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A Watchful Eye on Pharma

As vaccine programmes throughout the world are kicking into a higher gear and more of the population are receiving vaccinations, a watchful gaze over side effects, efficacy results, and bio/pharma companies in general has taken hold of the world’s media. AstraZeneca’s woes seem to be compounded, with Germany becoming the latest country to limit the use of the vaccine due to concerns over a rare blood-clotting disorder (1), despite regulatory authorities’ assurances that the benefits of the vaccine significantly outweigh any risks (2).

Yet, it is also important to remember that bio/pharma companies are not solely focused on COVID-19, even though a significant concerted effort has been made by the industry overall in this area over the past year. There is, in fact, a vast selection of drugs in development pipelines on the cusp of approval, some with the potential to become game-changers for certain disease areas that affect millions of people globally.

Exciting prospects ahead

According to Clarivate’s Drugs to Watch 2021 list, there are four new treatments in particular that have been deemed as likely to gain blockbuster status in the near future (3). All four in Clarivate’s list are predicted to deliver more than US $1 billion (€852 million) in sales within five years (3).

The four drugs highlighted by Clarivate are aducanumab from Biogen and Eisai, bimekizumab from UCB, relugolix from Takeda, and vericiguat from Bayer and Merck. These drugs are targeting Alzheimer’s disease, psoriasis, prostate cancer and female reproductive health issues (endometriosis and uterine fibroids), and chronic heart failure with reduced ejection fraction, respectively.

Several other prospective treatments in development were also highlighted within the report, which, despite not fitting within the inclusion criteria for Clarivate’s study, are considered to have ‘notable potential’ (3). These promising therapies included chimeric antigen receptor T-cell therapies idecabtagene vicleucel (Bristol Myers Squibb) and cilta-cabtagene autoleucel (Janssen), anti-inflammatory bardoxolone methyl (Reata), nerve growth factor inhibitor tanezumab (Pfizer and Lilly), monoclonal antibodies tralokinumab (LEO Pharma) and nemolizumab (Galderma), and experimental pneumonia vaccine PF-06482077 (Pfizer).

“In the last five years we have seen an increase in approvals of innovative medicines at a rate more than double what it was a decade ago,” said Mike Ward, global head of Thought Leadership, Life Sciences and Healthcare, Clarivate, in a press release (4). “A number of drivers have aligned to achieve this improvement, including greater insight into the biological roots of diseases, oncology research benefiting from the routine introduction of biomarkers to better target therapies, the emergence of gene and cell therapies, and efforts by regulatory agencies to introduce processes to accelerate medical assessment.”

The COVID-19 effect

Undoubtedly, COVID-19 has changed the bio/pharma landscape for the future. The general public have become more invested in details, such as clinical trials and supply chains, and have increased expectations for innovative solutions and therapeutic access, changes that have a likelihood of being permanent in the future (5).

Speed of development has been unprecedented during the COVID-19 pandemic, a reversion of which will be hard to accept for industry and patients. This change of development model, if it is to continue into the future, will require agreement and partnership with industry, regulators, and patients, as well as other stakeholders (3,5).

Key tools to aid with development timeline acceleration, which have found prominence during the pandemic, will be in the form of digital technologies. Whether it be increased digitalization of development and manufacturing, or the use of digital solutions to help with quality inspections and clinical trials, the industry’s pace in the direction of the digital transformation has quickened and will likely continue (5).

Finally, increased collaboration throughout the industry has proven to be invaluable during the COVID-19 pandemic. Cross-industry and cross-enterprise collaborations are anticipated to become the new normal, meaning that companies will need to adapt to this changing landscape (6).

References

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Long before the COVID-19 pandemic put a spotlight on supply-chain security and drug shortages, the pharmaceutical industry was working on advanced manufacturing technologies that could address supply challenges by increasing manufacturing flexibility and efficiency. Continuous manufacturing, for example, allows drug manufacturers to more easily adapt supply to demand, incorporate more automation, and have tighter control of quality. Continuous processes with smaller footprints provide mobility, creating the potential for distributed manufacturing—even bringing drug production to the point of care. The call for reshoring manufacturing may be answered in part by using these advanced technologies to produce APIs or to continuously manufacture drug products end-to-end, beginning with basic raw materials and ending with a finished drug form.

The United States government has been concerned for several years about drug shortages and the ability to manufacture essential medicines domestically. In October 2019, Janet Woodcock, director of the Center for Drug Evaluation and Research (CDER) at the US Food and Drug Administration, reported to Congress that only 28% of API facilities were located within the US, but suggested that advanced manufacturing technologies could enable competitive US-based production that would strengthen the security and reliability of the US drug supply (1). Woodcock noted then that CDER was working with the US Biomedical Advanced Research and Development Authority (BARDA) to develop a regulatory framework and the technology for mobile manufacturing of essential drugs at or near the point of care.

At the request of FDA, a committee assembled by the US National Academies of Sciences, Engineering, and Medicine (NASEM) researched emerging technologies that have the potential to modernize pharmaceutical manufacturing in the next five to 10 years. Continuous and intensified processes, as well as mobile and distributed manufacturing, were among the innovations that could improve quality and supply security highlighted by the committee in a report published in February 2021 (2). FDA’s Emerging Technology Team, the committee noted, has been successful in facilitating process development, but regulatory review continues to be perceived by industry as a barrier to modernization.

Although “reshoring” could be more complex in Europe than in the US, the pandemic illustrated the need for supply chain security in Europe as well and perhaps created new urgency to reduce dependence on bulk active ingredients and generic drugs sourced from India and China (3). Citing the need to balance reliance on API sourced from other regions,
in February 2020 Sanofi created a standalone company out of the combination of Sanofi’s API commercial and development activities at six of its European production sites (4). Sanofi said the spin-off company, to launch in 2022 with the name EUROAPI and headquarters in France, would allow greater agility to improve supply security (5).

Making chemical APIs

Continuous flow chemistry is one of the intensified, agile technologies showing promise, with some good manufacturing practice (GMP) lines running commercially and other novel processes in development. GlaxoSmithKline, for example, points to continuous manufacturing as a more efficient and environmentally friendly continuous manufacturing practice (GMP) lines showing promise, with some good intensified, agile technologies.

Making chemical APIs

Continuity Pharma. “Smaller footprint processes can also be designed to have greater operational efficiency in terms of energy and waste. The vision is to create more responsive systems that can produce drugs in an as-needed manner.”

Continuity Pharma, a start-up out of Purdue University, is using flow chemistry to develop a system that can continuously manufacture multiple APIs. Researchers are initially focusing on APIs that have common reaction processes to ease rapid changeover. Using US$1.5 million (€1.3 million) from DARPA granted in September 2020, the researchers are building proof-of-concept equipment that can produce at least 100 g/day with a maximum six-hour turnaround time between products by the end of the first year of the project, with shorter turnaround and higher volume the aim of the project’s second year, says Thompson. The portable, refrigerator-sized units will use continuous synthesis and purification by extraction, with a final polishing step by batch crystallization.

SRI International in California has developed its SynFin platform, a fully automated, small-scale synthetic chemistry system, primarily for discovery, under an earlier DARPA programme, and has developed continuous flow multistep processes for several APIs at a scale of grams per day. In August 2020, SRI was awarded a US$4.3 million (€3.7 million) DARPA contract to create a process modelling scale-up tool that would speed translation of these laboratory-scale processes into commercial-scale API production. SRI is working with a team at Rutgers University on the ProSyn digital twin tool and on scaling up to a modular platform that could be configured to produce a range of small-molecule APIs commercially in a “just-in-time” capacity model.

“To compete with the cost-effectiveness of offshore API manufacturing, innovative streamlined and automated systems are needed,” notes Nathan Collins, chief strategy officer of SRI’s Biosciences Division and the principal investigator for the ProSyn project. “Speed of development and manufacturing is also critical. As was highlighted by the pandemic, we need process development translated into manufacturing within months instead of years.”

The researchers plan to have proof-of-concept modules by the end of 2021 that can demonstrate the manufacturing process. Collins notes that they are concurrently building process validation tools to enable GMP production.

Snapdragon Chemistry, a 2014 spinoff from the Massachusetts Institute of Technology (MIT) based in Waltham, Mass., has developed flow-based API manufacturing processes. In mid-2020 the process development company doubled its R&D capacity and opened a lab to produce gram to kilogram-scale APIs and demonstrate its continuous flow manufacturing technology at pilot scale. The company began construction in January 2021 of a GMP drug substance manufacturing facility, which it plans to commission in November 2021. The facility would be set up for commercial production of APIs using processes designed by Snapdragon.

In a collaboration with BARDA that began in June 2020, the company developed a synthetic continuous manufacturing route for ribonucleotide triphosphates, which are a raw material for mRNA-based COVID-19 vaccines. Snapdragon is initiating discussions with prospective users, and the materials could be produced in the new commercial facility, says Matt Bio, CEO of Snapdragon.
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In a separate project launched in February 2021, Snapdragon is using a US$1.5 million (€1.3 million) DARPA grant to extend its technology to enable efficient US-based production of chemicals used in pharmaceutical production. “We’ve identified a set of chemical building blocks that can be produced on the same process-equipment setup using only programming changes, and we are developing a continuous manufacturing platform with next-generation automation to accomplish this goal,” explains Bio. The platform is currently in the R&D stage, and Bio anticipates that it could be commercialized within two years.

At Virginia Commonwealth University (VCU), the Medicines for All Institute (M4ALL) has developed continuous flow processing for APIs. The institute, founded in 2017 with funding from the Bill and Melinda Gates Foundation, has focused on expanding access to medications in developing countries by developing cost-saving production methods. These methods use high-yield reactions that don’t require isolating intermediates, explains Frank Gupton, CEO of M4ALL.

In May 2020, M4ALL partnered with Phlow Corporation to implement these continuous processes for US-based manufacturing of essential medicines, under a US$354 million (€301 million) contract Phlow received from BARDA. Continuous API production processes are currently being developed in the M4ALL labs, and the processes will then be scaled up to commercial manufacturing by Phlow, says Gupton. The aim is to have a full-scale manufacturing facility running by the end of 2022.

Continuous path for OSD drugs

While FDA has approved one API made using continuous flow chemistry, continuous manufacturing—starting with API and excipients and ending with finished drug—is now being used to produce six FDA-approved oral solid-dosage (OSD) drugs, including some previously made in batch manufacturing as well as new drugs approved with continuous OSD processes. Early adopters have worked through many challenges and are continuing to optimize these systems, which offer flexibility of scale and use process analytical technology and advanced process control to improve efficiency and quality.

Similar to API manufacturing, OSD continuous manufacturing lends itself to miniaturized processes; small-scale equipment can be run for a longer time period if more volume is needed. Pfizer’s PCMM [Portable, Continuous, Miniature, and Modular] system, for example, was originally developed in 2013 in a collaboration using GEA’s OSD continuous processing equipment and G-CON’s prefabricated cleanrooms, and is being used for development as well as commercial production. The small-footprint systems are designed as skid-mounted modules, which adds flexibility. The portability of PCMM could allow rapid deployment or redeployment, which could lend itself to new ways of manufacturing and distributing drugs (9). These successes in continuous OSD manufacturing have demonstrated that a regulatory pathway is possible, which is encouraging to other companies working on continuous processes.

End-to-end manufacturing

Fully end-to-end systems seek to encompass both API and final dosage form manufacturing in one integrated system. Technology developed at the Novartis–MIT Center for Continuous Manufacturing was demonstrated as a proof-of-concept end-to-end system in 2012. The technology is being leveraged by Novartis, which opened a facility in Basel, Switzerland in 2017, and US start-up company, Continuus Pharmaceuticals. Continuus designed and constructed a pilot plant using its Integrated Continuous Manufacturing (ICM) technology at its facility in Woburn, Mass., and in January 2021, the company was awarded a US$69.3 million (€58.9 million) contract from the US Department of Defense (DoD) to build a commercial ICM facility for US production of three critical APIs and their finished dosage forms. The facility is expected to be operational within two years.

Bayan Takizawa, co-founder and chief business officer of Continuus, says that the company plans to file applications to produce and market these drugs as its own products. Although there are not, as yet, any FDA-approved drugs made with a fully end-to-end line, Takizawa notes that the FDA’s Emerging Technology Team (ETT) is well informed of their advanced manufacturing platform through previous contract work the company performed for the Agency. Takizawa says that ICM provides several advantages. “The process is fully automated, which eliminates human errors that often translate into quality defects and increased costs,” he explains. “We eliminate the starts and stops that normally characterize pharmaceutical manufacturing, and the entire process is located in a single facility, where manufacturing operators are all in constant communication. This is very different from the currently fragmented model, where parts of the manufacturing process are performed by different companies that are not always aligned, often resulting in rework. Also important, we leverage novel technologies that enable integration and this seamless production.”

The commercial facility will have a capacity that is several times greater than that of the pilot line, with two end-to-end lines that will have the capacity to produce multiple APIs and drug products, including both sterile injectables and OSD drugs using a “multi-suite” design. With the extra capacity beyond future US government contract requirements, the company plans to produce and market its own drugs, as well as provide contract manufacturing services.

While the commercial facility will be a conventional, fixed-location facility, Continuus envisions that the ICM platform could also be used in a Mobile Pharmaceuticals (MoP) plant, which
would be housed in a prefabricated pod that could easily be transported and deployed across the globe to provide regional manufacturing and distribution.

**Small-scale end-to-end systems**

Innovators are prototyping other mobile manufacturing facilities using continuous, end-to-end manufacturing at small scales of finished drug forms for both small- and large-molecule drugs. Funding for several of these projects is coming from DARPA. Moderna, for example, received a DARPA grant in October 2020 as part of DARPA’s Nucleic Acids on Demand World-Wide (NOW) initiative to develop a mobile, end-to-end automated, GMP-quality manufacturing platform for mRNA vaccines and therapeutics for military and local populations in remote regions (10).

At the Center for Advanced Sensor Technology at the University of Maryland, Baltimore County (UMBC), professor and director Govind Rao and his team have developed the Biological Medicines on-Demand (Bio-MOD) platform, which uses a cell-free process to translate and continuously purify proteins. Bio-MOD is a “factory on a chip” that fits in a suitcase-sized container. Rao says the device has been demonstrated to reproducibly manufacture several drugs, including His-tagged granulocyte-colony stimulating factor, and that others are in progress. The system can also make nucleic acids. The group is looking for a partner to be a first adopter and take a target molecule through FDA approval. “The vision is to broadly license the technology so that it becomes a standardized production platform,” says Rao.

On Demand Pharmaceuticals (ODP) is developing proprietary Pharmacy on Demand (PoD) technology licensed from MIT to build a miniaturized, end-to-end medicine production system. The Rockville, MD-based company was founded with the mission of producing battlefield medicines, but the company envisions its portable, refrigerator-sized PoD technology being used for any localized manufacturing. ODP received a US$20 million (€17 million) contract award from DARPA in September 2020 to further develop its technology to produce critical APIs and final dosage forms. ODP has demonstrated that the technology can produce solid-dosage forms (diazepam, diphenhydramine hydrochloride, and ciprofloxacin hydrochloride tablets) and liquid formulations (lidocaine hydrochloride, atropine sulfate, as well as medicines used to treat critically ill COVID-19 patients requiring ventilation support). The technology can also be used to produce APIs or critical precursors as needed.

Although the units are small, they can produce significant volumes. “For some high-potency drugs, in which one dose is less than 10 mg, such as midazolam, one PoD running for 24 hours could produce as much as 2 million doses,” says Karl Stoever, chief external relations officer at ODP. The company expects, however, that its devices would make finished dosage forms of multiple drugs in a distributed manufacturing model. Stoever says that PoDs have demonstrated rapid turnaround time of approximately two hours for changeover from synthesis of one API to another.

The company moved into a 44,000-ft² facility in 2020 and is completing renovations in preparations for CGMP production. Stoever says that ODP has been working closely with FDA’s Emerging Technology Team and anticipates filing its first submission to the FDA in the next year, with additional product submissions to follow shortly thereafter.

In addition to domestic production capacity and working to ensure the protection of military service members, ODP sees its technology as useful for orphan drug and precision medicine markets. “Perhaps the most compelling use case for the PoD technology resides in addressing unmet needs in the world’s poorest communities,” adds Stoever. A flexible system for local manufacturing could meet a community’s need for a broad range of essential medicines.

**Challenges for novel processes**

Although regulators have expressed support for novel technologies, some regulatory barriers do still exist. For example, current GMP regulations depend on a conventional definition of a facility with a physical address; modular and mobile manufacturing does not fit this definition. Real-time release from portable systems with innovative process controls could also be a regulatory barrier. The NASEM committee, however, concluded that mobile, end-to-end systems “are becoming mature and robust enough to push the regulatory envelope within five to 10 years,” and that FDA would need to take a proactive approach and ease the regulatory burden if such systems were to be used (2).

“In a conservative industry, getting people to change the way they do things is a big challenge. But now there are drivers to innovate, so the question is how fast change will occur,” concludes Collins.

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Balancing the Art and Science of Topical Drug Formulation

Topical drugs are attractive to patients, but these complex products pose challenges for formulators.

The pharmaceutical market is dominated by oral solid and intravenous dosage forms. For some therapeutic compounds, however, conventional delivery is not possible due to drug degradation in the GI tract, drug instability, the inability to get the drug to the target site at the required concentration (bioavailability), or systemic side effects. For these drug substances, topical delivery may provide an attractive alternative to overcome these challenges in a rapidly growing marketplace.

With topical formulations, it may be possible to deliver the active ingredient to a specific site and avoid first-pass metabolism, changing drug concentrations in the bloodstream and patient-specific differences. In addition, topical products can be applied by the patient and often lead to increased medication adherence. “There can be substantial benefits to delivering a drug topically, even if the intended domain of action is systemic,” notes John M. Newsam, CEO of Tioga Research. “Topical formulations continue to be of substantial appeal to populations of patients affected by many different conditions. Where compliance and patient preference have been assessed, a topical or transdermal mode of administration is often preferred over all others.”

In addition to more traditional, passive, or molecular means of modulating the skin barrier using topical delivery formats such as emulsions, ointments, creams, sticks, gels, foams, sprays, and patches, active or physical methods compromising the skin barrier to enable delivery are being researched, including microneedles, iontophoresis, and electrophoresis, among others.

Complex formulations and many challenges

A topical or transdermal drug formulation must satisfy simultaneously, several different criteria, according to Newsam. The formulation must provide an appropriate level of delivery and/or permeation (in some cases, deposition), no irritation or sensitization of the skin, physical and chemical stability and compatibility with the container-closure system, suitable esthetics characteristics, and regulatory compliance.

“The ultimate objective of any formulator is to produce effective and patient-friendly formulations, and with topical formulations this can be a complex journey,” says Charles Evans, vice-president of pharmaceutical development at MedPharm. One of the major challenges that general formulators face, he adds, is the limited understanding of the complexity of topical formulations, particularly from an industry used to working on more conventional dosage forms such as oral solids and injectables.

Key challenges for topical formulation relate to the properties of the APIs in development today and include stability, low drug solubility, and permeability, observes Benjamin Goodyear, global technical marketing manager for BASF’s Pharma Solutions group. These challenges must be overcome while creating formulations with the right sensory characteristics and that are not irritating to the skin.

Many different types of excipients are required to achieve the desired performance properties of topical formulations, which has led to additional challenges. Presently, there is no separate approval pathway for novel excipients, so formulators generally are only willing to use excipients that are already used in approved pharmaceutical products. Most of these excipients were introduced many decades ago and often do not have the properties needed to satisfy the complex criteria required of topical drug products today, which has created a real need for novel materials. “Currently, discussions regarding possible routes of introduction for novel excipients that are separate from inclusion in a new drug application submission have been gaining some interest, but as of yet there is no clear method outlined by the regulatory authorities,” Goodyear says.

Cynthia A. Challener, PhD, is a contributing editor to Pharmaceutical Technology Europe.
The lack of novel excipients is a particular issue for the formulation of topical products based on biologics. Investment in biologics is increasing, and in 2019 they accounted for 35% of all dermatological investments, with topical-focused biologics contributing approximately 5–10% of this total, according to Evans. “While they offer targeted treatments for a multitude of conditions, their physicochemical properties mean that biologics are inherently difficult to deliver across the skin,” he explains.

For proteins, peptides, and nucleic acids, Newsam notes that in addition to the huge delivery challenge, maintaining the stability of the active(s) in the formulation, avoiding degradation in contact with skin, and realizing analytical methods with sufficient sensitivity are all potential issues. Even with complex small molecules, susceptibility to isomerization, hydrolysis, or reaction with classes of excipients can be a primary concern, he adds.

Formulators at small- to mid-sized pharmaceutical organizations, meanwhile, may be more inclined to use non-pharmaceutical grade materials during prototyping and scale up to cut development costs, not realizing that these materials do not always have the same performance as pharmaceutical-grade excipients, Goodyear observes.

In addition, introducing a safe and efficacious finished product into the global marketplace presents an overabundance of difficulties due to region-specific requirements mainly driven by the lack of harmonized testing protocols for complex semi-solid dosage forms, according to Goodyear.

There are further challenges for the development of topical generic formulations. To be approved as a generic drug, a topical formulation requires equivalency to a reference listed drug (RLD) product, which can be realized by preparing a material that is termed Q1/Q2/Q3 equivalent. In addition to having the same API at the same strength, the formulation has the same excipients (Q1) in the same relative concentrations (Q2), and the same microstructure (Q3). Developing such a formulation, Newsam comments, requires the development team to first complete a formulation deconstruction operation using a variety of analytical tools.

Bioequivalence can, however, be achieved with a formulation comprising a different slate of excipients. “This approach opens the interesting possibility of gaining patent protection for a generic topical product,” Newsam observes.

**Considerations for topical formulation development**

Formulating effective topical formulations attractive to patients requires consideration and prioritization of numerous variables. To address the challenges of solubility, permeability, and stability for topical formulations requires consideration of critical quality attributes. “A blend of solvents/ co-solvents may be used to improve solubility, chemical penetration enhancers can be employed to improve permeability, and anti-oxidants, preservatives, pH buffers, etc., can be used to stabilize the API, according to Vijendra Nalamothu, chairman and CEO of Tergus Pharma.

Topical formulations are typically disease-specific, so often certain other properties must be achieved or avoided. For instance, Nalamothu notes that for an acne formulation, oily excipients and comedogenic materials (those with a propensity to clog pores) should be avoided. Similarly, a psoriasis or eczema formulation should not contain drying or stinging agents such as alcohols and glycols.

More recently, Newsam notes that topical drug formulators have also begun to take into account the constitution of the microbiome that populates various human internal and external body surfaces. As an example, he points to the connection seen between the character of the skin microbiome and the appearance of skin lesions in atopic eczema patients. He also observes that selective topical antimicrobials are sought that do not cause harm to beneficial microbes. Pagoda Genomics (San Diego, Calif., USA), for instance, has developed the topical protein formulation Avlo, which exhibits selective activity against norovirus, the bane of many travelers, especially those on cruise ships.

All of these aspects must be considered in light of relevant consumer acceptance criteria such as smell, colour, and texture/aesthetic appeal, Nalamothu adds. “The excipients selected to ensure that a formulation treats the disease and appeals to the consumer cannot interfere with the stability and permeability of the API,” he states.

Developing improved formulations with better application, break, and/or finish but that contain the same therapeutic effect is often the goal for generic topical products, according to Goodyear. “Patients will be more likely to repurchase certain products that provide a better sensorial experience, especially when it comes to more aesthetically pleasing formulations,” he says.

Often *in-vitro* or *ex-vivo* models comprising fresh tissue such as eyes, skin, or cultured human epithelium have been shown to de-risk the development of the drug product for the patient, according to Evans. In combination with additional evidence, *in-vitro* permeation testing may be used to demonstrate bioequivalence of generic topical drug products by comparing the rate and the extent to which an active ingredient gets to the relevant site(s) of action, says Goodyear. “While clinical end-point studies can be utilized to evaluate bioequivalence, they are costly and the least sensitive method,” he adds.

In more recent US Food and Drug Administration-funded investigations, Goodyear notes that *in-vivo* cutaneous pharmacokinetics have been monitored in humans via dermal open-flow microperfusion.
and in preclinical studies with dermal microdialysis. "These methods have the potential to monitor the APIs accurately, sensitively, and reproducibly from topically applied drug products and ultimately can help reduce cost to the patient and increase the speed with which patients have these tests available," he observes.

Robust implementation of quality-by-design (QbD) and design-of-experiment (DoE) methodologies also serves as an effective strategy for de-risking formulation processing and development, according to Goodyear. Consideration of the scalability of the manufacturing process is crucial to success as well, Nalamothu asserts. "Formulators must consider the critical process parameters and develop robust critical quality attributes to make sure that the product developed in the lab has the same physicochemical properties as that of the product obtained from large-scale batches made in the manufacturing suite," he says.

**Best strategies leverage multi-faceted approaches**

With so many factors to consider when developing a topical formulation, the key to overcoming them is a multifactorial approach. "By integrating preformulation and formulation development closely with drug product design, key final product characteristics can be incorporated from a very early stage in the development," Evans asserts. Understanding elements such as route of delivery, site of action, potency, patient preferences, and even the target shelf life of a drug product ensures that development projects are cost and time-effective, he adds.

For Newsam, the preferred approach to topical drug development combines the use of various computational techniques to inform the formulation research programme, utilization of high throughput experimentation (HTE) tools in formulation innovation and DoE methods in formulation optimization. "Formulation innovation is more efficient if the focus is first on the property that is most taxing to match, which commonly is realizing the required level of skin delivery and permeation. As most molecules have low intrinsic skin permeability, we work to identify those particular combinations of excipients that are effective in permeabilizing the skin barrier, preferably in a manner that is more or less selective for the particular API," Newsam explains.

While software tools can help, there are no robust and broad means of predicting the effects of such excipient combinations; they must be measured. Because the space of excipient combinations is vast, HTE technologies have proven to be valuable in this endeavor.

Tioga Research’s approach begins with analyzing the physicochemical characteristics of the API followed by mining databases of formulation performance the company has accumulated over 19 years. Formulation composition spaces that have worked well for other molecules not dissimilar to the given API are identified as an initial platform for formulation fine-tuning. "This tailored formulation approach is quite suitable for new chemical entities where patentability is less critical," Newsam says.

Tioga has also developed a complementary approach referred to as Cascaded Screening methodology, which leverages the company’s HTE platforms. With this methodology, the skin permeation of an API from each of large numbers of different formulations is screened systematically, with the details informed by the molecular characteristics of the API. This approach, according to Newsam, has a remarkable success rate in yielding high-performing formulations that also prove patentable as novel compositions of matter.

When seeking to develop a "supergeneric"—or bioequivalent generic topical formulation with more attractive properties from a patient perspective—the formulator must first extract the quality target product profile (QTPP) from the patient and dermatologist viewpoints. "Defining the most important attributes about a topical treatment may shed some additional light on how product developers are able to enhance patient treatment outcomes in clinical settings," he explains. The bottom line, Goodyear stresses, is that most patients would rather use a soothing foam or cream formulation over a greasy ointment if given a choice.

**Repositioning opportunities tempered by challenges**

There is broad interest in drug repositioning or reformulation for a 505(b)(2) regulatory path, as it is considerably faster and less expensive than the 505(b)(1) for a new chemical entity. By reformulating a drug for the same or different indication, companies are offered a much quicker route to approval and these products do not require the same level of toxicological and clinical testing as new chemical entities. Safety and efficacy data from the literature or from studies by another organization can be used in support of the 505(b)(2) NDA. The risk of an unexpected safety issue arising is also substantially reduced, and this faster approach is approved and supported by regulatory agencies globally.

Reformulation as a topical product is often pursued if the existing molecule/dosage form is unable to meet patient needs due to less-than-optimized delivery kinetics and/or dosage strength, according to Nalamothu. Repurposing of an existing drug for a new indication is another reason for reformulating a drug substance as a topical product. Developing a topical formulation with an existing API can be challenging, though. Staying within the pharmacokinetic profile of the old dosage form and any efficacy/safety constraints is a primary
one, Nalamothu says. “In other words, the new dosage form has to meet the TPP of the unmet (clinical/commercial) need while providing or exceeding the efficacy and safety of the originator drug,” he explains. Tergus uses various tools such as in-vitro skin permeatio, and other in-vitro skin biology tests to conduct proof-of-concept studies and a QbD approach when developing these new formulations.

Another challenge relates to the patentability of these new topical formulations. There is, according to Newsam, therefore a huge value in establishing a robust basis for formulation composition of matter patentability. It is difficult, though, to realize this type of patentability value using conventional development techniques, he says. Tioga Research has found that using its proprietary HTE technologies and Cascaded Screening procedure typically yields formulation innovation and support for patentability.

“Historically, formulation composition-of-matter patents were not considered valuable because they could potentially be circumventable by minor changes in composition,” Newsam observes. “We have shown through the development of products such as PENNSAID 2% (US Patent 9,066,913), which was approved via a 505(b)(2) route, that it is possible to establish robust patent protection using this fresh approach to formulation innovation,” he asserts.

**Balance of art and science**

Formulators of topical drugs are faced with a number of technology, regulatory, and drug-compatibility limitations. Overcoming them requires access to a dynamic “ingredient tool box” and the knowledge and know-how to use these tools effectively, according to Goodyear. “Developing topical semi-solid formulations intended to elicit a pharmacological effect is a delicate balance between art and pharmaceutical science,” he states.

Both entry-level and experienced staff may gain additional knowledge by getting involved with other experts working in the topical pharmaceutical industry through participation in workshops and professional communities such as the American Association of Pharmaceutical Scientists Topical and Transdermal Community, Goodyear notes.

Considering product availability and patient access to be part of the treatment selection process during early development of new topical formulations may also yield more positive clinical outcomes and change the way we see skin treatments and clinical acceptance, Goodyear adds. He also notes that establishing best practices in-house that are aligned to FDA requirements will help reduce time to market while further enabling the development of formulations that patients will truly appreciate.

Newsam concludes by observing that the skin is an amazing barrier. “The outermost layer of the skin (the stratum corneum), which provides most of this barrier function, is some 15 microns thin. It is in a sense an extremely fragile medium for keeping potentially harmful agents outside the body and our molecular constituents maintained within.” He also comments that there is still much to learn about the skin and how it functions. “As we gain a greater understanding of this crucial organ, it is exciting to think about the broader spectrum of formulations that might be suitable for topical administration,” Newsam considers.
A

lthough approximations vary considerably in the literature (1–3), it is widely accepted that the costs associated with bringing a drug to market are substantial. Drug development can be made more cost-efficient by accelerating the development timeline, so that the speed-to-market is shortened, an aspect that can be aided with accelerated formulation strategies. “An accelerated formulation strategy offers many benefits,” says Andreas Seidl, chief operating officer, Leukocare. “Formulation development often can be taken off the critical path, allowing for better process development and project management. Furthermore, the overall project timelines from development until approval and commercial manufacturing can be accelerated.”

For Alexander Faude, director Process Science, Downstream Processing, Rentschler Biopharma, being able to take formulation development off the critical path provides the biggest benefit of accelerated formulation strategies. “Moreover, efficient processes that implement all the components discussed earlier will save time. This in turn will translate to a faster time-to-clinic which ultimately leads to a faster time-to-market,” he adds.

Although in agreement that the time efficiencies are the greatest potential benefit, Jeffrey Zonderman, chief commercial officer, RedShiftBio, also specifies that faster formulation studies provide opportunities for developers to gain a more complete understanding of the drug. “With the right technology in place, formulators can screen more drug candidates, assess a wider range of excipients and buffers, and rigorously apply quality-by-design (QbD) principles to develop a more robust product,” he notes. “So, acceleration can lead directly to an improved drug product with better stability and a higher chance of successful commercialization. Investing in information gathering at this stage can pay dividends in terms of a lower risk of product failure and a narrower field of higher quality candidates for further development.”

Seidl concurs that a successful accelerated formulation strategy can reduce the risks related to stability and adds that by closely interacting with other areas of the manufacturing process, such as downstream processing (DSP), it is possible to gain synergies across the whole process (e.g., integration of the formulation development results into DSP development). “Hence, risk reduction and yield increase are some advantages that result from this integration,” he says.

Key priorities for accelerated strategies

Formulation is clearly becoming more of a bottleneck in the biopharmaceutical drug development pipeline, explains Zonderman. “Strategies for accelerated formulation are consequently a growing focus for the industry and include the adoption of a QbD, knowledge-led approach, the greater use of computational modelling, and the identification of an optimal suite of orthogonal analytics,” he says. “The challenge is to enable more predictive formulation by combining the most appropriate analytical techniques with state-of-the-art modelling, to bring molecules through formulation more rapidly while at the same time driving down the risk of poorly formulated drugs proceeding further down the pipeline.”

A broad range of assays can be used to characterize therapeutic proteins, Zonderman continues. “Against a backdrop of accelerating formulation, identifying a suite of biophysical tools that work effectively together to generate the information required is therefore a vital and shared goal for many biopharmaceutical companies,” he emphasizes.

Some techniques that can provide valuable formulation insight, such
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as nuclear magnetic resonance and X-ray crystallography, are not necessarily compatible with accelerated strategies due to the amount of manual input required, particularly for data processing, Zonderman confirms. “Here, automation is a defining priority because it typically delivers rapid measurement, reduced manual input, and high throughput—all of which are crucial,” he says. “And it is important to note that the requirement for automation extends right through from sample preparation to data processing and handling.”

Using in-silico tools to narrow down the suitable excipients can greatly improve prediction of formulation strategies, asserts Marvin Kadisch, director Process Science, Upstream Processing, Rentschler Biopharma. “The application of in-silico tools is gaining increasing importance and will continue to do so in the near future,” he states. “This application will facilitate the efficient examination of excipient libraries (prior to design of experiment [DoE]) and overall prediction.”

Of high priority when approaching accelerated formulation strategies is the early availability of materials, notes Faude. “In addition to that, the process implemented for product development should be kept in mind, as this can greatly influence the final product, which in turn affects formulation development strategies,” he says.

Zonderman agrees that material availability and amount of material required for robust analysis early on are practical concerns, but he underlines that not everything is about practicality and speed. “The relevance and value of the information generated is critical,” he highlights. “Companies are looking to invest in complementary, orthogonal assays that slot together to generate understanding of the factors that influence formulation performance, notably drug activity and stability. The aim is to establish correlations that facilitate an efficient QbD approach.”

“Structural characterization is a vital aspect of this drive for fundamental knowledge, and an important analytical focus with secondary structure offers increased insight into the development of a robust understanding of protein behaviour, conformational stability, and aggregation pathways,” Zonderman asserts.

Identifying key degradation pathways and weaknesses of the molecule early on is certainly recommended, confirms Seidl. “With this information in hand, one can tailor formulation candidates with excipients that best address these weaknesses,” he adds.

Classically, several formulation candidates are tested at increased temperature in a short period of time to check for the impact of formulation on stability behaviour (aggregation, de-amidation, etc.) of the molecule. Components such as mechanical or freeze/thaw parameters can be included.”

Sequential testing (i.e., by applying clever DoE matrices) help to swiftly identify best performing excipients and combinations thereof, Seidl explains. “Using a confirmatory stability study as a last step, with the possibility to still be able to fine-tune formulations, further helps to reduce the risks and surprises at a later point in time,” he says. “With a proper data-set combining the accelerated aging and the confirmatory stability study, prediction quality of long-term storage is enabled and allows decisions on final formulation candidates already after only one month’s data of the formulations being tested in a confirmatory stability study.”

Implementation challenges
A lack of available material is challenging, according to Faude, who highlights the use of a staggered approach to accelerate the formulation process when early material is not available.

“One should start formulation development early with available material and continue sequentially with material received at later time points (representative of clinical material),” he says.

Kadisch underscores the fact that a lack of material affects analytical method development. “With limited availability of material, one often has to begin with generic methods and refine these along the way as more information becomes available,” he notes.

“Close collaboration with clients on accelerated formulation development is both a key factor and a potential challenge,” adds Seidl. “In general, the client knows the molecule best, and the information exchange with the client can be crucial when tailoring formulations for the specific molecule, for example, when based on previous findings of the client on aspects such as stability.”

Representative measurement at each stage of development is a major challenge raised by companies seeking to employ accelerated formulation strategies, Zonderman continues. “In early formulation this means measuring a parameter that is relevant to formulation behaviour with minimal amounts of sample, while in later formulation the problem may be the ‘dirtiness’ of the sample, including protein concentration and complexity,” he says. “Add to this requirement the need for precision/high sensitivity and you have a significant ask for instrumentation that is optimally suited to an accelerated formulation environment.”
When measuring structure, specifically protein higher-order structure—secondary, tertiary, and quaternary—drug activity can be defined, continues Zonderman. "Structural measurements also provide a sensitive indication of instability and insight into aggregation mechanisms," he says. "Measuring structure is therefore extremely useful when it comes to building a robust, fundamental understanding of the drug product for accelerated formulation."

However, this type of structural measurement demands technology that is capable of quantifying structure with sufficient sensitivity to generate useful information for a wide range of sample types, Zonderman asserts. "Unfortunately, techniques for secondary structure, including circular dichroism and conventional Fourier Transform infrared, have limitations when it comes to automated measurement in the presence of excipients, at high concentrations or, conversely at low concentrations," he notes.

Additionally, sample preparation for measurement purposes can compromise data, Zonderman highlights. If the sample is diluted, then the protein is no longer in the native environment of interest, the structure may be affected, and the resultant data jeopardized, he adds. "Switching between techniques, because, for example, one can be used at low concentration while the other is better for more clinically representative formulations, raises the issue that any differences observed may be related to artefacts of the techniques, rather than the drug," Zonderman states.

Tips and tricks
"Alignment of project goals at the start is vital for pragmatic risk assessment and to align on how much risk one is willing to undertake in accelerated strategies," emphasizes Faude. "A good strategy with defined goals and target achievement measures goes a long way in implementing a successful strategy. In addition, QbD plays a significant role, and an efficient analytical control strategy that is needed for approval is also equally important."

Consultation between an outsourcing partner and sponsor company is key when establishing project goals and a successful strategy, continues Faude. "It is essential to approach the project as a partnership, as a collaboration where the client and outsourcing partner are equally invested in project success," he explains.

Early alignment between the client and the formulation developer to determine the target product profile greatly facilitates the design of formulations to be applied, agrees Seidl. "Also, one should aim to know the molecule, as platform buffer systems may risk unwanted surprises at later stages of development," he says.

Therefore, an outsourcing partner’s experience in formulation development settings, strategies, and execution is vital for predefined targets by the client to be met, Seidl stresses. "Clear communication and consultation of the formulation development expert prior to project start enables smooth

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Identifying out-of-trend (OOT) and out-of-specification (OOS) results is a critical part of stability data evaluation. An OOT observation is a measurement of a lot under investigation that deviates from or is atypical of the expected trend of representative historical lots, while an OOS result falls outside of specification. A confirmed OOT may not yet be an OOS, but it is often a warning that underlying issues may be at play. In the past, researchers studying this topic used the terms “analytical alert” for OOT readings that result from an invalid analytical measurement and “process control alert” for an OOT degradation rate (slope) that resulted from some production-related event (1,2). A “compliance alert” occurs when an individual product lot’s degradation rate is steeper and projected to fall short of potency and/or impurity specification limits through the product’s shelf-life.

Instituting corrective and preventive action (CAPA) to minimize the impact of a confirmed OOT is a more proactive control strategy than simply waiting for OOS conditions to occur. For an analytical alert, re-testing can be done while unexpired sample and reagents are still available to replace those that were affected by the OOT result. This practice prevents an invalid test from becoming part of historical lot data, which will affect future trending and setting specification limits of new drugs based on stability data. For a process control alert that is also a compliance alert, the expiry of an investigated lot may be shortened based on statistical projection to avoid OOS if the lot is allowed on the market at its original shelf-life.

To identify the OOT early, trending should ideally be performed by quality control (QC) laboratory personnel who generate the measurements as close to real-time as possible. The OOT identification tools should be simple and practical so that they can be used by QC staffers who lack in-depth statistical training or access to a statistician. Simpler methods are crucial because the workload required for trending can be substantial, with multiple attributes to trend across several products. Moreover, the analyses must be repeatedly performed as new measurements are generated and added to the stability database.

There is no regulatory guidance spelling out which statistical techniques to use for OOT identification. Previously published methods such as regression control chart (RegCC), by time tolerance interval (ByTimeTI), slope-by-lot Control Chart (SlopeCC), prediction interval (PI), and Z-score have been described in the literature.

However, there had been no systematic evaluation of the effectiveness of each of these methods. The author has used simulation to compare these methods under varying scenarios of sample size, relative lot-to-lot and within-lot variation, and extent of analytical or process control OOT. Results verified that PI, Z-score, and RegCC were related methods with some slight variation in how each one worked. ByTimeTI was the least effective in detecting either types of OOT. SlopeCC was effective in detecting process control OOT, but, overall, RegCC performed best at detecting both analytical and process control OOT.

Citation
research described three approaches to identify analytical OOT and process control OOT (3). The RegCC method is applicable for both types of OOT, while ByTimeTI and Slope CC methods are applicable for process control OOT. Research that studied the use of all three methods for an actual product advocated their simultaneous use in order to get a visual image of the results (4). In the end, however, it recommended the Z-score method. Other research evaluated those three methods as well as the Z-score method (5). One study used historical estimates of intermediate precision for the RegCC method (2), and accounted for statistical significance in the slope method and also included prediction interval (2). Yet another study used a mixed effects model in a unified approach that incorporated both inter- and intra-lot variation to improve results (6).

All of this research has provided QC trending specialists with more information on statistical methods, their benefits and limits. The research described in this article was launched to provide a systematic and comparative evaluation of the effectiveness of statistical methods. The methods were compared by simulating various sample sizes, different levels of variation within and between lots, and varying extents of analytical and process control OOT. This article will describe details of the simulation study and results of the methods comparison.

**Materials and methods**  
**Statistical model for trended population.** The population considered were product lots, the attributes of which were assumed to vary linearly over time, and can be described using a random intercept, common slope model (Equation 1; refer to Appendix for complete definition of symbols):

\[ Y_{ij} = \mu + A_i + \beta \times T_{ij} + E_{ij} \quad \text{(Eq. 1)} \]

where \( i = 1, \ldots, I \) lots, \( j=1, \ldots, J \) timepoints for lot \( i \),  
\( A_i \sim \mathcal{N}(0, \sigma_A^2) \), \( E_{ij} \sim \mathcal{N}(0, \sigma_E^2) \) and \( A_i \) and \( E_{ij} \) are independent  
so that \( Y_{ij} \) at time \( T_{ij} \sim \mathcal{N}(\mu + \beta T_{ij}, \sigma_A^2 + \sigma_E^2) \).

This assumption is justifiable, because most of the attributes that are evaluated in stability programmes can be approximated by zero or first-order kinetics, which lend themselves to linear regression analysis. The release values at time of manufacture (intercepts) were expected to vary from lot to lot around some population mean \( \mu \) within some acceptable range (i.e., release limits). The rate of degradation over time (i.e., slope \( \beta \)) was expected to be common or fixed among the lots for a well-characterized and sufficiently controlled manufacturing process.

**Historical lots simulation.** To be a valid reference point for the subject lot being investigated, the historical lots should represent the population in terms of both analytical and process variations. Historical lots were simulated by drawing from a population described by Equation 1. Population parameters of \( \mu = 100 \) and \( \beta = -0.15/\text{month} \) were chosen to mimic a stability-indicating attribute such as potency. The full stability testing schedule was set at 0, 3, 6, 9, 12, 18, 24, and 36 months, with a shelf-life (expiry) of 36 months. Simulation parameters (i.e., sample size, intra-class correlation coefficient [ICC], and extent of analytical and process control OOT) that were set for the study design are listed in Table I.

The sample size of available historical lots depends on where the product is in its life cycle. A product that is still under development or just recently approved may only have a small sample of stability lots available for trending, while a mature commercial product may already have a large sample. In this simulation study, 6 and 30 historical lots were selected as small and large sample sizes, respectively. The ages of historical lots at the onset of trending were unbalanced, due to staggered enrollment into stability study as lots were manufactured. For simplicity, half of the total number of lots were assumed to have results up to 18 months (i.e., half of a 36-month expiry) and half to have results up to nine months.

The ICC, which represents the correlation among measurements in the same lot is defined in Equation 2, wherein \( \sigma_A^2 = \text{ICC} \) and \( \sigma_E^2 = 1 - \text{ICC} \) were used without affecting generality.

\[ ICC = \frac{\sigma_A^2}{(\sigma_A^2 + \sigma_E^2)} \quad \text{(Eq. 2)} \]

The ICC levels used in the study were 0.2 and 0.8. A data set with ICC=0.2 indicated that the between-lot (process) variability was small relative to within-lot (analytical) variability and vice-versa for ICC=0.8.

**Subject lot simulation.** The subject lot was simulated in two ways. If an analytical OOT was being identified, the subject lot was first drawn from the same population as historical lots. The initially drawn, in-trend subject lot results were then induced to be analytically OOT by subtracting \( k_{OOT} \times E_{ij} \).

<table>
<thead>
<tr>
<th>Table I. Levels for simulation parameters. OOT is out of trend.</th>
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<tbody>
<tr>
<td>Simulation parameter</td>
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<tr>
<td>---------------------------------------</td>
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<tr>
<td>Historical lots sample size (^a)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Intragroup correlation coefficient</td>
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<td></td>
</tr>
<tr>
<td>Extent of analytical OOT for subject lot ( k_{OOT} = E_{ij} ) subtracted from in-trend result</td>
</tr>
<tr>
<td>Extent of process control OOT for Subject subject lot (slope is ( \beta_{OOT} % ) steeper than ( \beta = 0.15 ))</td>
</tr>
</tbody>
</table>

\(^a\) – Historical sample size notation is [\# of lots (maximum age of lots in Months)]. For example, the small sample noted as [3(18),3(9)] consists of three stability lots at 0,3,6,9,12,18 months and three stability lots at 0,3,6,9 months.
Note that if an attribute wherein $\beta > 0$ (e.g., impurities) was being simulated, $k_{OOT} * E_{ij}$ was added instead. Only the latest subject lot result was induced to be analytically OOT while all the previous subject lot data were in-trend.

When the next result was generated, the previous analytical OOT would have been re-tested and replaced with an in-trend result. If a process control OOT was being identified, the subject lot was simulated using a slope that is $\beta_{OOT} \%$ steeper than the true population slope $\beta = -0.15$. The three levels of $k_{OOT}$ (0, 2, 4) and $\beta_{OOT} \%$ (0, 25, 50) listed in Table I represent no, moderate, and large extent of analytical and process control OOT, respectively.

**An example of simulated data.** An example of a simulated data set [small sample, ICC=0.2, subject lot either with $k_{OOT}=4$ or $\beta_{OOT}=50\%$] displaying the results for the full stability testing schedule is shown in Figure 1. The available historical lots (A1 to B3) and the subject lot (C) initially drawn to be in-trend at the onset of trending are the filled circles while the ones that have yet to be generated through the remainder of the stability study are the blank circles. The induced analytical OOT at each time $j$ of subject lot are the blue X’s while the induced process control OOT are the red Y’s. At the onset of trending, the subject lot C had a result at 0 month (i.e., release data) while the A and B historical lots had results up to 18 and 9 months, respectively. The stability data set was dynamically added as the measurements per the full testing schedule were generated. For example, when the three months result was generated for the subject lot C, the corresponding $9+3=12$ months results for the B lots would have also been generated. When the six months result for subject lot C was generated, the $18+6=24$ months results for the A lots would have also been generated, and so forth. Each subject lot result was assessed for OOT as it was generated.

**Methods to compare.** The methods summarized above were systematically compared using simulation. The mixed effects version (6) was excluded from the comparison because of the additional complexity it entails in analyzing random effects and the fact that it requires the use of more advanced statistical software, which may preclude most trending QC personnel from applying this method. The key steps in applying the methods, formulas, and which data are used to construct the trending limits are shown in Table II. The formulas for trending limits are two-sided, but only the lower limits were applied as appropriate for the attribute degrading over time examined in this paper.

The RegCC method uses common slope estimate $\hat{\beta}$ and root mean square error (RMSE) from fitting a separate-in-
tercepts, common slope (SICS) analysis of covariance (ANCOVA) model to the historical data. The random effect model (Equation 1) simplifies to a SICS model when lot is treated as a fixed effect. The RegCC trending limit at each time $j$ is calculated by adding to the subject lot intercept $Y_{0j}$, the average change up to that timepoint ($\bar{\beta} \times T_{Cj}$) and subtracting the product of standard normal quantile and root mean square error, $\frac{\alpha}{2\times \text{RMSE}}$.

As previously described (3), the trending limit for ByTime method is the constructed tolerance interval (TI) using the historical lots at each time $j$. For the SlopeByLot method, the trending limit is the control chart (CC) limit calculated from the individual historical lot slope estimates. For completeness of comparison in this paper, both TI and CC versions were applied to ByTime and SlopeByLot.

Similar to RegCC, prediction interval (PI) and Z-score methods are also based on fitting a SICS ANCOVA model to applicable data. The difference is that, in RegCC, all the subject lot data were excluded from model fitting. In PI and Z-score, only the latest subject lot measurement being evaluated if OOT was excluded from model fitting. The PI trending limit at time $j$ is the 100P% lower two-sided predicted interval at that timepoint for the subject lot. The Z-score trending limit at time $j$, $Z$, is the difference between actual and predicted value (i.e., residual) of the subject lot at that timepoint divided by RMSE. RMSE is the square root of the sum of squared residuals divided by the degrees of freedom of the error term from the ANCOVA output. Note that the Z divisor of RMSE used in this paper differs from other research/analyses/work which, instead, used the standard deviation of the residuals of only the subject lot (4,5).

### Simulation performance metrics

**Probability of correctly identifying OOT.** A data set was simulated and the seven methods listed in Table II were applied to assess whether the result under evaluation (i.e., the latest available subject lot result) was OOT. For all methods except Z-score, the result was flagged as OOT if it exceeded (i.e., was lower than)

<table>
<thead>
<tr>
<th>Method</th>
<th>How it works</th>
<th>Formula for limits</th>
<th>Data used to construct limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression Control Chart (RegCC)</td>
<td>• Fit data with SICS ANCOVA model. Use common slope estimate and RMSE to calculate limit by / Month. Compare each $Y_j$ against / limit.</td>
<td>$Y_{0j} + \bar{\beta} \times T_{Cj} \pm \frac{Z_{1-p}}{2} \times \text{RMSE}$</td>
<td>All lots, all months. Only the release (Month 0) data, $Y_{0j}$</td>
</tr>
<tr>
<td>By Time Tolerance Interval (ByTimeTI)</td>
<td>• Calculate Mean and SD by Month. Calculate TI by / Month. Compare $Y_j$ against / tolerance limit.</td>
<td>$\bar{Y}<em>j \pm k \frac{1-n-1-p}{2-n-1} \times \bar{\hat{\sigma}}</em>{Y_j}$</td>
<td>All lots at each month None</td>
</tr>
<tr>
<td>By Time Control Chart (ByTimeCC)</td>
<td>• Calculate Mean and SD by Month. Control limit by / Month. Compare $Y_j$ against / control limit.</td>
<td>$\bar{Y}<em>j \pm \frac{Z</em>{1-p}}{2} \times \bar{\sigma} \bar{\bar{\beta}}$</td>
<td>All lots at each month None</td>
</tr>
<tr>
<td>Slope By Lot Tolerance Interval (SlopeTI)</td>
<td>• Fit simple linear regression by Lot. Calculate Mean and SD of Historical Lot slopes. Calculate TI of Historical Lot slopes. Compare Subject Lot slope $\beta_c$ against slope tolerance limit.</td>
<td>$\bar{\beta} \pm k \frac{1-n}{2-n-1} \times \bar{\hat{\sigma}} \bar{\bar{\beta}}$</td>
<td>All months by lot. None</td>
</tr>
<tr>
<td>Slope By Lot Control Chart (SlopeCC)</td>
<td>• Fit ANCOVA. Calculate P 2-sided prediction interval at / Subject Lot Month under evaluation. Compare $Y_j$ against / prediction limit.</td>
<td>$Y_{Cj} \pm \text{StdErr}<em>{Y</em>{Cj}} \times \frac{Z_{1-p}}{2}$</td>
<td>All lots, all months. All months except latest time under evaluation.</td>
</tr>
<tr>
<td>Prediction Interval (PI)</td>
<td>• Fit ANCOVA. Calculate P 2-sided prediction interval at / Subject Lot Month under evaluation. Compare $Y_j$ against / prediction limit.</td>
<td>$Y_{Cj} \pm \text{StdErr}<em>{Y</em>{Cj}} \times \frac{Z_{1-p}}{2}$</td>
<td>All lots, all months. All months except latest time under evaluation.</td>
</tr>
<tr>
<td>Z-score (Z)</td>
<td>• Fit ANCOVA. Calculate prediction mean at / Subject Lot Month under evaluation $\bar{Y}<em>j$. Calculate Z score at / Subject Lot Month under evaluation. Compare abs($Z$) against $\frac{Z</em>{1-p}}{2}$ standard normal quantile</td>
<td>$Z_j = \frac{Y_{Cj} - \bar{Y}_{Cj}}{\text{RMSE}}$</td>
<td>All lots, all months. All months except latest time under evaluation.</td>
</tr>
</tbody>
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*) StdErr$_{Y_{Cj}} = \sqrt{\text{RMSE}^2 \times (1 + Y_{Cj} (X)^{3/4})}$ where X = design matrix.

The significance level $\alpha$ was fixed at 0.05. The proportion levels $P$ of 0.90, 0.95, and 0.99 were used in simulation but only the $P=0.95$ results are presented here, due to space limitations.
than) the trending limit. For Z-score, the result was flagged as OOT if the absolute value of $Z_j$ was larger than that of the $Z_{0.25}$ standard normal quantile. This OOT flagging was iterated 1000 times. The proportion of the iterations (i.e., probability) flagging the result as analytical or process control OOT was used as the primary metric in the simulation study. A method that has a low false alarm rate (i.e., probability of flagging a result as OOT when it is truly in-trend; also known as Type I error) and high detection rate (i.e., probability of flagging a result as OOT when it is truly OOT; also known as power) is preferred.

**Average ratio of the lower specification limit and lower trending limit.** Trending limits to identify an OOT serve as an early alert before the underlying issue(s) caused an OOS event. The OOT trending limit should not be so restrictive that it incorrectly flags an in-trend result as OOT. Neither should the limit be so wide that it exceeds the specification and fails its purpose as an early alert before an OOS occurs.

A “hypothetical” lower specification limit (LSL) at each time $j$ was calculated using Equation 3 to bound what would be “acceptable” at that time point. In practice, shelf-life specification limit is a fixed value that drug product has to conform to throughout its shelf-life. The ratio of the hypothetical LSL and the lower OOT trending limit at time $j$, averaged over 1000 iterations, was used as a secondary metric for the simulation study. This secondary metric helps in interpreting the results of the primary metric. For example, a low detection rate may be due to extremely wide trending limits that even exceed the specification (i.e., LSL/OOT limit >1). This ratio metric is only applicable to RegCC, ByTimeTI, ByTimeCC, and PI methods, whose limits are measures of individual results from the population, which are compatible to the concept of specification limit.

$$L_SL_j = \mu + \beta \times T_j - Z_{0.9973} \times \sqrt{\sigma_A^2 + \sigma_E^2}$$

*Equation 3*

**Results**

The probability of identifying as OOT the latest subject lot result (Y axis) becoming available from 3 to 36 months (X axis) were graphically summarized. Figures 2 and 3 compare the seven methods when subject lot was induced, at increasing extent from the top to the bottom row, with analytical or process control OOT, respectively. The four combinations of the sample sizes (small, large) and ICC levels (0.2, 0.8) were used to stratify results.

**Detection rates for analytical OOT.** To identify analytical OOT, the baseline case when the subject lot was in-trend was first

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**Figure 2. Probability of OOT identification for increasing extent of analytical OOT ($k_{OOT}$). OOT stands for out of trend.**

![Figure 2](https://example.com/figure2.png)
evaluated (Figure 2, \( k_{OOT} = 0 \)). PI had false alarm rates (or Type I error) below 5%. Type I error rates for RegCC were slightly inflated averaging around 12%. Z-score started with about 25% false alarm rates but flattened down to about 14% when more data became available. SlopeTI and SlopeCC started with elevated false alarm rates of about 30% and 40%, but those rates decreased to below 5% and 10%, respectively, with more available data. With moderate analytical OOT (\( k_{OOT} = 2 \)), RegCC and Z-score had comparable detection rates of about 45%, with PI lagging just slightly below. By-TimeTI performed worst, with detection rate as low as <5% for a small sample and ICC=0.8. ByTimeCC performed better than ByTimeTI but not as well as RegCC and Z-score. SlopeTI and SlopeCC started with high detection rates of 65 to 75% but those rates decreased as more data became available. With large analytical OOT (\( k_{OOT} = 4 \)), detection rates increased to at least 65% for most of the methods, with similar relative performances among the methods as \( k_{OOT} = 2 \). The sample size and/or ICC stratification level had only minimal effect on detection rates for RegCC, SlopeCC, PI, and Z-score, but had a major effect on ByTimeTI and ByTimeCC. Sample size affected SlopeTI results, with worse detection rates found for small sample size.

Detection rates for process control OOT. For identifying process control OOT, the baseline case when the subject lot was in-trend, process-wise (Figure 3, \( \beta_{OOT} = 0 \)), was simulated to be equivalent to in-trend, analytical-wise (Figure 2, \( k_{OOT} = 0 \)). The false alarm rates of the methods for these two in-trend cases were therefore identical. With moderate process control OOT (Figure 3, \( \beta_{OOT} = 25\% \)), detection rates of SlopeTI and SlopeCC started at about 30–40% but deteriorated as more data became available, except when the sample size was large and/or ICC=0.8. All the other methods started with low detection rates but improved as more data became available.

RegCC and SlopeCC had the best detection rates up to about 80% for ICC=0.8 when all data were available. The methods performed better when ICC=0.8 than they did when ICC=0.2, with little impact from sample size. ByTimeTI and SlopeTI performed worse on small than on large sample sizes. With large process control OOT (\( \beta_{OOT} = 50\% \)), the detection rates were increased, reaching as high as 100% for the best performing scenarios observed with \( \beta_{OOT} = 25\% \). SlopeTI and SlopeCC now monotonically improved with more available data, except for SlopeTI with small sample size at ICC=0.2, which deteriorated first before improving slightly.

Ratio of LSL and OOT trending limits. The width of the OOT limit relative to the hypothetical LSL (Equation 3) was evaluated by taking the ratio of the latter to the former. For a decreasing attribute, the OOT limit should be narrower (i.e., higher)
than the LSL if it is to be useful as an alert before OOS occurs. An LSL-to-OOT limit ratio of less than one is therefore desired. The ratios for RegCC, ByTimeTI, ByTimeCC, and PI with increasing extent of analytical and process control OOT are shown in Figures 4 and 5, respectively. For analytical OOT (Figure 4), ratios remained about the same regardless of $k_{\text{OOT}}$ extent and availability of data. This was because hypothetical LSL and OOT limit commensurately adjusted at each time $j$. The only exception was PI, in which case the ratio decreased slightly within the first 12 months and then remained flat.

The ratios were higher (i.e., OOT limits were closer to LSL) when ICC=0.2 than when ICC=0.8 except for ByTimeCC, in which case the ICC level had no impact. Sample size had no impact except for ByTimeTI where ratios at small sample sizes were higher than large sample sizes, even exceeding one (i.e., the OOT limit was wider than specification limit).

For process control OOT (Figure 5), the relative method performances were similar to those in Figure 4, with only a slight change in PI. The ratios for PI increased slightly with the extent of $k_{\text{OOT}}$ and also increased as more data became available.

**Discussion**

Previous research had indicated that RegCC can be applied to detect both analytical and process control OOTs, while ByTimeTI and SlopeCC are suitable for the latter type of OOT (3). The basis for this recommendation was not given. The current research directly compared prevailing methods to determine which one would perform best under varying scenarios. ByTimeTI performed the worst in detecting either analytical or process control OOT, which may be because the method uses only data at the evaluated time point, thus not utilizing data at other time points. It also ignores intra-lot correlation.

A supportive analysis was performed (results of which are not included in this article) that used TI for longitudinal data with random lot effects accounting for intra-lot correlation as described in prior research (7). This more complex TI method did improve upon ByTimeTI detection rates, but levels were still inferior compared to the results seen using other methods. TI is a direct function of sample size and can be excessively wide with small sample sizes. ByTimeCC eliminates the sample size-dependence of the tolerance multiplier $k_{1-n,3\sigma,n}$ by using $Z_{\alpha}$ and thus improving upon ByTimeTI detection rates. However, ByTimeCC still did not perform well at ICC=0.8.

Simulation results confirmed the unsuitability of the SlopeCC for analytical OOT as previously described in other research (3). Although the method has high detection rates with limited data, it deteriorates as more data become available. This is because an analytical OOT result that occurs at later time points usually do not substantially affect the slope estimate that it exceeds the slope control limits.
However, SlopeCC is very effective in detecting process control OOT, confirming results of prior studies (3). SlopeCC has high detection rates when there is still very limited data available, but these results may just be the baseline performance of the method.

The false alarm rates when the subject lot is in-trend are also high. This is expected since, when only very few time points are available, the subject lot slope estimate and constructed historical lots slope control chart limits are unreliable.

PI, Z-score, and RegCC are related methods in the sense that all use parameter estimates from a SICS ANCOVA model to construct their respective OOT trending limits. The results of the research described in this article indicate that PI, Z-score, and RegCC, ranked in increasing effectiveness although the differences between them were slight. They are versatile enough to detect both analytical and process control OOT.

Comparing RegCC versus PI, the RegCC limit is a modified form of confidence interval of the predicted means. It uses the 'observed' subject lot intercept \( \hat{Y}_{LO} \) and \( \frac{2}{\alpha^2} \) as a multiplier of RMSE to construct its limit. In contrast, PI uses the 'fitted' subject lot intercept \( \hat{Y}_{LO} \) and \( \frac{2}{\alpha} \) as a multiplier of RMSE. By definition, a prediction interval, which is what the PI method generates, should be wider than a confidence interval. This explains the slightly higher LSL-to-OOT limit ratio (i.e., wider limits) found with PI than with RegCC. The wider OOT limits for PI are less stringent, thus the method’s detection rates are lower than those for RegCC.

Contrasting RegCC versus Z-score, the two are conceptually similar, and the mathematical formulas that they use for trending limits represent different versions of the same expression. However, there are two differences: first, the respective use of observed versus fitted subject lot intercept; and second, the data used to generate RMSE for each model was fitted, but only on the subject lot results. In this study, the Z+ divisor used was the RMSE obtained after fitting the available historical and subject lot results (excluding the latest time point) with a SICS ANCOVA model. The RMSE, therefore, represents the deviation from this assumed inter-lot (i.e., common slope with varying intercepts) and intra-lot relationship. Using this RMSE to calculate \( Z^+ \), therefore, represents a more comprehensive implementation of the Z-score than found in prior research.

Detection rates for the best performing methods are only up to about 45% for a moderate extent of analytical OOT (Figure 2, \( k_{OOT} = 2 \)) and increase only up to about 65% at large analytical OOT (\( k_{OOT} = 4 \)). These magnitudes of induced analytical OOT are arbitrary and scaled as \( k_{OOT} E_y \) where \( E_{\text{y}} \sim \mathcal{N}(0, \sigma_y^2) \) and the total variance \( (\sigma^2 + \sigma_y^2) \) constrained to equal one per the ICC generalization. The relative performance among the methods is a more meaningful way to evaluate methods rather than the absolute values of the detection rates. To increase detection rates, the OOT trending limits can be rendered more conservative (e.g., by decreasing the proportion level used from \( P = 0.95 \) to \( P = 0.90 \)). The challenge, however, is finding the right balance of stringent OOT limits that increase detection rates but do not inflate false alarm rates.

Previous research on this subject noted that the trending limits need only be established once for each product (2). Updating limits is only necessary if changes are made to the product stability profile or to the analytical methods used, or to get more precise estimates when more lots are available to be included as historical data. As supportive simulation analyses in this research, the OOT limits for RegCC were calculated using only the historical data available at the onset of trending. The OOT limits were then “fixed” and used to trend the remaining subject lot method. These two differences are seen when RegCC is compared with PI. The RMSE for Z-score is slightly larger than that for RegCC. This is because both historical and subject lot results are included in the former (i.e., so there are more constraints) whereas only historical lots are used in the latter (i.e., fewer constraints). The larger RMSE results in smaller \( Z^+ \) and lower likelihood of flagging an OOT. This explains the slightly lower detection rates of Z-score compared to RegCC.
results without updating, even as additional historical data were being generated. This approach contrasts with the dynamic updating of RegCC OOT limits as used in Figures 2–5. The supportive analyses showed very little difference between the performances of fixed vs. dynamic RegCC OOT limits. Thus, the extra effort required to update RegCC OOT limits dynamically does not appear to be worthwhile, although, in some cases, periodic updating may be performed at some pre-determined frequency that is manageable for the QC personnel.

Previous research (4) examined the simultaneous use of RegCC, ByTimeTI, and SlopeCC to better visualize results, but eventually recommended Z-score. Implementing multiple methods simultaneously may be too taxing for trending personnel given the number of attributes and on-going, repeated analyses needed. If a single method can be universally applied to both types of OOTs, it will simplify the implementation of any stability monitoring program.

Among the methods compared in the scenarios studied in this research, RegCC performed the best in detecting both analytical and process control OOT with only a slight increase in Type I error rates. RegCC can be easily automated in an Excel spreadsheet to calculate limits at all times \( j \) after inputting \( \beta \) and RMSE from ANCOVA output. SlopeCC worked just as well, but only for detecting process control OOT. This method requires estimating individual slopes and constructing historical slope-control limits at each time \( j \) data cut-off. SlopeCC is therefore more complex to implement than RegCC, without resorting to statistically programmed codes for automation. The last supportive analyses performed was specifying \( \beta = 0 \) to simulate the data sets. The relative performances of the methods were similar to \( \beta = -0.15 \). This indicates that the OOT trending methods can be applied for both stability non-indicating and indicating attributes.

**Conclusion**

Studies used simulation to compare directly statistical methods that are typically used to identify analytical or process control OOT conditions under varying scenarios. Findings indicate that a tolerance interval-based version of either by time or slope by lot methods showed the worst performance. However, the slope by lot control chart is effective in detecting process control OOT.

Prediction interval, Z-score, and regression control chart are three related methods based on fitting a separate intercepts, common-slope ANCOVA model. The three are listed in order of their increasing effectiveness in detecting both analytical and process control OOT. The differences among them are only slight. Overall, the regression control chart performed the best among the seven compared methods, with only a slight increase in false alarm rates (or Type I error). The method is relatively simple to implement in Excel spreadsheets, requiring only ANCOVA model estimates from common statistical software. If a QC laboratory is constrained to use only one method for its stability trending programmes, the regression control chart is recommended. Using this method, QC staff can flag potential OOT in real-time, then consult a trained statistician to confirm OOT using more complex models, and institute CAPA as needed.

**References**


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Early-Stage Considerations for the Manufacture and Delivery of Vaccines

Although vials and prefilled syringes have different advantages, both find use in vaccine fill/finish.

Daniel Martinez is syringe product manager, Stevanato Group.

The speed of development of COVID-19 vaccines in response to the global pandemic has been extraordinary; the progress made by the pharmaceutical industry to create effective vaccines has been supported by fill/finish operations and packaging manufacturers to ensure the delivery and safe transportation of the vaccines.

With expected high demand for COVID-19 vaccines for the next few years, the pharmaceutical industry should assess the optimal vaccine delivery solutions as vaccine rollout continues and evolves.

New vaccines are initially packaged in vials—which are easier to handle in a laboratory environment than prefilled syringes or blow-fill-seal containers—and are simple and cheap. Commercial launch can be expedited by releasing a new vaccine in the same vial container used in early development, shelf life, stability, and extractables and leachables studies, eliminating the need to repeat tests for a different container, and saving time and expense needed to investigate alternative primary packaging options.

Vials are versatile; in addition to liquids, they can be used to package lyophilized and cryogenic products, as well as those in powder form. Vials can also be used to package multiple doses in a single container, an important consideration when global fill/finish capacity is in short supply, as was the case in 2020 when the COVID-19 pandemic struck. By using larger vials that can hold five or six doses—as was the case for the Pfizer-BioNTech and Moderna vaccines approved under US Food and Drug Administration (FDA) Emergency Use Authorization—the number of doses that can be filled using available filling capacity will be significantly higher than if single-dose packaging is used.

Multi-dose vials are the fastest route to deliver the billions of vaccinations needed in a short period of time to combat the pandemic. For non-pandemic applications, however, vaccine delivery typically transitions into another format for commercialization, most likely a prefilled syringe, as development moves into Phase II or Phase III clinical trials.

The shift to a prefilled syringe also is a lifecycle management tactic once a vial-packaged vaccine is established in the marketplace. The transition, however, requires analysis of the formulation, product stability, and consideration of how the vaccine will be administered to patients.

From vial to syringe

In general terms, glass vials are the preferred option for primary packaging when speed to market is the key driver in drug product development. A prefilled syringe is preferred if ease of use or patient experience are more important than speed or if minimizing product waste is important.

If a vaccine formulation is stable in liquid form, there are few challenges to change from a vial to a syringe format. If there are stability issues, reformulation will be required; this decision will be influenced by the nature of the vaccine, preferred shipping methods, and the container options.

Human factors should be addressed when considering a packaging format change. For example, during the COVID-19 pandemic, some patients avoided visits to healthcare providers, leading to more remote medical consultations. For patients requiring routine injections, the use of self-administered prefilled syringes may become a trend necessitating new safety systems or autoinjectors.

Currently any cryogenic product must be packaged in a vial. Internal research is underway at the author’s company to analyze how syringes are affected by a freeze-thaw cycle. If data show that efficiency and mechanical performance are not compromised, vaccines that need to be stored at frozen or cryogenic temperatures could be frozen as single doses in syringes. Prefilled syringes could facilitate administration to patients outside a mass vaccination center setting.

Multi-dose vials are the fastest route to deliver the billions of vaccinations needed in a short period of time to combat the pandemic. For non-pandemic applications, however, vaccine delivery typically transitions into another format for commercialization, most likely a prefilled syringe, as development moves into Phase II or Phase III clinical trials.
to patients. For example, FDA granted Pfizer-BioNTech permission to extract six doses of its COVID-19 vaccine from vials originally approved for five doses due to excess overfill [1].

Prefilled syringes, which contain the exact dose, reduce waste of the drug product, and are suitable for routine vaccinations such as influenza. While research is still in the early stages, it is expected that SARS-CoV-2 infections are likely to continue and COVID-19 vaccines may migrate to a single-dose prefilled syringe to reduce waste and avoid expiration of unused doses.

Several factors must be considered when converting from a vial delivery method to a prefilled syringe. If the product is not in liquid form, reformulation will be required. Product stability in the syringe must be established before pre-marketing studies commence.

A logistical hurdle when migrating from a vial-filled product into a prefilled syringe is identifying the capacity needed for the fill/finish operations. For innovator drug companies, fill/finish can be done in-house or by a contract manufacturing organization with the equipment and experience required to process the preferred container and the capability to handle that type of product. In-house manufacture may require investment in new capacity for a dedicated line for mass production of a single product or a flexible filling operation that can handle multiple products and different size vials. Flexible capacity has been crucial in the current efforts to fill COVID-19 vaccines.

**Vaccine solutions**

The popularity of different container types varies regionally and typically is influenced by the cost of shipping and warehousing. In North America and Europe, small formats such as 2R and 6R vials predominate, while in Latin America and Asia, larger multidose vials are more common. For COVID-19 vaccines, however, larger vials are being used globally because of the billions of doses required to achieve widespread vaccinations. In addition, vials are easier to transport because they pack more closely than syringes within blisters.

The most common size prefilled syringe is 1 mL with either a staked 5/8-in needle for intramuscular injection or a luer-lock adapter to allow a healthcare professional to select the appropriate needle. Currently, the market demands see an approximately 2:1 split between adapter and staked needle, according to market research by the author’s company. While the vast majority of staked needles are 25 gauge and 3 bevel, there is a trend towards using a 5-bevel needle to reduce the patient’s pain perception.

For other vaccines, approximately 40% of the vaccine containers used today are syringes, with a high growth both in volumes and value. For these syringes, a compound annual growth rate of 8% in volume and 5% in value is anticipated from 2020 to 2024 [2].

COVID-19 has changed the vaccine development landscape dramatically; more than 230 COVID-19 vaccines are in development; however, only three vaccines plan to launch in syringe format [3].

To date, syringe-filling capacity has not been impacted by high demand as much as vial-filling demand. By reprioritizing production space can be created to manufacture billions of vaccines to meet global needs. However, the author estimates that overall fill/finish capacity for vials and syringes is not sufficient.

Both formats have their advantages and should be considered as options at the start of development programmes. The COVID-19 pandemic has posed significant challenges to the vaccine fill/finish industry, and glass containers are playing a crucial role in getting vaccines to the general population. Depending on the future global needs for vaccinations, prefilled syringes may gain a significant market share.

**References**

2. IQVIA, Proprietary data.

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**CMOs boost vaccine fill/finish output**

Baxter BioPharma Solutions announced on 8 March 2021 that the company will provide fill/finish sterile manufacturing services and supply packaging for 60–90 million doses of the Moderna COVID-19 vaccine in 2021 from its Bloomington, Ind., USA facility.

“Baxter is honoured to provide our deep expertise in vaccine manufacturing to help partners like Moderna bolster the supply of their vaccine,” said Marie Keeley, vice-president, Baxter BioPharma Solutions, in a press release [1].

Catalent announced on 17 March 2021 that it will increase manufacturing capacity at its Anagni, Italy, site to accommodate the large-scale commercial supply of Janssen’s COVID-19 vaccine, including vial-filling, inspection, labelling, and packaging services. The company will fast-track the qualification and scale-up of an additional high-speed vial-filling line that is set to be operational in the fourth quarter of 2021 to support vaccine production through late 2022 [2].

**References**


—The editors of Pharmaceutical Technology Europe
In most isolators, surface decontamination is carried out with vaporized or sprayed hydrogen peroxide ($\text{H}_2\text{O}_2$) as the sporicidal agent. Direct spraying of micro-nebulized $\text{H}_2\text{O}_2$ into the isolator system produces a quick distribution with a smaller amount compared to vaporizing $\text{H}_2\text{O}_2$.

The decontamination cycle, using the selected agent, must be validated in accordance with GMP to generate a reliably aseptic atmosphere inside the isolator. The phrase “controlled with defined cycle parameters” does not mean solely the amount and time of the $\text{H}_2\text{O}_2$ in the isolator system; the following questions should be considered:

- How homogeneous and quick is the distribution of the sprayed or evaporated $\text{H}_2\text{O}_2$, including at difficult-to-reach positions (worst case observation)?
- If an uneven enrichment of the sprayed or evaporated $\text{H}_2\text{O}_2$ takes place, what consequences does this have for the decontamination cycle to be validated? Are surfaces exposed for a shorter or longer duration with a lesser or greater amount of $\text{H}_2\text{O}_2$, and what influence does this have?
- How is the concentration distribution of the $\text{H}_2\text{O}_2$ in the overall isolator system? Is a uniform $\text{H}_2\text{O}_2$ film created on all surfaces without undesirable droplets forming on surfaces?
- What materials are used in the isolator system and what is their influence on the surface decontamination? Stainless steel has a different decontamination factor (D-value) than glass or polymers, for example. These parameters need to be taken into account as well in the cycle to be validated.
- Are moveable parts located in the isolator system? How are transfers into and out of the isolator accomplished and what influence do these transfers have on the decontamination cycle?
- Are surfaces exposed during the production process and during

A revised draft of the European Union (EU) good manufacturing practice (GMP) Annex 1 was published in February 2020 (1), and a final version is expected in 2021. The GMP guidance includes several key points related to isolator design and operation (see Figure 1). As the author discussed in the first part of this article series, the design of an isolator is the basis for compliance with GMP in aseptic processing and in cleanability (2). Isolator design also affects surface decontamination.

### Surface decontamination

Hygienic design and cleaning to remove particulate and microbiological contamination are important attributes for surface decontamination of the inner surfaces of isolators and of their installations (e.g., a vial or syringe filling line, an incubator, or other utilities for manufacturing cell and gene therapies). Consider section 4.24 of the new EU Draft GMP Annex 1, which says (1):

For RABS [Restricted access barrier systems] and isolators systems, decontamination methods should be validated and controlled within defined cycle parameters. The cleaning process prior to the disinfection step is essential; any residues that remain may inhibit the effectiveness of the decontamination process:

1. For isolators, the decontamination process should be automated and should include a sporicidal agent in a suitable form (e.g., gaseous, aerosolized, or vaporized form) to ensure thorough microbial decontamination of its interior. Decontamination methods (cleaning and sporicidal disinfection) should render the interior surfaces and critical zone of the isolator free of viable microorganisms. (1)

This section has two messages in terms of isolators: decontamination methods should be validated and controlled within defined cycle parameters and the process should be automated and should use a sporicidal agent.
work in the isolator system that are not sufficiently exposed during the decontamination cycle?

The phrase “defined cycle parameter” also contains quality-by-design (QbD) attributes in regards to the variable parameters in the isolator system. Consider questions such as the following when developing a validated decontamination cycle:

- In which defined acceptance criteria (e.g., temperature range, relative humidity range, quantity and concentration of H₂O₂, air velocity and speed range if used) does the decontamination cycle function?
- Additionally what influence do the criteria have on the decontamination cycle? For example, what is the effect if the temperature is 15 °C instead of 20 °C or if the relative humidity is 30% instead of 40%?

**Automation**

Automation, including robotics, can make manufacturing of sterile products more efficient, quicker, and safer. Robotic systems are mentioned specifically in section 2.1 of the new EU Draft GMP Annex 1, which says (1):

> The manufacture of sterile products is subject to special requirements in order to minimize risk of microbial particulate and pyrogen contamination. The following key areas should be considered:

  i. Facility, equipment and process design should be optimized, qualified and validated according to the relevant sections of the Good Manufacturing Practices (GMP) guide. The use of appropriate technologies (e.g. Restricted Access Barriers Systems (RABS), isolators, robotic systems, rapid microbial monitoring systems) should be considered to increase the protection of the product from potential extraneous sources of particulate and microbial contamination such as personnel, materials and the surrounding environment, and assist in the rapid detection of potential contaminants in the environment and product. (1)

Robotic work processes are widely used in pharmaceutical packaging, and their use has been expanding; they are now employed for handling sterile products, free of any manual interventions. The International Society for Pharmaceutical Engineering (ISPE) DACH [Germany/Austria/Switzerland] Future Robotics special interest group (SIG) was founded by the author in 2019. The SIG concerns itself with possible new work processes for robotic systems, the facility of the future using robotics, and the

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regulatory requirements from the EU GMP Annex 1 Draft for using robotics. For example, a new work process for robotic systems is to place moulded parts in transport containers and transport them to the washing machine and, after washing, back to the isolator. This process needs to take into account transfers between individual cleanroom grades.

Another work process is aseptic filling and automated handling of sterile finished products by means of robotic systems in isolators as well as implementing their technical and regulatory compliance with current good manufacturing practice (CGMP). It is necessary, for example, to adapt viable monitoring to fully automated robotic solutions in aseptic manufacture. In the Draft Annex 1, the application of rapid microbial monitoring is mentioned, but the technical and organizational measures needed to replace the current monitoring methods using settle plates with rapid microbial testing methods need to be redefined. This topic will be addressed by the ISPE Future Robotics SIG in 2021.

**Containment requirements**

The production of highly active and hazardous substances in the biopharmaceutical industry has increased rapidly in recent years, requiring consideration of environment, health, and safety protection. Viral vectors, for example, require high protection for the employee as well as prevention of cross-contamination with other substances.

Consider section 4.14 of the new EU Draft GMP Annex 1, which says, regarding the topic of containment of highly active and dangerous substances (1):

"Particular attention should be paid to the protection of the critical zone. The recommendations regarding air supplies and pressures may need to be modified where it is necessary to contain certain materials (e.g. pathogenic, highly toxic or radioactive products or live viral or bacterial materials)…"

In addition to the pressure concept, the following aspects need to be taken into consideration for fill/finish of parenteral pharmaceuticals in an isolator system:

- Is there an Accepted Daily Exposure (ADE) or Permitted Daily Exposure (PDE) that sets a limit value for exposure, such as that found in the European Medicines Agency’s guideline on setting health based exposure limits (3)?
- This limit value is important for calculating cleaning limit values as well as for determining how to protect employees during manufacturing.
- Which biological safety level (BSL) is required, and which technical and organizational measures are necessary to achieve this BSL?
- Is this a monoproduction or a multi-purpose system? On a multi-purpose system the necessary cross-contamination requirements have to be observed. For products with an ADE/PDE, these requirements are determined by toxicologists. For viral vectors, there currently are no defined limit values, so a no-tolerance level from the previous to the subsequent product is assumed.

A quality risk management (QRM) process is needed that covers the requirements of GMP for product protection as well as protection of employees and the environment. A contamination control strategy CCS is derived from the QRM. Furthermore, prevention of cross-contamination and containment strategies must be developed.

**References**

1. EC, Draft Revision to “Annex 1, Manufacture of Sterile Medicinal Products,” (Brussels, 2020).

**More on isolator design and operation**

In Part 1 of this article series, “Understanding the Impact of Annex 1 on Isolator Design” (1), Richard Denk discusses what the European Union’s draft Annex 1 says about barrier systems, including restricted access barrier systems (RABS) and isolators. He details the importance of aseptic engineering design and describes how isolator design affects cleaning and decontamination.

**Reference**

Limitations and Advances in Dissolution Testing

Despite its importance in drug development, dissolution testing still has some limitations, but advances in automation and real-time monitoring are producing promising results.

Varying specifications
Dissolution rate specifications vary depending on the design of the dosage form. In 2018, the US Food and Drug Administration (FDA) updated its guidance on recommendations for dissolution testing and specification criteria for immediate-release dosage forms that contain highly soluble compounds (1). However, not all drug products are covered by this guidance, which is applicable only to solid orally administered immediate-release drugs that are intended to be swallowed. Generally, when measuring the dissolution rate for an immediate-release dosage form, a single-time point specification is used—exceptions may occur when evaluating slow-dissolving drugs where two time points can be used—with samples taken between 30 and 45 minutes, and when approximately 75–80% of the active ingredient is dissolved (2).

However, for modified-release dosage forms, multiple time points are specified for characterization of the in-vitro dissolution rate—for delayed-release forms, two time points are required, and for extended-release, at least three time points are required (3). For modified/extended-release dosage forms, dissolution testing should show the rate a solution is formed with the release of active ingredients over specified time intervals, explains Cho. Relevant guidance has been published to address the challenges of dissolution of modified or extended-release dosage forms (4,5).

When testing dissolution rates of solid-oral dosage form drug products, immediate-release and modified-release, standardized apparatus as described in the United States Pharmacopeia (USP) are employed (6). “USP General chapter <711> Dissolution includes four standardized apparatus: basket, paddle, reciprocating cylinder, and flow-through cell,” Cho notes. “Where specified in a monograph, USP dissolution tests are legal requirements.”

Yet, these standardized approaches are not without limitations, as have been previously reported in the literature (7–9). Issues relating to not only the equipment used in standardized approaches but also the analysts performing the tests can lead to dissolution profile variability.

Furthermore, for some dosage forms there are limited test methods described in the literature. As Speer et al. reported in 2019, for oromucosal film preparations with a modified release profile, despite there being a regulatory requirement to provide dissolution test data for this dosage form, there are no suitable recommendations of methods or established specifications available to follow (10). Therefore, when the test article may not respond to the standardized approaches and regulatory recommended methods, it may be necessary to employ new techniques or devices, Cho asserts.

Notable advances
“The most noticeable analytical method or technological advancement in recent years to aid with dissolution testing is automation,” confirms Cho. Although automated dissolution has been explored for some time, more recent advancements have been seen in the use of computerized software that aids with the regulatory audit trail required, he adds. For Cho, full dissolution automation—sampling station and analysis—and a paperless dissolution workstation will be important trends in the near future.

Recent research by Chi et al. described an automatic method for dissolution testing with high-
Dissolution Testing and Development

Contin. from page 19

SEC, the study needs to be thought hard about what sort of material must be utilized to ensure stability requirements for clinical early-stage material that matches time, confirms Kadisch. “Hence, and suit the client’s needs to save money,” he says.

To assure success in accelerated formulation strategies, the chosen strategy must be fit-for-purpose and suit the client’s needs to save time, confirms Kadisch. “Hence, early-stage material that matches stability requirements for clinical material must be utilized to ensure optimal time-to-clinic,” he says.

For Zonderman, it is important to think hard about what sort of information will be helpful when accelerating formulation. “For example, when it comes to stability studies, aggregate detection via SEC only sizes aggregates and does not measure structure, providing only limited insight. Secondary structure measurements can provide a way of detecting instability, Zonderman underscores. “Changes in elements of secondary structure, notably beta sheet content, are a reliable indicator of instability and incipient aggregation,” he adds. “By measuring secondary structure, it is therefore possible to accelerate stability studies by detecting instability much earlier, before aggregates form. At the same time, structural information provides insight into the aggregation pathway, facilitating mechanistic understanding and a QBD approach.”

Furthermore, it is imperative to understand the value of sensitivity—the ability to detect difference—affirms Zonderman. “A technique with high sensitivity will differentiate samples that a less sensitive technique classifies as identical. Higher sensitivity will therefore bring your understanding into sharper focus, enabling more precise structure-functionality relationships and more effective formulation optimization,” he states.

“One final point to make is that the analytical offering for biopharma is evolving fast, with many companies recognizing that it remains a work in progress,” Zonderman summarizes. “Keeping an open mind as to what might now be possible is vital when it comes to putting in place accelerated formulation strategies.”

References

project execution,” he says.

“Furthermore, the outsourcing partner should lead the client in the project but should also listen to clients’ needs.”

In other work, by Galata et al. in 2019, an artificial neural network (ANN) model was built to predict the dissolution profile of extended-release tablets (12). Using data collected by near-infrared and Raman spectroscopy, as well as the temporal resolution using high-speed capillary electrophoresis (HSCE) (11), in their work, the researchers’ proposed a system comprised of three components—a flow-through-cell for tablet dissolution, an automatic sequential injection with high-temporal resolution, and online separation/detection using HSCE. Validation of the methodology was performed according to FDA guidelines. Immediate and fixed-dose products were assessed by Chi et al. who found that with their fully integrated on-line automatic system it was possible to monitor dissolution with high-temporal resolution in real-time, which is promising particularly for fixed-dose combination drug products.

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References
Reducing Uncertainty of an Analytical Method through Efficient Use of Replication

For a robustly developed analytical method, a well-chosen replication strategy can effectively reduce the uncertainty.

Phil Borman, DSc, is senior fellow and director of product quality, GlaxoSmithKline; Timothy Schofield is owner and consultant, CMC Sciences, LLC; and David Lansky, PhD, is president, Precision Bioassay, Inc.

In January 2020, United States Pharmacopeia (USP) published a Stimulus to the Revision Process article in the Pharmacopeial Forum (PF) titled “Distinguishing the Analytical Method from the Analytical Procedure to Support the USP Analytical Procedure Life Cycle Paradigm” (1). One motivation behind the article was to encourage analytical scientists to consider the role of replication in the reduction of uncertainty in analytical measurement. More specifically, the PF article was directed towards the uncertainty of the reportable value that is impacted by both “between-run” and “within-run” replication. The authors consider the use of an analytical method to be the same as a ‘run’.

After publication of the PF article, it has become evident to the authors of this article that there are issues related to implementing the previous recommendations, such as ensuring compliance with compendial requirements and aspects of data processing for some methods. Examples of compendial requirements include the required minimum number of injections of a solution in high performance liquid chromatography (HPLC) to demonstrate system precision, and the assessment of similarity of test and standard samples in bioassay. These complicate consideration of replication within a method. This article considers strategies for replication within methods that conform to these (and other) constraints, improve precision via efficient replication, and challenge some norms.

Replication strategies should not be considered until an analytical method has been developed and confirmed to be generating data of a quality that is expected from its technology. The leverage of prior and platform knowledge is recommended to deduce the expected performance of an analytical method. While replication of factors can reduce the uncertainty of an analytical method, there are limits to how much the uncertainty can be improved and replication should not be used as a way of masking a poorly developed analytical method.

The core message of this article is to replicate those factors that have the greatest impact on analytical variability (2). It is incumbent upon the analytical scientist to estimate the relative impacts of factors that may introduce variability into the measurement.

With these principles in mind, this article will illustrate replication strategies for a separation method on a small-molecule API and a bioassay for a biopharmaceutical or vaccine where the variability of factors has already been estimated. Barriers to replication such as inappropriate interpretation of measurements from individual replicates will be discussed, while more suitable approaches will be recommended.

Statistical considerations and uncertainty

A general equation (Equation 1), which combines all the factors that can be replicated within a method, is used to generate the standard deviation of measurements from a method. Equation 1 uses a sum of variabilities (variance components symbolized $s^2_{\text{factor}}$) and the number of replicates (or unique levels, e.g., of intermediate precision factors such as analyst or equipment) of the individual factors ($n_{\text{factor}}$).

$$ s_{\text{Total}} = \sqrt{\sum s^2_{\text{factor}}/n_i}. \tag{Eq. 1} $$

Factors are often hierarchical in nature, such as replicate injections of multiple sample preparations in HPLC. In these cases, the degrees of replication are compounded leading to an adaptation of Equation 1 as shown in Equation 2:

$$ s_{\text{Total}} = \sqrt{\sum s^2_{\text{factor}}/n_i + \sum s^2_{\text{inter}}/n_{ij}}. \tag{Eq. 2} $$

Interpretation of the impact of within method replication is incomplete without consideration of two additional points. The first is the impact of factors that impact variability over the long-term use of a method. These between-
method (or between run) factors are defined by International Council for Harmonisation (ICH) Q2(R1) Validation of Analytical Procedures (3–4) as intermediate precision (5) (within laboratories) or reproducibility (between laboratories) factors and include laboratories, analysts, instruments, and reagent lots. In some methods these factors are negligible and can be ignored. Most notably the use of a standard can reduce the influence of these factors on variability. If non-negligible, the variability due to intermediate precision factors should be included in the calculation of $S_{\text{Total}}$. These intermediate precision factors may likewise be replicated to manage the total long-term variability of the reportable value. In this case, the reportable value could be a summary measure from replicate runs.

Another consideration is the translation of total variability to uncertainty. This can be expressed simply in Equation 3 as:

$$U = k \cdot S_{\text{Total}}.$$  \hspace{1cm} \text{[Eq. 3]}

Here $k$ is a statistical constant that is associated with "confidence". Thus, $k = 2$ is sometimes used to express the uncertainty associated with 95% confidence. The need to express total variability as uncertainty is required to evaluate a method’s capability against the target measurement uncertainty (TMU), which can be specified in the analytical target profile (ATP) (6–9). The ATP is used to define the performance requirements associated with the reportable value and is defined by USP <1220> as “a prospective description of the desired performance of an analytical procedure that is used to measure a quality attribute, and it defines the required quality of the reportable value produced by the procedure” (10). USP <1220> suggests that a replication design can be used that reduces uncertainty to the point of satisfying the ATP and further suggests that the replication design can be adapted to ensure conformance to the ATP after “qualification”.

Practical considerations often lead to non-hierarchical designs (perhaps with crossed rather than nested error terms) that are more difficult to understand and analyze correctly. It is also important to note that the variance models illustrated in the below examples (e.g., using Equation 5) assume not only independence among (random responses at different) levels of the hierarchical design, but also independence among replicates at each level. In most small-molecule assay systems, within-method replicates of a sample compared to the same set of standards, which may be correlated, are close enough to independent that the variance modelling approach used below provides valuable guidance. In bioassay, it is important to check on these assumptions. Worth noting, but outside the scope of this article, are designs that incorporate factors having an important impact on variability that are non-hierarchical (e.g., crossed).

**Example 1:**

**HPLC method using external standardization for small-molecule drug substance assay**

An external standard is commonly used where the concentration of the drug substance is quantified by comparing the response(s) obtained with the test sample solution(s) to the responses(s) obtained with standard solution(s) (11). Hofer et al. have previously highlighted that the variability of such HPLC assays range from 0.6–1.1% RSD [relative standard deviation] (12), which is an appreciable portion of a typical drug substance specification acceptance criteria (e.g., 98–102%). A suitable replication strategy is therefore desirable to reduce this variability to an acceptable level. Chatfield and Borman (13) describe the use of a precision to tolerance ratio (PTOL) that can be used to compare the analytical procedure variation to the gap between the process mean and specification limit. They suggest that the analytical procedure should ideally not take up more than 30% of the specification window. This equates to a target uncertainty $\leq 1.2\%$ for this example. This is one of many ways to specify the precision portion of the ATP.

The HPLC method (within-run) replication strategy should consider the number of preparations of the standard and sample solutions as well as how many times these solutions are injected onto the HPLC system. Equation 2 can be expanded using these factors to give Equation 4, which describes the uncertainty associated with a sample estimate compared to the standard from an HPLC assay method, where $n$, $n'$, $m$, $m'$ are the numbers of independent sample preparations, independent standard preparations, injections of each sample preparation, and injections of each standard preparation, respectively.

Intermediate precision factors (4–5) (e.g., analyst, instrument, or column) are not considered in this example as previous ruggedness studies (14–15) have shown these factors to have a negligible impact on the overall uncertainty (the use of external standardization significantly reduces the influence of these factors on variability).

For this example, a plausible sample and standard preparation standard deviation of 0.65 and an injection sample and standard deviation of 0.1 will be used to illustrate the impact of replication of these factors on the method uncertainty.

**Figure 1** displays four different designs (each with a different within-run replication design) for the factors expressed in Equation 4, and **Table 1** provides the constants that satisfy Equation 4 for these designs. The calculated uncertainty ($U$) for each design is shown in **Figure 2**.

$$U = k \cdot \sqrt{\frac{S_{\text{Sample Prep}}^2}{n} + \frac{S_{\text{Injection(Sample Prep)}}^2}{nn'} + \frac{S_{\text{Std Prep}}^2}{n'} + \frac{S_{\text{Injection(Std Prep)}}^2}{nn'}}.$$  \hspace{1cm} \text{[Eq. 4]}
Analytics

For the three samples shown in Design 4, two additional standards preparations (making a total of four) have been added, which results in a further improvement in method uncertainty (to 1.14). Where even more samples are being analyzed within a run, Design 4 allows the opportunity for increasing the number of standard preparations thus improving the uncertainty associated with all samples analyzed.

Design 1 represents a typical replication design strategy used by the pharmaceutical industry. It should be noted that the number of standard solutions and injections are often fixed and incorporated into the analytical method due to pharmacopeial expectations for checking system precision (17) (e.g., the Japanese Pharmacopoeia requires five degrees of freedom to be studied through six replicate injections). A predicted uncertainty of 1.31 was calculated for Design 1, which equates to 33% of the specification window (98–102%). This uncertainty does not quite meet the acceptance criteria in the ATP of the uncertainty taking up less than 30% (U≤1.2) of the specification window.

Interestingly, if no replication of injections were performed as in Design 2, then the overall uncertainty (1.32) is only very slightly increased compared to Design 1. Design 3 demonstrates that by adding just one additional standard preparation and balancing the injections across all samples and standards (while not compliant with pharmacopeial expectations, provides similar information for injection variability) the uncertainty can be reduced to 1.19 and thus just meets the uncertainty acceptance criteria in the ATP. Design 4 retains the pharmacopeial requirement of six injections at the start and end of the run and provides the opportunity to increase the number of standards depending on how many samples are analyzed. For the three samples shown in Design 4, two additional standards preparations (making a total of four) have been added, which results in a further improvement in method uncertainty (to 1.14).

Where even more samples are being analyzed within a run, Design 4 allows the opportunity for increasing the number of standard preparations thus improving the uncertainty associated with all samples analyzed.

### Table I.

<table>
<thead>
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<th>Standard prep StDev</th>
<th>Injection StDev</th>
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<th>m</th>
<th>n'</th>
<th>m'</th>
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<td>4</td>
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*Due to the imbalanced nature of the standard injections in Design 4, m' is not a round number and is determined from the harmonic mean of the number of injections across the standards (16).

### Figure 1.

Alternative replication designs for test sample and standard preparations for high performance liquid chromatography (HPLC) assay where S1, S2, etc., represents standard preparations; A1 and A2 represent test sample preparations of Sample A; B1 and B2 represent test sample preparations of Sample B, etc.; i1, i2, i3, etc., represents the replicate injections for each standard or test sample preparation.

### Figure 2.

Predicted uncertainty (U) of a reportable value for the different high performance liquid chromatography assay designs.
Example 2: Bioassay using relative potency for large-molecule drugs and vaccines

In bioassay, the potency of a test sample is computed relative to a standard to separate the variability inherent to the biological system (i.e., in cell culture or animals) from the measurement of interest and to scale measurements to a target of 1.0. This involves use of a dilution or concentration series of both sample and the standard and using a mathematical model (four parameter logistic or linear regression) to estimate the relative potency of the sample to the reference. Once a bioassay has been optimized, replication is utilized to have enough power to reliably pass similarity and to further reduce variability of the reportable value. Whether prospectively designed or not, all bioassays employ some form of replication.

Replication can occur both within and between runs of the method. Runs may be comprised of one or more bioassay plates and can extend to other factors that are associated with appreciable variability.

Two designs of a bioassay utilizing a hierarchical layout of runs, plates, samples, and a standard are illustrated in Figure 3 (the dilution or concentration series are not illustrated and may be in a non-hierarchical structure).

The two designs include the same numbers of standard and sample preparations, while Design 1 splits samples A and B from samples C and D between two runs, and Design 2 tests each sample in both runs.

These designs illustrate three levels of replication. Each design contains two runs with two plates in each run while the standard and samples are replicated within and across plates in each run of Design 1 and both across plates (within run) and between runs in Design 2. Relative potency can be determined for each sample on each plate. In general, a simple and useful model for uncertainty associated with the reportable value (the average across replicates of log relative potency) for both designs is given in Equation 5, where n, m, and l are the numbers of runs, plates performed in each run, and sample replicates on each plate in each run.

Equation 5 can be used to determine the uncertainty (U) associated with the two designs using variance components derived from log potency. It is noteworthy that both designs use a fixed number of standard replicates (2) as a part of the method. Different results will be realized from other methods that

\[ U = k \cdot \sqrt{\frac{S_{Run}^2}{n} + \frac{S_{Plate(Run)}^2}{nm} + \frac{S_{Replicate(Run,Plate)}^2}{nml}} \]

[Eq. 5]
use fewer (higher uncertainty) or more (lower uncertainty) standard replicates. **Table II** provides the constants for **Equation 5** associated with these configurations.

A total variability (intermediate precision) equal to 20% geometric coefficient of variation (%GCV) will be used for purposes of illustrating the uncertainty for the two designs. In bioassay, both the total variability and the relative proportions of the three sources of variability (runs, plates, and replicates) will differ from method to method. The calculated uncertainties using the constants in **Table II** and two distributions of standard deviations (of ln potency) corresponding to the three sources of variability are presented in **Table III**.

As illustrated in **Figure 4**, Design 2 is more effective in reducing uncertainty than Design 1 (i.e., there are meaningful reductions in uncertainty from 0.28 to 0.25 for standard deviation distribution 1, and from 0.24 to 0.21 for standard deviation distribution 2), which is a consequence of replicating the highest level factor (run).

Blue shading in the plot shows the intersection of the uncertainty requirement of the ATP with predicted uncertainties for the designs. Here uncertainty <30% (natural log uncertainty <0.26) is used for illustrative purposes. This will have been determined to ensure a low likelihood of generating an out of specification result due to imprecision.

**Discussion**

Prior to designing a replication strategy for an analytical method, system suitability criteria should be established. Alongside checks for characteristics such as specificity (e.g., ratio of peaks in a HPLC system) and sensitivity, system suitability may also include the acceptable variability among the individual results for the

<table>
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<th>m</th>
<th>l</th>
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<tr>
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</table>

**Table II. Constants in Equation 5 relating to two bioassay designs (where k=2, 95% probability).**

<table>
<thead>
<tr>
<th>Design</th>
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<th>s_Plate</th>
<th>s_Replicate</th>
<th>Uncertainty</th>
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<td>0.08</td>
<td>0.15</td>
<td>0.06</td>
<td>0.25</td>
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</table>

**Figure 4.** Predicted uncertainty \(U\) of a reportable value for different bioassay designs and sets of standard deviations (SD) for Run, Plate, and Replicate.

**Table III.** Uncertainties for two designs with different sets of standard deviations for Run, Plate, and Replicate.

<table>
<thead>
<tr>
<th>Design</th>
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</table>
factors (e.g., sample preparation, sample injection, etc.) being replicated. This can be obtained from prior knowledge about the analytical technology. A reportable value is only valid if such system suitability criteria are met.

Schofield et al. (1) and Foust et al. (19) highlight issues associated with existing regulatory guidance that makes implementing a replication strategy problematic. For example, the US Food and Drug Administration’s Guidance for Industry: Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production (20) and ICH Q1E: Evaluation for Stability Data (21) both indicate that individual measurements (rather than the averaged reportable value) should be compared against specification limits and if observed to fall outside, the reportable average of these measurements should be treated as an out-of-specification (OOS) result. System suitability criteria for acceptable variability among individual measurements should negate the need of this requirement. Comparing individual measurements against specification limits creates a disincentive for developing scientifically sound procedures. Increasing the replication improves product quality by reducing the uncertainty of results; however, the increased analytical testing generates a greater risk of observing OOS results.

The precision component of the ATP should refer to the reportable value that can be derived from the use of either an analytical method or an analytical procedure. The four designs shown in Example 1 and Design 1 in Example 2 exemplify derivation of the reportable result from an analytical method. An alternative strategy described by Schofield et al. (1) is where the analytical method is converted into an analytical procedure through replication of the entire analytical method (or a run) as in Design 2 in Example 2. Following this pathway leads to the question “when is the method good enough?” This might be answered by saying that the method is adequate when it has been developed using the best scientific principles and practices for the given technology, and that it performs as expected for that technology. This might be viewed as a quality-by-design approach that interprets method development as the practice of “building quality into the method,” through science and statistics (replication).

Some may argue that the use of replication may reduce the incentive to properly select and adequately optimize a method. A laboratory may, however, be faced with competing circumstances during method development. If, for example, a method is to be developed and then transferred to a laboratory or region with limited resources or lack of experience with more advanced technologies, a substandard technology might be selected, optimized, and the resulting analytical method replicated to a degree that satisfies the precision requirement for the reportable value. Here, as in the case of optimizing a technology and replicating to satisfy the ATP, the end justifies the means.

Conclusion
Once an analytical method has been developed it can be fine-tuned to reduce uncertainty (to meet the precision requirement in the ATP) by replicating the factors that give rise to most of the variation. Prior knowledge about technologies coupled with any precision studies performed on the analytical method can be used to predict the variability of method factors and guide which ones could benefit from being replicated. This article has demonstrated that certain replication strategies may be more appropriate than current compendial requirements. At the very least, improved replication strategies can be used in conjunction with replicating factors that are required to meet compendial requirements. Once an analytical method has been developed with a suitable replication strategy, system suitability limits should be established that should include the acceptable variability among the individual results for the replicated factors. With this in place, the practice of comparing individual measurements against specification limits is not necessary and should be discouraged. Consideration should also be given to using a stable control sample of known quality every time an analytical method is used or periodically if there are resource constraints. This allows for the predicted uncertainty estimated for the analytical method and/or procedure to be verified and adjusted over time if necessary.

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After Brexit there is an increased risk of the UK being exposed to counterfeit medicines, but regulations implementing blockchain as infrastructure technology could be the answer.

Felicity Thomas

Counterfeit pharmaceutical products are a global issue that has a significant financial impact and, more worryingly, poses potential dangers to patients’ health. “The topic of counterfeit medicines has been a concern for a number of decades, and it affects the Western nations as well as emerging markets,” explains Raja Sharif, CEO and founder of FarmaTrust—a provider of blockchain and artificial intelligence (AI) provenance systems for the pharmaceutical and healthcare sectors.

There is a general belief that counterfeit medicines is a low- and middle-income country issue rather than one that affects Europe and the United States, but if you look over the past five to 10 years, it becomes apparent that there are a lot of counterfeit medicines in Europe, and the issue is getting worse, Sharif continues. In fact, it is estimated by the European Union Intellectual Property Office (EUIPO) that nearly €10 billion of direct sales, within the pharmaceutical sector, are lost through counterfeiting per year (1).

Additionally, there is an increasing prevalence of purchasing medicines online, which is exacerbating the problem of counterfeit medicines. “Absolutely, there has been a trend for online purchases, a trend that was even recognized a number of years ago by Amazon who purchased an online pharmacy, PillPack,” notes Sharif. “But, irrespective of this trend, it mustn’t be underestimated that counterfeit drugs can be infiltrated into the national supply chains.”

Even looking at recent prosecutions by the Medicines and Healthcare products Regulatory Agency (MHRA) of the United Kingdom, it is clear to see that counterfeiters are using pill presses bought from China to make and distribute counterfeit medicines in the UK, Sharif underscores. “So, it is not just an online problem; although, online sales are of course much easier and the issue is getting worse, there are also cases of counterfeit medicines being purchased within UK pharmacies as well,” he says.

Legislation to tackle the issue

To tackle the issue of counterfeit medicines within the European Union (EU), a directive was formulated and published setting out various safety features that must be included on pharmaceutical packaging to assure product authenticity (2). “The issue of counterfeit medicines was obviously in discussion for a long time and resulted in what we now call the Falsified Medicines Directive (FMD) regulations in Europe,” confirms Sharif. “Essentially, what this directive allows you to do is to ensure that each packet of medicines is uniquely labelled, and then you scan the labels, which tell you whether there’s a duplicate or whether the product is actually genuine.”

In the United States, anti-counterfeiting measures have been enacted through the Drug Supply Chain Security Act (DSCSA) (3). “Both the EU and US systems generally require unique labelling, which is usually achieved by two-dimensional dot matrix codes,” says Sharif. “Some countries are using Quick Response codes these days as well as tamper-proof packaging, but legislations tend to require the matrix codes as a minimum.”

The main difference between the EU FMD and the DSCSA regulations is that in Europe, the labels are issued on the drug packaging line and then scanning of the unique identifier only happens when the product is scanned out of the pharmacy or hospital, explains Sharif. “Whereas the DSCSA regulations require data collection at each transfer of the chain of custody (i.e., when one company transfers the product to another or one person gives it to another person),” he states.

As there is not a requirement for tracing each step of the product’s
transition in Europe, there is a danger that counterfeit products can infiltrate the supply chain. It’s a race to the end, notes Sharif, if a counterfeit product with a duplicate unique label gets scanned first before the authentic product, it will be accepted to the pharmacy or hospital, he asserts.

Through working with the US Food and Drug Administration on blockchain pilots, Sharif reveals that it became apparent that if a product is tracked at each transfer of the chain of custody, not only is it possible to more easily prevent counterfeit medicines entering the supply but the process is more data-rich overall. “So, for data analytics purposes and efficiency gains, the DSCSA regulations are much better,” he adds.

UK at risk?
However, for the UK, which fully exited the EU on 1 Jan. 2021, the EU FMD legislation is no longer applicable. “The Brexit situation means that the UK has actually pulled out of the FMD process,” adds Sharif. As a result of the UK no longer being covered by the European legislation, the country is now exposed as there is no longer the ability to verify the unique labels within the central European verification system, he emphasizes.

At the time of writing, a bill concerning UK medicines regulations has gained Royal Assent (the final stage of bill passage in the UK)—the Medicines and Medical Devices Act 2021 (4). This bill is aimed at ways to make the UK more attractive in terms of innovative new solutions and technologies being approved, and it covers not just medicines and medical devices, but other aspects, such as clinical trials, states Sharif.

Sharif believes that a system akin to the US DSCSA would be most beneficial for the UK, to keep up its competitive landscape in terms of pharmaceutical anti-counterfeiting measures. “This route is potentially interesting given the fact that the UK’s regulator, MHRA, has been working closely with FDA and the Australian regulator post-Brexit,” he says. Much of the work that will be implemented into amendments to regulations and anti-counterfeiting measures in pharmaceuticals is expected to be delegated powers given to the parliamentary ministers, Sharif anticipates. For application, however, Sharif recommends tracking each transfer of custody and even the condition of the medicines as well, as that level of information will give a high level of patient safety.

Additionally, with such a data-rich approach, it is possible to improve demand planning and prevent medicine wastage, Sharif stresses. “For example, there are a lot of medicines that are wasted each year,” he says. “So, if you have a much more efficient ordering process, demand process, and planning process, the National Health Service can be saved billions of pounds, which can be spent on other vital areas.”

Blockchain benefits
A compulsory infrastructure technology should be implemented for anti-counterfeiting measures, in Sharif’s opinion. “One of the areas blockchain is particularly useful is to ensure the immutability and incorruptibility of data,” he says. “So, that means once a record is written, it cannot be changed. The record can be updated, of course, but the information on who updated it, when it was updated, and what the update was itself, will be available.”

Another aspect of blockchain that lends itself to anti-counterfeiting applications is the fact that it is very good at breaking down data silos, Sharif continues. “In a multi-stakeholder environment, such as track-and-trace solutions, you need to be able to allow different companies using different systems to be able to communicate with each other,” he says. “Blockchain is a very good interoperable, technology-neutral kind of solution, and so I would advocate using blockchain.”

In terms of any potential future anti-counterfeiting challenges, such as stricter requirements, blockchain is considered to be ‘future proof’. When looking at tools such as artificial intelligence or machine learning, Sharif highlights that there are potential limitations in terms of data corruptibility. As the solutions are only as good as the data that are input into them, should the data become corrupted then the results being obtained will be bad. Sharif confirms. Whereas, with blockchain solutions, data integrity is assured. Furthermore, blockchain platforms consume less energy than other technologies and will be required for automated processes, such as ordering and supply chain tracking.

A good example of how blockchain can aid with assurances of data integrity, particularly in the landscape of clinical trials, would be to take the recent approvals of COVID-19 vaccines, Sharif notes. MHRA was the first to approve a vaccine, as a result of the innovative way it considered data—assessing the clinical trial results in a rolling review capacity. “Imagine if MHRA could review the data digitally from a data platform with the assurance that the data hadn’t been altered, that would really revolutionize the way clinical trials are performed and data are approved by regulators,” Sharif summarizes.

References
With suppliers setting priorities, companies are receiving notifications (e.g., single-use materials, resins) to re-confirm and re-route deliveries. There’s the risk of over-ordering and creating safety stocks, which creates inefficiencies across the industry.

Recognizing how interconnected the bio/pharma industry is, and how shortages in one area affect other medicines, is critical to defining any solution for improving materials distribution and logistics.

People and ways of working: Impact of COVID-19

During the COVID-19 crisis, it’s been a challenge simply to keep manufacturing facilities open and running. One company with seven sites, all of which needed to be kept open, developed new ways of working. This experience has, of course, accelerated implementation of remote working practices, previously seen as having significant blockers.

Starting in mid-2020, the bioprocessing industry performed an impressive and rapid response to transfer and scale-up despite lack of clarity on basic information (e.g., on final formulation, stability data, dosage, and volumes). In addition, forecasting has been difficult; the volume and scales required for COVID-19 vaccines and therapies are a new experience for many companies. In one example, with work starting in mid 2020, the first commercial-scale vaccine material under good manufacturing practice (GMP) was produced in November 2020. However, this speed of response has resulted in less-than-optimal processes requiring further work to improve robustness of the process, and to respond to increasing demand volumes.

The industry also needs to consider ‘people resources’ capacity. Bio/pharma companies need people with the right education and pharma-specific skills. It is now near-impossible to source expertise across regions—both for manufacturing and support functions.

Monumental challenges are facing manufacturers, contract development and manufacturing organizations (CDMOs), and suppliers during the evolving situation in the manufacture of COVID-19 vaccines. BioPlan Associates interviewed front-line bioprocessing decision-makers to assess and highlight the general bioprocessing, raw materials, and supply chain challenges associated with COVID-19 vaccines, together with need for approaches to moderate risks and avoid delays. The following are key challenges for COVID-19 vaccine manufacturing and supply chains.

Availability of materials

The most critical factor raised in regard to COVID-19 vaccine manufacturing has been the availability of materials. While this has not been entirely unexpected, the delays and challenges have stressed the supply chain severely. From basic raw materials (e.g., buffers, resins, sodium chloride), to consumables (e.g., single-use bags, tubing, sterile filters) and fill/finish (e.g., vials, stoppers)—these all need to be available and in place for the system to function. Delays for delivery of some of these materials are forecast to be 12 or 15 months; sterile filters delivery times have been extended from 2–3 months to 9–12 months; lead-times also have extended for media and chemicals. And while some suppliers have the ability to expand with flex-capacity—a glass vials supplier is increasing output by five times—most do not.

Where suppliers can’t supply, they’re making priorities. The common view was that one needs to see the whole picture of demand with critical stock available for everyone to access. This ‘emergency’ supply chain management is new to the industry and is evolving. Suppliers are used to having known, predictable demand. As demand has increased considerably, old methods are now ineffective. For users, it is not straightforward to bring on and qualify new suppliers. There are some key materials (e.g., vials, syringes, stoppers, seals) from a limited number of suppliers, which limits flexibility to increase the supplier base.
Manufacturing capacity and supply chain
The overall view of those interviewed is that COVID-19 challenges are likely more centred on the fill/finish activities rather than drug substance manufacture. With low dosing, relatively small amounts of active product are needed to then manufacture large volumes of drug product.

Even before the pandemic, there has been experience of shortages of biological manufacturing equipment and supplies (e.g., bioreactors, single-use devices, analytical support), as organizations planned against a changing product and service portfolio. The ability to respond is now compromised, as travel restrictions make it difficult to have vendors on site to install and qualify new equipment (e.g., filling lines). One company BioPlan spoke with estimated a one-year delay to commission new facilities.

For processing capacity, the challenge is probably not stainless-steel vessels, because many vaccines are produced via single-use systems. In terms of global capacity, there does not seem to be an overall or strategic view of capacity availability and awareness of capacity builds; a better overview and coordination of demand and capacity across the bio/pharma industry is needed. The ad hoc approach to bioproduction of the various vaccines in development and in production has, and will continue to, lead to competition for raw materials, consumables, and supplies.

For COVID-19, the industry has come together in the short/medium term on the technical side, but this collaboration is limited in terms of resource allocation and supply of materials, both for COVID-19 and other medicines. The concern is how sustainable these technical collaborations may be in the longer term. What will normal capabilities be after the pandemic?

For example, the bio/pharma industry has focused on COVID-19 and this has been well supported by in-house quality assurance and regulatory functions and the regulatory authorities. This enabled accelerated approval and rapid product clearance for patients. COVID-19 vaccine production is creating significant bottlenecks in the biologics supply chain, and it’s unclear how this may be impacting the wider portfolio with other products in development and already in commercial supply.

The concern is how sustainable these technical collaborations may be in the longer term. What will normal capabilities be after the pandemic?

Existing and new business
Bio/pharma businesses continue to look to grow through increased capacity to meet existing customer demands (e.g., as CDMO service providers and to manufacture biosimilars). There has been significant new demand (drug substance and product) since mid-2020 in response to COVID-19. The ability to respond can be in direct competition to existing business, made more challenging with the high expectation on speed of response. From an overall business perspective, there’s clearly the challenge to respond to COVID-19, but also continue to supply other medicines and treat other ill patients.

Investors (including government) have been providing financial support and are taking risks to support the development of COVID-19 vaccines and therapies. It seems, however, that the cost of goods and sales price have not been significant drivers. The business is being driven by the need to reopen the world; this drive and investment has enabled access to, and speed of, the COVID-19 supply chain.

Increasing capacity demands
The overall view of manufacturers interviewed is that there may be sufficient (even if near 100%) available bioproduction capacity, if it could be efficiently coordinated. Despite capacity being available around the world, issues of intellectual property, regulatory factors, and quality challenges must be rapidly resolved in order to make capacity useable. The challenge here is how to become more flexible with how existing, available capacity is used and maintain a balance across the whole product portfolio.

For example, there’s the potential renaissance of the stainless-steel vessel as an enabler of mass production. Clients want to avoid shortages, and there seems some movement back to stainless rather than single-use systems, if technically feasible. There is the view from some that even if external capacity is available, some companies would still prefer to develop in-house, rather than have the delays (greater than six months) of qualification and technology transfer to a third party. For some organizations, there’s the preference to ramp up production capacity and scale up capabilities internally. There are, for example, some domestic facilities in China that have considered offering GMP large-scale capacity to the production of vaccines in order to create access to international markets. Despite the challenges in drug substance manufacture, the industry explicitly needs adequate fill/finish capacity to meet increasing global volumes for vaccines and other therapeutics that are being sidelined for vaccines production.

The bio/pharma industry has gained experience and efficiency over the past three decades, but nothing could have prepared it for the dramatic impact of the COVID-19 pandemic. Yet, it has risen to the crisis with remarkable speed. From development to vaccine delivery, the industry has created a scientific equivalent of the Wirtschaftswunder, the rapid reconstruction of German industry following World War II. Yet this has not been without impact on the underlying industry.
Outsourcing has increased due to accelerated development and approvals; processes are not developed for mass volumes. A solution is needed to improve capabilities to deliver better processes. This includes considering planning the process and the scale up in order to manage the capacity now, through, and after the pandemic. The same may be said about improving efficiencies across the supply chain. In discussion with industry experts, there’s the belief that there are opportunities to increase supply chain speed further in addition to the accelerated approvals seen via regulatory authorities’ fast-track approvals. Industry initiatives to optimize manufacturing and supply chains Most experts interviewed were not involved in or aware of significant initiatives underway to optimize supply chains; there is a lack of transparency to share issues and challenges.
information in real time. There was some variance in views as to whether the industry should coordinate ‘pandemic preparedness’; the industry has responded well with little apparent formal coordination. 

**COVID-19 response has accelerated implementation of new ways of working, but will these new approaches stick after the pandemic?**

There seems desire to align across the industry, but the reality is that companies are likely to still want to keep to their own practices. It’s been difficult to ‘predict the race’. With the variety of organizations and approaches, it would be very difficult to standardize longer-term preparedness. Some organizations have experienced significant growth due to COVID-19, but again this introduces more complexity to establish industry best practices and approaches.

Industry groups are working in this space (e.g., cross-industry forecasting). There are ongoing challenges to secure adoption of new ways of working, due to legacy regulatory and quality/compliance practices and established organizational culture. Some respondents expressed concern that after the emergency, the industry will go back to its normal ways of working.

**Conclusion**

The most critical factor raised regarding vaccine capacity has been the availability of materials. The industry must see the whole picture of demand with critical stock available for all stakeholders to access. With this holistic view, there should be better understanding of how supply chain challenges create shortages and how then to prioritize certain medicines and organization resources.

COVID-19 response has accelerated implementation of new ways of working, but will these new approaches stick after the pandemic?

The ability of a company to respond to COVID-19 can be in direct competition to existing business. It’s unclear what the impact is on the wider portfolio with other products in development and in commercial supply. Speed of response has resulted in non-ideal processes requiring further improvement to respond to increasing demand volumes. The solution is not just about making more capacity available, it’s more about capabilities to deliver better processes and supply chains.

Most experts interviewed were not involved or aware of significant initiatives underway to create long-term solutions to the problems that have surfaced regarding pandemic-level production challenges. Part of this is the result of a lack of transparency to share issues and information in real time.

The industry needs a better overview and coordination of demand and capacity and a network to solve problems together. Industry needs to become more flexible with how capacity is used—in particular, adequate fill/finish capacity to meet increasing global volumes, including new products in development. It is unlikely that this will be the last pandemic the bio/pharma industry will need to respond to.

**Reference**

later but sometimes forgot after the manufacturing run. In this simple exchange with the operator, you realize that the root cause of the repeat deviation is not a result of human error but a result of poor process flow.

Q: How much time should I allow for a deviation to be investigated?

A: The length of time it takes to complete an investigation depends on the complexity of the investigation. Simple deviations can be completed in a short time frame, but more involved deviations will take longer to complete. We recommend you set a time frame that is reasonable for your organization. For example, you might indicate that deviations will be investigated and completed between 30–60 days after the deviation was documented. This gives you some flexibility.

Q: Are out-of-specification (OOS) results considered deviations?

A: No. OOS results need to be investigated separately and quickly due to the potential impact to the product. If the root cause of the OOS cannot be attributed to laboratory error, you should initiate a deviation/investigation to determine what happened during product manufacturing that attributed to the erroneous laboratory result.

Reference

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We look forward to hearing from you.
Frequently Asked Questions on Deviations


Q. What is a deviation, and do all deviations need to be investigated?

A. A deviation is when there is a failure to follow the instructions guiding the performance of how an activity should be executed for optimal results. Simply put, deviations result from people not following their standard operating procedures (SOP), work instructions, batch record instructions, or other documents that explain what needs to be done in performing certain functions or tasks. Deviations relate to non-compliance and are a serious issue. While not all deviations are equal in their impact on product and quality, all should be investigated.

Q. What is a planned deviation?

A. In our opinion, there is no such thing as a planned deviation. Planned deviations were supposed to justify changes from SOPs that would be utilized to carry out the operation over a certain period of time. The current thinking by regulatory authorities is there is no such thing as a planned deviation. During a breakfast session at the 2018 PDA/FDA Joint Regulatory Conference, a representative from the US Food and Drug Administration (FDA) stated, “it’s a very strange term, and it kind of makes your skin crawl a little bit” (1). If you need to make a change to a procedure for a short period of time, we suggest you use the change control system to document the change. This may seem like a picky point, but until the change is evaluated for its impact on the process validation requirements and formally documented in a change control, encouraging the deviation’s continued use is supporting a posture of non-compliance.

Q. Why is human error not an acceptable finding for deviations?

A. The overuse of human error as a root cause to a deviation represents lost opportunities to reduce future issues by masking the identity of the true root cause. Herein is the human error in Human Error. The question that needs to be asked during the initial investigation into a deviation is what caused the employee to make the human error? Asking this question early in the investigation leads to better root cause identification and opportunities for continued improvement.

Let’s look at an example that might help clarify why human error can mask the real root cause of a deviation. Suppose, for example, you have a second shift manufacturing operator who continually forgets to sign a step in the batch record for a specific product. This operator is the only one who seems to have this issue. Your initial investigation into the first occurrence of the issue determines a root cause of human error. Because the operator works on the second shift, it is inconvenient to interview him directly, so you rely on the word of his supervisor that this was just a case of human error. You decide to retrain the operator on the proper use of filling out the form and skip the operator interview in order to complete the investigation and perform the retraining in the allotted 30-day time frame. This scenario repeats itself 10 times over the course of four months. You finally decide to interview the operator directly. When you talk to the operator, he informs you that in order to sign the batch record when it needs to be signed, he needs to exit the aseptic core, degown, sign the batch record, and regown, leaving the product unattended during that time. The operator tells you he chose to stay with the product and sign the batch record...
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