clean, so an amount of patience and focus is needed during a cleaning. To make sure agents are applied in overlapping strokes, it can be a challenge when the application is barely visible on the surface. The cleaners need to have ironclad focus.

Successful cleaning and disinfection comes from consistency and thoroughness. Practical-based training should be step-by-step and include an explanation of the 'why' behind the defined procedure. People are naturally curious, and whether they fully understand it or not, a trainer should give them the opportunity and chance to show that they understand the method(s). As in any training, encourage and accept all questions that people ask. The objective of this training is to embed that sense of ownership and create a 'speak-up' culture.

Cleanroom cleaners may have the hardest job to perform in a cleanroom. It's physically hard, and quite regularly these activities are performed on their own. A typical 'buddy system' within the cleanroom, which is to watch and provide feedback such as saying, "Hey, you've got exposed skin" or "Don't touch that bag on the shelf", is missing. Realizing this independent, yet critical lack of oversight, leads to the understanding of how important communication is between the cleaning team and the aseptic operations team. Environmental monitoring is not perfect and cannot detect all possible contamination points, so operations must rely on (cleanroom cleaning) people's integrity and honesty.

Where possible and appropriate, some firms have cleanroom cameras to monitor aseptic operations, including process simulations and off-shift activities. If present and operating, the cameras can be used for contamination investigations. Encouraging the speak-up culture and documentation of atypical situations is a way to reduce fear of the use of cameras by encouraging people to share potential deviations in procedures without negative personal consequences.

Selection of Cleaning Tools

The cleaning team and their supervisor should participate in any decision-making related to identifying the correct tools to use, and in some cases the disinfectant agents. Meeting with a supplier can accomplish this. The cleaning team should be able to handle the tools for weight and ergonomic feel for actual application use. This

would be followed by practicing use of the tools with an experienced application trainer.

Application of Cleaning and Disinfection Agents

Training should include an explanation of the importance of the cleaning step prior to disinfection. An explanation of the significance of cleaning of soil and/or residue prior to effective disinfection practices is valuable.

Cleaning is a physically demanding job, so part of that technique is knowing when you've reached either your mental or your physical capacity and being able to have enough robust staff that you can alternate people. That kind of understanding and open communication culture within the cleaning team would be vital. It can be beneficial to include breaks in a cleaning schedule; because if you don't pay attention to the GMP cleaners' emotional and physical state, then it's very possible to reduce the quality of the process.

Key soft skills are: the focus and the attention to detail. The repetition, being able to overcome that repetition and not drift is really difficult for cleanroom operators and the cleaners.

Discuss and/or create ways to help cleaners overcome that in your training program. One consideration is to give them tools, such as checklists that they could follow where they can evaluate their progress.

Build into the process a schedule where they can take a break. Ensure that you give the cleaning team enough time to complete the job without having to rush.

The technique for cleanroom cleaning is not hard when learned. Yet, it must be done and it must be done correctly. It's more important to focus on how they can maintain that technique once they've learned it and over such a large amount of time that it takes to accomplish the cleaning activity.

Training of the application of cleaning and disinfection agents can be shown by standing and starting in the furthest part of the room. The latter approach is common for a facility that is undergoing a regular cleaning and disinfection program. This could be different if, for example, after a shutdown when many people and materials have been moved through the external adjacent areas (e.g., hallways), the soil can be much higher than usual. In this case, cleaners may be asked to 'clean' their way into the aseptic core areas, and then subsequently follow the common practice of cleaning from the innermost to the outer areas. Also, express the rationale to the cleaning team for applying disinfection agents from high areas to low areas and then also backing your way out from the furthest point area to the exit, to show that correct procedure is not walking through what has been disinfected and preventing personal bioburden shedding over a place that was just cleaned. So, there is an order of procedural events intended for the way in which an area is cleaned.

One approach to practical-based training that has a history of success is a three-step process:

1. The cleanroom cleaner observes an experienced cleaner once, and then
2. the new cleaner performs cleaning under supervision, then when determined by supervision that they are ready,
3. the cleaner performs independently with an associate watching them without assisting, and then they can get documented approval.

The training needs to be continuous and regularly reinforced. It also can check and make sure that the trainees are up to date with everything that happens on a day-to-day basis and in operations. This is where a quality 'huddle' can come in handy on a daily or shift basis.

Contact Time and Surface Compatibility

Training cleaners about surface contact time should include the challenges, e.g., high velocity air movement that dries surfaces more rapidly, surface deviations that make access to contaminants more difficult, differences in surface tension that effect surface coverage, and different surface types that lead to disinfectant efficacy differences. A discussion about different methods of application (wipe, mop, spray and wipe, mechanical action, automated vaporization) can increase the confidence of the cleaners in the selected methods. It should also be highlighted that floors should not be walked on until the whole contact time has been achieved to allow the agent to be effective.

Also helpful is an explanation and discussion about the conditional use of sporicide compared to a regular disinfectant. Simple examples can be used. For instance, when something like a piece of equipment that goes outside of the facility, to get calibrated, and then is brought back into the cleanroom, explain that the common approach is to apply heavy wiping with a sporicide for increasing assurance of the removal of microbial contaminants. Another example could be to explain that sporicide should be used in an appropriate rotation to maintain control over fungi and bacterial spores.

Observing and Evaluating Cleaning Practices

Observing cleanroom cleaning can be a value-added activity for an aseptic operations supervisor. It's important to not only oversee a cleaning team but to watch how they perform the proceduralized activities, especially if the activities are performed on an off-shift. This can have great impact, because observing the cleaning team activity offers better insight, rather than just depending on what is documented in a cleaning activity logbook. Remember that people are only as good as the training and the communication that's been provided to them. That will have impact on what or how they are cleaning. Even in a multiple shift cleanroom operation, it is possible to not have sufficient oversight of the cleaning activity and this should be corrected.

Although this evaluation is a type of 'audit', it is important not to make the activity audit-like. It is better to explain to the cleanroom cleaners and their supervisor that normal cleaning practices should be evaluated periodically to help them do their jobs more consistently, efficiently and to seek improvement ideas from them. It is not a good idea to place them in a position where they feel they are being scrutinized and that their jobs are at stake. There are many benefits to good management of a cleaning evaluation, some of which include trust, integrity and developing a good quality relationship with those performing the cleanroom cleaning. The thinking should be, "We're all working towards the best quality process and when you are a part of it and I'm a part of it and we work together, then we'll make a better process".

Use of Environmental Monitoring (EM) Data for Cleaning Evaluation

EM data is used by the Quality Control/Quality Assurance group, within a production facility, to support many activities including cleaning. Yet, is there any sharing or communicating of that type of data with the cleaners?

The response and approach should be 'Yes'. It is important to recognize that if EM samples are taken immediately (and taken correctly) after cleaning there is direct relevance to the cleaning effectiveness. But, be aware and careful that if samples are not taken immediately, and any activity that occurs in the cleanroom(s) before EM samples are taken, this can reduce the direct relevance to cleaning and compound root cause identification.

An initial explanation to the cleaning team on how to understand EM data would be very useful. Microbial recovery can be a potential indication of a much bigger problem. The bigger picture, though, which could be the absence of recovered colonies after cleaning a larger surface area, is that it represents one small sample area. Also, a cleaning operator should not think automatically that a microbial recovery means that any one location needs to be cleaned better. There is a more holistic picture they should understand. A microbiologist can be very helpful in discussing and explaining what thinking and actions can be taken based on EM results and trends.

Training and Communication

Verbal communication is important, but documentation is also important because sometimes you're not going to be able to reach the people that you need to reach in a timely manner. Good Manufacturing Practices (GMP) is all about documenting at the time of the activity and if something goes wrong, it's better to document it immediately and then either justify it later or mitigate it later. But if we don't document, for example, the time or the aspect of what happened, we may lose that information. That's an important part of the job and a key skill set for someone who's working off-shift in a GMP process (such as a cleaning and disinfection activity) that has such a real impact on quality of your clean space.

For verbal communication, sometimes language barriers can be hard. If you have a cleanroom cleaning team that has multiple native languages it can be a challenge when training. Having someone that is bilingual within the team would be amazingly helpful.

Being very slow and deliberate in the training process and not rushing training is important when you have someone who has English as a second language and if the trainer only speaks English. A trainer in this situation should be creative and take adequate time to provide the training in a way that helps a person learn better and includes developing foundational understanding about what are the key takeaways the cleaners should learn. Subsequently the trainer should check their understanding before completion and then again later just to make sure the language wasn't an insurmountable barrier.

Some creative approaches to language differences are the use of videos to show and explain concepts and tasks. Sometimes the cleaners can review them on their own.

For documentation training, a good start is the introduction to appropriate words to use to describe a situation. An example might be: identify the location, explain in simple terms what was observed and if any action was performed prior to leaving the location. The objective is mainly to assist the individual with direction on how to describe a situation and document it accordingly. Explain to them the importance of clearly communicating to others in writing, as best as they can.

The Contract Model

Contract operating models are very common currently in the pharmaceutical industry, especially for functions like cleanroom cleaning.

For the contract model, there should be a policy or procedure for getting the cleanroom cleaners the specific application tools that they need, and clearly identify who owns the budget. This is important because while the cleaners are not client employees, they should be given the appropriate and adequate tools to use and have the personal protective equipment and training to ensure safe working conditions.

The necessary strong balanced relationship between the employees of a contract firm (cleaners and supervisor) and the management of their contract client firm must be trustworthy, successful and people-oriented. Remember, patients are the final recipients of this activity.

Supervision

Managing a cleanroom cleaning team depends on a strong leader and people developer, which is important because they have to work with a diversity of people.

Supervising full-time employees (FTEs) compared with a contract cleaning staff is quite different. For one, contract cleaning staff are likely working second or third shift, while the manufacturing operation staff and supervision may not be. This means that a manager responsible for the cleaning program needs to be willing to flex both hours

and communication style in order to stay current with the cleaning staff. When it comes to a day-shift staff, the same shift supervisor can easily speak to or send the cleaning staff an instant message to communicate, but for 2nd or 3rd shift staff, it's not so simple. In order to be inclusive, and to keep them current with the rest of the team and department news, it is necessary to put in a little extra effort, perhaps by creating online training packages, arranging quick huddles during their working hours, daily or weekly email updates, and videos so that they always have the same information as the manufacturing employees. Another difference is that the manufacturing supervisor overseeing the cleaning program may not be the cleaning team's only management. As contractors, they'll have their own company's management overseeing their work, so it's important to establish a healthy working relationship with their management. Arrange regular meetings with the contract cleaners' management in order to provide feedback on the employees, refresh expectations, and troubleshoot any issues. Also, consider inviting them to any client meetings that are held directly with the cleaning staff. This will reduce miscommunication and will ensure everyone is on the same page.

Initial and ongoing communications is important to develop a relationship between a manufacturing operation staff supervisor and a contract staff cleaning team. A manufacturing supervisor should invest some time getting to know their contract GMP cleaning team. Holding weekly or monthly meetings with them provides a way to learn their personalities and preferred communication styles. Keep in mind again that they may be working different hours from operations and the rest of the full-time staff, so be creative with how to communicate. Once you know your team, tailor communications to them. If they're more visual learners, perhaps consider making PowerPoint presentations or videos for team updates or training. If they like to discuss things in more of a round table setting, arrange quick huddles or check-in calls so that they have the opportunity to ask questions.

The field of parenteral manufacturing is always advancing, and this of course applies to 'GMP cleaning' as well. As equipment, technologies, and regulatory expectations evolve, so should be the approach to disinfection. Making it a priority for a supervisor and/or cleaning team members to regularly attend scientific conferences and vendor exhibitions is a helpful approach to keeping current, so that one can continuously explore developments in the cleaning space. Bring in vendors to give new product demonstrations, and always ensure the GMP cleaning staff are included. The cleaning staff are accountable for the cleaning and disinfection application; thus, they need to be included in any conversations regarding new processes, tools, or materials. It's in a supervisor's best interest to incorporate their feedback and ideas up front. Not only will this ensure a fit for purpose program that is designed right- first-time, but it will also ensure their active participation, ownership, and buy-in!

Building confidence and pride is an essential component of a contract cleaning team. Without first having a robust GMP cleaning program, all other sterility assurance behaviors and approaches are rendered less effective. In addition to basic GMP and facility disinfection training, a supervisor should talk regularly with the GMP cleaning staff about the relevant products and patients (i.e., what the products are indicated for, what living with that disease is like, etc.), mentioned in this article earlier about role ownership objectives. Making the work personal and meaningful increases their understanding of the important role they play and their commitment to quality. Helping the cleaning team achieve confidence and pride in their role is a fundamental objective of a supervisor.

Summary

The GMP cleaning program is absolutely critical to maintaining the microbiological integrity of a facility, operation, and ultimately the quality of the products. Therefore, the training, oversight, and culture of the GMP cleaning team is just as critical.

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Note: Contributions to this article by Lauren Sanmartin were written prior to her tenure with Gilead Sciences.

Reference

1. Technical Report No. 90 Contamination Control Strategy Development in Pharmaceutical Manufacturing, Parenteral Drug Association, 2023, Bethesda, MD
2. 2. USP 44-NF39, <1072> Disinfectants and Antiseptics, USP Convention

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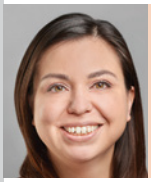
New Regulation to Reduce Contamination Risks in Sterile Manufacturing—and Ways to Adapt



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Both PDA TR 90 and the European Union's recently revised GMP Annex 1 require manufacturers of sterile pharmaceuticals to implement and document a holistic contamination control strategy (CCS) that covers the entire manufacturing chain across the facility. Within this CCS, the environmental monitoring program is an important basic part. Where aseptic operations take place, monitoring according to part 9 of Annex 1 should be frequent, using a combination of methods such as settle plates, volumetric air sampling as well as glove, gown and surface sampling with contact plates or swabs. The sampling plan parameters to consider should be based on a risk assessment and include the locations, number and frequency of samples to take, the sampling methods and practices (including incubation conditions) and the limits to set, including alert levels. Annex 1, although an EU regulation, also affects manufacturers elsewhere insofar as their exports to the EU are concerned, so it's worth taking a closer look at some of its changes.

Low-risk transfer of instrumentation

Moving equipment and materials is known to be a contamination risk in manufacturing. Annex 1 part 4.10 requires this risk of to be assessed, while part 4.15 calls for airflow patterns to be visualized to demonstrate there is no ingress from lower to higher grade areas. This is particularly important when using active microbial air samplers. For isolators, stationary systems specially adapted to this environment, like the **MAS-100 Iso NT® sampler**, are always preferable. If not an option, any sampler moved into a Grade A area should be easy to clean and disinfect and offer the option to have a HEPA filter mounted to its air outlet that retains almost all particles sized over 0.3 µm. This filter should have no significant influence on the airflow calibration and the microbial sampling efficiency.

A microbial air sampler's basic design matters too. Specific blower units with a brushless motor, like the MAS-100 NT® sampler uses, keep particle generation well below the Grade A limits. Validation documentation to demonstrate an instrument's suitability for controlled environments should be available from its manufacturer.

Physical separation to prevent contamination

Also relating to the transfer of equipment, Annex 1 part 4.12 states that all airlocks should be designed and used in a way that ensures physical separation and minimizes microbial and particle contamination between areas. The final stage of an airlock must, in the "at rest" state, be of the same cleanliness grade as the area to which it gives access. Annex 1 part 4.18 suggests that the entry of materials into isolators or RABS during processing (and after decontamination) be minimized and preferably supported by rapid transfer

technologies or transfer isolators. Space in isolators is usually very limited, so using its 190 mm alpha port to attach a transfer bag that contains a stock of contact and settle plates is proving popular. This space-saving storage concept can reduce the number of decontamination cycles and prolong production times. A single **gamma-irradiated IsoBag® DPTE BetaBag®**, for example, can safely hold up to 80 ready-to-use plates for air and personnel monitoring, allowing plate transfer through multiple connections. Triple-packed plates are the ideal choice to maintain aseptic conditions in Grade A (ISO 5) cleanrooms and isolators. This allows the outermost bag to be removed on advancing into the next higher-class area.

Some measures to avoid false results

Certain practices, while not risking contamination of the critical environment, may cause false positive test results. The immediate consequences for the manufacturer are much the same: an often lengthy and costly investigation into the root cause must be launched. Contamination can occur if, for example, plates open while being transferred for incubation between the sampling area and the lab. Lockable plates (e.g. of the **ICR_{plus} range**) help ensure that the lids stay safely in place.

Agar contact plates, however, are impractical for microbial testing of irregular surfaces such as equipment recesses, nooks, crevices, tubing and filling needles. Swabs that deliver a yes/no result are better suitable here. To minimize false positive results, unnecessary handling steps before and after sampling should be avoided. This is achieved by using a swab assembly that carries its own sterile growth medium and therefore has to be opened only once, like the **ICR-Swab**. Its medium comes into contact with the pre-moistened swab tip only after the system has been closed again.

Generally speaking, part 9.29 of the new Annex 1 calls for sampling methods and equipment to be fully understood and procedures put in place for correct operation and interpretation of results. The manufacturer of the culture media should be able to supply detailed validation data and application notes to help build the required documentation, for example on the efficiency of the media to neutralize sanitizers, the impact of air sampling on recovery, and recovery rates of dried versus fresh plates to determine the effects of water loss.

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Origin and Application of the 50-200% Range in the Bacterial Endotoxins Test

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Abstract

Laboratory analysts who have been performing compendial Bacterial Endotoxins testing (BET) for a number of years have come to accept the idea that the range of error of any BET compendial assay is 50-200%. The reality is, the real error of any of the compendial bacterial endotoxins tests is unknown. The following discussion examines the genesis, history, and application of the 50-200% range.

Introduction

The *Limulus* Amebocyte Lysate test was first described in the Federal Register fifty years ago (Federal Register, 1973). At that time, the only available test method was the gel clot test, which is still the current "referee" test in the harmonized Bacterial Endotoxins Test (BET) method (USP 2023). Since the introduction of the photometric assays in the late 1980s, the gel clot test has been replaced in most laboratories by the more quantitative kinetic assays - kinetic turbidimetric and kinetic chromogenic.

Today, the industry is looking at new alternate BET assays that are not dependent on animals, either as a test subject or as a source of reagent. Most of these alternate assays cite the same reference/calibration standards (RSE/CSE), the same standard curve ranges and the same basic assay tenets used by the compendial photometric assays. These alternative tests use several different platforms including the utilization of one or more recombinant proteins in the horseshoe crab's natural clotting cascade to measure endotoxins activity (Loverock et al, 2003; Mizumura, et al, 2017) and assays that use whole blood or Peripheral Blood Mononuclear Cells to measure cytokine responses to pyrogenic materials, including endotoxins (Hartung, et al, 2002). Most alternative assays report results in Endotoxin Units (EU), or a variation thereof called "EU Equivalents". In consideration of ongoing discussions regarding equivalence of the test results reported by these alternate methods to the current compendial assays, it's important to understand the origin and application of the 50-200% Positive Product Control recovery range.

Gel Clot

The gel clot test is the basic LAL assay that has served the pharmaceutical industry and has assured patient safety for 50 years. However, it suffers from three significant constraints:

- There is no continuous standard curve. Rather, the test reference is a discrete series of twofold dilutions of the calibration analyte (RSE or CSE) made to "bracket" the lysate sensitivity (reference Table 1).
- The test results are binary. After incubation, each tube containing either a standard or a sample + lysate reagent is removed from incubation and is slowly rotated 180°. If a firm gel has formed that maintains its integrity upon inversion, the test is positive. Anything else is negative.
- Analyst technique is important in the reading and interpretation of results. If the tube is not rotated in a smooth, slow and consistent motion, the fragile gel could be disturbed. Once disturbed, it will not re-form, causing a false negative test result.

Where did the concept of plus/minus one twofold come from? Table 1 represents a series of standards and results of a gel clot label claim verification study (USP, 2023). The test sensitivity (label claim or λ) is 0.25 EU/mL. The column entitled "RSE/CSE" represents the requisite twofold serial dilution of the calibration standard in Water for BET to bracket the label claim sensitivity of the reagent. The endpoint of each series is defined as the last positive tube followed by a negative one. Five possible sets of standard series results are provided in Table 1.

Table 1. Standard Series, Gel Clot

RSE/CSE EU/mL		Standard Series				
		A	B	C	D	E
2λ	0.5 EU/mL	+	+	+	+	-
λ	0.25 EU/mL	+	-	+	+	-
1/2λ	0.125 EU/mL	-	-	+	+	-
1/4λ	0.06 EU/mL	-	-	-	+	-

The endpoint for series A is 0.25 EU/mL and matches the label claim sensitivity "on the nose." However, if the analyst doesn't match the lambda endpoint exactly, there are only two ways to go – one twofold dilution up (series B where the endpoint is 0.5 EU/mL) or one twofold dilution down (series C where the endpoint is 0.125 EU/mL). A requirement to match λ exactly under the conditions of the gel clot test is rigid given the biological nature of the reagents, so the "rule" evolved to confirm the label claim plus or minus one twofold dilution because that's the best that the test can do. That sounds reasonable given the constraints of method and variability in sample preparation, but it's really a 50-200% swing relative to λ. Examples D and E are invalid because there are no endpoints.

Intuitively, the real endpoint of Series A lies somewhere between 0.25 EU/mL and 0.125 EU/mL, but the configuration of the test doesn't allow for that kind of resolution . Therefore, one twofold dilution is the resolution of the gel clot test, meaning that it is the best the test can do to resolve the difference between two values.

Perhaps "error" was a technically improper term to apply to this two-fold interval, although the terms accuracy and resolution are often used interchangeably. Accuracy describes how close a measured value is to the true value while resolution is the smallest distinguishable difference in results that the method can produce. While they are similar, and while accuracy is to a large degree dependent on resolution, they are not the same.

Photometric Tests

Upon their introduction, the photometric tests addressed and resolved the three major constraints of the gel clot test as well as the resolution issue. Rather than a discrete series of twofold dilutions, the photometric tests allowed for a continuous standard curve to be drawn from the kinetics of the activity of each diution in an RSE or CSE standard series. Figure 1 is a depiction of the kinetics of a series of calibration standards plotted as optical density (or color) as a function of time. The highest concentration in the series is on the left, and each of the subsequent curves represents decreasing concentrations of the standard.

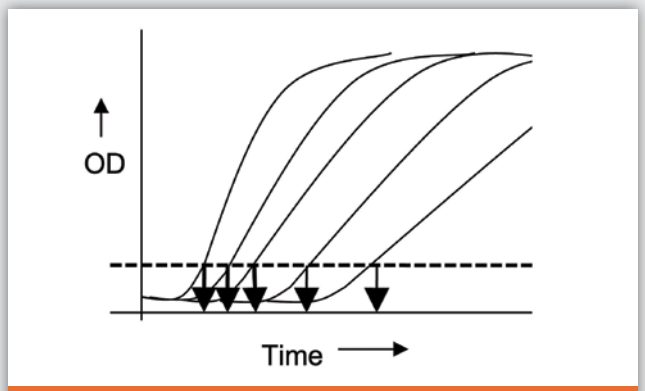
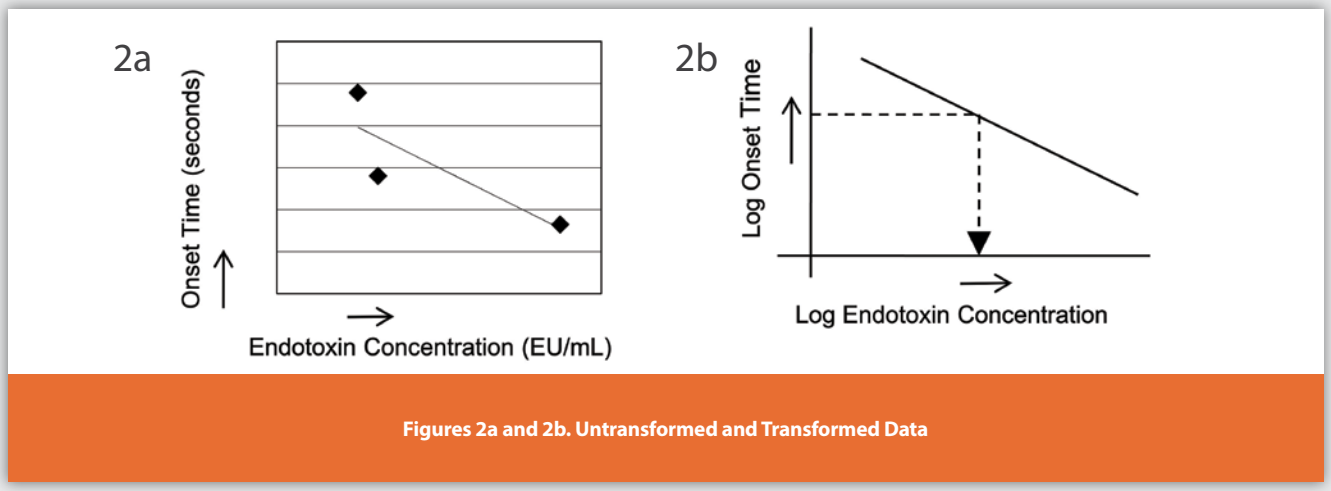


Figure 1. The kinetics of a series of calibration standards (adapted from Levin and Bang, 1968)

The dashed line in Figure 1 is called the Onset Optical Density, which is a pre-determined level of turbidity or color recommended by the manufacturer of the reagent. The downward arrows represent the time it takes for each standard to generate sufficient turbidity or color to reach the Onset OD. These individual times are called the Onset Times or Reaction Times. In Figure 1, the highest standard concentration has the shortest or quickest Onset Time. What is important to note is that each of these standards has a lag time and a reaction rate (slope) that are representative of the endotoxins activity: more concentrated (higher) calibration standard dilutions have a shorter lag time and react faster (steeper slope) than lesser concentration standards (Levin and Bang, 1968).



Figures 2a and 2b. Untransformed and Transformed Data

If the Onset Times for a series of standards are plotted as a function of endotoxin concentration, the graph looks like Figure 2a - hardly useful for routine endotoxins analysis. However, transforming the data by plotting the log of the onset time for each standard as a function of the log of its endotoxin concentration gives a straight line standard curve with a negative slope. Lower concentrations in the standard series take longer to reach the Onset OD (Figure 2b). Therefore, the convention in the harmonized BET Chapter <85> is to transform the data to establish a linear standard curve from which the endotoxins activity in a sample or a back calculation for a standard can be interpolated (dashed line in Figure 2b).

This transformation is most helpful in making a usable standard curve, but it reinforces the fact that a photometric test result is different than a gel clot result in that 1) it is dependent on time rather than gelation, and 2) due to the log transformation of the data, small changes in the onset time can have significant impact on the quality of the standard curve and ultimately the accuracy of the test result.

What is the "error" of the photometric tests? Dr. Ronald N. Berzofsky, formerly of Cambrex (now Lonza) suggested that the real error of a photometric test, based on reproducibility of reaction times for the calibration standard diluted in Water for BET, is about 10%. So where did 50-200% "validity criteria" for the photometric Positive Product Control (PPC) come from?

The accuracy and therefore the total error of any photometric test are dependent on the quality of the standard curve (slope, y-intercept and correlation coefficient), which means that the accuracy of the test result is ultimately dependent on the onset times (McCullough, 2011). Sources of onset time variability include but are not limited to the following:

- Dilution schemes and techniques
- Pipetting technique
- Uneven plate heating
- Endotoxin contamination, adsorption, or extractables/ leachables in accessories such as plates and pipette tips
- Timing of the addition of reagents to plates
- Product-specific interference

While the lysate as purchased and tested using Water for BET may exhibit an error of 10%, the real error of the test depends on the laboratory's level of assay understanding and state of control. Because of the number of potential input variables, the correct answer to the question of overall error in a photometric test is **"We don't know"**. What we do know is that defaulting to a 50-200% "error" or "validity" range for PPC recovery is scientifically unsupported.

The Interim Guidance on the use of kinetic tests provided by FDA in 1991 (now retired) was prescient in many ways. Italicized words are quotes from this document:

> *The performance characteristics (slope, y-intercept and correlation coefficient,) for the lysate lot sent by the manufacturer will not be valid. New performance characteristics have to be established for each lot by performing the procedures outlined in Appendix A (creation of a standard curve).*

When the number of reagent vendors and instruments were limited, the reagent manufacturer would add slope and y-intercept determinations on the reagent Certificate of Analysis and the user would have to match those values. As the number of vendors and instruments increased, it became apparent that the standard curve parameters could, and often did, change with the combination of reagent and instrument or from instrument to instrument. It is important to note that FDA recognized early on that standard curve parameters other than correlation coefficient were important and that they were dependent on individual laboratory conditions.

The Guidance went on to describe the Positive Product Control:

> *The calculated mean amount of endotoxin when referenced to the standard curve, minus any measurable endogenous endotoxin in the spiked drug product, must be within plus or minus 50% of the known spike concentration to be considered to neither enhance nor inhibit the assay. If there is no measurable endogenous endotoxin in the product, the value will usually be equal to or less than plus or minus 25% of the standard curve value.*

The original Guidance had no reference to the range of 50-200%, but made a distinction between recovery in samples with no detectable endotoxin background (± 25%) and samples with high levels of background endotoxin (± 50%). The reason for the two recommended recoveries is that high background (contamination) in a test sample will tend to mask a much lower PPC value.

However, neither the requirement to control the quality of the standard curve other than correlation coefficient, nor understand the potential impact of background endotoxins on the recovery of the PPC made it into USP <85>, as adding these requirements presented new challenges to the implementation of photometric methods in the QC laboratory. The 1991 Guidance was retired in 2012 with the publication of FDA's Question and Answer Guidance (FDA, 2012)

The gel clot reagent verification and the Test for Interfering Factors both make reference to the method's plus/minus one twofold test resolution (50-200% of lambda). However, there is no validity range for a PPC recovery in a gel clot test because the PPC level is set at 2 lambda (test resolution), and it MUST gel. Reagent verification and Test for Interfering factors are different in photometric tests, and are not constrained by test method resolution. The twofold resolution range was passed along to photometric PPC recovery to ease the path for a laboratory to move from the gel clot limits test to the more quantitative tests, and was justified at the time because all of the reagents came from the same source (Limulus lysate). In other words, the 50-200% PPC "validity range" is not related to any photometric assay attribute or capability.

In retrospect, that decision, while well intended to advance endotoxins testing beyond gel clot, perpetuated a misunderstood and scientifically unfounded parameter regarding variability and error in the photometric tests. What is important now is that QC laboratories that employ the BET understand the origin of 50-200% as a residual attribute of only the gel clot test and recognize that it does not define the error of any bacterial endotoxins test method.

Equivalence of Alternatives to <85>

Why is this information important to the assessment of alternatives to the compendial assays?

Figure 3 is an example of "relative recovery" calculations of a data set in the public domain comparing an alternative assay to a compendial photometric LAL test. For this exercise, the value for any one sample generated by the LAL test result is assumed to be correct given that it is a validated test in a compendial chapter numbered under 1000. The percent relative recovery for the alternate method is calculated by dividing the result obtained using the alternative test method by the result obtained on the same sample obtained by the LAL comparator. and multiplying the quotient by 100. For example, if the LAL test of a contaminated sample measures 46 EU/mL and the alternative test measures 42 EU/mL, the percent recovery of the alternative test to the compendial test is [(42 EU/mL) ÷ (46 EU/mL)] x 100 = 91% recovery. In other words, the alternative test measured 91% of the activity that the compendial test measured.

In figure 3, each column represents a single sample that was tested on both the compendial and the alternative tests. The height of the column represents the recovery of endotoxins activity relative to the LAL photometric, denoted as 100% by the dotted line. The shaded box represents 50-200% of endotoxins activity recovery relative to LAL. Graphically, all of the recoveries in Figure 3 appear to fall within the 50-200% range, so one might conclude that the proposed alternative test and the compendial test are therefore equivalent.

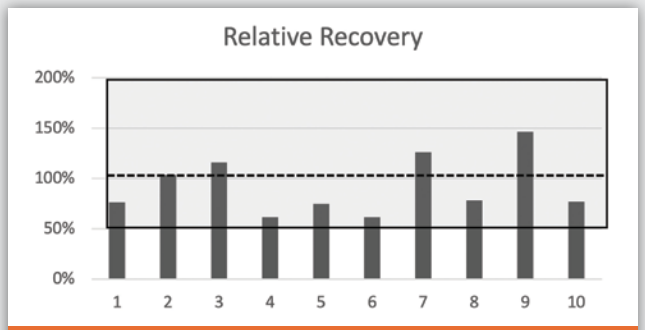


Figure 3. Relative Recovery

However, this inference is unfounded, as the data represented in this study not only reference a "validity range" that is scientifically unsupported for either method, but also does not contain a confidence interval or a justified equivalence interval, both of which are required for the assessment of true statistical analysis of equivalence (Hauck, et al, 2009).

Subjecting these same data to a statistical analysis of equivalence (two one-sided test, or TOST) reveals that the alternative method is not equivalent to the compendial test at an equivalence interval of 0.3 or ±30% and a confidence interval of 95%. So while relative recovery using a range of 50-200% seems on one hand to be a reasonable graphic way to compare test methods, it may not align with accepted, standard statistical analyses for equivalence.

Implications of the 50-200% PPC Recovery Range

The point of the PPC is to prepare the test article so that the calibration standard added to the prepared product acts like the analogous point on the calibration curve, which is standard prepared in water. The PPC is really one indicator of accuracy. For example,

- If the PPC in a prepared product recovers 58% of the actual test result, OR there are issues with the standard curve that affect spike recovery, we must assume that there is something going on in the sample that is interfering with the recovery, and that this same interference might affect the actual test result.

- Acceptance of the 50-200% range suggests that the laboratory also accepts that a reported test result is accurate to within the range of 50-200% of its reported value. Therefore, a test result of 14 EU/mL against a limit of 25 EU/mL would fail under the "validity" assumption because the is assumed to be accurate to within a 50-200% range of the reported value.
- Test results for kinetic tests and alternate tests are often reported out to three decimal places, which suggests a degree of accuracy is inconsistent with simultaneous acceptance of an "error"/"recovery range"/"verification range" of 50-200%.

Summary

In summary, 50-200% is neither the calculated error of any compendial BET assay nor is it an appropriate parameter for evaluating the equivalence of two test methods. The range was originally derived from the resolution of the gel clot test method that was applied to the kinetic PPC, based on the source of the reagent to ease the transition between gel clot and photometrics. Consequently, the true error of any photometric test is unknown, but variability, which can lead to inaccuracy, can be controlled almost exclusively in the laboratory by careful control of standard curve generation and sample preparation.

Literature Cited

Berzofsky, Ronald N. 2014. Test Variability: Artifacts and Attributes That Can Affect Endotoxin Recovery. Bacterial Endotoxin Summit. May, 2014

Federal Register January 1973. Federal Register Vol. 38, No. 8 p. 1404.

FDA. 1990. Interim Guidance for Human and Veterinary Drug Products and Biologials: Kinetic LAL Techniques (retired)

FDA. 2012. Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-pyrogen-and-endotoxins-testing-questions-and-answers

Hartung, Thomas, Members of the Human€ Pyrogen Test Study Group. 2002. Comparison and Validation of Novel Pyrogen Tests Based on the Human Fever Reaction. ATLA 30, Supplement 2, 49-51.

Hauck, W.W., A.J. DeStefano, T.L. Cecil, D.R. Abernethy, W.F. Koch, R.L. Williams. 2009. Acceptable, Equivalent, or Better: Approaches for Alternatives to Official Compendial Procedures. Pharmacopeial Forum 25(3): 772-778

Levin, J and F.B. Bang. 1968. Clottable Protein in Limulus: Its Localization and Kinetics of its Coagulation by Endotoxin. Thrombosis et Diathesis Harmorrhagica. 19(1): 186-197

Loverock, Bruce, Barry Simon, Allen Burgenson, Alan Baines. 2010. A Recombinant Factor C Procedure for the Detection of Gram-negative Bacterial Endotoxin. Pharmacopeial Forum, Stimulus to the Revision Process. Vol. 36(1).

McCullough, Karen Zink. 2011. Constructing and Interpreting Standard Curves for Quantitative BET Assays. In: The Bacterial Endotoxins Test A Practical Guide. Karen Zink McCullough, editor. DHI Publishing, River Grove, Illinois.

Mizumura, Hikaru, Norihiko Ogura, Jun Aketagawa, Maki Aizawa, Yuki Kobayashi, Shun-ichiro Kawabata, Toshio Oda. 2017. Genetic engineering approach to develop next-generation reagents for endotoxin quantification. Innate Immunity. 23(2): 136-146

United States Pharmacopeia. 2023. <85>, "Bacterial Endotoxins Test"

Author Biographies

Terry Munson *is a former FDA Microbiologist, Compliance Officer and Director of Sterile Drug Compliance in FDA's CDER Office of Compliance. He was the lead author for the 1987 "Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products and Medical Devices" as well as the 1991, "Interim Guidance for Human and Veterinary Drug Products and Biologicals: Kinetic LAL Techniques" After retiring from FDA, he served as the Technical VP for Parexel Consulting. He is also a former member of the USP Expert Committee, Microbiology General Chapters. He is now retired.*

Karen McCullough *is Managing Member of MMI Associates, LLC, a consulting company specializing in Pharmaceutical Microbiology, with a focus on the Bacterial Endotoxins Test, and also in Quality Systems development, particularly for start-up ATMP companies. She has held positions as Director of Microbiology, Sr. Director of Quality Compliance and VP of Quality Operations in pharmaceutical, medical device, cell therapy, and CDMO orgaizations. She is a former member of the USP Expert Committee, Microbiology General Chapters.*

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LUBRITAB® RBW – Direct Compression Tableting vs. Standard Lubricants

Aim of the study

The goal of this study was to compare the lubricant functionality of LUBRITAB® RBW with that of the lubricants magnesium stearate, sodium stearyl fumarate (PRUV®), and hydrogenated vegetable oil (LUBRITAB®).

The tablet model chosen used microcrystalline cellulose (MCC) and ibuprofen. With respect to tableting issues, ibuprofen is a challenging active ingredient – it is sticky, has a low melting point, exhibits poor flow, and is generally formulated as a high dose active.

Each lubricant was studied at five different levels. A portion of each blend was withdrawn from the blender at five different blending times, and compacted at five separate compression forces. This was done to develop a thorough understanding of their behavior in this tablet model.

LUBRITAB® RBW

LUBRITAB® RBW (Rice Bran Wax) is an all-natural, gluten-free, plant-based, clean label lubricant that is suitable for nutraceutical applications. Derived from the bran layer found in rice grains, LUBRITAB® RBW serves as a lubricant in tablet and capsule formulations at recommended concentrations of 0.25 – 2% (w/w), and may be used in combination with an anti-adherent.

LUBRITAB® RBW has multiple advantages:

- The only clean label lubricant
- Sustainably sourced
- Comparable use level to commonly used lubricants
- Robust tablets
- Low risk of overblending
- Minimal impact on tablet disintegration
- Inert for improved API stability

Formulation

The tablet formulation consisted of ibuprofen, EMCOCEL® 90 M (MCC) as binder, and either LUBRITAB® RBW, magnesium stearate, PRUV® SSF or LUBRITAB® HVO as lubricant. The amount of MCC was adjusted to make up for the different lubricant levels used and maintain a consistent blend size and drug concentration. USP/NF grade ibuprofen and magnesium stearate were used for the study.

Formulation	mg/Tablet	Contribution (%)
Ibuprofen	400.0	80.00
EMCOCEL® 90 M	95.00 – 99.375	19.00 – 19.875
Lubricant	0.625 – 5.00	0.125 – 1.00
Total	500.0	100.0

Excipients

LUBRITAB® RBW is a clean label lubricant that is intended for nutraceutical formulations. Magnesium stearate is the most commonly used tableting lubricant. PRUV® SSF is a tablet lubricant specifically designed for formulations in which other lubricants lead to formulation and/or manufacturing challenges. LUBRITAB® HVO is a liquid film lubricant that can also be applied as an auxiliary tabletmbinder. EMCOCEL® 90 M is a microcrystalline cellulose grade with good flow and compactibility characteristics.

Procedure

Blending

Blends consisting of five separate levels of each lubricant type were prepared, with the lubricant sieved through a 20-mesh screen and added to the ibuprofen-MCC premix in a low shear mixing vessel, and the blending time set

for up to 60 minutes. After the initiation of blending, a portion of each blend (enough to make a representative number of tablets) was withdrawn at each of 5 intervals (5, 15, 30, 45 and 60 minutes). Each blend was compacted at five different compression forces, on an instrumented tablet press, using ½ inch round, flat-face tooling.

Equipment	
Low Shear Mixer	Glen Mills Turbula
Tablet Press	Piccola Rotary Instrumented Tablet Press
Hardness Tester	Sotax Model HT10 Hardness Tester
Tablet Characteristics	
Tablet Weight	500.0 mg
Tablet Shape	0.5000" round flat faced
Tablet Height	2.7 - 4.3 mm

In all cases, with all lubricants at all conditions, some "filming" of the punches was observed, highlighting the sticky nature of ibuprofen. Punches were cleaned before continuing with each variant. Picking was observed in the blends with magnesium stearate.

Tablet hardness and ejection force were measured for each variant. Ejection force values within each lubricant level and each blending time were generally low (100 – 200 N) and comparable across all four lubricants indicating satisfactory lubricant functionality (Table 1).

Table 1. MCC/Ibuprofen Tablets: Ejection Forces after 5 Minutes

	Magnesium Stearate	PRUV® SSF	LUBRITAB® HVO	LUBRI-TAB® RBW
Lubricant Concentration	**Ejection Force [N]**			
0.125%	177	166	161	168
0.25%	182	185	164	156
0.50%	168	207	153	171
0.75%	215	191	157	164
1.0%	123	153	105	131

Figure 1 shows similar ejection force and tablet hardness values for LUBRITAB® RBW, magnesium stearate and LUBRITAB® HVO at 0.5% lubricant after 5 minutes of blending, with slightly higher ejection forces (but similar tablet hardness values) with PRUV® SSF. The increase in ejection forces with increased tablet hardness is to be expected as increased compaction force increases die-wall adhesion.

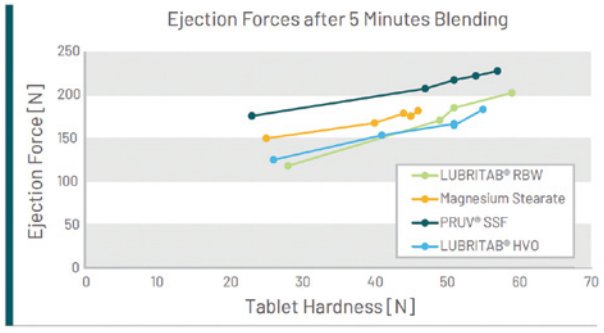

Figure 1. Effect of Lubricant Choice on the Ejection Forces of MCC/ Ibuprofen Tablets after 5 Minutes Blending.

Figure 2 shows similar ejection force and tablet hardness values for LUBRITAB® RBW, PRUV® SSF and LUBRITAB® HVO at 0.5% lubricant after an hour of blending, with similar ejection forces (but lower tablet hardness values) with magnesium stearate, due to decreased inter-particulate cohesion from lubricant over-blending.

Figures 3-6 show three-dimensional contour plots for each lubricant at 15 kN compression force, indicating tablet hardness across lubricant levels and lubricant blending times. When comparing tablet hardness values for the lowest levels of lubricant blended for the shortest time

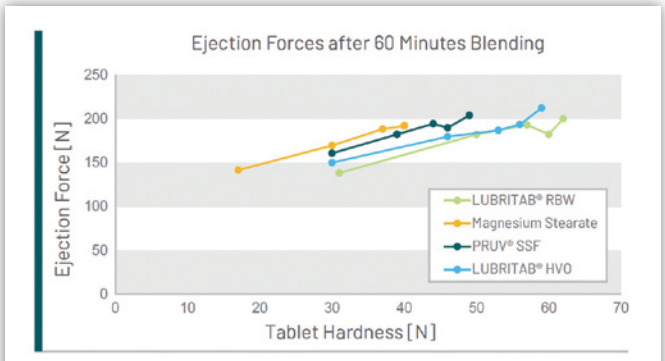

Figure 2. Effect of Lubricant Choice on the Ejection Forces of MCC/ Ibuprofen Tablets after 60 Minutes Blending.

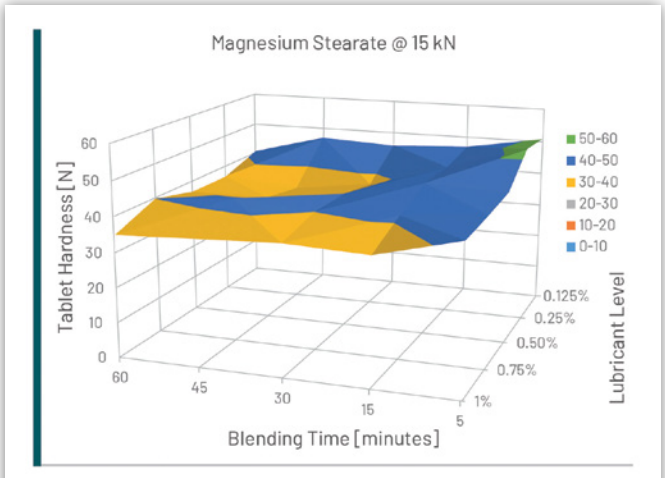

Figure 3. Effect of Lubricant Level and Blending Time on the Tablet Hardness of MCC/Ibuprofen Tablets Lubricated with Magnesium Stearate.

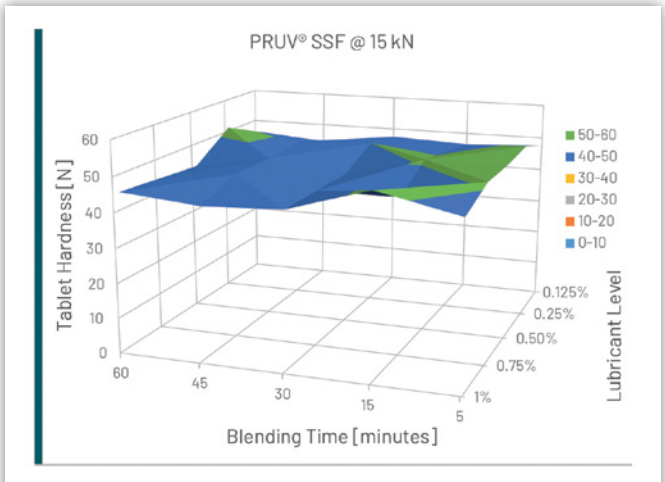

Figure 4. Effect of Lubricant Level and Blending Time on the Tablet Hardness of MCC/Ibuprofen Tablets Lubricated with PRUV® SSF.

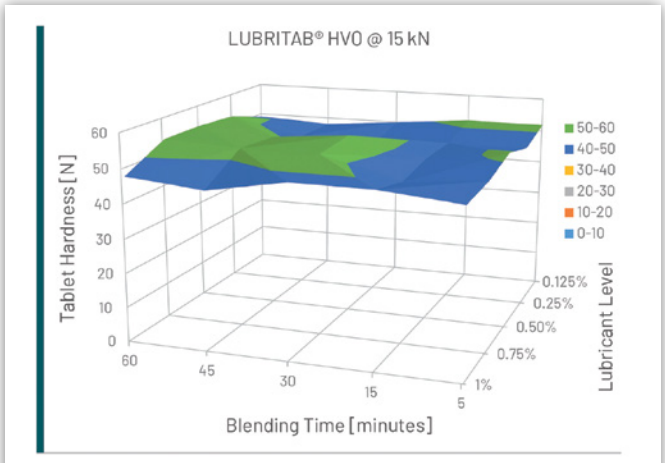

Figure 5. Effect of Lubricant Level and Blending Time on the Tablet Hardness of MCC/Ibuprofen Tablets Lubricated with LUBRITAB® HVO.

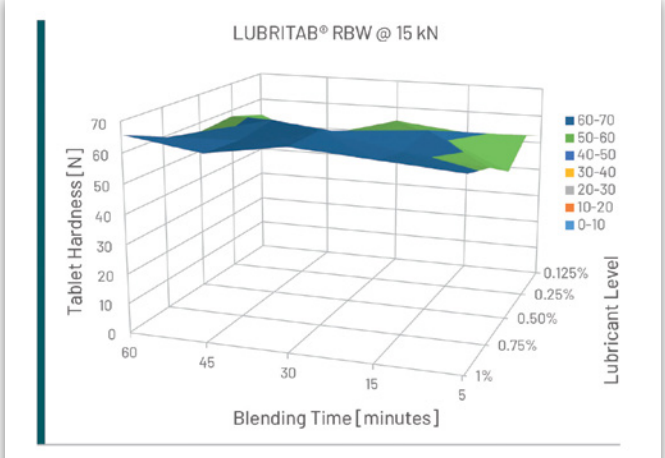

Figure 6. Effect of Lubricant Level and Blending Time on the Tablet Hardness of MCC/Ibuprofen Tablets Lubricated with LUBRITAB® RBW.

with the highest levels blended for the longest time (see also Figure 7), tablets lubricated magnesium stearate show a substantial drop in tablet hardness (~16 N), while tablets lubricated with PRUV® SSF, LUBRITAB® HVO show a minor drop in tablet hardness (~2-4 N). Tablets lubricated with LUBRITAB® RBW, on the other hand, show a marked increase in tablet hardness (~13 N)

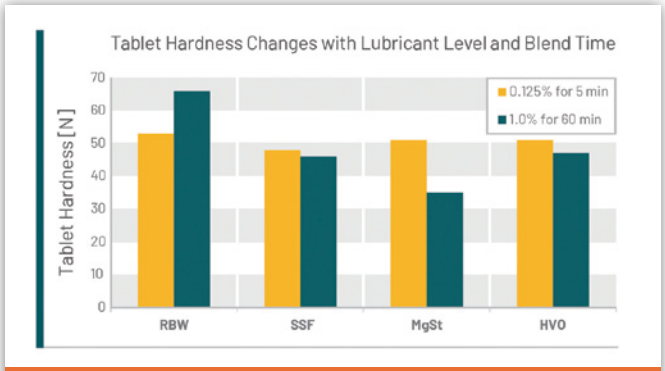

Figure 7. Effect of Lubricant Level and Blending Time on the Tablet Hardness of MCC/Ibuprofen Tablets at 15 kN Compression Force.

Conclusion

The main functions of a tablet lubricant are to reduce adhesion of the tablet post compression, minimize ejection forces, and reduce punch face adhesion. An ideal tablet lubricant would achieve these functions without any negative impact on the compaction and disintegration / dissolution of the resulting tablets.

Contrary to what is typical of lubricants, tablets lubri- cated with LUBRITAB® RBW showed an increase in tablet hardness of with increasing compression forces and blending times. This suggests that LUBRITAB® RBW is acting as a binder to some degree (without negatively impacting ejection forces).

When compared to PRUV® SSF, LUBRITAB® HVO and magnesium stearate, LUBRITAB® RBW demonstrated comparable and in some cases superior die wall lubri- cation (lower ejection force) and comparable reduction of punch face adhesion, in the ibuprofen tablet model studied.

Find out more about LUBRITAB® RBW on https://www.jrspharma.com/pharma_en/products/promo/lubritab-rbw.php

Key Words: Lubricant, Direct Compression, Clean Label

JRS Products: LUBRITAB® RBW, PRUV® SSF, LUBRITAB® HVO, EMCOCEL® 90 M, EXPLOTAB® SSG

What Makes LUBRITAB® RBW Special

An ideal tablet lubricant	Magnesium Stearate	PRUV® SSF	LUBRITAB® HVO	LUBRITAB® RBW
Facilitates ejection of the tablet from the die (lowers ejection force)	+++	+++	+++	+++
Minimizes adhesion of the tablet ingredients to the punch faces	+	+++	++	++
No impact on tablet hardness	-	++	++	+++

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LUBRITAB® RBW

Rice Bran Wax

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LUBRITAB® RBW (Rice Bran Wax) is the innovative all-natural lubricant designed to meet growing clean label demands. Derived from rice, this plant-based ingredient is gluten-free, and offers a sustainable solution for tablet and capsule formulations.

Benefits

- The only clean label lubricant
- Sustainably sourced
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- Robust tablets
- Low risk of overblending
- Minimal impact on tablet disintegration

Application

- Direct compression
- Capsules
- Wet granulation
- Dry granulation
- Continuous manufacturing

Packaging

- Available in 25 kg drums, 5 kg pails

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AN INTERVIEW WITH...

Robert Cartee, PhD

Senior Director Biopharmaceutical Services

SGS North America Inc.

1. Can you give us a brief overview of SGS and the services the company provides to help pharmaceutical companies test, develop, and manufacture cell and gene therapies?

As a leading global Testing, Inspection, and Certification (TIC) company, SGS supports pharmaceutical and biotech companies in the development of Cell and Gene Therapies (C&GT) by providing comprehensive testing of raw materials, drug substances, and drug products through all phases of product development. Our testing laboratories across the globe have a broad portfolio of techniques and expertise and operate at the highest quality under global GMP compliance. SGS also has a Phase 1 clinical unit and bioanalytical testing services that support C&GT products. SGS works collaboratively with C&GT developers utilizing a business model that best suits their needs.

2. What type of testing is required for cell and gene therapy products?

Like all biopharmaceutical products, C&GT products require testing throughout their production from testing of raw materials and production cell lines to in-process testing during manufacturing to the quality control release testing and stability of drug substance and drug product lots. Extensive testing is required to determine the identity, safety, purity and potency of these materials throughout all stages of production. SGS provides a comprehensive panel of analytical testing to support most stages of C&GT production that include evaluation of physicochemical properties such as appearance, pH and osmolality, determination of identity and content of raw materials and final products, quantitation and determination of both product and process impurities, testing of cell lines and final products for contamination with endotoxin and adventitious agents, and evaluation of potency through a variety of cell-based and in vitro methods.

3. How does testing cell and gene therapies play a crucial role in ensuring their safety and efficacy before they are administered to patients?

Like any other therapeutic agent, C&GTs, are evaluated for a number of critical attributes, including safety and efficacy, prior to patient

administration. Since many C&GT products incorporate cellular production systems, potential contamination from host-related components and adventitious agents that pose a safety concern must be considered and carefully monitored by robust testing methods. Furthermore, general manufacturing processes also require assessment, including sterility, extractables and leachables, endotoxin, mycoplasma, and bioburden. Lastly, since many C&GTs involve changing the genetic make-up of the target cell (i.e. adding or editing genes), determining any off-target genetic modifications is becoming a key part of product safety. In addition to carefully evaluating the safety of a product, measuring a products potency is key to ensuring its potential efficacy when administered to a patient. These potency assays often involve in vitro cell-based assays that can quantitatively measure the functional outcome of the therapy.

4. What are the potential risks and adverse effects associated with cell and gene therapies, and how does rigorous testing help in identifying and mitigating these risks?

Risk assessment of C&GT products relies heavily on a detailed understanding of the product, its mode of action and the manufacturing/delivery processes. These risks include contamination by harmful agents (biologic and chemical) that stem from the raw materials, cell lines, and production process. Additionally, there are risks associated with off-target effects of the product, undesired immunogenicity that can lead to reduced product efficacy or cross reactivity, heredity implications, as well as potential passing of vector from patient to an untreated individual. Most of these risks, however, can be mitigated by thoroughly evaluating the quality and consistency of cell lines and raw materials; screening cell lines for adventitious agents, such as viral, bacterial, mycoplasma, fungi, prions, or parasites, and developing sensitive methods for evaluating any off-target impacts of the product.

5. In what ways can testing cell and gene therapies help researchers and clinicians gain valuable insights into the mechanisms of action and optimal dosing regimens for these innovative treatments?

Evaluating the potency of C&GT as part of release testing and stability is critical for understanding the product dose level required to elicit the desired impact in a cell. These assays usually involve an in vitro cell-based assay where the cells are treated with varying dose levels of product followed by a quantitative readout of the desired functional impact of the therapy on the cell. As the products progress through the clinical evaluation, this potency assay can be correlated with the clinical outcome influencing dose level and frequency.

6. What are the long-term implications of inadequate testing or skipping essential preclinical and clinical trials when it comes to cell and gene therapies?

While long-term risks require evaluation for many therapeutic classes, C&GT's may be particularly prone to such events since many are intended to achieve a prolonged response and, as such, long-term exposure may produce unpredictable or unexpected delayed negative consequences for the patient. As such, comprehensive analytical and pre-clinical testing is critical in order to minimize delayed risks such as malignancy, impaired gene function, autoimmune-like reactions, reactivation after latency and infection, and resistant infections.

7. Looking ahead how will SGS continue to bring innovation and value to its customers who are developing cutting edge treatments such as cell and gene therapies?

Since many of the technologies being developed for C&GT are relatively new and only now being tested clinically, the understanding of how their quality attributes contribute to their safety and efficacy is constantly evolving. SGS's collaborative approach in client interactions allows us to continually improve assays sensitivity and robustness, as well as develop new methods to meet regulatory needs. Furthermore, SGS recognizes the promising future of C&GT products in helping patients with debilitating and previously untreatable diseases and the need for rapid but accurate and sensitive testing. To meet this need, SGS has invested in state-of-the-art equipment and personnel with specialized expertise to reduce assay turn-around-times without sacrificing quality.

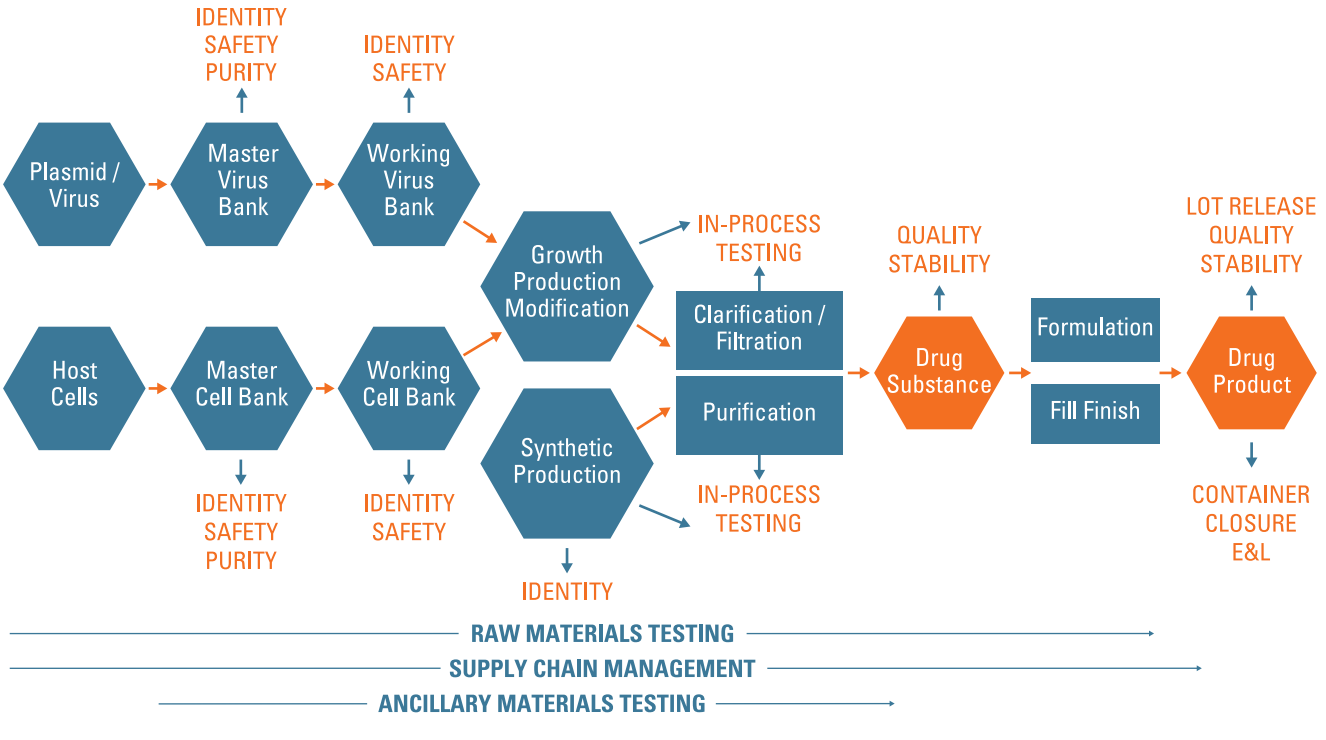
Cell & Gene Therapy

Health Inspired, Quality Driven.

How SGS Supports C&GT Manufacturing

C&GT product manufacture requires comprehensive testing regimens which must address key aspects of Safety, Identity, Strength/Potency, Purity and Quality. SGS can address the analytical demands of all these categories, including development (de novo), transfer, stability and release.

- SGS offers one of the most comprehensive analytical support packages for the manufacture of C&GT products.
- Partnering with SGS provides access to an exceptional testing portfolio and expertise found within our key biopharmaceutical pillars of biosafety, characterization and QC & release.
- SGS's long history in biopharmaceuticals, wide geographical footprint and superior client service support combine to provide an outstanding partner for your C&GT outsourcing needs.



IDENTITY
Confirm presence and structural characteristics of therapeutic vector

SAFETY
Ensure that products are free of detectable contaminating agents

QUALITY
Ensures product consistency and standard

PURITY
Establishes a product free from physical, biological and chemical contamination

POTENCY
Defines the therapeutic activity of the drug product

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  This session is chaired by Jaimie Dufresne of YYZ Pharmatech and John Wasylyk of Bristol Myers Squibb

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Regulatory Submissions for Rapid Microbiological Methods (RMMs)

Jeanne Moldenhauer

Vice President
Excellent Pharma Consulting, Inc.

Background

More than twenty years ago, companies started introducing rapid microbiological methods (RMMs) to the industry. Some call these methods alternative microbiological methods. Rapid or alternative microbiological methods refer to techniques that enable faster and more efficient detection, enumeration, and identification of microorganisms compared to traditional methods. Note: Some companies choose to separate identification methods from this category, due to the differences in validation and implementation of these methods. RMM methods have the potential to reduce testing time, increase sensitivity, improve accuracy, and enhance overall efficiency in the analysis of microbial contamination in pharmaceutical, food, and environmental samples.

Salespeople came to companies promising sterility tests that took substantially less time than the 14-day test currently required. Other salespeople indicated that these new methods could have a more sensitive test method, for example single cell detection.

When these individuals were queried, it became evident that many did not have a good understanding of the regulatory and testing requirements that had to be met, prior to a pharmaceutical company's ability to implement these methods. As a result, companies were slow to move to these newer methods. Additionally, RMM manufacturers drastically underestimated the time it would take to get their systems into full implementation at regulated companies.

As an individual in a company becomes interested in RMMs, they go to their management to get approval to purchase and/or investigate these methods. One of the first questions from management personnel is often some version of whether the regulators will accept these methods.

Contrary to common industry perceptions, the regulators have embraced RMMs. The use of RMMs supports quality and safety objectives of the regulatory agencies, as these systems may obtain more information on how the process is operating earlier in the manufacturing process. The regulators have requirements that testing be performed to verify performance and equivalence, or superiority of the methods when compared to traditional methods.

Hindrances to Acceptance by the Pharma Industry

Many individuals think that the regulators are the reason that RMMs are not yet used extensively.

Dr. Michael J. Miller published a great article on the regulatory acceptance of rapid methods. "After all was said and done, the participants came to a universal agreement that it really wasn't the regulators who were not accepting RMMs, but rather it was primarily their internal departments like regulatory affairs. When pressed on their reasons, the following themes were disclosed why companies are still hesitant about implementing RMMs:"[1]

- 'A desire not to change approved regulatory dossiers or marketing authorizations"
- "A fear of new methods and in general, change"
- "Being too conservative and not open to thinking outside the box"
- "Perception that the cost of implementing RMMs and submitting variations to dossiers would be prohibitive"
- "Companies do not want their scientists talking with regulators."

Major Issues with Perceived Lack of Acceptance by Regulators

At the time when RMMs were introduced, the regulatory group at companies was typically comprised of chemists, with few if any, microbiologists. The chemists in these company departments were not used to dealing with microbiologists. In general, when people do not know a topic well, they tend to be extra conservative. In talking to various people about validation conditions, there were concerns with how much data would be needed to convince regulators to allow implementation of these methods. Additionally, chemists are used to very tight controls and limits for chemistry methods. Most microbiology methods would have significant issues meeting these types of tight limits. In testing these parameters for RMMs, most often it is the conventional method which fails the validation criteria.

As stated earlier, many companies were concerned with "not knowing the expectations for a submission to the Agency for a new RMM." In many cases, the concern was that an extensive validation strategy could be evaluated, and numerous studies performed, and the regulators would not accept or agree with the validation strategy that was used. This could result in significant costs to the company and delay the acceptance of the intended usage of the new technology. This one is also tied to a fear of loss of reputation by pushing for a methodology and major project which then is not accepted by the regulatory agency.

In general, microbiology budgets have very few pieces of equipment that are in the tens of thousands of dollars. Fear of the regulators not accepting your methods after spending such large amounts of money is also prohibitive to many potential users.

Applicability of RMMs in Pharma Companies

Today there are numerous RMMs available, several instantaneous methods for environmental monitoring methods, growth and instantaneous methods for water monitoring, a variety of potential sterility test methods, methods for conducting specific bioburden and other test methods, endotoxin test methods, and identification methods. Identification methods have been widely accepted and implemented in pharma, as the regulatory implementation requirements are quite different.

Often the focus has been on sterility testing, as most companies felt that this methodology had the highest potential to show a significant cost savings (avoidance) due to the elimination or reduction of the 14-day incubation time period.

Resources Available to Aid in the Development of Validation Requirements

Industry was one of the first to respond to support the use of RMMs by publishing guidance on the topic of selecting, testing, and evaluation of RMMs. The Parenteral Drug Association has published a technical report on this topic.[2] Since then, this document has been revised and updated to include new information.[3] At the time of writing this article, another revision to this Technical Report is in progress.

Both the United States Pharmacopeia (USP)[4] and the European Pharmacopoeia, called Pharm Europa (Ph. Europa)[5] issued monographs on the appropriate validation criteria to be met for rapid or alternative methods.[6] The Japanese Pharmacopeia also has requirements for RMMs.

Additionally, there are numerous articles published by a variety of early adopters of RMMs that provide guidance on validation of these methods. Dr. Miller has also published many articles referring to the comparison of different validation criteria, e.g., USP vs. Pharm Europa vs JP, or the comparison of compendial requirements to those in PDA's Technical Report No. 33.

Comparability Protocols

A comparability protocol is a written agreement between the Regulatory Agency and the User Company. This document allows a company to submit a written protocol to the Regulatory Agency for review, prior to executing the work, to ensure that it will be considered acceptable if done in agreement with the prior approved protocol. This submission is accomplished as part of a pre-approval supplement to the product regulatory submission. This allows for the company to submit their protocol and know whether FDA will accept the data if you meet all the requirements of your testing protocol as specified in the Comparability Protocol.

One of the suggestions by The United States Food and Drug Administration (FDA) to address this type of concern was to allow companies to submit a "Comparability Protocol" to the Agency for rapid microbiological methods. The final guidance for the Comparability Protocol process has been issued. This would allow companies to receive feedback from the Agency prior to submission of data for review.

An advantage of using comparability protocols for the submission is that while the comparability protocol was submitted to the FDA as a prior approval supplement, the Agency generally allowed a significantly shorter regulatory path for implementing the change. Among the most attractive options were the equivalent to the Changes Being Effective – 0 Days (CBE-0), i.e., the company needed to notify the FDA that the change was completed and was being implemented.[7]

An example of the typical contents of a comparability protocol is included in Figure 1.

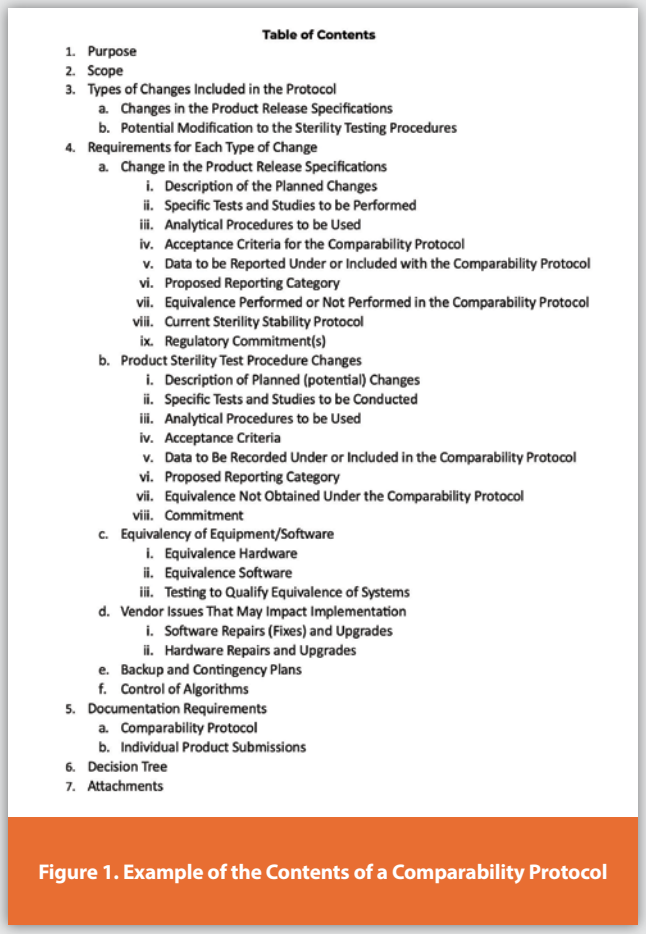

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Figure 1. Example of the Contents of a Comparability Protocol

While this is allowed for the United States, comparability protocols are not applicable for other regulatory agencies., however, another regulatory pathway is open for approval of these methods.

Europe's Alternative to Comparability Protocols

While Europe does not specifically have a program called Comparability Protocols, a new guidance document was issued entitled Guidance for Post Approval Change Management Protocols (PACMPs).[8] While not specifically the same as the US document, it allows the user to submit their validation documents and obtain regulatory review and approval prior to implementation of the change.

The stated review times for prior approval supplements appear to be shorter than the corresponding approval times for the FDA, approximately two months versus four months.

Another potential option to gain information on the acceptability of a validation approach to rapid microbiological methods is the EMEAs Guidance for Scientific Advice.[9] This procedure allows for review of validation approaches prior to conducting the testing. There is a charge associated with this service, but it can be useful in assessing whether it may be feasible for the company to pursue the validation methodology.

Early Adopters and Successful Implementation of Methods

Most of the early adopters of RMMs pursued rapid sterility testing for aseptically processed products. This was attractive to companies that already had implemented parametric release for their terminally sterilized products. Parametric release allowed for elimination of sterility testing and use of controlled manufacturing parameters to ensure product sterility. The first company to implement parametric release for terminally sterilized drugs was implemented in 1983.

The main technologies available for sterility testing are summarized in Table 1.

An ophthalmics company was the first to obtain FDA approval for a rapid sterility test of their aseptically filled drug products. This company predominantly had preserved products, lowering the risk of a potential non-sterile unit. This approval was obtained in four months from the date of the submission (i.e., equivalent to the expedited review time for submissions). No deficiencies were obtained for the submission package. This submission utilized a ScanRDI system. This approval required a Special Report (like a CBE-0) to be used to notify FDA of the implementation of the new testing method.

This submission was followed in short order by a company that manufactured generic drugs that were aseptically filled drug products. Like their predecessor, they had already implemented parametric release for terminally sterilized products and were using the ScanRDI. This submission was also approved four months from the date of the submission, with no deficiencies. It also required a special report be submitted for implementation.

Several compounding pharmacies wanted to implement rapid microbiological methods, as their products were frequently used in patients prior to the completion of sterility testing. Due to the high number of small product batch sizes, systems like the BacT/

Table 1.

	Technology	Growth/No Growth	USP Media	Incubation	Enumeration or Presence/Absence (P/A)
Scan RDI	Viability substrate Solid phase cytometry	No Growth	No	<4 hrs @40C	Enumeration
BAC T Alert	pH change	Growth	No, proprietary	5-7 days 20-25 and 30-35C	P/A
Celsis	ATP detection	Growth	Yes	5-7 days 20-25 and 30-35C	P/A
Milliflex Rapid	ATP detection	Growth	No, Scheadlers Blood Agar	5-7 days 20-25 and 30-35C	Enumeration

Alert and the Bactec were desirable, due to the system designs, e.g., automated identification of sterility test positives, minimum change in testing methods, and ability to process many samples simultaneously. These systems were also routinely used in blood processing operations. Some of these pharmacies utilized comparability protocols, while others worked through FDA directly to review validation test plans.

Interactions with Regulatory Agencies During the RMM Implementation Process

The FDA has identified Dr. Bryan Riley in CDER, as an expert in RMMs. It is possible to request a meeting with Dr. Riley to discuss potential validation methods and to discuss your RMM strategy. Many find it useful to provide examples of test plans prior to the meeting.

Alternatively, a comparability protocol can be submitted for formal review and comment.

If there are discrepancies between the company and the Agency, it is useful to discuss what potential responses may mitigate the circumstances.

For European companies, it may be useful to ask for formal scientific advice in accordance with the established guidance. There is a charge for this service.

Conclusions

In the last twenty years, many improvements have been made in providing guidance to the validation and implementation of RMMs. Regulatory agencies have established procedures to obtain guidance and opinions on proposed validation strategies. Many company personnel have identified that it is really their own companies that are hindering the adoption of these new methods.

Take courage and try these methods out. They are worth the risk. In most cases, regulators are not the problem. We are!

References

1. Miller, M.J. (2017) The regulatory acceptance of rapid microbiological methods. European Pharmaceutical Review. 22(2). Downloaded from: The regulatory acceptance of rapid microbiological methods (europeanpharmaceuticalreview.com) on June 9, 2023.
2. PDA (2000) Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods. Parenteral Drug Association. Bethesda, MD.
3. PDA (2013) TR33 Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods, Technical Report No. 33. Revised 2013.
4. USP (2016) Validation of Alternative Microbiological Methods. United States Pharmacopeial Convention. US Pharmacopeia. <1223>. USP 39/NF34: 1616.
5. Ph. Europa (2017) Alternative Methods for Control of Microbiological Quality, European Directorate for the Quality of Medicines & HealthCare. Pharmeuropa; Chapter 5.1.6. Supplement 9.2
6. JP (2016) (The Japanese Pharmacopoeia, Seventeenth Edition (JP17). the MHLW Ministerial Notification No.64]http://jpdb.nihs.go.jp/jp17e/ JP17_REV_1.pdf (mhlw.go.jp)
7. Food and Drug Administration. Comparability Protocols for Human Drugs and Biologics: Chemistry, Manufacturing, and Controls Information. Guidance for Industry. U.S. Department of Health and Human Services, Rockville, Maryland. 2016. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM496611.pdf
8. European Commission. Regulation No 1234/2008 concerning the examination of variations to the terms of marketing authorizations for medicinal products for human use and veterinary medicinal products. 2008. http://ec.europa.eu/… and European Commission. Regulation No 1234/2008 concerning the examination of variations to the terms of marketing authorizations for medicinal products for human use and veterinary medicinal products. 2008. http://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/reg_2008_1234_cons_2012-11-02/reg_2008_1234_cons_2012-11-02_en.pdf
9. European Medicines Agency. Human Medicines Research and Development Support. Guidance for applicants seeking scientific advice and protocol assistance. EMA/691788/2010 Rev. 7. 2014. http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/10/WC500004089.pdf

The Value and Benefits of Rapid Mold Detection in the Pharmaceutical Industry:

Importance of Accurate Microbial Identifications and Proper Contamination Control for Effective Mold Remediation

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Introduction

In the pharmaceutical industry, maintaining a clean and sterile environment is of utmost importance to ensure product quality, safety, and efficacy. Mold contamination poses a significant risk to pharmaceutical manufacturing facilities as it can compromise the integrity of products and potentially harm patients. Rapid, accurate mold detection and identification, with proper contamination control play pivotal roles in identifying and mitigating mold-related issues promptly. This article highlights the value and benefits of rapid mold detection within a day, emphasizes the importance of accurate microbial identifications, and underscores the significance of performing quality control disinfection protocols for effective mold remediation in the pharmaceutical industry.

The Value of Rapid Mold Detection

Timely Response and Mitigation

Rapid mold detection enables pharmaceutical companies to identify mold contamination early, allowing for immediate response and mitigation measures. Detecting mold within a day provides an opportunity to prevent further spread and minimize the potential impact on products, equipment, and facilities. Early intervention reduces the need for extensive remediation and helps maintain production schedules.

Cost Savings

Detecting mold contamination quickly can result in significant cost savings for pharmaceutical companies. Timely identification and remediation reduce the risk of product loss, minimize the need for facility shutdowns, and limit the expenses associated with extensive mold remediation. Rapid mold detection ultimately helps preserve resources and protect the company's bottom line.

Regulatory Compliance

Pharmaceutical manufacturing facilities must adhere to strict regulatory guidelines and standards. Rapid mold detection allows companies to promptly address mold contamination issues, ensuring compliance with regulatory requirements. By demonstrating a commitment to quality control and contamination prevention, pharmaceutical companies maintain regulatory compliance and avoid potential penalties or product recalls.

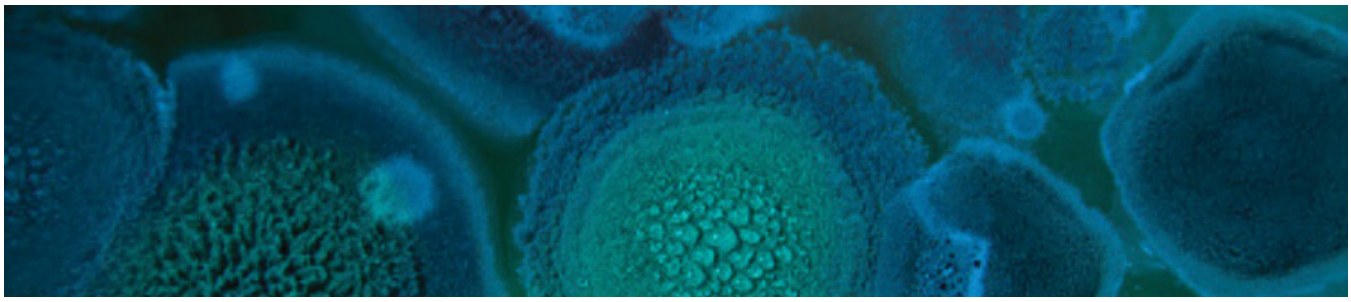
The Benefits of Accurate Microbial Identifications

Targeted Remediation Strategies

Accurate microbial identifications are crucial for developing targeted and effective mold remediation strategies. Different types of mold require specific remediation approaches, as some may be more resistant to certain disinfection methods than others. By accurately identifying the mold species present, pharmaceutical companies can tailor their remediation efforts to eradicate the specific mold strains effectively. Additionally, cleaning procedures may be revised to ensure more frequent risk management of known potential mold contaminants.

Patient Safety, Quality Assurance, and Risk

Accurate microbial identifications play a crucial role in ensuring patient safety within the pharmaceutical industry by contributing to



comprehensive risk assessment and management processes, as well as maintaining product safety and quality assurance. Certain molds have the potential to produce toxins or exhibit allergenic properties, posing health risks to both workers and end-users of pharmaceutical products. By accurately identifying the specific mold species, companies can evaluate associated risks thoroughly and implement appropriate control measures to protect the well-being of personnel and consumers. Additionally, accurate microbial identifications are vital for maintaining product safety, as some molds can produce metabolites or mycotoxins that may contaminate pharmaceutical products, compromising their safety and efficacy. Through the identification of mold species and the assessment of their potential impact on products, pharmaceutical companies can take necessary actions to ensure product safety and, ultimately, protect the health of patients.

Root Cause Analysis

Identifying the precise mold species responsible for contamination facilitates root cause analysis. Determining the source of the mold infestation is vital to prevent its recurrence. Accurate microbial identifications help identify the source of the contamination. Addressing the root cause reduces the likelihood of future mold contamination events, enhancing overall facility cleanliness and product integrity.

Proper Contamination Control and Quality Control Disinfection Protocols

Contamination Prevention

Implementing proper contamination control measures is critical to prevent mold contamination in pharmaceutical manufacturing facilities. This includes maintaining stringent cleanliness standards, regular inspections, and monitoring environmental conditions such as temperature and humidity. Adhering to robust contamination control protocols minimizes the risk of mold growth and transmission, safeguarding product integrity.

Disinfection Protocols

Quality control disinfection protocols are essential for effective mold remediation. After mold identification, pharmaceutical companies must establish appropriate disinfection protocols that target the identified mold species. These protocols may involve the use of disinfectants specifically formulated to eliminate molds, following recommended contact times and concentrations. Proper disinfection protocols help ensure thorough mold eradication and prevent recontamination.

Validation and Verification

Validation and verification of disinfection procedures are crucial to confirm the effectiveness of remediation efforts. Pharmaceutical companies must conduct post-remediation assessments to ensure that mold has been effectively eliminated. This may involve microbial testing, air sampling, and surface swabbing to confirm the absence of mold and verify the success of the disinfection protocols.

Training and Education

Proper contamination control and quality control disinfection protocols require well-trained personnel. It is essential to invest in comprehensive training programs to educate employees on best practices for mold prevention, detection, and remediation. By ensuring that staff members are knowledgeable and competent in contamination control procedures, pharmaceutical companies can maintain a proactive approach to mold management.

Conclusion

Rapid mold detection, accurate microbial identifications, and proper contamination control are indispensable components of mold management in the pharmaceutical industry. Timely detection allows for immediate remediation, resulting in cost savings, regulatory compliance, and minimized production disruptions. Accurate microbial identifications facilitate targeted remediation strategies, risk assessment, and root cause analysis, enhancing product safety and quality assurance. Proper contamination control and quality control disinfection protocols are essential for preventing mold contamination and effectively eradicating mold when detected. By prioritizing these practices, pharmaceutical companies can uphold their commitment to product integrity, consumer safety, and regulatory compliance, ultimately safeguarding their reputation and contributing to a healthier society.

Risk Analysis for the Adoption of the Recombinant Factor C Reagent for Bacterial Endotoxin Testing

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Introduction

The issue at hand is not whether to include the recombinant Factor C (rFC) reagent in the U.S. Pharmacopoeia, but rather how to implement this change with minimal risk. The authors suggest that a gradual implementation approach, based on a risk analysis of the potential consequences during the transition planning phase, could help facilitate this transition smoothly. Factors that can help preclude potential risks will be explored, including sample type, examination of process microflora, reagent sample combined interference profile, pass-fail frequency examination, reagent quality awareness, and use of corroborating data.

Why should we consider a risk analysis before the adoption of the rFC reagent for bacterial endotoxin testing? The risk assessment can provide a systematic framework for the decision-making to transition from the traditional reagent (LAL) to the now well-established alternative reagent (rFC). If the benefits are self-evident, disadvantages absent or minimal, the risk of releasing a pyrogenic lot is very low, i.e., a decision equivalency of rFC to the Limulus Amebocyte Lysate (LAL)-based assay, and uncertainty around the transition low, then the U.S. Pharmacopeia should proceed with the addition of the rFC reagent to <85> *Bacterial Endotoxin Tests*.

The Case for rFC

The use of a sustainable recombinant protein in lieu of the harvesting of natural proteins from the horseshoe crab has begun to be implemented in earnest by many large pharmaceutical companies to provide sustainability and supply chain security advantages.[1,2] The advantages accrue to the environmental conservation efforts as well regarding ensuring that supply chains are reinforced against potential reagent supply chain difficulties including (worldwide and localized) availability as well as protection against price increases.

RFC alternative validation activities have been performed ad *infinitum* since the original validation in 2010[3] with now over twelve years of hindsight the added value for each additional user performed alternative validation is very low. Compendial status would remove the need to perform studies where the outcomes are already well

known. Yes, the linearity, accuracy, and precision etc. are important but they are also readily demonstrable and have been repeated enough times to show compendial level repeatability. There is little value in its repeat performance because issues that may arise are not method related, but rather are likely relative to specific sample types and/or unique process microflora. In risk assessment, the emphasis is placed on demonstrating real-world sample-specific recovery.

Risk Analysis

After a general overview of risk analysis relative to BET, a discussion of the major elements of risk-based decision-making will follow. Figure 1 identifies many of the critical analytical inputs for Bacterial Endotoxin Testing in a fishbone diagram.

Tables 1, 2, and 3 below assign risks in terms of probability of test failure for different sample types, likelihood of detection of bacteria endotoxin exceeding the limit, and estimated risk of severity of a contamination event.

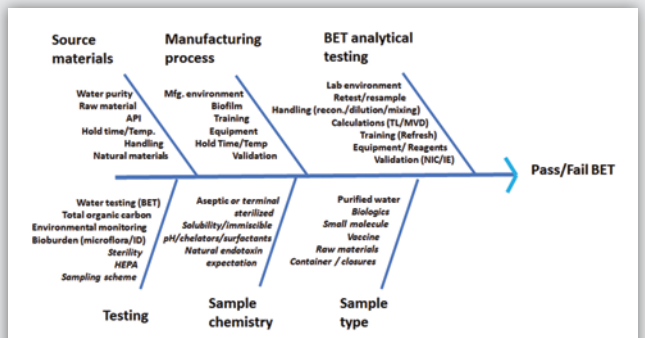


Figure 1. Considerations of Critical Analytical Inputs for Bacterial Endotoxin Testing.

A high-level comparison of the rabbit pyrogen and the bacterial endotoxin tests is found in Table 4.

Table 1. Estimated Probability of Failure in Terms of Manufacturing Process and Testing History

Probability of Failure	Water For Injection	Ingredients	Cell Culture Media	Buffers & Solutions	In-process Materials	Finished Products
High						
Moderate			+		+	
Low		+		+		+
Very Low	+					

Table 2. Estimated Likelihood of Historical Endotoxin Detection

Likelihood of Detection	Water For Injection	Pharmaceutical ingredients	Cell Culture Media	Buffers & Solutions	In-process Materials	Finished Products
Remote						
Very Low						
Low						
Moderate						
High		+	+		+	+
Very High				+		
Almost Certain	+					

Table 3. Estimated Severity of Effect of Endotoxin Contamination with Different Test Samples

Severity of Effect	Water For Injection	Pharmaceutical ingredients	Cell Culture Media	Buffers & Solutions	In-process Materials	Finished Products
Hazardous (Without Warning)						
Hazardous (With Warning)						
Very High						+
High					+	
Moderate	+		+			
Low		+				
Very Low				+		
Minor						
Very Minor						
None						

Table 4. Comparison of the Rabbit Pyrogen Test and LAL-based and rFC-based Bacterial Endotoxin Test Methods					
Parameters	Rabbit Pyrogen Test	LAL- gel clot method	LAL-Turbidimetric method	LAL-Chromogenic method	rFC Fluorescent Method
Animal Involvement	Animal-based test	Animal-derived reagent	Animal-derived reagent	Animal-derived reagent	Recombinant Protein reagent
Measurement	Rectal Temperature	Semi-Quantitative	Quantitative	Quantitative	Quantitative
Time to result	3 to 4 Hours not including Sham Testing	60 minutes	Endpoint – 60 minutes	Endpoint – 60 minutes Kinetic – Variable	Endpoint – 20-60 minutes Kinetic – Variable
Kinetic – Variable	Endpoint – 60 minutes	Heating Block & Thermometer	Plate Reader	Plate Reader	Plate Reader
Kinetic – Variable	Endpoint – 20-60 minutes	Low pyrogen water	Low pyrogen water or glucans-inhibiting buffer	Low pyrogen water or glucans-inhibiting buffer	Low pyrogen water
Kinetic – Variable	None	Single Lyophilized vial	Single Lyophilized vial	Single Lyophilized vial & a colorimetric reagent	Three-part Liquid Reagent
Instrumentation	Rectal Thermocouples & recorder	Heating Block & Thermometer	Plate Reader	Plate Reader	Plate Reader
	Syringes	Depyrogenated Test Tubes	Clear 96 Well Plates	Clear 96 Well Plates	Blackened 96 Well Plates
Diluent	None	Low pyrogen water	Low pyrogen water or glucans-inhibiting buffer	Low pyrogen water or glucans-inhibiting buffer	Low pyrogen water
Reagent Format	None	Single Lyophilized vial	Single Lyophilized vial	Single Lyophilized vial & a colorimetric reagent	Three-part Liquid Reagent
Standard	Sham Test	Reference Standard Endotoxin	Reference Standard En-dotoxin	Reference Standard Endotoxin	Reference Standard Endotoxin
Consumables	Syringes	Depyrogenated Test Tubes	Clear 96 Well Plates	Clear 96 Well Plates	Blackened 96 Well Plates

Specific Elements to be Considered in Precluding Potential Risks

Analytical efforts that can help preclude potential risks in any risk analysis will be explored, including (i) sample type, (ii) examination of process microflora, (iii) reagent-sample combined interference profile, (iv) pass-fail frequency examination, (v) reagent quality considerations and (vi) use of corroborating data.

(i). Sample type

The introduction of a BET method, here the rFC reagent, has different impacts associated with different sample types tested. The lowest risk of the introduction of the method is with water for injection (WFI), the finished product has the highest risk in terms of impacts of failure in terms of sample complexity, cost, and patient safety. The other sample risks lie in between these two bounds. With WFI, double-distilled water would have a lower risk of failure than WFI produced by reverse osmosis. As the European Union until very recently did not authorize the use of reverse osmosis, U.S. manufacturers used distillation to sell their products globally. WFI meets strict chemical and microbiological standards and has no added substances, thus reducing the risk.

In quality testing for finished products the expectation is that the test should be performed with aliquots from beginning, middle, and end vials and the original vials should be saved for failure analysis purposes. After all, the surest indication of a lab-induced failure is a retesting of the original vials from which the original aliquots were taken. Care should be taken to aseptically remove aliquots (to pool) from those vials and to preserve those vials (minus aliquots) at the appropriate storage temperature.

Many drug companies have begun to test purified water samples routinely using rFC as a first step in gaining sustainability and supply chain assurance improvements given the ease of such a switch. The ease of change is facilitated by the lack of regulatory impediments (inspectional rather than pre-submission based), even with the current USP status as an alternative method, it is even easier with EP compendial status.

Consider that monoclonal antibody biologic products that do not have sterile downstream processes, are routinely monitored for endotoxin and bioburden. Since bioburden testing has an associated limit of NMT 10 CFU per 100 mL based on EU GMP requirements, then meeting this level ensures that the absence of febrile-level events[4] provided further processing bioburden controls remain in place.

As a collective effort, we should seek common ground. The transition could be pharmaceutical-grade water in the first phase with the method published in USP <1231> *Water for Pharmaceutical Use,* validation requirements for non-lysate methods as published in a revised <1085> to facilitate the transition and after the development of a larger body of industry experience, add rFC reagent as an option to lysate in <85> *Bacterial Endotoxin Tests* after the standard enhancement/inhibition and linearity testing.

Revision to a harmonized compendial chapter will require the agreement of the U. S. Pharmacopoeia, European Pharmacopoeia, and Japanese Pharmacopoeia. At the June 2022 Pharmacopoeial Discussion Group (PDG) meeting, the Japanese Pharmacopoeia., who incidentally is designated as the coordinating pharmacopoeia for the chapter, was not ready to add the use of rFC reagent to the harmonized chapter and indicated they were sponsoring a comparative study to provide additional evidence of equivalency. It should be noted that any test sample test that is not water for Injection is a specific sample test and therefore should not impede method acceptance.

(ii) Examination of process microflora

The most critical concern of testing is the potential of the generation of false negative results in finished product testing. If there is internal concern about the detection coverage of all Gram-negative bacterial

endotoxin types, an examination of the bacterial microflora types available from bioburden testing can be performed. Uncommon Gram-negative bacteria can be checked and verified in side-by-side testing with rFC and LAL reagents.

Obtaining false positive bacterial endotoxin tests, such as the detection of glucans using LAL, can be problematic in that lots of products suitable for use are rejected and the product is not available to treat patients. Also, the pharmaceutical manufacturer will experience an economic loss, see their brand impacted, and may have to contend with unhappy clinicians and their patients impacted by product supply problems. The out-of-specification investigation may possibly uncover that this is a false positive result by investigative testing with a LAL-based assay and even conducting a rabbit pyrogen test.

How can we best detect false negative and positive reactions? Analytical efforts to preempt any possibility of false negative results can focus on process microflora (available from process bioburden data). In the very rare instance of potential diminished detection of specific bacterial endotoxin in specific process settings, the previously mentioned check of any unique endotoxin types or endotoxin-like PAMPs (*Sphingomonas*[5] or *Francisella*[6] or *Heliobacter pylori*) can be made to verify the detection capability via specific LAL and rFC reagents-from both a false positive and false negative perspective. *Heliobacter pylori* has been pointed to as a species underestimated by rFC,[7] however, the likelihood of finding *H. pylori* in any manufacturing process is virtually nil given its fastidious growth requirements. The low reactivity of *H. pylori* endotoxin has been theorized to be a mechanism of persistence in human gastric ulcers.[8]

As part of change control requirements multiple lots may be tested in parallel with both the LAL-based and rFC-based methods. The method suitability requirements as found in USP <85> may be sufficient to detect false negative and positive reactions.

(iii) Reagent-sample combined interference profile

The product interference profile is determined relative to the Maximum Valid Dilution (MVD) of a product with a specific reagent as per USP <85> and is generated by performing a non-interfering concentration (NIC) test followed by validation close to the lowest product concentration level that overcomes interference. This is an important consideration when comparing results from different reagents that may have different abilities to overcome interference mechanisms. This includes pH, buffering capability, matrix background response including glucan cross-reactivity, and endotoxin aggregation properties. Performing tests at the same dilution with different reagents will not necessarily provide a comparison that overcomes inhibition for both tests at different dilutions. The test that retains inhibition will likely show significantly reduced values relative to the test that has overcome inhibition (a test that is neutral or shows enhancement). Some LAL manufacturers have suggested that such a comparison test should be viewed as "under detection" in the test with the lower result whereas simple method development work needs to be performed to overcome inhibition in this test. This is the purpose of the NIC testing as required in USP <85>.

(iv) Pass-fail frequency examination

To make an initial comparison, we can construct a simple pro versus cons table. Secure representative samples from a range of sample types (See Table 1) and test replicate samples using both test methods and make a statistical comparison of the total samples and the samples by sample type. Lastly, spike known amounts of bacterial endotoxin into a common sample matrix to confirm percent recovery, the limits of detection and quantification, linearity, and pass/fail rates around a bacterial endotoxin limit for a range of products. The major advantages of the rFC reagent are its expected greater lot-to-lot consistency, non-reliance on an animal source, and insensitivity to cellulose-derived glucans. When using highly sensitive assays, there may be a statistical difference between the numerical results obtained using the various methods when analyzing the same sample, but there will be no difference in the frequency with which the bacterial endotoxin limit for the same product will be passed or failed. As recommended in USP <1223>, the results should be comparable with a non-inferiority test applied to the data.[9] Other critical issues are the breadth of sample type evaluated and the number of samples tested to provide sufficient statistical power to the analysis.

(v). Reagent quality considerations

The LAL reagent is a biological product licensed by the Center for Biologics Evaluation and Research (CBER), Food and Drug Administration purified from the hemolymph of the North American Horseshoe Crab, Limulus polyphemus, whereas recombinant Factor C (rFC), is a biotechnology-manufactured version of the same Factor C protein that is the triggering LAL biosensor that does not require licensing by CBER or CDER. Some in our industry believe this difference in regulatory oversight increases the routine test risk. However, LAL reagent has been historically regulated due to its status as a blood-derived product, it is the only compendial test reagent so regulated.[10] Although not regulated, as any other biomanufacturing process, the rFC production undergoes drastic quality control checks to assess its quality along the process. Drug companies can audit any reagent manufacturer as well as track various reagent performance characteristics over time as reagent lots change. For example, reaction times for a BET may change significantly from batch to batch and users should monitor these changes. For fluorescent methods, any change in the reagent batch will trigger a new gain test to ensure continued optimal performance [delta relative fluorescence units(dRFU's)].

(vi) Use of corroborating data

Corroborating data may include the use of bioburden data and associated bacterial identification to verify that unusual flora does not present false positive or false negative possibilities within the process flow.

Another set of corroborating data is associated with purified water testing for Total Organic Carbon (TOC). TOC while not directly correlated with bacterial endotoxin, an increase can be viewed as an indicator of bacterial growth in the form of accumulating biofilm.

Experience with the Transition from the Rabbit Pyrogen Test to the LAL-Bacterial Endotoxin Test

What can be learned from the transition from the Rabbit Pyrogen Test to the LAL-Bacterial Endotoxin Test? The history of this transition is well described in the literature.[11] The Rabbit Pyrogen Test was used for over 30 years to test and release pharmaceutical and biological products. The challenges were vastly greater in this transition than in the current transition. They included manufacturing the reagent, establishing reference and control standards, standardizing the tests, establishing the threshold pyrogenic level for bacterial endotoxin, developing a regulatory framework, implementing a globally recognized reference standard, setting bacterial endotoxin limits for different parenteral products, and demonstrating equivalency of the two methods. The LAL to rFC transition is less challenging and may eventually be viewed as a like-to-like reagent substitution.

Patient Safety Risk from Parenteral Drug and Medical Devices Contaminated with Bacterial Endotoxin

A recent review article[12] addressing the period of 2011 to 2021 using FDA data from Good Manufacturing Practice non-compliance observations, product recalls and the Adverse Event Reporting System clearly demonstrated an absence of industry issues with bacterial endotoxin. For example, with the 188 GMP compliance observations reported, 70% and 30% were related to laboratory testing and manufacturing respectively with 56% associated with finished drug product testing. In contrast, 95% of the endotoxin-related recalls were associated with medical devices. These findings reinforce the effectiveness of the Bacterial Endotoxin-LAL assay. All current indications are that rFC will present a continuation of this well-established safety record.

Potentially Confounding Issues

Another minor risk associated with the implementation of the rFC-based assay is an upward or downward shift in the historic bacterial endotoxin results so adverse trending rules may need to be reset. It is recognized that testing within the MVD will confirm that the lot does not exceed the bacterial endotoxin limit but to quantify the bacterial endotoxin level test should be conducted at the lowest possible dilution to overcome inhibition or enhancement of the assay (as per 2012 FDA Q&A Guideline). Many laboratories routinely select a dilution 2- or 10-fold lower than the MVD when testing the product and trending the results.

Confounding issues associated with BET testing in general include differences found in naturally occurring endotoxin and highly purified reference standard endotoxin. It also includes enhancement of results by glucans in test materials tested by LAL (variable as affected by various LAL formulations), the simplification due to the application of the concept of the threshold pyrogenic effect (using purified LPS), the assumption that endotoxin is homogeneously distributed throughout a product, the presence of non-endotoxin febrile responses in patients (routine for some immune modulating biologics), and the non-adherence of clinicians to the recommended dosage when administering the drug (see gentamicin episode).[13]

Frequency of Testing and Sample Type

It is estimated that over 70 million Bacterial Endotoxin Tests are conducted globally annually. As an estimated 90% plus of the reagents sold are animal-derived, the transition could relieve potential disruptions to the bacterial endotoxin testing market if the LAL reagent comes to be in short supply.

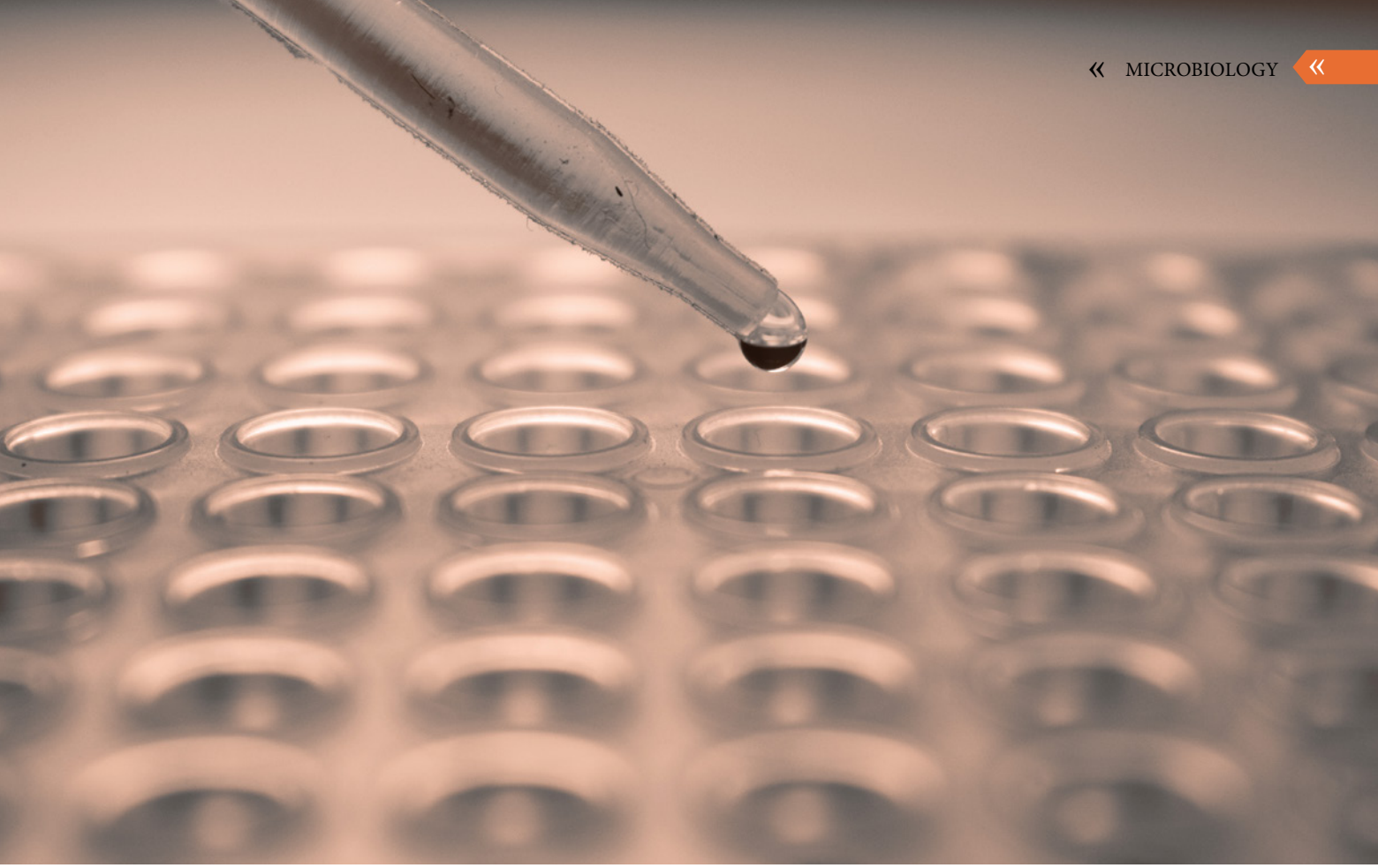
It is revealing to explore the percentage of Bacterial Endotoxin Tests in support of pharmaceutical-grade water, pharmaceutical ingredients, cell culture media, buffers, in-process samples, and finished products during the manufacture of three major industrial manufacturing sectors – small molecules, large molecules, and cellular therapies. Rough estimates made by the authors are found in Table 5.

Table 5: Estimated Distribution of Bacterial Endotoxin Tests by Sample Type

Test Samples	Small	Large Molecules	Cellular Therapies
Molecules	Large Molecules	Cellular	30%
Therapies	10%	3%	5%
Pharmaceutical-grade water	80%	70%	30%
Pharmaceutical ingredients	10%	3%	5%
Buffers and solutions	2%	2%	5%
Cell culture media	N.A.	5%	15%
In-process Materials	3%	15%	30%
Finished products	10%	5%	10%
Total Tests Conducted (%)	50%	40%	10%

Review of the Existing Literature Supporting the Transition from the LAL to the rFC-based Bacterial Endotoxin Test

A comprehensive review of the peer-reviewed literature on the equivalency of LAL and rFC-based Bacterial Endotoxins Test supports the transition.[14]



Conclusions

Recombinant Factor C (RFC) reagent use has the potential to bring positive new dimensions to the current BET test paradigm including test modernization, increased supply chain security and sustainability in support of the horseshoe crab as a keystone species as well other dependent species (such as shorebirds that need the HSC egg mass as foodstuff for their rigorous migratory journeys).

Based on risk analysis principles and a review of the published literature, the authors will be interested to see the actions taken by the new USP Microbiology Expert Committee. The overview of stepwise risk assessment considerations and potential preclusion exercises proposed here are intended to support those discussions and decisions.

References

1. Guest, M., Duncanson, P., Capper, K., & Wong, D. (2022). A strategic approach to optimisations of testing bacterial endotoxins. Euro. Pharma. Rev., 24 August
2. Sustainability Bond Allocation and Impact Report, Eli Lilly & Co., 2021.
3. Loverock B, Simon B, Burgenson A, Baines A. (2010). A recombinant factor C procedure for the detection of Gram-negative bacterial endotoxin. Pharm. Forum, 36:321–329
4. Microbial Monitoring for Biological Drug Substance Manufacturing: An Industry Perspective. Corresponding author: David Bain, Facilitator, BPOG Corresponding author contact information: BPOG, 5 Westbrook Court, Sharrow Vale Road, Sheffield, S11 8YZ, United Kingdom, david@biophorum.com. Published in a 'Special Section' of the PDA J. Pharm. Sci & Technol, in May/June 2015.
5. Shahar, E., Emquies, K., Bloch, I. et al. (2023) Endotoxin-free gram-negative bacterium as a system for production and secretion of recombinant proteins. Appl. Microbiol. Biotechnol. 107:287–298
6. Okan, N. A. and D. L. Kasper (2013) The atypical lipopolysaccharide of Francisella, Carbohydr. Res. 378: 79–83.
7. Stevens, I et al (2022) Advanced recombinant cascade reagent PyroSmart NextGen® for bacterial endotoxin test as described in the pharmacopeias, BPB Reports 5:105-114
8. Leker, K., Lozano-Pope, I., Bandyopadhyay, K. et al. Comparison of lipopolysaccharides composition of two different strains of Helicobacter pylori . BMC Microbiol. 17, 226
9. Validation Strategy for New Recombinant Factor C Users, Evelyn Der, Senior Scientist, Roche Genentech, QC Technology Innovation and Implementation Group, South San Francisco, CA, USA. Carmen Marin, QC Scientist Microbiology, F. Hoffmann La Roche Ltd, Kaiseraugst, Switzerland. Viviane Grunert da Fonseca, PhD, Non-clinical Statistician, Roche, MSAT (Manufacturing, Science and Technology), Penzberg, Germany. Lindsey Silva, PhD, QC Director, Roche Genentech, Microbiology, South San Francisco, CA USA., Amer. Pharm. Rev., Feb. 28, 2022.
10. Berzofsky, R.N. (2004) Does Endotoxin Testing = FDA Licensing? Cambrex (now Lonza), vol. 104, issue no. 1
11. Cooper, J. F. 2011 Discovery and acceptance of the bacterial endotoxins test In The Bacterial Endotoxins Test – A Practical Guide edited by Karen Z. McCullough DHI/PDA pp1-14
12. Tidswell, E. C. (2023) A non-trivial analysis of patient safety risk from parenteral drug-and medical device-borne endotoxin. Drugs in R & D 23:65-76.
13. Endotoxin-Like Reactions Associated with Intravenous Gentamicin -- California, 1998, MMWR, October 23, 1998 / 47(41):877-880. https://www.cdc.gov/mmwr/preview/mmwrhtml/00055322.htm#:~:text=Parenteral%20antimicrobials%20such%20as%20gentamicin,effects%20when%20infused%20into%20humans.
14. Bolden, J., et al (2020) Current available recombinant alternatives to horseshoe crab lysates: Are they comparable for the detection of environmental endotoxins? A review. PDA J. Pharm. Sci. & Technol. 74 (5): 602-611

The Need for Speed and Confident Decision Making

Implementing Rapid Micro Methods (RMMs) Throughout Production

Briana Nuñez

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Pharmaceutical and biopharmaceutical manufacturers need faster data related to microbial contamination in all aspects of production. Relying on growth-based methods that take days is not fast enough to make actionable changes when drugs and potentially patient lives are at risk. Implementation of Rapid Microbiological Methods (RMMs) such as the new Sievers rapid bioburden analyzer can provide timely trend analysis and early warnings of contamination events in various stages of the manufacturing process. From screening raw materials in the receiving docks to at-line monitoring of the water system, and including final quantitative results from the QC lab, nearly every area of a manufacturing facility can benefit from monitoring contamination control processes with a rapid bioburden analyzer.

Ultra-sensitive, high throughput testing is possible with the Sievers bioburden system, a RMM that has a strong correlation to plate counts. This enables manufacturers to quickly detect microbial contamination and have confidence in their actions to ensure safety, quality, and compliance.

Screening of Raw Materials in Receiving

Maintaining acceptable quality of a final product relies on the quality of the incoming raw materials. Raw materials used in manufacturing are susceptible to microbial contamination and must be assessed before they can be released to production. Using the novel technology

WHERE TO DEPLOY RMMs:

- Raw material testing
- At-line monitoring - water loops, elution buffers, effluent from purification columns, and other process steps
- Cleaning validation
- Environmental monitoring
- Final product testing

WHAT TO GAIN:

- Better process understanding and control
- Increased speed of decision making and risk mitigation
- Improved data integrity and data management
- Improved contamination control strategy and protection of assets (chromatography and purification solutions, etc.)

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in the Sievers rapid bioburden analyzer, these raw materials can be analyzed in less than 45 minutes without extensive incubation to determine contamination levels. Instead of transporting samples to the lab to complete microbial limit testing - which requires waiting days for results - simple to complex, filterable raw materials can be analyzed immediately after receiving. Rapid screening of incoming materials decreases the time needed before releasing raw materials into production with assurance of their microbial levels.

At-line Monitoring

Implementation of the Sievers rapid bioburden system in close proximity to a sampling site provides at-line monitoring for faster information related to contamination events, sanitization of assets, etc. Some applications that would benefit from at-line rapid monitoring include CCPs, or Critical Control Points, on water loops. CCPs are often found on the return leg of a water loop and indicate the quality of the water found within the interior of the water loop. Monitoring CCPs provides an early warning of a contamination event inside the water loop.

Another critical application is at-line monitoring of buffers or effluent from purification columns. Over sanitizing purification columns reduces their effective lifespan, causing underutilization of a high value asset. Without rapid microbial detection, columns are assumed to be contaminated and sanitized at the harshest level. Instead, accurate and fast detection using RMMs enables proper sanitization protocols suitable for contamination levels inside the column. This can significantly increase the lifespan of the asset.

Inside the QC Lab

The Sievers rapid bioburden analyzer is considered to be an alternative method to the compendial plate count. Like any alternative method, a validation will initially be required to prove suitability for purpose in release testing. For quicker adoption, users can opt to explore a range of applications such as kill-rate studies, preservative efficacy testing, quantitative testing of reference microorganisms,and culture media qualification in parallel to existing QC methods. Doing so will allow the new instrument to evolve into a validated alternative method based on the user's application priority.

Conclusion: Improving Speed and Decision Making with RMMs

The Sievers rapid bioburden analyzer can be used for many applications, in addition to water testing. Implementing rapid bioburden testing in various locations inside a manufacturing site can play a key role in decision making and manufacturing agility, resulting in better contamination control strategies, cost savings, and speed.

Whether for faster release of raw materials, optimization of protocols to prolong the lifespan of expensive assets, or near real-time monitoring of products in the QC lab, rapid microbiological methods can quickly provide critical information to maintain control of production. Faster determination of whether a contamination event has occurred allows for immediate action to preserve and protect products, giving manufacturers confidence that their products meet the highest quality standards for customers.

Design And Evaluation of a Disinfectant/Sporicide Combination Triple Sanitization And In-Situ Disinfectant Validation

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Abstract

There remains debate on both how to conduct a triple clean evaluation as well as what data generation is optimal during the process in order to demonstrate in situ effectiveness of a disinfection program. A triple clean can be performed at any time following a worst-case event such as a shutdown or equipment failure at which times the microbial challenge is expected to be much higher than typically encountered in a cleanroom environment.

In this study, we outlined a triple clean remediation as a quaternary ammonium compound disinfectant used twice followed by a peracetic acid/hydrogen peroxide sporicide. This regimen allows for excellent microbial remediation as a long-term disinfectant rotational program which can be demonstrated by observing reductions in microbial bioburden during the process of reinstituting the cleanroom for use. During a triple clean event, the worst-case conditions present in the cleanroom give an excellent indicator of future performance of a rotational program when significantly lower bioburden challenges are expected.

Introduction

There is increasing focus on an overall, well thought out contamination control strategy (CCS). As the CCS is developed and implemented across the cleanroom site it is critical to have an effective cleaning and disinfection program. Annex I conveys that the disinfection process should be validated.[9] Validation studies should demonstrate the suitability and effectiveness of disinfectants in the specific manner in which they are used. Therefore, a well-planned CCS will include disinfectant field trials to support the validation of the disinfectants and sporicides being implemented onsite.

This disinfectant in-situ validation is one of the key activities for evaluating the effectiveness of the cleaning and disinfection program.[1,5] The most common definition of the triple cleaning process is to use the disinfectant two times followed by the sporicide.[2,3,4] There

are some other processes utilized in the industry for bringing the facility online after a worst-case event such as fogging application, VHP application or a 9X cleaning.[3]

A triple clean consists of an in-situ evaluation following a worst-case event, including a facility shut down, maintenance work or power failure. After these events, bioburden levels in a cleanroom environment are expected to be at their highest when compared to standard operation.

The qualification process typically begins with a disinfectant efficacy study with in-vitro studies where the efficacy of sanitizers/disinfectants/sporicidal agents are evaluated on representative cleanroom substrates with in-house isolates, or reference strains (for a new facility). The in-vitro tests simulate as closely as possible the actual cleanroom conditions such as temperature, cleanroom substrates, and the type of microorganisms. After the sanitizers/disinfectants/sporicidal agents successfully pass the efficacy tests, the qualification process moves into stage 2, in-situ testing, where the performance of the sanitizers/disinfectants/sporicidal agents will be evaluated under the actual worst-case conditions in the cleanroom. Stage 3 of the process is the continuous monitoring and trending of the environmental data over time to ensure the cleaning and disinfection program continues to be effective at controlling bioburden in the cleanroom environment.

In this case study from a pharmaceutical manufacturer, the ISO-5, ISO-7 and ISO-8 cleanrooms in this facility needed to be shut down for engineering and construction work. The engineering and construction work involved equipment installation, room pressure modification, and facility construction including shutting down HVAC systems that supply HEPA filtered air to the cleanrooms. The site conducted Environmental Monitoring Performance Qualification (EMPQ) in order to re-establish their cleanroom status after the maintenance shut down.

The goal of this testing was to evaluate the effectiveness of the triple cleaning process involving two applications of a quaternary ammonium compound disinfectant, followed by the application of a peracetic acid/hydrogen peroxide sporicide under these worst-case conditions.

Materials and Methods

This evaluation consisted of two separate events performed with the same regimen of products. The two evaluations, Evaluation I and Evaluation II, were performed after separate shutdown events approximately four months apart with the redundant testing designed to incorporate different seasons, microbial load and personnel entry requirements. During the maintenance shutdown prior to Evaluation II, workers were not required to wear cleanroom PPE and gowning materials, which could affect the initial microbial load. Both evaluations were performed in the same room areas with the same disinfectant and sporicide regiment. The regimen consisted of first cleaning all debris and residual construction materials. Once the debris and materials were removed, baseline counts were determined as it is critical to understand the existing bioburden after the shutdown occurs. The area was then disinfected with a quaternary ammonium compound ready to use disinfectant followed by baseline sampling of all surface sites. A second application of a quaternary ammonium compound ready to use disinfectant then occurred with surface sampling after treatment. Finally, the area was disinfected with a peracetic acid/hydrogen peroxide sporicide as a final step of the triple clean and all surfaces sampled.

All cleaning and disinfection were performed using a two-bucket system. The two-bucket system consists of a stainless-steel trolley system equipped with color-coded buckets, a DuoPress™ mop wringer, Duo™ Plus frame, extendable Universal Handle and individually sterile wrapped microfiber laminated mop.

The test phases (i.e., T=0, T=1, and T=2) were intended to assess the change in bioburden levels following each cleaning phase. The recovered growth was characterized, including Gram staining, to understand the nature of the microorganisms (i.e., fungal, spore-forming, non-spore forming) and changes between each baseline phase.

Environmental monitoring was performed on three consecutive days with cleaning and disinfection performed between T0 and T1 sampling and before sampling on T2 and T3 (Refer to Figure 1). All environmental monitoring locations remain the same for T0 (baseline monitoring), after the 1st sanitization (T1), after the 2nd sanitization (T2) and after the 3rd sanitization (T3), including floor surface, wall surface, counter/equipment surface and air viable.

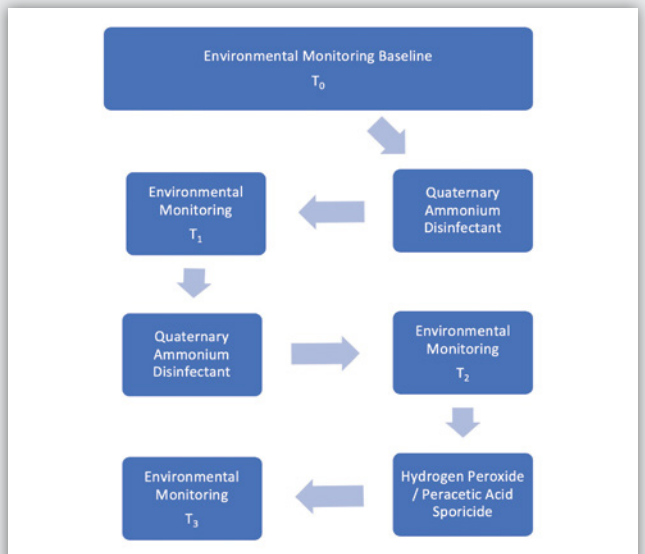


Figure 1. Triple Clean and Environmental Monitoring Plan

Evaluation I and Evaluation II followed the same procedures and product utilization. The environmental sampling points were conducted at worst case and high traffic areas in the cleanroom

operation. The environmental monitoring consisted of RODAC® plating, settle plating, active air sampling, and swabbing at these worst-case locations in the cleanrooms.

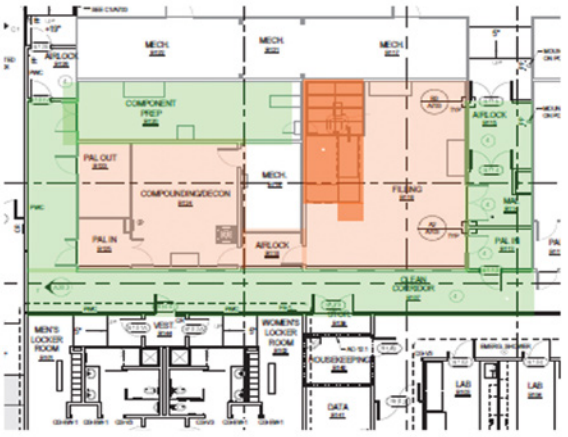


Figure 2. Facility Layout

For each evaluation, the media plates used were Trypticase™ Soy Agar (TSA) Sterile Plates with Lecithin and Polysorbate 80, RODAC® plates, and Trypticase™ Soy Agar Settling plates. All lots of media plates were tested for Growth promotion and passed. The plates used for Environmental Monitoring of this study were stored in a 2-8° refrigerator.

Immediately after sampling, all media plates were incubated between 20 to 25 °C for no less than 72 hours. After initial incubation, all media plates were transferred to an incubator set between 30 to 35 °C for no less than 48 hours. After all of the sampled plates were pulled from the 30 to 35° incubators, they were read using the colony counter and examined visually for morphology and Gram stained.

Post-incubation plates

Results

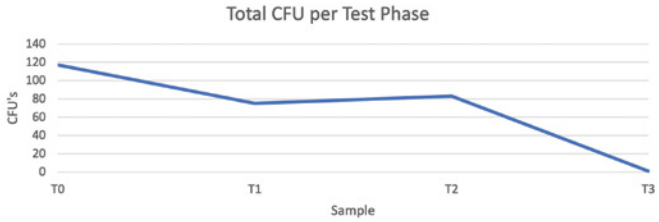
The tables below summarize the air and surface data from the T=0, T=1, and T=2 sampling results, as expressed in both bacteria and mold recoveries expressed as CFU.

Evaluation I.

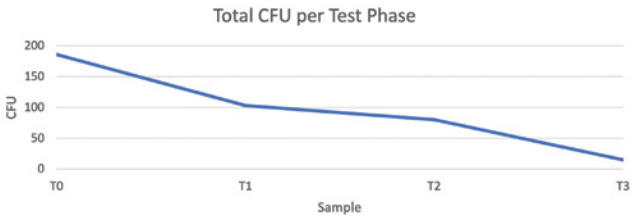
Summary: Sample Collection and Test Results

Room	ISO Class	Samples				CFU count				CFU/Plate			
		T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
B107 Clean Corridor	8	16	16	16	16	10	8	10	0	0.63	0.50	0.63	0.00
B120 Prep Room	8	12	12	12	12	9	5	6	0	0.75	0.42	0.50	0.00
B123 Material Entrance	7	5	5	5	5	0	0	0	0	0.00	0.00	0.00	0.00
B125 Aseptic Gowning	7	6	6	6	6	4	3	0	0	0.67	0.50	0.00	0.00
B124 Compounding Room	7	16	16	16	16	15	7	5	0	0.94	0.44	0.31	0.00
B118 Pass Through	7	6	6	6	6	4	2	3	0	0.67	0.33	0.50	0.00
B116 Filling Suite	7	19	19	19	19	24	30	21	1	1.26	1.58	1.11	0.05
B116 Laminar Flow Hood	5	3	3	3	3	2	0	0	0	0.67	0.00	0.00	0.00
B116 Behind Curtain	5	10	10	10	10	10	2	0	0	1.00	0.20	0.00	0.00
B116 Fill Machine	5	14	14	14	14	29	3	0	0	2.07	0.21	0.00	0.00
B116 Isolator Finger Tips	5	13	13	13	13	4	0	0	0	0.31	0.00	0.00	0.00
B114 Material Exit	7	6	6	6	6	0	0	0	0	0.00	0.00	0.00	0.00
B113 Personnel Exit	7	6	6	6	6	6	15	37	0	1.00	2.50	6.17	0.00
Overall	NA	132	132	132	132	117	75	83	1	0.89	0.57	0.62	0.01

For each of the thirteen sample areas, the number of microorganisms present decreased significantly from the initial baseline levels with only one site showing a single colony present of all 132 samples taken after the triple clean process. When all sites are pooled, the data suggest an initial reduction resulting from the use of the quaternary ammonium disinfectant with a reduction to nearly zero with the final application of the sporicide.



Similar to Evaluation I, Evaluation II showed a progressive reduction in total microbial CFU's recovered over the application of the two disinfectants and the sporicidal treatment. This evaluation had a higher initial value of CFU recovered and demonstrated a more pronounced reduction between the first and second disinfectant step. Despite some larger initial values as predicted for this evaluation, the counts for 11 of the 12 sample sites after the sporicidal treatment still demonstrated between 0-4 CFU present. There was an outlier in the data set from the B125 Personnel Entrance sample which demonstrated an increase in microorganisms from a single CFU after the second disinfectant treatment to 27 CFU after the subsequent sporicide application. Since a sporicide could not increase the number of microorganism present, the outlier was omitted from the graph below. The investigator in the study theorized that the outlier could be a result of unauthorized foot traffic during the study.



Discussion

A triple clean event serves to return an area to operational conditions following a worst-case event such as a shutdown, room failure or maintenance intervention. Following these events, a cleanroom or controlled surface will have higher levels of bioburden present than typically expected during normal operation as a result of uncontrolled access and environmental conditions. Additionally, the triple clean activities can provide excellent documentation of the overall expected success of a disinfectant program. Three main elements often comprise a well-designed disinfectant qualification and implementation program. These include in vitro analyses (Disinfectant Efficacy Studies), in situ evaluation (triple clean studies) and active interpretation and surveillance through environmental monitoring.

These elements are key components to an overall Contamination Control Strategy to demonstrate the suitability and effectiveness strategy of the disinfectant program in place.

In this study, we followed the regimen of disinfectant/disinfectant/sporicide to show the reduction of microorganisms per application after a shutdown event. This approach combines the surfactant-based one-step cleaning/disinfection of a quaternary ammonium compound disinfectant with the enhanced sporicidal and fungicidal efficacy of an effective PAA/hydrogen peroxide sporicide.

Evaluation II.

Summary: Sample Collection and Test Results

Room	ISO Class	Samples				CFU count				CFU/Plate			
		T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
B116 Behind Curtain	5	12	12	12	12	20	1	2	1	1.67	0.08	0.17	0.08
B116 Filling Suite	7	21	21	21	21	40	5	6	3	1.90	0.24	0.29	0.14
B118 Air Lock	7	6	6	6	6	5	1	3	1	0.83	0.17	0.50	0.17
B124 Compounding Room	7	18	18	18	18	17	18	1	2	0.94	1.00	0.06	0.11
B125 Personnel Entrance	7	6	6	6	6	11	13	1	27	1.83	2.17	0.17	4.50
B123 Material Entrance	7	5	5	5	5	4	2	0	0	0.80	0.40	0.00	0.00
B120 Component Prep	8	13	13	13	13	21	18	18	4	1.62	1.38	1.38	0.31
B113 Personnel Exit	8	6	6	6	6	10	2	0	0	1.67	0.33	0.00	0.00
B114 Material Exit	8	6	6	6	6	2	1	3	0	0.33	0.17	0.50	0.00
B107 Clean Corridor	8	18	18	18	18	55	51	47	4	3.06	2.83	2.61	0.22
Fill Machine	5	20	20	20	20	11	4	0	0	0.55	0.20	0.00	0.00
Isolator Glove Fingertips	5	13	13	13	13	1	0	0	0	0.08	0.00	0.00	0.00
Overall	NA	144	144	144	144	197	116	81	42	1.37	0.81	0.56	0.29
Overall	NA	132	132	132	132	117	75	83	1	0.89	0.57	0.62	0.01

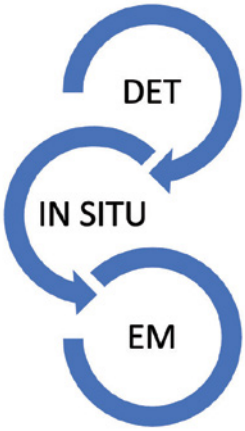


Figure 3. Three key elements of a disinfectant/sporicide qualification

The similar results obtained from Evaluation I and Evaluation II, despite being conducted months apart with each demonstrating a unique bioburden, further suggest the robustness of the disinfection program. Although the design of the studies were identical, inherent variability exists during microbiological evaluations, so the observed results demonstrating the same trend from the use of the product rotation adds additional confidence that the products selected will function well under normal conditions.

Conclusion

A progressive reduction in microbial count was observed from baseline T0 to T3 for each sample site within the ISO-5, ISO-7 and ISO-8 cleanrooms. The total recovered CFU after the final disinfection using the peracetic acid/hydrogen peroxide sporicide gave very low counts with zero CFU's recovered in 17 out of 25 samples.

This case study demonstrates the effectiveness of the triple cleaning process involving two applications of a Quaternary Ammonium Disinfectant, followed by the application of a peracetic acid/hydrogen peroxide sporicide under these worst-case conditions which serves as an excellent indicator of future performance of this regimen. The case study supports the sites disinfectant validation program and is an integral part of their ongoing CCS which can be assessed routinely for ongoing improvements. The disinfectant field trials are always an important part of a robust CCS which encompasses all facets of the cleanroom operation.

References

1. Polarine, J. and Walker, T. Evaluation of a Quaternary Ammonium Ready-to-Use (RTU) Disinfectant and Hydrogen Peroxide/Peracetic Ready to Use (RTU) Combination Sanitization Regimen for Cleanroom Start-Up. American Pharmaceutical Review, May/June 2020.
2. Polarine J., Kroeger, B. HOW ISSUES RELATED TO UTILITIES, SURFACES AND PRACTICES IMPACT CLEANROOM ENVIRONMENTS, Contamination Control in Healthcare Product Manufacturing, Volume 4 PDA/DHI Publishing, June 1, 2016
3. Polarine J., Kroeger, B. Bringing Cleanrooms Online Initially and After a Worst-Case Event, PDA Poster at PDA Annual Meeting 2015, March 10, 2015.
4. Polarine J., Kroeger B., Start-up of cleanrooms, initially and after a worst-case event., Clean Air and Containment Review, August 15, 2017.
5. IEST-RP-CC-018.5 Cleanroom Cleaning and Sanitization: Operating and Monitoring Procedures., IEST May 2021.
6. Ildikó no Ziegler, Judit no Borbély-Jakab, Lilla no Lilla Sugó and Réka J. Kovács. Revision of Viable Environmental Monitoring in a Developmental Pilot Plant Based on Quality Risk Assessment: A Case Study. PDA Journal of Pharmaceutical Science and Technology January 2017.
7. Anne Marie Dixon, Ch. 11, Cleaning of Non-Product Contact Surfaces in Cleaning and Cleaning Validation for the Pharmaceutical and Medical Device Industries, Vol. 1 Basics, Expectations, and Principles. Paul L. Pluta, Ed., PDA, Bethesda, MD, and DHI Publishing, LLC, River Grove, IL. 2009.
8. PDA Technical Report Number 70, "Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities".
9. Annex I Manufacture of Sterile Medicinal Products. August 2022.

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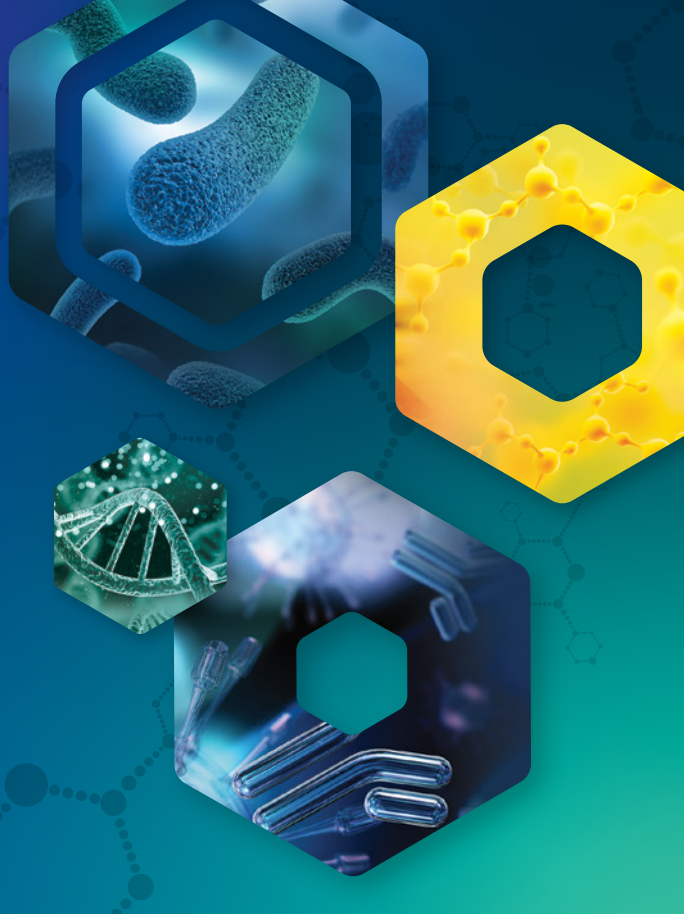

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Designing an Environmental Monitoring Solution for GMP Applications

Mark Hallworth
Life Sciences Senior GMP Scientist, Life Science Division
Particle Measuring Systems

Introduction

With the release of the new EU GMP Annex 1 revision, a review of current practices is required to ensure that the installed monitoring system, chosen to meet the needs of Annex 1, complies with the requirements. This chapter will review the needs of Annex 1 with systems designs currently being installed.[1]

Cleanroom Classification

Pharmaceutical cleanrooms are classified according to the particle concentration of the air that is required to meet the cleanliness criteria for the manufacturing process being performed. The determination of the cleanroom class is a process based on actual statistically valid measurements, and a function of the filtration and operations status of the room, it is, in essence, a calibration of the room to ensure it meets its intended classification, it is not, primarily, a function of risk of application.[2]

There are three measurement phases involving particle counting in cleanrooms:

As Built: a completed room with all services connected and functional but without production equipment or personnel within the facility.

At Rest: all the services are connected, all the equipment is installed and operating to an agreed manner, but no personnel are present.

Operational: all equipment is installed and is functioning to an agreed format and a specified number of personnel are present, working to an agreed procedure.

The airborne particle count test is performed by counting particles at defined grid locations within the cleanroom. The test points should be equally spaced throughout the room and at work height to demonstrate the quality of the air cleanliness at the work area.

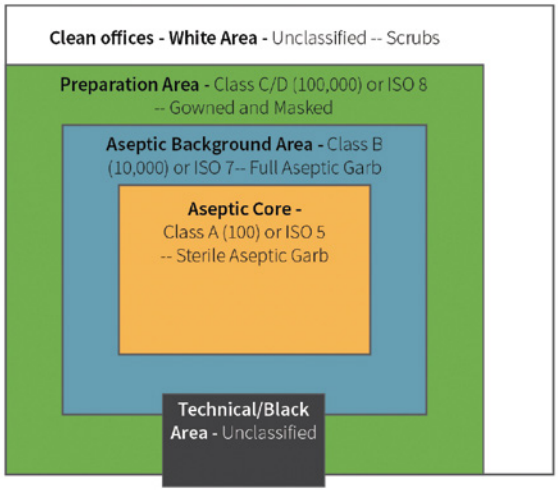

Figure 1. Diagram of Typical ISO Ratings in Areas of Cleanrooms

Pharmaceutical cleanrooms typically operate at Class 5 (most aseptic areas), Class 7 (surrounding areas), or Class 8 (support areas). See Figure 1 on the following page for a visual representation.

Pharmaceutical Cleanroom Utilization

Once a cleanroom has been tested for compliance to cleanroom classification typically using a Light Scattering Aerosol Particle Counter (LSAPC), the classification achieved dictates which production activities can be performed in that cleanroom or clean air device. The FDA defines two areas.

1. **Critical Areas** contains products that, if exposed, are vulnerable to contamination, these areas are designated Grade A (ISO5), within the Annex 1 document. To maintain product assurance, it is essential that the environment in which aseptic operations are conducted be controlled and maintained at an appropriate quality.
2. **Supporting Clean Areas** are used for all other activities outside the critical core, these are designated as grade B/C/D within the Annex 1 and are typically a lower risk to finished product contamination.

Once a cleanroom or clean air device has been proven to meet the requirements for cleanliness from a certification perspective, it must also demonstrate that this control can be maintained throughout production periods. The environment needs to be rigorously monitored to ensure that there is full and constant awareness of current conditions, including the detection of periodic events which could be catastrophic if gone unnoticed. Constant monitoring creates a continuous flow of information, resulting in a large quantity of data which can be used to watch for trends.

The manufacturing facility should therefore have a comprehensive environmental monitoring program, which includes monitoring for nonviable and viable airborne particulates, surface viable contamination and, in the aseptic areas, and personnel. These procedures should address frequencies and locations for the monitoring sample points, warning and alarm limits for each area, and corrective actions which need to be undertaken if any of the areas show a deviation from expected results. Actions taken when limits are exceeded should include an investigation into the source of the problem, the potential impact on the product, and any measures required to prevent a recurrence.

Contamination Control Strategy

A Contamination Control Strategy (CCS) will include the environmental monitoring program and should be implemented across the facility. The CCS should define critical control points as part of a risk assessment and assess the effectiveness of the controls and monitoring measures used to manage risks associated with

contamination. The CCS should be reviewed frequently, especially during the early phases of implementation, and it should be updated to drive continuous improvement of the monitoring and control methods, ultimately improving overall quality of process.

Elements that should be considered as part of a Contamination Control Strategy will include:

i. Design of the plant and processes.
ii. Premises and equipment.
iii. Personnel.
iv. Utilities.
v. Raw material controls.
vi. Product containers and closures.
vii. Vendor approval –key suppliers.
viii. Outsourced services, such as sterilization, ensure the process is operating correctly.
ix. Process risk assessment.
x. Process validation.
xi. Preventative maintenance.
xii. Cleaning and disinfection.
xiii. Monitoring systems - the introduction of scientifically sound, modern methods that optimize the detection of environmental contamination.
xiv. Prevention – trending, investigation, corrective, and preventive actions (CAPA).

The scope of a Facility Monitoring System should encompass those identified in the list above (i, ii, iii, ix, x, xii, xiii and xiv), many of the CCS considerations should be included in the Environment Monitoring (EM) program.

Environmental Monitoring Requirements

Table 1. EU GMP Annex 1 room classification table (Annex 1 2022)

EU GMP grade	At Rest		in Operation	
	Maximum number of particles permitted/m3 equal to or greater than the tabulated size			
	0.5 µm	5.0 µm	0.5 µm	5.0 µm
A	3520	Not Specified (a)	3520	Not Specified (a)
B	3520	Not Specified (a)	352,000	2930
C	352,000	2,930	3,520,000	29,300
D	3,520,000	29,300	Not Predetermined (b)	Not Predetermined (b)

(a) Classification including 5um particles may be considered where indicated by the CCS or historical trends.
(b) For grade D, in operation limits are not predetermined. The manufacturer should establish in operation limits based on a risk assessment and routine data where applicable.

Monitoring should be performed using suitable techniques that meet the needs of the Risk Assessment; for many of the monitoring requirements

of lower classification areas, a portable instrument can be deployed and used.

However, the grade A area should be continuously monitored (for particles ≥0.5 and ≥5 µm) with a suitable sample flow rate (at least 28.3 LPM / 1CFM) so that all interventions, transient events, and system deterioration is captured.

The system should frequently correlate each individual sample result with alert levels and action limits at such a frequency that any potential excursion can be identified and responded to in a timely manner. Alarms should be triggered if alert levels are exceeded. Procedures should define the actions to be taken in response to alarms, including the consideration of additional microbial monitoring.

The requirement for continuous monitoring within the Grade A is satisfied by using point of use dedicated sensors; these are connected to a central monitoring software application that can send alarm outputs to operators within the cleanroom or messages to concern groups. These alert and alarm excursions are also permanently recorded in the audit trail of the system.

One aspect of the system that needs to be determined is the location of the sample point(s); these should be determined following a documented Environmental Monitoring Risk Assessment (EMRA) and include the following information:

- Sampling locations
- Frequency of monitoring
- Monitoring method used and
- Incubation conditions (e.g. time, temperature(s), aerobic

and/or anaerobic conditions). and be based on the following inputs from site:

- Detailed knowledge of; the process inputs and final product, the
- Facility, equipment,
- Specific processes,
- The operations involved,
- Historical monitoring data,

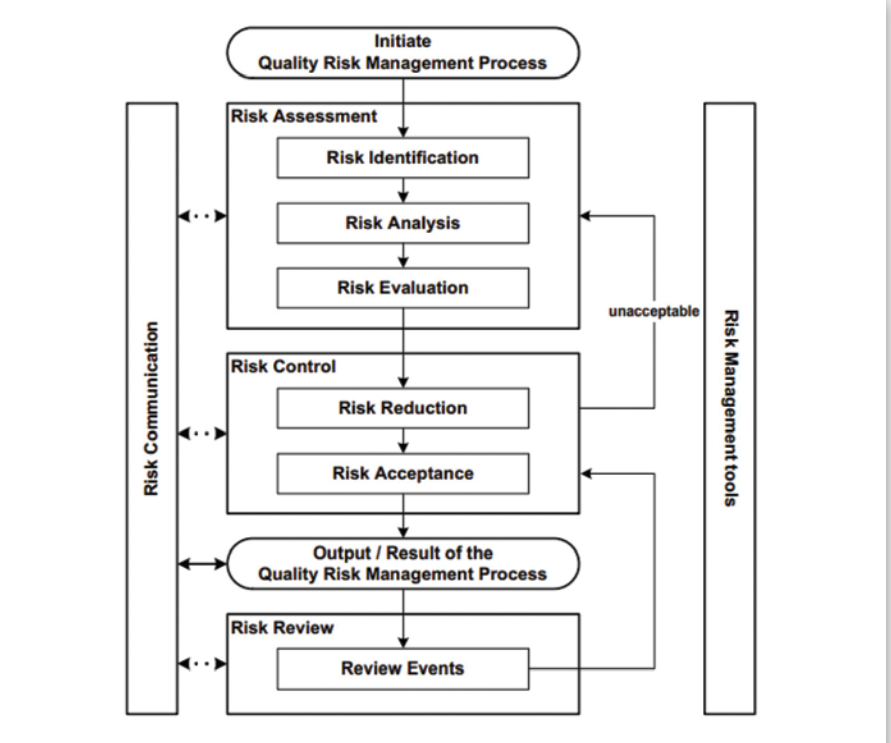

Figure 2. Steps for Risk Identification and Analysis

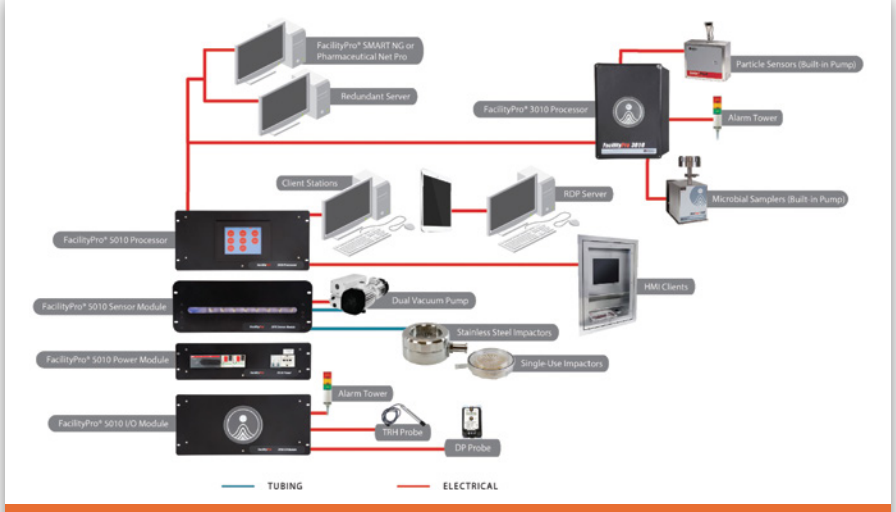

Figure 3. Example Facility Monitoring System (FMS) Setup

- Monitoring data obtained during qualification and
- Knowledge of typical microbial flora isolated from the environment.
- Air visualization studies should also be included Suitable sample point locations are also impeded by:
- Physical installation of sample probe
- Physical installation of instrument
- Tubing length, bends and bends radii between the two

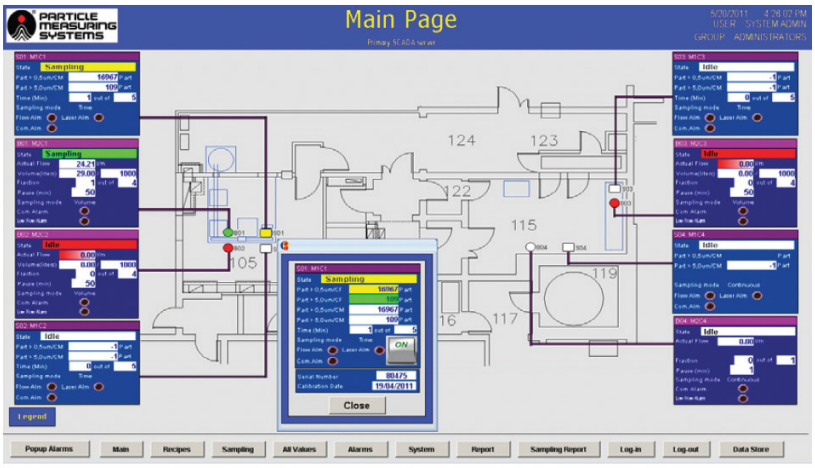

Figure 4. Main Page of Facility Monitoring Software

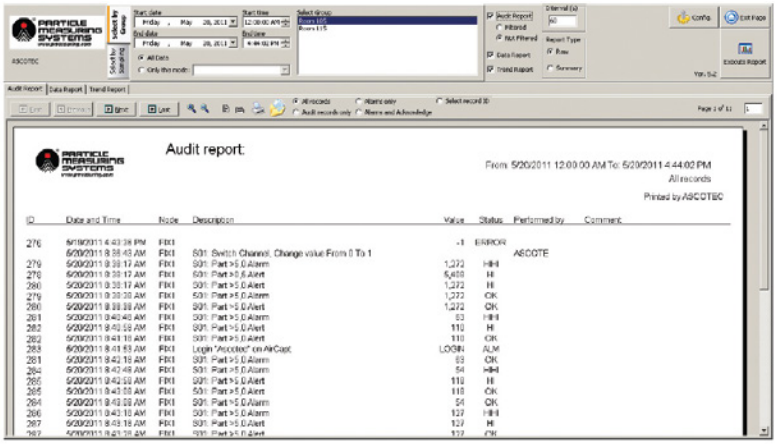

Figure 5. Report Generator

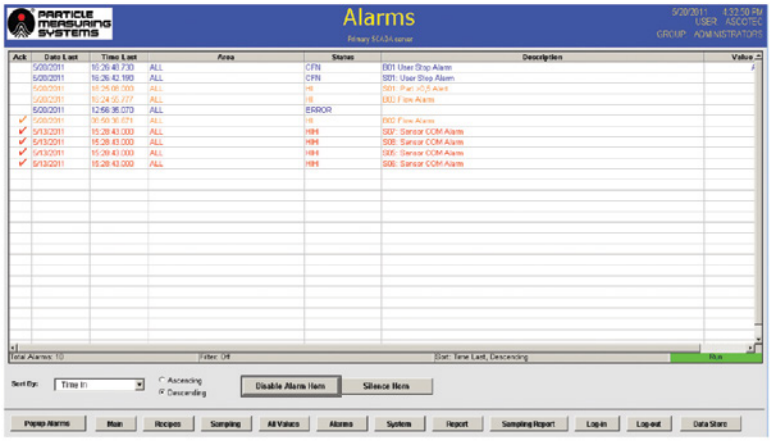

Figure 6. Alarms Page

Typical Automated Continuous Monitoring System

Instrumentation used in constructing an integrated solution will typically include:

Particle Counting – The need for continuous data requires a dedicated sensor at each location that samples continuously during the set-up and production phases of manufacturing. The sensor(s) send data back to a central processing component that is used to manage response processing, data buffering, and sensor controls. The sensor can have an internal pump or a remote vacuum source; both are controlled using the central interface within the software application.

Microbial Sampling – Where a risk has identified the need for total particle counting, there is an associated requirement for microbial sampling. The sample head only is placed within the environment, ensuring that any exhaust is managed by the system and not emitted locally within the critical space. Microbial samplers are fixed flowrate devices (typically 25 LPM), and this flow control is performed either locally (using a dedicated device) or centrally (using the same central vacuum source as the particle counter sensor sub-system). Start and stop controls are performed via the software interface.

Alarm Beacons – These additional devices allow for local annunciation (visible and audible) and can alert operators within the controlled space if a system is out of tolerance. Additional information can be achieved by situating a remote interface within the viewing space of the operators; these can also be interactive if they are within the clean core of the facility.

Central Software System - The system is designed with Industrial Automation architecture, which consists of a central processing system that collects data from field sensors and controls remote devices while communicating with a SCADA (Supervisory Control and DataAcquisition) software package. The following features should be available to interact effectively with the system and data reports.

Data and Status information Displays - The main page is used for visualization of the facility layout with current data and status information for each sample point.

Data, status, and sampling information can also be viewed for each dedicated area on a single screen.

User and Area segregation - According to CFR 21 Part 11 and Annex 11, single user access shall be controlled and managed to ensure each individual operated as described in the user Standard Operating Procedures and, according to the role, responsibility and training received.

The SCADA software should also ensure proper segregation of data whenever multiple departments are connected and controlled by the same supervising system.

Report generator - The SCADA software requires a data report generator capable of providing human readable reports for all recorded data such as audit trails (events), data/statistical summaries, and trend charts. The system should be capable of retrieving data historically as defined in the site User Requirement Specification for the associated system. Using filters for data, time, location, and batch, data should be readily accessed and, where required, exported or printed to support the release of product.

Alarms - The alarms display provides date, time, area, description, value, etc. for alarms and provides an alarm acknowledgment function. The alarms display also offers the capability to sort alarms by different criteria. Defining the alarm set point within the software is based upon the limits table in Annex 1 and based on historical data for each sample location.

Annex 1 (2022) also notes in Section 9, that

> *"The occasional indication of macro particle counts, especially ≥ 5 µm, within Grade A may be considered to be false counts due to electronic noise, stray light, coincidence loss etc. However, consecutive or regular counting of low levels may be indicative of a possible contamination event and should be investigated. Such events may indicate early failure of the room air supply filtration system, equipment failure, or may also be diagnostic of poor practices during machine set-up and routine operation"*

Therefore, when considering alarm rationale, it should also take into account the frequency of event and not singularly the magnitude; these factors should be considered within the CCS.

Additional information and alarming strategies can also be found in ISO 14644-2:2015, paragraph B.3.1; Environmental Monitoring Systems should allow for seamless and validated configuration of an "N of M" strategy to ensure sequences of out of specification events are promoted to alarms when the conditions are met.

As with all integrated systems, especially those using a central software package, the validation is a significant element on the timeline of any installed project. The review and circulation of documents can take several weeks where multiple departments are involved, and the start to finish time of a project should be discussed with the installation project team to ensure it meets the site requirements for shutdown accessibility.

Conclusion

Monitoring of pharmaceutical aseptic production environments is well established and the changes presented in the revision of Annex 1 (2022) do not change many aspects of the requirements for monitoring. The formal risk assessment and inclusion of data within a CCS create a more comprehensive addition to the continuous systems installed under past regulations. More emphasis is given to establishing the correct sample locations and techniques based on risk and reviewing data to support product release. This emphasis is however an enhancement to the documentation requirements more than the traditional expectations of a continuous system.

References

1. European Commission. The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use: Annex 1, Manufacture of Sterile Medicinal Products, Annex 1 (2022).
2. International Standards Organization. Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness by particle concentration, ISO Standard No. 14644-1:2015 (2015).

Author Biography

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Editor: Noelle Boyton

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Microbiology

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Executive Director of Microbiology & Sterility Assurance
Quality Executive Partners, and Owner of VVF Science, LLC

In general, in the last year have there been any significant or interesting developments in microbiology tools and technologies.

Timothy Francis, Senior Technical Specialist for the LAL Division of FUJIFILM Wako Chemicals U.S.A. Corporation: From the viewpoint of the pharmaceutical industry clients that I work with, one of the most impressive developments I have seen is the advancements made in customized solutions for pharmaceuticals to provide specific solutions to individual needs. For example, this year has seen the development of several pharmaceutical businesses that use viral agents, such as AVV or bacteriophage, to manufacture customized drugs for unique recombinant drug products. There also has been great innovation in drug development as pharmaceutical formulations developed by computer intelligence have been undergoing development tests over the last year.

Meg Provenzano, Product Manager- Biodetection, Veolia Water Technologies & Solutions - Sievers Instruments: The publication of the revision of Annex 1 has certainly been an interesting development. The document encourages the adoption of rapid methods and continuous monitoring systems in order to minimize risks of contamination. Rapid Microbiological Methods are encouraged for Environmental Monitoring, Personnel Monitoring, and Facilities Monitoring. Annex 1 also encourages manufacturers to have a quality Contamination Control Strategy (CCS) in place in order to define all critical control points and assess the effectiveness of controls and the associated methods and frequency of monitoring. Implementing RMMs within a facility allows manufacturers to monitor any contamination much faster than traditional methods. The Annex 1 guidelines align with recent interesting developments related to RMM technologies. For example, with certain technologies for rapid bioburden testing, results

can be obtained not only faster, but also with a strong correlation to culture-based methods. Adopting such a system can help users address microbial contamination in near real time.

How has the growth of Advance Therapy Medicinal Products (ATMPs) changed the way pharma companies approach their microbiology efforts. What have you noticed?

Francis: The increase in pharmaceuticals such as Advanced Therapy Medicinal Products have created new complex needs for quality control testing that may have not been apparent for conventional pharmaceuticals. For example, pharmaceuticals made for a specific purpose that will not be manufactured in large numbers may need unique strategies for the quality control testing requirements needed for release. Regulatory bodies such as the USP have acknowledged the need for customized testing patterns for unique products manufactured in small lot numbers such as this.

Provenzano: With the industry moving toward personalized medicine, it means that Rapid Microbiological Methods (RMMs) are going to be important for ATMPs as well. Some of these therapies involve taking cells directly from the patient, administering therapy to the cells, and then returning them back into the patient's body. It is critical that microbes are not introduced into the patient causing further illness, and speed is also important in order to avoid delays that can also impact patient safety. Implementing technologies such as RMMs and endotoxin testing systems that are fast, fully compliant, and easy to use can provide confidence to all parties involved - including the most important party, the patient.

Vanessa Vasadi-Figueroa, Executive Director of Microbiology & Sterility Assurance, Quality Executive Partners, and Owner of VVF Science, LLC: Absolutely, the growth of ATMPs has helped the field of industrial microbiology progress in gaining familiarity with shorter turnaround times and managing risk. For some organizations, this means implementing rapid microbiological methodologies, and for others it means bringing in new technology for real time process monitoring or automated release testing. The hard work with this progression is not only the test methods themselves- these have existed for 15-30 years now - but rather it's becoming more comfortable with assessing risk, as well as understanding and managing the risks for our process and products. I see us owning risk analysis more than we have done in the past and interjecting ourselves earlier into the product development cycle. Microbiology is also more involved in facility, equipment, and process design than ever before. We have ATMPs to thank for that, as they have pushed us forward, and we are seeing ripple effects throughout other sectors in our industry such as sterile compounding and traditional aseptic processing. Preventing contamination and reducing turnaround time for its analysis makes good sense, both in the technical and business side. It's certainly better for getting safe product out the door, especially when on the critical path for delivery to individual patients, drug shortages areas or locally compounded drugs.

Looking at regulatory and compliance issues – have there been any recent changes/updates related to microbiology operations that affect how pharma companies conduct international operations?

Francis: In my area of quality control testing for endotoxins in pharmaceuticals, the most pertinent area in which regulatory changes have been made and hopefully continue to be made is in the pharmacopeial acceptance of recombinant reagents for bacterial endotoxin testing. The development of several different recombinant reagents and their continued acceptance in end-product QC testing has led to all three of the pharmacopeial bodies to accept or formally consider these reagents. Although here in the USA recombinant LAL reagents are not compendial, It is encouraging that continued efforts are being made by the USP to provide a compendial chapter on recombinant LAL reagents.

Vasadi-Figueroa: It goes without saying that the release of Annex 1 in August 2022 has rippled throughout the industry in a way that I hadn't seen a regulatory change do so (in a long time). Pharma companies now have a heightened sense of global responsibility with respect to contamination control, but it's up to each company to act appropriately. While the requirement itself to prevent contamination from entering a process—and controlling its presence once detected—isn't novel, the requirement hadn't been mandated in such an organized and compelling manner like this latest release. What this regulatory update accomplished was to distribute the strategic control of contamination throughout every aspect of the pharmaceutical manufacturing business. As a microbiologist, I find this exhilarating and somewhat validating, but I would suppose others are a bit less thrilled. With enforcement imminent, I think companies are demanding better quality from their suppliers, asking tough questions to facility engineers, implementing better production controls, and educating the workforce on sterility assurance principles. Microbiological controls are becoming a more shared responsibility (think fewer microbiologists running around demanding action), and so all we can do now is continue to build strength on these controls and see how they are enforced by global regulators.

As the industry, and the world, slowly moves passed the pandemic, did the pandemic cause any significant changes in how companies approach their microbiology efforts? Do you see any changes in supplies, staffing, services?

Francis: After the pandemic, supply chain issues definitely created a higher reliance on the need for products, reagents, and accessories that could be reliably sources. I believe that pharmaceutical companies have begun to prioritize solutions that are resilient to global and local disruptions. In my industry specifically, I think this has given rise to a greater valuing of in-house testing. Overall, a positive aspect is a greater redundancy in in-house production. However, a negative of

this new approach is that many global connections that existed before the pandemic need to be rebuilt. In-person business seems to have decreased but is definitely increasing.

Vasadi-Figueroa: I would like to think that the pandemic brought visibility to the science of microbiology and an awareness to microbes in the world all around us. For some, this meant implementing better hand sanitization practices and cleaning methods to stay safe, but for others, it meant intellectually distancing themselves from the science of it all to stay sane (which meant differently safe). Then there are the people in between, following the rules and doing their best, one day at time. These polarizing mentalities most definitely impacted our industry because people are the very fabric of our workforce. Whether they are from small, hardworking cities in American to large, bustling metropolises in Asia, people bring with them a newfound perception towards these tiny things called microbes. The diligence with respect to masking and gloving has been eroded, so even seasoned staffing have varying degrees of interest in doing it properly. New hires also find it challenging to adhere to stringent cleanroom controls, but the potential for contamination is still very real in the production environment. This translates to difficulties finding adequate staffing, or it may mean that companies are experiencing high turnover rates for persons working in the manufacturing or laboratory areas. This has only compounded the staffing complexities experienced prior to the pandemic.

Finally, what long-term changes has the pandemic left on the industry in general and microbiology specifically?

Francis: In general, the world of pharmaceuticals seems more greatly disconnected than previously. This could be a combination of the lack in regulatory harmonization with the new recombinant reagents, as well as the rebuilding of the global business post pandemic. However, a great positive that has resulted has been many specific innovations in both drug development, manufacture, and testing. For example, with the increase in reagent options as well as newer innovative instruments and manufacturing technology, the pharmaceutical world has increased in its innovative power. A specific push to this innovation was definitely the role the pharmaceutical industry played in the fight to end the pandemic.

Provenzano: The pandemic created opportunities for people to work remotely, including some QA and QC personnel. In these cases, remote data sign off is important along with electronic signatures. Manufacturers need software that allows for data to be signed at times from a home office, thus resulting in faster product release. In the case of endotoxin testing, for example, the Sievers Eclipse allows for a client/server installation so the reviewer can sign off on endotoxin results of up to 21 samples from the comfort of their home. The pandemic also encouraged manufacturers to find and utilize other digital solutions, so many companies are joining the digital revolution and evaluating and/or implementing technologies faster than the adoption seen in the past.

Vasadi-Figueroa: There is something about the pandemic that enabled our industry to <begin> to let go of our rigid ways of the past and embrace a more flexible way of working. I don't intend that to mean working remotely, although in some cases, it's still quite feasible. What I intend to convey is that we must incorporate new ways of working or being more agile in the pharma workplace. We can achieve this by building resilience in our workforce by training, educating, and organizing work in a manner that keeps people truly engaged. Gone are the days where you show up to work and do one thing, rinse and repeat each day of the week. Employees expect to learn new skills, contribute to a team in a meaningful way, and do different things over the course of their tenure. For example, if you start in the Micro lab as an EM analyst, that's great (we all started this way) but folks expect to migrate to other parts of the lab. Rather than waiting for a performance plan, companies need to rotate people from the start of their work plan by teaching them new skills and new ways of thinking critically. This builds resilience within the analyst, but also for the laboratory and greater company goals.

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Sterility Assurance for Single-Use Systems

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Introduction

Sterility assurance is a critical aspect of pharmaceutical manufacturing, which commonly includes single-use systems. Single-use systems offer numerous advantages, including reduced contamination risk, increased operational efficiency, and improved flexibility. However, ensuring the sterility of these systems requires careful consideration of various factors. In this overview, we will delve into the key topics that pharmaceutical manufacturers should be aware of when addressing sterility assurance for single-use systems.

1. Regulatory Guidelines

Pharmaceutical manufacturers must stay abreast of regulatory guidelines set forth by authorities such as the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the International Organization for Standardization (ISO). These guidelines provide the framework for sterilization validation, including the choice of methods, acceptable sterility assurance levels (SALs), and documentation requirements.

2. Sterilization Methods

Different sterilization methods are available for single-use systems, and each has its own advantages and limitations. Steam sterilization (autoclaving) is commonly used in the pharmaceutical industry due to its effectiveness, low cost, and compatibility with various materials, but is not commonly used for single-use systems due to the high temperatures required and the impact on many polymers that are part of the systems. Although other common sterilization methods like ethylene oxide (EO) and electron beam irradiation could be explored, the complexity and density of the single-use systems creates difficulties in using these methods. The most common methods used are gamma and X-ray irradiation, and they have served the single-use systems industry well for many years. Manufacturers should understand the strengths and limitations of each method and select the most appropriate one for their specific single-use systems. Since radiation sterilization is the most common method applied to single-use systems, most of the information below will be focused on radiation.

3. Material Compatibility

Single-use systems consist of various components, such as tubing, filters, and connectors, made from different materials. Manufacturers must evaluate the compatibility of these materials with the chosen sterilization method to ensure that sterilization does not compromise their integrity or performance. Compatibility testing may involve assessing factors such as material stability, leachables, and changes in physical or mechanical properties. This evaluation might influence whether the sterilization process could include some overkill, or should be optimized to result in a more gentile cycle for the materials.

4. Validation Studies

Validation studies are crucial for establishing appropriate sterilization parameters for single-use systems. These studies involve determining the ideal combination of temperature, time, and pressure (for most sterilization processes), or the minimum radiation dose to achieve the desired sterility assurance level (SAL). Validation protocols should include worst-case scenarios and stress conditions to ensure the robustness of the sterilization process.

5. Bioburden Assessment

Bioburden refers to the microbial load present on single-use systems before sterilization. For radiation sterilization, conducting a thorough bioburden assessment is essential for determining the initial level of contamination and establishing an appropriate sterilization dose. This assessment involves sampling, enumeration, and some degree of microorganism characterization which might include identification of microorganisms present, as well as evaluating the potential influence of bioburden on the sterilization process.

6. Sterility Assurance Level (SAL)

The SAL is a measure of the probability of a product remaining non-sterile after sterilization. Expressed as 10^{-n}, where "n" represents the desired level of sterility assurance, manufacturers must define the appropriate SAL based on the risk associated with the specific application. The chosen sterilization method must be capable of achieving the selected SAL, which is typically 10^{-6} (1 in a million), but alternative SALs can be selected under certain circumstances (see AAMI ST67 and ISO 19930).

7. Dose Mapping

Dose mapping studies are essential for verifying the uniformity of radiation sterilization throughout the entire single-use system. Particularly important for large or complex systems, dose mapping involves placing radiation sensors (called dosimeters) at various locations within the system to ensure that all areas receive the minimum sterilization dose. This process helps identify potential low-dose areas that may require adjustments in the sterilization process.

8. Product Integrity Testing

After sterilization, it is crucial to assess the integrity of single-use system components to ensure they have not been compromised. Product integrity testing may involve evaluating parameters such as seal strength, particulate matter, leachables, and container closure integrity. These tests help confirm that the single-use system remains intact and capable of maintaining its sterile condition.

9. Residuals and Residues

Many sterilization processes can leave behind residual sterilization agents or reaction by-products on single-use systems. One of the benefits of radiation sterilization is that there are no residuals, so no testing regarding that topic is necessary. When using sterilization processes other than radiation, manufacturers must assess the presence and impact of these residuals to ensure they comply with safety and quality standards. Residual testing may involve chemical analysis and toxicological evaluations to verify the absence of harmful residues that could pose risks to patients or affect product stability.

10. Packaging and Storage Considerations

Packaging and proper storage are critical for maintaining the sterility of single-use systems until their intended use. Manufacturers should consider the influence of packaging materials on sterilization processes, as well as factors like moisture control, gas permeability, and compatibility with the chosen sterilization method. Although packaging can have an influence, such as those just listed, on most sterilization methods, rarely does packaging have any influence in radiation sterilization.

11. Verification of Sterility

Users of single-use systems often are asked, or desire, to verify the sterility of the single-use systems they are using. Performing tests for sterility on some systems does not verify the sterility of other systems they use, and is not the appropriate way to address this question. The proper way to verify sterility is to perform a technical diligence audit of the single-use system supplier, ensure the sterilization process is validated and maintained per an appropriate method (usually an ISO standard), and demonstrate that the sterilization process has been correctly applied (usually through a review of the certification of irradiation).

Conclusion

Sterility assurance is an essential aspect of ensuring the safety and efficacy of single-use systems in pharmaceutical manufacturing. The eleven topics discussed in this overview can help pharmaceutical manufacturers navigate the complexities of sterilization validation effectively. Staying up to date with regulatory guidelines, understanding sterilization methods, assessing material compatibility, conducting validation studies, and performing rigorous product testing are key steps in ensuring appropriate sterility assurance of single-use systems.

DNA: The Code to Life

Saanvi Vavilala, Savaas Iqbal, Neelam Sharma and Hemant Joshi

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Discovering the Code to Life

Code is a system of words, letters, or signs used to represent a message in a secret, shorter or in a convenient form. DNA (deoxyribonucleic acid) is such a code to all living cells in the universe. DNA holds the instruction manual for each living thing for anything from a blade of grass to bacteria, animals, and humans. But what exactly is DNA, and how did we discover it?

Even centuries ago, scientists knew that there was some microscopic molecule inside organisms that encoded its characteristics - causing similar traits to be passed down to offspring. However, scientists were unsure about what this molecule could be. In the 1950s, scientists named the "gene" to be this very microscopic molecule. This elusive "gene" was capable of replicating with little to no error, a biological machine that is key to all life as we know it.

The chemistry, structure, and replication of genes were unknown to scientists. Only after all the main groups of macromolecules—proteins, sugars, and lipids—were ruled out, was an amazing discovery about to occur. Watson & Crick are credited to have discovered the double helix structure of DNA (Figure 1).

Figure 1. A typical double helix structure of DNA

Editor's Note: Hemant Joshi and his colleagues at Tara Innovations are frequent contributors to *American Pharmaceutical Review* and *Pharmaceutical Outsourcing* magazines. Tara offers summer internships to students interested in science. During their internship students learn how science is applied to real-world situations.

American Pharmaceutical Review is proud to publish this article written by two of Tara's 2022 interns.

Through rigorous study of genetics, biochemistry, chemistry, physical chemistry, and X-ray crystallography—the secrets to modern molecular biology were uncovered. Numerous contributions were also made by a British chemist Rosalind Franklin and a New Zealand-born biophysicist Maurine Wilkins. Franklin's discovery allowed for the final pieces of the puzzle to come together, with Photo 51, an x-ray crystallography image captured by her PhD student, RG Gosling (Figure 2).

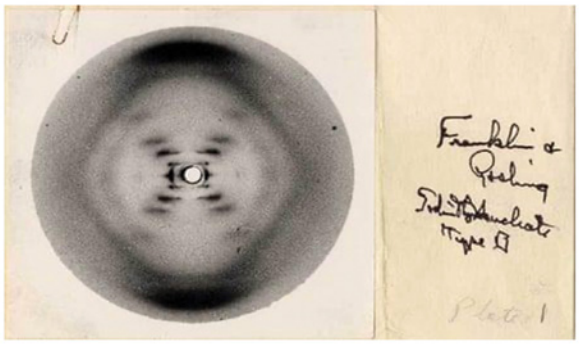


Figure 2. Photo 51 by RG Gosling. An X-ray based fiber diffraction image of a paracrystalline gel composed of DNA fiber.

What is DNA?

DNA stands for Deoxyribonucleic Acid. This large and intimidating word actually tells us a lot about DNA's structure. The prefix "deoxy" means that there is no oxygen atom attached to one of the carbon atoms, and this fact differentiates DNA from its closely related relative, RNA, or ribonucleic acids. The "ribo" refers to the ribose sugars that make up the sugar-phosphate backbone of a nucleotide, as can be seen below (Figure 3). Finally, the actual nucleic acids are what create the genetic code, and in DNA, they are 'A', 'T', 'C', 'G' or Adenine, Thymine, Cytosine, Guanine, respectively. These nucleotide bases are the fundamental units of the genetic code. Bases A, G, C and T are found in DNA while RNA (Ribonucleic acid) includes a fifth base, Uracil (U). Thymine and Uracil have similar chemical structures, except for the methyl group found in Thymine on the fifth carbon (C5) of the heterocyclic six-membered ring.

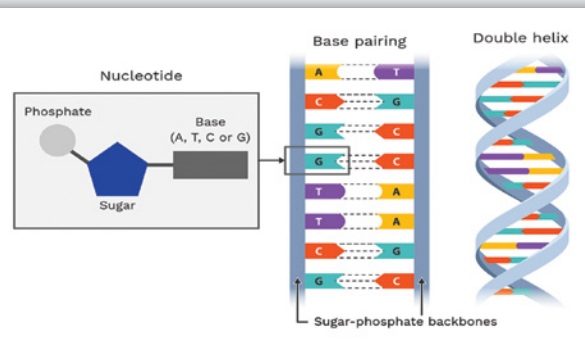


Figure 3. Basic structure of DNA and its components

The double helix structure is supported by a phosphate-sugar backbone, which provides structural support and prevents strands from breaking apart.

One of the key components to DNA are nucleotides. These nucleotides are the building blocks of DNA. The structure consists of 3 parts: phosphate, pentose (5-carbon) sugar, and a nitrogenous base. These bases then create unique sequences that can code for something entirely different if even just one base is changed. There are two categories of nitrogenous bases, pyrimidines, and purines (Figure 4). Pyrimidines are smaller and pair with the larger purines. Each nitrogenous base pairs with specific other nitrogenous bases.

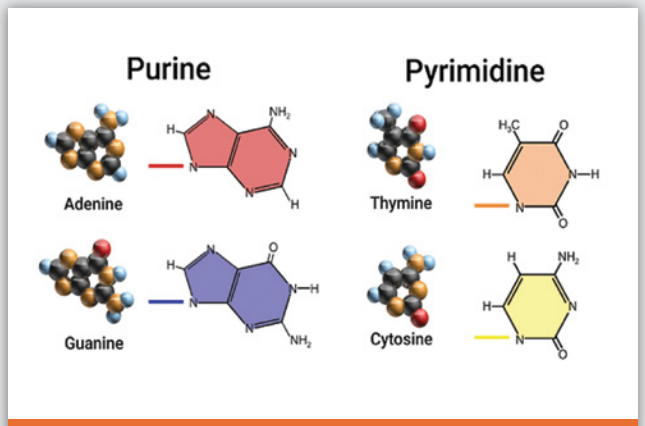


Figure 4. Chemical structures of the Nucleobases of DNA.

The purine Adenine pairs with the pyrimidine Thymine, and the purine Guanine pairs with pyrimidine Cytosine.

DNA and Genes

As described above, DNA is a long-chain of nucleotides, and genes are small stretches of DNA. An organism's genome is the entire collection of DNA and can contain thousands of genes. Genes are so important because they provide the instructions for building proteins, which conduct all sorts of functions, from digesting food to carrying oxygen through our bloodstream. Gene encodes an amino acid sequence of a specific protein. Genes are part of DNAs and RNAs.

How Else Can We Use DNA?

DNA is unique to each and every individual. The human genome contains 3,000,000,000 base pairs arranged in a unique sequence — meaning that DNA can be used to identify any individual. DNA decides traits – a characteristics that is caused by genetics. Traits can be various kinds – personality, physical features, value system, likings etc. Mainly, genes determine traits of an organism. Babies get genes from both parents. As a results, they get traits from their both parents. This fact becomes extremely useful when solving crimes. A person can be identified from even the smallest sample of saliva, blood, urine, or hair!

Additionally, DNA testing can help scientists learn about human history through analyzing migration patterns, disease outbreaks, and pathogens. In the medical field, DNA is used to recognize predisposition to diseases, vaccine development, and cancer therapy. DNA can also be used for cloning, which is the process of making identical genetic offspring by reusing existing DNA.

All of these endeavors became possible due to the Human Genome Project, which lasted 13 years, from 1990 to 2003. It was an ambitious project that was aimed at discovering the sequence of the entirety of human DNA—all 3 billion base pairs! Now that we have access to such a valuable information, it is possible to do all of the processes mentioned above, from DNA finger-printing and catching criminals to creating genetic clones.

Future of DNA Fingerprinting and Gene-Editing

After DNA sequencing became quick, easy, and accessible, scientists began to look for ways to change this code in a precise way. In 2012, American biochemist - Jennifer Doudna, French scientist - Emmanuelle Charpentier, discovered CRISPR-Cas9 (Clustered, Regularly-Interspaced Short Palindromic Repeats), a technology that allows scientists to cut and edit genomic sequences with high accuracy. CRISPR itself was first found in bacteria, which used this mechanism to splice and edit their own genetic sequences to remove viral infections. By combining CRISPR with the protein Cas9, Doudna and Charpentier were able to create a technology with endless possibilities, most notably the ability to genetically modify crops, livestock, and even human beings. Major ethical considerations come into play with editing human DNA. These modifications can be passed down from generation to generation, possibly subjecting the entire human population to the creation of "designer babies," or the concern that people would begin to choose their children's eye color, height, athletic ability, and more.

With gene-editing technology, creating genetically-modified organisms (GMOs) becomes even easier. GMOs are typically found in produce, common medicines, and animal products. Since the 1970s, organisms have been modified for various purposes. The labeling of GMO products, the safety of consumption, and the ethical considerations of consuming GMOs have been notoriously controversial. Many nations restrict the consumption of certain genetically-modified organisms, and many people believe that altering what is natural is something that shouldn't't be done. On the other hand, proponents of GMOs argue that creating better, stronger crops and medicines that can help the world's people is more important than these concerns.

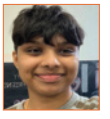
DNA has influential applications in the field of medicine too. For example, DNA is used to design vaccines. Since viruses can't reproduce on their own, they enter human cells and use human genetic materials in the cells to replicate themselves. Viruses replicate so quickly and as a result, they evolve extremely quickly, making them more virulent or weaker. Therefore, new vaccines are constantly being made to keep up with the changing genetic sequences of the invading virus. DNA vaccines use engineered DNA to induce an immunologic response in the host against bacteria, parasites, viruses, and potentially cancer.

Many drugs are not effective in patients or patients experience severe side effects due to some medicines and these might be related to their DNA. We are moving towards more personalized medicines. We are moving away from the conventional "one drug and one dose fits all" approach. We will be focusing on our DNA in the future to develop personalized medicines.

In the future, we can expect to see newer and more complex gene-editing tools, capable of engineering solutions to century-old problems. For example, scientists have already begun to develop cures to seizure disorders caused by mutations in the HCN1 gene and other long-term treatments for genetic disorders such as gene therapy. While nobody truly knows what the future may hold, we can say with certainty that DNA's importance and the presence of gene-editing is here to stay.

Author Biographies

Saanvi Vavilala *graduated from the Academy for Biotechnology at Mountain Lakes High School, and will be majoring in cell and molecular biology at the University of Texas at Austin while pursuing a career in biological laboratory research.*



Savaas Iqbal *graduated from the Academy for Biotechnology at Mountain Lakes High School, and will be pursuing STEM at Cornell University's College of Engineering.*

EDITOR'S TOP TECH

Bioreactors To Support Bioprocess Development

Expanded BioXplorer range of bioreactors features two new automated parallel bioreactors: BioXplorer 400XL and BioXplorer 400P. The new systems will further enable the efficient and cost-effective development and optimization of bioprocesses. The BioXplorer 400P is designed for use at pressures up to 10 bar, allowing for increased cell density and product yields. Optimized for syngas fermentation, the BioXplorer 400P combines easy to use software with modular hardware to give complete control of four high-pressure bioprocesses simultaneously. The BioXplorer 400 XL features 8 configurable bioreactors, which can be controlled independently or in parallel. Its modular design allows accurate and precise additions of liquids and gases, controlled by up to four sets of peristaltic pumps and up to two sets of mass flow controllers respectively, with software enabling control of all eight bioprocesses at once. Both bioreactors are available for use with either magnetic or direct agitation, to support enhanced cell densities and product yield.

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Understanding and Resolving Drug Shortages in the Pharmaceutical Industry

David Edwards
Chief Revenue Officer (CRO)
MasterControl

People undergoing treatment for attention deficit/hyperactivity disorder (ADHD), certain cancers, various infectious diseases, and pain management may find themselves on a medication waiting list due to a worldwide drug shortage. Various drugs such as Adderall, often prescribed for those with ADHD, as well as cisplatin and carboplatin, used in chemotherapy for several different cancers, are all in short supply. Because there are no alternatives for many of the drugs on the shortage list, patients need to either skip treatments or take lower dosages of the medications. In some cases, there are alternative treatments, but most are either not as effective or can lead to adverse outcomes.[1,2]

The U.S. Food and Drug Administration (FDA) has a mandate to protect public health by ensuring the safety, efficacy, and security of human and veterinary drugs and biological products by enforcing current regulations through product surveillance programs and by performing regular compliance inspections. This poses challenges for the regulatory watchdog because the agency also bares the responsibility of ensuring that there are enough medications and therapies to go around. Despite the efforts of the FDA and other global regulatory agencies to ensure the ample supply of safe and effective medications, there are still alarming numbers of public health concerns stemming from drug shortages.

Dissecting the Drug Shortage Issue

Pharmaceutical product development is one of the most innovative sectors. Companies in this industry continue to push more breakthrough products and advanced therapeutics into mainstream health care. But these medical advancements don't offer much value until they get manufactured into the products that improve patients' lives. The shortfall in drugs has been an ongoing concern for decades. That said, mitigating the shortages and the resulting impact of not having a sufficient supply of the medications necessary for patient care is a high priority for the FDA. In the 2020 report "Drug Shortages: Root Causes and Potential Solutions," the FDA cited that drug shortages continue to pose a real challenge to public health, particularly when the shortage involves critical drugs that have no alternatives.[3]

When it comes to drug shortages, fingers tend to point in various directions in an attempt to identify root cause. For example, COVID-19 was menacing in a lot of ways, making it an easy target of blame for many societal infirmities. Most of the limitations of medical supplies can largely be attributed to the pandemic. During the global lockdown, factories shut down in order to prevent the spread of the virus. Also, regulatory agency employees were tethered to their home offices due to travel restrictions and concerns for the health and safety of the inspectors. This made it more difficult for regulators to keep up with inspection and approval timelines.

Another culprit could be the profitability, or rather lack of, in producing generic medications. With generic products, manufacturers can only compete on price. Due to the high cost of manufacturing generics, companies have little incentive to produce the less-profitable products, so they either discontinue production or opt out of launching the product lines altogether. Manufacturers of generic drugs face intense price competition, uncertain revenue streams, and high investment requirements, all of which limit potential returns.[4]

As with Adderall, there has been a substantial increase in demand for many pharmaceutical products. Some cancer drugs are prescribed to over 500,000 new patients per year. Manufacturing drugs requires raw materials from suppliers that are often located in a variety of countries, which can create another supply chain bottleneck. Multiple companies that produce the same products may all get their raw materials from the same supplier. When supply shortages or supply chain obstacles occur for any reason, it impacts all manufacturers of the product.

The Root Cause of Drug Shortages

While there may be a number of factors involved in the deficit in drug inventories, according to the FDA, the main contributors to the drug shortages are quality and manufacturing issues. For a 2020 report on drug shortages published by the FDA, the organization assembled a team of economists that examined a sample of 163 drugs in the agency's database that first went into shortage between calendar years 2013 and 2017. The results revealed that of the 163 drugs in shortage, 62% went into shortage after supply disruptions occurred due to manufacturing or product quality problems.[5]

Pharmaceutical manufacturing firms inherently focus their efforts on compliance with current good manufacturing practices (cGMPs), which include standards for material systems, equipment and facilities, production, laboratory, packaging and labeling, and a quality system. These standards are foundational and set only a minimum threshold that companies must achieve to be compliant with regulatory guidelines. While this approach is usually sufficient for approvals, it does not incorporate the more advanced levels of quality or manufacturing management needed for public health products.

In a typical manufacturing environment, employees are still creating and expediting reports and documents on paper. This poses a number of issues and delays. Depending on the purpose of the documents they may need to be treated to be used in a laboratory or clean room. Paper documents also need to be scanned to create electronic versions for

audit purposes. During document review cycles, quality personnel or other stakeholders often discover problems, deviations, or data entry errors that should have been identified much earlier or should not even exist, resulting in the product having to be reworked or scrapped. The issues that go unnoticed usually get flagged by inspectors.

In June 2022, the FDA issued a Form 483 containing six observations to a pharmaceutical company. The observations cited that the company did not evaluate the operating ranges for critical process parameters, such as temperature and packaging process/filling configuration. A non-assessment during a validation led to the distribution of products manufactured with non-validated equipment configuration and operational parameters. The list of infractions also pointed out that "procedures applicable to the quality control unit are not in writing and fully followed. Some standard operating procedures (SOPs) relating to the use and cleaning of storage vessels and sampling and testing were not followed and/or no documentary evidence was kept."[6]

In July 2022, an inspection of an antibiotics manufacturer revealed lapses in the company's drug ingredients production processes. The resulting Form 483 called out the facility's quality unit for failing to ensure that the company's active pharmaceutical ingredients (APIs) were up to par. The reprimand also cited that the company's "quality unit dropped the ball when it came to document control and electronic records plus management of its computer systems."[7]

Most of the bottlenecks, production errors, missing or incomplete documents, and other mishaps along the supply chain stem from using paper in production processes. To get products developed and delivered, companies implement systems and processes designed to catch errors and prevent defective products from going out the door. They have teams that perform extensive testing of materials at all stages of production. Then there are teams that review records multiple times to catch and resolve errors. A typical batch record used in a biotech company's manufacturing operation can contain up to 45,000 manual entries — each entry is an opportunity for an error. The amount of oversight companies put into document reviews is necessary for compliance and to ensure the quality, safety, and efficacy of all products. But a significant amount of time goes into the duplicated efforts. Data from a 2021 McKinsey report states that in pharmaceutical manufacturing approximately 30% of the staff's time is spent on documentation-related activities.[8]

Human errors are a costly burden on companies as they often result in a product rejection or recall. Even in cases where the error is considered minor, it still requires an investigation and follow-up testing to confirm the identification and resolution of the root cause. Pharmaceutical products are complex, often involving multiple sites, so additional testing is often not feasible. And as medicines become more costly, the value of the material wasted increases. The financial impact of production errors is measured in the costs of materials and other manufacturing resources, lost sales, and monetary penalties, which ultimately lead to product shortages.

Taking Steps to Resolve Drug Shortages

It's important to have an understanding of the various contributors and causes of drug shortages. Still, the situation will persist until the pharmaceutical industry is on board with a resolution. To mitigate drug shortages, there is a need for more advanced strategies and technologies in manufacturing management.

Speed, quality, and patient safety are high priorities in an industry where there is little to no margin for error. Using manual, paper-based processes, companies forfeit the necessary agility and computing power needed to effectively oversee critical aspects of manufacturing, such as collecting data, creating reports, and delivering manufacturing instructions to the shop floor for timely product releases.

The real benefits of a digital transformation are manifest in how it supports workers. A digitized manufacturing operation connects people, processes, and systems. This fosters faster and more confident decisions and provides companies with more leverage in operations, quality, and regulatory compliance. A digital transformation can be completed in a progressive process called the Digital Maturity Model (Figure 1 illustrated below), where each phase advances the company toward a fully optimized manufacturing operation.

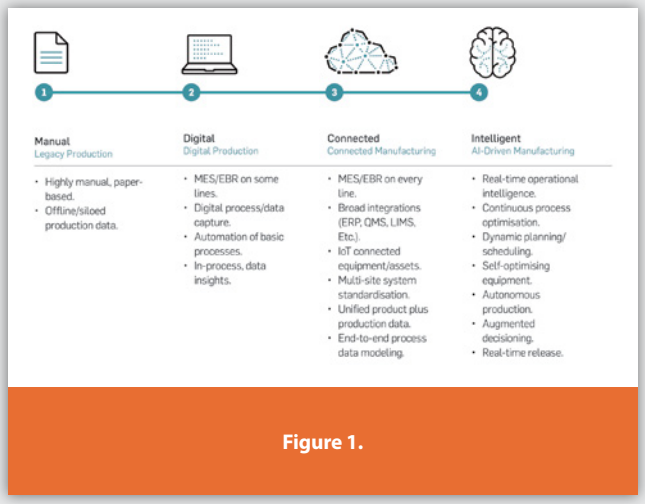

Figure 1.

The basic principle is that connecting machines and systems creates intelligent networks along the value chain. For instance, machines would be able to predict failures and trigger maintenance processes autonomously. Companies improve efficiency, enhance quality, and ensure compliance with regulatory requirements.

The overarching impetus of digitizing manufacturing operations is to get paper out of production in order to mitigate errors, achieve compliance, and move products out the door faster. But many companies in pharma manufacturing are lagging behind in modernizing their operations. Recent research on the implementation

of technology in life sciences product manufacturing revealed that 57% of companies have a digital manufacturing solution in place; however, only 9% have it functioning on all lines and in all sites. In the same survey, respondents said their manufacturing operations struggle with process inefficiency (64%), compliance issues (64%), preventable human errors (63%), excessive rework or scrap (59%), and delayed review and release (57%). Digital transformation has become a strategic imperative. Companies not pursuing the transition risk facing increased operating costs, falling behind competitors, and losing their market position.[9]

How Digitization Is Improving Drug Availability

Efficient processes are a prerequisite for any company to continue achieving its growth and scalability goals. When data is spread across disconnected systems, it's next to impossible to analyze and operationalize it in context with other cross-functional data. Digitizing operations bridges these gaps by fully integrating the organization's quality management system (QMS), enterprise resource planning (ERP), manufacturing execution system (MES), etc. Stakeholders can automatically transfer data to other systems and functional areas and make real-time line adjustments to minimize misallocation of resources and streamline production processes. By integrating the disparate enterprise applications in your manufacturing IT ecosystem with a fully digital production record system, manufacturers glean truly actionable, data-driven performance insights to achieve significant productivity improvements.

Figure 2.

It's essential to seek out ways to improve processes and reduce lead times. Digitized batch record processes and modern cloud-based batch record systems are transforming the pharma landscape and offering pharma manufacturers new opportunities to dramatically improve operations. A digital transformation typically boosts productivity by between 50% to 100%. Average-performing labs could achieve an even larger productivity improvement of 150% to 200% percent of their current rates. In some cases, digitization and automation have resulted in a more than 65% reduction in overall deviations and over 90% faster closure times. They can also prevent major compliance issues, which can in themselves be worth millions in cost savings.[10]

Today more than ever pharmaceutical manufacturers are striving to expand their contributions to the value chain. The sheer size of the pharmaceutical industry makes it important to stay up to date with pharmaceutical industry trends. Manufacturing companies are feeling the pressure to determine how advanced technology fits into their business models and the right ways to leverage its possibilities. It's critical to evolve faster in order to stay current with the new trends driving the industry. The implementation of advanced technologies and best practices that improve efficiency, speed, and adaptability gives enterprises a competitive edge and ensures their long-term relevance in the industry.

References

1. "The Adderall Shortage: Why It's Still Happening and What to Do if You Can't Get Your Meds," Fran Kritz, Everyday Health, May 4, 2023.
2. "A National Drug Shortage Creates Care Challenges in Oncology," Killian Meara, Drug Topics (Voice of the Pharmacist), May 25, 2023.
3. "Drug Shortages: Root Causes and Potential Solutions," A Report by the Drug Shortages Task Force, U.S. Food and Drug Administration (FDA), 2020.
4. "Factors Involved in U.S. Drug Shortages," Elsen C. Jacob, U.S. Pharmacist, June 18, 2020.
5. Supra Note 3.
6. "Exclusive: U.S. FDA Flags Quality Lapses At Glenmark Pharma's Baddi Facility," Monal Sanghvi, BQ Prime, July 26, 2022.
7. "Antibiotics Pro Centrient Runs Afoul of FDA at Indian Manufacturing Plant," Fraiser Kansteiner, Fierce Pharma, Dec. 14, 2022.
8. "Operations Can Launch the Next Blockbuster in Pharma," Ulf Schrader, McKinsey & Company, Feb. 16, 2021.
9. "The State of Digital Maturity in Pharma and Medtech Manufacturing," MasterControl, 2023.
10. "Digitization, Automation, and Online Testing: Embracing Smart Quality Control," Norman Carra, Evgeniya Makarova, et al., McKinsey & Company, April 14, 2021.

Author Biography

Dave Edwards *brings more than two decades of manufacturing, general business management, sales, and customer service experience to MasterControl, a global provider of software solutions that enable life sciences and other regulated companies to deliver life-changing products to more people sooner. Edwards most recently served as the Chief Operating Officer at 3form, a leading manufacturer of translucent building materials in the architecture and design industry. Prior to 3form, he worked at Danaher Corp., a Fortune 500 manufacturing conglomerate, and at TenFold Corp., an enterprise software company. Edwards holds an MBA from Harvard Business School and a bachelor's degree in economics from the University of Utah.*

Overview

SciX2023 conference marks its 50th year providing cutting edge presentations in the field of analytical chemistry and allied science. Organized by FACSS family of international organizations, the Golden Anniversary will bring to the forefront exciting sessions covering everything from A to Z in analytical chemistry and spectroscopy. Multiple Award Sessions and Plenary talks covering a range of topics from AI to Multimodal Spectroscopy to Biophotonics are sure to grab everyone's attention. Special focus on Early Career Researchers and Women in Analytical Science, accompanied with the many networking opportunities will sure make this a valuable and unforgettable event.

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- **Vibrational Spectroscopy to Support Pharmaceutical Manufacturing**
  This session is chaired by Patrick Wray of Bristol-Myers Squibb and Sergei Kazarian of The Imperial College London
- **Media integrity in BioPharma**
  This session is chaired by Alan Ryder of Nanoscale Biophotonics Laboratory
- **Transmission Raman for Pharma Applications**
  This session is chaired by Julia Griffen of Agilent Technologies
- **Nanomedicine Applications**
  This session is chaired by Zahra Rattray of The University of Strathclyde
- **Measurement of proteins and modifications towards Precision Medicine**
  This session is chaired by John Marshall of The Toronto Metropolitan University and John Wasylyk of Bristol Myers Squibb
- **Emerging plasmonic nanoparticles for drugs and pharmaceutical analysis**
  This session is chaired by Malama Chisanga of The University of Montreal
- **Vibrational Spectroscopy in Devoloping Biologics & Cell and Gene Therapy**
  This session is chaired by Kevin Dahl of Particlese-Consultant LLC
- **Small moleule analysis in Biopharma**
  This session is chaired by Karl Burgess of The University of Edinburgh and Roy Goodacre of The University of Liverpool
- **Analysis of Proteins, Antibodies, Biologicals and Nucleic Acids**
  This session is chaired by Jaimie Dufresne of YYZ Pharmatech and John Wasylyk of Bristol Myers Squibb

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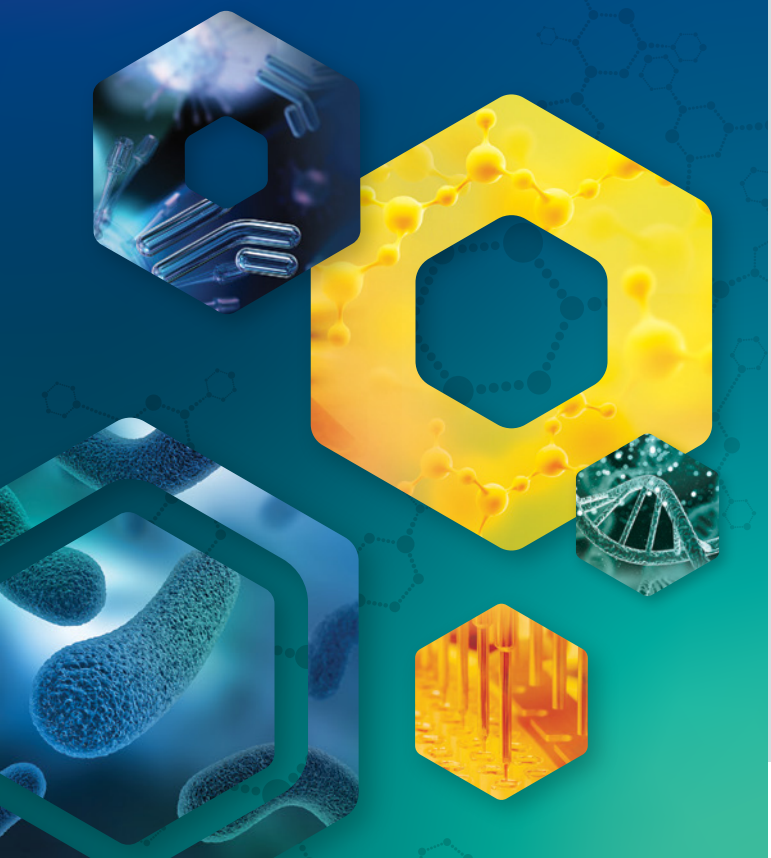

THE FOLLOWING IS A SAMPLING OF WHAT IS TO COME AT PharmSci 360.

SUNDAY, OCTOBER 22

PharmSci 360 opens on Sunday at 5 pm with a **plenary talk** from Dr. Thomas Hartung, M.D., Ph.D. He is currently the Doerenkamp-Zbinden Chair for Evidence-based Toxicology in the Department of Environmental Health and Engineering of the Johns Hopkins Bloomberg School of Public Health and Whiting School of Engineering, Baltimore.

Celebrate the start of an exciting program at our **Welcome Reception** at the nearby Hyatt Regency Orlando's Plaza Ballroom at 6:30 pm.

MONDAY, OCTOBER 23

Start the day by attending one of our **symposia**. "Accelerating the Drug Development Process through Formulation and Delivery Strategies" examines the development and production of drug products while "Advanced Manufacturing and Emerging Technologies in Pharmaceuticals and Biopharmaceuticals" features presentations on the latest manufacturing technologies.

The morning continues with a **Speaker Spotlight presentation** on process analytical technology (PAT) solutions in continuous manufacturing with insights from Thomas De Beer, Ph.D.

Afternoon keynotes include a presentation that compares and contrasts computational and experimental methods for particle design and a presentation that looks at bioanalytical strategies.

Make new connections at upcoming firms during **happy hour in the Start-Up Pavilion**.

TUESDAY, OCTOBER 24

Begin the second full day of PharmSci 360 with a **choice of symposia**. "Advanced Modelling and Predictive Approaches in Drug Development, Manufacturing and Analysis" includes presentations exploring AI and machine learning, continuous manufacturing, deep learning models, and digital twins. "Enabling Excipients and Approaches for Advancing Formulation and Delivery" examines new approaches to formulation.

The symposia "Harnessing the Power of AI/ML in Drug Development" promises to be a "can't miss" session with a presentation diving into the current regulatory environment.

Afternoon keynotes address twenty-first century excipient taxonomy, long-acting injectable formulations, and use of process modeling and digital twins for monoclonal antibody bioprocessing.

Don't miss the **Speaker Spotlight** from USP's Catherine Sheehan! She provides information on USP excipient standards.

End the day by attending a **Hot Topic panel** on ICH M10.

WEDNESDAY, OCTOBER 25

On the final day of PharmSci 360, participate once again in a **selection of symposia**. "Novel Modalities and Cross-Modality Considerations in Manufacturing and Analysis" explores regulatory requirements for extractables and leachables testing in combination products, statistical methods, and submicron particles. "The Bioanalytical Lab of the Future" examines metabolomics, biomarkers, assays for cell and gene therapies, and proteomics.

Fernando Muzzio, Ph.D., offers insights into the future of advanced manufacturing in a **Speaker Spotlight**.

Afternoon Keynotes address hot advancements in science. U.S. FDA's Jason Rodriguez, Ph.D., highlights high-resolution analytical tools for enhancing regulatory science. Another Keynote looks at microfluidic technology as a drug delivery technology.

After a busy three-and-a-half days, **Julie DeMartino, Ph.D., closes out PharmSci 360**. She is the founder and CEO of AcheneRx, a pharma consulting firm focusing on drug discovery and development of novel treatments for debilitating and progressive diseases.

Before you leave, join us for the **Closing Reception** from 5:30 pm to 6:30 pm and say farewell to your colleagues and your new industry connections.

In addition to these sessions, each full day includes career development resources, Rapid Fire presentations and opportunities to visit poster presentations.

FOR MORE INFORMATION and to access the full program, visit www.aaps.org/pharmsci360.

Pharmaceutical P.I.N. POINTS
Patent Innovation News

The purpose of this column is to highlight and summarize recent key patents in the pharmaceutical arena issued by the US Patent Office in May 2023.

Neelam Sharma, MS,
Lakshmi Lavanya Kundurthy, BE and
Hemant N. Joshi, PhD, MBA*

Tara Innovations, LLC
*www.tara-marketing.com, *hemantjoshi@tarainnovations.com*

Solid Dispersion Forms of Rifaximin; P. Golden and M.A. Kabir; Salix Pharmaceuticals, Inc., USA: U.S. Patent # 11,660,292; May 30, 2023.

The incidence of liver disease is on the rise and will continue to be a major health burden. Cirrhosis is a major cause of much of the chronic liver disease (CLD). The management of cirrhosis is based on disease severity. The use of rifaximin in preventing complications of cirrhosis is supported by multiple lines of clinical and experimental evidence. It has now been discovered that certain pharmaceutical compositions comprising solid dispersions of rifaximin effectively reduce the hospitalization time and prevent mortality associated with complications of liver disease. The present disclosure provides pharmaceutical compositions comprising solid dispersions of rifaximin as well as methods for their manufacture, and therapeutic uses associated with complications of liver disease. Present patent discloses solid dispersions comprising rifaximin and hydroxypropyl methylcellulose acetate succinate (HPMC-AS) wherein the HPMC-AS is present in an amount of from about 10 wt % to about 60 wt %.

Composition Comprising Lactic Acid Bacteria Improved in Intestinal Adherence by Coating with Silk Fibroin; B. Heo, Y. Kim, W. Kim, M. Seo, B. Kim, and I. Choi; CKD Bio Corp, Seoul, Korea; U.S. Patent # 11,642,318; May 9, 2023.

Lactic acid bacteria are a group of bacteria that produce lactic acid by using sugars as energy sources. Lactic acid bacteria are found in humans and are widely distributed in natural systems. Lactic acid bacteria are microorganisms that produce no harmful substances in human or animal intestines and have a beneficial function of preventing decay in the intestines. The present invention is to provide a composition containing lactic acid bacteria coated with silk fibroin and cellulose. Also, it provides a method for enhancing viability, storage stability, resistance against acid or bile, and intestinal epithelial cell adhesion of lactic acid bacteria. Conventional techniques construct only a physical protective barrier outside a lactic acid bacteria body by multi-stage coating and thus, retains the limitation of being unable to identify an effect on the coherence of lactic acid bacteria to intestinal epithelial cells upon the uptake of the lactic acid bacteria. In contrast, the present invention provides a method in which lactic acid bacteria is coated with silk fibroin extracted from cocoons, whereby the lactic acid bacteria are improved in stability under a storage and distribution condition as well as having remarkably increased stability and intestinal adherence particularly under an intestinal environment.

Assays and Reagents for Antimicrobial Susceptibility Testing; B. Spears and K. Flentie; Selux Diagnostics, Inc., USA; U.S. Patent # 11,649,477; May 16, 2023.

Antimicrobial resistance (AMR) has emerged as a major threat to public health globally. Phenotypic antimicrobial susceptibility testing (AST) of microorganisms is critical for informing physicians of appropriate therapeutic regimens. Using current methods, AST determination is time consuming. There is an urgent need for a more rapid and reliable test to improve infection diagnosis and support evidence-based antimicrobial prescribing. Present patent describes systems and methods for AST in which variances in anionic charge of microbes are taken into account. Cationic surfactants may be used to sensitize otherwise resistant microorganisms to polycationic antibiotics, such as polymyxins. Since microorganisms gain polycationic antibiotic resistance through mutations that decrease surface anionic charge, the susceptibility of a microorganism to a polycationic antibiotic may be indicative of its surface charge. In order to enable electrostatic interactions with the microorganism surface, a cationic surfactant may be applied to increase the anionic charge of the microorganism.

Pharmaceutical Compositions Containing Anti-Beta Amyloid Antibodies;

S.A. Lantz, K. Gupta, S. Sule, and A. Zunic; Biogen MA Inc., USA; U.S. Patent # 11,655,289; May 23, 2023.

Aβ is a peptide generated from the metabolism of amyloid precursor protein (APP). These have a tendency to aggregate into dimers and oligomers. Patients with higher amounts of anti-Aβ antibodies are likely to show reduced cognitive impairment. The patent describes a pharmaceutical composition comprising an anti-beta amyloid antibody, arginine, methionine, histidine, and a thiol-containing antioxidant. Antibody formulations degrade due to mechanical stress during manufacture. Polysorbate 80 seemed to protect the antibody from agitation-induced stress. Formulations containing arginine and methionine were found to be more effective.

Nanocrystals, Compositions and Methods That Aid Particle Transport in Mucus;

A. Popov, E.M. Enlow, J. Bourassa, C.R. Gardner, H. Chen, L.M. Ensign, S.K Lai, T. Yu, J. Hanes and M.Yang; The Johns Hopkins University, USA; U.S. Patent # 11,642,317; May 9, 2023.

Mucus layers in various places in the body serves to protect tissues against pathogens, allergens and debris. Mucus tends to clear foreign substances by its turn-over. Polymer-based mucus penetrating particles (MPP) can encapsulate various therapeutic agents and enable their delivery. The current patent describes specially coated nanoparticles. The surface altering agents comprised a triblock copolymer comprising a hydrophilic block-hydrophobic block-hydrophilic block configuration. The method allows very high drug loading. The patent described various kinds of dosage forms delivering medicines to different parts of body.

Blend Compositions for Oral Administration as a Rapidly Dissolving Powder and/or Suspension;

A. Favara and M. Karetny; Marenda Pharmaceuticals, LLC, USA; U.S. Patent # 11,642,312; May 9, 2023.

Patient acceptance of medications, often referred to as patient adherence, has been reported to average 50% in developing countries around the world. For example, oral tablet formulations can sometimes cause nausea and vomiting in patients. Further, unpleasant taste may be a challenge in administering a medicine particularly for children. Formulations which can be administered both in dry powder form and in suspension by adding a minimum quantity of water to dissolve the dry powder are not available on the market. Powder, solution, and suspension dosage forms typically utilize a different set of excipients to achieve acceptable physical properties. Also, taste masking of active pharmaceutical ingredients (API) is typically dependent on type, concentration, and processing method of excipients. Accordingly, the inventors herein have succeeded in devising formulations that can be administered either as a dry powder or in a small quantity of water. The new formulations provide improved taste and mouthfeel to facilitate ease of administration and patient adherence. Disclosed is a dry powder oral formulation, a chewable, swallowable, and/or orally disintegrating tablet that includes an API, a lecithin powder, a galactomannan, one or more sweetening agents, one or more flavoring agents and an organic acid in a pharmaceutically acceptable preparation. Also disclosed is an excipient composition in the absence of an API and methods of making and using the formulations and compositions.

Darunavir Combination Formulations;

U.A.C. Delaet, P.E.H. Heyns, E.M.J. Jans, R.J.M. Mertens, and G.V.D. Avoort; Janssen Sciences Ireland Ltd., IE and Gilead Sciences, USA; U.S. Patent # 11,654,150; May 23, 2023.

Darunavir is an antiretroviral medication used to treat HIV/AIDS. Tablets of Darunavir with a dose of 600 mg have a total weight of over one gram and they are hard to swallow. Direct compression leads to inferior formulations due to limited gliding and flowing capacity. The present invention describes granulation of darunavir, which allows high drug loading in the tablet. Hypromellose in water is sprayed on darunavir powder and the granules are dried. The granules are further mixed with MCC, silicone dioxide and a disintegrant before making tablets. The formulation also contains GS-9350, an inhibitor of cytochrome P450 3A isoform, a pharmacokinetic enhancer.

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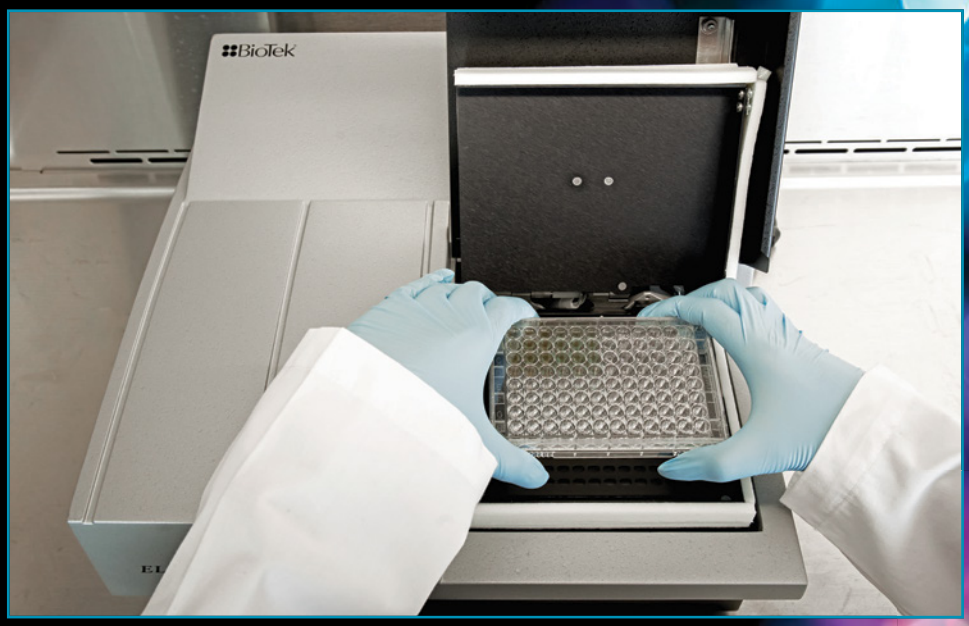

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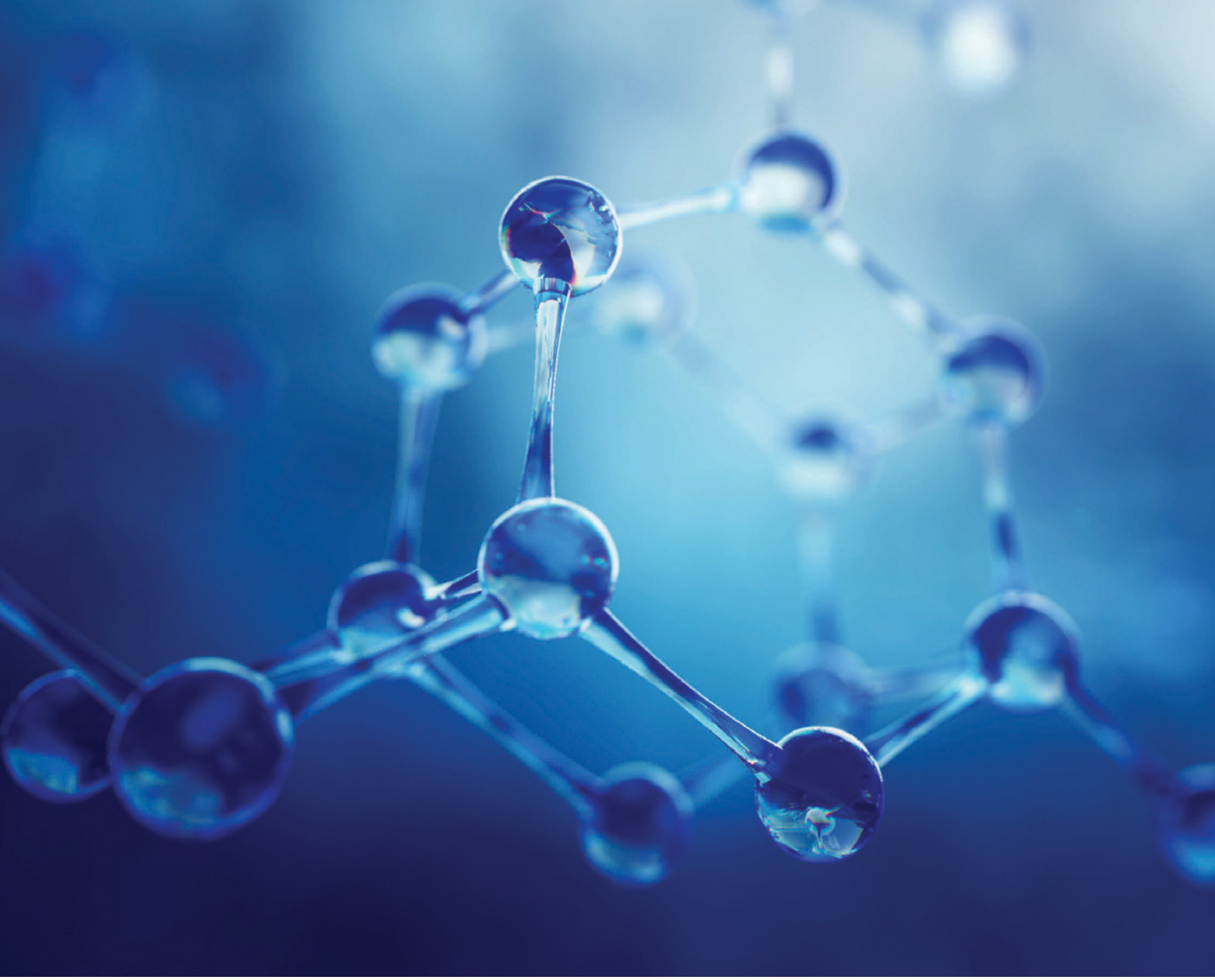

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