PDA Journal of Pharmaceutical Science and Technology

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CONTENTS

EDITORIAL
Editor’s Note
Richard Levy 367

RESEARCH
A Vial Container Closure System Performance Optimization Case Study Using Comprehensive Dimensional Stack-Up Analyses
Anthony Bucci, Le Ho, Lauren Orme, and Qingyu Zeng 368

Determining the Clearance of Degraded Protein via a Monoclonal Antibody Purification Process in Support of Cleaning Carryover Limits in Multiproduct Facilities
Pao Lin Che, Alison P. Bailey, Chi Yan J. Tam, Melissa Alvarez, and Adeyma Y. Arroyo 377

Comparison of Limulus Amoebocyte Lysate and Recombinant Factor C Assays for Endotoxin Detection in Four Human Vaccines with Complex Matrices
Marine Marius, Frédéric Vacher, and Thierry Bonnevay 394

CASE STUDY
Environmental and Personnel Monitoring Programs—A Risk-Based Case Study of Cutibacterium acnes
Edward C. Tidswel and Kenneth Boone 408

REVIEW
A Semiquantitative Risk Assessment Methodology Fit for Biopharmaceutical Life Cycle Stages
Ajay Babu Pazhayattil, Naheed Sayeed-Desta, Shu Chen, and Marzena Ingram 423

COMMENTARY
Steam Sterilization Chemical Indicators Are Not Adequate for Monitoring Real Steam Sterilization Cycles
Paulo Roberto Laranjeira, Rafael Queiroz De Souza, Jeane Aparecida Gonzales Bronzatti, and Kazuko Uchigawa Graziano 435

The Goldilocks Challenge—Controlling Uncertainty When Setting Product Specifications
Richard K. Burdick and Julia C. O’Neill 439
CONTENTS continued

Continuous and Effective Microbiological Air Monitoring in Critical Environments: A Comparison of Analytical Methodologies 446
Gilberto Dalmaso, Anna Campanella, and Paola Lazzeri

Industry One-Voice-of-Quality (1VQ) Solutions: Effective Management of Post-Approval Changes in the Pharmaceutical Quality System (PQS)—through Enhanced Science and Risk-Based Approaches 456
Emma Ramnarine, Anders Vinther, Kimberly Bruhin, Christina Tovar, and Marcello Colao

PDA PAPER
Controls to Minimize Disruption of the Pharmaceutical Supply Chain During the COVID-19 Pandemic 468
EDITOR’S NOTE

July-August 2020 Issue

This issue of the PDA JPST includes a diverse collection of papers focusing on packaging science—container closure integrity, cleaning and carryover limits in an mAb process, endotoxin testing, environmental and personnel monitoring, product specification setting, risk assessment and post-approval changes. It also features the first work product of the PDA COVID-19 Task Force, a commentary on minimizing the effects of the virus on the pharmaceutical supply chain.

On the research side, a paper on a vial container closure system (CCS) performance optimization study uses a parallel design computer program that can model the stack-up process for any quantity of vial CCS components. The authors propose that this program can also aid troubleshooting and ensure that a packaging process is operating within the optimal CCS performance window.

For those of you who follow endotoxin testing, as well as vaccines, one paper compares LAL and recombinant Factor C endotoxin testing assays in human vaccines with complex matrices. We also have a risk-based EM case study focused on the control of Cutibacterium acnes in parenteral manufacture.

Another article introduces a semi-quantitative risk determination tool that can be used to systematically evaluate material attributes and/or process parameters to scientifically establish “reliable, robust and efficient risk assessments” applicable in biopharmaceutical development and manufacturing.

Three commentaries included in this issue cover steam sterilization chemical indicators, balancing risk when setting product specifications, and continuous microbiological air monitoring in critical environments.

The fourth commentary focuses on the management of post-approval changes. Authored by members of the One-Voice-of-Quality collaboration, the paper proposes specific science- and risk-based solutions that converge to support industry alignment and the standardization of post-approval changes within the pharmaceutical quality system.

Finally, we offer a very timely and useful PDA Paper authored by members of the newly formed PDA COVID-19 Task Force. This paper discusses the challenges our industry is facing during the COVID-19 pandemic as well as GMP controls that can help manufacturers minimize the spread of the virus and the disruption of the pharmaceutical supply chain.

Until the next issue, please contact me any time at journal@pda.org with any questions you may have or ideas you would like to contribute.

Regards,

Richard Levy
Editor-in-Chief

A Vial Container Closure System Performance Optimization Case Study Using Comprehensive Dimensional Stack-Up Analyses

ANTHONY BUCCI, LE HO, LAUREN ORME, and QINGYU ZENG*

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ABSTRACT: Compatible vial container closure system (CCS) components in combination with a proper capping process are crucial to ensuring reliable performance, maintaining container closure integrity (CCI), and achieving CCS visual acceptance. CCI is essential for parenteral packaging and must be maintained throughout the entire sealed drug product life. In order to build the most robust CCS performance, many variables, including component selection, fit, function, and capping processes, must be set according to the actual dimensions of the CCS components used. However, conventional CCS stack-up calculations are based on dimensional engineering data and its tolerance from CCS component drawings without consideration of the real statistical distributions and their resultant impact on the risk of CCS end performance. CCS dimensional variations may lead to capping failure, resulting in CCS visual defects, CCI failure, and potentially costly destruction of an entire CCS production batch. In this paper, we demonstrated a comprehensive approach utilizing real CCS component dimensional data as a statistical input for CCS dimension stack-up calculations to calculate the actual CCS end performance window and the CCS’s quantitative failure risk to determine the CCS’s optimal sealing performance and visual acceptance under different stopper compression percentages. We examined two vial CCSs differing by the stopper as a case study. Each component was measured and included in comprehensive dimensional stack-up calculations. The resulting statistical distributions were used to examine component variability and stack-up assemblies at multiple stopper compressions and to identify the optimal CCS based on the performance window generated from the real data. Using this data-driven approach, we quantitatively identified that as little as 5% stopper compression difference could impact the CCS chosen. More importantly, comprehensive dimensional stack-up calculations can assist in selecting the best vial CCS and appropriate stopper compression, as well as troubleshoot processing concerns and ensure operation within the optimal CCS performance window.

KEYWORDS: Container closure system (CCS), Residual seal force (RSF), Container closure integrity (CCI), Stopper, Vial, Cap, Seal, Capping, Modeling, Performance Window, Dimensional Testing.

Introduction

A popular container closure system (CCS) configuration for parenteral drug products is composed of three major packaging components: a glass vial, a rubber stopper, and an aluminum seal (1). The final parenteral product is determined by not only the CCS components (vial, rubber stopper, and aluminum seal) but also by the capping process. After capping, the sealed drug product must maintain container closure integrity (CCI) throughout its shelf life. A CCI breach from oxygen, carbon dioxide, or microbiological ingress can risk the drug product quality or patient safety. Therefore, selecting the appropriate vial, rubber stopper, and aluminum seal, together with a capping and sealing process optimized around those components, is essential for maintaining CCI.

A recent publication (2) provided a general overview of holistic considerations to ensure CCI performance. There are many test methods currently used to assess CCI performance. Although these CCI test methods evaluate leakage of the system, residual seal force (RSF) testing is commonly used in industry as a complementary measurement to confirm seal tightness. RSF is the force that a rubber closure flange exerts against the
vial land seal surface of an assembled, capped CCS (2, 3, 4, 5, 6, 7, 8, 9). The resultant RSF values are directly influenced and controlled by the selection of the rubber stopper, vial, and seal as assembled during the capping process setup (10, 11, 12). Additionally, the RSF is included in USP <1207> (4) as a seal quality test for parenteral vial assemblies.

In prior study (13), it was shown that there is a correlation between the RSF and the CCI. Generally, as shown in Figure 1, a high RSF CCS will lead to better average helium leak rate (AHLR) results than those of a low RSF CCS. By tuning the process to achieve a high RSF, it is possible to remain below the maximum allowable leak limit (MALL), maintain CCI, and reliably produce a high-quality CCS. There is a limit to what constitutes a high RSF, however, as shown in Figure 2A–D. When extremely large forces are applied to the rubber stopper, the viscoelastic nature of the material results in deformation of the rubber, such as stopper dimpling (Figure 2B). It is also possible to damage the seal or cause the vial to fail. Examples of seal damage include skirt wrinkling (Figure 2A) or buckling damage to the aluminum during crimping (Figure 2C, D). Although not pictured, excessive force can also crack the glass vials. To avoid these defects and maintain CCI, it is essential to remain within the performance window where there is a balance between the stopper compression limit (Figure 2 purple vertical lines) and seal skirt overhang length limit (Figure 2 red horizontal lines). Each product has a unique performance window that must be identified and adhered to.

The stack-up calculation is particularly helpful in this regard, defining the amount of aluminum available for crimping a vial system. If the overhang is too small, undercrimping can occur, and the vial system may not assemble properly, threatening CCI. Overcrimping, which results in too much overhang, increases the likelihood of CCS visual defects like those in Figure 2. Both situations are unacceptable and affect visual acceptance as well as potentially CCI. The conventional stack-up calculation is usually based on dimensions specified in component engineering drawings to estimate whether adequate sealing will occur at a given stopper compression. In addition, deciding on an ideal overhang length and stopper compression is often based on past experiences without consideration of the actual CCS dimensional variations. This can lead to costly manufacturing defects especially if entire lots of product need to be destroyed because of uncertainty in the resultant CCS performance and/or visual inspection rejection.

We have previously reported on the need to consider each component in the CCS as a statistical variable when designing a pharmaceutical capping process (13). It is known that CCS components can exhibit a range of dimensions as specified on the engineering drawings. However, few consider the dimensions of each physical piece received and instead rely on the reported engineering specifications. Additionally, components are often sourced from different suppliers and have limited information on compatibility. Relying only on the drawing dimensions limits the ability to fully understand the variability within a population of components. As a result,
it is difficult to assess the likelihood of successful assembly, capping, and sealing for a given CCS. As empirically experienced in the pharmaceutical industry, the result over time is unexpected failure, which manifests as CCI failure, visual defects, or incomplete sealing.

In this work, we showed an approach utilizing real CCS component dimensional data as statistical input to calculate the actual CCS end performance window and its quantitative failure risk in terms of its sealing performance and visual acceptance. A case study examined two CCSs differing only in the stopper. We quantified how the stopper flange thickness, vial flange thickness, and seal skirt length varied and calculated the exact statistical distributions illustrating the range of sizes for each component. Examining the component and stack-up statistical distributions for the two vial CCSs, we showed that it is possible to identify the CCS dimensional window at any stopper compression. Lastly, the same statistical distributions could be used to not only identify the condition that suits a process but also quantify the data-driven risk assessment of being outside the performance window over a range of conditions as well. In this way, the best CCS can be selected and applied for application-specific needs. This approach can also be applied for troubleshooting.

**Experimental Testing Setup**

Two vial CCSs were evaluated in this study. Two 20 mm serum stoppers from different manufacturers were selected for comparison. The seal and glass vial were kept constant and a standard 20 mm aluminum I-seal without a flip-off cap and an ISO 6R tubular glass vial were used. For each of the four components, 500 pieces were obtained from a single batch and each seal length (Figure 3C), stopper flange thickness (Figure 3B), and vial flange thickness (Figure 3A) was measured in the laboratory in ambient conditions and its value was entered into a database. The dimensions were measured using a combination of vendor specific quality control procedures, which are proprietary, including digital gauges and optical comparators. A custom high-performance computer code was then developed to calculate the seal skirt overhang length and the resulting statistical distribution for every combination of components at various stopper compressions. The seal skirt overhang length was defined as the amount of aluminum available to tuck under the vial flange after compressing the CCS and was calculated as shown in Figure 3.

The number of ways that the components could be assembled for each CCS at a particular stopper compression was given by combinatorics as \(A \times B \times C\), where
Figure 3
Schematic representation of a container closure system vial system showing the vial flange (A), the stopper flange (B), the overall seal skirt length (C), and the seal skirt overhang length (D).

\[ A = B = C = 500, \text{ or } 125,000,000 \text{ potential CCS combinations.} \]

Histograms generated with this approach reported the probability of each possible seal skirt overhang length for a given stopper compression percentage and were saved into a database. The database information was then retrieved and viewed using a graphing utility where the performance window was overlaid and explored. The custom high-performance computer program was then generalized to accept any number of components, as well as utilize cloud-based parallel computing to efficiently handle data sets of any size. The methodology does not have any limit to the sample size as long as the computer network infrastructure can host all of the data. Practically, there is almost no limit to how much data can be stored in a computer network, and our methodology took full advantage of this. The 500 pieces for each of the four components from a single batch were just for the case study to comprehensively show how the methodology works by using real data for calculation of the CCS end performance window.

Experimental Testing, Results, and Discussion

This work focused on:

1. data analysis of the CCS component dimensional data,
2. exploration of the CCS performance window, and
3. quantification of the risk of falling outside the performance window.

We previously reported (13) on the need to treat each of the components in a vial system—the rubber stopper, aluminum seal, and glass vial—as statistical variables. This need was a result of the variability within the component manufacturing processes that generated a range of critical dimensions. During the capping process, these variable dimensions might cause visual defects and/or possible CCI failure. Here we addressed the variability in dimensions by identifying and quantifying those qualities and used this information to minimize their adverse effects as part of a case study. Additionally, CCS component variability statistics could then be used as input for pharmaceutical manufacturing process development and optimization.

Typically, CCS component dimensions are measured by the manufacturer and reported as the average and standard deviation of the seal skirt length, vial flange thickness, or stopper flange thickness. This information is based on manufacturer-specific quality control procedures. Here, we assumed that the 500 pieces of each component represented a single, complete batch. By measuring each piece, we reported the precise component average and standard deviations in Table I, eliminating the uncertainty typically encountered. Based on the averages and standard deviations of the stoppers, we saw a small difference between the average flange thicknesses with Stopper1 exhibiting nearly threefold greater variability. However, this statistical summary, although more accurate than only using engineering specifications, did not describe the true range of stack-up configurations because there was no way to quantify how component-to-component variability influenced the CCS assembly.

After examining the top-level information provided by the component averages and standard deviations in Table I, we looked at the individual distributions associated with each CCS. These histograms illustrated the probability of a given component size in the unassembled state, providing additional insight into the components used in CCS1 and CCS2. In Figure 4, we show the individual component distributions for the four components used in this study.

During the computer calculations, every piece was accounted for, and the range of values on the x-axis for each histogram in Figure 4 spanned the smallest dimension through the largest. Therefore, a finite probability exists for every value on the x-axis. Examining the seals (Figure 4D), the probability of a piece having a skirt length of 7.478 mm was 0.002. Additionally, the average seal skirt length for the 500 pieces was 7.579 mm and its probability was approximately 12%. Examining the
stopper and vial, similar percentages lay at the calculated average of the parts. The repercussions of this are profound when designing a pharmaceutical capping process. A process setup based on the component averages alone might exhibit uncertainty in the size of up to 85% of the components received. Using a comprehensive dimensional stack-up calculator, we captured the dimensions of every piece, ensuring that each one was accounted for and available as input for further process development and optimization.

The stack up and resulting seal skirt overhang length was dependent on the stopper compression. Examining the stack-up results at 25% compression, a common target, for CCS1 (Figure 5A) the variability in the stack up could be as much as 0.607 mm, with the seal skirt overhang length ranging from 0.801 mm to 1.408 mm. CCS2 (Figure 5B) exhibited a variability of 0.445 mm, with seal skirt overhang lengths ranging from 1.093 mm to 1.538 mm. Once again, we saw the need for pharmaceutical manufacturing processes to account for a range of values instead of the component averages.

### TABLE I
Summary Data from the Two Container Closure Systems Evaluated. Five Hundred Pieces of Each of the Four Components Were Measured and Used During the Comprehensive Calculation

<table>
<thead>
<tr>
<th>Selection</th>
<th>Information</th>
<th>Individual Component Average (mm)</th>
<th>Component Standard Deviation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stopper Flange Thickness</td>
<td>Stopper1</td>
<td>3.554</td>
<td>0.076</td>
</tr>
<tr>
<td>Seal Skirt Length</td>
<td>Seal1</td>
<td>7.579</td>
<td>0.037</td>
</tr>
<tr>
<td>Vial Flange Thickness</td>
<td>Vial1</td>
<td>3.784</td>
<td>0.018</td>
</tr>
<tr>
<td>Stopper Flange Thickness</td>
<td>Stopper2</td>
<td>3.311</td>
<td>0.028</td>
</tr>
<tr>
<td>Seal Skirt Length</td>
<td>Seal1</td>
<td>7.579</td>
<td>0.037</td>
</tr>
<tr>
<td>Vial Flange Thickness</td>
<td>Vial1</td>
<td>3.784</td>
<td>0.018</td>
</tr>
</tbody>
</table>

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Figure 4

Statistical distributions for CCS1 stopper flange thickness (A), CCS2 stopper flange thickness (B), vial flange thickness (C), and seal skirt length (D).
of simply an average or target. Using our comprehensive dimensional stack-up calculations, it was possible to quantify the percentage of assembled and compressed systems that should fall within a specific range. Identifying the most common assembly, it was 22% with an overhang of 1.153 mm for CCS1 and 23% with an overhang of 1.304 mm for CCS2. Interestingly, these values were similar to the calculated stack up based on the averages, 1.129 mm and 1.312 mm for CCS1 and CCS2, respectively. The drawback to using the averages, as noted previously, is that there is no indication of how many assembled systems meet this criterion. For the 125,000,000 possible combinations of components that were measured for each CCS, we could now say that up to 22% will meet the seal skirt overhang length calculated from the average component dimensions. A process designed around the averages alone will once again exhibit extreme variability, here with uncertainty of the size in approximately 78% of the assembled and compressed CCSs. However, using our comprehensive stack-up calculator approach, we can now precisely identify the percentage of assemblies that meet a given seal skirt overhang criterion. In fact, taking this one step further, our approach can determine the range of seal skirt overhang lengths that encompasses any amount of the data. To achieve a minimum guaranteed correct assembly of 95% of all components, 95% fell between a seal skirt overhang length of 1.056 and 1.249 mm for CCS1 (Figure 5A). For CCS2 (Figure 5B), 97% of the seal skirt overhang lengths fell between 1.210 and 1.398 mm. By extension, this analysis could

Figure 5

Results of the comprehensive stack-up analyses for CCS1 at a fixed stopper compression of 25% (A) and for CCS2 at fixed stopper compressions of 25% (B).
be performed for any portion of the data, and the histograms tuned to provide as much information as needed. This breadth of dimensional stack-up data was previously unavailable when evaluating a CCS. Now, using our comprehensive dimensional stack-up calculations based on real testing data, it is possible to take variables into account systematically when choosing a CCS together with a proper capping process. This permits a robust examination of any CCS selection so that the most informed decision possible can be made. These same techniques can also be applied to explore existing processes to account for previously unknown CCS component variability to aid troubleshooting.

When choosing the best CCS, understanding and identifying the performance window is key. There must be a maximum and minimum seal skirt overhang that is determined based on the requirements of the drug product. These limits are dependent on how much risk is acceptable for a specific drug product with regard to sealing performance and visual acceptance. For our case study in Figure 6, the performance window was selected to illustrate how small changes in rubber stopper compression could affect whether CCS1 (red) or CCS2 (green) would be selected. As an example of the case study, the performance window for overhang length was selected to be between 0.75 and 1.5 mm while achieving a stopper compression from 15% to 30%. Examining the statistical distributions within the performance window, we can select the best CCS and utilize sound data-driven quality by design (QbD) decision making. For 100% of the CCS components to assemble properly, at a compression of

Figure 6

Statistical distributions for CCS1 (red) and CCS2 (green) overlaid on the performance window at fixed stopper compressions of 15% (A), at fixed stopper compressions of 20% (B), at fixed stopper compressions of 25% (C), at fixed stopper compressions of 30% (D), and legend (E).
20% (Figure 6b), CCS2 (green) is the best choice, whereas at 25% compression (Figure 6c), CCS1 (red) is ideal.

Expanding the scope to identify ranges of conditions that best fit CCS1 or CCS2, as little as 5% compression separates the two. According to Table II, at a target compression of 20%, CCS1 had a 0.1% chance of stacking up incorrectly. It is common to see slight variations in stopper compression owing to the capping process setup as well as in-process operator modifications. For processes that may undercompress or for slight in-process modifications that reduce the stopper compression, we saw that CCS1 will begin to fail at nearly 30% as you approach 15% stopper compression. For reference, based on the average stopper flange thickness, the difference between 15% and 20% compression in CCS1 was 0.177 mm (0.007 in), which was only half the reported standard deviation of the stopper. On the other hand, CCS2 exhibited a zero chance of failure from 15% to 25% compression, indicating that it was more robust to in-process variability or slight in-process modifications that affect stopper compression. Therefore, for processes and CCI that require rubber stopper compression approaching 30%, CCS1 was the best choice, whereas for applications requiring compression of 25% or less, CCS2 would be recommended.

### Conclusion

This comprehensive CCS dimensional stack-up case study examined two vial CCSs with each having 500 individual stoppers, seals, and vials. A high-performance computer program designed to calculate the stack-up assembly calculated every possible combination of vial CCS components and determined the probability for each of the 125,000,000 combinations of components at stopper compressions ranging from 0% to 50%. A total of 250,000,000 combinations of stopper, seal, and vial were calculated for the two CCS for any compression. Using the calculated statistical distributions, we mapped out the vial CCS performance window as well as the quantitative risk for falling outside the performance window and risking visual defects and/or CCI failure. We showed that based on component averages alone, approximately 20% of the data falls at the reported average, leading to an uncertainty in the size for up to 80% of the components entering a process. Using this real data approach, we also identified what the most probable assembly was for each CCS as well as the range of seal skirt overhang lengths encompassing any percentage of assemblies in the performance window at any stopper compression. Lastly, at stopper compressions in a window ranging from 15% to 30%, we precisely determined the probability of remaining in the performance window as well as the range of stopper compressions that each CCS was best suited for. As little as 5% stopper compression was the difference between a failure rate of 0% versus 30%, reinforcing the fact that CCS performance is influenced by both component and capping process variability. Our computer program was developed to generalize the stack-up process for any quantity of vial CCS components and can efficiently process large amounts of data because of its parallel design backed by powerful database infrastructure. In general, our comprehensive approach of stack-up calculations can assist in selecting the best CCS and appropriate stopper compression, as well as troubleshoot processing concerns and ensure a process is operating within the optimal CCS performance window.

### Acknowledgments

Thanks to Anand Ramachandran and Christoph Edmunds for information technology support. Thanks to Wayne Curry, Erik Anderson, Cathy Zhao, and Fran DeGrazio for review and suggestions to this paper.

### Conflict of Interest Declaration

The authors declare that they have no competing interests.

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**TABLE II**

At Each of The Four Compressions Within the Performance Window, the Probability of Falling Outside the Performance Window Is Listed for Each Container Closure System (CCS)

<table>
<thead>
<tr>
<th>System Number</th>
<th>Stopper Compression (%</th>
<th>Probability of Falling Outside the Performance Window</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCS1</td>
<td>15</td>
<td>0.333</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.000</td>
</tr>
<tr>
<td>CCS2</td>
<td>15</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.305</td>
</tr>
</tbody>
</table>
References


Determining the Clearance of Degraded Protein via a Monoclonal Antibody Purification Process in Support of Cleaning Carryover Limits in Multiproduct Facilities

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ABSTRACT: Cleaning validation acceptance criteria in multiproduct facilities are established using maximum allowable carryover calculations. Carryover calculations incorporate the shared equipment surface area between two products to ensure that an acceptable limit for residue from the previously manufactured product to the subsequent product is determined. The shared surface area can be limited to areas where carryover presents the highest risk to product quality or patient safety. In these cases, specifically for biologic drug substance manufacturing, the shared surface area is limited to equipment after the purification process based on the assumption that the purification process would remove potential product fragment residues from the previous product. Until now, this assumption has been based on empirical knowledge without experimental data quantifying the clearance or removal of potential residues. We present a three-part study that determined the effects of cleaning conditions on selected monoclonal antibodies (mAbs) and the generation of degraded fragments and evaluated the clearance of both the degraded mAb1 in a laboratory setting and the degraded fragments in the presence of a subsequent product, assessing the risk of co-purification. Several analytical techniques were used, including gel electrophoresis, capillary zone electrophoresis/laser-induced florescence detection, and liquid chromatography-mass spectrometry. Protein fragment generation was demonstrated for five different mAbs from different immunoglobulin G subclasses. The clearance of the degraded fragments in the absence and presence of the subsequent product was determined by calculating fold clearance and log reduction value (LRV) for each chromatography step. The data showed that the fragments generated during cleaning could be removed by the purification process. The fold clearances were determined to be values of 5400 (3.7 LRV) in the absence of subsequent product and 4428 (3.6 LRV) in the presence of subsequent product. The results supported the removal of product residues from shared surface areas by the purification process in multiproduct biologic drug substance manufacturing facilities.

KEYWORDS: Cleaning, Carryover limits, Degraded protein clearance.

1. Introduction

Cleaning validation programs in multiproduct manufacturing facilities include an assessment of the carryover of process residues from a previously manufactured product (Product A) to the subsequent or next manufactured product (Product B). Carryover assessment is performed by calculating the total mass of residues of Product A allowed in a batch of Product B based on batch size, maximum therapeutic dose, and acceptable daily exposure. This amount is generally referred to as the maximum allowable carryover (MAC), which may be calculated as described by the Parenteral Drug Association Technical Reports 29 and 49 (1, 2).

The calculated MAC is used to establish acceptable carryover limits. The MAC is the total mass of residues of Product A allowed on the surface area of equipment that is shared in the manufacture of both Product A and Product B. The carryover limit is calculated by dividing the MAC by the shared surface area, resulting in a mass per unit surface area limit. The carryover limit
can also be calculated in terms of the mass per unit rinse volume by incorporating the rinse volume used during cleaning of the shared equipment. Thus, determining the shared surface areas and rinse volumes in multiproduct facilities are important requirements for establishing carryover limits.

The shared surface area used in carryover calculations can be based on the entire manufacturing process, from cell culture production to purification and final formulation. Depending on the manufacturing equipment scale, the calculated shared surface area can be on the order of $10^6 \text{ cm}^2$, which results in carryover limits that cannot be measured even with highly sensitive assays. This is a highly conservative approach that does not take into consideration exposure to cleaning conditions and the potential for previous product removal during the subsequent purification process.

Taking the potential for previous product removal into consideration, the shared surface area used in carryover calculations for the subsequent product can be limited to the surface area of the manufacturing equipment where further clearance of previous product removal is not expected. In other words, the surface area of equipment used in the purification steps and upstream of the purification steps can be removed from the carryover calculation as these steps are expected to clear the previous product-related residues. This approach is supported by International Conference on Harmonisation (ICH) guideline Q7, which states that cleaning validation “should be directed to situations or process steps where contamination or carryover of materials poses the greatest risk to API [active pharmaceutical ingredients] quality,” and that validation of cleaning procedures may not be necessary for equipment “where residues are removed by subsequent purification steps” (3).

The expected clearance or removal of previous product-related residues by a purification process can be justified based on available knowledge or understanding of purification performance. Purification of biologic drug substance products involves multiple steps designed to remove process- and product-related impurities. The clearance of process-related impurities is generally achieved through various chromatography steps. A typical purification process for monoclonal antibodies (mAbs) produced in Chinese hamster ovary (CHO) cells consists of three column chromatography steps that include protein A affinity chromatography, followed by cation exchange (CEX) chromatography and anion exchange (AEX) chromatography (4). These steps are required for the clearance of process- and product-related impurities to ensure the safety and the purity of the final product. Examples of impurities that are effectively cleared by chromatography include CHO proteins (CHOP; also known as host cell proteins) and viruses. Clearance of CHOP by the different chromatography steps has also been extensively documented. For example, viral clearance studies using AEX chromatography have shown clearance of both enveloped and nonenveloped viruses achieving log reduction values (LRVs) >4 across different AEX conditions (8–10).

The removal of CHOP and viruses by the purification process demonstrates the potential for the clearance of cleaning-related protein residues that may be present as carryover from the previous production run. It is expected that these residues, like CHOP and viruses, will also have varying size and charge properties. That is, protein residues potentially present on the equipment surface are exposed to caustic and/or high-temperature solutions during the cleaning procedures, which results in protein degradation and generation of fragments with a range of sizes and charges. Although the purification of degraded protein residues can be expected, we are not aware of any published studies that confirm this.

Here, we present a three-part study that demonstrates the degradation of selected mAbs when exposed to cleaning procedures typically employed in a manufacturing setting. Carryover residue removal throughout the various purification steps was quantified to determine the clearance of the protein residue. Part I of the study establishes the effects of the cleaning conditions on selected mAbs and the generation of degraded fragments. Parts II and III focus on evaluating the clearance of degraded mAbs in a laboratory setting with three chromatography steps. The degraded fragments were independently processed through each step to obtain effective clearance of the degraded fragments in each step. Part II investigates the clearance of the degraded fragments, which resemble the carryover protein after exposure to typical cleaning conditions. Part III investigates the clearance of the degraded fragments in the presence of another mAb, assessing the risk of co-purification. To ensure that the experimental conditions
represented a worst-case scenario from the standpoint of co-purification, two immunoglobulin G1 (IgG1) mAbs (mAb1 and mAb2) with similar properties (i.e., molecular weights, isoelectric points, and extinction coefficients) were selected.

The data presented herein provide experimental evidence in support of limiting the shared surface area used in carryover calculations to equipment or steps for which clearance or removal of previous product-related residues is not expected. This study provides a science-based rationale for removal of upstream shared equipment surface area up to, and including, the purification steps in carryover limits calculations by demonstrating mAb degradation by the cleaning procedure and quantifying the degraded fragments clearance through the purification processes.

2. Materials and Methods

The three-part study approach is summarized in Figure 1. Part I focused on degradation effects across different mAbs. Part II evaluated the clearance of degraded mAb1 through the purification processes. Part III evaluated the clearance of degraded mAb1 in the presence of mAb2 through the purification processes.

### 2.1. Part I: Degradation Effect across mAbs

#### 2.1.1. Monoclonal Antibody Stocks

To study the degradation effects across different mAbs, five mAbs that were expressed in CHO cells and produced at Roche network facilities were selected. The study stocks included two IgG1 mAbs, one IgG1 bispecific mAb (BsAb), one IgG2 mAb, and one IgG4 mAb.

#### 2.1.2. Degraded mAb Preparation

Each of the five mAbs was diluted to 375 μg/mL in purified water (PW). An equal volume of 1.0 N sodium hydroxide (NaOH) was added such that the final concentration of the caustic cleaning solution was 0.5 N NaOH. Each mAb was treated in 0.5 N NaOH at 20°C for 30 min. It has been reported that the combination of heat and a caustic cleaning agent was most effective at protein degradation (11). In our study, the ambient caustic treatment condition, 0.5 N NaOH at 20°C for 30 min, was selected as it is considered a less effective cleaning condition in protein degradation, and therefore, the worst case compared to that at elevated temperature. At the end of the incubation, PW was added to each mixture to reach a final protein concentration of 125 μg/mL. Caustic treated and nontreated samples were loaded into the sodium dodecyl sulfate–polyacrylamide (SDS-PAGE) gel immediately upon preparation.

![Figure 1](image-url)

**Three-part study approach.**

<table>
<thead>
<tr>
<th>Part I: Degradation effects</th>
<th>(mAb1, mAb2, mAb3, mAb4, mAb5) + caustic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part II: Clearance of Degraded mAb1</td>
<td>MabSelect SuRe</td>
</tr>
<tr>
<td>Part III: Clearance of Degraded mAb1 + mAb2</td>
<td>MabSelect SuRe</td>
</tr>
</tbody>
</table>
tion of the caustic treated samples was not performed, because we had previously observed precipitation of the protein when neutralized. The protein concentrations used to demonstrate degradation were selected to enable visualization of the mAb fragments on the SDS-PAGE gel while minimizing saturation of the signal intensity.

2.1.3. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. Non-reduced sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed on a 4%–20% precast polyacrylamide gel (BioRad Laboratories, Inc., Hercules, CA) with a running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3). Caustic treated and non-treated protein samples were mixed with 4× Laemmli sample buffer (BioRad) and incubated at 80°C for 10 min. Each lane of the precast gel was loaded with 3 μg of protein sample. Electrophoresis was carried out using an electrophoresis chamber (BioRad) with a power supply (Hoefer, Inc., Holliston, MA) set at 200 V for 40 min.

Once electrophoresis was completed, the gel was placed in fixing solution (50% methanol, 7% acetic acid) for 30 min and incubated in SYPRO Ruby stain (Invitrogen, Thermo Fisher Scientific, Waltham, MA) for at least 3 h. After staining, the gel was placed in washing solution (10% methanol, 7% acetic acid) for 30 min and rinsed with PW. The gel was imaged on a ChemiDoc MP Imaging System (BioRad).

2.2. Part II: Clearance of Degraded mAb1 through the Purification Processes

Three chromatography steps were performed using the AKTA Explorer 100 chromatography system (GE Healthcare Life Sciences, Uppsala, Sweden) at ambient temperature. The AKTA system was programmed to run established cycles for the three chromatography steps, namely MabSelect SuRe chromatography step (pre-elution, preregeneration, pre-equilibration, load, wash 1, wash 2, wash 3, elution, regeneration, equilibration, and storage), Poros XS chromatography step (equilibration, load, wash, elution, sanitization, and storage), and Capto Adhere chromatography step (equilibration, load, postload wash, regeneration, sanitization, and storage).

2.2.1. Chromatography System Setup. Protein A chromatography, CEX chromatography, and multimodal AEX chromatography were performed using a laboratory-scale system for the mAb2 purification process. Protein A chromatography was packed with MabSelect SuRe resin (GE Healthcare Life Sciences) in a 1.5 cm inner diameter (ID) glass column (Omnifit, Diba Industries, Inc., Danbury, CT) with a 10–12 cm bed height resulting in a 17.7–21.2 mL final column volume. CEX chromatography was packed with Poros XS resin (Applied Biosystems, Oxford, MA) in a 1.0 cm ID glass column (Omnifit) with a 22–27 cm bed height resulting in a 17.3–21.2 mL final column volume. Multimodal AEX chromatography was packed with Capto Adhere resin (GE Healthcare Life Sciences) in a 0.66 cm ID glass column (Omnifit) with an 18–22 cm bed height resulting in a 6.2–7.5 mL final column volume. Both MabSelect SuRe and Poros XS were operated in bind and elute mode, whereas Capto Adhere was operated in flow-through (F/T) mode.

For each chromatography step, a control run was performed with mAb2 without the degraded mAb1 spike. Product yield and chromatograms were evaluated after each control run to be representative of the large-scale mAb2 purification process. All purification studies used parameters, buffers, resins, and pooling criteria equivalent to those used in the large-scale mAb2 purification process, with the exception of protein load density, which was prepared for the different experiments (degraded mAb1 only; and degraded mAb1 with intact, not degraded mAb2).

2.2.2. Load Preparation. To determine the clearance of degraded mAb1 fragments, the load for each chromatography step was prepared by spiking degraded mAb1 fragments in phosphate-buffered saline (PBS) or respective elution buffers. Degraded mAb1 fragment samples were prepared by treating mAb1 with 0.5 N NaOH at 20°C for 30 min.

A load density calculation was performed to avoid overloading and allow for purification assessment before and after processing. In order to determine the load density for each chromatography step, the maximum and the target load density for each resin were reviewed. The load density of the degraded mAb1 fragments was calculated as the difference between the target and the maximum load for each step to maintain the same approach for Part II and Part III of the study. The calculated load mass was spiked in PBS for the MabSelect SuRe column; in MabSelect SuRe elution buffer for the Poros XS column, and in Poros XS
elution buffer for the Capto Adhere column. The pH and conductivity of the load was adjusted to the target load condition and passed through 0.22 \( \mu \)m filters (Corning, Inc., Corning, NY) before loading onto the column.

As no product peak was present in the degraded mAb1 load, the pre-elution, elution, and F/T steps were collected based on the expected volumes from a standard mAb2 chromatographic run. Fractions from each chromatography step were analyzed to determine the degraded mAb1 clearance. For capillary zone electrophoresis/laser-induced florescence detection (CZE-LIF) analysis, sample aliquots were taken without pH adjustment and stored frozen at \(-70^\circ\)C, per established protocol, before sample analysis. For SDS-PAGE analysis, fractions collected from each of the chromatography steps were at different pH thus aliquots were pH adjusted to 6–8 before sample analysis.

2.2.3. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. Protein samples were prepared by collecting fractions during the MabSelect SuRe, Poros XS, and Capto Adhere chromatography steps. Each lane of a precast gel was loaded with pH-adjusted protein sample from the various fractions.

Non-reduced SDS-PAGE analysis was carried out as described in Section 2.1.3 except that the gel was loaded with 35 \( \mu \)L of protein samples, which were collected from each fraction of the purification chromatography steps.

2.2.4. Capillary Zone Electrophoresis/Laser-Induced Florescence Detection. CZE-LIF analyses were carried out using a Beckman PA800+ capillary electrophoresis system (SCIEX, Framingham, MA) coupled with a laser-induced fluorescence (LIF) detector at 488 nm excitation. Samples collected from each of the chromatography steps were buffer exchanged into 25 mM sodium bicarbonate using NAP-5 desalting columns (GE Healthcare). The buffer-exchanged samples were treated with potassium cyanide and FQ dye reagent [3-(2-furoyl) quinolone-2-carboxaldehyde] and incubated at 70°C. The dye–protein reactions went through a second desalting column equilibrated with 10 mM sodium phosphate to remove excess dye reagent. The samples were mixed with 0.2% SDS solution to facilitate SDS-protein complexes formation. The samples were loaded onto a 31 cm \( \times \) 50 \( \mu \)m ID fused-silica capillary (SCIEX) by hydrodynamic injection, separated by electrophoresis, and monitored by LIF detection.

2.3. Part III: Clearance of Degraded mAb1 in the Presence of mAb2 through Purification Processes

2.3.1. Chromatography System Setup. Scale-down chromatography was performed as described in Section 2.2.1, with the exception that degraded mAb1 was spiked into the mAb2 loads in place of PBS or respective chromatographic elution buffers.

The degraded mAb1 load mass, which was calculated as described in Section 2.2.2, was spiked in the mAb2 harvested cell culture fluid (HCCF) for the MabSelect SuRe column, in the mAb2 protein A pool for the Poros XS column, and in the mAb2 CEX pool for the Capto Adhere column. In addition to the degraded mAb1 spike, all mAb2 loads were calculated at the target load density for each resin. The pH and conductivity of the load was adjusted to the target load condition and passed through 0.22 \( \mu \)m filters (Corning). Load and pool samples were collected from each chromatography step, pH adjusted to 6–8, and stored frozen at \(-70^\circ\)C. The load and pool samples from each of the chromatography steps were analyzed using liquid chromatography–mass spectrometry (LC-MS) to determine the level of degraded mAb1 clearance in the presence of mAb2.

2.3.2. Load Preparation. For the clearance study of the degraded mAb1 fragments in the presence of mAb2, the load materials for the three chromatography runs consisted of degraded mAb1 spiked into mAb2 HCCF, degraded mAb1 spiked into mAb2 protein A pool, and degraded mAb1 spiked into mAb2 CEX pool. With the exception of the additional degraded mAb1 fragments, the load materials for the three chromatography runs were prepared to be representative of the process loads at each chromatography step.

The degraded mAb1 was prepared by treating mAb1 with 0.5 N NaOH at 20°C for 30 min. All mAb2 load materials were taken from Roche manufacturing production runs. The mAb2 HCCF was generated by collecting cell culture fluid from the CHO cell culture production and removing cells and debris from the harvest cultures by centrifugation and depth filtration. The mAb2 protein A pool was generated by purifying HCCF using protein A chromatography. The mAb2 CEX pool was generated by purifying the pH-adjusted protein A pool using CEX chromatography.
2.3.3. Intact Protein Mass Spectrometry Analysis. To investigate the effect of the cleaning conditions on mAb1 integrity, mAb1 was treated with 0.5 N NaOH at 20°C for 30 min and adjusted to pH 9.0 ± 0.1 using acetic acid. The solution was then diluted with high-performance liquid chromatography (HPLC)-grade water to obtain a mAb1 concentration of 0.5 mg/mL. One microliter of the diluted sample, equivalent to 500 ng of mAb1, was desalted by reversed-phase high-performance liquid chromatography (RP-HPLC) using an Agilent PLRP-S 2.1 × 150 mm, 8 µm, 1000 Å column (Agilent Technologies, Inc., Santa Clara, CA) and Waters Acquity ultra-high-performance liquid chromatography (UHPLC; Waters Corporation, Milford, MA) before analysis by electrospray ionization mass spectrometry (ESI-MS). The sample components were separated with a two-step linear gradient using an aqueous mobile phase of 0.1% formic acid in water and an organic mobile phase of 0.1% formic acid in acetonitrile. The HPLC flow rate and column temperature were held constant throughout the chromatographic run and were set to 300 µL/min and 77°C, respectively. The analytes were detected on Synapt G2-Si time-of-flight mass spectrometer (Waters Corporation) operating in positive ionization mode using a source temperature of 120°C, capillary voltage of 3.0 kV, and a cone voltage of 120 V. Mass spectra were acquired over the m/z (mass-to-charge ratio) range of 500–4000 with a spectral acquisition rate of 10 Hz.

2.3.4. Peptide Mapping by Liquid Chromatography-Mass Spectrometry. To quantitate the amount of degraded mAb1 fragments in the presence of mAb2, degraded mAb1 was serially spiked into mAb2 and analyzed by tryptic peptide map LC-MS analysis. A 200 µL aliquot of each standard, as well as load and pool samples collected from the three chromatography steps, were denatured with buffer containing 7 M guanidine HCl, 250 mM Tris, and 1 mM EDTA, pH 7.5. The sample was then reduced at 37°C for 1 h with 10 mM dithiothreitol (DTT) followed by carboxymethyl of the free sulfhydryls with the addition of 34.8 µmol iodoacetic acid at ambient temperature for 20 min in the dark. Excess alkylating reagent was then quenched with 30 µmol of DTT. Reduced and carboxymethylated samples were then buffer exchanged into digestion buffer (25 mM Tris, 1 mM calcium chloride, pH 7.5) using gel filtration columns according to the manufacturer’s protocol (NAP 5 column; GE Healthcare Life Sciences). The desalted sample was then digested with trypsin at a 1:25 (enzyme:substrate) ratio for 1.5 h at 37°C. Proteolysis was halted with 10% formic acid to obtain a final formic acid concentration of 0.2% (v/v). The resulting tryptic peptides were separated by reversed-phase ultra-high-performance liquid chromatography (RP-UHPLC) on a Waters Acquity UPLC Peptide CSH (2.1 mm × 150 mm, 1.7 µm, 130 Å) C18 column (Waters Corporation). Separation was achieved using a linear gradient at a flow rate of 300 µL/min and a column temperature of 77°C. The aqueous and organic mobile phases contained 0.1% (v/v) formic acid in mass spectrometry-grade water and acetonitrile, respectively. The eluting peptides were detected on-line by ESI-MS using an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific Inc., San Jose, CA) operating in positive ionization mode.

LC-MS/MS data was collected by data dependent acquisition with MS1 resolution of 60,000 and a scan range of 200 to 2000 m/z, and MS2 on the top three most intense ions with dynamic exclusion enabled. The complementarity-determining region (CDR) peptides of the degraded mAb1 were monitored by extracting the ion chromatograms of the most abundant charge states.

2.4. Clearance Quantification

The degraded fragment clearance by each of the three chromatography steps was expressed in two ways: fold clearance, which is the total degraded fragment mass ratio between the load and pool (eq 1); and LRV, which is the log₁₀ of the fold clearance (eq 2). The total degraded fragment mass in the load and pool fractions were obtained by multiplying the degraded fragment concentrations (mg/mL) in the load and pool samples by the sample volumes (mL).

\[
\text{Fold Clearance Degraded mAb1} = \frac{\text{Total Mass of Degraded mAb1 in Load}}{\text{Total Mass of Degraded mAb1 in Pool}} \tag{1}
\]

\[
\text{LRV Degraded mAb1} = \log_{10}(\text{Fold Clearance Value Degraded mAb1}) \tag{2}
\]

3. Results

Table I summarizes the experiments of this three-part study: Part I presents SDS-PAGE results across five different mAbs; Part II provides clearance results for degraded mAb1; and Part III provides clearance results for degraded mAb1 in the presence of mAb2.
3.1. Part I: Degradation Effect across mAbs

To evaluate the degradative effect of the cleaning conditions on various mAbs, five mAbs were treated with 0.5 N NaOH at 20℃ for 30 min and analyzed for degree of degradation by SDS-PAGE. The five mAbs selected for this study included various IgG subclasses: IgG1 (mAb1, mAb2), IgG1 BsAb (mAb3), IgG2 (mAb4), and IgG4 (mAb5) with similar properties, that is, molecular weights, isoelectric points, and extinction coefficients (Table II). The degradation profiles for the five mAbs were generated after NaOH treatment; untreated mAbs were included as controls. Complete degradation was indicated by the absence of a distinguishable product band on SDS-PAGE near the 150 kDa control. Figure 2 shows that all five mAbs, regardless of the IgG subclasses, were completely degraded when treated with NaOH as evidenced by the absence of a product band on the SDS-PAGE gel. Instead, smears between 20 kDa and 50 kDa and bands below 15 kDa were observed. The consistency in the degradation profiles across the five mAbs demonstrated that the cleaning condition used (0.5 N NaOH at 20℃ for 30 min) was effective at degrading a variety of IgG mAbs.

3.2. Part II: Clearance of degraded mAb1 through purification processes

To study the removal of the degraded fragments by the three chromatography steps, degraded mAb1 load mass was spiked in PBS for the MabSelect SuRe column, in protein A elution buffer for the Poros XS column, and

TABLE I
Summary of the Three-Part Study Approach and Analytical Results Expected for Each Part

<table>
<thead>
<tr>
<th>Study Part</th>
<th>Selected mAbs</th>
<th>Purification Chromatography Steps</th>
<th>Analytical Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part I: Degradation effect across mAbs</td>
<td>mAb1, mAb2, mAb3, mAb4, mAb5</td>
<td>MabSelect SuRe</td>
<td>SDS-PAGE Gels</td>
</tr>
<tr>
<td>Part II: Clearance of degraded mAb1 through purification processes</td>
<td>Degraded mAb1</td>
<td>MabSelect SuRe, Poros XS, Capto Adhere</td>
<td>SDS-PAGE Gels, CZE-LIF, Peptide Mapping</td>
</tr>
<tr>
<td>Part III: Clearance of degraded mAb1 in the presence of mAb2 through purification processes</td>
<td>Degraded mAb1 + mAb2</td>
<td>MabSelect SuRe, Poros XS, Capto Adhere</td>
<td>LC-MS</td>
</tr>
</tbody>
</table>

mAbs is monoclonal antibodies.
SDS-PAGE is sodium dodecyl sulfate–polyacrylamide gel electrophoresis.
CZE-LIF is Capillary Zone Electrophoresis/Laser-Induced Fluorescence Detection.
LC-MS is liquid chromatography–mass spectrometry.

TABLE II
Characteristics of the Selected Monoclonal Antibodies (mAbs) Evaluated in the Degradation and Clearance Study

<table>
<thead>
<tr>
<th>mAb</th>
<th>Immunoglobulin G (IgG) Subclasses</th>
<th>Molecular Weight (kDa)</th>
<th>Isoelectric Point (pI)</th>
<th>Extinction Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAb1</td>
<td>IgG1/mAb</td>
<td>144</td>
<td>8.8–9.3</td>
<td>1.67</td>
</tr>
<tr>
<td>mAb2</td>
<td>IgG1/mAb</td>
<td>145</td>
<td>8.8</td>
<td>1.62</td>
</tr>
<tr>
<td>mAb3</td>
<td>IgG1/BsAb</td>
<td>146</td>
<td>9.3</td>
<td>1.56</td>
</tr>
<tr>
<td>mAb4</td>
<td>IgG2/mAb</td>
<td>150</td>
<td>6.4</td>
<td>1.50</td>
</tr>
<tr>
<td>mAb5</td>
<td>IgG4/mAb</td>
<td>145</td>
<td>6.5</td>
<td>1.43</td>
</tr>
</tbody>
</table>
in CEX elution buffer for the Capto Adhere column. After pH adjustment and filtration, the actual degraded mAb1 load mass for the respective columns was 1/8, 1/68, and 1/31 of the target load mass (Table III). Partitioning of degraded mAb1 in the absence of mAb2 was examined on all three chromatography steps. Fractions were collected at all stages of each chromatography step and evaluated by SDS-PAGE for the degree of degradation and analyzed by CZE-LIF for the total protein content.

3.2.1. MabSelect SuRe Chromatography. Figure 3 depicts the degradation of mAb1 under the specified cleaning condition and subsequent clearance by MabSelect SuRe. When compared with the distinct untreated product band at 144 kDa in the SDS-PAGE gel (Figure 3A, Lane 1), the absence of the product band in the load lane (Figure 3A, Lane 2) indicated complete degradation of mAb1 after treatment with NaOH. Inspection of the collected fractions from MabSelect SuRe chromatography showed that most of the fractions, including the

![Sodium dodecyl sulfate–polyacrylamide gel electrophoresis data for the five monoclonal antibodies untreated and treated with the selected cleaning condition (0.5 N NaOH at 20°C for 30 min).](image)

**TABLE III**
Comparison between the Actual Degraded mAb1 Load Mass and the Target Protein Load Mass for Each Chromatography Step

<table>
<thead>
<tr>
<th>Chromatography Column</th>
<th>Target Load Mass (mg)</th>
<th>Degraded mAb1 Load Concentration&lt;sup&gt;a&lt;/sup&gt; (mg/mL)</th>
<th>Load Volume (mL)</th>
<th>Actual Load Mass (mg)</th>
<th>Ratio (Actual/Target)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MabSelect SuRe</td>
<td>631</td>
<td>0.32</td>
<td>245.73</td>
<td>79</td>
<td>1/8</td>
</tr>
<tr>
<td>Poros XS</td>
<td>1296</td>
<td>0.09</td>
<td>208.65</td>
<td>19</td>
<td>1/68</td>
</tr>
<tr>
<td>Capto Adhere</td>
<td>1068</td>
<td>0.39</td>
<td>90.00</td>
<td>35</td>
<td>1/31</td>
</tr>
</tbody>
</table>

<sup>a</sup>Degraded mAb1 load concentration was measured by capillary zone electrophoresis/laser-induced fluorescence detection analysis.
elution pool, appeared as smears throughout the gradient of the gel. In addition, bands \( \leq 50 \text{kDa} \) as well as high molecular weight (MW) species \( > 250 \text{kDa} \) were observed (Figure 3A, Lanes 3 to 11). Comparing the degraded mAb1 load in Figure 3A with the degraded mAbs in Figure 2 showed that the fragment MW distribution profile was consistent, with the exception of the high MW species presence in the degraded mAb1 load. These high MW species likely formed after pH adjustment of the degraded material in the load.

Total protein content measured by CZE-LIF demonstrated a mass balance of 100% of the degraded fragments collected in the fractions compared with the mass loaded onto the column (Figure 3B). Furthermore, 83% of the degraded fragments were found in the load F/T and regeneration phases. The presence of fragments in the elution pool suggested retention of the degraded mAb1 fragments on the MabSelect SuRe column until the fragments were washed out by the NaOH regeneration (Figure 3A). The mass of degraded fragments detected in the elution pool was 15 times lower than that in the load, thus reflecting the fold clearance achieved by the MabSelect SuRe chromatography.

3.2.2. Poros XS Chromatography. Figure 4 depicts the degradation of mAb1 under the specified cleaning condition and subsequent clearance by Poros XS. When compared with the distinct untreated product band at 144 kDa in the SDS-PAGE gel (Figure 4A, Lane 1), the absence of the product band in the load lane (Figure 4A, Lane 3) indicates complete degradation of mAb1 after treatment with NaOH. Figure 4A also shows bands that are \( < 15 \text{kDa} \) in the load, load F/T, wash, and elution pool lanes (Lanes 3, 4, 5, and 7, respectively) based on collected fractions from Poros XS chromatography. In addition, a smear and multiple bands can be observed in the sanitization lane (Figure 4A, Lane 8).

The total protein content measured by CZE-LIF demonstrated 61% of the degraded fragments collected in the fractions compared with the mass loaded in the column (Figure 4B). Even though 39% of the degraded mAb1 mass was unaccounted for, it is unlikely that the degraded fragments will co-elute with the subsequent product owing to the low level of degraded fragments observed in the elution pool. Additional studies may be required to understand the recovery of degraded fragments in this particular chromatography step.
Most of the degraded fragments remained bound (via nonspecific interaction) during the elution phase and were removed by stripping the column with NaOH sanitization. The sanitization phase accounted for 57% of the degraded mAb1 mass loaded on the column. The level of degraded fragments detected in the elution pool was 90 times lower than that in the load, thus reflecting the fold clearance achieved by the Poros XS chromatography.

### 3.2.3. Capto Adhere Chromatography

Figure 5 depicts the degradation of mAb1 under the specified cleaning condition and subsequent clearance by Capto Adhere. When compared with the distinct untreated product band at 144 kDa in the SDS-PAGE gel (Figure 5A, Lane 1), the absence of this band in the load lane (Figure 5A, Lane 3) indicated complete degradation of mAb1 after treatment with NaOH. Inspection of the collected fractions from the Capto Adhere results showed that the F/T pool lane bands (Figure 5A, Lane 5) were <15 kDa, whereas a smear and multiple bands were observed in the regeneration and sanitization lanes (Figure 5A, Lanes 6 and 7).

Total protein content measured by CZE-LIF demonstrated 90% recovery of the degraded fragments collected in the fractions compared with the mass loaded in the column (Figure 5B). Despite the apparent low level of degraded fragments shown on the SDS-PAGE gel (Figure 5A), 25% of the degraded fragments were present in the F/T pool (Figure 5B). This can be explained by the large volume of buffer used during the F/T pool step, which diluted the concentration of the degraded fragments as seen in the gel. Most of the degraded fragments remained bound (via nonspecific interaction) during the F/T pool phase and were removed by stripping the column with NaOH sanitization. The regeneration and sanitization phases accounted for 60% of the degraded mAb1 mass loaded on the column. The level of degraded fragments detected in the F/T pool was four times lower than that in the load, thus reflecting the fold clearance achieved by the Capto Adhere chromatography.

### 3.2.4. Calculating Fold Clearance of Degraded mAb1 Fragments

The fold clearance and LRV of the degraded fragments achieved at each chromatography
step was calculated using eq 1 and eq 2, respectively, and summarized in Table IV. The results showed fold clearances and LVRs of 15 and 1.2 (MabSelect SuRe), 90 and 2.0 (Poros XS), and 4 and 0.60 (Capto Adhere) respectively. Overall clearance across the three chromatography steps was calculated as 5400 and LRV as 3.7.

3.3 Part III: Clearance of Degraded mAb1 in the Presence of mAb2 through the Purification Processes

3.3.1 Peptide Mapping by Liquid Chromatography-Mass Spectrometry. Because the CZE-LIF assay is a nonspecific total protein assay incapable of distinguishing degraded mAb1 from other various protein species (such as degraded mAb1, mAb2, and mAb2 process- and product-related impurities), LC-MS-based intact protein analysis was initially selected to differentiate the degraded mAb1 from mAb2. To verify that intact protein MS analysis can distinguish between degraded and intact mAb, we first performed the analysis on degraded and intact mAb1. Figure 6 shows the degradation of mAb1 by the NaOH cleaning condition using intact protein MS analysis. When compared with the distinct protein charge envelope between 2000 and 3400 m/z in the intact mAb1 (Figure 6A), a left shift of protein charge envelope to lower m/z and relative abundance indicated

**TABLE IV**
Fold Clearance of the Degraded Fragments in the Absence of mAb2 Demonstrated through the Purification Processes

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total Mass of Degraded mAb1 (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MabSelect SuRe</td>
</tr>
<tr>
<td>Load</td>
<td>79</td>
</tr>
<tr>
<td>Pool</td>
<td>5.2</td>
</tr>
<tr>
<td>Degraded fragments fold clearance</td>
<td>15</td>
</tr>
<tr>
<td>Degraded fragments LRV**</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**LRV** is log reduction value.
fragmentation and complete degradation of mAb1 after the NaOH cleaning condition (Figure 6B). However, because of the sensitivity limitations of intact protein MS analysis, quantification of the degraded mAb1 in the presence of mAb2 was performed by monitoring mAb1 CDR peptides via peptide mapping. This approach provided specificity in detecting degraded fragments of mAb1, as CDR peptides are part of the variable region and unique to each antibody. Because mAb1 was degraded, the clearance of each peptide could be affected by its physiochemical properties. To quantitate the amount of degraded mAb1 fragments and to evaluate the clearance of degraded mAb1 in each chromatography step, a wide concentration range of degraded mAb1 was spiked into mAb2. The signal from each specific CDR peptide was then used to generate calibration curves (Figure 7) that were used to quantify the degraded fragments in each chromatography step.

Quantification of the degraded fragments was done by measuring the concentrations of the six CDR peptides of mAb1 in the load and pool fractions of each chromatography step. All CDR peptides showed a limit of detection (LOD) at 5 µg/mL, except for CDR2 and CDR6 with the LOD at 10 µg/mL. The CDR peptide with the highest protein concentration in the pool fraction was selected to calculate the worst-case or highest mass of degraded mAb1 fragments. CDR4, CDR5, and CDR1 were selected for MabSelect SuRe, Poros XS, and Capto Adhere worst-case fold clearance calculations, respectively. Table V summarizes these results, with the selected CDRs marked in the cells.

3.3.2. Calculating Fold Clearance of Degraded mAb1 Fragments. The results from Table V were used to calculate the total mass of degraded mAb1 in the load and pool fractions of each chromatography step. The fold clearance and LVR of the degraded mAb1 fragments across each chromatography step were calculated using Eq 1 and Eq 2, respectively, and summarized in Table VI. The results showed fold clearances and LVRs of 18 and 1.3 (MabSelect SuRe), 82 and 1.9 (Poros XS), and 3 and 0.4 (Capto Adhere), respectively. The overall clearance across the three chromatography steps was calculated as 4428 and the LRV as 3.6.

3.3.3. Evaluating Potential Interaction between Intact mAb2 and Degraded mAb1 Fragments. The potential interaction of degraded mAb1 fragments and intact mAb2 can be assessed by comparing the clearance data in the absence of mAb2 and in the presence of mAb2. Table VII shows the comparison of fold clearances and LRVs for the
Figure 7

Standard curve of the mAb1 complementarity-determining region (CDR) peptides prepared by spiking a wide concentration range of degraded mAb1 in 5 mg/mL of mAb2. The limits of detection of all CDR peptides were determined at 5 µg/mL, except for CDR2 and CDR6 which were determined at 10 µg/mL.
chromatography steps in the absence and presence of mAb2. This comparison can be used to determine if the presence of mAb2 impacted the clearance of mAb1 using individual step results as well as the overall results. Each chromatography step clearance showed very similar results in the absence and presence of mAb2 (e.g., MabSelect SuRe results were 15 vs. 18 for fold clearance and 1.2 vs. 1.3 for LRV). Additionally, the sum of the LRVs for the degraded mAb1 fragments across all steps, in the absence of mAb2 (sum = 3.7) was comparable to the sum of the LRVs in the presence of mAb2 (sum = 3.6).

4. Discussion

Shared equipment surface area is one of the variables used to calculate the MAC of Product A into Product B in pharmaceutical manufacturing. Limiting the shared surface area to the highest-risk equipment can be supported by understanding the purification potential of the unit operations through the final purification step.

The results of this three-part study showed that mAbs are degraded when exposed to the cleaning condition typically used in manufacturing, generating a degradation profile that is significantly different from the intact mAb as demonstrated by MS analysis. Exposure to the cleaning condition results in fragmentation of the mAb via hydrolysis of the amino acid chains. The degree of fragmentation is dependent on the distribution of the cleavage sites in a given mAb and can vary based on the cleaning conditions, that is, pH, temperature, and duration of exposure. Fragmentation is also dependent

### TABLE V
Quantification of the Complementarity-Determining Region (CDR) Peptide Concentrations in Each of the Chromatographic Steps

<table>
<thead>
<tr>
<th>Chromatography Step</th>
<th>CDR Peptide Concentration (μg/mL)</th>
<th>Volume Collected (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CDR1</td>
<td>CDR2</td>
</tr>
<tr>
<td>MabSelect SuRe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Load</td>
<td>355</td>
<td>276</td>
</tr>
<tr>
<td>Elution pool</td>
<td>120</td>
<td>123</td>
</tr>
<tr>
<td>Poros XS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Load</td>
<td>105</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Elution pool</td>
<td>5</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Capto Adhere</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Load</td>
<td>201</td>
<td>278</td>
</tr>
<tr>
<td>F/T pool 1</td>
<td>64</td>
<td>59</td>
</tr>
<tr>
<td>F/T pool 2</td>
<td>56</td>
<td>51</td>
</tr>
</tbody>
</table>

- Limit of detections of the liquid chromatography–mass spectrometry peptide mapping were found to be 5 μg/mL for all CDR peptides with the exception of CDR2 and CDR6 with a LOD of 10 μg/mL.
- For the MabSelect SuRe chromatography step, CDR4 was analyzed as the worst-case scenario for the clearance calculation.
- For the Poros XS chromatography step, CDR5 was analyzed as the worst-case scenario for the clearance calculation.
- For the Capto Adhere chromatography step, CDR1 was analyzed as the worst-case scenario for the clearance calculation.
- F/T is flow through.

### TABLE VI
Fold Clearance of the Degraded mAb1 Fragments in the Presence of mAb2 Demonstrated through the Purification Processes

<table>
<thead>
<tr>
<th>Total Mass of Degraded mAb1 (mg)</th>
<th>MabSelect SuRe</th>
<th>Poros XS</th>
<th>Capto Adhere</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load</td>
<td>CDR4</td>
<td>CDR5</td>
<td>CDR1</td>
</tr>
<tr>
<td>218</td>
<td>24.6</td>
<td>26.8</td>
<td></td>
</tr>
<tr>
<td>Pool</td>
<td>11.9</td>
<td>0.3</td>
<td>9.8</td>
</tr>
<tr>
<td>Degraded fragments fold clearance</td>
<td>18.3</td>
<td>82</td>
<td>2.7</td>
</tr>
<tr>
<td>Degraded fragments LRV</td>
<td>1.26</td>
<td>1.9</td>
<td>0.43</td>
</tr>
</tbody>
</table>

- LRV is log reduction value.
on the secondary and tertiary structures, which can influence access to specific cleavage sites. However, once the primary structure of a protein is disrupted through hydrolysis, the secondary to quaternary structures are also changed (12). In this study, the degradation results indicated that the caustic cleaning condition was effective at degrading a variety of mAbs. All five mAbs with different IgG subclasses were completely degraded when exposed to 0.5 N NaOH at 20°C for 30 min. This result was consistent with previous findings showing that caustic chemicals such as NaOH are effective at degrading a variety of mAbs (11).

The study results also showed that the protein purification process has the potential to remove high levels of cleaning-related protein residues. All three chromatography steps provided clearance of degraded fragments using a load concentration that is significantly higher than what would be expected in manufacturing. In this study, the load concentration was selected so that degraded mAb fragments could be detected in the load and the pool and clearance could be estimated. The differences between this study and what is expected in manufacturing can be determined using the ratio of the potential concentration expected in manufacturing to the load concentration used in this study. For example, rinse samples after cleaning are typically about 1 ppm, (or 1 μg/mL) total organic carbon in biologics manufacturing (13). Based on this concentration of carbon, the load concentration into a chromatography column would be at this level, that is, 1 ppm carbon or lower as dilution is likely to occur as a result of additional flushes during processing. In this study, the load concentration varied from 0.09 to 0.39 mg/mL for the different chromatography steps as shown in Table III. Using these values, and assuming that a typical mAb contains approximately 50% total organic carbon, the load concentration in this study, that is, 0.09 to 0.39 mg/mL, is therefore 45- to 195-fold higher than the typical cleaning limit in biologics manufacturing.

Clearance of the degraded fragments through the purification process was demonstrated in the absence and in the presence of the subsequent product. When the degraded fragments were applied to the three chromatography steps in the absence of the subsequent product, a majority of the fragments either flowed through the chromatography column or were stripped off during the NaOH sanitization step. The fold clearance of the degraded fragments at each of the three chromatography steps was 15 for MabSelect SuRe, 90 for Poros XS, and 4 for Capto Adhere. When the degraded fragments were applied to the three chromatography steps in the presence of the subsequent product, comparable fold clearances of the degraded fragments, 18, 82, and 3, respectively, were observed. These similar fold clearance values suggest that there was minimal interaction between the cleaning-related protein residues and the subsequent product; thus, carryover of the previous product bound to and co-eluted with the subsequent product is not likely.

Although clearance was demonstrated, the acceptable clearance of cleaning-related protein residues to support surface area removal has not been established. Experimental evidence of clearance when degradation is demonstrated or supporting the removal of shared surface area has not yet been published. However, a discussion on acceptable clearance of degradants by purification steps has been presented with reference to a three-log clearance expectation based on different cleaning evaluations (14). In the study presented here, the cumulative fold clearance of degraded fragments was calculated to be between 4428 and 5400 across the three chromatographic steps. This is equivalent to three to four-log clearance of the cleaning-related protein residues.

### Table VII

<table>
<thead>
<tr>
<th>Chromatography Step</th>
<th>Fold Clearance</th>
<th>LRV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mAb2 (−)</td>
<td>mAb2 (+)</td>
</tr>
<tr>
<td>MAbSelect SuRe</td>
<td>15</td>
<td>18.3</td>
</tr>
<tr>
<td>Poros XS</td>
<td>90</td>
<td>82</td>
</tr>
<tr>
<td>Capto Adhere</td>
<td>4.0</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*a* mAb2 (−) refers to degraded mAb1 clearance in the absence of mAb2; mAb2 (+) refers to degraded mAb1 clearance in the presence of mAb2.

*b* LRV = log reduction value.
residues, consistent with the aforementioned three-log clearance expectation. Additionally, these results are confounded by factors such as the loading density and analytical techniques used; clearance of the degraded protein residues could be expected to be at least three-log based on the documented purification potential for other impurities (i.e., CHOP, endotoxin) using similar purification processes.

Finally, the clearance data in this study were limited to a specific mAb1 and mAb2 combination and, as such, there are limitations. For example, only two products were evaluated, the purification process could change, and the cleaning conditions must be effective in degrading potential product residues to ensure removal. However, although only two products were evaluated in this study, the property differences between the intact and the degraded mAbs were significant enough to enable a high degree of degraded protein residue clearance via purification by the chromatography processes. For example, SDS-PAGE data generated in Part I of the study across five mAbs showed differences in size between the intact and degraded mAbs. Other published studies also showed similar size profiles after exposure to different cleaning conditions (11). Moreover, removal of other protein residues such as host cell proteins has consistently been shown at a very high level for chromatography-based purification processes across the industry. In regards to purification process changes, in this study, fold removal was based on a load that was orders of magnitude greater than what is expected in an actual manufacturing process. In an actual process the load is significantly smaller, as there are multiple opportunities for soil removal, that is, cleaning and changeover procedures with multiple rinses, before the subsequent product (Product B) is exposed to the shared equipment surface area. Thus, although a specific purification step may change, the purification potential across all steps is expected to be similar, in alignment with what has been documented for other impurities, that is, virus and CHOP. With respect to the cleaning conditions, this is an important aspect to consider in implementing this approach. The caustic condition used in this study is one example showing complete degradation of the evaluated mAbs. Variations in caustic conditions with sufficiently high pH, exposure time, and/or high temperature can also achieve the degradation profiles evaluated in this study. For new types of molecules other than mAbs, studies can be performed to verify the effect of the cleaning conditions and support the purification assumptions.

Additionally, for mAbs in general, these studies can also be performed if cleaning conditions have a pH, temperature, and/or exposure time significantly less than those used here.

5. Conclusions

This three-part study demonstrated the clearance of cleaning-related protein residues in a typical purification process for mAbs produced in CHO cells. It showed that the caustic cleaning condition used was effective at degrading a variety of mAbs with different IgG subclasses. The study also showed that the clearance of previous product residues can be expected to be at least three to four logs based on the demonstrated effectiveness of the purification process and the documented clearance of other impurities (5–10). The experimental data presented in this study provide a science-based rationale that supports limiting the shared surface area to equipment where previous product residues clearance is not expected or equipment with the highest risk of carryover to the subsequent product.

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Conflict of Interest Declaration

The authors declare that they have no competing interests.

References


Comparison of *Limulus* Amoebocyte Lysate and Recombinant Factor C Assays for Endotoxin Detection in Four Human Vaccines with Complex Matrices

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**ABSTRACT:** Endotoxins, heat-stable lipopolysaccharides from Gram-negative bacteria, are potential contaminants that can be introduced during manufacturing of pharmaceutical products, including vaccines. Parental pharmaceutical products undergo endotoxin testing because endotoxins are pyrogenic in humans and can induce severe physiological reactions. Currently, animal-derived *Limulus* amoebocyte lysate (LAL) assays are widely used. Assays using recombinant factor C (rFC), a nonanimal-derived reagent, have been proposed as alternatives. Some components in the matrices of pharmaceutical products can interfere with these assays. We compared two LAL- and two rFC-based assays for endotoxin detection in four complex human vaccine matrices. We showed that the results for the rFC-based assays were at least equivalent to those for the LAL-based assays, although the rFC-based assays were found to be adequate but slightly less suitable for one of the products that contained proteases as the methods used to inactivate the proteases reduced the assay performance. Likewise, LAL was adequate but less suitable for another product that contained glucans. The rFC assays offer a number of benefits, including compliance with the principles of the 3Rs, i.e., replacement, reduction, and refinement of animal testing by safeguarding animal welfare and promoting more ethical and sustainable use of animals for testing. After they are fully validated, as per the compendial requirements, they could be considered as suitable replacement assays for the detection of endotoxin in the manufacturing processes of pharmaceutical products. In summary, we demonstrated that both LAL and rFC assays are adequate for testing and releasing four vaccine products.

**KEYWORDS:** Endotoxin testing, LAL-based endotoxin assay, recombinant factor C (rFC) endotoxin assay, human vaccines.

**Introduction**

Endotoxins are heat-stable lipopolysaccharides (LPSs) from Gram-negative bacteria. When injected into the human body (e.g., via the intravenous, intrathecal, intramuscular, or subcutaneous routes), endotoxin is seen as an indicator of the presence of bacteria by the immune cells, via an innate reaction. These cells respond with a pyrogenic reaction that can be responsible for severe physiological reactions. In addition to inflammation, endotoxins can also induce a septic shock response (1). However, although LPS is the most potent inducer of cytokine production in septic shock, this is not entirely specific to Gram-negative bacteria as septic shock can also be caused by trauma, antigenic immune response, and so forth (2–4). Gram-negative bacteria have been reported to be responsible for 50% of sepsis cases (5). Hence, parenteral products are tested for endotoxin contamination as detailed in various pharmacopeias and specific vaccine monographies (6–9).

For nearly 40 years, animal-derived *Limulus* amoebocyte lysate (LAL) assays have been widely used for the detection of bacterial endotoxins in the pharmaceutical industry. These assays are routinely used within Sanofi Pasteur for vaccine release testing. In the presence of endotoxin, factor C in the LAL is activated by binding to the endotoxin, and this induces a cascade of reactions (Figure 1) (10). In the kinetic chromogenic LAL assay, a clotting enzyme, via factor G, cleaves a

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peptide that has the chromogen para nitroanilide (pNA) attached at its terminal to release the pNA, which produces a yellow solution that absorbs light at 405 nm. The time (in seconds) needed to reach a predetermined absorbance of the reaction mixture is measured to determine the concentration of endotoxin. One of the potential drawbacks of the LAL assay is that other substances, such as (1→3)-β-glucans, which can come from cellulose-based devices commonly used in vaccine manufacturing processes, for example for purification or sterile filtration, can also activate the LAL cascade and thus lead to false-positive results (11). Glucan-blocking buffers can be used to block the factor G pathway of the endotoxin cascade and have been reported to effectively prevent these false-negative reactions (12–13).

Recombinant factor C (rFC) is a nonanimal-derived reagent that has been proposed as an alternative to LAL. Recombinant FC assays can be based on fluorescent endpoint assays, with the main receptor being rFC instead of factor C from horseshoe crabs (Figure 1). Unlike in the past, endotoxin rFC assays are now available from different manufacturers, reducing concerns about a single-source supply. In addition, the enzymatic reaction for rFC is much simpler than that for the LAL-based assay, as can be seen in Figure 1. Another potential advantage is the more consistent interbatch performances of rFC assays, which have uniform and predictable behavior toward potential interfering substances with none of the biologic variability inherent in LAL assays, resulting from the production process for rFC. Recombinant FC assays have been shown to be insensitive to (1→3)-β-glucans, which therefore reduces the rate of false-positive results, and the shorter enzymatic reaction results in less interference from the matrix (14).

The increased demand for endotoxin testing (driven by maturing Asia Pacific markets and growing needs from pharmerging countries) could have an impact on horseshoe crab conservation (15–17). Reducing the use of LAL-based assays would be aligned with the principles of the 3Rs, that is, replacement, reduction, and refinement of animal testing (18). The United States Pharmacopeia (USP), European Pharmacopoeia (Ph. Eur.), and other regulatory authorities recognize rFC assays as an alternative endotoxin detection method to the prescribed LAL-based assays (7, 19, 20). Since July 1, 2016, rFC assays have been accepted for use as an alternative to the compendial LAL assay as indicated in the Ph. Eur. (20). However, it is necessary to demonstrate that “the method is appropriate for the given substance or product and gives a result consistent with that obtained with the prescribed method . . .” (21). The alternative assay should be validated as described in USP

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**Figure 1**

Principle of *Limulus* amoebocyte lysate (LAL) and recombinant factor C (rFC) assays showing that FC is activated by endotoxin in both assays (10).
Validation of Compendial Procedures in the USA and Chapter 5.1.6.: Alternative Methods for Control of Microbiological Quality in the Ph. Eur. (19, 22–24).

For these reasons, we evaluated four different assays (two LAL-based and two rFC-based) for the detection of endotoxins in four complex human vaccine matrices and we report our findings here.

Materials and Methods

Endotoxin Assays

We evaluated two kinetic chromogenic LAL-based endotoxin assays, Kinetic-QCL (KQCL; Lonza, Walkersville, MD) and ENDOSAFE-MCS (Charles River, Charleston, SC). ENDOSAFE-MCS is a miniaturized, automated version, similar to the KQCL assay, but which uses a multiple-cartridge system and an internally archived standard curve to determine concentrations. Both assays were performed as described in the supplier’s instructions. The sensitivities of the KQCL and ENDOSAFE-MCS assays are 50–0.005 EU/mL and 0.5–0.005 EU/mL, respectively.

For the KQCL assay, the released chromogenic product (yellow) was measured at 405 nm with a BioTek ELX808 (BioTek Instruments, Inc., Winooski, VT) absorbance microplate reader coupled with WinKQCL software (Lonza). The samples were tested in duplicate and the mean was used for calculations. The concentrations were determined using standard curves fitted with a polynomial regression (power curve) model.

We also evaluated two rFC assays, ENDOZYME II and ENDOLISA (bioMérieux). The ENDOZYME II assay is a fluorescent endpoint 96-well microplate assay, which was not commercially available at the time of this study, although it is now available. Endotoxin binding activates the rFC to create a moiety that cleaves a synthetic substrate resulting in the generation of a fluorescent compound. The fluorescence was measured with a BioTek FLX800 microplate reader (BioTek Instruments, Inc.) at time zero and after 1 h incubation at 37°C using excitation and emission wavelengths of 380 nm and 440 nm, respectively. Each sample was tested in triplicate and the mean was used for calculations. The standard curve was analyzed using a 4-parameter logistic regression model for 1/10 dilutions from 50 to 0.005 EU/mL (25).

The ENDOLISA assay is a solid-phase, endpoint fluorescence microplate assay using a recombinant bacteriophage-derived capture molecule that has high affinity and high specificity for the conserved core region of LPS that enables it to bind endotoxin variants (26). After the endotoxin was bound on the solid phase, the sample matrix, with potentially interfering components, was removed by washing, thus facilitating reliable quantification of the endotoxin in the sample. The bound endotoxin was then detected using a fluorogenic substrate. The fluorescence was measured with an FLX800 microplate reader at time zero and after 1 h incubation at 37°C using excitation and emission wavelengths of 380 nm and 440 nm, respectively. Each sample was tested in triplicate and the mean was used for calculations. The standard curve was analyzed using a 4-parameter logistic regression model for 1/10 dilutions from 50 to 0.05 EU/mL (26).

Reference Standard Endotoxin

The reference standard endotoxin (RSE) was diluted with 5 mL of endotoxin-free water (vortexed for 30 min as per supplier instructions) to give a final concentration of 2000 EU/mL (batch 5.1: European Directorate for the Quality of Medicines and HealthCare (EDQM)/Council of Europe, France). RSE was used to spike undiluted test samples (hard spike). For this, 50 μL of a 2000 EU/mL RSE solution was added to 950 μL of sample, resulting in 1 mL of spiked sample with an endotoxin content of 100 EU/mL. The control standard endotoxin (CSE) supplied in each assay kit was used to prepare the positive product controls (PPCs) (diluted samples).

Complex Vaccine Matrix Samples

Four proprietary human viral and bacterial vaccines were selected for their different complex matrices (Sanofi Pasteur, Marcy l’Étoile and Val de Reuil, France). The routine release tests for all four had been performed with the KQCL assay. Product A was an attenuated live viral vaccine containing proteases. Product B was an inactivated viral vaccine with no interference for the endotoxin assay. Product C was an
inactivated bacterial vaccine potentially containing natural endotoxin content from the Gram-negative antigens it contained. Product D was an inactivated viral vaccine with a strong red color that can interfere with the yellow endpoint product in classical LAL chromogenic kinetic assays.

Assay Conditions

The conditions for each assay were tested before the comparisons were performed. One of these conditions is the dilution necessary to minimize interference (inhibition or enhancement) from matrix substances while respecting the maximum valid dilution (MVD). The MVD is calculated by dividing the endotoxin limit (i.e., the maximum acceptable endotoxin concentration in the undiluted sample) by the assay sensitivity (i.e., the lowest standard concentration, e.g., 0.005 EU/mL for ENDOZYME II). The dilutions used for the KQCL assay were those used routinely in our laboratory. For the other assays, the dilutions investigated ranged from no dilution to 1/10,000 (Table I).

We investigated the following pretreatments for the inactivation of proteases: denaturing by heating at 75°C for 15 min, a protease inhibitor, higher dilutions in endotoxin-free water, and dilutions in 0.5 M endotoxin-free NaCl. Product C contained β-1,3-glucan, which is a glucose polymer of varying molecular weight that can be present in preparations derived from yeast and cellulosic material, including hemodialysis filters. When present in sufficient quantities, β-1,3-glucan can produce a positive LAL result in the absence of endotoxins by activation of the glucan-sensitive G pathway (Figure 1). The presence of β-1,3-glucan in Product C was confirmed using the Glucatell assay kit (Associates of Cape Cod, Inc., East Falmouth, MA). Therefore, we added a β-1,3-glucan blocker diluent to block the G pathway and minimize the false-positive reaction.

The dilutions for spiked and unspiked samples were prepared from the same aliquot at the same time for use in all four assays. As prerequisite for method comparability, three independent runs per method were performed by two technologists with each sample in duplicate for the LAL assays and in triplicate for the rFC assays. Only one run was performed for the investigation of pretreatments for Product A.

The assay validity criteria were the coefficient of correlation \( r > 0.980 \) and a percentage of recovery of PPC 50%–200%, based on the Ph. Eur. 2.6.14, USP <85>, and JP 4.01 (6, 8, 9). In addition, the coefficient of variation (CV) for the samples and spikes was \(<10.00\% \) for KQCL and \(<25.00\% \) for ENDOSAFE-MCS and \(\leq 25.00\% \) and \(\leq 20.00\% \) for samples with ENDOLISA and ENDOZYME II, respectively, based on the manufacturers’ recommendations (8, 9, 25–28).

Outcomes for Comparison of Endotoxin Assays

Using the optimal conditions identified, the assay sensitivities for all four assays were compared. The endotoxin content was expressed in EU/mL. We evaluated the RSE recovery in all four products and in water to calculate the percentage RSE recovery as: \[ 100 \times \frac{\text{sample endotoxin reportable results at time } t}{\text{water control reportable results at time } t}. \] The average percentage RSE recovery was calculated using the average results in the calculation. The high concentration spike and the PPC recoveries were estimated as the percentage of the added endotoxin that was recovered and had to be between 50% and 200% to validate the test results.

Results

Optimization of Assay Conditions for Product A

Because Product A was known to contain proteases, preliminary assays were performed to assess their impact on the results from the LAL and rFC-based endotoxin assays. We observed a positive signal with only the rFC-based assays: the endotoxin signals for untreated, unspiked Product A were 31 EU/mL and 0.6 EU/mL with ENDOZYME II and ENDOLISA, respectively (Table II). The shapes of the sample rFC curves were different from the shapes of the standard curves, unlike the KQCL curves, which were the same shape. In addition, a signal, corresponding to 0.80 EU/mL, was detected with the ENDOLISA assay in wells containing samples of Product A, with and without the RSE spike, when rFC was not added. This signal was because of the protease in Product A that cleaved the fluorogenic substrate, leading to a false-positive result. The comparison of wells with and without added rFC showed that the potential residual endotoxin was \(<0.01\) EU/mL. This indicates that the false-positive results in rFC were because of nonspecific activation.
<table>
<thead>
<tr>
<th>Product</th>
<th>MVD&lt;sup&gt;a&lt;/sup&gt;</th>
<th>KQCL&lt;sup&gt;b&lt;/sup&gt;</th>
<th>ENDOSAFE-MCS</th>
<th>ENDOZYME II</th>
<th>ENDOLISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Assay sensitivity: 0.005 EU/mL</td>
<td>Assay sensitivity: 0.05 EU/mL</td>
<td>dilution: 1/100, assay sensitivity: 0.005 EU/mL</td>
<td>dilution: 1/100, assay sensitivity: 0.05 EU/mL</td>
<td>dilution: 1/10, assay sensitivity: 0.05 EU/mL</td>
</tr>
<tr>
<td>Product A</td>
<td>200,000</td>
<td>20,000</td>
<td>dilution: 1/100, assay sensitivity: 0.005 EU/mL</td>
<td>dilution: 1/100, assay sensitivity: 0.05 EU/mL</td>
<td>dilution: 1/10, assay sensitivity: 0.05 EU/mL</td>
</tr>
<tr>
<td>Product A pretreated&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>dilution: 1/10 heat-treated, further dilution: 1/10 (total 1/100), assay sensitivity: 0.005 EU/mL</td>
<td>dilution: 1/10 heat-treated, further dilution: 1/10 (total 1/100), assay sensitivity: 0.05 EU/mL</td>
<td>dilution: 1/10 heat-treated, further dilution: 1/10 (in total 1/100), range assay sensitivity: 0.005 EU/mL</td>
<td>dilution: 1/10 heat-treated, assay sensitivity: 0.05 EU/mL</td>
</tr>
<tr>
<td>Product B</td>
<td>10,000</td>
<td>1000</td>
<td>dilution: 1/100, assay sensitivity: 0.005 EU/mL</td>
<td>dilution: 1/100, assay sensitivity: 0.05 EU/mL</td>
<td>dilution: 1/10, assay sensitivity: 0.05 EU/mL</td>
</tr>
<tr>
<td>Product C</td>
<td>96,000</td>
<td>9600</td>
<td>dilution: 1/1000, assay sensitivity: 0.005 EU/mL</td>
<td>dilution: 1/100, assay sensitivity: 0.05 EU/mL</td>
<td>dilution: 1/100, assay sensitivity: 0.05 EU/mL</td>
</tr>
<tr>
<td>Product D</td>
<td>5000</td>
<td>500</td>
<td>dilution: 1/100, assay sensitivity: 0.005 EU/mL</td>
<td>dilution: 1/100, assay sensitivity: 0.05 EU/mL</td>
<td>dilution: 1/100, assay sensitivity: 0.05 EU/mL</td>
</tr>
</tbody>
</table>

<sup>a</sup>MVD is maximum valid dilution.

<sup>b</sup>Dilutions defined and used in routine testing.

<sup>c</sup>Tested in one run.
Different pretreatment methods for Product A were therefore tested to minimize the expected impact of the proteases on false-positive results. Heat treatment (75˚C for 15 min) to inactivate the proteases did not prevent signals in rFC-based assays and resulted in substantial endotoxin masking in spiked samples, possibly owing to exposure of hydrophobic surfaces by protein denaturation, which would drastically increase particle motility (Table II). Pretreatment of Product A with 0.5 M NaCl did not prevent protease binding to the ENDOLISA microplate. The PPC recovery was 161%, close to that obtained with samples diluted in water (155%), and the decreased endotoxin signal was insignificant (1/10: 0.06 vs. 0.08 EU/mL in 0.5 M NaCl and water, respectively). Six concentrations of benzamide hydrochloride (a serine protease inhibitor) (5, 10, 25, 50, 75, and 100 mM) were tested in the ENDOLISA assay. The maximum concentration of benzamidine hydrochloride that can be tolerated by the ENDOLISA assay is 100 mM and concentrations of 1–10 mM are sufficient to inhibit most serine proteases. There were no significant differences between the results when Product A was diluted in water or in benzamidine hydrochloride, suggesting that either the benzamidine hydrochloride was washed off during the assay or that the protease present was not a serine protease.

When Product A was diluted 1/100 and 1/10,000 in water for the ENDOLISA and ENDOZYME II assays, respectively, the assay results were <5 EU/mL and <50 EU/mL, respectively. When Product A was diluted to 1/100 in 0.5 M NaCl for the ENDOZYME II assay, the PPC recovery was <50%. The results for spike recovery (PPC) were within the prespecified limits for all four methods in the samples without and with heat treatment (Table II).

None of the other pretreatments evaluated for Product A (i.e., higher dilutions in LAL reagent water, dilutions in 0.5 M endotoxin-free NaCl, use of antiprotease) removed the signals. However, the ENDOLISA assay gave lower results than the ENDOZYME II assay, probably because the washing steps removed the protease (about 50 times more efficient).

**Sensitivity of Endotoxin Detection Assays**

The results obtained with all four endotoxin detection assays were below the required internal product specifications for all four products, with the most sensitive assays being KQCL in Product D and water, and ENDOZYME II in Products A and B and water (Figure 2). ENDOSAFE-MCS gave the least sensitive results in Products A and B, and ENDOZYME II in Products C and D.

**Percentage Recovery of Reference Standard Endotoxin**

The recovery of PPC from water was 70%, 178%, 69%, and 106% with the KQCL, ENDOSAFE-MCS, ENDOZYME II, and ENDOLISA assays, respectively. The percentage recovery of RSE from water (vs. the theoretical recovery) was 92%, 67%, 84%, and 75% for the KQCL, ENDOSAFE-MCS, ENDOZYME II, and ENDOLISA assays, respectively. The percentages of RSE recovered from the vaccines, calculated as a percentage of the recovery from water, were all within the required limits of 50% to 200% (Figure 3). The average RSE recovery for Product B was >100% for all four

![Table II](image-url)

**Table II**

Results From One Assay for Samples of Product A (No Pretreatment or Heated to 75˚C for 15 min) without and with an Endotoxin Spike

<table>
<thead>
<tr>
<th></th>
<th>KQCL</th>
<th>ENDOSAFE-MCS</th>
<th>ENDOZYME II</th>
<th>ENDOLISA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unspiked</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Product A</td>
<td>&lt;0.5</td>
<td>100</td>
<td>&lt;5</td>
<td>195</td>
</tr>
<tr>
<td>Heated Product A</td>
<td>&lt;0.5</td>
<td>110</td>
<td>&lt;5</td>
<td>135</td>
</tr>
<tr>
<td><strong>Spiked</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Product A</td>
<td>118</td>
<td>86</td>
<td>109</td>
<td>188</td>
</tr>
<tr>
<td>Heated Product A</td>
<td>41</td>
<td>99</td>
<td>27</td>
<td>132</td>
</tr>
</tbody>
</table>

Vol. 74, No. 4, July–August 2020 399
assays and was >180% with the KQCL and ENDOZYME II assays.

Endotoxin Content Detection

With the exception of Product C with the KQCL assay, all endotoxin concentrations were below the assay’s quantification limit (Table III). For Product C, an average endotoxin concentration of 10 EU/mL was obtained with the KQCL assay, but the concentrations were below the sensitivity threshold with the other assays, suggesting a potential enhancement of endotoxin concentration by (1→3)-β-glucans or another component (one of the vaccine valences is produced on yeast). In the presence of a β-
TABLE III
Endotoxin Concentrations and Mean Percentages of Spike (Positive Product Control; PPC) Recovery (As a Percentage of Recovery from Water) for Each of the Four Assays with the Four Products (Product A, Unheated). The Results Shown Are the Average Results from Three Independent Runs by Two Technologists

<table>
<thead>
<tr>
<th>Assay</th>
<th>KQCL EU/mL</th>
<th>PPC (%)</th>
<th>ENDOSAFE-MCS EU/mL</th>
<th>PPC (%)</th>
<th>ENDOZYME II EU/mL</th>
<th>PPC (%)</th>
<th>ENDOLISA EU/mL</th>
<th>PPC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unspiked products</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Product A</td>
<td>&lt;0.5</td>
<td>100</td>
<td>&lt;5</td>
<td>161</td>
<td>36</td>
<td>106</td>
<td>0.7</td>
<td>135</td>
</tr>
<tr>
<td>Product B</td>
<td>&lt;0.5</td>
<td>109</td>
<td>&lt;5</td>
<td>159</td>
<td>&lt;0.5</td>
<td>109</td>
<td>&lt;0.5</td>
<td>120</td>
</tr>
<tr>
<td>Product C</td>
<td>10</td>
<td>93</td>
<td>&lt;5</td>
<td>139</td>
<td>&lt;50</td>
<td>93</td>
<td>&lt;5</td>
<td>99</td>
</tr>
<tr>
<td>Product D</td>
<td>&lt;0.5</td>
<td>68</td>
<td>&lt;5</td>
<td>115</td>
<td>&lt;12.5</td>
<td>99</td>
<td>&lt;5</td>
<td>84</td>
</tr>
<tr>
<td>Spiked products</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Product A</td>
<td>125</td>
<td>74</td>
<td>107</td>
<td>176</td>
<td>84</td>
<td>89</td>
<td>82</td>
<td>137</td>
</tr>
<tr>
<td>Product B</td>
<td>196</td>
<td>78</td>
<td>136</td>
<td>163</td>
<td>182</td>
<td>74</td>
<td>107</td>
<td>93</td>
</tr>
<tr>
<td>Product C</td>
<td>142</td>
<td>87</td>
<td>114</td>
<td>162</td>
<td>133</td>
<td>95</td>
<td>93</td>
<td>98</td>
</tr>
<tr>
<td>Product D</td>
<td>79</td>
<td>57</td>
<td>92</td>
<td>144</td>
<td>148</td>
<td>97</td>
<td>90</td>
<td>91</td>
</tr>
</tbody>
</table>

glucan blocker, the endotoxin concentration detected was, on average, 41% lower in unspiked Product C with three independent runs of the KQCL assay. The mean PPC was 59%, which is within the variability of the method (i.e., 50%–200%). Using the Glucatell assay, we found 153 pg/mL of beta-glucans in Product C. An average endotoxin concentration of <5 EU/mL was obtained with the ENDOSAFE-MCS assay. The differences in result obtained with the LAL-based assays could be because of different lysate sensitivity to reactive material such as (1→3)-β-glucans (13). The average PPC for Product D was >90% in all assays except for the KQCL assay where it was 57%, which, although low, is a valid result.

The endotoxin concentrations detected by the assays in all four unspiked products were all below the company’s internal product-specific release limits, although the endotoxin concentrations were >10 EU/mL with the ENDOZYME II assay for Products A, C, and D (Figure 4). The highest endotoxin concentration with the KQCL assay was in Product C (10 EU/mL) compared with <1 EU/mL for the other products, but this was below the company’s internal product-specific release limit.

Discussion

Our results showed that the rFC-based endotoxin assays, ENDOZYME II and ENDOLISA, are suitable for the detection of endotoxin in four products with different matrix characteristics. There was no trend for either the LAL or rFC assay results to be higher or lower in the unspiked products, although false-positive results are possible for both as discussed following. The RSE spike recovery (100 EU/mL) was within the required limits of 50% to 200% for all products and all assays. The RSE spike recoveries in the ENDOSAFE-MCS and ENDOLISA assays were closer to 100% than those for the other two assays and, compared with the ENDOLISA assay, the recovery with the ENDOSAFE-MCS assay was higher, possibly owing to the use of an internally archived standard curve in the cartridge.

The results obtained with the LAL-based and the rFC-based assays were within the company’s internal product specifications. One advantage of the rFC-based assays is that the presence of β-1,3-glucan, which can come from the manufacturing processes for these products, was not detected, unlike in the LAL tests in which a glucan blocker needs to be added to prevent false-positive signals. We showed that all four endotoxin platforms could be used for the different products tested (Table IV). They all provided results that were below the company’s product-specific release limits. In addition, they achieved the Pharmacopeia and Sanofi Pasteur internal assay validity criteria, although some assays were more suitable for some matrices owing to
the absence of false-positive results and lower sensitivity to interference from the matrix.

False-positive results can be obtained with LAL assays owing to various factors such as the presence of blood products, polynucleotides, and (1→3)-β-glucans (13, 29, 30). (1→3)-β-glucans contain glucose polymers of varying molecular weight linked primarily through (1→3)-β glycosidic linkages. If sufficient amounts of glucans with a particular molecular weight are present, a positive LAL response, that is not an endotoxin-mediated response, may be observed. The factor G pathway is activated most efficiently by linear (1→3)-β-D-glucans whereas chains containing branches are less effective, and short oligosaccharide chains (two to seven glucose units) do not activate factor G at all (4, 31, 32). A β-glucan blocking effect was first reported for a high-molecular-weight β-glucan, and then a low-
molecular-weight β-glucan was also reported as a β-glucan blocker (33). The factor G pathway can be blocked by the addition of low-molecular-weight glucans, which can competitively block the LAL alternate pathway at specific concentrations (US Patent 5,155,032) (4, 34).

We showed that the signal observed for Product A in the rFC-based assays (ENDOLISA and ENDOZYME II) is because of the presence of proteases. This is supported by the “positive” result obtained in the absence of rFC. It is possible that assessing different pretreatments for Product A with the ENDOLISA and ENDOZYME II assays could improve the results. For example, the impact of protease on the results with ENDOLISA could be reduced by higher dilutions of Product A in water, which would result in a weaker signal, but this would not completely eliminate the interference from the presence of

---

**Figure 4**

Endotoxin quantification in the four unspiked products with the four endotoxin detection assays.

**TABLE IV**

Summary of Recommended Assay Taking into Account the Characteristics of the Different Human Vaccine Matrices

<table>
<thead>
<tr>
<th>Product (specificity of matrix)</th>
<th>KQCL</th>
<th>ENDOSAFE-MCS</th>
<th>ENDOLISA</th>
<th>ENDOZYME II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product A (presence of proteases)</td>
<td>✓ⁿ</td>
<td>✓</td>
<td>LSⁿ</td>
<td>LS</td>
</tr>
<tr>
<td>Product B (no interference)</td>
<td>LSⁿ</td>
<td>✓</td>
<td>✓</td>
<td>LS</td>
</tr>
<tr>
<td>Product C (natural endotoxin; complex)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Product D (colored-matrix)</td>
<td>LS</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

ⁿSuitable assay(s) (no false-positive results, minimization of interference from matrix on results).

ⁿⁿLS is less suitable assay(s).
proteases. Although the result for the unspiked or spiked sample could have been subtracted from the sample tested without added rFC to improve the assay sensitivity in this setting, it would not be feasible to do this routinely for product release in a quality-control laboratory. Thus, the ENDOZYME II was not suitable for testing Product A under the conditions tested. Because the LAL-based assays worked well for Product A, it would seem that the rFC-based assays may not be suitable for this particular Product A protease-containing sample without further testing.

The RSE recovery from Product B was >180% with KQCL and ENDOZYME II, indicating important interference from the product matrix in these two assays and making them less suitable for this product. All assays can be used for Product C. Product D showed an inhibitory effect in the KQCL assay, although the spike recovery was valid (>50%). This is because of the matrix formulation, which contains a red substance that interferes with the measurement of the yellow chromogenic product, making the KQCL assay less suitable.

The RSE recovery from water was best with the ENDOZYMA assay, 106% compared with 69%, 70%, and 178% with the ENDOZYME II, KQCL, and ENDOSAFE-MCS assays, respectively. This could be because of the different concentration range that is used for the ENDOLISA assay, which requires a higher PPC spike of 5 EU/mL vs. 0.5 EU/mL with the other assays, as the higher spike can give a better percentage recovery. For the ENDOSAFE-MCS method, the 0.78 EU/mL spike is not added by the operator as it is already present in the cartridge. The high percentage of overlap in water could be because of the cartridge batch used or the technique itself.

We also demonstrated that there was less interference from the matrices in the ENDOZYME II assay than in the KQCL assay. However, it is difficult to compare the levels of interferences from the matrices with the different methods because we also observed differences with the water controls owing to the parameters of each assay, such as the value of PPC and the preparation of standard curve, which were not identical.

Results from one multicenter study showed that the requirements for validation of compendial methods for specificity, precision, accuracy, linearity, range, and quantitation limit were satisfied for the rFC assay and were equivalent to the USP photometric procedures for endotoxin measurement (35). In another study, an rFC-based endpoint fluorescence assay was validated and compared with a LAL-based assay using 10 drug products, 6 drug substances, 2 pharmaceutical formulation excipients, and pharmaceutical production grade water (14).

A summary of the characteristics of the four assays compared in this study, based on our results and information from the manufacturers, can be found in Table V. One of the main advantages of the rFC-based assays is their enhanced specificity for endotoxin. The spike recovery is statistically more robust and their sensitivity range, 0.005 EU/mL to 5 EU/mL, is suitable for endotoxin detection for pharmaceutical products (29). They also do not require samples to be substantially diluted and will, therefore, potentially reduce the number of invalid results arising from the high dilutions required for the KQCL assay. The ENDOSAFE-MCS assay can provide rapid results (e.g., 20 to 30 min for 5 samples with one module) if only a few samples need to be tested, however microplates have higher throughput and up to 21 samples can be tested using one microplate. The ENDOLISA assay takes the longest time to perform, but it is suitable for samples with matrices that have high interference and it is, therefore, the most adapted for troubleshooting purposes.

The growing need for endotoxin assays has led to fears of excessive demands for LAL and thus potential pressure on the horseshoe crab populations from which the LAL is obtained. When rFC-based assays became available, there was only one commercial supplier of rFC reagents, but now there are other suppliers. Hence, rFC being a nonanimal-derived reagent can help to overcome any potential shortage of LAL while supporting the aims of the 3Rs initiative in terms of more ethical and sustainable use of animals for testing (36).

The pharmacopeia requires that replacement assays are more scientifically relevant, which is the case for the rFC assays as they show high specificity and have a simpler enzymatic reaction than the cascade reaction in LAL-based assays and should, therefore, be less sensitive to interference (37). In January 2019, the Ph. Eur. launched a public consultation on a new General Chapter, 2.6.32: Test for Bacterial Endotoxins Using Recombinant Factor C (rFC). For the time being, this will not be referenced in individual monographs but will be a standalone chapter.
In addition, the American USP Microbiology Expert Committee organized a workshop in June 2019 to consider new reference endotoxin standards and the requirements for the inclusion of new endotoxins test methods (39).

To conclude, our results from this study demonstrated that the results from the rFC-based assays, ENDOZYME II and ENDOLISA, gave comparable results to those obtained with LAL-based assays. However, samples containing

<table>
<thead>
<tr>
<th>TABLE V</th>
<th>Comparison of Characteristics for Two <em>Limulus</em> Amoebocyte Lysate (LAL; KQCL and ENDOSAFE-MCS) and Two Recombinant Factor C (ENDOZYME II and ENDOLISA) Endotoxin Assays, Using Information from the Assay Manufacturers and from This Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Origin of reagent</strong></td>
<td><strong>KQCL</strong></td>
</tr>
<tr>
<td>Lot-to-lot reproducibility$^a$</td>
<td>Animal</td>
</tr>
<tr>
<td>Assay range with maximum sensitivity (EU/mL)$^a$</td>
<td>50–0.005</td>
</tr>
<tr>
<td>pH range$^a$</td>
<td>6 to 8</td>
</tr>
<tr>
<td>PPC$^b$ spike (EU/mL)</td>
<td>0.50</td>
</tr>
<tr>
<td>Standard range</td>
<td>Performed manually</td>
</tr>
<tr>
<td>Glucan reaction</td>
<td>False positive</td>
</tr>
<tr>
<td>Matrix effects</td>
<td>Enhancement (RSE$^c$ recovery) for Product B</td>
</tr>
<tr>
<td>Equipment/software</td>
<td>ELX808/win KQCL v4.02</td>
</tr>
<tr>
<td>Time to result</td>
<td>2 h</td>
</tr>
<tr>
<td>Regulatory status</td>
<td>Pharmacopeia method</td>
</tr>
</tbody>
</table>

$^a$Manufacturer’s information.  
$^b$PPC is positive product controls.  
$^c$RSE is reference standard endotoxin.

(38) In addition, the American USP Microbiology Expert Committee organized a workshop in June 2019 to consider new reference endotoxin standards and the requirements for the inclusion of new endotoxins test methods (39).
proteases that can liberate the colored substrate or fluorophore in the absence of endotoxin are problematic for both the LAL-based and the rFC-based endotoxin assays. The rFC-based assays offer a number of additional benefits and can be considered as suitable alternative assays for the detection of endotoxin. Our results confirmed that all four methods (two LAL, two rFC) are essentially comparable and are suitable to release all four products.

Acknowledgments

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Conflict of Interest Declaration

All authors are employees of Sanofi Pasteur.

References


15. Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Management; Carmichael,


CASE STUDY

Environmental and Personnel Monitoring Programs—A Risk-Based Case Study of Cutibacterium acnes

EDWARD C. TIDSWEL1,* and KENNETH BOONE2

1Merck, West Point, PA 19486; and 2The Maurice R. Hilleman Center for Vaccine Manufacturing, Merck, Durham, NC 27712. © PDA, Inc. 2020

ABSTRACT: In the aseptic manufacture of parenteral drug products and low bioburden, cell, and gene therapy products, the control and monitoring of environmental- and personnel-associated microorganisms is an imperative for the confirmation of controlled conditions and the assessment of microbial risks. Environmental and personnel monitoring programs exist to assure product quality and serve as one of the several means of removing the emphasis on finished drug product testing. Therefore, these programs must adequately assess these risks and identify situations in which increased microbial risks occur. The major source of microbial risks in the controlled clean room environments for parenteral drug product manufacture are personnel. Modern microbial analytical methods, including metagenomic analysis, have identified a greater abundance of Cutibacterium acnes; traditional culture-based monitoring fails to consistently recover and assist in the identification of the potential risk that this microorganism represents. This review provides a case-study assessment of this microorganism in the context of parenteral manufacture for the purpose of assisting in the deciding the necessary controls and the potential monitoring addressing this microbial risk.

KEYWORDS: Microbiological risk, Cutibacterium acnes, Culture medium, Incubation conditions, Monitoring, Controls.

Introduction

Environmental and personnel monitoring both serve critical functions in the assessment of the adequacy of the pharmaceutical and biotechnology manufacturing environments and the aseptic compounding pharmacies. Both forms of monitoring operate in a fundamentally identical fashion; application of risk-based sampling of locations for microorganisms, the culture-based growth of any microorganisms recovered by sampling, their accurate enumeration (1), comparison to specification, and finally a decision regarding any potential impact. These programs are primarily geared to monitor microorganisms originating from the most common and frequent source—personnel and their inherent microbiome. Although there is no absolute relationship directly linking environmental microbial load and product quality, the clean room environment and personnel must meet certain levels of bioburden control to contribute to the overall assurance of product quality. Failure of controls and high levels of environmental and personnel bioburden may result in contaminated product, the loss of sterility, and if administered, patient morbidity and mortality owing to infection. It is well recognized that environmental monitoring and personnel monitoring (2) are inherently constrained and are not absolute indicators of adequacy; they are however important elements of quality and sterility assurance upon which firms heavily rely. In consideration of this, it is imperative that these essential monitoring programs are sufficiently designed and operated for their intended purpose of gauging clean room and personnel bioburden. One key premise is that the growth medium and subsequent incubation conditions are adequate for measuring this risk. It is not necessary for monitoring programs to have the capability to grow all microorganisms but rather a capacity to grow sufficient and representative microorganisms that illustrate overall risk. Adequacy of the program is therefore predicated upon the appropriate choice and use of microbiological growth medium. To date, the industry has routinely used tryptic soya agar (TSA) and aerobic incubation as the gold standard growth medium for environmental and personnel monitoring programs. The

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choice of TSA and aerobic incubation is based upon the assumption that this medium is the optimum for consistently growing microorganisms representative of the species and the quantity shed from personnel. Over the last few years, the National Institutes of Health (NIH) Human Microbiome Project (HMP, URL: http://hmpdacc.org), its subsequent second phase, the Integrative Human Microbiome Project (iHMP, https://www.hmpdacc.org/ihmp/), and related scientific endeavors (3) have illustrated that our skin-borne microbiome is complex (4) and not only keeps us healthy (5) but may also cause ill health through dysbiosis (6). The HMB has demonstrated that Cutibacterium acnes is one of the most abundant skin-borne microorganisms (7). Several inherent characteristics make this an organism with profound potential risk in aseptic processing environments, and yet the industry standard medium of TSA will not sufficiently support the growth of this microorganism. The industry’s environmental monitoring and personnel monitoring programs could be oblivious to the risk from this organism. The authors believe that a risk-based consideration is necessary for firms to appropriately evaluate the controls and monitoring programs and provide the basis for their optimization. To this end, this review presents a risk-based case study of the microorganism C. acnes, its location and inclusion in the microbiome, the literature documenting the recovery and growth of C. acnes, associated FDA noncompliance observations, and potential risks to aseptic manufacturing.

The Microorganism Cutibacterium acnes

The Gram-positive aerotolerant anaerobic bacillus C. acnes is a pleomorphic nonmotile bacterium first described by Orla-Jensen in 1909 (8). A single bacterium is 0.4–0.5 μm wide and 0.8–0.9 μm long (9), and they are frequently microscopically observed in chains of two or more cells. As a member of the Actinobacteria, Propionibacteriales, it was initially classified into the genus Corynebacterium in 1923 before transfer to the genus Propionibacterium in 1933 (9) and then inclusion into the new genus Cutibacterium in 2016 (10).

Although this lipophilic microorganism is usually regarded as a strict anaerobe, it can tolerate oxygen and grows at reduced rates (11). Cutibacterium acnes may survive as long as 8 months under anaerobic conditions and prefers low redox potential environments (12).

The application of early phenotypic methods, including serological agglutination, cell wall sugar analysis, bacteriophage, and fermentation profiling revealed three C. acnes clades termed type I, II, and III (13–16). Cutibacterium acnes type I clade can be subdivided into closely related phylotypes: IA1, IA2, IB, and IC; phylotype IA1 has been associated with acne, whereas types II and III are generally isolated in association with healthy skin (7).

Cutibacterium acnes possesses the potential to generate virulence factors that may assist in the adhesion and colonization of habitats on the human skin. These virulence factors include hyaluronate lyase (17), sialidases, and endo-glycoceramidases putatively involved in host tissue degradation (18).

Cutibacterium acnes hydrolyzes triglycerides in sebum and releases free fatty acids, including lauric and linoleic acid, as well as short-chain fatty acids (e.g., propionic acid) that contribute to an acidic skin pH and antimicrobial activity (19, 20). Cutibacterium acnes is also known to produce bacteriocin-like molecules that may be responsible for its successful colonization in the follicle and on the skin surface (21, 22).

The whole genome sequencing of C. acnes (strain DSM 16,379) describes 2,560,265 base pairs coding for a repertoire of genes that support impressive adaptive capabilities; including the capacity to grow under microaerobic as well as anaerobic conditions (18). The genes encode all of the components for the conservation of energy by oxidative phosphorylation, substrate-level phosphorylation via the Embden–Meyerhof pathway and pentose phosphate pathway, and several amino acid–degrading pathways. This array of metabolic pathways and catabolic options equip C. acnes with a significant degree of flexibility in surviving and/or proliferating in environments with changing oxygen concentrations. Surprisingly, several gene sequences demonstrate similarity to immune reactive proteins shared by Mycobacterium tuberculosis and Mycobacterium leprae (18); the authors not only question why, the general significance, but also what specific consequence, if any, this might have in our field.

Cutibacterium acnes and the Skin

Metagenomic analysis (23) of the human body has permitted the accurate identification and mapping of the diverse microbial communities and their interactions hitherto impossible with culture-based methodologies. Thus, we are now very much aware of our own inherent microflora, or more accurately our “microbiome” (24). The HMP (25) has illustrated the abundance of micro-
organisms that reside on and in our bodies and in particular the surface and subsurface layers of our skin. This in turn has illuminated two major concerns for the pharmaceutical microbiologist. Firstly, the human skin microbiome is extraordinarily large and diverse in microorganisms; their interactions representing complex abundant sources of microorganisms capable of contaminating controlled environments, processes, and products. One simple numeric illustration is that up to $10^{11}$/m² bacteria inhabit the human skin (26). Secondly, most microorganisms that inhabit the skin are either in a quiescent state or unrecoverable by traditional culture media and conditions rendering this microbiological risk for the most part unseen by the standard means of monitoring environments, personnel, processes, or products. The complexity and dynamics of the human microbiome and the newly understood risks to pharmaceutical manufacturing and control are exemplified by *C. acnes*.

In general, we can divide the skin into three broad regional habitats relevant to our skin microbiome: (1) moist regions including the axilla, perineum, and toe webs; (2) oily or sebaceous areas including the head and neck; and (3) dry areas such as the forearms and legs. Whereas certain abundant species of microorganisms are recovered in one or two of the skin regions, *Cutibacterium* has been recovered from all areas of healthy individuals (27, 28). Although ubiquitous to all regions of the skin, *C. acnes* also appears to be the predominant species in oily, sebaceous skin areas (29). The skin’s sebaceous glands manufacture a hydrophobic mixture of lipids (30) termed sebum and purposed to lubricate and protect the skin and hair. As such, the chemical constituents of the lipid rich sebum are regulated and altered depending upon several factors, such as environmental conditions (e.g., temperature) and age. Although sebum generally exerts antimicrobial activity, *C. acnes* hydrolyzes the triglycerides present in sebum, releasing free fatty acids that promote bacterial adherence, and then colonizes the sebaceous units (6). The hair follicle and sebaceous glands of the skin represent hospitable environments for *C. acnes*, which communicate using autocrine signal molecules to modulate biofilm generation along the lumen of the hair follicle and the hair follicle itself (31). A simplified illustration of a hair follicle and the location of *C. acnes* is provided in Figure 1. It is a valid assertion that cells of *C. acnes* are commonly physically “coated” with sebum and that they benefit from its protective properties. A propensity to generate biofilm appears linked to certain phylotypes of *C. acnes* (32) and therefore has recently been associated with the causation of acne vulgaris (7, 33). In the follicular environment, *C. acnes* exhibits niche competition with other microorganisms, such as *Staphylococcus* species, and is known to produce a thiopeptide antibiotic, cutimycin (34). In contrast to the classical assertion that *C. acnes* is the infectious agent causing acne vulgaris, contemporary science provides evidence that *C. acnes* is not only ubiquitous to all skin locations but is also equally abundant in both unaffected and acne-affected follicles (7). It could be feasible that the choice of the skin sampling techniques might bias the conclusion regarding the abundance of *C. acnes* on the skin surface versus follicular niches; however, Hall et al. (35) compared different techniques and concluded that there was negligible difference. *Cutibacterium acnes* is a commensal microorganism that, although associated with acne vulgaris, is a well-recognized opportunistic pathogen associated with implant surgery (36–39) yet conversely mutualistically assists in the health and homeostasis of the skin (40).

**Culturing Cutibacterium acnes**

The majority of studies regarding *C. acnes* are in the clinical context of infection evaluation, investigation, and clinical infection control. Few studies exist regarding the isolation, recovery, and culturing of *C. acnes* in pharmaceutical manufacturing environments. *Cutibacterium acnes* is known to grow on a variety of media and under various incubation conditions; culture media it grows on include blood agar, chocolate agar, Brucella agar, and brain heart infusion broth, whereas *C. acnes*
fails to grow on MacConkey agar with lactose (41). The
ideal culture temperature of C. acnes is 30˚C–37˚C (42).
The variety of media that permit the recovery and
growth of C. acnes may reflect the metabolic diversity
of C. acnes; however, extended incubation conditions of
10–13 days might be required for culture media that are
suboptimal for C. acnes (41, 43). In clinical infections
studies, the limitation of the incubation time to 7 days
has resulted in the failure to associate and identify the
presence of C. acnes (44). Other authors have also dem-
onstrated a similar incubation period of 6–10 days can
be adequate to recover C. acnes (45). A review of the
literature illustrates the fastidious nature of C. acnes in
preferring a complex growth medium rich in soluble
growth factors derived from blood or animal tissue
digests. Blood agar (46) or brain heart infusion (47, 48)
agars are commonly the media of choice and provide
the necessary growth factors. The absence of these
growth factors would prevent recovery of isolates of C.
acnes or at minimum result in retarded growth rates.
Hyde (49) reported that the inclusion of Tween-80 in the
media also assists the growth of C. acnes. Furthermore,
C. acnes demonstrates a clear preference for anaerobic
growth conditions and low redox potential environments.
Recovery and growth under aerobic conditions is gener-
ally reported as slow (11, 50); however, supplementation
of the environment with 5% (v/v) CO₂ is beneficial. 
Rieber et al. (43) demonstrated an increased speed of re-
cover of C. acnes with supplemented nutrient-rich
media. The authors pointed out the importance of main-
taining a continuous anaerobic environment during cul-
turing, otherwise growth is impaired. Table I summarizes
the source literature describing those culture conditions
successfully used for culturing of C. acnes on agar plates.
Details quantifying the precise numbers of colony form-
ing units (CFUs) or recovery efficiency from the various
media are not included. These details would be valuable
in gauging the different abilities of the media to recover
C. acnes; however, the diverse, varied, clinical contexts
of the methods and sampling make this impossible. In
general, we can conclude that successful consistent recov-
er of C. acnes on agar requires a complex medium and
anaerobic incubation at 30˚C–37˚C for at least 7 days.
Other authors reported the recovery and culturing of C.
acnes in liquid broth such as thioglycollate broth (43, 57).

Adequacy of Industry Standard Monitoring and
Sterility Testing For Cutibacterium acnes

It is important to recognize the inherent constraints of
all environmental and personnel monitoring programs
in that they are imperfect “snap shots” of microor-
isms in the environment and on personnel. One of the
greatest constraints of environmental and personnel
monitoring programs is that they will only detect a
small fraction of the total microorganisms present (58).
Monitoring programs are neither quantitatively precise
nor encompassing all of microorganisms. The cadence
of sampling, sample location, technique, and choice of
growth media and incubation conditions for the recov-
ery of microorganisms are all factors of compromise
chosen with a specific end purpose in mind. All envi-
ronmental monitoring and personnel monitoring pro-
grams are purposed to assist in the assessment of the
adequacy of the controls protecting the environment,
process, and product from extraneous sources and vec-
tors of microorganisms. An adequate environmental
and personnel monitoring program should therefore be
an early diagnostic of potential end product risk, incor-
porating the optimum choice of factors to fulfill that
purpose. Effective environmental and personnel moni-
toring programs must therefore be more effective in
recovering and identifying potential risk than the lim-
ited end product testing program is as a means of
detecting contamination. No single program is capable
of comprehensively demonstrating the complete array
of microorganisms and the associated risks to environ-
ment, process, or product; however, all programs must
be adequate in describing those risks. As such, the
diagnostic effectiveness of environmental and person-
nel monitoring programs is primarily dependent upon
the choice of growth media and the incubation condi-
tions to adequately capture those risks by the recovery
of appropriately representative microorganisms. In this
respect, the adequacy of environmental monitoring and
personnel monitoring programs relies upon the recov-
ery of microorganisms that are indicative of this risk
and the assumption that the recovery and the patterns
of recovery for these microorganisms are a sufficient
marker. All effort dedicated to optimized sampling fre-
quency and location are all confounded if the choice of
growth medium is inadequate for recovering microor-
ganisms. As we understand far more clearly the actual
types and populations of microorganisms in the clean
room environment (59, 60) and upon personnel, the
selection of the growth media to recover microorgan-
isms that are adequately representative of overall
microbiological risk must be subject to reassessment.
To this end, almost all environmental and personnel
monitoring programs use the industry standard media
of trypticase soy agars for the recovery of bacteria and
Sabouraud dextrose agar for the recovery of fungi. A
A cursory review of the literature providing data supporting the choice of the monitoring media and the incubation conditions is summarized in Table II. None of these studies evaluated by comparison the recovery of human commensals such as *C. acnes* by inclusion of alternative growth media and incubation conditions. A comparison of the industry-standard agar-based media and incubation conditions per Table II with the data in the prior section (see Table I) clearly shows that *C. acnes* would seldom be consistently recovered by the monitoring programs. Although inconsistent and irregular recovery of *C. acnes* has been reported on

### TABLE I
Peer-Reviewed Literature Reporting the Agar Growth Medium and Incubation Conditions Successful in the Recovery of *Cutibacterium acnes*. No Comparison or Quantification Relative or Otherwise to the Ability of Each Regimen to Recover *C. acnes* Is Included Here

<table>
<thead>
<tr>
<th>Medium</th>
<th>Temperature</th>
<th>Incubation Duration</th>
<th>Aerobic/Anaerobic</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robertson's cooked-meat broth</td>
<td>Not stated</td>
<td>Not stated</td>
<td>Anaerobic</td>
<td>51</td>
</tr>
<tr>
<td>Trypticase soy blood agar (TSA) plates, containing 5 mg/L hemin and</td>
<td>37°C</td>
<td>7 days</td>
<td>Anaerobic10% CO₂, 10% H₂, and</td>
<td>52</td>
</tr>
<tr>
<td>50 μg/L vitamin K</td>
<td></td>
<td></td>
<td>80% N₂, or aerobically in the</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>presence of 5% CO₂.</td>
<td></td>
</tr>
<tr>
<td>Blue lactose plates under aerobic conditions</td>
<td>37°C</td>
<td>7 days</td>
<td>Aerobic</td>
<td>44</td>
</tr>
<tr>
<td>Columbia sheep blood agar, Colistin-nalidixic acid (CNA) blood agar,</td>
<td>Not stated</td>
<td>7 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MacConkey agar, Crowe agar (chocolate agar supplemented with</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bacitracin and IsoVitaleX)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brucella agar (in-house sheep blood agar plates with hemin and vitamin</td>
<td>Not stated</td>
<td>7 days</td>
<td>Anaerobic</td>
<td></td>
</tr>
<tr>
<td>K1), Kanamycin-vancomycin agar (laked sheep blood Brucella agar plates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with kanamycin and vancomycin), Phenylethyl alcohol agar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain Heart Infusion agar</td>
<td>37°C</td>
<td>4 days</td>
<td>Anaerobic</td>
<td>32</td>
</tr>
<tr>
<td>Brain Heart Infusion agar</td>
<td>37°C</td>
<td>3 days</td>
<td>Anaerobic</td>
<td>53</td>
</tr>
<tr>
<td>Blood agar (Trypticase soy agar with 5% sheep blood), chocolate agar</td>
<td>37°C</td>
<td>28 days</td>
<td>Aerobic with 5% CO₂</td>
<td>41</td>
</tr>
<tr>
<td>Brucella agar (with blood, hemin, and vitamin K)</td>
<td>37°C</td>
<td>28 days</td>
<td>Anaerobic</td>
<td></td>
</tr>
<tr>
<td>Chocolate agar supplemented with Polyvitex</td>
<td>35°C</td>
<td>21 days</td>
<td>Aerobic with 5% CO₂</td>
<td>54</td>
</tr>
<tr>
<td>CDC blood agar with a gentamicin disk (ANA CDC)</td>
<td>37°C</td>
<td>7 days</td>
<td>Aerobic with CO₂</td>
<td>55</td>
</tr>
<tr>
<td>Trypticase soy agar containing 5% sheep blood (Oxoid), chocolate</td>
<td>36°C</td>
<td>14 days</td>
<td>Aerobic</td>
<td>56</td>
</tr>
<tr>
<td>agar (Becton Dickinson), MacConkey II agar (Becton Dickinson)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schaedler agar containing vitamin K1 and 5% sheep blood (Becton</td>
<td>36°C</td>
<td>14 days</td>
<td>Anaerobic</td>
<td></td>
</tr>
<tr>
<td>Dickinson)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
aerobically incubated TSA supplemented with Tween-80 (4–7 days incubation at 35˚C–37˚C), the authors postulate that a skin-associated growth factor may have aided recovery on the growth medium (67).

The absence of *C. acnes* from clean room microflora profiling assessments (68, 69) is likely not because of the physical absence of this organism but rather because of the inability of the growth medium to recover this microorganism. Also, some environmental monitoring technologies, such as active air samplers, may fail to deposit the microorganisms or may impart forces that damage the microorganisms and render them unculturable. *Cutibacterium acnes* has a moderate level of oxygen tolerance, superoxide dismutase and catalase activity, and a capacity to endure in aerobic environments for at least many hours (70–72). It would seem that industry standard environmental and personnel monitoring programs have adopted TSA as the preferred growth medium and incubation conditions tailored to aerobic mesophilic microorganisms. In so doing, the programs could be erroneously designed in their failure to recognize that a more abundant microbiological risk to the process and the product may be present as microorganisms that would not fit in this category and could therefore fail to be recovered. *Cutibacterium acnes* would likely not be consistently recovered by this industry standard, and yet contemporary scientific literature suggests that this microorganism is one of the most, if not the most, prevalent skin-borne microorganism. It appears somewhat ironic that monitoring regimens specifically chosen with the rationale of recovering the microorganisms most likely present from human origin could be failing to do so by the exclusion of the most abundant because of the selection of the growth medium and the incubation conditions.

TABLE II
Representative Literature Supporting the Choice of the Current Industry Environmental and Personnel Monitoring Growth Media (Agars) and Incubation Conditions

<table>
<thead>
<tr>
<th>Growth Medium</th>
<th>Incubation Temperature</th>
<th>Aerobic/Anaerobic Incubation</th>
<th>Incubation Duration</th>
<th>Purpose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone soya agar with 1% (w/w) glucose</td>
<td>25˚C</td>
<td>Aerobic</td>
<td>5 days</td>
<td>Environmental monitoring for recovery of bacteria and fungi</td>
<td>61</td>
</tr>
<tr>
<td>Tryptone soy agar</td>
<td>30˚C–35˚C</td>
<td>Aerobic</td>
<td>5 days</td>
<td>Environmental monitoring for recovery of bacteria and fungi</td>
<td>62</td>
</tr>
<tr>
<td>Ravan medium</td>
<td>20˚C–25˚C</td>
<td>Aerobic</td>
<td>Up to 21 days</td>
<td>Environmental and personnel monitoring</td>
<td>63</td>
</tr>
<tr>
<td>Tryptic soy agar</td>
<td>30˚C–35˚C</td>
<td>Aerobic</td>
<td>3 days</td>
<td>Environmental monitoring, total aerobic count and mold counts&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64</td>
</tr>
<tr>
<td>Sabouraud dextrose agar</td>
<td>20˚C–25˚C</td>
<td>Aerobic</td>
<td>7 days</td>
<td>Environmental monitoring, bacteria and fungi</td>
<td>65</td>
</tr>
<tr>
<td>Tryptone soy agar</td>
<td>20˚C–25˚C and 30˚C–35˚C&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Aerobic</td>
<td>≥5 days and ≥2 days</td>
<td>Environmental monitoring bacteria and fungi</td>
<td>65</td>
</tr>
<tr>
<td>Sabouraud dextrose agar</td>
<td>20˚C–25˚C</td>
<td>Aerobic</td>
<td>8 days</td>
<td>Environmental monitoring fungi</td>
<td>66</td>
</tr>
</tbody>
</table>

<sup>a</sup>These conditions yielded the highest recovery for total aerobic counts and for molds, respectively; however, the authors point out that their data support the use of other conditions as acceptable.

<sup>b</sup>The sequence of different temperatures may be optimized depending on the environment and the process.
pink when the Eh is above −51 mV. The appropriately applied sterility test is therefore likely to recover the presence of viable cells of C. acnes within a test sample. The industry standard application of environmental and personnel monitoring (TSA culture medium incubated aerobically) together with end product sterility testing (including thioglycollate medium to recover anaerobes) may be fundamentally flawed and of ill design. As they stand, the identification of microbiological risk through environmental and personnel monitoring clearly fails the intended purpose of prospective early signaling of risk and patient safety and relies more upon the statistically and technically flawed sterility test (73).

### FDA Form 483s concerning *Cutibacterium acnes*

Although FDA Form 483 observations frequently fail to include comprehensive details and an accurate context for the quality or compliance issue identified, they do assist in assessing purportedly significant microbiological risks. A review of recent FDA Form 483 observations reveals that *C. acnes* features in several quality and compliance issues spanning at least two decades and across a diverse number of pharmaceutical and biotechnology firms. The risk that *C. acnes* represents to aseptically manufactured product is neither limited by the type of sterile product manufactured nor by the manufacturing process. This organism possesses a number of unique features that confer a remarkable potential for product and patient risks. A summary of many (but not all) of those FDA Form 483 observations that specifically identify *C. acnes* by name as a sterility assurance and patient risk are provided in Table III.

Reviewing the details contained in each Form 483 in Table III, we may make several conclusions:

1. *Cutibacterium acnes* has been and continues to be recoverable in controlled clean room environments. Clean room environments that are in many ways managed and controlled according to current Good Manufacturing Practices. Controls include appropriate gowning and garbing and clean room sanitization practices. Recovery of this microorganism has been achieved by firms adopting growth media and incubation conditions similar to those discussed previously.
2. *Cutibacterium acnes* is not routinely and consistently recoverable on the typical industry standard culture medium of TSA and the associated aerobic incubation durations and temperatures (see previous). Nor have firms appropriately qualified their environmental and personnel monitoring programs to demonstrate the recovery of this aerotolerant anaerobe.

3. Sterility test fails associated with *C. acnes* are not rare nor extraordinary events but are commonly associated with the personnel and the intervention activities performed during manufacturing or sterility testing.

4. Firms continue to fail to associate *C. acnes* with significant sterility assurance risks and fail to accommodate appropriate monitoring genuinely capable of measuring or assessing this risk. It is quite possible that there exists a pervasive misconception that anaerobes cannot endure our aerobic environment, requiring anaerobic conditions to pose a risk, and do not represent viable contamination risks to sterile product.

In addition to the Form 483s summarized in Table III, the authors reviewed and identified several other related nonconformance reports that did not reference *C. acnes* by name but did reference the inadequate consideration of anaerobic conditions within the personnel and the environmental monitoring programs. It is highly likely that many firms beyond those listed here have and are, knowingly or unknowingly, experiencing patient risk from this microorganism.

**Patient Risk from Cutibacterium acnes**

During aseptic manufacturing processes, microorganisms represent a hazard for the sterile end product in which contamination with a single microorganism constitutes a loss of sterility, nonconforming product, and a potential for adverse patient impact. At a fundamental level, microorganisms originating from a specific source gain access into the manufacturing processor or the finished drug product by a transfer process.

Humans represent the major source of microbiological hazards to all aseptically manufactured products. Each individual human possesses approximately $10^{13}$ bacteria (26) with an average skin surface area of 1.5–2.0 m² (26 m² if the follicular surface area is included (74)) and hosts up to $10^7$ CFU/cm² (75) as determined using culture-based techniques. As previously mentioned, *Cutibacterium* and *C. acnes* may be more abundant than other microorganisms, represent a higher proportion of the population of different species, and are exceptionally stable members of the skin microbiome (76). Fully gowned personnel discharge 1–2 CFU/m³ air in clean rooms of 90 m³ volume and volumetric air change rate of 20/h (77, 78), shedding 0.22–0.38 CFU/s (79, 80). Therefore, the quantity of *C. acnes* entering the environment is likely significant. It should be noted, however, that these data for CFU shed from gowned personnel were derived using growth media (TSA) purposed to recover aerobic microorganisms (79). It is quite feasible that the actual microbiological risk and the actual microbiological shedding from personnel has been underestimated. Many specific steps of transfer are recognized; however, mechanistically, the transfer of microorganisms from a source to a vulnerable process or product may occur from either airborne transfer or touch contamination, the transfer from a contaminated surface to another surface (81). Recent data demonstrate that the surface-to-surface transfer efficiency is of a magnitude of approximately 15%–35% within the constraints of experimental parameters (82). Furthermore, residual skin bacteria left on objects can be matched to the individual who touched the object (83). Airborne transfer has been effectively modeled (84). The risk assessment of aseptic processes was well described in the seminal work of Whyte and Eaton (85, 86) and related frameworks (87). The application of these methodologies clearly illustrate the potential magnitude of microbial risk posed by *C. acnes*, and that *C. acnes* could remain a risk “unseen” by the industry standard environmental and personnel monitoring programs. An additional and important consideration of risk from this organism is that the “coating” of sebum over cells of *C. acnes* provides an additional protective barrier that is perhaps not so prevalent on other skin-borne microorganisms. This hydrophobic envelope affords physical and diffusional (88) protection to *C. acnes* that may extend to disinfectants and chemical sterilants.

In terms of direct patient impact, *C. acnes* is a significant causal agent of certain clinically relevant infections (89). As previously stated, *C. acnes* is the cause of patient morbidity and mortality as an opportunistic infectious agent, mostly in association with implant surgeries (36–39). Specific isolates of *C. acnes* are linked to disease (90, 91); furthermore, phylotypes IC and II have been specifically associated with opportunistic infections of deep tissue (92). The authors have found no corroborated reports of infection from parenteral products contaminated with this microorganism;
however, there are many literature references concerning blood products, the infusion of blood products, the associated recovery of *C. acnes*, and potential patient risk. Care is warranted when evaluating many of these reports as the study methodologies adopted do not always consider the specific culture requirements needed for recovery of *C. acnes*. For example, Cunningham and Cash (93) evaluated the microbial contamination of 1000 platelet concentrates stored at 20°C and did not report the recovery of *C. acnes*. Closer examination of the methods illustrates that the duration of the incubation of the tests (96 h in total) was unlikely to recover *C. acnes*. Jacobs et al. (94) reported on the relationship between bacterial contamination and patient impact by surveying platelet infusions; *C. acnes* was not featured as a recovered contaminant. Unsurprisingly the recovery methodology, specifically the 48 h duration of the culture incubation, was insufficient to recover *C. acnes*. Current practice in the culturing of platelet concentrates reflects these culture requirements; generally, a maximum duration of 5–7 days using the rapid microbiological test technology BacT/Alert (95) confirms the occasional presence of *C. acnes* (96). A recent study (97) stated that 95% of contaminated platelet units contained *C. acnes*; this study employed anaerobic culture for 7 days incubation using the BacT/Alert rapid test system. There is unequivocal data that evidences that *C. acnes* is a recognized bacterial contaminant of platelet concentrates and is implicated in transfusion-transmitted bacterial infections (98, 99). Nevertheless, Brecher and Hay (99) are clear in stating “Although a few cases of *Propionibacterium*–contaminated units have been infused into patients, no long-term sequelae have been reported. Debate continues as to the value of an anaerobic culture in this context.”

A recent comprehensive NIH study evaluated several modern rapid sterility test technologies versus the compendial referee method using 118 isolates (77 bacteria, 8 yeasts, and 33 molds) representing 93 organisms from a NIH cGMP facility, 3 organisms from contaminated product, and 22 reference strains; *C. acnes* was not included (100). The absence of *C. acnes* is noteworthy and may indicate that this microorganism is not a concern in the NIH cGMP facilities and contaminated sterile products or simply that the monitoring and test conditions are not conducive to recovery. All these facts together regarding the clinical recovery and significance of *C. acnes* may indeed suggest the potential presence of this microorganism in aseptic manufacturing environments, which could result in unsterile product. However, the severity of the patient impact from infusion of the microorganism into the blood stream is questionable.

**Conclusion**

Over the last few years, metagenomic analysis (101) and other modern nonculture-based methods have illustrated an accurate picture of the human microbiome, controlled clean room environments (102), and the abundance of the microorganism of *C. acnes*. Recent advances in our understanding of the clean room microbiota coupled with a detailed mapping of the human microbiome warrant scientifically and risk-based optimization of pharmaceutical clean room control and environmental and personnel monitoring programs. These advances lead us to challenge the dogma (103) that skin-borne microorganisms recoverable upon the industry standard TSA are adequate and accurate representatives of the overall clean room microbiology. The microorganism *C. acnes* continues to be featured in FDA Form 483 observations, providing another insight into the relevance of *C. acnes*. This microorganism also possesses several unique characteristics in terms of its aerotolerance, abundancy, inability to grow on TSA aerobically but ability to grow in the thioglycollate sterility test, and its association with protective hydrophobic sebum. These characteristics imbue *C. acnes* with significant challenges in terms of its monitoring, recovery, and potential resistance to sterilants/sanitizing agents. We therefore suggest that environmental and personnel controls and monitoring programs carefully consider this microorganism in the context of the data and information provided in this review. To this end, the authors are careful to avoid a recommendation to simply add greater environmental monitoring efforts to preexisting programs; such additional activities might have the unintentional consequences of increasing the introduction of *C. acnes* into clean rooms. One alternative might be to remove those environmental monitoring activities that could be valueless in their consistent absence of microbial recoveries and replace them with media conditions “tuned” to the recovery of *C. acnes*. Other options would be to gather data using environmental monitoring and incubation conditions conducive to recovering *C. acnes* on a less frequent basis, with minimal additional burden and yet an evaluation of the potential risk from this microorganism. Ideally, modern microbiological monitoring technology should replace our current industry standard growth-based methods entirely removing the constraints of sampling, recovery, and increased personnel presence associated with...
surveying the clean room for \textit{C. acnes}. Real-time spectrophotometric technologies such as biofluorescence particle counters are available, proven (104), and implementable (105) for this precise purpose. Although environmental monitoring has value and all efforts should be made to ensure it is appropriately diagnostic (fit for purpose), the risks posed from \textit{C. acnes} are always more effectively addressed by controls preventing its potential access to the clean room, process, and product. In other words, complete physical segregation from the source (application of isolators with no human interventions) and optimized gowning, cleaning, and sanitization.

**Conflict of Interest Declaration**

The authors declare that they have no competing interests.

**References**


92. Lomholt, H. B.; Kilian, M. Population Genetic Analysis of Propionibacterium acnes Identifies a


A Semiquantitative Risk Assessment Methodology Fit for Biopharmaceutical Life Cycle Stages

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ABSTRACT: This paper introduces an innovative risk assessment tool, a semiquantitative risk determination (SQRD) method designed to address risk on the operational and organizational level with a distinct patient safety perspective. Quality Risk Management (ICH Q9) is a systematic process for the assessment, control, communication, and review of risks to the quality of the drug (medicinal) product across the product life cycle. SQRD is a systematic data-driven risk assessment tool. It is of practical significance to have a risk assessment tool that directly links to patient safety attributes. The SQRD methodology has six distinctive steps that are customized to address patient impact and non-patient impact quality attributes. The target was to develop and utilize an advanced risk assessment tool that is reliable, robust, objective, and data-driven. SQRD can be applied to batch production, continuous process, or a hybrid of the two, and at any stage of the product life cycle such as early development, pilot formulation development, process validation, or commercial manufacturing. The output of SQRD can help in shaping and optimizing the product control strategy. The exercise enables systematic mitigation of the identified risks. The proposed SQRD tool systematically evaluates data and scientifically establishes reliable, robust, and efficient risk assessments.

KEYWORDS: Risk assessment, Quality by design, Pharmaceutical quality systems, Validation.

Introduction

A risk assessment is a systematic process for organizing and evaluating information to support a risk decision that is made within a quality risk management system. The process consists of the identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards (1). In the pharmaceutical industry, a technical risk assessment encompasses risks associated with various aspects of the drug substance or product including, but not limited to, early development, development of a synthetic route, drug substance process design, drug product formulation development, drug product process design, and process scale-up. The U.S. Food and Drug Administration (FDA) encourages the use of modern pharmaceutical development concepts and quality risk management at all stages of the manufacturing process life cycle (2). As per the FDA guidance on process validation, the life cycle concept encompasses product and process development, qualification of the commercial manufacturing process, and maintenance of the process in a state of control during routine commercial production (3).

Quality risk assessment, an integral component of the process validation life cycle, can be used during the process design (Stage 1), process qualification (Stage 2), and continued process verification (Stage 3) stages to identify and mitigate unexpected risks. Pharmaceutical cGMPs for the 21st Century—A Risk-Based Approach, an FDA initiative, recommends the use of modern risk-management tools and concepts that are expected to enable risk-based decision-making with sound science throughout the product life cycle (4). In light of this initiative, this current paper proposes an alternate risk assessment tool that has potential use in the biopharmaceutical manufacturing industry. The purpose of the alternate technical risk assessment methodology is to facilitate science-based decision-
making in a systematic, objective, and efficient manner as recommended in the ICH Q9 Quality Risk Management guideline (1).

According to the ICH Q9 guideline, quality risk management should utilize science-based risk evaluation to ultimately protect patient safety and ensure a level of risk-management effort commensurate with the level of risk. These two primary principles require appropriate risk assessment tools to be chosen and employed during various scenarios of the life cycle. Besides simple techniques such as flowcharts/maps, fishbone diagram (Ishikawa diagram), or check sheets, other risk assessment tools such as fault tree analysis (FTA), failure mode and effects (and criticality) analysis (FMEA/FMECA), preliminary hazard analysis (PHA), hazard analysis and critical control points (HACCP), and a hazard and operability study (HAZOP) are commonly applied during the risk assessment process. Certain tools are appropriate at specific stages of development. For example, a PHA tool might be best utilized when minimal data is available at early formulation development stages (5). However, the pharmaceutical industry may require further customization to accommodate the unique nature of pharmaceutical process development requirements and product quality attributes. The scales used to measure are binary (pass or fail) or ordinal (such as FMEA Risk Priority Number – RPN) in nature (6).

Most of the critical quality attributes (CQAs), for example assay and dissolution, are presented in continuous numerical fashion. It is extremely important for the sake of both patient safety and for business organization operations efficiency to capture CQA performance trends. Intuitively, it is beneficial and helpful to have quantitative measure of the risk profiles. HACCP aims to manage risks associated with physical, chemical, and biological hazards owing to the design, development, production, and use of the product. It is of practical significance to have similar risk assessment tools that directly and explicitly link to patient safety. The semi-quantitative risk determination (SQRD) methodology currently under discussion is designed to encompass the two previously mentioned desired elements. Quality risk management is about an overall systematic process for the assessment, control, communication, and review of risks to the quality of the drug (medicinal) product across the product life cycle (Table I). SQRD is a systematic data-driven risk assessment tool that can be utilized before and post generation of sufficient product/process design of experiments (DoE) data.

Risk assessment processes involve risk identification, risk analysis, and risk evaluation. The SQRD methodology has incorporated six distinctive steps that are customized to treat patient safety (patient impact CQA) as the ultimate target and utilize an innovative risk-estimation approach that is reliable, robust, objective, and data-driven.

In general, the six steps involved in SQRD are:

1. Define the project scope and review the product/process information.
2. Identify a CQA of a product manufactured by the process.
3. Identify a plurality of material attributes (MAs) (e.g., active pharmaceutical ingredient [API] particle size, bulk density) and/or a plurality of process parameters (PPs) (e.g., compression force, press speed) that are considered to have potential impact on the CQA.
4. Classify each of the MAs and/or PPs into a risk category using a decision tree for risk category determination.
5. Determine the overall risk score and ratio for the CQA based on risk factors designated for each of the risk categories and count of the MAs and/or PPs in each of the risk categories.
6. Visualize the outcome of the SQRD using various types of charts.

These steps are illustrated in the flow chart in Figure 1.

The performance of a particular CQA is considered a function of all pertinent critical MAs (CMAs) and critical PPs (CPPs) that are considered to have potential significant impact toward the CQA results. The relationship between CMAs, CPPs, and CQAs is depicted in Figure 2 and is the foundation behind the concept of designing the SQRD methodology.

The SQRD methodology can be applied to batch production, continuous process, or a hybrid of the two, at any stage of the product life cycle such as early development, pilot formulation development, or commercial manufacturing process, to determine product risk, process risk, and scale-up risk for the risk associated with product life cycle changes.
**TABLE I**  
Risk Assessment Steps and Proposed Tools for Drug Substance

<table>
<thead>
<tr>
<th>Category</th>
<th>Risk-Gauging Steps</th>
<th>Facilitator/Collaborators</th>
<th>Examples of Recommended Quality Risk-Management Tools</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Synthetic route selection assessment</td>
<td>R&amp;D</td>
<td>Scientific rationale.</td>
</tr>
<tr>
<td></td>
<td>Impurity, stress studies risk assessment</td>
<td>R&amp;D/Analytical</td>
<td>Scientific rationale, Checklist.</td>
</tr>
<tr>
<td></td>
<td>Initial quality by design (QbD) process development risk assessment</td>
<td>R&amp;D</td>
<td>Fault tree analysis (FTA), Failure mode and effects (and criticality) analysis (FMEA/FMECA).</td>
</tr>
<tr>
<td></td>
<td>Occupational health toxicity/potency categorization</td>
<td>Toxicologist/R&amp;D, Technology transfer</td>
<td>Preliminary hazard analysis (PHA), hazards analysis and critical control points (HACCP)</td>
</tr>
<tr>
<td></td>
<td>Registered starting materials (RSM) and key material supplier qualification</td>
<td>Quality/R&amp;D</td>
<td>Ishikawa diagram, FMECA, Risk ranking and filtering.</td>
</tr>
<tr>
<td>2</td>
<td>Post QbD process development risk assessment</td>
<td>R&amp;D</td>
<td>FMEA/FMECA, Semiquantitative risk determination (SQRD).</td>
</tr>
<tr>
<td></td>
<td>Pre analytical quality by design (AQbD) method development risk assessment</td>
<td>Analytical</td>
<td>FTA, FMEA/FMECA</td>
</tr>
<tr>
<td></td>
<td>Pre scale-up risk assessment</td>
<td>R&amp;D/Technology transfer</td>
<td>FMEA/FMECA, SQRD</td>
</tr>
<tr>
<td></td>
<td>Facility/Equipment assessment</td>
<td>Engineering/Technology transfer</td>
<td>FMEA/FMECA</td>
</tr>
<tr>
<td></td>
<td>Method transfer risk assessment</td>
<td>Analytical/Quality</td>
<td>FMEA/FMECA</td>
</tr>
<tr>
<td></td>
<td>Pre-clinical risk assessment</td>
<td>Clinical/R&amp;D</td>
<td>Checklist, FTA, Hazards and Operability Studies (HAZOP)</td>
</tr>
<tr>
<td></td>
<td>Stability study risk assessment</td>
<td>Analytical/R&amp;D</td>
<td>FTA</td>
</tr>
<tr>
<td>3</td>
<td>Drug master file submission risk assessment</td>
<td>RA/Quality</td>
<td>Checklist</td>
</tr>
<tr>
<td></td>
<td>Post scale-up/pre-process performance qualification (PPQ) risk assessment</td>
<td>Technology transfer/Operations, Quality</td>
<td>SQRD</td>
</tr>
<tr>
<td></td>
<td>PAI audit risk assessment</td>
<td>Quality/Technology transfer, R&amp;D</td>
<td>Checklist</td>
</tr>
<tr>
<td>4</td>
<td>Risk-based determination of Stage 2 PPQ batches</td>
<td>Technology transfer/Quality</td>
<td>Statistical # of batch estimation tools</td>
</tr>
<tr>
<td></td>
<td>Post PPQ risk assessment</td>
<td>Technology transfer/Quality</td>
<td>SQRD</td>
</tr>
<tr>
<td></td>
<td>Risk-based determination of Stage 3a batches</td>
<td>Technology transfer/Quality</td>
<td>Statistical # of batch estimation tools</td>
</tr>
</tbody>
</table>
Scope Determination and Product/Process Review

Gathering product, and/or process knowledge is a fundamental requirement for SQRD methodology.

1. A conventional risk identification process might start by addressing the “what might go wrong?” question by identifying hazards and possible consequences based on systematic use of information from historical data and input from subject matter experts (SMEs) and stakeholders. The SQRD methodology decides to predefine the “what might go wrong?” question specifically to be “what CMAs and/or CPPs are likely to impact the CQAs of the product?” The

<table>
<thead>
<tr>
<th>Category</th>
<th>Risk-Gauging Steps</th>
<th>Facilitator/ Collaborators</th>
<th>Examples of Recommended Quality Risk-Management Tools</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Post Stage 3a risk assessment</td>
<td>Technology transfer/Quality</td>
<td>SQRD</td>
</tr>
<tr>
<td></td>
<td>Ongoing annual product review/ continued process verification (CPV) risk assessment</td>
<td>Quality</td>
<td>FMEA/FMECA</td>
</tr>
<tr>
<td></td>
<td>Investigation risk assessment</td>
<td>Quality</td>
<td>Ishikawa diagram</td>
</tr>
<tr>
<td></td>
<td>Change implementation risk assessment</td>
<td>Quality</td>
<td>Ishikawa diagram</td>
</tr>
<tr>
<td>6</td>
<td>Data visualization</td>
<td>R&amp;D/Engineering/ Technology transfer/ Analytical/Quality</td>
<td>Data visualization tools</td>
</tr>
</tbody>
</table>

Table I (continued)

Figure 1

Semiquantitative risk determination (SQRD) process steps.
goal of this alternate method is to shift the focus toward risks more related to CQAs that are critical to the end users of the drug product.

2. Convert the concept of “hazards” vs. “consequences” into a definite context of “CMA/CPP” vs. “CQA, patient safety.” Thus, the risks identified are manageable and can be mitigated.

During the scope determination and product/process review stage, the objective and scope of the current assessment is identified and confirmed among project owners and stakeholders. A process flow map or fishbone diagram may be constructed and amended when appropriate to cover all operations and controls in the process under evaluation. For products developed with a non-quality by design-based approach, gap assessment can be utilized to determine if historical data collection is needed before further assessment is carried out. The data can be utilized via statistical constructs and modeling to determine the CMAs and CPPs with direct impact to non-label and label claimed CQAs (7). SQRD can be done at any stage of the product life cycle such as the early development, formulation development, or commercial manufacturing process. The manufacturing process life cycle stage that the current SQRD is pertinent to is clearly defined to meet the corresponding regulatory framework and expectations. For instance, FDA/Therapeutic Products Directorate (TPD)/European Medicines Agency (EMA) requirements on various drug applications and any subsequent submission activities (such as scale-up and post-approval changes) or any international guidance (International Conference on Harmonisation [ICH], World Health Organization [WHO]) on pharmaceutical manufacturing/quality system practice (8). In order to be thorough and comprehensive, it is recommended to have the scope of the SQRD encompass the background details, for example for drug products—formulation of the product, the process design, and the scale-up of the associated commercial process.

**CQA Identification**

Attributes can be a physical, chemical, biological, or microbiological property or characteristic of either an in-process product or a finished product. There are two major categories of quality attributes: label claim quality attributes, which have direct end user impact, and non-label claim quality attributes, which have no direct end user impact but may indirectly impact the label claim quality attributes. The SQRD apply individual decision logic for each. Examples of the label claim and non-label claim quality attributes appear in Table II.

**TABLE II**

<table>
<thead>
<tr>
<th>Category of Quality Attributes</th>
<th>Examples (May Vary Product to Product)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Label claim quality attributes</strong></td>
<td>Assay, concentration, content uniformity, dissolution, drug release, fill weight, sterility assurance level, potency</td>
</tr>
<tr>
<td><strong>Non-label claim quality attributes</strong></td>
<td>Adhesion, assay per spray, blend uniformity, bulk density, cold flow, disintegration, droplet size, extractable volume, friability, hardness, integrity, osmolality, particle count, particle size distribution, particulate matter, peel, pH, sheer, specific gravity, spray pattern, tack, torque, unit weight, viscosity, or weight variation</td>
</tr>
</tbody>
</table>
A process flow map or fishbone diagram from a formulation process and/or an optimization process can be used to facilitate the identification of potential MAs (drug substance and excipients) and PPs that will potentially impact the CQAs for the risk assessment. Identifying proper MAs and PPs for risk assessment is a collaborative effort of the multifunctional team of SMEs. Following is a brief list of examples of MAs and PPs for both solid and liquid dosage forms (Table III).

### TABLE III
**Material Attribute (MA), Process Parameter (PP) Examples**

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA</td>
<td>Active pharmaceutical ingredient (API) – particle size, stability, solubility, Biopharmaceutical Classification System class Excipient – quantity, grade, viscosity, particle size, surface morphology/surface area, density, impurities/degradation products</td>
</tr>
<tr>
<td>PP</td>
<td>Solid dosage form – compaction force, screen size, roller speed, roller type, roller gap, compression speed/force, coating gun spray rate, coating pan speed, coating pan temperature Liquid dosage form – mixing speed/time, temperature, compounding time, hold time, tubing type, filer type, purge volume/time, fill volume</td>
</tr>
</tbody>
</table>

**Material Attribute/Process Parameter Identification**

A process flow map or fishbone diagram from a formulation process and/or an optimization process can be used to facilitate the identification of potential MAs (drug substance and excipients) and PPs that will potentially impact the CQAs for the risk assessment. Identifying proper MAs and PPs for risk assessment is a collaborative effort of the multifunctional team of SMEs. Following is a brief list of examples of MAs and PPs for both solid and liquid dosage forms (Table III).

**Material Attributes/Process Parameters Classification and Risk Factor Assignment**

As per ICH Q9 guidance (1), risk analysis is the qualitative and/or quantitative linking of probability of occurrence, severity of harms, and sometimes detectability of the hazard (based on the tool used) associated with the product/process. Proper determination of these terms oftentimes requires extensive information collection and can be very subjective based on various perceptions of different functional groups. Numerical values or surrogate descriptors for probability, severity, and detectability are usually assigned as discrete ordinal numbers or as qualitative descriptors (low, high). It is important to recognize that the magnitude of these numbers is hardly meaningful on a numerically proportional scale. An event with probability number of 2 does not necessarily translate into a probability twice as likely to occur as an event with probability number of 1. Furthermore, it is also mathematically flawed to multiply ordinal scale numbers. The validity of such multiplication (e.g., the risk number = 2 × 3 × 4) has already been under debate for some time (9, 10). For instance, if both probability numbers are 2, an event that has a severity number of 5 and a detectability number of 1 can have the same risk number as an event that has a severity number of 1 and a detectability number of 5. In order to overcome this issue, in SQRD methodology, a hybrid numbering system of ordinal risk categories that represents the combination of probability and severity is created. It can be presented in the form of a decision tree (Figures 3, 4). All MAs and PPs that are subject to the risk assessment process will be examined against the decision tree and assigned an ordinal risk category accordingly.

The first decision tree is for MAs and PPs that are identified to be related to non-label claim quality attributes (no direct patient impact). The second decision tree is for those that are directly related to label claim quality attributes (direct patient impact such as assay, content uniformity, and dissolution). Each of the categories is assigned a risk factor that is twice that of the corresponding categories from the non-label claim quality attributes (0, 2, 4, 6, 8, or 10) (Table IV).

Collaborative fact–based decision-making is needed among the project owner and the SMEs to classify each of the identified MAs, PPs, and their interactions into the appropriate risk category. Product history such as outcome from early development trials is extremely valuable and important (3). Informed opinion based on literature review and/or previous experience on similar product/process is also very helpful. Compared to the conventional probability and severity numbers, one distinctive feature of these risk factors is that they are established almost exclusively based on data. As the first step, science-based decision-making is required to categorize the MA/PP of interest into the Critical or Not Critical group. Subsequently, the MA/PP of interest can be sorted into one of the subgroups within the two main groups simply based on if it was evaluated already and if it was mitigated.
Another notable characteristic of the alternate risk assessment is that it treats probability of occurrence and severity of the consequence as a dynamic combination factor rather than two unrelated static entities, which is not typical. For example, by varying a critical PP such as compression force without proper control, tablet hardness would be significantly impacted. This would represent a scenario of a risk factor of 5 when supporting evidence is not available from the process. A mitigation plan can be implemented where the effect of compression force on tablet hardness is studied and a proper operating range is proposed. As a result, the risk factor is adjusted to 3. An objective measure like the SQRD risk factor will automatically account for the overall risk profile. Last but not least, the conventional detectability component is also implied to some extent in the alternate SQRD risk factor system. Specifically, for

Figure 3

Quality attributes with no direct end user impact.

Figure 4

Quality attributes with direct end user impact.
those MAs that are evaluated with preexisting specification and testing requirements, it should be easily detected and quantified in a validated measurement system. For MAs and PPs that are not evaluated for the specific product/process or if similar product/process data is used, there is a possibility that their detectability is at question and this is reflected in the relatively greater risk factors associated with them.

### Computing Risk Ratio and Constructing Risk Assessment Charts

When all the CMAs and CPPs are assigned a risk factor, the overall risk score and risk ratio of the process/product can be computed using any data compilation and validated software system. The risk score for each risk category is calculated by multiplying the count of total MA/PP factors per category with the risk factor for that risk category. A greater risk score indicates a greater level of risk for that CQA within the particular process under review. The overall risk score of the CQA will be the sum of the risk scores for all the risk categories. The value of the risk score is directly related to the number of MAs/PPs included in the risk assessment. Therefore, to draw meaningful conclusions, comparison has to be done among risk scores that are calculated using a comparable number of total MAs/PPs. For instance, comparison can be made on risk scores for the same CQA of two similar products at a particular product life cycle stage (higher strength vs. lower strength).

Unlike the risk score, which cannot be interpreted in isolation/out of context, the risk ratio is a more independent indicator that can be compared among different situations. The overall risk ratio is computed as the sum of the count of CMAs and/or CPPs under the categories “critical not evaluated with set point” and “critical not mitigated” divided by the sum of the count of CMAs and/or CPPs under the categories “critical mitigated”, “critical not evaluated with set point”, and “critical not mitigated”. In other words, the risk ratio represents the portion of the CMAs/CPPs that are not evaluated and/or not mitigated out of the total CMAs/CPPs that are identified. The risk ratio is calculated from the following equation:

\[
\text{Risk Ratio} = \frac{\text{Sum of Count of CMA/CPP factors (Critical/Not Evaluated with Set Point + Critical/Not Mitigated)}}{\text{Sum of Count of CMA/CPP factors (Critical/Mitigated + Critical/Not Evaluated with Set Point + Critical/Not Mitigated)}}
\]

The risk ratio is a positive fraction ranging between 0 and 1. The critical limit of the risk ratio can be established based on the existing operational/business/corporate risk tolerance, which are often set as a balance.
between company strategy and regulatory/quality/legal requirements (11). Table V is an example where the calculated risk ratio for the process is further classified into several risk levels. Organizations can establish different thresholds as appropriate.

Based on the computed risk ratio (Figure 5), risk assessment charts can be constructed for the CQA with the MAs and/or PPs. The chart should provide an indication of the risk category for the MAs and/or PPs and the ranking of the risk level/significance for each of the MAs and/or PPs. A typical risk assessment chart can be a heat map (Figure 6) or a Pareto chart. Other types of charts that can be used include bar chart, bubble chart, cone chart, pie chart, radar chart, scatter chart, surface chart, and prioritization matrix.

Based on the outcome of the risk assessment chart, one or more CMAs and/or CPPs can be further identified for optimization. DoE can be performed under the guidance of the SQRD output to expedite the optimization process. Following optimization, the SQRD assessment can be repeated until the risk ratio is reduced to medium risk or low risk. The risk ratio can be used to guide the future data collection and to determine the need for additional testing/designed studies. It can be a critical piece of supporting information for creating control strategy and process modifications of the product.

Based on the heat map and risk ratio for each stage, top CMAs and CPPs can be selected for optimization of the process to minimize/eliminate the risk identified. Following optimization, the SQRD technical risk assessment is repeated until the risk ratio is reduced to medium risk or low risk. As product development is an iterative process that involves multiple risk assessments, adequate access and traceability of SQRD reports are important for product knowledge management and for effective application of the life-cycle approach. The recommendation for mitigation action and post SQRD risk assessments are documented within the firm’s quality management system (QMS). SQRD is an effective alternate risk assessment methodology for biopharmaceutical life cycle stages.

### Potential Applications

The SQRD methodology can be used by various functional areas of the organization for determining formulation risk, functionality design risk, process risk, scale-up risk, and any other risks associated with product life cycle changes for CQAs with or without direct product end user impact. It is suitable to be used for the biopharmaceutical industry as well as other industries that require similar types of risk assessment (i.e., food, cosmetic, medical device). It can provide input to guide the Quality by Design (QbD) initiatives during the development stage and mitigate the product/process risk before the commercialization stage. The output of SQRD can help to shape the control/monitoring strategy and to enable systematic mitigation of identified

<table>
<thead>
<tr>
<th>Risk Ratio</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.25</td>
<td>Low risk</td>
</tr>
<tr>
<td>0.25–0.75</td>
<td>Medium risk</td>
</tr>
<tr>
<td>&gt;0.75</td>
<td>High risk</td>
</tr>
</tbody>
</table>

**Table V**

<table>
<thead>
<tr>
<th>Category</th>
<th>Count of CMA/CPP Factors in Each Category</th>
<th>Risk Factor</th>
<th>Risk Score for Each Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Critical</td>
<td>0</td>
<td>0</td>
<td>0x0 = 0</td>
</tr>
<tr>
<td>Not Critical/ Evaluated</td>
<td>31</td>
<td>1</td>
<td>31x1 = 31</td>
</tr>
<tr>
<td>Not Critical/ Not Evaluated</td>
<td>26</td>
<td>2</td>
<td>26x2 = 52</td>
</tr>
<tr>
<td>Critical/ Mitigated</td>
<td>41</td>
<td>3</td>
<td>41x3 = 123</td>
</tr>
<tr>
<td>Critical/ Not Evaluated</td>
<td>16</td>
<td>4</td>
<td>16x4 = 64</td>
</tr>
<tr>
<td>Critical/ Not Mitigated</td>
<td>2</td>
<td>5</td>
<td>2x5 = 10</td>
</tr>
</tbody>
</table>

**Figure 5**

Example semiquantitative risk determination (SQRD) technical risk assessment during Stage 1 (Process Design).
risks. Following is a list of examples of areas where the alternate SQRD methodology can be applied (12):

1. Development (e.g., analytical/product/process development, establish specification/control).
2. Quality management (e.g., change management/control, continuous improvement, defects/deviations, annual review, complaints & recall management, auditing).
3. Production (e.g., validation, in-process sampling/testing, production planning).
4. Laboratory control and stability (e.g., validation, methods development, out-of-specification [OOS] investigation and retest period/expiry determination).
5. Facilities/equipment/utilities system management (e.g., design, qualification, environmental control, maintenance).
6. Materials management (e.g., assessment and evaluation of suppliers and contract manufacturers, storage/logistics and distribution conditions).
7. Packaging and labeling (e.g., design of package and container closure system, label controls).
8. Regulatory activities (external/authority, internal scrutiny/audit pre- and post-approval).

Quantified risk profiles such as the ones developed with SQRD can help to accelerate the decision-making process in the fast-paced industrial environment. SQRD can be updated in a real-time fashion. Once the SQRD platform for a particular quality attribute is established, the risk assessment can be updated continuously and efficiently with an audit trail supported software and thus reduce additional time for rework/repeat risk assessment for complex multiunit operations. Also, compared to failure/hazard analysis, it is not the failure/hazard that will be prioritized but rather the

**Figure 6**

Example scale-up semiquantitative risk determination (SQRD) heatmap: critical process parameter (CPP) vs critical quality attribute (CQA).
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk focus</td>
<td>Deviation of critical quality attributes (CQAs) that can link to end user safety</td>
<td>Failure modes</td>
<td>Process faults/deviations</td>
<td>Harm (hazards, faults)</td>
<td>Hazards</td>
</tr>
<tr>
<td>Tool approach</td>
<td>Top-down approach; identify factors that impact the CQA and their extent of impact.</td>
<td>Bottom-up approach</td>
<td>Top-down approach; consider what causes a failure</td>
<td>Bottom-up approach</td>
<td>Bottom-up approach</td>
</tr>
<tr>
<td>Input sources</td>
<td>Historical data, subject matter expert (SME) technical input or product and process data.</td>
<td>Historical data, SME technical input or product and process knowledge</td>
<td>Output from other hazard analysis; Comprehensive process and cause-effect understanding</td>
<td>Highly dependent on SME input</td>
<td>Comprehensive understanding of process and controls</td>
</tr>
<tr>
<td>Outcome indicator</td>
<td>Semiquantitative – risk score and risk ratio</td>
<td>Semiquantitative – risk priority number</td>
<td>Qualitative – failure path and estimated probability</td>
<td>Qualitative risk rating</td>
<td>Qualitative</td>
</tr>
<tr>
<td>Risk ranking</td>
<td>Yes, by ordinal numbers</td>
<td>Yes, by ordinal numbers</td>
<td>Yes</td>
<td>Yes, semiquantitative (critical/noncritical)</td>
<td></td>
</tr>
</tbody>
</table>
projected product risk potentially linking to end users. Because in pharmaceutical manufacturing failure/failure mode/hazard are not easily identified and defined, product/process risks are more relevant and practical to be defined and assessed. Table VI is a comprehensive comparison between the SQRD approach versus some of the conventional risk assessment tools.

Regulators have concluded that modern quality systems, along with risk assessment tools that enable application of product/process knowledge, can help simplify the regulatory prior approval requirements for post-approval changes to facilities, equipment, and processes (13). In line with the FDA and ICH Guidance on Process Validation, Quality Risk Management and Quality by Design (14, 15), this SQRD tool systematically evaluates the need for additional characterization requirements. The goal is to ensure risks are eliminated, mitigated, and controlled before commercialization and marketing to patients.

Conflict of Interest Declaration

The authors declare that they have no competing interests.

References


COMMENTARY

Steam Sterilization Chemical Indicators Are Not Adequate for Monitoring Real Steam Sterilization Cycles

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ABSTRACT: Chemical indicators are commonly used in hospitals to monitor steam sterilization conditions, indicating that medical devices are safe to be used. The results are stored for future evidence in the event of an infection incident root cause analysis. This type of indicator is also becoming an option for cycle monitoring in pharmaceutical steam sterilizers, improving cycle control. They are constructed and tested according to published standards, but contradictory results between chemical indicators and cycle printouts have a critical impact on process control. We found that Type 6 chemical indicators used in steam sterilizer cycles did not perform according to their intended use, showing an “approved” result in a “failed” cycle (a false positive). This study demonstrated that Type 6 chemical indicator specifications are not adequate for monitoring steam sterilizers. A change in standards is therefore needed.

KEYWORDS: Chemical indicators, Cycle monitoring, Process indicators, Biological indicators, Steam sterilization, Medical device.

Introduction

Chemical indicators (CIs), especially Types 5 and 6, are designed to react to all steam sterilization cycle critical process variables, showing a pass result only if all stated conditions are met. In most hospital institutions around the world, they are the key instrument used to pass a sterilized medical device for clinical use (1). The performance of these indicators is guided by international standards and challenged using standardized equipment, known as a biological indicator Equipment resistometer (BIER) vessel (2, 3). CI Type 5 is an integrator, mimicking biological indicator performance, with its results integrated during the cycle (1). CI Type 6 is an emulator, which will only show a pass result when the specified temperature and time are achieved in the exposure phase (4). Based on these characteristics, it is expected that a CI Type 5 will integrate toward its pass area if the temperature and time are present in a cycle, and a CI Type 6 will only react during the exposure phase, showing a pass when total exposure time and temperature are reached (5).

Commercially available steam sterilizers have their cycles and performance specified according to the international standard ISO 17665. However, only the exposure phase is stipulated, leaving the conditioning and drying phases undefined, which allows wide variation in the duration of the come-up time in the conditioning phase (6). Reports from the field, where cycles have been canceled because of failures or intentionally, have raised questions about the effectiveness of these indicators, especially when, in qualified steam sterilizers, both Type 5 and Type 6 CIs reached the end point (pass) despite the exposure phase not being completed according to physical indicators (the temperature and time printout of the sterilizer).

Objectives

The study objective was to determine the effectiveness of those Type 5 and 6 CIs that have been certified as compliant with current international standards and to compare the results between chemical and physical indicators when applied to qualified steam sterilizers. The intent of this study was not to compare different CIs manufactured. This has been referenced in many

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articles and an important one is referenced in this document (4, 5), which does the comparison. The results from this study, when added to other CI papers, will have a complete rationale that should support a revision of current standards.

Methods

Thermal qualification reports according to ISO 17665-1 of 95 steam sterilizers from different hospitals in Brazil were analyzed to determine the average common come-up ramps and a minimum of 3 min was determined. This time was programed into the BIER vessel cycle to simulate a real steam sterilization cycle come-up ramp and was compared with the standard 10 s come-up ramp. A Type 6 CI from the same manufacturer and in two different lots with a pass specification of 134°C and 4 min exposure was chosen, and a Type 5 CI from the same manufacturer and two different lots with a pass specification of 121°C and 22.9 min exposure, or 135°C and 1.9 min exposure, with results presented by a moving front window instead of a color change. Triplicate studies were conducted in a BIER vessel, with the temperature adjusted to 134°C, using three Type 5 CIs and three Type 6 CIs for each cycle configuration. Cycles 1, 2, and 3 were programed with a 10 s come-up ramp, and the exposure time was set to 2, 3, and 4 min respectively. Cycle 4 had the come-up ramp adjusted to 3 min by controlling the pressure increase over time, linear until reaching the exposure phase, which was set at 3 min. Cycle 5 was identical to cycle 4, but the exposure was reduced to 2 min. All cycles had a single vacuum pulse at the conditioning phase, according to the standard.

Results

The CIs performed according to stated values in all cycles with the 10 s come-up ramp. In Cycle 2, a difference was observed between the Type 5 and Type 6 CIs: Type 5 CIs showed a pass result because of the integration of the temperature and time from the start of the exposure phase, whereas Type 6 CIs showed a failed result because only the required temperature was reached, but the time was too short (Table I).

In Cycles 4 and 5, with the 3 min come-up ramp (simulating an actual hospital sterilizer), with 3 min and 2 min exposures, respectively, at 134°C, Type 6 CIs should have shown failed results but instead passed with exposures below the end user labeled specification instructions of 134°C and 4 min exposure. Type 5 CIs, which had a stated value of 135°C for 1.9 min, all passed with a 3 min exposure but 2 out of 9 failed with a 2 min exposure.

Discussion

CIs are constructed according to ISO 11140-1 and have their efficiency tested in the BIER vessel, which only simulates the exposure phase of a sterilizer, not the real sterilization cycle. Also, steam sterilizer process validation standard ISO 17665 does not establish come-up ramp criteria, which allows qualified equipment to have come-up ramps in dry load cycles ranging from 3 to 12 min. For liquid sterilization, the come-up ramps can be even longer than the exposure phase, and CIs cannot be used. Comparing both equipment standards, an important difference was noticed in the conditioning phase. In the BIER vessel, a 10 s come-up time was needed, and for the steam sterilizer there was no performance requirement (Figure 1). CIs end point performances are only obtained in BIER vessels. With a 3 min come-up ramp, both CIs did not perform according to end user instructions for use specification, but Type 5, which is an integrator, could produce viable end point results at a maximum 3 min come-up ramp. Type 6 CIs were not adequate for come-up ramps longer than 10 s.

### TABLE I

<table>
<thead>
<tr>
<th>Cycles at 134°C</th>
<th>Type 5</th>
<th>Type 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle #</td>
<td>Come-up time</td>
<td>Exposure time</td>
</tr>
<tr>
<td>1</td>
<td>10 s</td>
<td>2 min</td>
</tr>
<tr>
<td>2</td>
<td>10 s</td>
<td>3 min</td>
</tr>
<tr>
<td>3</td>
<td>10 s</td>
<td>4 min</td>
</tr>
<tr>
<td>4</td>
<td>3 min</td>
<td>3 min</td>
</tr>
<tr>
<td>5</td>
<td>3 min</td>
<td>2 min</td>
</tr>
</tbody>
</table>

*All cycles were run in triplicate with three chemical indicators of each type inside the chamber, placed in a holder that kept all of them in the same upright position.*
The intent of this study was not to compare different CI makes as this has already been done (4, 5). The results from this study support a revision of CI standards ISO 11140-1 and ISO 18472 (addressing the BIER vessel cycle configuration), where the conditioning phase of real steam sterilizers is taken into consideration in the specification of CIs, and performance tests should solve the unexpected results observed in our study. This revision should also take into consideration the limitation of CI Type 6 color change interpretation, were a color reference to determine if a pass condition was obtained may vary between users, and the precise exposure time is hard to determine (4). To improve CI monitoring techniques, a revision of ISO 17665, addressing the impact of the come-up ramp duration on the CI results will help end users better understand how to correctly choose the indicator to monitor the sterilization cycle. Until the standards are revised, we recommend that thermal qualification technicians limit the come-up ramp to 3 min in their sterilization process qualification, allowing healthcare professionals to use a Type 5 CI (combined with biological and physical indicators results) to ensure adequate cycle monitoring, while observing local regulations and recommendations. The use of a Type 6 CI may cause the end user to clear a load for clinical use that was not processed according to medical device instructions with strict time and temperature requirements.

Conclusion

We do not advocate the complete elimination of Type 6 chemical indicators. Rather, the intent was to demonstrate that current standardized testing conditions do not accurately simulate the operations of a real sterilizer and that chemical indicators are not providing the result each manufacturer states in their instructions for use. Because resistometer cycles were developed for characterization of biological indicators, if configured correctly, and considering the chemical reaction characteristics, CIs may be used in qualified sterilizers. Our recommendation is achievable because a Type 6 CI with a 7 min exposure will be able to react correctly to cycle failures when used in a 4 min exposure. This evidence should help convince users and manufacturers that a revision of the standards and resistometer cycles is required.

Acknowledgments

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Conflict of Interest Declaration

The authors declare that there are no known conflicts of interest associated with this publication and no competing interests exist.
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The Goldilocks Challenge—Controlling Uncertainty When Setting Product Specifications

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ABSTRACT: Product specifications are ideally based on knowledge of patient needs or requirements of subsequent manufacturing steps. However, in most applications, knowledge of patient needs is neither precise nor comprehensive enough to fully define specifications. The prevailing practice is to base specifications on process experience, setting limits to assure consistency of future results with initial results representative of clinical material. Developers of new medicines are often required to set initial product specifications and other limits when only small amounts of process experience have been accumulated. Product developers and health authority reviewers share the mandate to protect patients from harm and assure the effectiveness of medical products, which motivates a tendency to set limits very tight. But although tighter limits give the impression of tighter control, limits alone accomplish no reduction in the variation that exists in established processes and test methods. Limits that are too tight do not represent the natural variability of the process and test methods. Unnaturally tight limits will result in a high number of excursions beyond the limits, potentially causing discards, supply disruptions, and higher cost of goods sold. In this article, we demonstrate how to deliberately control the probability of having intervals that are too tight during the early manufacturing process.

KEYWORDS: Specifications, Quality by Design (QbD), Tolerance interval, Product life cycle management, Control strategy, Process capability, Statistical process control (SPC).

1. Introduction

For several years now, there has been a surge in submissions for therapies on accelerated approval pathways. For example, the Center for Drug Evaluation and Research used at least one expedited development and review method to speed approval for 61% of all novel drugs approved in 2017 (1). These products are often directed at treating unmet medical needs, such as certain cancers and some diseases with a genetic cause. They are frequently also classified as orphan drugs, with a very small patient population for a rare or even ultrarare disease. Maintaining patient safety remains just as high a priority as it is for nonaccelerated products. However, speeding these novel products to patients at commercial scale rises in priority compared to traditional products. The time lines for commercialization are compressed to meet urgent demand. Nowhere does this time compression have more of an impact than for setting product specifications.

Guidance documents outline the basic requirements for setting specification limits for pharmaceutical products (2–6). Setting specification limits for any particular quality attribute requires thoughtful consideration of all available knowledge of the product’s action in patients and review of all accumulated experience from clinical trials and precommercial development and manufacturing. The translation of this knowledge and experience into numeric limits for critical quality attributes is not fully prescribed by guidance. The key considerations are patient safety and efficacy, but direct links between product characteristics and patient outcomes are often elusive. According to Woodcock (7):

“The limits on quality attributes are often chosen empirically to ensure production of batches that resemble the batches tested in the clinic. However, this approach will only ensure consistent clinical performance if the relationship between those limits and the clinical outcome is
understood. Without this understanding, the limits could be overly wide, unnecessarily tight, or completely irrelevant to clinical performance.”

The ranges used in clinical trials provide a starting point for defining specifications, but pivotal trials do not typically include batches of widely varying quality. The safe and acceptable range may be broader than the clinically qualified range (8). For traditional products, specifications are informed by process characterization studies, process performance qualification limits, clinical history, and manufacturing experience (9). In our experience supporting submissions for new drug products, it is expected that at least 30 lots be available to characterize the variability in the manufacturing process. The minimum number 30 is not a definitive cutoff based on statistical theory but rather is a reasonable starting point when establishing expected performance for a new process or test method (10). This can be shown empirically by considering the expected width of a confidence interval for a process variance. As sample size increases, the expected width of the confidence interval will decrease indicating less uncertainty about the true value of the process variance. Figure 1 shows the expected length of a 95% confidence interval on a process variance divided by the true value of the variance as a function of the sample size.

Figure 1 suggests that there is a considerable decrease in uncertainty when the sample size increases in the range from 3 to 20 lots, but that after 20–30 lots, there are diminishing improvements in the precision of the variance estimate.

When products are accelerated, there may be far <30 lots available at the time of submission. Typically, there may be as few as 15 full-scale lots completed and tested when specifications are initially established, and in some cases, even <15 lots.

One acceptable strategy for managing specifications on an accelerated pathway is to establish initial specifications with all available data at the time of submission. These initial proposed specifications are accompanied by a formal commitment from the sponsor to update the specifications after obtaining some fixed number of additional lots if commercial experience warrants. Once sufficient lots to fully characterize the process variance have been obtained (e.g., ≥30), it is quite common to compute “three-sigma” specification limits. These are limits where the specification range is formed by adding and subtracting three times the standard deviation of the manufactured lots to the mean of the lots. Residual uncertainty about process variation remains high until all sources of variability have fully manifested. Setting initial specifications with three-sigma limits with fewer than 30 lots sets up the possibility of very narrow initial limits, which will be difficult to reproducibly meet. The goal is to find initial limits that are neither too tight nor too loose and update as additional manufacturing experience is gained.

2. Finding “Goldilocks Specification Limits”

The classic tale of Goldilocks describes the experiences of a young girl visiting a cabin in the woods, where she finds the belongings of a family of three bears. One item is always too extreme (e.g., either too hot or too big), the second is too extreme in the other direction (e.g., either too cold or too small), but the third is “just right”. This Goldilocks framework is useful in thinking about specification setting—specification limits should be neither too tight nor too wide, but “just right”. In this context, intervals that are too wide will not detect significant changes in the manufacturing process. Intervals that are too tight will result in false out-of-specification (OOS) signals when the process is running as designed. Statistical methods provide some useful approaches for developing these Goldilocks limits.

Specification limits based on manufacturing experience typically consist of a measure of the process center
(e.g., the average), an estimate of the expected future variation (e.g., the standard deviation), and a constant $K$. These measures are combined into the interval

$$\bar{Y} \pm K \times S$$  \hspace{1cm} (1)$$

where $\bar{Y}$ is a sample average, $S$ is a sample standard deviation, and $K$ is a constant.

Three-sigma limits (reference intervals) are often used to define specification limits. These limits are defined by eq 1 when $K = 3$. For a normal population, 99.73% of the data fall within three standard deviations of the true process mean. Thus, values outside this range can be considered atypical. However, the percentage of values between the three-sigma limits estimated from any sample is generally $<99.73\%$, because the true values of the process mean and standard deviation are unknown. Rather, the mean and standard deviation are estimated with the sample mean $\bar{Y}$ and sample standard deviation $S$ for a given sample of size $n$. Because of the inherent uncertainty in the sample estimates, the assumptions that underlie the three-sigma model are not met exactly. As will be shown, three-sigma specification limits based on relatively small sample sizes provide limits that are usually too tight. This results in false OOS signals when the process is operating as expected. Often such excursions have substantial consequences up to and including discard of acceptable material.

Goldilocks limits for early manufacturing lots align in principle with the first phase of control chart monitoring. Montgomery (11) states that in phase I, process data is gathered to construct trial control limits. Control limits in phase I are primarily used to establish the ranges that represent a state of statistical control. Phase II begins once a minimum of 20 or 25 representative process results have been produced to establish long-term limits. Although long-term limits may be established with as few as 20 lots, in our experience with regulated products, we have found a minimum of 30 to be a more acceptable baseline. The objectives in Phase II are to identify atypical results. Woodall (12) notes that the use of control charts in Phase I (and by extension here, initial specification limits) is usually iterative.

Thus, the challenge of setting specification limits when working with accelerated products (and temporary limits with nonaccelerated products) is to determine a value of $K$ that controls the probability of excursion at a reasonable (Goldilocks) level when the process is operating as designed. During this time, ranges of typical long-term performance are being established.

We propose using “appropriately calibrated” tolerance intervals for developing Goldilocks limits based on early manufacturing experience. “ Appropriately calibrated” tolerance intervals are those that provide an acceptable probability of false OOS signals throughout early manufacturing.

A tolerance interval has the form of eq 1 with the value of $K$ dependent on sample size ($n$), a desired long-run containment percentage or “coverage” (100$P\%$), and a level of statistical confidence (100(1 – $\alpha\%$)). The level of statistical confidence, for example, 100(1 – $\alpha\%$) = 95\%, expresses the long-run probability that the computed tolerance interval will contain at least 100$P\%$ of the future process values. When the process is running as planned, the long-run probability of a false OOS signal is 100(1 – $P\%$) for the given level of confidence.

An approximate formula for $K$ with confidence 100(1 – $\alpha\%$) and coverage of 100$P\%$ recommended by Howe (13) is

$$K = \sqrt{\frac{(1 + \frac{1}{n})Z^2_{(1+P)/2} \times (n - 1)}{\chi^2_{\frac{\alpha}{2}, n-1}}}$$  \hspace{1cm} (2)$$

where $n$ is the sample size, $\chi^2_{\frac{\alpha}{2}, n-1}$ is the chi-squared percentile with $n - 1$ degrees of freedom and area $\alpha$ to the left, and $Z_{(1+P)/2}$ is a standard normal percentile with area $(1+P)/2$ to the left.

The coverage of three-sigma limits estimated using small samples can be approximated for comparison to the theoretical coverage of 99.73\%. Equation 2 can be reorganized to determine the coverage of three-sigma specification limits by setting $K = 3$ and solving for $P$ with given values of $n$ and $\alpha$. In particular, eq 2 is reorganized to solve for $P$ as

$$P = 2 \times \Phi \left( \frac{3 \sqrt{n \chi^2_{\frac{\alpha}{2}, n-1}}}{n + 1(n - 1)} \right) - 1$$  \hspace{1cm} (3)$$

where $\Phi(\cdot)$ is the area in a standard normal curve to the left of ($\cdot$). For example, $\Phi(-1.96) = 0.025$. Table I reports the values of 100$P\%$ and 100(1 – $P\%$) using eq 3 for several values of $n$ with a 95% level of confidence.
For \( n = 30 \), the coverage of 97.9% is close to the theoretical value of 99.7% for three-sigma limits. However, for lesser values of \( n \), the value of 100\( P\% \) can be quite low as demonstrated for \( n = 3 \) and \( n = 5 \). The estimated coverage is less than the theoretical coverage of 99.7% for three-sigma limits with small \( n \) because of the uncertainty in estimating the mean and the standard deviation.

The 97.9% coverage for three-sigma limits based on 30 lots translates to an OOS rate of 2.1% (with 95% confidence). The use of three-sigma specifications with a sample of at least 30 lots seems quite reasonable. Consistent with Figure 1, the variance is reasonably characterized at this point. We propose that Goldilocks limits require that intermediate specification limits before 30 lots have the same probability of OOS as the 2.1% at 30 lots. In particular, the recommended Goldilocks limits are defined as tolerance intervals with 95% confidence and coverage of 97.9% for all sample sizes <30. Some might consider the alternative of using 95% tolerance intervals that contain the theoretical three-sigma coverage rate of 99.73%. Calculations shown in Table II using eq 2 demonstrate that these “Papa Bear” 99.73% intervals are wider and carry a higher probability of failing to detect large shifts, even at \( n = 30 \). Because of the great amount of uncertainty in the standard deviation for samples of <5 lots, we do not recommend using statistical intervals to set specifications with <5 lots.

One other approach sometimes advocated for specifications is to use the range from the minimum observed value to the maximum observed value. With an assigned confidence level of 95%, the proportion of future values that can be expected to fall in this min–max range is determined by solving for \( P \) in the equation

\[
0.95 = 1 - nP^{n-1} + (n-1)P^n
\]

See Section 5.3 of Meeker et al. (14). This calculation does not assume a normal distribution and can be considered a conservative or worst-case estimate. This approach carries an especially high level of probability of false OOS signals as shown in Table III. We do not recommend using min–max intervals for defining specifications.

### 3. Example

The proposed strategy for setting and updating limits is illustrated with a simple example. A manufacturer plans to submit an accelerated product to a regulatory agency that will include \( n = 15 \) lots at the time of filing. A formal commitment is made to update the

### Table I

<table>
<thead>
<tr>
<th>Sample Size, ( n )</th>
<th>100( P% )</th>
<th>Probability of OOSa</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>44.4%</td>
<td>55.6%</td>
</tr>
<tr>
<td>5</td>
<td>75.2%</td>
<td>24.8%</td>
</tr>
<tr>
<td>10</td>
<td>91.8%</td>
<td>8.2%</td>
</tr>
<tr>
<td>15</td>
<td>95.3%</td>
<td>4.7%</td>
</tr>
<tr>
<td>20</td>
<td>96.7%</td>
<td>3.3%</td>
</tr>
<tr>
<td>30</td>
<td>97.9%</td>
<td>2.1%</td>
</tr>
<tr>
<td>100</td>
<td>99.2%</td>
<td>0.8%</td>
</tr>
</tbody>
</table>

aOOS is out of specification.

### Table II

<table>
<thead>
<tr>
<th>Sample Size, ( n )</th>
<th>OOS Ratea</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6.0%</td>
</tr>
<tr>
<td>10</td>
<td>4.0%</td>
</tr>
<tr>
<td>15</td>
<td>3.5%</td>
</tr>
<tr>
<td>20</td>
<td>3.2%</td>
</tr>
<tr>
<td>30</td>
<td>3.0%</td>
</tr>
</tbody>
</table>

aOOS is out of specification.

### Table III

<table>
<thead>
<tr>
<th>Sample Size, ( n )</th>
<th>Coverage with Min–Max Limitsa</th>
<th>OOS Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>13.5%</td>
<td>86.5%</td>
</tr>
<tr>
<td>5</td>
<td>34.3%</td>
<td>65.7%</td>
</tr>
<tr>
<td>10</td>
<td>60.6%</td>
<td>39.4%</td>
</tr>
<tr>
<td>15</td>
<td>72.1%</td>
<td>27.9%</td>
</tr>
<tr>
<td>20</td>
<td>78.4%</td>
<td>21.6%</td>
</tr>
<tr>
<td>25</td>
<td>82.4%</td>
<td>17.6%</td>
</tr>
<tr>
<td>30</td>
<td>85.1%</td>
<td>14.9%</td>
</tr>
</tbody>
</table>

aDoes not assume a normal distribution.
specifications after 30 lots if commercial experience warrants. Suppose that initial limits are desired during manufacturing when \( n = 5 \) lots are obtained, and that updates are planned after collection of 10 and 15 lots. The run chart in Figure 2 presents a sequence of five simulated protein concentration values (mg/mL) from a normal distribution with mean 65 mg/mL and standard deviation 2 mg/mL.

This sample of five values has a sample mean of 63.66 mg/mL and a sample standard deviation of 1.67 mg/mL. The computed three-sigma limits are

\[
L = 63.66 - 3 \times 1.67 = 58.7 \\
U = 63.66 + 3 \times 1.67 = 68.7
\]

Referring to Table II, the value of \( K \) that provides the Goldilocks limits (95% tolerance interval with 97.9% coverage) for \( n = 5 \) lots is \( K = 6.0 \). The value of the Goldilocks limits after five lots is \( L = 63.66 - 6 \times 1.67 = 53.6 \) and \( U = 63.66 + 6 \times 1.67 = 73.7 \).

Figure 3 shows these computed limits and the next five observations to be monitored with these limits. These five values are simulated from the same distribution as the first five values. The three-sigma limits are
represented by the red dashed lines and the Goldilocks limits by the black solid lines.

Note that Lot 7 falls outside the upper three-sigma limit (red-dashed line) in Figure 3, but is contained in the Goldilocks limit. This excursion occurs even though the process has not changed. Lot 7 is consistent with the rest of the manufactured lots and falls outside the three-sigma limits only because the initial limits based on just five lots were set too tight.

The first 10 lots are now used to construct specification limits for Lots 11–15. These limits and all initial 15 lots are shown in Figure 4.

The three-sigma limits are shown as red dashed lines and the Goldilocks limits as solid black lines. All values are now contained within both intervals, and the difference between the two intervals has lessened. Finally, Figure 5 shows all 15 lots and the updated specifications based on all 15 lots. All values are contained in both limits.

Table IV reports how the specification intervals change over the course of the sample collection.

Note first how the sample mean has quickly centered on the true process value 65 mg/mL, whereas the sample standard deviation is more variable and is still not equal to the true value of 2.0 mg/mL. This is typical because standard deviation estimates have relatively greater uncertainty than mean estimates for a given sample size. This is the primary reason why three-sigma limits can sometimes be too tight. As in this example, the first sample of five values underestimated the true standard deviation (which has a value of 2.0 in the simulation), and the resulting specification was too tight to capture all of the subsequent five lots. Also notice in Table IV that as the number of lots increases, the difference between the width of the three-sigma and Goldilocks intervals decreases. Recall that the Goldilocks intervals are designed to equal the three-sigma limits once 30 lots have been collected.

4. Summary and Recommendation

When specifications are set based on manufacturing experience with a few lots, the probability of false OOS results can be controlled by maintaining the same coverage probability throughout the early manufacturing process. The Goldilocks 95% tolerance interval with coverage 97.9% aligns with the coverage for three-sigma limits based on 30 lots—a minimum sample size for which process variation can be suitably characterized. These limits provide accommodation for residual uncertainty in establishing specifications based on small initial experience.

Conflict of Interest Declaration

The authors declare that they have no competing interests.

References


COMMENTARY

Continuous and Effective Microbiological Air Monitoring in Critical Environments: A Comparison of Analytical Methodologies

GILBERTO DALMASO, ANNA CAMPANELLA*, and PAOLA LAZZERI

Particle Measuring Systems, 5475 Airport Blvd., Boulder, CO 80301 © PDA, Inc. 2020

ABSTRACT: Manufacture of sterile products must strictly follow carefully established and validated analytical methods of manufacture and control. Based on this consideration, we evaluated scientific literature describing settle plates and active air sampler monitoring effectiveness. A contamination control strategy should be implemented by pharma manufacturers, especially for aseptic productions, to assess the effectiveness of environmental monitoring and demonstrate that the process is under statistical control. It is of key importance for microbiological monitoring data to correlate as best as possible with total particle monitoring data so that each batch release is reliably supported.

KEYWORDS: Settle plates, Clean room classification, Annex 1, cGMP, 14698, Clean room monitoring, Clean room contamination.

Introduction

Clean rooms are controlled areas where contamination levels are monitored and managed to meet a defined cleanliness level. In good manufacturing practice (GMP)-defined clean rooms, microbial contamination is a critical parameter and must be controlled.

The most universally applied method for clean room air cleanliness classification was recommended in 1963 by Federal Standard (FS) 209. In FS 209, the number of particles ≥0.5 μm measured in one cubic foot of air classified the room (i.e., 1 particle classified the room as Class 1, 10 particles as Class 10, etc.). In the same period, scientific publications concerning healthcare facilities suggested that most airborne particles carrying microorganisms associated with human dispersion were found on particles with 4±20 μm diameters. This contamination has traditionally been detected using settle plates.

For more than 10 years, regulatory authorities have highlighted their expectations concerning microbiological continuous process air monitoring in Grade A (ISO 5) and Grade B (ISO 7) areas with the settle plate method as a reference. Essentially the physical deposit of particles on a surface, the settle plate/4 h sampling method is still referenced in European Union (EU) current good manufacturing process (cGMP) guidelines in which microbial contamination limits are defined. However, cGMPs frequently include the inherent limitations of different environmental monitoring methods. Settle plate monitoring is a method not validated with a specific ISO standard or regulatory norm. Comparatively, active air monitoring is validated in accordance with ISO 14698 for its physical and biological efficiency.

A large portion of sterile products require manufacturing to be done in aseptic areas to reduce the risk of microbiological contamination. Products labeled as sterile are manufactured by aseptic processing or by terminal sterilization in the final container. These products include pharmaceutical sterile products, bulk sterile drug substances, sterile intermediates, excipients, and, in some cases, medical devices. To mitigate the risk of contamination, regulatory agencies have strongly recommended that pharmaceutical manufacturers implement a solution that separates sterile products from personnel. This has resulted in barrier systems that are now widely used in sterile pharmaceutical manufacturing departments. Scientific knowledge and advanced technologies offer increased safety level solutions to prevent the risk of sterile products microbial contamination (1, 2).

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doi: 10.5731/pdajpst.2019.010181
The cGMP guidelines note that it is impossible to expect a microbial contamination level of zero if personnel are in the clean room (3). The expectation concerning surfaces in aseptic areas is not to have a total absence of microorganisms but instead a very low level of microbiological contamination within the aseptic room as a whole. A sterile environment must have a complete absence of viable microorganisms or organisms to be considered as such, so aseptic areas cannot be considered truly sterile. Sterile pharma products must be safe for patients, in compliance with the recent guideline requests, and also in compliance with the auditors’ approach to microbial monitoring criticality in aseptic processes. In the purest microbiological sense, an aseptic process is one that prevents contamination by the exclusion of microorganisms. Routine microbial monitoring must demonstrate aseptic processing environments operate in an adequate state of control. The methods used are critical components of an effective contamination risk control strategy.

New technologies (i.e., laser-induced fluorescence) can monitor clean room microbial contamination levels continuously and reliably. However, traditional growth-based solutions (i.e., settle plates) are still the most common air-monitoring approach although having a limited period of use. Active microbial air samplers must be validated in compliance with ISO 14698-1:2003. They monitor from 10 to 40 min in continuous mode to sample one cubic meter (one thousand liters) and are configured for flow rates starting at 25 lpm to \(>100\) lpm. To provide evidence that a continuous sampling of clean room air is performed, pharmaceutical manufacturers widely use settle plates, even if scientific and regulatory experts agree that they are a nonquantitative and nonvalidatable method.

Considering scientific and technological advances, as well as the robustness of the reference methods for calibration and verification, can active air samplers provide a method that is reliable enough for continuous monitoring to replace the use of settle plates? To answer this question, how pharmacopoeia limits were defined for air cleanliness classification and monitoring and their scientific principles must be examined. These limits were defined nearly 60 years ago, so are they still scientifically reliable and applicable to today’s manufacturing?

The primary goal of the authors’ research was to clearly understand and describe the scientific assumptions of airborne microbial contamination recovery rates for settle plates.

### Scientific Background on Size Distribution of Airborne Particles and the Effectiveness of Settle Plates

Airborne viable and nonviable particles vary in size, shape, and density. Their size is normally given as an equivalent particle diameter or the spherical diameter of unit density that settles in air at the same velocity as the particle under consideration.

Airborne biological particles, also referred to as bioaerosols, are a complex mixture of different components, from simple organic molecules with dimensions in the nanometer range, to viruses, bacteria, bacteria spores, mold spores, hyphae, and pollen with diameters of \(\geq100\) \(\mu\)m. These components become airborne as single particles or aggregates. See Figure 1 for relative particulate sizes.

In 1884, Hesse revealed experimentally that airborne bacteria occur mainly in “colonies”, whereas mold spores are found detached (4, 5). Widely discussed is whether it is expedient to determine the count of bacteria in a given volume of air or just the number of microbe-carrying particles (MCPs) (6). Both approaches have been applied in the research that has followed since.

Without a standard reference for airborne particles, general approximations like the geometric equivalent diameter are used to obtain a particle’s size, shape, and density. This necessary estimation must be considered when we evaluate the reliability of studies that aim to determine the effectiveness of microbial monitoring methods. The effectiveness of settle plate recovery was estimated several years ago on the basis of a specific standardized parameter referring to available scientific assumptions based on static conditions of air in the environment. However, this does not represent the environment in pharmaceutical clean rooms where dynamic conditions are present with several air changes per hour based on classification. For example, in an ISO 5 (Class A) environment, air is controlled to a laminar state ranging between flow rates of 40 and 800 feet/min and 240 to 480 air changes per hour.

Several accredited authors support the adherence of actual pharmacopoeia limits for microbes to cGMP parameter definitions. One of the most referenced publications regarding the size distribution of airborne particles and microorganisms was written by W. C. Noble, Lidweix and Kingston in 1963. In this publication, the
authors observed that organisms associated with human disease or carriage are usually found on particles in the range of 4–20 μm equivalent diameters. In the described experimental conditions, settle plates can give evidence of airborne microbes with diameters >10 μm. Specifically, they noted that “in most cases an arithmetic-normal distribution appeared to fit the data reasonably well, where the median equivalent particle diameter was greater than 10 microns” (7).

Several recent studies provide evidence that settle plates primarily detect bigger, heavier airborne microorganisms or particle-carrying microorganisms with average diameters >10 μm (1, 8). In contrast, studies of active microbial air sampler physical efficiency and cut-off size (also known as a D50 value) widely use polystyrene latex particles ranging from 0.5 to 9.8 μm in aerodynamic size, with a focus on the minimum size (9, 10).

One of the top experts in airborne particle distribution is W. Whyte, University of Glasgow UK, whose publications are widely referenced in both the literature and cGMP pharmacopoeia (e.g., USP <1116> (3) mentions one Whyte publication from 1985 and ISO 14644-17:2018 (11) mentions five Whyte publications published between 2010 and 2016). The settle plate counts corresponding to airborne concentrations specified in EU guidelines were originally derived from an equation published by Whyte in 1986 (12) and data published in the Parenteral Society in 1989. In Whyte’s study, standard parameters were defined and a single particle deposition velocity of 0.46 cm/s was used as a reference.

In a 2005 publication, Whyte concluded settle plates were a “fundamental method of measuring the number of microbe-carrying particles that will deposit onto a given area in a given time. There is therefore no need to determine its collection efficiency” (13). In 2016, the European Journal of Parenteral & Pharmaceutical Science in partnership with Whyte and T. Eaton reassessed and suggested improvements to EU cGMP’s Annex 1, specifically for how airborne concentration and settle plate counts of MCPs contribute to the grade of a pharmaceutical clean room (14). Using more accurate deposition velocities, the EU cGMP maximum concentrations can be revised to provide more accurate settle plate counts. This opinion is promoted by several authors.

Airborne Particle Deposition Velocity Standard Calculation as a Microbial Monitoring Method Effectiveness Parameter

In 2000, a detailed study evaluated the velocities of variable-diameter particle sedimentation (15). The authors concluded that the dynamic behavior of an aerosol is influenced by several factors, both physical (i.e., Brownian motion, electrical gradient, particle density, thermal gradients, humidity, and ventilation) and biological. Air friction was also a major influencer of the motion of particles with different dimensions.

To define a standard sedimentation velocity, a particle is simplified to an uncharged sphere with dimension and density such that deposition is influenced mainly by the gravitational field and environment (i.e., a uniform temperature of 25 °C and no perturbation). Under these static conditions, the particles in the air sediment with a constant velocity according to eq 1:

\[
V_c = \frac{2}{9} r^2 g \frac{p - p_o}{\eta}
\]  

Figure 1

Relative particulate sizes.
where $V_c$ is the contamination velocity or settling velocity of one colony-forming unit (cfu), $r$ is the particle radius, $g$ is the acceleration due to gravity, $p$ is the particle density, $p_a$ is the air density, and $\eta$ is the air viscosity.

From 2015 to 2016, Whyte et al. wrote a series of articles that discussed the deposition of airborne particles onto critical surfaces in clean rooms (16). In the first article published in 2015, the authors reviewed the various mechanisms of particle deposition on surfaces in clean rooms and concluded that the most important were gravitational settling, turbulent deposition, electrostatic attraction, and, for particles less than $\frac{1}{2}$ to $0.5 \mu m$, Brownian diffusion (17). Experiments were carried out with 10 $\mu m$ particles and demonstrated that >80% of the deposition was by gravitational sedimentation.

In the second article (18), the authors described a clean room investigation concerning the relationship between the airborne particle concentration and the particle deposition rate (PDR), where both are related by the deposition velocity of particles in air according to eq 2:

$$PDR_D = C_D \times V_D$$ (2)

where $C_D$ is the airborne concentration of particles of size $D$ in $\mu m$ and $V_D$ is the deposition velocity of particles of size $D$ in $\mu m$.

In the third article in this series, the authors proposed the following airborne cleanliness equation for clean room classification using the ISO 14644-1 standard:

$$C_n = 10^N \left( \frac{0.1}{D} \right)^{2.08}$$ (3)

where $C_n$ is the maximum permitted concentration per cubic meter of airborne particles that are equal to and greater than the considered particle size, $N$ is the ISO class number, and $D$ is the considered particle size in micrometers.

If the critical particle size that causes contamination is within the normal range of particles used in ISO 14644-1 (i.e., $\geq 0.1 \mu m$ to $\leq 5 \mu m$), the maximum particle concentration can be calculated, and the ISO class can be found in ISO 14644-1.

The authors noted that particles with cumulative counts $>5 \mu m$ are not given in this table and therefore it was necessary to calculate the ISO class by use of the following equation:

$$N = \log \left( \frac{C_n}{\left( \frac{0.1}{D} \right)^{2.08}} \right)$$ (4)

An important clarification by the authors of this study was that the deposition velocities given in Table I, except for those for the 0.3 and 0.5 $\mu m$ particles, were obtained in ISO Class 8. This study found an inverse relationship between the PDR and the particle concentration: as the PDR increases, the particle concentration decreases. Smaller particles were more likely to be swept from the clean room with little time to deposit, whereas larger particles would still deposit with gravity. An increase in the turbulent intensity of the air may be a contributing factor. The authors clarify that, for a range of particles between $\sim 5 \mu m$ and $30 \mu m$, the deposition velocities would be expected to increase 1.7-fold if applied to an ISO Class 7 clean room, about threefold if applied to an ISO Class 6 clean room, and about fivefold if applied to an ISO Class 5 clean room. Particles sized at 0.3 $\mu m$ or 0.5 $\mu m$ were expected to be less influenced by gravity, with the same deposition velocity applied over the range of clean room cleanliness classes.

The deposition velocity of airborne MCPs in different clean room cleanliness conditions has been investigated by Whyte and Eaton (14). It was found that the deposition velocity increased as the cleanliness of the clean room increased, and the results of the study are given in Table II.
Because of its impact on particle sedimentation, the deposition velocity should be modified for different ventilation conditions. Whyte and Eaton, using the set of more accurate deposition velocities, reassessed the relationship between the airborne concentration and the settle plate counts of MCPs used in Annex 1, and suggested that EU cGMP maximum concentrations should be revised to provide more accurate settle plate counts.

Microbial Active Air Sampler Efficiency Validation: International Standard Requirements

ISO 14698:2003’s Annex B describes a technique for determining the collection efficiency of samplers used for counting airborne microbes. The collection efficiency of microbial air samplers is considered in two ways: physical efficiency and biological efficiency.

1. **Physical efficiency** is the ability of the sampler to collect various sizes of particles.

2. **Biological efficiency** is the efficiency of the sampler in collecting microbe-carrying particles.

Physical efficiency is the same for inanimate particles, particles carrying microorganisms, and particles that are microorganisms. Biological efficiency is expected to be lower than physical efficiency, because it depends on the survival of the collected microorganisms and the collection medium to support their growth. The test method described in Annex B is mainly concerned with physical efficiency.

The experimental method for determining physical efficiency involves a test aerosol generated and diffused in a test chamber where relative humidity and temperature are defined. The test aerosol can be generated using a *Bacillus subtilis* var. *niger* (NCTC 10073) spore suspension with polystyrene spheres or another type of nonviable particle. No matter the type, the results are similar. However, in some samplers it is impossible to detect all nonviable particles, whereas if microbes are used, they will grow into colonies that can be easily seen and identified.

To determine biological efficiency, *Staphylococcus epidermidis* (NCTC11047 – ATCC 14990) can be used to represent a human-related contamination strain. Because of the collection efficiency variation caused by sprayed solutions and collection conditions, this method is considered less reliable than the one for determining physical efficiency.

Any test must be conducted in parallel with a membrane-filter air sampler to obtain the efficiency of the sampler as in eq 5:

$$\text{Efficiency of sampler (\%)} = \frac{\text{test sampler count}}{\text{total count (from membrane sampler)}} \times 100 \tag{5}$$

As outlined in ISO 14698:2003’s Annex A, the selection of the microbial contamination air sampling device to be used in a risk zone is dependent on the purpose of the sampling. In addition, the device should have an impact velocity (speed of the air hitting the culture medium) that is a compromise between:

1. A high enough velocity to allow the entrapment of viable particles down to approximately 1 μm, and

2. A low enough velocity to ensure viability of particles by avoiding mechanical damage or the break-up of clumps of bacteria or micromycetes.

In the life science industry, the ISO standard’s recommendation has generally been a sampler at or near 50% physical recovery with a D50 of 1 μm. However, microbial samplers with a D50 of <1 μm are widely recognized. Because we know that aerosolized, microbe-carrying particles are 10–20 μm in size, why is good performance down to 1 μm important? As it turns out, small particles are more difficult to collect than larger macroparticles (particles >5 μm). From a microbiological standpoint, 1 μm is the size of most common species of individual bacteria. Fungal particles are usually 2–5 μm, and *Bacillus anthracis* spores have a size range from 0.65 to 2.0 μm in aerodynamic diameter with about 60% of spores in the 1.1 to 2.0 μm range.

TABLE II
Deposition Velocity of Microbe-Carrying Particles (MCPs) in Relation to Airborne Concentrations

<table>
<thead>
<tr>
<th>Concentration of MCPs per cubic meter</th>
<th>0.1</th>
<th>0.5</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deposition velocity (cm/s)</td>
<td>3.55</td>
<td>2.04</td>
<td>1.61</td>
<td>0.92</td>
<td>0.73</td>
<td>0.42</td>
<td>0.33</td>
<td>0.26</td>
<td>0.19</td>
</tr>
</tbody>
</table>

PDA Journal of Pharmaceutical Science and Technology
Particle Capture by Active Air Samplers

If a stream of gas undergoes a sharp change in direction, the particles it transports will tend to continue in their original direction, especially as the ratio of their mass to their linear dimensions increases. Particles having different dimensions and densities will thus follow different trajectories and may be collected separately. When a jet of air is accelerated through a nozzle, the particles it transports are carried at the same speed as the fluid and follow its flow line (see Figure 2). If the fluid flow lines rapidly change direction at the nozzle output, the particle trajectories will appreciably depart from the airflow lines, depending on the inertia associated with the particles. In other words, the particles will tend to run in a straight line and if they find a surface in their path, they can adhere to it and thus be captured.

Active air impactors are designed to sample particles in the air or other gas through a collision with a solid surface. The impactor’s geometry ($W, T, S$ in Figure 3) is designed to have laminar flow into the nozzle ($Re < 2300$) with a velocity as high as possible and a D50 as low as possible.

Settle Plate Definition and Background Analysis

To reiterate, settle plates provide evidence of MCPs whose average diameter can be considered $>10 \mu m$. Because of particle deposition by gravitational sedimentation, turbulent airflow must be considered the most appropriate condition for particle collection on passive surfaces such as settle plates. In ISO 14698-1:2003’s Annex C, the settle plate definition states that passive microbial air sampling devices such as settle plates do not measure the total number of viable particles in the air, but rather the rate at which viable particles settle on surfaces.

Often, settle plates are recommended as a method for continuous microbiological air monitoring in critical areas because of their required handling is limited in comparison to that of active samplers. However, for several years, ready-to-use impactors using agar plates have been available on the market. Single-use impactors in particular are designed to simplify and reduce handling while limiting operator contamination risk. The device’s D50 complies with ISO requirements as well as laboratory best practices. It has also proven to be a reliable performer in long-term sampling. Instruments using alternative microbiological methods make it possible to perform critical area continuous monitoring that detects even the smallest microbe-carrying particles with a lower related risk of contamination than that of settle plates.

Discussion of Suggested Improvements: Comparison of the Two Methods

As reported in the new EU GMP Annex 1 draft (19):

“Processes, equipment, facilities and manufacturing activities should be managed in accordance with QRM principles that provide a proactive means of identifying, scientifically evaluating and controlling potential risks to quality. Risk assessments should be used to justify alternative approaches to those specified in this Annex only if these alternative approaches meet or surpass the intent of this Annex.

Quality Assurance is particularly important, and manufacture of sterile products must strictly follow carefully established and validated methods of manufacture and control.”
Several studies have attempted to compare the values of microbial concentrations in air obtained through both active and passive sampling methods, but with inconsistent results. In some cases, there was significant correlation, whereas in others there was none (7, 9, 20–22).

Gowning: Impact on the Microbial Concentration in the Air

Often an overlooked variable, the sterile gown should absolutely be considered when evaluating air contamination. In some cases, it is the only barrier between the operator and the product. In Reinmüller and Ljungqvist’s article (23), the authors analyzed the total particles and microorganisms originating from people using different clean room garments and clothing conditions.

Table I in the article provides the ratios between the total number of airborne particles (≥0.5 μm, ≥5 μm) per cubic meter and the number of aerobic cfu per cubic meter for the clothing systems used in aseptic production of sterile products (23).

- The ratio for particles ≥ 0.5 μm was in the range of a maximum of 1.81 × 10³ to a minimum of 0.72 × 10³.
- The ratio for particles ≥5 μm was in the range of a maximum of 32.4 to a minimum of 14.1.

The gowning area is a high-impact variable for product quality, considering the following:

“Within the cleanroom, people are the main source of airborne microbial contamination. Results from performed tests show no significant differences of the released airborne particulate contamination levels among small variations in accessories such as with and without goggles, different face masks and different sizes of hoods. Results from a comparison of two types of cleanroom underwear used with the cleanroom coverall show significantly lower levels of released airborne particulate contamination when long sleeved cleanroom undershirts were used in combination with long-legged cleanroom underpants.

Values presented indicate that a relationship exists between the number of particles equal to and larger μm than 0.5 per volume unit of air and the number of aerobic airborne CFU per volume of unit air. In a typical cleanroom environment, with people dressed in modern cleanroom clothing systems as the main contamination source, it seems to be possible to establish a relationship at a ratio of approximately 1.500 to 1” (23).

Scientifically sound technologies are needed for clean room air monitoring to monitor the number of microorganisms present in the air caused by people and clothing systems.

Settle Plates vs Continuous Microbial Active Air Sampling

Based on the considerations discussed previously, settle plates are not recommended in Grade A areas because they do not detect low concentrations of microorganisms and offer low sensitivity when combined with high airflow rates. Settle plates are possibly acceptable only in Grade B, C, and D areas where air movement (turbulence) allows for an increase in MCPs to be deposited.

Owing to modern clean room design (i.e., restricted access barrier systems, isolators, etc.) and clothing features, in addition to the improved quality of materials used (i.e., wipes, packaging, bags, etc.), MCPs detected in clean room environments are primarily in the size range of 0.5–5 μm. For this reason, continuous microbial active air sampling in Grade A is recommended as a replacement for the combination of settle plates and single or intermittent active air sampling activities. The following table shows the comparison between the two systems.

Table III highlights that active microbial air sampling is an improved approach compared to settle plates and traditional volumetric air samplers in Grade A continuous monitoring applications.

Rationales for Monitoring Different Grades

The qualification of pharmaceutical clean rooms is a fundamental step for their use in the production of medicines where patient safety is key. Microbiological qualification provides verification of microbiological contamination in the air while these medicines are manufactured. Following qualification and its positive outcome, pharmaceutical companies must design a monitoring plan that documents and demonstrates that during batch product production, the quality of the air
is in accordance with the specifications established following validation. Through monitoring, there can be microbiological control of air.

The rationales for monitoring Grade A areas (ISO 5 critical areas) and Grade B areas (ISO 7) in aseptic production are different and take into consideration the following details:

1. Grade A areas include the product, product contact materials, and the surrounding environment in contact with the product (i.e., air). For this reason, they are considered extremely critical areas and subject to high air monitoring frequency. This can include continuous monitoring during all production phases including setup, surface monitoring at the end of production, and on operator clothes and gloves following any critical intervention. In these areas, air, surfaces, and operators must have no microbiological contamination.

2. Grade B areas are used to protect Grade A areas and include the presence of operators in a variable number depending on the production process. Here, microbiological monitoring takes on a different meaning for frequencies and action limits. The trend analysis of these areas should demonstrate the process and microbiological aspect is under statistical control. Trend analysis should consider the seasonal variability to define random trends of the process.

Beyond these minimum expectations, microbiological monitoring should be comparable with total particle monitoring. Total particles are not limited to inert particles, but also include:

1. Particles containing an unknown number of microorganisms on their surface.

2. Microorganisms that are themselves particles and therefore detectable by the particle counter.

### TABLE III
Comparison of Methodologies

<table>
<thead>
<tr>
<th>Component</th>
<th>Settle Plates</th>
<th>Continuous Microbial Active Air Sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous monitoring</td>
<td>Both can be used to monitor all phases of production.</td>
<td>Volumetric samplers measure the number of microorganisms in a given volume of air pulled in from the environment which then impact on agar media.</td>
</tr>
<tr>
<td>Measuring the concentration of microorganisms in the air</td>
<td>Settle plates do not measure the number of microorganisms in the air. They measure the number of microorganisms settling from the air onto a known surface area within a known time in a turbulent environment.</td>
<td>This is a quantitative method. The results can be compared in terms of time and air quality.</td>
</tr>
<tr>
<td>Quantitative method</td>
<td>Settle plates are not a quantitative method. Their results are often reported as the number of microorganisms per settle plate, with the size of the plate and the time exposed often not reported.</td>
<td>These systems have a higher probability of detecting a low concentration of microorganisms.</td>
</tr>
<tr>
<td>Detect low concentrations of microorganisms</td>
<td>Settle plates do not detect low concentrations of microorganisms and provide very low sensitivity owing to the high airflow rate in Grade A.</td>
<td>The correlation of data is very useful because the speed of air of the two systems is similar (e.g., a total particle counter with 28 LPM flow combined with a single-use impactor and active air sampler with 25 LPM flow).</td>
</tr>
<tr>
<td>Comparison of microbiological and particulate data</td>
<td>The correlation of data is not definable because of the two methods’ differences (e.g., total particle counter vs settle plate).</td>
<td>The correlation of data is very useful because the speed of air of the two systems is similar (e.g., a total particle counter with 28 LPM flow combined with a single-use impactor and active air sampler with 25 LPM flow).</td>
</tr>
<tr>
<td>Validation</td>
<td>This method is not validated.</td>
<td>This device is validated according to ISO 14698-1.</td>
</tr>
</tbody>
</table>
In accordance with cGMP, total particle monitoring is continuously performed for the duration of the manufacturing process. To be comparable, microbiological monitoring should also be continuous. Continuous airborne particle monitoring provides key information on the amount and size of total particles present in the air at a given sampling point. It is therefore important to have a strategy for both total particle and microbiological monitoring that utilizes validated methodologies in accordance with pharmacopoeia or international standards. The strategy should also allow for quantification of the microorganisms present in the same area as the total particle sampling. Doing so will aid in determining the potential correlation between events and provide the data necessary for investigation of a possible out-of-action limit.

**Conclusion**

The settle plate method depends on the deposition of MCPs and microorganisms on the surface of the agar plate. The method is strictly qualitative without the possibility of determining its collection efficiency. This method should not be used in Grade A areas (ISO 5) where it is common to have high air speeds and a high number of air changes. These parameters severely limit the ability for stand-alone particles, particles containing microorganisms, and stand-alone microorganisms to settle. Its use is more practical in static environments (i.e., outdoors) or where there are infrequent air changes.

Continuous microbiological monitoring of air in critical areas with validated methodologies, such as active air samplers with recovery rates of ≥70%, should be more widely used. This contamination control strategy satisfies the regulatory demand for better process knowledge and greater sterility assurance of the released product.

**Terms and Definitions**

**Active air impactor:** device designed to sample particles in the air or other gas, through a collision with a solid surface (24).

**Microbial active air monitoring:** Monitoring of air in the environment using an active air impactor.

**Passive air monitoring:** Monitoring of air in the environment using settle plates. The contaminants fall down on the agar plates following the environmental airflow; Viable particle: particle that consists of, or supports, one or more live microorganisms (24).

**Conflict of Interest Declaration**

The authors are affiliated with the Particle Measuring Systems Advisory Team, long-standing experts in the subject matter discussed in this manuscript. They are not involved in the selling of Particle Measuring Systems’ products.

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COMMENTARY

Industry One-Voice-of-Quality (1VQ) Solutions: Effective Management of Post-Approval Changes in the Pharmaceutical Quality System (PQS)—through Enhanced Science and Risk-Based Approaches

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ABSTRACT: Post-approval changes are inevitable and necessary throughout the life of a drug product—to implement new knowledge, maintain a state of control, and drive continual improvement. Many post-approval changes require regulatory agency approval by individual countries before implementation. Because of the global regulatory complexity, individual post-approval changes usually take years for full worldwide approval even when they reduce patient risk, improve compliance, or enhance the manufacturing process or test methods. This global complexity slows down continual improvement and innovation and can cause drug shortages and current good manufacturing practices compliance issues. Manufacturers that market products globally experience the greatest challenge and risks in their daily operations because of this post-approval change complexity. A global problem needs a global solution. This paper has been sponsored and endorsed by senior quality leaders (Chief Quality Officers/Heads of Quality) from >20 global pharmaceutical companies who have collaborated to speak with “One-Voice-Of-Quality” (1VQ). The paper provides two specific solutions that lay the foundation for an aligned and standardized industry position on the topic of effective management of post-approval changes in the pharmaceutical quality system (PQS). This document represents the 1VQ standard approach for the steps necessary to establish and demonstrate an effective quality system to fully leverage a risk-based approach to post-approval changes as laid out by ICH Q10 Annex 1. Implementation of the solutions presented in this paper can help achieve a transformational shift with faster implementation of new knowledge, continual improvement, and innovation through post-approval changes. The Chief Quality Officers/Heads of Quality are inviting other companies to join the 1VQ (contact either Emma Ramnarine or Anders Vinther) and other stakeholders to join the dialog.

KEYWORDS: Pharmaceuticals, Post-approval change (PAC), ICH Q10, Pharmaceutical quality system (PQS), ICH Q12, Science and risk-based approach.

Context and Current State, May 2020

This paper lays the foundation for an aligned and standardized industry position on the topic of effective management of post-approval changes (PACs) in the pharmaceutical quality system (PQS). Senior quality leaders (Chief Quality Officers/Heads of Quality) from more than 20 global pharmaceutical companies have collaborated to speak with “One-Voice-Of-Quality” (1VQ). The first two solutions identified in the One-Voice-of Quality (1VQ) Concept Paper “Solving the Global Continual Improvement and Innovation Challenge: How an Effective Pharmaceutical Quality System Can Transform Post-Approval Change Management” (1), published in the PDA Journal of Pharmaceutical Science and Technology, are presented here. This document represents the 1VQ standard approach for the steps necessary to establish and demonstrate an effective quality system to fully leverage
the risk-based approach to PACs as laid out by ICH Q10 Annex 1. Demonstrating a detailed understanding, effective implementation, and compliance with ICH Q10 will allow companies to overcome barriers to continual improvement and innovation. Additionally, it will help reduce drug shortages in the global environment by allowing faster implementation of PACs and reducing the PAC burden on both industry and regulators. This paper also provides the foundation for implementation of ICH Q12. It is intended to drive a paradigm shift from a country-specific and “one size fits all” approach to an enhanced science and risk-based approach for approval expectations focused on patient safety and product availability.

PACs are inevitable and necessary throughout the life of a drug product—to implement new knowledge, maintain a state of control, and drive continual improvement. Many of these PACs require regulatory agency approval by individual countries before implementation. Owing to global regulatory complexity, individual PACs often take years for full worldwide approval, even when they reduce patient risk, improve compliance, and/or enhance the manufacturing process or test methods. The consequence of this can ultimately lead to potential drug shortages for patients and possible compliance risks for companies.  

The current COVID-19 pandemic, although an exceptional situation, is challenging pharmaceutical companies and regulators alike in making life-saving decisions for patients in unprecedented ways to ensure drug products are available with no shortages. The global impact of COVID-19 has demonstrated that diseases know no borders, and solutions to fight such diseases need be global in nature to be timely and effective. It has also underscored the necessity to transform our current national or regional-based systems and processes whereby changes to manufacturing and testing of drug products already marketed or for new indications, can be implemented quickly. The highly complex global regulatory framework for managing PACs is simply not capable of dealing with a crisis like the COVID-19 pandemic, and systems have to be bent to prevent drug shortages. Opportunities to learn from and adopt new ways of working that emerge from the COVID-19 crisis should be integrated into transforming how patient needs are met by making products available with the highest sense of urgency, by an industry that is capable of globally implementing improvements in a timely manner.

The 2005 ICH Q10 Concept Paper (2) recognized the challenge with global filing of PACs, including: “Delays may occur in the availability of medicines to patients around the world” and “Delays in the implementation of innovation and continual improvement for existing products may occur due to different expectations in the three regions”. To address these issues caused by the PAC global complexity within the current regulatory framework, a solution to reduce the size of this challenge has already been described in ICH Q10 Pharmaceutical Quality System. The benefits of the ICH Q10 guideline upon completion and implementation, as stated in the Concept Paper, include “Encourage industry to improve manufacturing processes”, “Facilitate innovation and continual improvement”, and “Encourage a science and risk-based approach to quality decisions”.

The ICH Q10 guideline was approved by the ICH parties in 2008 (3). Annex 1 of the document describes potential opportunities to enhance science and risk-based regulatory approaches to PACs as follows: When a company can “demonstrate effective PQS and product and process understanding” this is an opportunity to “optimize science and risk-based PAC processes to maximize benefits from innovation and continual improvement”. Since the ICH Q10 approval in 2008, no regulatory guidance has been made available on what the measures for an effective PQS are and how to demonstrate effectiveness of a PQS. Current regulatory mechanisms and guidance for PACs also do not consider the company’s latest product and process knowledge when determining the type of filing required to implement the change. Further, the effectiveness of the company’s PQS to manage PACs is not considered during the assessment of individual PACs or during inspections. The 1VQ Concept Paper addresses these challenges. It details the perceived problem, strategic importance of the topic, actions proposed, deliverables, and issues to be resolved.

This document expands on the main deliverables from the 1VQ Concept Paper. It outlines how PACs can be effectively managed in the PQS utilizing enhanced

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1 Enhanced risk-based approach: For companies—risk assessments are updated with the latest product and process knowledge, regardless of filing geography (science knows no borders). For regulators—effectiveness of the PQS and current product/process knowledge (vs. general risk understanding) is used in risk-based decision-making for PACs.

science and risk-based regulatory strategies that are aligned with ICH Q10; this could allow more changes to be managed in the PQS or via notification pathways, instead of by prior approvals. It identifies specific PQS elements to further develop and define for managing PACs in the PQS, provides points to consider for PACs for each of these elements, and how the effectiveness of PAC management in the PQS can be demonstrated. It includes a standard risk-based assessment of PACs that incorporates latest product and process knowledge at the individual change level.

**Purpose**

Although this document is intended foremost to define a standardized approach to demonstrate effective management of PACs in the PQS using product and process knowledge in industry, it is also an opportunity to encourage dialog with and among regulators on this topic, thus ultimately resulting in global regulatory harmonization for managing PACs. In order for these 1VQ solutions to deliver the value envisioned, it is essential for regulatory agencies to accept an enhanced science and risk-based approach to managing PACs. This can require changes to current practices, regulations, and/or guidelines. Upon implementation, this approach can further reduce the regulatory burden for PACs and allow regulatory agencies and companies to focus on the changes that are a higher risk to product quality as it relates to patient safety and efficacy. Health authorities relying on each other for assessments of the same PAC submitted by the company to multiple countries would further reduce the challenge both industry and regulators face.

Although this document is based on ICH Q10—and hence applicable to countries that are members of ICH—the full benefit for patients and companies in terms of reduced drug shortages and enhanced innovation will only be achieved when health authorities around the world engage in a dialog on PAC management complexity with industry and with each other. This document is written to encourage a convergence and harmonization dialog between the industry 1VQ and regulators. Upon adoption of the solutions presented in this paper, companies will be able to gain the benefits of implementing latest product and process knowledge to improve quality, ensure a sustainable supply, and ultimately reduce the potential for drug shortages.

Prior to reading this document, reading ICH Q9 (5), ICH Q10 (3), ICH Q12 (6), the “One-Voice-Of-Quality” Concept Paper (1), and the PIC/S paper “PIC/S Recommendation on How to Evaluate/Demonstrate the Effectiveness of a Pharmaceutical Quality System in relation to Risk-based Change Management” (4) is recommended.

**Background**

Implementation of an effective PQS is essential for a company to achieve product realization, maintain a state of control, and facilitate continual improvement (3).

As commercial product experience and knowledge is gained, changes are generally needed to improve daily operations, manufacturing processes, and the control strategy. PACs are thus a natural and essential part of a product’s commercial life cycle. PACs are needed for many different reasons, such as (but not limited to):

1. upgrading aging facilities and equipment;
2. maintaining current good manufacturing practice (cGMP) compliance and a state of control;
3. evolving regulatory requirements;
4. new technologies;
5. supplier changes; and
6. acquisition of new knowledge about products and processes (e.g., monitoring of product quality controls and trends, post market surveillance, adverse event reporting, annual product review, etc.).

To better serve patients, PACs should be implemented in a timely manner. However, today many PACs require regulatory prior approval that can take years before full implementation worldwide. Moreover, the accumulation of multiple PACs awaiting regulatory approvals with time lines that cannot always be predicted increases the potential for drug shortages.

Additionally, sometimes companies and quality leaders find themselves in a dilemma when a PAC is needed to maintain cGMP compliance in certain countries while the change requires approval in the same or other countries before implementation. This dilemma of cGMP
compliance vs. regulatory conformance poses a complexity for timely and effective PAC management. The intended enhanced science and risk-based approach cannot be used to justify noncompliance with cGMP requirements. Companies should remain compliant with cGMP requirements while using the enhanced science and risk-based approach to determine regulatory strategy and manage conformance to global registrations. Regulatory filings should be kept current on a regular basis.

Utilizing ICH Q10 for Effective Management of PACs

When PACs are introduced, the combination of an effective PQS, product and process understanding, use of quality risk management (QRM), and a mature quality culture should ensure that product quality, patient safety, and adequate supply to patients are maintained. ICH Q10 (3) states that when a company can “demonstrate effective pharmaceutical quality system and product and process understanding”, this is an “opportunity to optimize science and risk-based postapproval change processes to maximize benefits from innovation and continual improvement” (ICH Q10 Annex 1). However, ICH Q10 does not provide specific details on how each of the quality system elements and key enablers can be further defined and detailed to effectively manage PACs in the PQS. This document provides enhanced science and risk-based guidance on how companies can effectively manage PACs within the PQS, building on the principles laid out in the ICH Q10 Guideline, by adding specific PAC-related details for each of the two enablers and the four quality system elements. Figure 1 depicts the PQS elements, enablers, and principles discussed in ICH Q10 that can support effective management of PACs through the PQS.

The PQS elements include: the process performance and product quality monitoring system (PPPQMS), the corrective action and preventive action (CAPA) system, the change management system, and management review. The enablers include: knowledge management (KM) and QRM.

Figure 2 depicts how a company can maintain a state of control and facilitate continual improvement through a PQS that (1) captures triggers/signals for changes or corrective and preventive actions, (2) manages these within the PQS, and (3) verifies them for effectiveness. All of this information should be utilized to determine the regulatory filing approach for a PAC.

Building an effective PQS is the responsibility of the company, one that extends beyond having a license or

Figure 1

Utilizing ICH Q10 for effective management of post-approval changes.
a cGMP certificate to manufacture medicinal products. Being compliant with cGMP is a critical requirement and a prerequisite to gain the benefits of regulatory flexibility and timely PAC management. The framework should extend to also include PACs in outsourced operations and supplier management, to ensure that these are also planned, managed, and controlled by the company’s PQS and communicated appropriately. In order to achieve the benefits of ICH Q10 Annex 1 through an enhanced science and risk-based approach, companies are encouraged to implement and demonstrate these 1VQ solutions within their PQS (e.g., the quality manual or quality plan).

The effectiveness of the company’s PQS to manage PACs for each manufacturing site and across multiple sites should be considered during the assessment of individual PACs and can be evaluated during health authority inspections. Management should conduct reviews of the PQS to effectively manage PACs. This includes developing performance indicators and allocating adequate resources and budget for continual improvement and planning, implementing, and monitoring PACs. Additional management responsibilities include accountability for the overall PAC management strategy, including implementation of the 1VQ solutions, ensuring that internal audits, change mechanisms (or change management system), and QRM enable proactive assessment and mitigation of risks in the PQS, and for developing and maintaining the desired quality culture at all levels in the company.

**PQS Enablers**

ICH Q10 describes QRM and KM as enablers of the PQS because they:

1. facilitate product realization, state of control maintenance, and continual improvement and
2. enable a company to successfully and effectively implement ICH Q10.

Therefore, structured KM and QRM (as described in ICH Q9 (5)), should be implemented and integrated throughout the product life cycle and into the four PQS elements, and appropriate resources should be allocated by management accordingly.
ICH Q10 defines KM as “a systemic approach to acquiring, analyzing, storing and disseminating information related to products, manufacturing processes and components.” In practice, KM aggregates existing and newly acquired information to inform risk management and guide PAC decisions. Examples include knowledge gained from PPPQM, deviations, trends, complaints, recalls, product quality reviews, and management reviews. Development studies, including designs of experiments, should also be considered for gaining new knowledge, as well as, but not limited to, the use of enhanced data analysis and analytics, statistical tools, and mathematical and predictive models. The expanded access to and use of technical and operational information, combined with increased competency of employees based on latest product and process knowledge, enables faster implementation of new knowledge to continually improve the quality and availability of a product during its commercial phase.

To enable effective PAC management, KM should be utilized as part of the PQS. KM should incorporate both explicit and tacit knowledge with an aim to further understand the risks and benefits of a given PAC. For example, product and process knowledge should serve as an input to the control strategy to better understand relationships between parameters and attributes. The same inputs may be used during risk management of PACs.

The elements of KM should be defined in the PQS and maintained through appropriate mechanisms to enable ready access to product and process knowledge. Methods for information capture and dissemination should be systematic and standardized. Management should take an active role in the promotion and utilization of KM, defining roles, expectations, and incentives to maintain the robustness of the system and timely implementation of new knowledge. Learning interventions, after-action reviews (“lessons learned”), job shadowing, and active expert networks are some examples of processes and tools that require active promotion to maintain their viability and benefit to PAC management. As ICH Q10 describes KM as an enabler of the PQS, review of new knowledge should occur in the context of identifying candidates for PACs as well as when reviewing change requests.

Effective QRM should provide a patient-centric decision-making framework to ensure that systematic and proactive risk-based and data-driven decision-making is used for all PACs. This includes decisions related to whether or not to proceed with a PAC based on an appropriate risk-benefit balance, how to control risks that might be introduced by a PAC, and regulatory conformance strategy for the PAC based on risk level.

The elements of the PQS and the enablers should collectively drive identification of risks to product realization, state of control, or the need for continual improvement. It is important to demonstrate product and process understanding to identify the level of risk and manage the control strategy accordingly. QRM should help identify changes that can reduce the risk of product and process failures and issues and/or improve process performance. Effective QRM should ensure that no unacceptable risks are introduced to product quality and/or patient safety as a result of the PAC. At a minimum, the PAC should not increase risks beyond current levels.

A risk assessment based on current product and process knowledge, the control strategy, and the product life cycle should be performed for identified PACs. The risk assessment of the PAC should assess potential risks and benefits to all relevant products, processes, and/or systems that might be impacted by that change. A specific PAC may be categorized differently depending on the level of knowledge, risk controls, and PQS effectiveness. The outcomes of the risk assessment should drive change planning, prioritization, implementation, and time lines. The rigor of the risk assessment associated with a PAC should be commensurate with the complexity and/or criticality of the change.

Residual risks or any unintended consequences of the change (during and after change implementation) should be assessed to ensure that they have been managed to acceptable levels for impacted products, processes, and systems. As appropriate, residual risks and the effectiveness of the change should be monitored post-implementation (via relevant ongoing review/monitoring systems), to ensure that a state of control is maintained.

A process/mechanism should be established to capture, manage, and track key risks to product quality, efficacy, and safety for implemented and pending PACs.

ICH Q10 Annex 1 provides the opportunity for risk-based regulatory oversight when an effective PQS can
be demonstrated. Therefore, QRM should also help determine the change category based on the risk level; it should distinguish changes that require regulatory approval reporting from changes that can be managed solely in the PQS. In certain cases, the risk assessment may be shared and discussed with regulators in a post-approval change management protocol or product life cycle management document, to proactively align on change categorization.

**PQS Elements**

The sections following describe how the four PQS elements shown in Figure 1—PPPQMS, CAPA system, change management system, and management review—should be utilized to support effective management of PACs.

**Process Performance and Product Quality Monitoring System (PPPQMS)**

An effective PQS should include an enhanced PPPQMS that proactively ensures the process and product remain in a state of control and are continually improved as appropriate, to provide increased assurance of product quality and process performance. Product quality reviews should include a summary evaluation of process performance and product quality.

Although ICH Q10 identifies high-level principles for the monitoring program, additional details can provide increased insights into determining the effectiveness of the program. An enhanced PPPQMS may include:

1. Tools for measurement of process and method performance including process capability, that is, statistical process controls.
   ○ Use statistical tools to establish and monitor process and analytical method capabilities and ensure a high degree of confidence that the process and methods are capable and continuously improved, as needed.
   ○ Establish control charts for evaluating trends that warrant additional investigations.
   ○ Provide tools to measure method performance including frequency of invalid results.

   ○ Establish limits beyond which additional evaluations are performed to identify sources of variation and appropriate corrective or preventive actions.

   ○ Perform process performance monitoring in near real-time to enable early detection of process drifts/unexpected variability/trends and react in a timely manner to prevent quality issues or failures.

2. Periodic evaluation with cross-disciplinary subject matter experts to monitor trends and/or deviations in process and method performance and integrate information from product complaints, audits/inspections, and the pharmacovigilance program.

3. Identification of PACs needed or desired to maintain a state of control, ensure product availability, and drive continual improvement of product, processes, and the control strategy.

4. A quality plan to identify, communicate, and implement key quality objectives to drive continual improvement within the PQS.

5. Escalation of significant issues or trends (e.g., product impact, cross-product, and cross-facility issues) for management review and potential changes to the quality plan.

6. Enhanced monitoring and sampling of product quality following major changes including notification to the pharmacovigilance program.

**Corrective Action and Preventive Action System (CAPA)**

The design and use of the CAPA element of the PQS should result in product and process improvements. An effective CAPA system monitors and manages unintended risks and consequences of PACs and should enable appropriate actions that can be taken to correct problems and prevent their recurrence. The CAPA system also provides insight into how the PQS can be improved.

Corrective actions (CAs) can be driven by an unanticipated event such as a complaint investigation, product rejection, nonconformance, recall, deviation, audit, regulatory inspection finding, QRM, and adverse trend from process performance and product quality monitoring. For each of these, it is expected that a thorough investigation and root cause analysis is conducted.
Preventive actions (PAs) can be driven by continual improvement initiatives as new product and process knowledge is gained. These PAs are designed to anticipate and prevent issues, deliver low rates of deviation, and emphasize the need to learn from deviations, deviation trends, and complaint/recall incidents.

CAs and PAs may identify the need for PACs to maintain or improve the assurance of product safety, efficacy, and supply. An effective CAPA program monitors and verifies the effectiveness of any CAPAs associated with PAC initiatives. Unintended risks or consequences should be addressed in a timely manner.

Change Management System

Prioritization of changes should be considered and regularly reviewed as part of management responsibilities to ensure that the company maintains a state of control and for resource planning. Additionally, in considering PACs to implement, management should ensure product availability to patients during and post completion of such changes. Where the supply chain contains multiple locations providing the same product, management should ensure that there is consistency in the change being implemented at different locations as relevant for national and regional regulatory filings.

Effective change management should result in improved product quality, process performance, state of control, and product availability. Change management should rely on a data-driven, enhanced science, and risk-based assessment of changes. Human factors should also be considered when proposing and implementing a change.

The QRM principles outlined in ICH Q9 (5) should be used during all steps of the change management process—change proposal, change evaluation, change implementation, change review, and closure. Based on the outcomes of the quality risk assessment, an appropriate regulatory reporting category (prior approval, notification, or not reportable) should be proposed. Figure 3 describes the overall flow for risk-based assessment of PACs and determination of regulatory reporting category.

Step 1: Change Proposal: When a PAC is proposed and entered into the change management system, the potential quality, safety, and efficacy (QSE) and legal/regulatory impact of the change needs to be considered during the initial high-level impact assessment. This can be assessed by using the following risk questions: what might go wrong when changing from the current situation to the proposed one? Why could this happen? This initial impact assessment should consider existing product and process knowledge (including process performance and variability) and current control strategies.

If the initial impact assessment indicates that

1. there is no additional potential QSE risk associated and there is no legal/regulatory impact per local/regional regulation, the change can be processed to the next step without the need to perform a detailed quality risk assessment. Additionally, the change can be categorized as a non-reportable and managed within the company’s PQS. Rationale supporting this decision to manage the change internally with no regulatory submission/reporting should be clearly documented within the change management system.

2. there might be a potential QSE risk OR a potential legal/regulatory impact, a more detailed risk assessment needs to be performed to define the reporting category of the change.

Step 2: Change Evaluation:

A. Quality Risk Assessment: If the initial impact assessment concludes that there might be a potential impact associated with the change, or if the potential impact is unclear, a quality risk assessment should be performed. When assessing potential risks of the change, any potential impact (direct or indirect) on the identity, strength, quality, purity, or potency of the product should be considered, based on current product/process knowledge and the control strategy; some examples of risk questions include:

- Can the change impact product safety?
- Does the change impact a critical quality attribute, a critical process parameter and/or a critical material attribute?
- Can the change potentially affect conformity of the product to current specifications?
- Can the change potentially affect the purity of the product? Can the change introduce a new potential source of contamination or increase an existing potential source of contamination (e.g., including adventitious agents)?
Can the change potentially affect the potency of the product (i.e., the ability of the product to yield a given result)?

Can the change potentially affect the homogeneity of the product?

Can the change potentially impact the sterility of the product?

Can the change potentially impact the stability of the product?

Can the change impact the performance of an analytical method?

Can the change affect any of the above for another product or process?

The rigor of the risk assessment may vary and should be commensurate with the complexity and potential adverse impact of the change. Regardless of the tool used, the risk assessment should categorize the various risk levels based on the current product/process knowledge and risk controls. Changes should be evaluated by experts with relevant technical, scientific, and quality competencies and background. Peer or independent reviews can be done in teams like change review boards. A decision about acceptance or mitigation of the identified risks needs to be made before implementation of the change and documented in the change record, including appropriate rationale.

B. Assignment of Regulatory Reporting Category: Consistent with ICH Q12 (6), it is recommended that:

- High-risk changes are categorized as prior-approval and as such require regulatory authority review and approval prior to implementation.
- Moderate- to low-risk changes are communicated to the regulatory authority as a formal notification that takes place within a defined period of time before or after implementation, according to regional requirements.

The quality risk assessment (performed in step 2A) should be used to determine the level of risk associated with a change. Additional factors may also play a role as part of the evaluation. Possible documentation approaches include narrative evaluation, decision tree, checklists, and so forth. Rationale supporting the proposed regulatory
reporting category should be documented in the change management system. In certain circumstances in which the risk level and recommended change category is not commensurate with the local/regional regulations, companies should consider their strategy for regulatory conformance to implement the change.

**Steps 3 and 4: Change Implementation, Review, and Closure:** Change implementation, review, and closure should be performed per the change management process. Outcomes of impact and risk assessments should be integrated into the overall change implementation plan. After implementation of the change, residual risks should be assessed and managed to acceptable levels before change closure; any unintended consequences or risks introduced as a result of the change should be evaluated, documented, and handled adequately through effectiveness verification mechanisms. In case several changes are introduced at the same time or are related to each other, the company should assess the cumulative effectiveness of the changes.

After change closure, relevant risk assessment tools/documents are updated post effectiveness assessments. Other elements of the PQS, in particular the process performance and product quality monitoring system, should be used post closure for the ongoing review/monitoring of the risks associated with the change as well as for continuous process verification.

All PACs should be included and assessed as part of the periodic product quality review process, which should ensure that the regulatory filing information is consistent with all implemented PACs. ICH Q12 (6) provides additional details of PQS change management. The PIC/S paper “PIC/S Recommendation on How to Evaluate/Demonstrate the Effectiveness of a Pharmaceutical Quality System in Relation to Risk-Based Change Management” (4) provides a practical checklist tool that can be used by a company and inspectors to evaluate the effectiveness of a company’s PQS in relation to risk-based change management.

**Management Review**

Management review is comprised of oversight activities including product and process performance monitoring and PQS effectiveness. Effective management review should include a review of PAC initiatives, their timely implementation, intended objectives, and outcomes. Management review should include an assessment of the effectiveness of PACs management in the PQS.

Management review can be organized in a tiered structure that links the PQS with specific product/process reviews as appropriate. Performance indicators should be defined that allow management to understand the capability of the internally managed PAC process and the successful implementation of PACs. Management should decide which specific PAC-related performance indicators will be implemented, tracked, and acted upon by the company. Examples include:

1. KM: PACs initiated because of new knowledge.
2. QRM: Unacceptable risks introduced as a result of PACs, risk reduction because of PACs, health authorities that have accepted the company’s PQS for managing PACs.
3. PPPQMS: PACs related to preventive or continual improvement measures, recurring deviations, or adverse trends.
4. CAPA: PACs with unintended risk or consequence, CAPA effectiveness.
5. Change Management: PACs that did not meet intended objectives, adherence to PAC implementation timelines, or PAC effectiveness.
6. Management Review: review performance indicators for each PQS element, percentage of PACs covered in the PQS without requiring prior approval vs overall PACs, inspectional or internal audit findings related to PAC management.
7. Management Responsibilities: PQS effectiveness conclusion from management review, actual vs planned resources for PACs, timeliness of PAC implementation, survey assessment of quality culture/mindset, drug shortages.

The preceding are some examples. For several of these examples the company could report and discuss an actual number or percentages or both.

Management should be vigilant and aware of the cumulative impact of changes to a product over time.

Audit and internal inspection findings related to implemented PACs serve as an input to the review. Management should ensure that responses or actions related to any such findings are appropriate. If the objectives of
PAC initiatives are not achieved, effective management review ensures that formal CAPA action plans are developed and implemented, and that lessons learned are captured and incorporated into future PAC activities.

The management review should provide visibility of the status of in-progress PACs as well as any other PACs that are pending to evaluate any potential impact on product availability and ensure that a state of control is maintained.

Management review outputs and decisions should be documented. Continual improvement input should be driven by outputs of the management review process.

**Conclusion**

This 1VQ document describes how a company can leverage the PQS to effectively manage PACs through an enhanced science and risk-based approach. For each of the four quality system elements and the two enablers, it provides guidance to realize the opportunities outlined in ICH Q10 Annex 1, to manage more PACs within the PQS without increasing the risk to the patient and drug product QSE. Establishment of an effective PQS can achieve the objectives of realizing product, maintaining a state of control, and facilitating continual improvement.

The benefits of applying the principles described in this document are:

1. continual improvement with timely implementation of many PACs;
2. enhancing product availability and mitigating potential drug shortages;
3. focusing regulatory resources on PACs that may have a potential to impact product quality as it relates to safety and efficacy;
4. eliminating regulatory approvals for low-risk changes that can be handled by an effective PQS; and
5. faster implementation of innovative technologies.

Full implementation of this enhanced science and risk-based approach for managing PACs will require dialog and discussion with regulatory agencies and further changes to current national or regional regulations and guidance pertaining to managing PACs.

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Conflict of Interest Statement

The authors declare no conflict of interest related to the content of the article.

References


**PDA PAPER**

**Controls to Minimize Disruption of the Pharmaceutical Supply Chain During the COVID-19 Pandemic**

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**ABSTRACT:** This article reviews currently available scientific literature related to the epidemiology, infectivity, survival, and susceptibility to disinfectants of Coronavirus, in the context of the controls established to meet good manufacturing practice (GMP) regulations and guidance, and the public health guidance issued specifically to combat the COVID-19 pandemic. The possible impact of the COVID-19 pandemic on the pharmaceutical supply chain is assessed and recommendations are listed for risk mitigation steps to minimize supply disruption to pharmaceutical drug products. Areas addressed include a brief history of the COVID-19 viral pandemic, a description of the virus, the regulatory response to the pandemic, the screening of employees, the persistence of the virus on inanimate surfaces, cleaning and disinfection of manufacturing facilities, the use of GMP-mandated personal protective equipment to counter the spread of the disease, the role of air changes in viral clearance, and approaches to risk assessment and mitigation. Biological medicinal products have a great record of safety, yet the cell cultures used for production can be susceptible to viruses, and contamination events have occurred. Studies on SARS-CoV-2 for its ability to replicate in various mammalian cell lines used for biopharmaceutical manufacturing suggest that the virus poses a low risk and any contamination would be detected by currently used adventitious virus testing. The consequences of the potential virus exposure of manufacturing processes as well as the effectiveness of mitigation efforts are discussed. The pharmaceutical supply chain is complex, traversing many geographies and companies that range from large multinationals to mid- and small-size operations. This paper recommends practices that can be adopted by all companies, irrespective of their size, geographic location, or position in the supply chain.

**KEYWORDS:** COVID-19 pandemic, SARS-CoV-2 virus, Drug shortage, Supply chain, Employee screening, Risk assessment, Risk mitigation.

**Introduction**

During academic training, every microbiologist learns about pandemics and their impact on society. However, this bookish knowledge, although foundational, does not prepare one for all the potential contingencies and scenarios that a pandemic can present. As of writing this article, there is no approved drug to treat COVID-19 infection and a shortage of testing capabilities to identify the virus in people exhibiting symptoms of infection and to screen for viral antibodies to determine infected individuals who have recovered and might have immunity and return to the workplace. It should be noted that several health authorities have given emergency authorization for use of certain drugs and antibody tests. Further, because of the global increase in demand and the disruption in transportation, the COVID-19 pandemic has resulted in major shortage of medicines needed to treat symptoms of the disease, manage pain, or to prevent or control secondary infections. See the Food and Drug Administration (FDA) Drug Shortage website for additional information (1). In addition, the pandemic has created a shortage of supplies of disinfectants and sanitizers and personal protective equipment (PPE) such as masks and gowns needed to meet good manufacturing practice (GMP) standards and associated standard operating procedures (SOPs).

Over the last 20 years, the world has experienced outbreaks of major novel respiratory infectious diseases such as the SARS (Severe Acute Respiratory...
Syndrome) outbreak (2002), influenza H1N1 pandemic (2009), and MERS (Middle East Respiratory Syndrome) outbreak (2012), and now the COVID-19 pandemic (2020). Pandemics can disrupt pharmaceutical supply in multiple ways at a time when medicines and vaccines are critically needed to control the pandemic and treat medical conditions not related to respiratory illness in other patients. Unavailability of raw materials, manufacturing supplies, shutdown of transportation systems, and most importantly employee absenteeism owing to infections, suspected infections, or fear of infections can disrupt supply. The U.S. FDA published Guidance for Industry—Planning for the Effects of High Absenteeism to Ensure Availability of Medically Necessary Drug Products (2) in March 2011, that is, following the 2009 Influenza H1N1 pandemic. More recently, the Medicines and Healthcare products Regulatory Agency, United Kingdom (MHRA) (3) and the World Health Organization (WHO) (4) have published guidance on flexibilities in response to the COVID-19 pandemic. Since the last pandemic in 2009, there has been a massive global increase in the use of social media platforms as a source of information, which may or may not be accurate and can result in poor decisions that can impact the drug supply.

This article reviews currently available scientific information related to coronaviruses and disinfectants from peer-reviewed journals, authenticated sources such as the U.S. federal Centers for Disease Control and Prevention (CDC), FDA, WHO, major health authorities, and national and international GMP standards. Although the article is primarily directed toward pharmaceutical drug manufacturing, the content should be useful to all manufacturers of over-the-counter drug products, consumer health products, cosmetics, and medical devices. The question asked is how potential risks can be identified and mitigated to protect our manufacturing facilities, employees, drug products, and customers from this pandemic respiratory virus? It should be noted that the COVID-19 pandemic is fast moving, and a tremendous amount of new scientific knowledge is being created every day. The authors have endeavored to make recommendations based on an anticipated progression of the pandemic in general terms, although the effect of the pandemic locally and internationally may vary. In addition, local laws, regulations, and other requirements will inform each company’s specific response to the pandemic.

**Potential Risks That Can Result in Drug Shortages**

The potential risk associated with COVID-19 can be categorized into direct risk posed by the virus to employees and to product and indirect risk to manufacturing and distribution activities materialized by policies and controls promulgated by local, state, and national governments to control the pandemic (Figure 1).

To assess the direct risk posed by COVID-19, it is important to understand the epidemiology, infectivity, and susceptibility to disinfection.

**Epidemiology of the COVID-19 Pandemic and Related Outbreaks**

The 2019 novel coronavirus that was identified as the cause of an outbreak of respiratory illness is now referred to as the COVID-19 pandemic with the infectious viral agent designated as SARS-CoV-2. It was
first detected in Wuhan, China, on December 12, 2019 (5) and reported to the WHO China Country Office on December 31, 2019 (6). By mid-January, COVID-19 spread to Thailand, Japan, and Korea and then to Europe.

As the rapid spread of SARS-CoV-2 in the U.S.A. is well documented in the published literature, the authors have used the U.S. as the basis for discussion. The first documented U.S. case was a 34-year-old man who traveled to Wuhan, China to visit family, returned, and reported to an urgent care clinic in Snohomish County, Washington on January 19, 2020, in response to a health alert from the U.S. CDC (7). On March 11, 2020, the WHO declared Coronavirus Disease 2019 (COVID-19) a pandemic (WHO Statement, 2020) as it had spread to multiple countries with high prevalence of community transfer. On January 31, 2020, the U.S. federal Health and Human Service (HHS) issued a declaration of a public health emergency related to COVID-19 and mobilized the Operating Divisions of HHS (8). In addition, on March 13, 2020, the President of the United States declared a national emergency in response to COVID-19 (9).

By the end of March, there were 240,000 confirmed U.S. cases with over 7000 deaths, resulting in the declaration of a national emergency by the President of the United States, with orders for nonessential workers to work from home. By the end of April, there were over 1 million U.S. confirmed cases and 60,000 deaths. Pharmaceutical employees involved in production and testing activities were deemed essential globally; health authorities issued requests for pharmaceutical industry to work to avoid drug shortages.

Description of the SARS-CoV-2 Virus

Coronaviruses are lipid-enveloped, single-stranded, positive sense RNA viruses, with 26 to 32 kilobases, belonging to the genus Coronavirus, which includes several relatively benign, seasonal, common cold viruses and three new, more virulent coronaviruses: severe acute respiratory syndrome coronavirus (SARS-CoV), which emerged in the human population in 2003; Middle East Respiratory Syndrome coronavirus (MERS-CoV), which emerged in humans during 2012; and SARS-CoV-2 that emerged in late 2019 and became the COVID-19 pandemic in early 2020 (10–12).

Enveloped viruses like SARS-CoV-2 are highly susceptible to cleaning agents and disinfectants, above ambient temperature, and usually do not survive on inanimate surfaces beyond 2 days. This will be discussed in more detail in the section on persistence of SARS-CoV-2. The individual coronavirus particles are around 0.125 μm in diameter. N95 face masks are rated to capture 95% of particles down to 0.3 μm. This means that viral particles may still potentially get through this protection. The virus appears to be dispersed as larger droplets or aerosols and the multiple layers of the face masks largely mitigates this risk. In contrast, HEPA filters are 99.97% effective at 0.3 μm and thus are much more efficient than face masks.

Sources of the SARS-CoV-2 Virus

Zoonotic respiratory viruses initially emerge by animal-to-human and then largely by human-to-human transmission and to a lesser degree surface-to-human transmission. Dense human populations coexisting with dense chicken, duck, and pig populations as found in the People’s Republic of China, as well as the consumption of wild animals as food, favors the emergence of novel respiratory viruses (13, 14). There is little or no evidence that the coronavirus is either a foodborne or waterborne viral pathogen.

Foodborne viral pathogens are responsible for a larger number of illnesses than bacterial pathogens annually. They may be classified into three main groups of viral illnesses: viruses that cause gastroenteritis, for example, norovirus and rotavirus; enterically transmitted hepatitis viruses, for example, hepatitis A; and enteroviruses, for example, poliovirus (15). Notably foodborne viruses are not associated with respiratory infection.

According to a March 2020 WHO Interim Guidance (16), although SARS-CoV-2 persistence in drinking water is possible, there is evidence from surrogate human coronaviruses that they are not present in surface or ground water sources or transmitted through contaminated drinking water. The coronavirus, an enveloped virus with a fragile outer membrane, does not survive in the environment and would be susceptible to filtration and chlorine treatment before water distribution.

Because SARS-CoV-2 may be shed in fecal matter (up to 10% confirmed presence in diarrhea) with some
earlier reports of fecal-to-oral transmission (17, 18), this route of transmission should not be treated casually, and personnel hygiene should be emphasized in manufacturing facilities.

Public health experts have ruled out the possibility of insect-to-human transmission.

**Personnel Health and Safety**

The biggest risk to the community at large, and as such to the pharmaceutical supply chain, is human-to-human transmission of the coronavirus. Protecting the health and safety of employees should be the top priority of companies. The following points should be considered and as appropriate built into the pandemic response plans developed by individual companies.

**Social Distancing and Work from Home**

Certain local, state, and national governments and their health authorities have issued social distancing guidelines that may include a mandatory stay at home order with a caveat to exclude “essential employees,” that is, employees necessary to keep critical services and activities in operation. To our knowledge, all governments combating the pandemic have classified “pharmaceutical employees” as essential workers. As such, pharmaceutical companies generally have the liberty to determine what employees, if any, should work from home and who should report to work. The authors recommend companies develop a comprehensive contingency plan to identify essential activities and the employees needed to execute such activities. Support staff in quality assurance (QA), regulatory affairs, purchasing, human resources, research and development, planning, sales and marketing, and general management largely may work from home. Staff directly employed in manufacturing operations, quality control testing, engineering and maintenance, security, warehousing, and shipping must be on site and potentially expose each other, facilities, and products to viral contamination.

The employees identified as essential will depend on the manufacturing sites ability to conduct GMP activities remotely via a 21 CFR Part 11 compliant information technology (IT) system. For example, sites that have completely electronic batch records may not need to have a QA batch record reviewer on site, but those that have paper records or hybrid paper and paperless systems may need to include such an employee on the list of essential employees.

Furthermore, the determination of which employees are considered essential will depend, in part, on the level of community spread. For example, site auditors may not be considered essential if the virus threat level is high within the local community, but as the threat decreases, auditors may be included in the list of essential employees.

Employees working off-site, using communication tools like mobile telephones, e-mail, and videoconferencing, can conduct many support activities in the pharmaceutical industry. This will significantly reduce the numbers of employees on-site. In terms of the drug product supply chain, such employees may be viewed as nonessential, whereas those employees (operators, as well as managers) directly involved in product manufacturing, testing, and distribution are viewed as essential. However, this distinction is not clear-cut. For example, it may be possible to delay a supplier audit of a pharmaceutical ingredient used in the manufacture of an essential drug product, annual GMP training for packaging operators, or even marketed product stability testing. Determining what are nonessential activities and what are essential must be approached in a well-considered manner. The authors recommend that companies develop pandemic contingency plans that mandate essential activities and provide justification and timelines for the completion of activities that are delayed as nonessential. These plans should be approved by their quality control unit and may, importantly, be subject to regulatory review.

**Identification of Infected Employees**

As we believe that the biggest risk to the supply chain is person-to-person viral transmission followed by surface-to-person transmission, the most important risk mitigation will be excluding infected employees, especially those manufacturing, sampling, and testing drug products, from the pharmaceutical workplace to maintain the workforce and not potentially cause product contamination.

GMP regulations, for example 21 CFR 211.28 d states:

Any person shown at any time (either by medical examination or supervisory observation) to have an apparent illness or open lesions that
may adversely affect the safety or quality of drug products shall be excluded from direct contact with components, drug product containers, closures, in-process materials, and drug products until the condition is corrected or determined by competent medical personnel not to jeopardize the safety or quality of drug products. All personnel shall be instructed to report to supervisory personnel any health conditions that may have an adverse effect on drug products.

As such, pharmaceutical companies are required to have procedures in place and a training program for employees to self-report and supervisors to observe and detect illness.

The major symptoms of COVID-19 infection are dry cough, fever, and difficulty breathing, but asymptomatic sufferers may shed the virus (19). All employees who self-identify as sick must be encouraged to stay home and report their status to their immediate supervisor and seek medical help. Examples of high-risk factors include sick family members and friends, recent foreign and domestic travel, attendance at events with large crowds such as arena concerts and professional sport games, frequenting places of worship, schools, restaurants, social clubs, and bars, dwelling in high-density population cities and towns, and commuting using public transportation.

To maximize the possibility that employees will self-report potential illness, the authors recommend reviewing sick-leave policies for all employees, including temporary and contract workers. We also recommend that companies enhance general awareness about the symptoms of COVID-19 by reinforcing the need for self-reporting and supervisory vigilance.

Screening Pharmaceutical Employees at the Point of Entry to Manufacturing Facilities

Recent epidemiological studies of COVID-19 suggest that infected individuals may remain asymptomatic or pre-symptomatic for up to 2 weeks (21). Therefore, in addition to reinforcing GMP requirements to self-report illness, companies should consider instituting procedures to screen employees entering manufacturing facilities. Such procedures need to be developed in accordance with government-mandated requirements and employee rights to privacy, avoidance of discrimination, and respect for existing employee contracts and union agreements.

On April 23, 2020, the U.S. Equal Employment Opportunity Commission (EEOC) issued an update to its guidance that now expressly acknowledges that employers may test employees for COVID-19 and conduct temperature-screening measures without violating the provisions of the Americans with Disabilities Act (ADA) or the Rehabilitation Act (20). According to the EEOC, “an employer may choose to administer COVID-19 testing to employees before they enter the workplace to determine if they have the virus.” Further, as stated by the EEOC:

Consistent with the ADA standard, employers should ensure that the tests are accurate and reliable. For example, employers may review guidance from the U.S. Food and Drug Administration about what may or may not be considered safe and accurate testing, as well as guidance from CDC or other public health authorities, and check for updates. Employers may wish to consider the incidence of false-positives or false-negatives associated with a particular test. Finally, note that accurate testing only reveals if the virus is currently present; a negative test does not mean the employee will not acquire the virus later.

The EEOC guidance applies only with respect to the two specified federal statutes in the U.S. We recommend that companies in the U.S. also consider the applicability of relevant state and local law and consult legal counsel as needed before initiating a mandatory employee-testing program. Companies outside the U.S. must obviously consider applicable national and local laws.

Also, in the U.S., the federal Occupational Safety and Health Administration (OSHA) recommends that companies develop an Infectious Disease Preparedness and Response Plan (22). The OSHA updated recommendations state the following:

If one does not already exist, develop an infectious disease preparedness and response plan that can help guide protective actions against COVID-19. Stay abreast of guidance from federal, state, local, tribal, and/or territorial health agencies, and consider how to incorporate those recommendations and resources into workplace-specific plans. Plans should consider and address the level(s) of risk associated with various worksites and job tasks workers perform at those sites.
How can employees be screened? Depending on the nature of the workforce, and the confidence and trust management has in the workforce, companies should decide whether to institute mandatory testing and/or institute self-screening or screening during entrance into the plant site.

If screening is performed during entrance to your plant sites, the employees’ entry should be staggered to maintain social distancing and prevent delays. In addition to any mandatory COVID-19 testing that may be adopted, the authors believe in their expert opinion that the screening may consist of the following activities:

1. Monitor the body temperature of all employees. If self-monitoring is permitted, employees could check their body temperature using a personal clinical thermometer just prior to leaving for work. Employees should not report to work and should seek medical advice immediately if their body temperature is above 100.4°F/38°C. If screening is conducted at the plant entrance, a trained screener should use a calibrated handheld or wall-attached no-touch thermometer. Employees with a body temperature exceeding 100.4°F/38°C should be segregated and not allowed to enter the facility.

2. Conduct brief interviews of potentially SARS-CoV-2-infected employees to identify those with the common symptoms of fever, dry cough, and difficulties breathing.

3. Take a travel and contact history of a suspected infected employee for the past 14 days to determine the potential for contamination of the manufacturing facility and infection of other employees.

4. Segregate any potentially infected employees and encourage them to get medical care through their regular physician or neighborhood medical center. It would be useful to provide employees with healthcare contact information.

5. Obtain a commitment from the employee to report their medical status and results of any testing for the presence of the coronavirus when they update their sick leave status according to company policy.

6. Determine whether the infected employee’s coworkers within social distancing need to be quarantined from the workplace.

7. Determine the potential impact on the manufacturing operation as a result of employee absences.

8. When an employee is confirmed positive for the coronavirus, clean and disinfect the locations directly impacted in their workspace and other high-traffic areas like bathrooms, break rooms, hallways, and entrances.

9. Review of the frequency of employee exclusions by a local oversight committee to determine the need for self-quarantine of potentially infected employees, institute additional risk mitigations, and determine the potential impact to the manufacturing schedule.

The recommended screening listed as 1 through 6 will have recognizable limitations. Multiple screening methods will most likely be more effective than a single method. Clinical thermometers may be in short supply during a pandemic. Studies on the use of infrared thermal image scanners in influenza and COVID-19 airport screening (23–25) to identify infected travelers showed that, compared to virus testing, they might detect less than half of those infected because of the viral incubation period and asymptomatic individuals. Screening each day of entrance to the workplace as compared to a departure or arrival screening at an airport will increase its effectiveness. A recent publication on the presenting characteristics of 5700 patients hospitalized with COVID-19 in the New York City area (26) found that only 31% had an elevated temperature, 17% rapid breathing, and 43% rapid heart rate. No information on the incidence of body ache and coughing was provided. This variability in symptoms will make screening more challenging. Based on these findings, companies are cautioned not to place too much reliance on only temperature screening. Another report from a Northern California hospital system indicated that the chief symptoms in 377 adults when presenting in the emergency department were 49% shortness of breath, 34% fever, and 32% cough (27).

As testing for the SARS-CoV-2 virus to detect infected individuals and the antibody to detect individuals who have been infected and recovered becomes widespread, companies should consider offering testing on a voluntary basis as an effective tool for keeping their workforce. Based on a PDA membership survey (In press) apparently most employees would accept this offer of screening. The best method of managing this testing will become apparent as infectious disease experts gain
more experience managing the pandemic and the subsequent return to work.

**Facility and Process Management**

**Persistence of Coronavirus on Inanimate Surfaces**

Human viruses cannot multiply outside of the body and will not survive on inanimate surfaces for long. An analysis of 22 studies on the persistence of human coronaviruses other than SARS-CoV-2 virus (Table 1) reveals that they may persist on metal, glass, or plastic for up to 9 days (range 2 h to 9 days), but they are readily inactivated within 1 min by disinfectants and sporicides such as 62%–71% ethanol, 0.5% hydrogen peroxide, or 0.1% sodium hypochlorite (28).

The studies summarized in Table I have their technical limitations as they used related coronaviruses but not SARS-CoV-2, different types of inoculum preparations, high inoculum levels, different storage conditions, and reverse transcription polymerase chain reaction assays as a measure of survival and not infectious units determined by cell culture methods. However, until additional studies are conducted with the SARS-CoV-2 virus, these will be indicative and will contribute to our analysis.

Other agents used in the pharmaceutical industry, including the antiseptics 0.05%–0.2% benzalkonium chloride and 0.02% chlorhexidine digluconate, are less effective, requiring a contact time of up to 10 min (28). These findings will strictly limit the ability of novel coronaviruses to contaminate the pharmaceutical supply chain.

A more recent March 17, 2020, letter to the New England Journal of Medicine analyzed the aerosol and surface stability of SARS-CoV-2 (COVID-19) and

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**TABLE I**

Persistence of Coronaviruses on Inanimate Surfaces (28)

<table>
<thead>
<tr>
<th>Type of Surface</th>
<th>Virus(^a)</th>
<th>Inoculum (Viral Titer)</th>
<th>Persistence (Temperature)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steel</td>
<td>MERS-CoV</td>
<td>(10^5)</td>
<td>48 h (20°C)</td>
</tr>
<tr>
<td></td>
<td>HCoV</td>
<td>(10^3)</td>
<td>8–24 h (30°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 d (21°C)</td>
</tr>
<tr>
<td>Aluminum</td>
<td>HCoV</td>
<td>(10^3)</td>
<td>2–8 h (21°C)</td>
</tr>
<tr>
<td>Wood</td>
<td>SARS-CoV-1</td>
<td>(10^5)</td>
<td>4 d (RT)(^b)</td>
</tr>
<tr>
<td>Paper</td>
<td>SARS-CoV-1</td>
<td>(10^6)</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10^5)</td>
<td>3 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10^4)</td>
<td>&lt;5 min (All at RT)</td>
</tr>
<tr>
<td>Glass</td>
<td>SARS-CoV-1</td>
<td>(10^5)</td>
<td>4 d (RT)</td>
</tr>
<tr>
<td></td>
<td>HCoV</td>
<td>(10^3)</td>
<td>5 d (21°C)</td>
</tr>
<tr>
<td>Plastic</td>
<td>SARS-CoV-1</td>
<td>(10^5)</td>
<td>≤5 d (22–25°C)</td>
</tr>
<tr>
<td></td>
<td>MERS-CoV</td>
<td>(10^5)</td>
<td>48 h (20°C)</td>
</tr>
<tr>
<td></td>
<td>SARS-CoV-1</td>
<td>(10^7)</td>
<td>6–9 d (RT)</td>
</tr>
<tr>
<td></td>
<td>HCoV</td>
<td>(10^5)</td>
<td>4 d (RT)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10^7)</td>
<td>2–6 d (RT)</td>
</tr>
<tr>
<td>Polyvinylchloride</td>
<td>HCoV</td>
<td>(10^3)</td>
<td>5 d (21°C)</td>
</tr>
<tr>
<td>Silicon Rubber</td>
<td>HCoV</td>
<td>(10^3)</td>
<td>5 d (21°C)</td>
</tr>
<tr>
<td>Surgical Glove (Latex)</td>
<td>HCoV</td>
<td>(5 \times 10^3)</td>
<td>≤5 d (21°C)</td>
</tr>
<tr>
<td>Disposable Gown</td>
<td>SARS-CoV-1</td>
<td>(10^6)(10^3)(10^4)</td>
<td>2 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>≤8 h (All at RT)</td>
</tr>
<tr>
<td>Ceramic</td>
<td>HCoV</td>
<td>(10^3)</td>
<td>5 d (21°C)</td>
</tr>
<tr>
<td>Teflon</td>
<td>HCoV</td>
<td>(10^3)</td>
<td>5 d (21°C)</td>
</tr>
</tbody>
</table>

\(^a\)MERS is Middle East Respiratory Syndrome virus; HCoV is Human Coronavirus; SARS-CoV-1 is Severe Acute Respiratory Syndrome virus.

\(^b\)RT is room temperature.
compared this stability to that of SARS-CoV-1, its most closely related coronavirus (29). The authors of the letter reported 10 experimental conditions, conducted in triplicate, involving the two viruses in five environmental conditions, that is aerosols, inoculated plastic, stainless steel, copper, and cardboard. SARS-CoV-2 remained viable as measured by median tissue culture infectious dose for 50% of the cells to be infected (TCID\(_{50}\)) in the aerosol suspension for the 3 h duration of the experiment with a reduction in infectious titer from \(10^{3.5}\) to \(10^{2.7}\) TCID\(_{50}\)/mL, representing a half-life of 1.1–1.2 h. In a controlled environment with many air changes per hour, the virus would be readily removed from the air.

The frequency of cleaning and disinfection of an area in a GMP manufacturing facility will depend on the intensity of traffic in the area and the exposure of personnel to drug product manufacturing. This frequency may range from weekly, to daily, to before and after each shift. It should be emphasized that cleaning to remove grime and product residues prior to the application of disinfectants is critical for their best efficacy. Table II, although not exhaustive as the U. S. Environmental Protection Administration (EPA) List N (34) which contains 75 agents, provides useful information on representative commercially available products, their active ingredients, contact times, and antiviral claims.

Cleaning and disinfection are considered critical GMP processing steps especially in sterile product manufacturing subject to process validation (see 2004 FDA Aseptic Processing Guideline). Guidance on the qualification of individual disinfectants and sporicidal agents may be found in USP <1072> Disinfectants and Antiseptics (35). Media reports highlight the shortage of disinfectants and hand sanitizers. Under the current circumstance, it is the expert opinion of the authors that on an interim basis, alternate suppliers may be identified and a like-for-like substitution made, forgoing process validation and a vendor audit to make up for the shortage. Critical elements for making this like-for-like selection of an alternative source of a disinfectant include reputation of the supplier, active ingredient, active ingredient concentration, EPA and other national registration, efficacy claims, whether the disinfectant formulation is diluted before use or a ready-for-use product, and sterilization by gamma irradiation. To alleviate the shortage of hand sanitizers, the FDA has authorized the in-house production of alcohol hand sanitizers (36), but the authors believe that these materials should not be used in the critical ISO 5 aseptic processing areas.

The coronavirus was more stable on plastic and stainless steel than on copper and cardboard. Although the virus could be detected for up to 72 h, its titer was greatly reduced (from \(10^{3.7}\) to \(10^{0.6}\) TCID\(_{50}\)/mL after 72 h on plastic and from \(10^{3.7}\) to \(10^{0.6}\) TCID\(_{50}\)/mL after 48 h on stainless steel). On copper, apparently because of Cu\(^{2+}\) toxicity, no viable SARS-CoV-2 was measured after 4 h and on cardboard, no viable SARS-CoV-2 was measured after 8 h (29). This means, that in the event that controls established to exclude an infected employee fail, and in the event that packaged product is exposed to the virus, the plastic container used for primary packaging and the cardboard used for secondary packaging should not carry infectious coronavirus because of the amount of time the drug products will be advancing through the supply chain. Therefore, pharmaceutical products are very unlikely to pose any risk of infecting pharmacists dispensing, medical staff administering, and patients taking such products.

The relationship between temperature and relative humidity and the survival of coronaviruses in aerosols and on surfaces has been investigated. Enveloped viruses were found to survive longer at lower temperatures and humidity and may persist longer in refrigerators and cold rooms (30–32). The efficacy of pasteurization (63°C for 30 min) was demonstrated with MERS-CoV in camel, goat, and cow milk with the virus titer reduced from \(10^{5.5}\) to \(<10^{0.5}\) TCID\(_{50}\) (33). Clearly, the heat sensitive SARS-CoV-2 virus will not survive sterilization processes used in sterile product manufacturing.

**Inactivation of Viruses Owing to Routine Cleaning and Disinfection**

The coronavirus may be physically removed from a surface with a particle-free wipe, inactivated by detergents in cleaning agents, or inactivated by disinfectants and sporicides. In addition to routine cleaning and sanitization programs established to meet GMP requirements designed to protect product, companies should establish cleaning and sanitization of surfaces in non-GMP areas, including hallways, bathrooms, offices, and other common areas, to protect the health and safety of employees during the pandemic. The controls, qualification, and documentation requirements for GMP activities should be well-established and subject to change control and/or planned deviation. Such controls are not required for sanitization programs designed for the non-GMP areas.
<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Product Name</th>
<th>Company</th>
<th>Contact Time (Minutes)</th>
<th>Formulation Type</th>
<th>Emerging Antiviral Claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen Peroxide</td>
<td>Accel (Concentrate)</td>
<td>Virox Technology</td>
<td>5</td>
<td>Dilutable</td>
<td>Yes</td>
</tr>
<tr>
<td>Phenolic</td>
<td>LpH</td>
<td>Steris</td>
<td>10</td>
<td>Dilutable</td>
<td>Yes</td>
</tr>
<tr>
<td>Phenolic</td>
<td>VespheNe II se</td>
<td>Steris</td>
<td>10</td>
<td>Dilutable</td>
<td>Yes</td>
</tr>
<tr>
<td>Sodium Hypochlorite</td>
<td>CloroxPro™ Clorox® Germicidal Bleach</td>
<td>Clorox Professional Product Co.</td>
<td>5</td>
<td>Dilutable</td>
<td>Yes</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>DECON-AHOL WFI® Formula 70%</td>
<td>Veltek Associates</td>
<td>NA</td>
<td>RTU</td>
<td>No</td>
</tr>
<tr>
<td>Hydrogen Peroxide and Peracetic Acid</td>
<td>DECON-SPORE 200® Plus</td>
<td>Veltek Associates</td>
<td>NA</td>
<td>RTU</td>
<td>No</td>
</tr>
<tr>
<td>Quaternary Ammonium;Ethanol</td>
<td>Lysol® Disinfectant Spray</td>
<td>ReckitBenckiser LLC</td>
<td>5</td>
<td>RTU</td>
<td>Yes</td>
</tr>
<tr>
<td>Hydrogen Peroxide</td>
<td>Oxy-1 Wipes</td>
<td>Vorox Technology</td>
<td>0.5</td>
<td>Wipes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ethanol</td>
<td>PURELL Professional Disinfectant Wipes</td>
<td>GOJO Industries</td>
<td>5</td>
<td>Wipes</td>
<td>Yes</td>
</tr>
<tr>
<td>Sodium Hypochlorite</td>
<td>Clorox Healthcare® Bleach Germicidal Wipes</td>
<td>Clorox Professional Products Co.</td>
<td>3</td>
<td>Wipes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Viral Clearance by HVAC Systems and HEPA Filters in Clean Rooms

Warehouses, offices, laboratories, and manufacturing and packaging areas in a pharmaceutical manufacturing plant will be served by heating, ventilating, and air conditioning (HVAC) systems to maintain targeted temperature, humidity, and numbers of air changes appropriate for each of these areas. In addition, clean rooms and other controlled areas where sterile drug products are manufactured are supplied with high-efficiency particulate air (HEPA)-filtered air to meet specified air cleanliness levels as well as more stringent requirements for temperature, relative humidity, space pressurization, and number of air changes per hour to prevent product contamination.

In general, the level of environmental control, the PPE worn by clean room operators, and the cleaning and disinfection program will make it unlikely that the coronavirus will persist in clean rooms and contaminate sterile drug products (37). This leaves questions around areas served solely by conventional HVAC systems without HEPA filtration.

The 2014 American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) Position Document (38) highlights that some infectious diseases including those caused by coronaviruses are transmitted through the inhalation of airborne infectious particles, which can be disseminated through buildings by pathways that include ventilation systems. This transmission may be reduced using dilution ventilation, directional airflow, room pressure differentials, source capture ventilation, air filtration, and ultraviolet germicidal irradiation (UVGI), as well as appropriate cleaning and disinfection practices.

The ASHRAE document addresses control strategies (38). In a practical application, a combination of the individual interventions will be more effective than a single intervention in isolation. It is recommended that a heating and air-conditioning engineer be consulted on the implementation of these strategies. Pharmaceutical companies may consider improving particle filtration for the central air handler, adding upper-room UVGI units, increasing the outdoor ventilation rates, and avoiding the use of a lower ventilation rate motivated solely by reduced energy consumption.

Small aerosolized particles, <10 μm, generated by talking, coughing, or sneezing will be suspended in the air and transported into the lower respiratory tract during breathing whereas larger drops, 10–25 μm, will fall through the air and accumulate on horizontal surfaces (39). Aerosols may be transported some distance by sideways airflows in non-classified rooms, whereas vertical laminar airflow with floor-level exit registers will sweep the air clean in classified clean rooms. Table III provides information on the number of air changes per hour.

We believe that pharmaceutical manufacturing conducted in classified areas (ISO 8 to ISO 5) will provide environmental conditions that will adequately clear viral particles potentially shed by employees. Nonclassified areas where non-sterile drug products are manufactured and all packaging and labeling areas will need to be assessed for the number of air changes (ventilation rate) to facilitate viral clearance and for their cleaning and disinfection practices. Based on this risk assessment, changes may be necessary.

Hand Sanitization

Hand washing and sanitization are an essential component of GMP controls designed to protect product. In addition to these routine controls, additional hand sanitization stations should be established in non-GMP areas to mitigate risk to employees.

### Table III

<table>
<thead>
<tr>
<th>Class of Clean Room</th>
<th>Airflow Type</th>
<th>Average Velocity (Ft./Min.)</th>
<th>Air Changes/hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISO 8 (Class 100,000)</td>
<td>N/M</td>
<td>1–8</td>
<td>5–48</td>
</tr>
<tr>
<td>ISO 7 (Class 10,000)</td>
<td>N/M</td>
<td>10–15</td>
<td>60–90</td>
</tr>
<tr>
<td>ISO 6 (Class 1,000)</td>
<td>N/M</td>
<td>25–40</td>
<td>150–240</td>
</tr>
<tr>
<td>ISO 5 (Class 100)</td>
<td>U/N/M</td>
<td>40–80</td>
<td>240–480</td>
</tr>
</tbody>
</table>

N is Non-unidirectional; M is Mixed Airflow; U is Unidirectional. Note: 10 ft/min equals 3.048 m/min.
TABLE IV
Appropriate Personal Protective Equipment for Routine Pharmaceutical Manufacturing (40)

<table>
<thead>
<tr>
<th>Protective Clothing</th>
<th>Non-Sterile Manufacturing Areas</th>
<th>Sterile Manufacturing Areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant uniform or plant uniform with overalls for high-risk product and environment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hair/beard coverings</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Safety glasses</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Dedicated shoes or shoe coverings</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Gloves</td>
<td>Yes (if in direct product contact)</td>
<td>Yes</td>
</tr>
<tr>
<td>Face masks</td>
<td>Yes (if in direct product contact)</td>
<td>Only if manufacturing high-potency, toxic drugs or infectious biological agents</td>
</tr>
<tr>
<td>Enclosed respirators</td>
<td>Only if manufacturing high-potency, toxic drugs or infectious biological agents</td>
<td>Only if manufacturing high-potency, toxic drugs or infectious biological agents</td>
</tr>
<tr>
<td>Sterile clean room uniforms (coveralls), hoods, sleeves, goggles, face masks, and gloves</td>
<td>No</td>
<td>Yes, if in critical aseptic processing area</td>
</tr>
</tbody>
</table>

Personal Protective Equipment

As COVID-19 infected individuals cannot be absolutely excluded from our manufacturing sites, PPE will become more important. A decision should be made whether the workplace wearing of face masks would be mandatory or limited to those activities in which personnel have direct contact with drug products. Table IV addresses the appropriate PPE to be worn during non-sterile and sterile drug product manufacturing.

The efficacy of different grades of face masks in entrapping COVID-19 viral particles has not been fully established. The level of exposure to coworkers, processing equipment, and pharmaceutical drug products may determine the choice from homemade face masks to cone masks, to medical face masks to N95 grade face masks (respirators).

The specifications for different grades of face masks obtained from commercial supply catalogs may include:

2. Bacterial Filtration: >92%, >95%, or >99% @ 1.0 μm particle size retention
3. Reduced Particle Generation: Layers ultrasonically welded to limit particle shedding
4. Effective Pore Size: 1.0, 0.45, or 0.1 μm
5. Usage: Disposable, single use, or re-usage
6. Sterility: Non-sterile or sterile

FDA Regulation of Face Masks as Medical Devices

The FDA regulates face masks and respirators when they meet the definition of a device under section 201 (h) of the federal Food, Drug, and Cosmetic Act (FD&C Act). Generally, face masks fall within this definition when they are intended for a medical purpose, including for use by health care professionals (41). These classifications are useful in determining which devices should be used in a pharmaceutical manufacturing facility.

The FDA defines these devices that may be suitable for use in a GMP manufacturing facility as follows:

Face Mask—A mask, with or without a face shield, that covers the user’s nose and mouth and may or may not meet fluid barrier or filtration efficiency levels.

Surgical Mask—A mask that covers the user’s nose and mouth and provides a physical barrier to fluids and particulate materials. The mask meets certain fluid barrier protection standards and Class I or Class II flammability tests.
N95 Respirator—A disposable half-mask filtering face piece respirator (FFR) that covers the user’s airway (nose and mouth) and offers protection from particulate materials at an N95 filtration efficiency level per 42 CFR 84.181. Such an N95 FFR used in a health care setting is regulated by the FDA under 21 CFR 878.4040 (FDA product code MSH) and is either a Class II device that is exempt from premarket notification requirements under section 510(k) of the FD&C Act or is a Class II cleared device.

NIOSH Approved N95 Respirator—An N95 respirator, approved by National Institute for Occupational Safety and Health, that meets filtration efficiency level per 42 CFR 84.181.

The following are devices suitable for use in a clinical setting when medical staff treat COVID-19-infected patients but not in pharmaceutical manufacturing:

Face Shield—A face shield is a device used to protect the user’s eyes and face from bodily fluids, liquid splashes, or potentially infectious materials. Generally, a face shield is situated at the crown of the head and is constructed with plastic to cover the user’s eyes and face.

Filtering Face Piece Respirator—A filtering face piece respirator (FFR) is a device that is a disposable half-face-piece nonpowered air-purifying particulate respirator intended for use to cover the nose and mouth of the wearer to help reduce wearer exposure to pathogenic biological airborne particulates.

Surgical N95 Respirator—A disposable FFR used in a health care setting that is worn by health care personnel (HCP) during procedures to protect both the patient and the HCP from the transfer of microorganisms, body fluids, and particulate material at an N95 filtration efficiency level per 42 CFR 84.181. A surgical N95 respirator is regulated by the FDA under 21 CFR 878.4040 (FDA product code MSH) and is either a Class II device that is exempt from premarket notification requirements under section 510(k) of the FD&C Act or is a Class II cleared device.

Given the CDC recommendation that in addition to social distancing, face masks should be worn when people leave their places of residence, pharmaceutical companies should require employees reporting to work to be wearing face masks and should supply nonmedical masks to be worn on company premises until this recommendation is lifted. These face masks would be replaced at least twice a day and disposed as potential biohazard waste.

In manufacturing areas where pharmaceutical ingredients, packaging components, intermediates, and finished products are exposed to workers, the PPE recommendations found in Table IV would be strictly followed.

Pharmaceutical companies as high-volume users of face masks may employ strategies to conserve these devices when they are in short supply. These include allowing employees to use homemade cloth face masks to enter their facilities and continue to wear them in office areas, allowing the removal of face masks in isolated offices that maintain strict social distancing, and, when strictly necessary, the reuse of surgical masks after decontamination. On April 9, 2020, the FDA issued an Emergency Use Authorization (EUA) for the emergency use of a Steris Corporation vapor-phase hydrogen peroxide sterilization system for decontaminating compatible N95 respirators for reuse by medical personnel to prevent potential exposure to pathogenic airborne particulates when the respirators are in short supply because of the COVID-19 pandemic. The data submitted by Steris supported up to 10 sterilizations and reuse. It was noted that cellulose-based face masks are incompatible with hydrogen peroxide (42).

Vaccines against the SARS-CoV-2 Virus

The availability of safe and effective vaccines would help relax employee screening, social distancing practices, and the use of PPE in non-GMP areas. More than 40 different vaccines are in development globally with at least two now in Phase I human safety trials as of April 22, 2020. Vaccine clinical development is a complex process, which requires large clinical trials and establishing the long-term safety of the vaccine. The history of vaccine development is replete with examples of safety signals emerging post approval as a result of unexpected immunological response post immunization, for example, Rotashield and Dengvaxia. Further, it should be noted that there is no approved vaccine for SARS-COV-1 and for MERS, outbreaks that occurred in 2002 and 2012, respectively. Because of the widespread disease, recruiting large number of subjects for clinical trials is not expected to be a hurdle for COVID-19 vaccines, but this may change as the pandemic recedes. Despite all-out efforts, Dr. Anthony Fauci, Director of the U.S. National Institute of Allergy
and Infectious Diseases, predicts that the availability of a vaccine will take a year to a year and a half, at least (43).

**Regulatory Responses**

*Regulatory Response to the COVID-19 Outbreak*

On February 11, 2020, when the WHO formalized the name of the current outbreak as COVID-19, the FDA immediately added this official disease on their website. This new FDA Webpage will soon fill up with a wide range of guidance documents and directives in a very short time. This COVID-19 outbreak has generated an unprecedented response from the FDA and other U.S. regulatory agencies to remove many regulatory hurdles that were hindering the Industry response efforts to monitor and treat this pandemic. These dramatic changes from the global compliance norm reflect the seriousness of this viral threat to our medical supply industry along with the safety of those who work in these industries and those who may be patients in need of these pharmaceutical medical products. Among these U.S. federal agencies are the FDA, the CDC, OSHA, and the NIH. Their general contribution to the mitigation and relief of regulatory formalities will be briefly described following. In a February 14, 2020, press release, the FDA announced that “if a potential shortage or disruption of medical products is identified by the FDA, we will use all available tools to react swiftly and mitigate the impact to U.S. patients and health care professionals”. At this time period, the FDA press releases were focused on the impact of medicine and product coming from China owing to the COVID-19 outbreak that was occurring in that area of the world. It is very unlikely at that time that the pharmaceutical industry or regulators knew how significantly the subsequent global pandemic would impact their routine activities. Subsequently, there has been a significant list of FDA initiatives to minimize the shortage of essential medicine within the U.S. because of active pharmaceutical ingredient (API) unavailability for the manufacturing of the finished products (FDA announcement date February 27, 2020). Included in this list are the following guidance documents and directives and their date of issuance:

1. Critical human drug shortages can be mitigated with lengthening the expiration dates. (FDA, February 27, 2020).

2. A new policy for certain laboratories that develop and begin to use validated COVID-19 diagnostics before the FDA has completed review of their EUA request (FDA, February 29, 2020).

3. The FDA and Federal Trade Commission (FTC) issued seven warning letters to companies for selling fraudulent COVID-19 products. The products cited in these warning letters are teas, essential oils, tinctures, and colloidal silver. (FDA, March 9, 2020).

4. Coronavirus (COVID-19) Update: The FDA issues Guidance for Conducting Clinical Trials. The FDA is aware that protocol modifications may be required, and that there may be unavoidable protocol deviations owing to COVID-19. This would eventually include expedited early vaccine trial for the development of a COVID-19 vaccine (FDA, March 18, 2020).

5. The FDA and National Institutes of Health (NIH) have begun a randomized controlled trial for the treatment of COVID-19 patients with the investigational antiviral drug Remdesivir; interleukin-6 receptor inhibitors; as well as the application of convalescent plasma and hyperimmune globulin, antibody-rich blood products that are taken from blood donated by people who recovered from the virus infection (FDA, March 19, 2020).


7. The FDA provides maximum flexibility to importers seeking to bring PPE into the U.S. with minimal disruptions during the importing process. The agency provided instructions to manufacturers on how to inform the U.S. Customs and Border Protection with specific advisement to expedite regulatory clearance. (FDA, March 24, 2020).

8. The FDA issues an EUA to allow for the emergency use in health care settings of certain ventilators, anesthesia gas machines modified for use as ventilators, and positive pressure breathing devices modified for use as ventilators (FDA, March 27, 2020).
9. The FDA establishes a new program to expedite the development of potentially safe and effective life-saving treatments. The program is known as the Coronavirus Treatment Acceleration Program (CTAP). This public-private approach is cutting red tape, redeploying FDA staff, and working day and night to review requests from companies, scientists, and doctors who are working toward therapies. (March 31, 2020).

10. The FDA issued a new EUA for non-NIOSH-approved respirators made in China, which makes KN95 respirators eligible for authorization if certain criteria are met. (FDA, April 3, 2020).

The authors of this review applaud the FDA in providing a more flexible regulatory response to the pandemic.

However, caution should be mentioned as to the temporary nature of these allowable regulatory shortcuts during this pandemic, and the return to standard practice should be documented to prevent any regulatory citations made by health authority inspections as the urgency of this historic event diminishes over time.

European Union Regulatory Expectations during the COVID-19 Pandemic

As with the FDA in the United States, we are seeing a strong regulatory response to the COVID-19 pandemic from other regions of the world (43). For example, the combined organizations of the European Commission (EC), Heads of Medicines Agencies (HMA), and the European Medicines Agency (EMA) have published a Notice to their Stakeholders. The document is entitled “Questions and Answers on Regulatory Expectations for Medicinal Products for Human Use During the COVID-19 Pandemic” (EU Q&A document, April 2020). We will not discuss the entire Q&A in this review article, but we will highlight some key responses that the European Union (EU) expressed in their handling of deviations in manufacturing and importation of finished products and API as they relate to GMP and good documentation practice (GDP) issues during this pandemic.

Among some of the temporary changes include: (1) measures should be put in place to ensure the validity of GMP certificates that support manufacture and importation of medicinal products into the EU should be extended to avoid disruptions in the availability of medicines, with a liberal time extension to sites located inside the EU; (2) with the difficulty to perform on-site GDP inspections, the validity of GDP certificates will be extended until the end of 2021 with no further company action required; (3) remote batch certification and remote audits of API manufacturers have been expanded, even for those EU companies previously disallowed from this process; and (4) in case of imports of investigational medicinal products from outside of the EU, the companies quality department should ensure that the quality of the batch is in accordance with the terms of the clinical trial authorization and meets EU GMP requirements. The EMA recommended to make this assessment remotely; the companies need to review documents including batch records, in-process test reports, validation status of facilities, the results of any analyses performed after importation, stability reports, storage and shipping conditions, and so forth. Most gratifying was the response to the question can quality requirements be waived/adapted for medicines intended to be used for the treatment of COVID-19 patients? The short answer to this question was “No” but with the EMA offer that if manufacturers were having difficulties performing the compliance quality control steps, they were invited to contact the competent authorities and “to present an adapted control scheme based on a risk-based approach”. There was additional information to help navigate the regulatory hurdles posed by the restrictive travel conditions during this pandemic, so a review of the entire document is suggested for those manufacturing and conducting business in the EU geographic areas.

Overall Risk Assessment

Risk Analysis Tools

A number of risk analysis tools, including quality risk management, may be used when assessing risk factors. Tables S-I to S-VI (see Appendix) show an example of a hazard analysis and critical control points (HACCP) program approach (originating in the food industry), which summarizes the common inputs, identifies the various risks with ratings from low to high, and suggests common risk mitigations or critical process control points. Risk assessment of the specific steps in the supply chain for a representative non-sterile and sterile drug product, that is, activity, risk level, critical control point, and mitigation are provided (44).

Steps analyzed included staff recruitment, procurement of pharmaceutical ingredients and packaging components, facility design and operation, cleaning and
disinfection, utilities, manufacturing processes, packaging and labeling, warehousing, shipment, dispensing, and patient usage.

Drug Shortages

A major responsibility of the pharmaceutical industry and regulators is to anticipate and meet the changed demands for drug products driven by patient treatment and disruption to our supply chain. This statement appeared recently on the FDA website https://www.fda.gov/drugs/drug-safety-and-availability/guidance-notifying-fda-permanent-discontinuance-or-interruption-manufacturing-under-section-506c-fdc.

“Due to the COVID-19 pandemic, FDA has been closely monitoring the medical product supply chain with the expectation that it may be impacted by the COVID-19 outbreak, potentially leading to supply disruptions or shortages of drug and biological products in the U.S. The guidance, Notifying FDA of a Permanent Discontinuance or Interruption in Manufacturing Under Section 506C of the FD&C Act, is intended to help applicants and manufacturers provide the agency with timely and informative notifications about changes in the production of certain drugs and biological products. In urging the submission of these notifications, the guidance may assist in our efforts to prevent or mitigate shortages of such products, including under circumstances outside of the COVID-19 public health emergency.”

Many pharmaceutical drug products have the potential to be in short supply because of increased demand to treat hospitalized COVID-19 patients. Shifting production schedules to meet this increasing demand will help but may create backorders for other needed drugs. The disruption to the supply chain because of absenteeism of production and testing personnel, warehousing and shipping of packaging components, pharmaceutical ingredients and finished products, and imposition of national trade barriers to the free distribution of pharmaceuticals are all serious concerns.

Shortages have been reported for drugs that are used to keep patients’ airways open, as well as antibiotics, antivirals, and sedatives (45–47). In March 2020, orders for broad-spectrum antibiotics like azithromycin and antivirals like ribavirin have tripled; medicines for sedation and pain management like fentanyl, midazolam, and propofol have increased by 100%, 70%, and 60%, respectively.

Risk Assessment

The authors believe that as SARS-CoV-2 is a communicable human respiratory virus, the largest risk to the supply chain is absenteeism among line employees preventing the manufacture, testing, and distribution of drug products and not product contamination.

There are gaps in our knowledge of the epidemiology of the COVID-19 pandemic around identifying at-risk populations and the role of antibodies in preventing repeat infection that when filled will help manage our workforce (48).

The cell culture-based manufacturing processes of biological medicinal products can, and has in several instances, been infected by viruses (49). The susceptibility of the currently used manufacturing platforms, such as cell lines CHO, HT1080, and HEK 293 for the new SARS-CoV-2 has already been tested though, and the cell lines were found nonpermissive, that is do not support viral growth, to this new virus (Kreil, 2020 Personal Communication). See Table V for a summary of the lack of capacity of the coronavirus to grow in commonly used cell lines.

The detectability of the new SARS-CoV-2 has also been tested, and the cell line panels used in standard in vitro adventitious virus testing as required by regulatory guidance (ICH Q5A (R1)) were found capable of revealing virus presence (Kreil, 2020 Personal Communication). As might have been expected from experience with the earlier SARS-CoV, the Vero cell line was highly susceptible to infection, which was easily visible by development of a cytopathic effect (49).

The three main risks for viral contamination in cell culture for therapeutic production are the cell source, materials used in cell culture, and exposure of the process stream to the operators or environment with viral clearance, that is, inactivation or removal from the product, being most important in reducing the risk of virus contamination of the finished product. The reader is referred to the Consortium on Adventitious Agent Contamination in Biomanufacturing (CAACB) study for more details on risk mitigation (49).

Discussion

The objective of our discussion will primarily focus on the unique current conditions and problems associated
with the controls to minimize disruption of the pharmaceutical supply chain and to highlight other factors that we may not have had the available information to include in this review publication. Future data and experiences will eventually fill in the gaps of our current understanding and control of the COVID-19 pandemic.

The 2020 COVID-19 global pandemic meets all the characteristics of “A Black Swan”, what the best-selling author Nassim Nicholas Talab defined as an event with low probability, extreme impact, unforeseen, and retrospective predictability (51). Dr. Talab, a renowned Professor of Risk and Decision Science, proposed the Black Swan theory to explain highly improbable events, and the bias in decision-making introduced by past experience and by pockets of knowledge not available to all decision makers.

**Low Probability Events**

As per the WHO, in 2016 lower respiratory infections were the deadliest communicable disease, causing 3 million deaths worldwide, and were the fourth overall on the top 10 global causes of death (52). As per the CDC, in 2017 influenza and pneumonia were the leading cause of communicable disease, causing 55,672 deaths in the U.S., and were the eighth overall on the top 10 causes of death (53). Although scientists understood the possibility of another novel, highly contagious, and deadly respiratory virus outbreak, past experience gained in managing SARS-CoV-1, H1N1 influenza, and MERS outbreaks that had limited spread may have introduced a bias among some scientists, policy makers, and the public at large in assessing probability and underestimating the rapid spread of the COVID-19 outbreak into a pandemic.

**Extreme Impact Events**

The medical impact of a pandemic has been well studied by scientists; however, the impact of mitigation strategies such as social distancing, and the impact of a global end to transportation and the resulting economic activity was perhaps not well understood and anticipated. As such, the scientific, medical, business, and legal communities had to scramble to find quick solutions and remedies to both the direct and indirect deleterious impact from this pandemic. The essential nature of the pharmaceutical industry to combat pandemics and its higher level of knowledge and awareness, regulatory requirement to have business continuity plans, regulatory relief, and GMP controls has resulted in relatively less impact on the manufacturing activities of pharmaceutical companies as compared to many other manufacturing and service industries.

**Unforeseen Events**

The complexity of the supply chain, the increased demand for PPE, and the inability of essential employees to commute to work during stay-at-home orders were unforeseen domino effects. When national catastrophes (i.e., tornadoes, hurricanes, forest fires) occur in certain regions of a country there are generally local, state, or federal contingency plans in place to mitigate or coordinate the multiresponse to that effected area. Pandemics being relatively rare events, and each having unique characteristics and speed of spread, can result in many unanticipated challenges.

### TABLE V
Capacity of SARS-CoV-2 to Replicate in Primate and Human Cell Lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Cell Line Origin</th>
<th>SARS-CoV Replication</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>Chinese Hamster Ovary</td>
<td>Incompatible</td>
<td>(Kreil, 2020 Personal Communication)</td>
</tr>
<tr>
<td>HEK293</td>
<td>Human Embryonic Cells</td>
<td>Incompatible</td>
<td>(Kreil, 2020 Personal Communication)</td>
</tr>
<tr>
<td>HT1080</td>
<td>Human fibrosarcoma</td>
<td>Incompatible</td>
<td>(Kreil, 2020 Personal Communication)</td>
</tr>
<tr>
<td>A549</td>
<td>Human Adenocarcinoma Cells</td>
<td>Incompatible</td>
<td>50</td>
</tr>
<tr>
<td>HUH</td>
<td>Human Liver Cells</td>
<td>Moderate</td>
<td>50</td>
</tr>
<tr>
<td>HEK293T</td>
<td>Human Embryonic Cells</td>
<td>Moderate</td>
<td>50</td>
</tr>
<tr>
<td>VERO</td>
<td>African Green Monkey Kidney Cells</td>
<td>High</td>
<td>50</td>
</tr>
</tbody>
</table>

*Incompatible is no growth, no cytopathic effect (CPE); Moderate is growth but no CPE; High is growth and CPE.
Retrospective Predictability

As the pandemic progresses, it is easy to connect the dots and conclude that the pandemic was predictable and that different decisions were required. The fast spread of a novel virus requires decision-making on limited information and decisions need to be reevaluated as new information becomes available. There needs to be a deliberate and educated infectious disease preparedness and response plan built into business continuity plans for the next inevitable pandemic.

Limited Viral Characterization and Transmission Factors

One of our knowledge gaps as it relates to this infectious virus is our inability to identify asymptomatic infected individuals and exclude them from the pharmaceutical workplace without widespread testing for the virus and its antibodies. This would be followed by the problem with a lack of follow-up contact investigations. Lastly, a more definitive assessment of the efficacy of PPE, especially face masks, against the SARS-CoV-2 virus is needed.

Current Industry Manufacturing Practices That Are Low Risk

In general, because of the reduced ability of the lipid-enveloped virus to survive (but not proliferate) outside the human body, clean room environmental controls, cleaning and disinfection programs, and the PPE employed in the pharmaceutical industry are adequate to prevent COVID-19 viral contamination of our sterile products manufactured in GMP-compliant facilities and do not need to be changed. Our review includes recent information that the COVID-19 virus does not grow in the conventional manufacturing cell lines used for the proliferation and production of biologically based pharmaceutical products (Kriel, 2020 Personal Communication).

Risk Mitigation Steps That May Need Reevaluation

Areas that may need a closer risk assessment may be environmental conditions and controls in non-sterile product manufacturing rooms and all product labeling and packaging areas. The engineering and operational standards of the HVAC systems supplying these workspaces should be reviewed and, if necessary, improved. Parameters that may need to be assessed may include the number of air changes per hour, cleaning and disinfection programs, and the PPE worn in these areas. Because of the low risk of viral contamination of our GMP-controlled pharmaceutical equipment and manufacturing rooms, no product testing for the presence of the COVID-19 virus is recommended. Until COVID-19 is better understood, employees holding staff positions should work from home. Protocols for the eventual integration of the total work force back to pre-COVID-19 activity need to be written and reviewed, which should include medical and legal staff to allow for the gradual and specific viral monitoring with a cognizant determination for those who are at highest risk to this virus. The reliability of the medical platform for making these determinations should be assessed. For example, the benefit of measurable antibody blood titers to the COVID-19 virus may not be a 100% reliable factor for preventing reinfection by this virus.

Critical Risk Mitigation Steps That Should Be Evaluated

Pharmaceutical companies must aggressively screen their employees for COVID-19 infection and remove those infected, in a timely manner, from the workplace. These actions are necessary to avoid absenteeism because of continuing infection or reinfection of critical employees and the general work staff and a loss of employees to manufacture, test, and distribute essential drug products. A more definitive list of the risk mitigation steps recommended by the authors will be presented in the next section.

Conclusions

The authors, from their point-of-view as microbiologists, have attempted to review the risks and recommend mitigation steps that pharmaceutical companies, depending on their circumstances, should consider implementing in their manufacturing facilities.

The PDA has established a COVID-19 task force with broader representation of the disciplines within our membership that expand on areas over and above this review.

Recommendations

The following risk mitigation steps to minimize the impact of COVID-19 on the pharmaceutical supply
chain based on the risk classification in Figure 1 are recommended.

Direct Risks Posed by the COVID-19 Pandemic

Personal Health and Safety

1. To facilitate social distancing, employees able to do their job from home should be allowed to do so while employees directly involved in the manufacture, testing, packaging, and distribution of pharmaceutical product should be screened for potential COVID-19 infection and if suspected to be infected should be excluded from the workplace.

2. Nonessential visitors should be denied entry to all manufacturing facilities.

3. Additional hand sanitization stations should be installed in non-GMP areas.

4. Wearing of face masks by all employees in non-GMP areas should be considered after careful evaluations of the risks and benefits and would not be governed by GMP procedures.

5. If a safe and efficacious COVID-19 vaccine is available, it should be made widely available to the manufacturing employees of pharmaceutical companies.

Product Quality

1. Meeting GMP requirements related to PPE and excluding at risk employees from manufacturing activities will provide adequate assurance.

2. Testing for the presence of COVID-19 in manufacturing facilities and products is not recommended.

3. Pharmaceutical GMPs should be strictly maintained.

4. Determine if mammalian cell lines used for biopharmaceutical production are not susceptible to the COVID-19 virus.

5. Pharmaceutical companies are encouraged to actively monitor recommendations from the U.S. CDC an FDA, the EMA, and the WHO and make changes to their policies and procedures as the COVID-19 pandemic recedes.

GMP Manufacturing

1. Environmental controls, the appropriate use of PPE, and cleaning and disinfectant practices, especially in warehouses, non-sterile manufacturing areas, and packaging lines should be reviewed and updated if necessary.

2. Manufacturing and testing schedules can be adjusted and additional shifts added to facilitate social distancing and to ensure essential drug products are not in short supply.

Availability of Supplies

1. Strategies for conserving face masks and other PPE should be implemented.

2. Enhance collaboration with suppliers, educating them about their importance to the pharmaceutical industry.

Indirect Risks Posed by the COVID-19 Pandemic

Availability of Employees

1. Repurpose employees for critical activities.

2. Supply alternative transportation for employees who commute to work using public transportation.

Transportation Infrastructure

1. Distribute finished goods using dedicated transportation in place of common carriers.

Regarding the availability of raw materials, for example, drug substances, excipients, solvents, processing supplies, and packaging materials:

1. Strengthen existing supplier relations.

2. Seek alternative suppliers.

Acknowledgments

The authors wish to thank T. Cosgrove, Esq., and A. Caruso for their assistance in writing the article, and the members of the PDA COVID-19 Task Force for their review and helpful comments.
Disclaimer

The opinions and suggestions made by the authors in this manuscript do not necessarily reflect the policies and requirements of their affiliated companies.

References


34. EPA List N: Products with Emerging Viral Pathogens and Human Coronavirus Claims for Use against SARS-CoV-2: Date Accessed: 03/28/2020


Appendix

HACCP Approach to Risk Assessment
A comprehensive risk assessment of the different parts of the pharmaceutical supply chain may be found in Tables S-VI–S-XI. The four columns in each table address the input or activity, potential risk identification, assigned risk rating (low, moderate, or high), and existing critical process controls and recommended risk mitigations. The risk assessment model used is based on food-based HACCP principles that may be unfamiliar to some people working in the pharmaceutical industry (45). These represent the informed opinions of the authors with an emphasis on the risk assessment process and an attempt to capture all relevant aspects of the steps in the supply chain.

TABLE S-I
Risk Assessment—Management of Human Resources during COVID-19 Pandemic

<table>
<thead>
<tr>
<th>Activity</th>
<th>Risk Identification</th>
<th>Risk Rating</th>
<th>Risk Mitigation/Critical Process Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruitment of new staff</td>
<td>Local; Regional; National; International</td>
<td>Low to Moderate</td>
<td>Remote interviews; Limit domestic and international travel; 14-day quarantine prior to starting work</td>
</tr>
<tr>
<td>Training new hires and existing employees</td>
<td>Lack of information; Issues not addressed in corporate policies and procedures</td>
<td>Moderate to High</td>
<td>GMP compliance; Coronavirus awareness training; Symptoms identification</td>
</tr>
<tr>
<td>Deployment of employees</td>
<td>Domestic; International</td>
<td>Moderate to High</td>
<td>Staff functions conducted from home; Restrictions on nonessential domestic and international travel; deferment of large staff meetings</td>
</tr>
<tr>
<td>Management and Supervision of Employees</td>
<td>Staff and line functions</td>
<td>Moderate to High</td>
<td>Illness recognition; Monitoring with thermal sensors; Universal wearing of non-medical face masks on the job</td>
</tr>
<tr>
<td>Employee Attendance</td>
<td>Employees working sick; Loss of human resources; Inability to commute</td>
<td>Low</td>
<td>Flexibility in working hours; Stress importance of staying home when ill; Provide sickness benefits; Add company sponsored transportation when public transportation is unavailable</td>
</tr>
</tbody>
</table>
### TABLE S-II
Risk Assessment—Management of Manufacturing Materials during COVID-19 Pandemic

<table>
<thead>
<tr>
<th>Materials</th>
<th>Risk Identification</th>
<th>Risk Rating</th>
<th>Risk Mitigation/Critical Process Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceutical Excipients</td>
<td>Excipients derived from plant, animal, or mineral origin; Excipients of synthetic or semisynthetic origin</td>
<td>Low</td>
<td>Supplier awareness of potential COVID-19 risk; Standard duration of transit and hold times</td>
</tr>
<tr>
<td>Drug Substances</td>
<td>Drug substance of synthetic or semisynthetic origin</td>
<td>Low</td>
<td>Standard duration of transit and hold times</td>
</tr>
<tr>
<td>Packaging Components</td>
<td>Glass vials, stoppers and seals (Sterile products)</td>
<td>Low</td>
<td>Standard duration of transit and hold times; automation of component handling; washing, depyrogenation and sterilization (sterile products)</td>
</tr>
<tr>
<td></td>
<td>Plastic container, heat induction seals and caps (non-sterile products)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labeling Materials</td>
<td>Labels and package inserts</td>
<td>Low</td>
<td>Reduce handling during label identification and reconciliation</td>
</tr>
<tr>
<td>Incoming Potable Water</td>
<td>Ground or surface water</td>
<td>Low</td>
<td>Communication with local water authority</td>
</tr>
</tbody>
</table>

### TABLE S-III
Risk Assessment—Testing of Incoming Pharmaceutical Ingredients, Packaging Components, Intermediates, and Finished Products during COVID-19 Pandemic

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling of incoming materials</td>
<td>Human intervention; Limited environmental controls in warehouses</td>
<td>Moderate</td>
<td>Personal protective equipment; sampling booths; labeling sampled containers</td>
</tr>
<tr>
<td>Transportation to the testing area</td>
<td>Transition through the facility</td>
<td>Low</td>
<td>Disinfection of the surface of containers, drums, shrink-wrap, and pallets</td>
</tr>
<tr>
<td>Sample testing</td>
<td>Personnel handling</td>
<td>Low</td>
<td>Personal protective equipment; limited access to testing area; environmental controls</td>
</tr>
<tr>
<td>Sample disposition</td>
<td>Disposal</td>
<td>Low</td>
<td>Controlled destruction of samples</td>
</tr>
</tbody>
</table>
TABLE S-IV
Risk Assessment—Management of Plant Utilities during COVID-19 Pandemic

<table>
<thead>
<tr>
<th>Materials—Plant Utilities</th>
<th>Risk Identification</th>
<th>Risk Rating</th>
<th>Risk Mitigation/Critical Process Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceutical-grade water</td>
<td>Incoming potable water</td>
<td>Low</td>
<td>Maintain existing microbial monitoring program</td>
</tr>
<tr>
<td>Pharmaceutical-grade plant air</td>
<td>Distribution lines and delivery nozzles</td>
<td>Low</td>
<td>Assure sanitary design</td>
</tr>
<tr>
<td>Compressed gases</td>
<td>None</td>
<td>Low</td>
<td>Maintain existing microbial monitoring program</td>
</tr>
<tr>
<td>HVAC system</td>
<td>Poor temperature, humidity, and air exchange; Recirculation and lack of segregation</td>
<td>Low to Moderate</td>
<td>Reduced recirculation in more critical areas; Higher air change rates; High room ultraviolet germicidal irradiation</td>
</tr>
<tr>
<td>Domestic and clean steam</td>
<td>None</td>
<td>Low</td>
<td>None</td>
</tr>
<tr>
<td>Vacuum</td>
<td>Discharge</td>
<td>Low</td>
<td>Review vacuum discharge</td>
</tr>
<tr>
<td>Washing facilities</td>
<td>Poor design and opportunities for cross-contamination</td>
<td>Low to Moderate</td>
<td>Access to detergents, hot water, and hand sanitizing agents; Segregation of clean and dirty materials</td>
</tr>
<tr>
<td>Waste and sewage disposal</td>
<td>Poor separation</td>
<td>Moderate</td>
<td>Backflow elimination; Segregation of clean and dirty materials</td>
</tr>
</tbody>
</table>
# TABLE S-V
Risk Assessment—Representative Non-Sterile Drug Product (Compressed Tablet) during COVID-19 Pandemic

<table>
<thead>
<tr>
<th>Manufacturing Process</th>
<th>Risk Identification</th>
<th>Risk Rating</th>
<th>Risk Mitigation/Critical Process Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incoming material sampling and testing</td>
<td>Sampling is an invasive</td>
<td>Moderate</td>
<td>Sampling booths; Personal protective equipment (PPE)</td>
</tr>
<tr>
<td></td>
<td>process</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warehousing</td>
<td>Limited environmental control</td>
<td>Low</td>
<td>Improved environmental control</td>
</tr>
<tr>
<td>Ingredient weighing</td>
<td>Weighing is an invasive</td>
<td>Low to Moderate</td>
<td>Evaluate weighing booths and PPE</td>
</tr>
<tr>
<td></td>
<td>process</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excipient size reduction</td>
<td>Equipment cleaning</td>
<td>Low</td>
<td>Upgrade COP and CIP operations</td>
</tr>
<tr>
<td>Blending</td>
<td>Equipment cleaning</td>
<td>Low</td>
<td>Upgrade COP and CIP operations</td>
</tr>
<tr>
<td>Granulation—Dry</td>
<td>Equipment cleaning</td>
<td>Low</td>
<td>Upgrade COP and CIP operations</td>
</tr>
<tr>
<td>Granulation—Wet</td>
<td>Equipment cleaning</td>
<td>Low</td>
<td>Upgrade COP and CIP operations</td>
</tr>
<tr>
<td>Compression</td>
<td>Equipment cleaning</td>
<td>Low</td>
<td>Upgrade COP and CIP operations</td>
</tr>
<tr>
<td>Bulk Tablet Storage</td>
<td>None</td>
<td>Low</td>
<td>None</td>
</tr>
<tr>
<td>Packaging and labeling</td>
<td>Packaging operations are labor intensive</td>
<td>Low to Moderate</td>
<td>Evaluate HVAC Systems; Automation of component handling; PPE</td>
</tr>
<tr>
<td>Finished goods warehousing</td>
<td>Poor environmental control</td>
<td>Low</td>
<td>Improved environmental control</td>
</tr>
<tr>
<td>Shipping</td>
<td>Lack of chain of custody</td>
<td>Low</td>
<td>Dedicated carriers</td>
</tr>
</tbody>
</table>

Clean Out-of-Place (COP) and Clean In-Place (CIP).
<table>
<thead>
<tr>
<th>Manufacturing Process</th>
<th>Risk Identification</th>
<th>Risk Rating</th>
<th>Risk Mitigation/Critical Process Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incoming material sampling and testing</td>
<td>Sampling is an invasive process in a warehouse</td>
<td>Moderate</td>
<td>Sampling booths and personal protective equipment (PPE)</td>
</tr>
<tr>
<td>Warehousing</td>
<td>Limited environmental control</td>
<td>Low</td>
<td>Improved environmental control (IEC)</td>
</tr>
<tr>
<td>Ingredient weighing</td>
<td>Weighing is a labor-intensive process</td>
<td>Low to Moderate</td>
<td>Evaluate weighing booths and PPE</td>
</tr>
<tr>
<td>Bulk solution preparation</td>
<td>Solution preparation is a potentially invasive process</td>
<td>Low to Moderate</td>
<td>Use of Restricted Access Barrier Systems (RABS) and isolators</td>
</tr>
<tr>
<td>Packaging component preparation</td>
<td>Component loading and unloading is a potentially invasive process</td>
<td>Low</td>
<td>Automation of component handling; Depyrogenation and sterilization of vials and stoppers</td>
</tr>
<tr>
<td>Sterile filtration and aseptic filling and Sealing</td>
<td>Aseptic processing is a potentially invasive process</td>
<td>Low</td>
<td>Use of RABS and isolators</td>
</tr>
<tr>
<td>Visual inspection</td>
<td>Inspection is an invasive process</td>
<td>Low</td>
<td>Handling automation; IEC; PPE</td>
</tr>
<tr>
<td>Packaging and labeling</td>
<td>Packaging is an invasive process</td>
<td>Low</td>
<td>Handling automation; IEC; PPE</td>
</tr>
</tbody>
</table>

TABLE S-VI
Risk Assessment—Representative Sterile Drug Products (Liquid-filled Stoppered Glass Vials) during COVID-19 Pandemic