PHARMACEUTICAL ONLINE

CLEANING & STERILIZATION IN PHARMACEUTICAL MANUFACTURING

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Sterility assurance, cleaning validation, and environmental monitoring continue to be hot subjects during pharmaceutical regulatory inspections and aseptic processing industry discussions. This e-book provides best practices of all aspects of this field.

The first article summarizes ASTM E3263-20, Standard Practice For Qualification Of Visual Inspection Of Pharmaceutical Manufacturing Equipment And Medical Devices For Residues. The second article delves into the seven rules to follow for cleaning verification and validation of multipurpose API plants. Next, the e-book shares a better approach to aseptic process simulation for lyophilized products. The following article uses data to calculate the process capabilities of cleaning processes.

The second half of the e-book provides best practices for different aspects of a sterility assurance program, including the sterility assurance program itself, risk-based environmental monitoring of modern drug product facilities, trending in environmental monitoring programs, and trending environmental monitoring data. Regulatory agencies have written warning letters and observations regarding environmental monitoring and trending programs, so these are key issues for regulatory compliance.

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INTRODUCTION TO ASTM E3263-20: STANDARD PRACTICE FOR QUALIFICATION OF VISUAL INSPECTION OF PHARMACEUTICAL MANUFACTURING EQUIPMENT AND MEDICAL DEVICES FOR RESIDUES

By Andrew Walsh, Ralph Basile, Ovais Mohammad, Stéphane Cousin, Mariann Neverovitch, and Osamu Shirokizawa

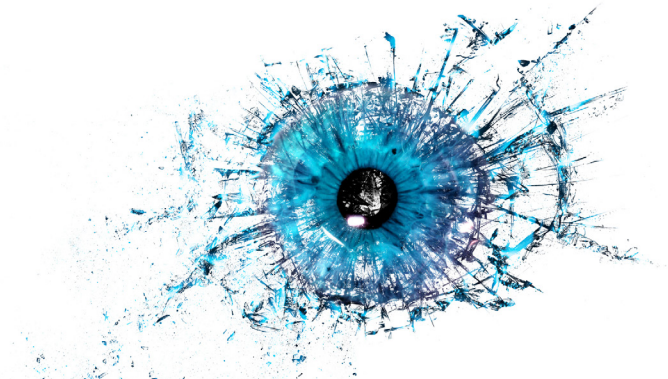
PART OF THE CLEANING VALIDATION FOR THE 21ST CENTURY SERIES

Visual inspection has been widely used for many years by the pharmaceutical, biologics, and medical device industries after cleaning to release manufacturing equipment and devices. However, visual inspection has never been demonstrated to be an effective, reliable, or safe method to use for these inspections. Recently, the European Medicines Agency (EMA) issued a Q&A[1] to its guideline on determining health-based exposure limits (HBELs) that describes what criteria have to be met for visual inspection to be acceptable to the EMA for release of manufacturing equipment. Some form of guidance or a standard has been needed to guide these industries on how to meet these criteria and demonstrate that operators/QA inspectors are capable and qualified to accurately assess the absence or presence of residues on manufacturing equipment or medical devices. This article discusses the development and publication of a new ASTM International (American Society for Testing and Materials) standard practice for the qualification of visual inspection.

HISTORY AND REGULATORY PERSPECTIVES ON VISUAL INSPECTION

U.S. regulation has required the "inspection of manufacturing equipment immediately before use" since 1979.[2] While this regulation did not specify that the inspection should be "visual," in practice, pharmaceutical manufacturers have been releasing equipment based on a visual inspection for many years and industry and regulators have come to see this inspection as a visual inspection requirement. However, throughout all this time only a few studies on visual inspection have been performed, with varying results reported. Another misinterpretation of this statement has resulted in many companies only inspecting the equipment "immediately before use," after the cleaned equipment has been reassembled. After reassembly, large portions of equipment surfaces may not be visible, so they cannot be inspected and confirmed as visually clean. Visual inspection should take place after the cleaning process and ***before*** any reassembly of equipment.

In 1993, an article[3] was published that mentioned that spiking studies indicated that many compounds were visible at



approximately "100 µg per 2 x 2-inch swab area" (or approximately 4 µg/cm²). This 4 µg/cm² value was quickly adopted by many companies as an "industry standard," although no data or any other supporting information were provided by the authors. Another article following in 1994 claimed that residues could be seen down to 1 µg/cm² by using an additional light source.[4] A subsequent article in 2000 claimed to see residues down to approximately 0.4 µg/cm² for several compounds.[5] A series of studies[6,7] examining several different compounds found a range from 0.4 to >10 µg/cm². Three studies using a different spiking technique that spread the residue evenly over the surface found detection limits for one residue at levels of 3, 5, and 7 µg/cm². These detection limits were calculated following an ICH Q2[8] approach and were found to be influenced by several factors, including training.[9] In 2010, Ovais Mohammad proposed using a statistical approach (logistic regression) to these spiking studies to more accurately derive the visible threshold.[10]

While extensive studies and analysis of the ability of visual inspection to identify the presence of residues have not been performed to date, regulatory agencies appear to be more flexible with regard to its use. The EMA's 2015 update to Annex 15[11] now states that "It is not ***generally*** acceptable for this criterion alone to be used," indicating that visual inspection could be used ***alone*** for cleaning validation ***under certain circumstances***. Aware of the significance of this statement, the recent ASTM E3106 Standard Guide[12] provided the following guidance to support this Annex 15 statement:

> *"Using visual inspection alone for validation may be acceptable only when a **Risk Assessment** has shown that the **risk is low and 100 percent of the equipment surface can be inspected** under appropriate viewing conditions."* (emphasis added)

However, it should be understood that regulators are highly unlikely to accept visual inspection ***alone*** for cleaning validation unless manufacturers have exceptional justification, such as a very low-hazard product, and will still likely expect some analytical testing to confirm acceptable cleaning during the cleaning validation phase. Where visual inspection can most likely be used ***alone*** is in subsequent cleaning process validations (verifications) for new low-risk products on multi-use equipment where prior satisfactory validation studies of the cleaning process have already been performed.

Moving even further in this direction, Pharmaceutical Inspection Co-operation Scheme (PIC/S)[13] has now stated that "...spiking studies should determine the concentration at which most active ingredients are visible," indicating that these health agencies are expecting to see visual inspection being used more frequently as a semi-quantitative tool and have requirements for its use. This statement specifically about active ingredients (APIs) has led some manufacturers to set up visual inspection and the related training activities to focus only on active ingredients. Any compound that is identified as a hazard in the Risk (Hazard) Identification step and found to be a risk in the Risk Analysis step may need to be included in a visual inspection qualification and training program. This is especially true for medical device manufacturing, as cleaning agents and processing aids may be the compounds identified as risks and not APIs.

Expanding further on this, on April 16, 2018 the EMA posted an update to its draft Q&A on the guideline for setting health-based exposure limits. In this final version, two new questions and answers appeared (Q7 and Q8) that are directly applicable to the use of visual inspection. These Q&As state:

Q7. Is analytical testing required at product changeover, on equipment in shared facilities, following completion of cleaning validation?

A: Analytical testing is expected at each changeover unless justified otherwise via a robust, documented Quality Risk Management (QRM) process. The QRM process should consider, at a minimum, each of the following:

- *the repeatability of the cleaning process (manual cleaning is generally less repeatable than automated cleaning);*
- *the hazard posed by the product;*
- *whether visual inspection can be relied upon to determine the cleanliness of the equipment at the residue limit justified by the HBEL.*

Q8. What are the requirements for conducting visual inspection as per Q&A 7?

A. When applying visual inspection to determine cleanliness of equipment, manufacturers should establish the threshold at which the product is readily visible as a residue. This should also take into account the ability to visually inspect the equipment, for example, under the lighting conditions and distances observed in the field.

Visual inspection should include all product contact surfaces where contamination may be held, including those that require dismantling of equipment to gain access for inspection and/or by use of tools (for example mirror, light source, borescope) to access areas not otherwise visible. Non-product contact surfaces that may retain product that could be dislodged or transferred into future batches should be included in the visual inspection.

Written instructions specifying all areas requiring visual inspection should be in place and records should clearly confirm that all inspections are completed.

Operators performing visual inspection require specific training in the process including periodic eye sight testing. Their competency should be proven through a practical assessment.

So, the regulatory requirements for implementing visual inspection as one of the tools available for cleaning validation are now pretty well defined by the EMA and all that was needed was detailed guidance on how to satisfy these criteria. The new ASTM 3263 was written specifically to provide the necessary guidance for establishing qualified visual inspection programs to comply with these newly clarified regulatory expectations.

ASTM E55 AND F04 COMMITTEE COLLABORATION

In 2017 members of the ASTM E55 Committee on Manufacture of Pharmaceutical and Biopharmaceutical Products and members of the ASTM F04 Committee on Medical and Surgical Materials and Devices collaborated on writing a new Standard Guide that resulted in the E3219 *Standard Guide for Derivation of Health Based Exposure Limits (HBELs)*.[14] The team that developed the E3219 also included members of the E3106[12] team. The collaboration on the E3219 had been successful, and this team discussed collaborating on other standards that would benefit both pharmaceutical and medical devices and other industries and, in particular, for the qualification of visual inspection.

Therefore, in March of 2019, a new Work Item (WK67425) was initiated on the ASTM website and a collaboration area for the WK67425 was created. The original E3219 and E3106 teams were expanded to include several more pharmaceutical and medical device industry stakeholders to work on this standard (Table 1).

Table 1: WK67425 Collaboration Area Team Members

Team Member	Company	Industry Expertise
Ralph Basile	Healthmark Industries Co	Medical Device
Dhanapal Boopathy	Zimmer Biomet	Medical Device
Stéphane Cousin	GSK Vaccines	Pharmaceutical
Delane Dale	Confluent Medical Technologies	Medical Device
Parth Desai	Nostrum Laboratories Inc.	Pharmaceutical
Jayen Diyora	Alnylam Pharmaceuticals	Pharmaceutical
Christophe Gamblin	Theraxel	Pharmaceutical
Igor Gorsky	ConcordiaValsource, LLC	Pharmaceutical
Jove Graham	Geisinger Center for Health Research	Medical Device
Jessica Graham, Ph.D., DABT	Bristol Myers Squibb	Pharmaceutical
Barbara Kanegsberg	BFK Solutions LLC	Medical Device
Reto Luginbuehl	Blaser Swisslube AG	Medical Device
Spiro Megremis	American Dental Association	Medical Device
Mariann Neverovitch	Bristol-Myers Squibb	Pharmaceutical
Mohammad Ovais	Pharmaceutical Consultant	Pharmaceutical
Rodney Parker	Stryker	Medical Device
Vimal Sachdeva	World Health Organization	harmaceutical
Stephen Spiegelberg, Ph.D.	Cambridge Polymer Group	Medical Device
Norma Turner	Cambridge Polymer Group	Medical Device
Andrew Walsh	Center for Pharmaceutical Cleaning Innovation	Pharmaceutical

GOALS OF ASTM E3263

The goals for this new standard practice are to specifically provide instructions for qualification of visual inspection for residues on pharmaceutical manufacturing equipment and medical devices. This new standard would provide guidance for achieving the following six goals:

1. An approach that applies the science-based, risk-based, and statistics-

based concepts and principles introduced in Guides E3106[12] and E3219.[14]

2. An approach for qualifying the inspection of equipment for cleanliness in accordance with 21 CFR 211.67(b).[2]
3. An approach for qualifying the visual inspection of equipment for cleanliness in accordance with European Medicines Agency (EMA) Annex 15.[11]
4. An approach for qualifying the visual inspection (and visual threshold) of equipment for cleanliness in accordance with the EMA's Q&A Guidance (Q&A 7 and Q&A 8).[1]
5. An approach that would apply the risk-based principles introduced in ICH Q9[15] so that the level of effort, formality, and documentation for cleaning validation would also be commensurate with the level of risk.
6. An approach for releasing manufacturing equipment and manufactured medical devices or cleanliness that is compatible with the U.S. FDA's guidance on its Process Analytical Technology Initiative.[16]

SCOPE OF ASTM E3263

The E3263 provides statistically valid procedures for determining the visual detection limit (also called the visual threshold) of residues and for the qualification of operators/QA inspectors to perform the visual inspection of pharmaceutical manufacturing equipment surfaces and medical devices. E3263 applies to pharmaceuticals, including active pharmaceutical ingredients, finished dosage forms, over-the-counter (OTC) drugs, veterinary medicines, biologics, clinical supplies, medical devices, cosmetics, and consumer products. E3263 can be used for all types of chemical residues (including APIs, biological substances, intermediates, cleaning agents, processing aids, machining oils, etc.) that could remain on manufacturing equipment surfaces or the surfaces of medical devices.

THE E3263 STANDARD PRACTICE GUIDE

The Procedure section of E3263 contains guidance on six main elements. The first element discusses what initial criteria must be met in order to implement a visual inspection program.

1. Initial Criteria for Establishing Qualification Programs for Visual Inspection:

1. Calculation of MSSR – MSSRs (Maximum Safe Surface Residues) must first be calculated for all equipment to be inspected, as it is necessary to determine the minimum level of residue that must be detectable by the visual inspection. The MSSR, expressed in mass units per surface area (for example, µg/cm²), is calculated using the following equation (from ASTM E3106):

$$MSSR = \frac{MSC}{TSA}$$

Where:

MSC = Maximum Safe Carryover

TSA = Total Surface Area (of shared equipment or medical device)

2. Selection of Surfaces for the Qualification Study – the steps to take in selecting the materials of construction for visual inspection studies
3. Selection of Products/Compounds for the Qualification Study – the steps to take in selecting the compounds/products (e.g., APIs, cleaning agents, machining oils, etc.) for visual inspection studies
4. Preparation of Surrogate Surfaces or Devices – how to prepare the surrogate surfaces (e.g., coupons, equipment parts, medical devices, etc.) for use in visual inspection studies
5. Surrogate Surface Storage and Handling – how the surrogates' surfaces should be handled
6. Viewing (Lighting) Conditions – what the lighting requirements are for performing the visual inspection studies

2. Inspector Training – This section discusses what is necessary to train operators/QA inspectors for visual inspection and maintain their qualified state.

3. Determination of Visual Residue Limits (VRL) – This section discusses how to identify the lowest spiked residue level (visual threshold) that is most likely to be seen by all trained operators/QA inspectors for the product/compound of a spiked coupon study. This spiked residue level (visual threshold) should be the starting point for inspector qualification studies. In cases where the VRL

is determined in a study using a small number of inspectors (e.g., N=4) the VRL may not be statistically justifiable. A method for setting scientifically and statistically justifiable VRLs using logistic regression analysis (Figure 1) was developed by Ovais Mohammad to provide a meaningful determination of the VRL.[17]

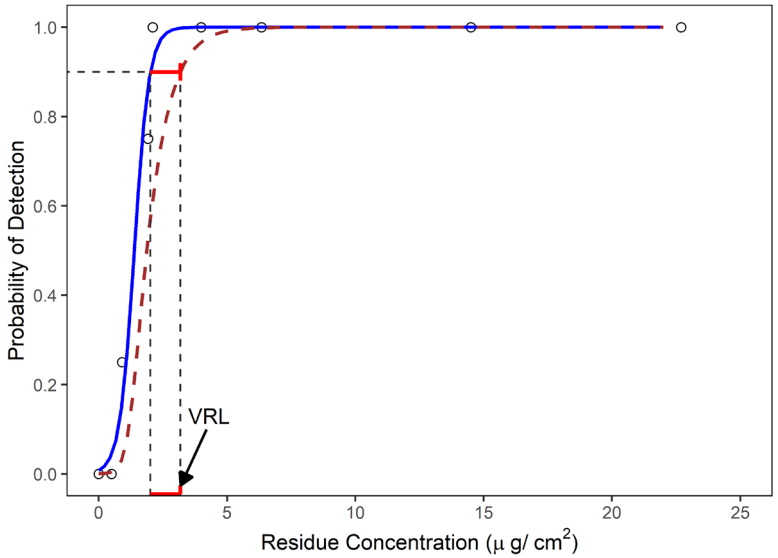


Figure 1 – Determination of VRL using Logistic Regression Analysis: *The solid blue line is predicted probability of detection and the brown dashed line is the lower 95 percent confidence bounds for the predicted probabilities. In this example, VRL represents the residue concentration at the lower 95 percent confidence for 90 percent probability of detection. (Reprinted from ASTM E3263-20 "Standard Practice for Qualification Of Visual Inspection Of Pharmaceutical Manufacturing Equipment And Medical Devices For Residues", copyright ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428, USA, www.astm.org.)*

4. Qualification of Operators/QA inspectors Using Attribute Agreement Analysis – This section discusses how to set up a visual inspection study to qualify any number of operators/QA inspectors by analyzing inspection results as attribute/binary data (i.e., clean/dirty).

5. Acceptance of the VRL for Cleaning Validation – This section discusses how to determine whether visual inspection is appropriate for use by comparison to the MSSR using the Visual Detection Index (VDI).[18]

6. Documentation – This section discusses what key documents are necessary for establishing a visual inspection program.

SIGNIFICANCE OF HBELs FOR VISUAL INSPECTION

Probably the most important consideration for the implementation of visual inspection is the HBEL of the compound being considered. The 1/1,000th of a dose and 10 ppm limits have been shown to be overly conservative with low-hazard compounds and not restrictive enough with high-hazard compounds, which does not reflect the risk-based approach of ICH Q9.[18] This error in logic extends to the use of visual inspection. A simple analysis will demonstrate this.

The MSSRs for the 304 drug compounds in the article cited above[19] were calculated for both HBELs and the 1/1,000th of a dose / 10 ppm combination using the parameters shown in Table 2. The total equipment surface area was chosen to be typical of a packaging line, which can be considered one of the more appropriate areas for visual inspection.

Table 2: Parameter Assumptions for MSSR Calculations

Parameter	Value
Batch Size	100 kg
Maximum Daily Dose	10 g
Total Equipment Surface Area	25,000 cm²

The MSSRs for both HBELs and the 1/1,000th of a dose / 10 ppm combination were plotted as shown in Figure 2. A reference line for a visual residue limit at 10 µg/cm² has been added and a box drawn to contain the compounds that are below or possibly too close to this visual residue limit to allow for visual inspection. It should be obvious from this graph that many, if not most, compounds could not be considered for visual inspection.

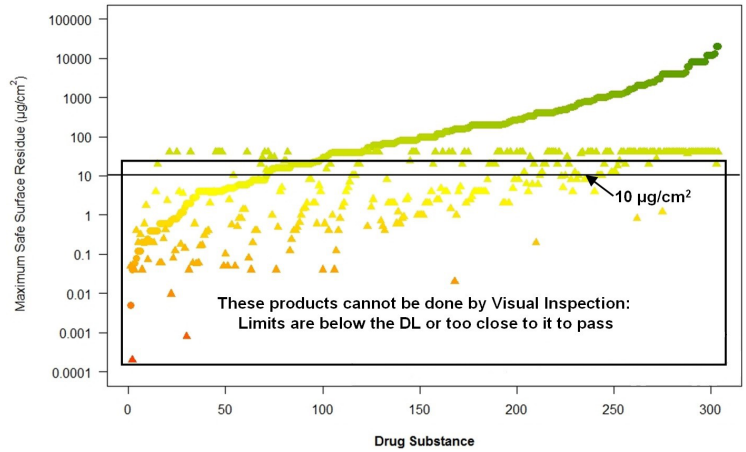


Figure 2 – Comparison of MSSRs for HBELs and 1/1,000th & 10 ppm: *The o symbols represent the MSSRs based on HBELs and the Δ symbols are their corresponding MSSRs calculated from the 1/1,000th or 10 ppm limits. (DL = Detection Limit) X-axis indicates the number of compounds. Data plotted in the "R" statistical programming language by Ovais Mohammad.*

Figure 3 shows the same graph with the Δ symbols for the MSSRs for the 1/1,000th of a dose / 10 ppm combination removed. The box is now drawn to contain the compounds that are above this visual residue limit and would allow for visual inspection. It should be obvious from this graph that, with the HBELs, there are many compounds, in particular low-risk compounds, that could possibly be considered for visual inspection.

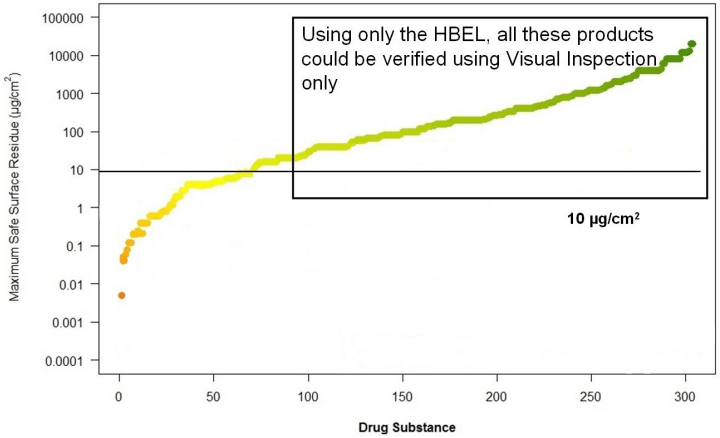


Figure 3 – MSSRs for HBELs with 1/1,000th & 10 ppm MSSRs Removed: *Data plotted in the "R" statistical programming language by Ovais Mohammad.*

SUMMARY

While the EMA's new Q&A 7 and Q&A 8 may have been a surprise for many in the industry, they were added to allow companies with products found to be low risk (based on their HBELs) the option of using visual inspection at product changeovers. Hopefully, from the discussion above, it is clear that companies must finally let go of holding on to the historical 1/1,000th dose and 10 ppm limits and implement the HBEL in order to take advantage of visual inspection.

At the same time, some companies have already started moving to visual inspection for release of equipment by simply stopping swab/rinse testing without any QRM program in place, any adequate justification, or any qualification of their operators/QA inspectors. These are unacceptable practices that will inevitably lead to regulatory action resulting in significant costs and reputational damage for these companies. Implementing procedures as described in E3263 should prevent this. However, E3263 cannot be implemented completely independent of the ASTM E3106 and E3219 standards and must be coordinated with these guides.

Again, as stated in the "History And Regulatory Perspectives On Visual Inspection" section, visual inspection ***alone*** will most likely find initial use in cleaning process validations on multi-use equipment for new low-risk products. At least initially, this will require that prior satisfactory validation studies of the cleaning process already exist. The 6-step QRM process described below would be an appropriate procedure to implement visual inspection.

1. The HBEL of the new product must be derived by a qualified toxicologist and compared to the HBELs of the existing portfolio of products using the Toxicity Scale.[20] If the hazard (toxicity) level is acceptable, the product can move to step 2.
2. HBEL-derived cleaning (swab) limits should be calculated for the new product, compared to the existing cleaning data for the equipment, and its potential Process Capability Score (Cpu Score) calculated.[21] If the Cpu Score is acceptable then the product can move to step 3.
3. The "Cleanability" of the new product is measured and compared to the existing "Hardest-to-Clean" product.[22] If the Cleanability of the new product is acceptable then the product can move to step 4.

4. The visual detection limit (visual threshold) should be determined for the new product and the Visual Detection Index (VDI) calculated.[18] If the VDI is acceptable then visual inspection ***alone*** could be justified.
5. Qualification of all operators/QA inspectors to visually inspect the new product[23]
6. Visual inspection of 100 percent of (disassembled) equipment surfaces should be performed and documented after each batch of the new product is manufactured.

The authors believe that the new E3263 standard provides the science-, risk-, and statistical-based guidance and the tools needed for companies to implement the use of visual inspection within a QRM program that meets the criteria promulgated in the EMA's new Q&A 7 and Q&A 8.

PEER REVIEW

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CLEANING VERIFICATION & VALIDATION OF MULTIPURPOSE API PLANTS: 9 RULES TO FOLLOW

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Cleaning validation continues to be a hot topic during regulatory inspections and industry discussions. The cleaning required in an active pharmaceutical ingredient (API) plant between one manufacturing process and the next can present a huge challenge. This challenge is greatest when multipurpose plants are used, which are configured, cleaned, and reconfigured to manufacture a wide variety of intermediates and APIs. I've experienced many examples of poor practice and best practice during my involvement in the design, execution, and assessment of API plant cleaning methods, analytics, and validation principles and protocols. This article provides a sample of important rules to follow when verifying and validating cleaning the cleaning process for multipurpose API plants.

1. KNOW YOUR LIMITS.

First, it's important to understand and determine the scientifically derived acceptance criteria you will need to demonstrate you have achieved when cleaning between one manufacturing process and the next. The acceptance criteria have evolved over the years, from the initial 1,000th of the dose (worst case is the minimum dose) of the contaminating molecule, per dose (worst case is the maximum dose) of the receiving product. This was based on the principle at the time that a patient receiving 0.1x the dose of the contaminating molecule will not experience an adverse reaction; however, a safety factor of 10 was then included, together with an additional robustness factor of 10, hence the 0.001 or 1,000th criteria. It is no longer acceptable to calculate a maximum allowable carryover (MACO) based on the adjustment of an LD_{50}. The calculated criterion was often translated into a default limit of 10 ppm of the contaminant per minimum batch size of the next production (unless the calculated dosage criteria were lower). In the past, this 10-ppm limit was found to be acceptable in the majority of situations.

Exceptions could be made for manufacturing highly potent APIs, where the calculation of acceptance criteria recently evolved further, to require the use of health-based exposure limits (HBELs). HBEL calculation requires input from qualified toxicologists and usually results in limits that are higher (less stringent) than the limits applied in the past, unless the API is

highly potent, which would probably require the use of dedicated facilities to avoid cross-contamination.

Once a MACO has been calculated for a specific changeover, it needs to be translated into the analytical limits that can be applied to rinse and swab samples taken from the cleaned equipment. This requires calculating the surface area inside each piece of equipment that becomes contaminated and is required for reuse in a different manufacturing process. Typically, qualified engineers provide internal surface area calculations for each piece of equipment, resulting in inspectable documentation showing the m^2 total for each item and for the total equipment train required for reuse.

The analytical limits are calculated by dividing the MACO (per minimum batch size of the next production in grams [g]) by the combined surface area of all the equipment being reused. This is relatively easy for direct surface (swab) analysis; however, rinse analysis is not a direct measure of the contamination remaining on a cleaned surface and requires care. Swab test limits are typically calculated as g or mg of contaminant per 100 cm^2 of swabbed surface area (10 cm x 10 cm). A rinse sample provides an indication of the contaminant removed (dissolved) by the rinsing conditions and not specifically the amount of contaminant remaining on the surfaces. It is therefore preferable to combine the rinse analysis with the results of a rinse efficiency study performed in the laboratory (as described below).

2. DON'T DOUBLE UP.

In some API manufacturing sites, the equipment used to manufacture the crystallized API (usually isolated as a wet cake) is in a different building than the equipment used to dry and offload the finished API. A common mistake is to apply the entire MACO to the equipment in the first facility and again in the second facility, resulting in the output potentially containing two times the MACO. The MACO to surface area calculation must be applied to the complete equipment train from input to dried output.

3. MAXIMIZE THE RECOVERY.

If rinse samples are tested as an indirect measure of cleaning effectiveness, the rinsing conditions, e.g., the solvent mix, temperature, agitation, and time, should be optimized and the recovery efficiency determined by performing laboratory studies. A known amount of representative residue should be spiked onto the surfaces of an equivalent test item, e.g., a stainless steel or glass beaker or flask, dried (as the worst-case challenge to the rinse), and subjected to the rinsing conditions. Samples of the rinse should be taken at intervals and tested to quantify the concentration of the residue rinsed from the surface. The results can be used to determine the optimized rinsing conditions and also the recovery efficiency when using the rinse samples as an indication of the cleanliness of the surfaces.

The analysis of direct surface (swab) samples is accepted as the most reliable indication of surface cleanliness. However, like rinse analysis above, the efficiency of the swab test conditions also needs to be optimized and determined. The choice and use of a swab media that is absorbent and does not generate background interference during analysis is preferred. Similar to the rinse study, a known amount of representative residue is spiked onto a representative surface area, e.g., stainless steel, glass, plastic, etc. A useful approach is to spike the acceptance criteria amount (R) onto a marked 10 cm x 10 cm area and also spike 2xR and R/2 onto additional marked 10 cm x 10 cm areas of the same material. In this way, the swab test and analytical conditions are demonstrated for efficiency and linearity across the range of results, R/2 to 2xR, which should be sufficient.

The moistened swab dissolves the residue from the test surface and retains some of this solution on the swab for analysis. However, a quantity of the residue solution is left behind on the surface. This can be recovered by using a dry swab immediately after the moistened swab, followed by analysis of both swabs combined. In this approach, the use of a non-volatile solvent for the swab testing is preferred. If a volatile solvent must be used, using a second swab immediately after the first can improve the recovery in a similar way. The recovery study described above provides three results for the efficiency of the residue removal, which should be comparable if the conditions are suitable. The results are then used to determine a correction factor, which is applied when calculating the amount of residue remaining on a surface after cleaning. A recovery of less than 50% should not be accepted, and the conditions should be further optimized. The optimized "wet then dry" principle described above typically provides recoveries of 80% or more.

4. FOLLOW THE MAP.

A “residue map” should be prepared that lists the composition of the residue remaining inside each piece of equipment after use. Note: Only the equipment used for the later stages of synthesis will contain the API molecule; therefore, the analysis used to determine the cleanliness of earlier equipment may need to look for residual raw materials, reactants, and intermediates instead.

Where a multistage synthetic route is used to manufacture an API, the carryover of residue to the next stage might represent the presence of raw materials, reactants, or contaminants with respect to that next stage; therefore, the residue map should be used to determine the appropriate cleaning limits for changeover from one stage to the next. Typically, the changeover to non-consecutive stages requires cleaning to a higher standard.

5. VALIDATE THE DIRTY HOLD TIME.

After the offload of the last batch, the equipment should be left “dirty” for the worst-case time period (delay) before the start of the cleaning. This should be stated in the cleaning validation protocol.

6. CONFIRM THE APPROPRIATE METHOD OF CLEANING.

When mapping the composition of the residue remaining in the equipment after use, it is useful to examine the design and use of the equipment to determine where residue might be retained and present a challenge to the cleaning conditions available for use. This will determine which equipment can be cleaned in-situ (while connected) and which equipment will require dismantling to expose the surfaces for cleaning.

The initial cleaning typically involves contacting the contaminated surfaces with a cleaning solution, which is then passed through the equipment chain from top to bottom. The cleaning solution composition should be chosen by examining the solubility of the residue composition shown on the residue map under trial conditions of temperature, time, and agitation. If organic solvents cannot be used, aqueous reagents may be trialled and applied, although the output residue composition might be different due to reaction or derivatization that needs to be incorporated into the analytical strategy used to examine surface cleanliness.

The use of (food-grade approved) detergents is seen as introducing contamination and therefore the removal of the detergent after cleaning is an additional requirement that needs to be included. The use of rinsing studies and rinse analysis using total organic carbon (TOC) methodology is an option. In this case, the optimized rinse should remove the detergent until the rinse and the input water have an equivalent TOC output.

Typically, ancillary equipment used directly during manufacturing, such as condensers, pumps, samplers, etc., should be cleaned using conditions that are optimized to ensure maximum surface contact and residue removal. Flexible hoses should initially be cleaned while connected to the equipment. Each flexible hose should be uniquely identified by a code that is clearly shown on a label and/or stamped onto the hose. A register should be maintained that shows the duty and usage history of each hose. If flexible hoses are used for (non-waste) slurry or suspended solids transfer, these should be dedicated for use due to the challenges associated with cleaning and the risk of cross-contamination during reuse in a different duty. A best practice is to clean all flexible hoses separately using a recirculated cleaning solution that is pumped through the hose offline from the plant, using a dedicated cleaning assembly. The interior cleanliness after cleaning should be demonstrated by swab testing each end and inspection of the internal surfaces using an endoscope. If an endoscope is not available, a worst-case study can be performed, which involves cutting the hose into sections after cleaning, followed by the swab testing of each section. Of course, this destroys the hose; however, if the worst case is chosen correctly, this needs to be done only once.

The (manual) cleaning of disassembled equipment is difficult to replicate and therefore typically cannot be validated, although I visited one firm that had installed large automatic washing machines, which are used following a validated loading pattern, cleaning agents, and fixed programming of conditions to avoid the need for manual cleaning and verification of cleanliness after cleaning. If manual cleaning cannot be avoided, the method of cleaning should be carefully described to be as reproducible as possible, with scripted training for the operators involved. After cleaning, the manually cleaned equipment should be verified as clean when dry by visual inspection followed by swab testing. If only

a random selection of the cleaned equipment is tested, consideration must be given to re-cleaning all the equipment if a single item does not pass the acceptance criteria.

7. ENSURE THE STANDARD OF CLEANLINESS IS ACCEPTABLE.

The initial criterion for all cleaned equipment is "visually clean when dry." The reliability of visual inspection, however, is influenced by factors that require careful control:

- The eyesight of the inspectors, who must pass eyesight proficiency checks at specific intervals.
- The clarity of the view of the surface to be inspected. Vessels and other large items of equipment must be opened for inspection. The internal surface areas requiring visual inspection must be described and indicated, e.g., by using diagrams or photographs. A best practice is to illuminate the surface for inspection using a beam of light (torch/flashlight) reflected from a mirror (preferably not made from glass), carefully angled and positioned at the end of a telescopic pole. In this way, it is possible to inspect inlets and outlets, together with some otherwise difficult to see areas of the equipment, e.g., the dome and venting of vessels, seals, and the outer surfaces of dip-pipes, etc.
- The personnel performing the visual inspection must be trained and certified as competent (preferably by QA) to perform the inspection reliably and reproducibly, using the carefully derived and described conditions, equipment, and methods.

If the equipment is not visually clean, it must be re-cleaned and re-inspected (with the generation of the appropriate documentation to track and record all of this additional work). If, after validation, the equipment is not visually clean after cleaning has been completed, a deviation must be initiated to document and investigate the exception and take action accordingly, which might trigger the need for further cleaning optimization and re-validation.

If the equipment is "visually clean when dry," analysis can then be performed to determine if the standard of cleanliness is acceptable for reuse or not. This will typically involve rinse and direct surface swab analysis. Rinse analysis might also be used during optimization to determine if the equipment is sufficiently clean to move to examination by "visual inspection when dry." The location of swab testing positions should be determined after a study of the design and use of the equipment, to identify the locations that represent the "most difficult to clean" areas. The locations should be described using diagrams or photographs and training should be completed and recorded for the personnel who obtain the swab samples.

8. DETERMINE AN ACCEPTABLE CLEAN HOLD TIME.

When the equipment has been demonstrated as cleaned to the required standard, the length of time during which it can remain sealed and empty before reuse should be determined. This typically involves determining how the equipment surfaces become microbiologically contaminated over a worst-case period of time and, hence, whether a sanitizing rinse should be applied before reuse. The cleaned and held equipment must be confirmed as still visually clean when dry before reuse.

9. CONTINUE VERIFICATION POST-VALIDATION.

The above requirements are described in a validation protocol and practical compliance is described and demonstrated in a validation report. Thereafter, the use of identical, reproducible cleaning methods should not require further assessment, although it is typical that partial (or even full) analytical verification continues post-validation.

The minimum criteria post-validation should be "visually clean when dry" as described above. The validation approach can use a "worst-case" demonstration of cleaning effectiveness, based on a combination of factors such as the lowest MACO, the residue potency and solubility, and the difficulty in cleaning specific pieces of equipment. Alternatively, each specific cleaning can be validated after the equipment has been configured and used for a specific manufacturing process. I have also seen the use of a default worst-case standard for all cleaning, followed by a check, based on the reconfiguration of the equipment for reuse, that the residue remaining in the reconfigured full equipment train (based on the analytical data obtained) does not exceed the relevant MACO for the specific changeover before the plant is released for use by QA.

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A BETTER APPROACH TO ASEPTIC PROCESS SIMULATION FOR LYOPHILIZED PRODUCTS

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Proposed 2020 revisions to EU Annex 1[1] with respect to aseptic process simulation (APS) for lyophilized products have prompted extensive discussions on best practices for process simulation of the lyophilization unit operation. This article serves to address these expectations and present a risk-based, holistic best practice approach for APS for lyophilized drug products.

Annex 1 revision excerpt (2nd draft, Sec. 9.35):

The process simulation test should imitate as closely as possible the routine aseptic manufacturing process and include all the critical manufacturing steps, specifically:

i. *Process simulation tests should assess all aseptic operations performed subsequent to the sterilization and decontamination cycles of materials utilised in the process to the point where the container is sealed.*

ii. *For non-filterable formulations, any additional aseptic steps should be assessed.*

iii. *Where aseptic manufacturing is performed under an inert atmosphere, the inert gas should be substituted with air in the process simulation unless anaerobic simulation is intended.*

iv. *Processes requiring the addition of sterile powders should use an acceptable surrogate material in containers identical to those used in the process under evaluation.*

v. *Separate simulations of individual unit operations (e.g., processes involving drying, blending, milling and subdivision of a sterile powder) should generally be avoided. Any use of individual simulations should be supported by a documented justification and ensure that the sum total of the individual simulations continues to fully cover the whole process.*

vi. *The process simulation procedure for lyophilized products should represent the entire aseptic processing chain including filling, transport, loading, chamber dwell, unloading and sealing under specified, documented and justified conditions representing worst case operating parameters.*

vii. *The lyophilization process simulation should duplicate all aspects of the process, except those that may affect*

the viability or recovery of contaminants. For instance, boiling-over or actual freezing of the solution should be avoided. Factors to consider in determining APS design include, where applicable

- *The use of air to break vacuum instead of nitrogen.*
- *Replicating the maximum interval between sterilization of the lyophilizer and its use.*
- *Replicating the maximum period of time between sterilization and lyophilization.*
- *Quantitative aspects of worst-case situations, e.g., loading the largest number of trays, replicating the longest duration of loading where the chamber is open to the environment.*

Both the 2020 revision and the current EU Annex 1 Manufacture of Sterile Medicinal Products (March 2009)[1] specify that "The process simulation test *should imitate as closely as possible the routine aseptic manufacturing process*." The words "as closely as possible" are of critical importance for lyophilization as certain aspects of the "routine aseptic manufacturing process" for lyophilization would adversely affect the media itself and/or microbial recovery. First, with freezing temperatures of -50°C or below and secondary drying temperatures as high as 60°C, the normal shelf temperature extremes during the lyophilization cycle are well outside the recommended storage temperature for the nutrient medias of 2 to 25°C.[2] Best practice is, therefore, to load the media filled containers on shelves precooled as close as possible to the normal product loading temperature, but within the recommended media storage range of 2 to 25°C.[3, 4] For example, APS for a product normally loaded onto shelves precooled to -50°C would use shelves at 5°C (the target temperature for 2 to 8°C storage to avoid excursions below 2°C) throughout the simulation. Conversely, a product loaded onto ambient shelves (without shelf temperature control) would be subsequently controlled at a setpoint equal to the normal aseptic area room temperature, such as 20°C, throughout the rest of the simulation. Second, the typical 20 to 1,000 µbar (0.02 to 1.0 mbar) chamber pressures used during lyophilization would quickly boil away the media. To avoid boiling the media, the chamber pressure during APS must not drop below the equilibrium vapor pressure of the media (essentially water, 32 mbar at 25°C) at the loading temperature. Best practice, therefore, is to maintain the chamber pressure between approximately 100 and 200 mbar during simulation of sublimation and secondary drying.

The proposed 2020 Annex 1 revision further specifies that "The process simulation procedure for lyophilized products should represent the entire aseptic processing chain under specified, documented and justified conditions representing worst case operating parameters." A risk analysis for each step in the lyophilization process is presented below in Table 1.

Table 1: Relative risk of contamination by lyophilization process step.

Step	Relative Risk	Rationale
Transport from filling	Low	Grade A or unidirectional flow cart
Loading (fully automated)	Low	Grade A with no human intervention
Loading (fully manual)	High	Manual handling of open containers
Evacuation	Low	Pre-sterilized and closed system with vapor slowly exiting the open contain
'Chamber dwell' (simulation of sublimation and secondary drying)	Low	Pre-sterilized system with successful pre- and post-leak rates and reduced differential pressure. No vapor flow from sublimation or upstream pressure control leaves no mechanism for transport of organisms into the container (see discussion below)
a Aeration	High	Violent air turbulence with vapor entering the containers
Unloading	Low	Closed containers under Grade A airflow
Transport to capping	Low	Closed containers under Grade A airflow

a – While the risk to products stoppered under full vacuum is low, the risk to the APS remains high.

RECOMMENDED APPROACH AND RATIONALE

The level of risk during loading is a function of the loading method. Fully manual loading, with human operators inside the Grade A loading area who come into direct contact with the trays or containers, must be

considered high risk and the maximum load simulated in each APS. This may be achieved by loading a sufficient number of empty trays or trays with unfilled containers in addition to the media-filled containers to achieve the maximum commercial loading. Conversely, fully automatic loading, with no direct operator intervention (except allowed interventions), is considered low risk and would require only media-filled units and a sufficient number of empty, stoppered containers to fully load the last shelf with media-filled containers. Semi-automatic loading systems, where additional measures have been implemented to reduce the risk of fully manual loading, must be individually assessed and classified accordingly with a documented rationale for the APS design. As with the filling operation, corrective interventions permitted during commercial operations must be included in the challenge; specific interventions for each APS will be defined in the protocol but if product probes are used during commercial operation, this practice must be encompassed by the APS. Given that probed product containers are routinely rejected during normal production, media-filled containers with product probes must likewise be rejected after unloading and not incubated as part of the APS. Loading begins with the first opening of the chamber door and ends when the door is closed and/or the lyophilization cycle initiated. During operations where the chamber door is closed (fully or partially to minimize airflow) periodically during filling/loading, the maximum number of door openings and the total time that the door would normally remain open with containers exposed to the room should be encompassed during the APS. The time the door is closed should be captured as part of the filling operation. Similarly, unloading begins when the chamber door is opened to begin unloading and ends when the last container has been sealed. Alternatively, if unloading and capping are performed as separate unit operations with an intermediate hold, the end of unloading may be defined as removal of the last container from the chamber, with the maximum allowable storage time captured as part of the capping operation.

Shelf temperature and chamber pressure limitations that are required to avoid damaging the recovery of contaminants (i.e., adversely impacting viability or recovery) during primary and secondary drying, or “chamber dwell,” substantially alter the level of microbial risk as compared to the normal commercial process. In both cases, pre-sterilization of the lyophilizer assures sterility prior to loading. However, while vapor flow during normal product lyophilization provides a mechanism to transport organisms potentially present in the chamber into the containers, the only possible movement within the chamber during APS is slow evaporation of the media. Furthermore, while the upstream pressure control (gas injection) during the normal product process provides additional turbulence to transport organisms throughout the chamber during the cycle, the 100 to 200 mbar pressure required to prevent boiling of the media is well above the typical operating range of these systems such that nitrogen injection pressure control cannot be used during APS. With no mechanism to transport particulates into the containers during chamber dwell, the microbial risk drops to near zero and the length of the chamber dwell becomes immaterial. In addition, with some cycles lasting up to a week or longer, chamber dwells simulating the full cycle duration risk damaging media due to evaporation, particularly considering that the evaporation rate increases as chamber pressure decreases. Moreover, the higher chamber pressure required to avoid boiling the media results in a lower pressure differential between the lyophilizer chamber and the external environment such that the worst-case commercial conditions for microbial ingress during chamber dwell cannot be replicated in the APS.

Finally, the violent air turbulence during chamber aeration at the end of the cycle for both the normal commercial process and the APS may easily distribute any contamination present throughout the chamber and potentially into the media-filled units, particularly given that vapor is now flowing into the containers. Thus, aeration presents a high risk of microbial contamination during APS of the lyophilization process. Therefore, best practice to assure worst-case APS of the lyophilization unit operation is to pull the partial vacuum and then aerate to atmospheric pressure multiple times. As full vacuum would boil off the media, three partial vacuums are recommended. While a short hold after breaking vacuum and before the next partial vacuum adds value by allowing any disturbed particles to settle, holding the partial vacuum longer risks damaging the media by evaporation. Note that while stoppering under full vacuum substantially reduces the risk to product containers during aeration, full aeration must be performed prior to stoppering during APS to avoid inhibiting microbial recovery due to lack of oxygen such that aeration remains the greatest risk of failure.

In summary, the greatest risk of microbial contamination during lyophilization is, by far, redistribution of any potential contamination by the turbulent airflow during aeration, particularly given that air is flowing into the

containers. As full vacuum would boil off the media, three partial vacuums are recommended to assure a worst-case simulation. The steps in the recommended approach are listed in Table 2.

Table 2: Summary of steps in the recommended APS process.

Step	Notes
1. 1. Load the lyophilizer as per typical product loading while ensuring maximum number of door openings and the maximum open-door duration.	If it is a deviation from the normal loading process, the practice of explicitly loading top, middle, and bottom shelves during APS is unwarranted and of little value because the air turbulence during vacuum break assures distribution of any particulates throughout the entire chamber. In addition, where automated loading/unloading systems are used, any deviation from the normal loading pattern is not representative of the qualified process.
2. Reduce chamber pressure to between 100 and 200 mbar.	Chamber pressures significantly below 100 mbar must be avoided to prevent boiling the media. AIR must be used to facilitate microbial growth (except anaerobic APS).
3. Allow pressure to stabilize (-1-2 minutes), and then aerate to atmospheric pressure with sterile filtered Al R	N/A
4. Wait a certain amount of time to allow settling of any disturbed particles.	N/A
5. Repeat steps 2, 3, and 4 (second evacuation/aeration).	N/A
6. Repeat steps 2, 3, and 4 (third evacuation/aeration).	N/A
7. Stopper and unload as per typical product unloading.	Media-filled containers should not be stoppered under partial vacuum as this could inhibit microbial growth

CONCLUSION

This paper describes a best practice method for media fill simulation of the lyophilization unit operation. While previous literature presented a regulatory perspective for aseptic media fill qualification evaluating vial size and fill volume parameters,[7] the method described in this paper uses a holistic risk-based process analysis to ensure a clear worst-case APS based on sound scientific rationale and a comprehensive understanding of how the process limitations required to avoid damaging the media inherently alter the APS. This method also offers a clear operational advantage in that, by eliminating the chamber dwell during APS, the operational capacity of the lyophilizer (typically the capacity-limiting factor in the manufacture of lyophilized products) is improved.

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CALCULATING THE PROCESS CAPABILITIES OF CLEANING PROCESSES: A PRIMER

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With the publication of the ASTM E3106 Standard[1] in 2017, the pharmaceutical industry began the movement to science-, risk-, and statistics-based approaches to cleaning process development and validation. Due to this movement, process capability has become a critically important measure for demonstrating the acceptable performance of cleaning processes.[2] Process capability is also vital for measuring the risk associated with these cleaning processes[3] and ultimately for determining the level of effort, formality, and documentation necessary for cleaning validation.[4] Clearly then, an understanding of calculating process capability and how to apply it to cleaning processes is essential to implement the science and risk-based approaches of ASTM E3106. This article will explain what process capability is, the various techniques that can be used for calculating process capability, and how it should be applied to cleaning processes.

INTRODUCTION TO PROCESS CAPABILITY

Process Capability (C_p) is simply the ratio of the spread of the process data (its variability) to the spread of the specifications for that process. Basically, it is a measure of how well the spread of the data can fit within their specification range. A process is said to be capable when the spread of its data is contained within its specification spread. The smaller the spread of process data is than the specification spread, the more capable a process is. These C_p concepts are illustrated in Figure 1:

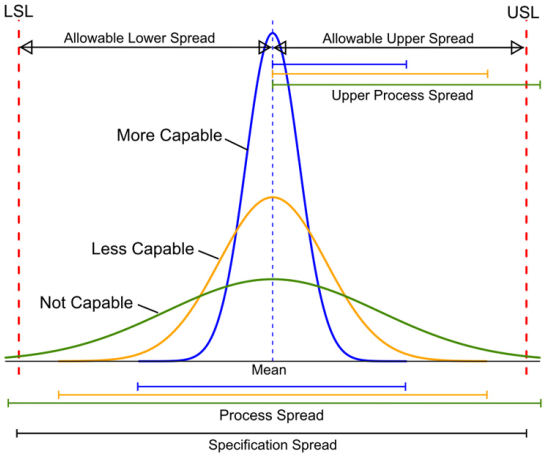

Figure 1: Comparisons of Process Spread to Specification Spread

A number of indices are used as measures of process capability or process potential. Among them are the widely used C_p, C_{pk}, C_{pl}, and C_{pu}. These four standard indices are defined in Table 1 below using the process mean (μ), process standard deviation (σ), lower specification limit (LSL), and upper specification limit (USL):

$C_p = \frac{\text{Specification Spread}}{\text{Process Spread}} = \frac{USL - LSL}{6\sigma}$	**Process Capability** (Equation 1)
$C_{pk} = \min[C_{PL}, C_{PU}]$	**Process Capability Index** (Equation 2)
$C_{PL} = \frac{\text{Allowable Lower Spread}}{\text{Lower Process Spread}} = \frac{\mu - LSL}{3\sigma}$	**Process Capability Index (Lower)** (Equation 3)
$C_{PU} = \frac{\text{Allowable Upper Spread}}{\text{Upper Process Spread}} = \frac{USL - \mu}{3\sigma}$	**Process Capability Index (Upper)** (Equation 4)

Table 1: Standard Indices of Process Capability

The indices C_p and C_{pk} are used as capability measures for processes that have both upper and lower specification limits (i.e., the specification limits are two-sided). On the other hand, for processes that only have lower or upper specification limits (i.e., the specification limit is one-sided, such as with cleaning data), C_{PL} and C_{PU} are used as measures of their capability and C_p is not. The indices C_{PL} and C_{PU} compare the lower and upper spread of the process data to the distance from the data's center (i.e., mean) to the upper specification limit and to the lower specification limit, respectively. (Note: When working with one-sided specifications, C_{pk} does not refer to the minimum value of C_{PU} and C_{PL} but to whichever the one-sided limit is upper or lower.)

Figure 2a shows hypothetical data with a mean of 100 and a standard deviation (σ) of 15 analyzed against a specification range of 25 to 175, which results in a C_p of 1.67. This is a good result. However, data are rarely centered exactly within the specification range and are normally closer to one specification limit than the other. The standard C_p calculation will not reveal this. As an extreme example, Figure 2b shows that 100% of the data can be outside of the specification range and the C_p calculation will be the same. It should be understood that process capability as measured by C_p can only reveal if the process is ***potentially capable*** of meeting the specification range — not that it does meet the specification.

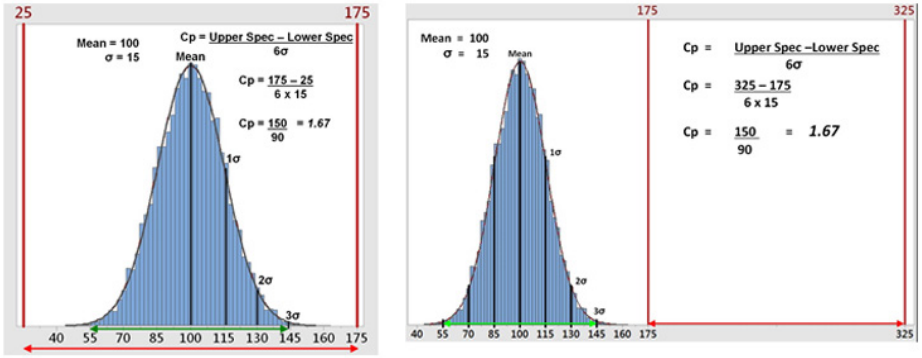

Figure 2a: Example 1 of Process Capability (C_p) *- This graph shows hypothetical data with all the data well within the specification range (25 to 175), which yields a C_p of 1.67.* ***Figure 2b: Example 2 of Process Capability (C_p)*** *- This graph shows the hypothetical data but with all the data outside the specification range (175 to 325), which still yields a C_p of 1.67.*

An improvement on the C_p calculation that provides better information about the process is the C_{pk} (process capability index) calculation. The C_{pk} index has been designed particularly for processes that are not centered within the specification range. This index only looks at the distance of the mean to whichever specification limit is closest to the mean. In these cases, either the C_{pu} (for upper specification limit (USL)) or C_{pl} (for lower specification limit (LSL)) is used, and these are simple ratios of the difference of the data's mean from the upper or lower specification limit to half of the spread of data (its variability).

Figure 3 shows plots and process capability calculations for two hypothetical data sets. The data set on the top has a mean of 100 that is centered between the upper and lower specification limits and a standard deviation of 15. As can be seen, the capability indices C_p and C_{pk} are the same for these data. Whereas the lower data set in Figure 3 has a mean of 85 that is not centered between the upper and lower specification limits; it has the same standard deviation of 15. In this case, as the mean is shifted toward the lower specification limit, the C_{pk} value is less than the C_p value. This shift makes the data set look better against its upper specification limit but worse against its lower specification limit.

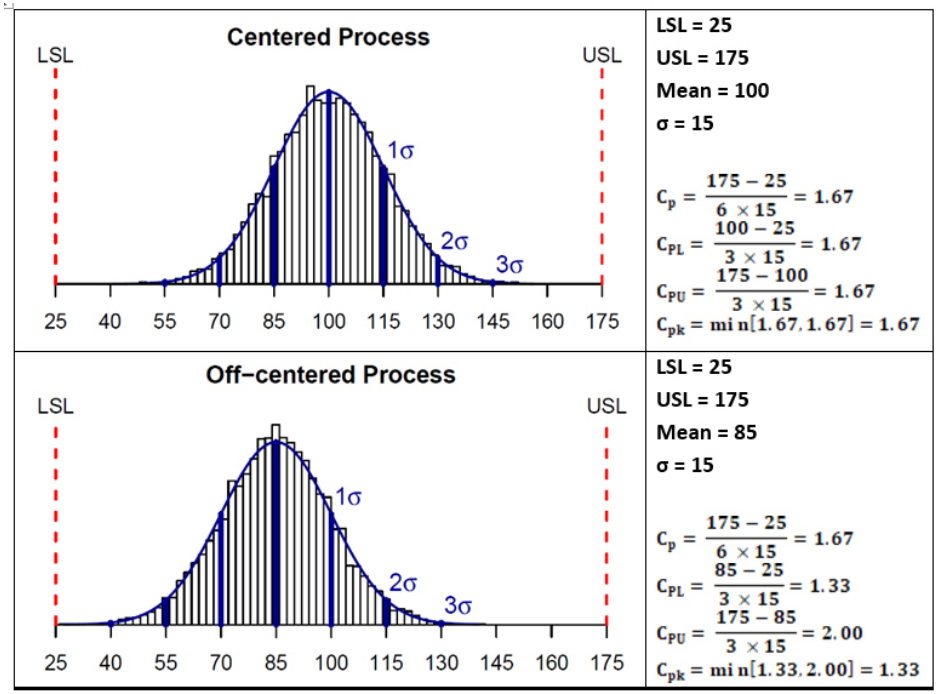

Figure 3: Examples showing calculations of Process Capability indices

ASSUMPTIONS FOR ESTIMATING PROCESS CAPABILITY

As seen from the formulas above, calculating these process capability indices (PCIs) is simple and straightforward. However, it should be noted that these calculations are based on the assumptions that the process data are normally (or approximately normally) distributed and that the process is stable (the absence of significant trends such as drifts and/or stationarity).

For cases where the process data is not normally distributed or may not be expected to be (e.g., swab results obtained from different locations during a cleaning qualification/verification run where the collection of samples is not done in a particular sequence or order), a different set of indices, known as process performance indices (PPIs), are used for comparing process data to specification limits. These indices, denoted by P_p, P_{PK} P_{PL}, and P_{PU}, are used in place of C_p, C_{PK} C_{PL}, and C_{PU} respectively, to measure compliance to specifications using a detrended estimation of sigma. The formulas for calculating these indices are the same as the ones used for calculating PCIs, the only difference being the type of standard deviation used. In estimating PCIs, group or short-term standard deviation (the one estimated using control charts) is used, whereas overall or long-term standard deviation (i.e., the standard deviation of all the values) is used when estimating PPIs.[5] Similarly, when the process data are not normally distributed (e.g., microbial data), different formulas that are based on percentiles of the fitted or empirical distribution are used for calculating PCIs or PPIs. These modified indices are interpreted the same way as the standard PCIs discussed earlier. For ease of understanding, only the notations used for standard PCIs will be used in this article.

REQUIREMENTS FOR PROCESS CAPABILITY

In Six Sigma or Operational Excellence programs, the values generated by these process capability calculations are considered critical in interpreting how acceptable a process is. The guidelines that are widely used for these values are shown in Table 2.

C_p/C_{pk} Value	Six Sigma Definitions[6]
$C_p < 1.0$	Unacceptable or "not capable"
$1.0 \leq C_p < 1.33$	Fair
$1.33 \leq C_p < 1.66$	Acceptable
$1.66 \leq C_p < 2.0$	Exceptional
$C_p = 2.0$	Goal of Six Sigma

Table 2: Six Sigma Definitions of Process Capability Values

The goal of these so-called "Six Sigma" programs has been to develop or improve manufacturing processes such that they have an additional three standard deviations (sigma) of room on both sides of their process data, which mathematically calculates to a C_p of 2.0. It should be noted that, in practice, many companies have been satisfied just to reach Five Sigma (C_p = 1.66) and feel that striving for Six Sigma (C_p =2.0) is not worth the extra cost and effort. Achieving a process capability of 1.66 is considered very good.

There are other recommended values, depending on the process being measured. For processes with two-sided specifications, Montgomery[7] recommends minimum values of C_p/C_{pk} of 1.33 for existing processes, 1.50

for new processes or for existing processes involving a critical attribute/parameter (e.g., safety or strength), and 1.67 for new processes involving a critical attribute/parameter (e.g., safety or strength). For processes with one-sided specifications, he recommends minimum values of C_{pu}/C_{pl} as: 1.25 for existing processes, 1.45 for new processes or for existing processes involving a critical attribute/parameter (e.g., safety or strength), and 1.60 for new processes involving a critical attribute/parameter (e.g., safety or strength).

Like other statistical parameters that are estimated from sample data, the calculated process capability values are only estimates of true process capability and, due to sampling error, are subject to uncertainty. Hence, to address these uncertainties, it is prudent to compare lower or upper confidence limits of the estimated process capability indices to the capability requirement when making decisions pertaining to process capability. Almost all statistical software in use today can provide confidence intervals for process capability values. For the two example data sets shown in Figure 3, 95% one-sided lower bounds for C_{PU} were estimated to be 1.47 (centered process) and 1.75 (non-centered process). Since this value is lower than 2.0, we cannot conclusively infer that the process has met that Six Sigma goal.

CLEANING PROCESS CAPABILITY

As we have seen, process capability is an important process performance measure that is relatively simple to calculate, as it only requires the mean and standard deviation of data from the process and the specification limits for that process. So, in the case of a cleaning process, the process capability of the cleaning process can be calculated from the mean and standard deviation of the swab or rinse data from the cleaning process and the Health Based Exposure Limit (HBEL)-based cleaning limits for the swab or rinse data.

To determine process capability (C_{pu}) of a cleaning process, the terms in this equation can be substituted with the values estimated from cleaning (swab or rinse) data and the HBEL-based cleaning limit as shown in the example in Equation 5.

$$\mathbf{Cpu}_{\mathbf{Cleaning}} = \frac{\mathbf{Cleaning\ Limit}_{\mathbf{HBEL}} - \mathbf{Mean\ of\ Cleaning\ Data}}{\mathbf{3\sigma\ of\ Cleaning\ Data}} \quad \text{(Equation 5)}$$

Statistical software such as Minitab, JMP, "R," and many others are capable of performing process capability analyses and graphing the results, such as the example report in Figure 4.

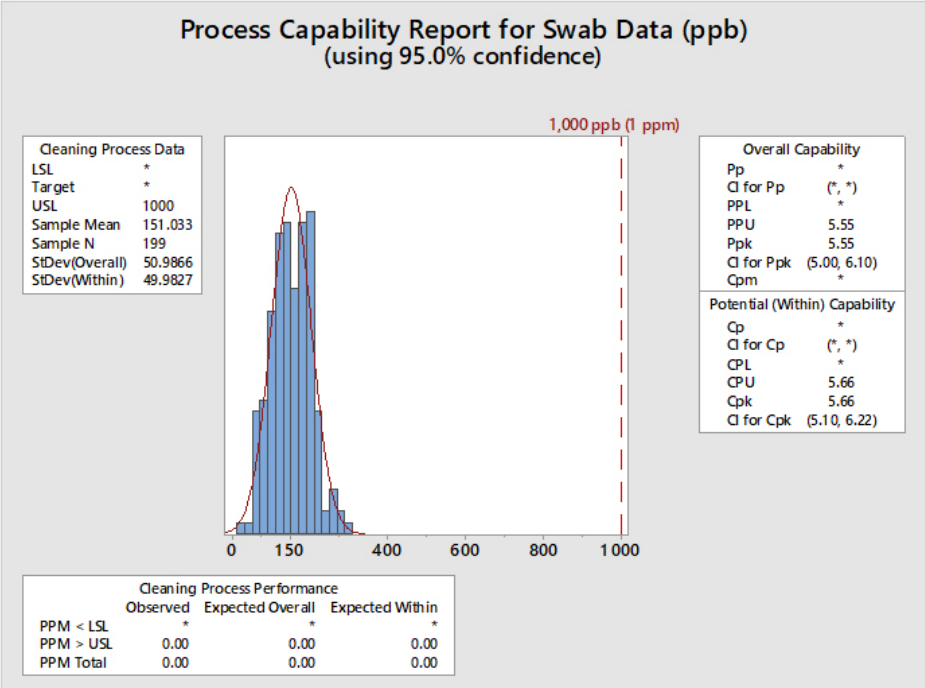

Figure 4: Example of Process Capability Analysis in Minitab for Hypothetical Swab Data *- This graph shows the process capability of swab sample data (N=199) with a mean of 151 ppb and a standard deviation of 50 ppb. The hypothetical HBEL-based limit is 1,000 ppb or 1 ppm. The process capability is shown as the process performance upper (P_{PU}) for the overall capability with a value of 5.55 and as process capability upper (C_{PU}) for the potential (within) capability with a value of 5.66. Minitab provides the option of calculating confidence intervals for the process capability values since the mean, standard deviation, and N are all known. This option is especially useful when there is a small number of samples. The confidence intervals for the C_{PU} are 5.10 and 6.22, meaning that based on the number of samples (N), the C_{PU} could be as low as 5.10 or as high as 6.22. The graph also shows the number of observed and expected failures per million (PPM &GT USL) is 0.00.*

Also, since process capability values are derived from the mean and standard deviation, upper and lower confidence intervals can be calculated for these process capability indices. In cases where there may be varying or small sample sizes (e.g., where N is typically < 25) it is prudent and recommended to report and use the lower confidence limit of the C_{pu} from these calculations instead of just the C_{pu} itself. Almost all statistical software in use today can provide confidence intervals for process capability values. Figure 4 shows an example using Minitab 17. In the textbox on the right, from the output from Minitab reports, the C_{pu} as 5.66 and additionally reports that the 95% confidence intervals (CI) for the C_{pu} range from 5.10 to 6.22. (Note: Minitab reports the CI for the C_{pk}, which is either the C_{pu} or the C_{pl}; in this case it is the C_{pu}.)

Minitab can also report the expected number of failures out of a million based on the process capability analysis. In this example, the lower textbox reports, based on these data, that there are 0.00 possible failures out of 1 million (i.e., exceeding the upper specification limit). This indicates that there is a very low probability of a cleaning failure in this example.

PROCESS CAPABILITY OF NON-NORMAL DATA

It is important for the reader to understand that the calculations (in equations 4 and 5) for estimating process capability are based on the assumption that the process data are normally (or approximately normally) distributed. However, not all cleaning data follow a normal distribution. For example, while swab data for total organic carbon analysis are frequently normally distributed, HPLC data are frequently not. Before performing any statistical analysis of cleaning data, it is important to determine whether the data are normally distributed or not. If the cleaning data are not normally distributed, the following options can be used in these situations:

1. Transform the data to a Normal distribution. When this strategy is followed, limits should be also transformed accordingly.
   - The Box/Cox Transformation (power) raises the data to either the square, the square root, the log, or the inverse.
   - The Johnson Transformation selects an optimal transformation function.
2. Identify the data distribution and evaluate the data using a non-normal distribution model.[8,9]
   - Lognormal, Gamma are some examples
3. Evaluate the data using a non-parametric method.
   - Empirical percentile method

When using option 2, it is necessary to identify which of the many non-normal distribution models to use. Minitab has a test (Individual Distribution Identification) that can evaluate data against 12 different and common distributions. Based on these evaluations, the best-fitting distribution model can be selected. For example, Figure 5 shows a graph generated by Minitab comparing four distributions (normal, lognormal, gamma, and exponential). The data set being analyzed is randomly generated data from a gamma distribution. The interpretation of the graphs is based on a visual examination of how well the data fall along the expected (red) line and whether the P-value of the Goodness of Fit Test for the distribution is greater than 0.05. The red line plots where the data should fall if they follow that particular distribution. If the data do not fall along the red line, then the data are unlikely to be from that distribution. If the data appear to follow the red line, the next criterion to check is the P-value. By convention, the P-value should be greater than 0.05, indicating there is at least a 1:20 chance that the data follow this distribution, and it is considered safe enough to analyze the data using this distribution.

In Figure 5, the plots for the normal and lognormal distributions are clearly off their red lines and also have P-values of <0.005. Consequently, these distributions would be appropriately rejected as good models for analyzing these data. The gamma and exponential plots closely follow their red lines and appear very similar to each other. Examination of their P-values reveals the gamma to be 0.250 and exponential to be 0.522. Both distributions pass the criteria for selection.

But should the exponential distribution be selected since it has the higher P-value even though we know the data came from a gamma distribution? The higher P-value does not indicate that the exponential is the best distribution to select; it only means that the distribution of the data is not significantly different statistically from the exponential distribution and there is just less evidence to reject the exponential distribution as a good fit for the data. The choice of which distribution to use should be based on where the data were

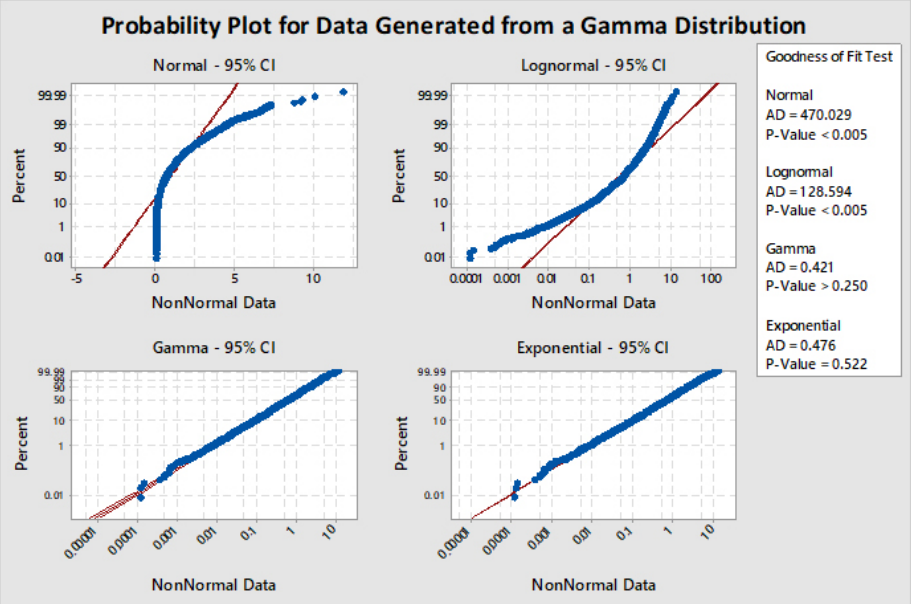

Figure 5: Individual Distribution Identification *- This graph shows the evaluation of a data set of 10,000 random values generated from a gamma distribution. Minitab allows for testing the data against 12 distribution models and two transformations (Box-Cox and Johnson). The evaluation is performed both*

collected from and what type of distribution the data would be expected to follow. It should also be understood that the gamma distribution is actually a "family" of distributions and, depending on its shape and scale parameters, the gamma distribution can closely follow the exponential distribution, which explains the results in Figure 5.

Figure 6 shows process capability analyses of the data from Figure 5 using the gamma distribution (left) and the exponential distribution (right). It is immediately evident that the data are well described by both distributions and the process capability results are basically identical. In this case, and in many other cases, the choice of the distribution model is not critical. In addition, a visual inspection of the data may be helpful when deciding among multiple distributions that statistically fit the data.

As an alternative to using non-normal distributions, there are different formulas based on percentiles of the fitted/empirical distribution that can be used for calculating process capability. If the distribution of process data is known,

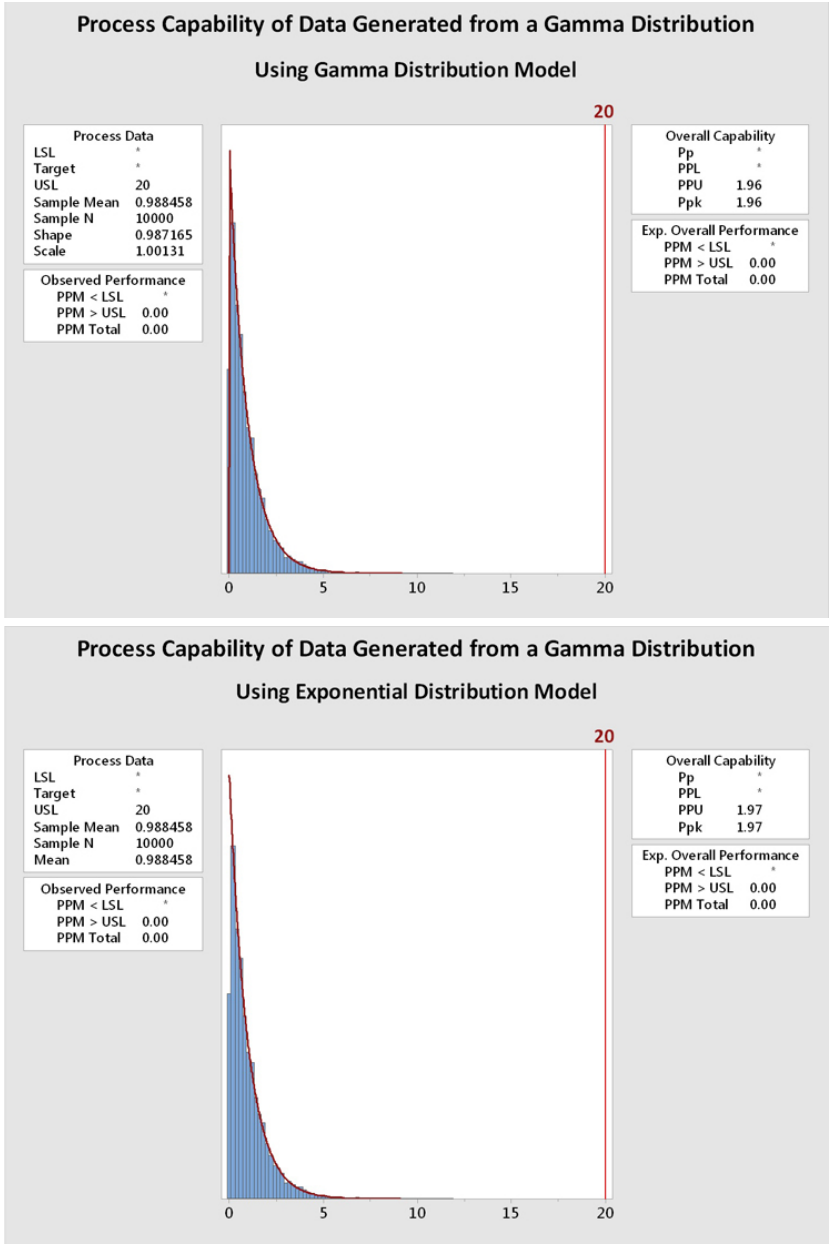

Figure 6: Process Capability Analysis of Data Generated From a Gamma Distribution using Gamma and Exponential Distributions *- These graphs show the process capability evaluation of the data set of 10,000 random values generated from a gamma distribution using both gamma and exponential distributions. There is no difference in the analysis of the data. This is due to the*

or can be assumed, the formula in Equation 6 is used for calculating process capability index C_{pu}. In the equation, P_{50} and $P_{99.865}$ are the 50th (median) and 99.865th percentiles of the specified distribution, respectively.[6]

$$C_{pu} = \frac{USL - P_{50}}{P_{99.865} - P_{50}} \quad \text{(Equation 6)}$$

Similarly, when the underlying distribution of process data is unknown, a non-parametric (distribution-free) variant of C_{pu} calculated by empirical percentile method proposed by McCormack et al, can be used.[8] The C_{pu} calculation using this method is shown in Equation 7. In the equation, P_{50} and $P_{99.5}$ are the 50th (median) and 99.5th percentiles of the empirical distribution, respectively.

$$C_{pU} = \frac{USL - P_{50}}{P_{99.5} - P_{50}} \quad \text{(Equation 7)}$$

This approach can only be used when sample size is ≥100. A macro for calculating non-parametric capability indices based on McCormack's approach is available on Minitab's website.[10]

These modified indices are interpreted the same way as the standard C_{pu} discussed earlier. Examples of decision trees to identify the calculation method for C_{pu} are given in Appendices I and II.

"ALL MODELS ARE WRONG, BUT SOME ARE USEFUL"

It may be surprising for some readers to realize, as we saw in Figure 5, that more than one distribution model may be used to analyze data instead of the "correct one." Actually, this is perfectly acceptable as, in truth, there is no "correct one" or "absolutely true" model. The quote starting this section is typically attributed to the statistician George E. P. Box, and this quote is considered famous, primarily among statisticians.

George E. P. Box expanded on this idea, stating:

> *"Remember that all models are wrong; the practical question is how wrong do they have to be to not be useful."*[12]

Box's comment is most relevant to this article since there are voices in the cleaning validation community claiming that process capability is not appropriate for cleaning or cannot be determined since cleaning data are non-normal or simply that statistics is unnecessary for cleaning. Consequently, there has been hesitancy to adopt these techniques out of fear that the analysis may be calculated incorrectly and serious errors made. However, comparisons of process capabilities calculated using multiple distributions have shown that such errors are minimal if an appropriate selection process has been followed.[13] Appendices I and II offer two decision trees that can be followed to minimize errors in selecting models.

CONCLUSION

Many pharmaceutical companies have implemented Six Sigma programs in the past 10 years or more and have come to understand the significance and value of process capability analysis. These companies have also come to understand that process capabilities less than 1.00 are inadequate. They also understand that process capabilities greater than 1.66 are within reach and the benefits of achieving this level of process capability is very much worth the effort. For many cleaning processes it is possible to easily achieve process capabilities of greater than 10! In fact, the suggested values for Six Sigma programs in Table 2 are not appropriate for cleaning processes, as they are easily capable of much higher process capabilities.

The assessment of process capabilities provides important knowledge and understanding about the cleaning process and allows for a quantitative measure of the risk to the patient from carryover of residuals. Understanding the level of risk that cleaning process capability can provide can also facilitate implementing the second principle of ICH Q9 and justify a "level of effort, formality and documentation commensurate with the level of risk" for the cleaning validation process.[4] The knowledge and understanding gained from such cleaning process capability estimates can even justify the use of simpler analytical methods such as visual inspection for cleaning verification or validation.[14, 15]

PEER REVIEW

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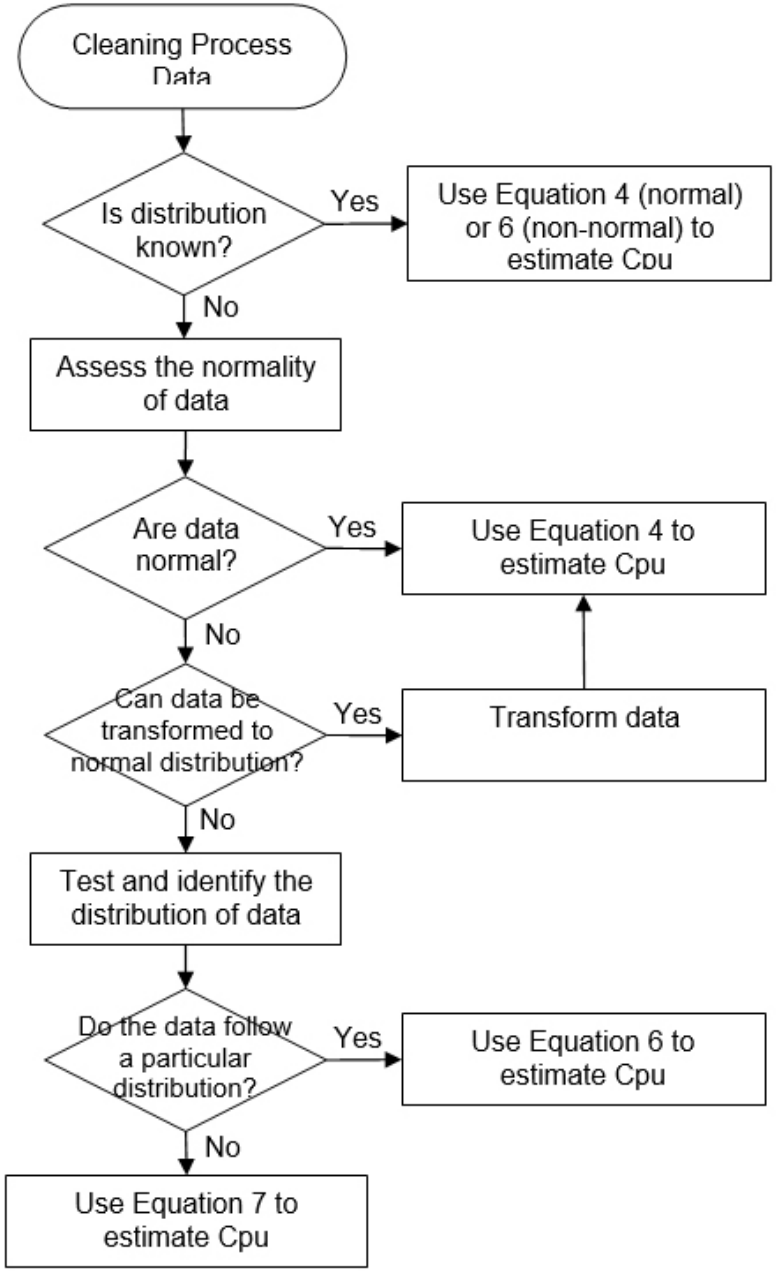
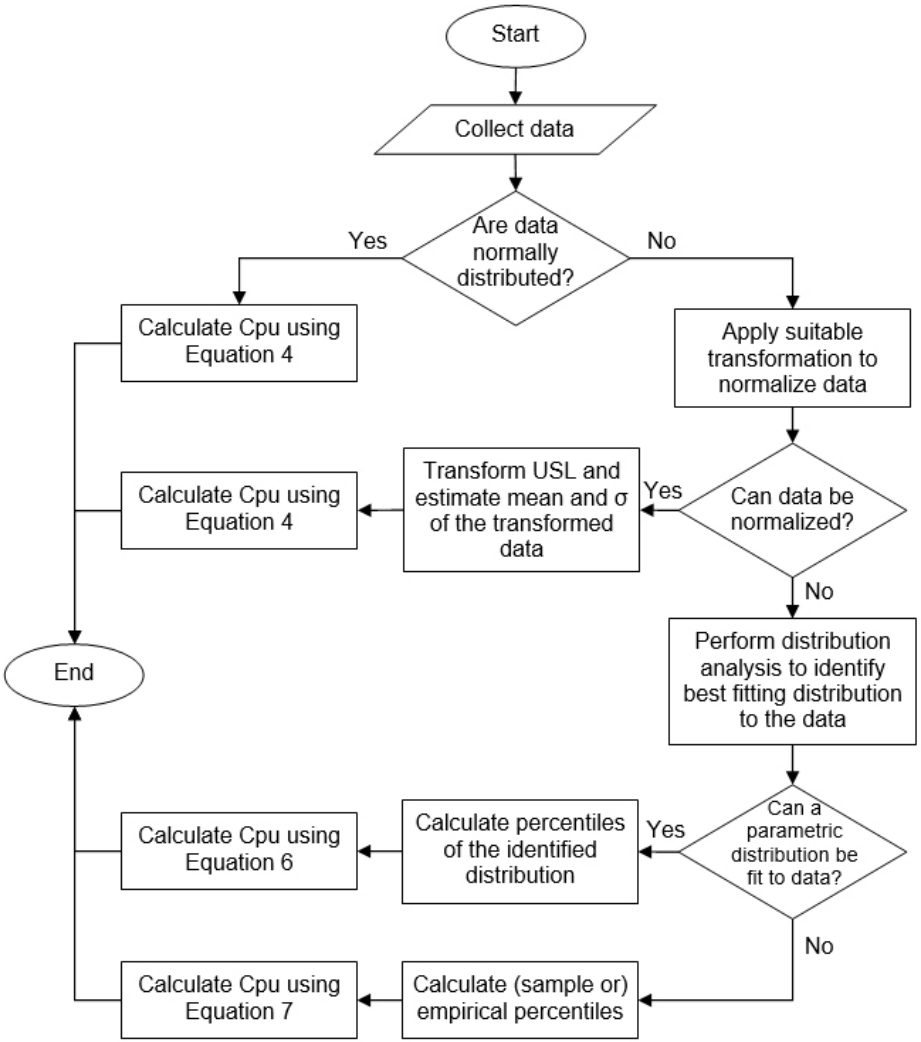
APPENDIX I

Decision Tree to Identify the Calculation Method for Cpu (Example 1)



APPENDIX II

Decision Tree to Identify the Calculation Method for Cpu (Example 2)



THE ESSENTIAL COMPONENTS OF A STERILITY ASSURANCE PROGRAM

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Regional Manager, PSC Biotech

Sterility assurance is a level of confidence that a particular product or unit that is purported to be sterile is sterile. Sterility cannot be demonstrated without the destruction of every unit of product produced. Therefore, sterility assurance is achieved through multiple practices and procedures. This article discusses the different variables of contamination control that help to increase confidence in sterility assurance.

USP <1211&GT is a general information chapter on sterility assurance. The chapter states that "an item is deemed sterile only when it contains no viable microorganisms."[1] It is well known in the industry that the sterility testing model described in USP <71&GT Sterility Tests has limitations. The test only indicates that the subset of articles from a lot that are tested are sterile. The test is destructive in that every unit that is tested is either consumed or no longer sterile after the test is performed. To help ensure consumer safety, additional measures must be put into place to add assurance that the entire batch or lot of products manufactured is sterile. This is accomplished using a sterility assurance program.

Many companies have sterility assurance programs in place but may not have their processes labeled as a sterility assurance program. Figure 1 from USP <1211&GT shows several factors that influence sterility assurance. The factors listed in Figure 1 should be considered for their impact on the sterility of the final product.

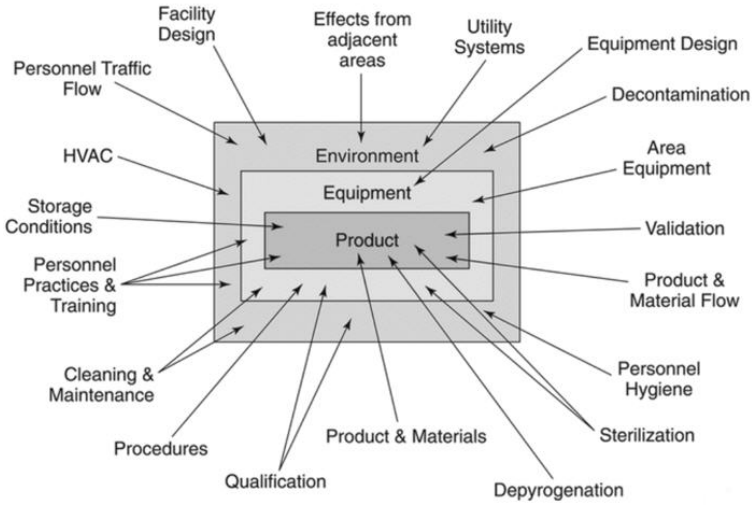

Figure 1: Influences on Sterile Products.

The first step in developing a sterility assurance program is to list each step in the process, beginning at the point of use and ending in sterile storage.[2] Each step should be evaluated for ways to prevent contamination in the manufacturing process or environments.

A sterility assurance program should be fit for purpose for the product or device that is being manufactured. A holistic sterility assurance program for an aseptic manufactured product could include the following components.

PERSONNEL

Personnel must be properly trained, educated, and/or supervised to be involved with aseptic processing. The training records must be maintained. Training concepts should include the importance of proper aseptic technique and cleanroom behaviors. It must be recognized that humans are the primary source of contamination in the cleanroom environment. Retraining and qualification of personnel should be done on a routine basis to keep personnel sensitized to the importance of aseptic technique.[3]

PERSONAL HYGIENE AND SANITATION PRACTICES

There must be procedures and training that govern personnel hygiene, sanitation, aseptic technique, aseptic behavior in the cleanrooms, and aseptic gowning practices. Personnel must adhere to sanitation and health precautions designed to avoid contamination of the test, environment, and/or product. Personnel must also adhere to gowning and personal protective equipment procedures. If an employee is feeling ill, they must inform their supervisor of any health or medical condition that may have an adverse effect on a test, product, or environment. Personnel must also be monitored for microbial growth and undergo gowning qualification training to ensure aseptic status of the manufacturing or testing environment.

PERSONNEL FLOW

There must be procedures and practices regarding personnel flow. Personnel must follow established entry and exit routes to prevent cross contamination. The routes should include different levels of gowning for each grade of the cleanroom environment. These routes must also be established in standard operating procedures (SOPs) and understood by personnel.

PROCEDURES

All procedures for aseptic processing and subsequent quality control testing must be written in accordance with good manufacturing practices (GMPs). Procedures must remain up to date, accurate, and revised when warranted.

PRODUCT AND MATERIAL FLOW

Like the procedures and practices regarding personnel flow, there must be procedures and practices for product and material flow to prevent cross contamination. The routes should include levels or methods of sanitization of products, materials, and/or waste as they enter or exit the cleanroom areas. These routes must also be established in standard operating procedures and understood by personnel.

EQUIPMENT

The use and preparation of equipment for aseptic processing must be documented in SOPs. It must be designed appropriately for the intended use and housed in a manner to prevent cross contamination. Equipment used in the generation, measurement, or assessment of data and equipment used for facility environmental control must be of the specified design and capacity to function according to GMPs. The equipment must be suitably located for operation, inspection, cleaning, and maintenance. It must be inspected, cleaned, and maintained. Equipment used for the generation, measurement, or assessment of data must be tested, calibrated, standardized, and/or sterilized.

STERILIZATION

The use and sterilization of equipment, components, or other materials for aseptic processing must be governed in SOPs. This could include purchasing items that are ready to use or preparing the items for use in-house.

DEPYROGENATION

Likewise, the use and depyrogenation of equipment, components, or other materials for aseptic processing must be governed in SOPs. This could include purchasing items that are ready to use or preparing the items for use in-house.

DECONTAMINATION

Decontamination practices for aseptic processing must be documented in SOPs. This could include chemically sanitizing equipment to take into the cleanrooms, wiping items down with disinfectants, or using decontamination devices such as vaporized hydrogen peroxide (VHP) generators or autoclaves.

FACILITY DESIGN

The design of the facility should be documented on maps and flow diagrams to help personnel in their daily tasks. The facility must be constructed to prevent microbial contamination. This could include items like differential pressure cascades, the use of classified areas, and pass throughs to name a few items.

There must be separate areas available for the storage and quarantine of materials. The warehouse must be neat, clean, and orderly, with temperature/humidity controls where appropriate. Cardboard or other items containing cellulose fibers should not be allowed in clean areas as they could be a source of mold contamination.

Laboratory practices must also be implemented to prevent microbial contamination from outside of cleanrooms. This could include changing uniforms and shoes and using proper aseptic gowning practices.

Automated or separative manufacturing designs such as isolators or restricted barrier access systems (RABS) can be utilized in the manufacturing areas to prevent cross contamination.

High efficiency particulate air (HEPA) and heating, ventilation, and air conditioning (HVAC) systems should be used with differential pressure cascades, temperature controls, and humidity controls to prevent microbial contamination. If the temperature is too hot or humid, people could sweat, compromising their cleanroom gowning. In addition, when pressure cascades are not controlled properly, microbes could enter the cleanrooms. Excessively humid environments can increase the potential for fungal contamination.

EFFECTS FROM ADJACENT AREAS

The effects from adjacent areas should also be considered. If an adjoining room has microbial contamination, that contamination could migrate into the inner core of the cleanrooms. Transition areas should be monitored and controlled.

SUPPLIER QUALIFICATIONS

Qualifying suppliers is an important approach to control items that are purchased sterile and ready to use. It is important that vendors are trusted to provide quality supplies to maintain sterility assurance of the final product that is being manufactured. Supplier qualifications must be governed by SOPs.

VALIDATION AND QUALIFICATIONS

Clean areas must be validated and maintained. This should include environmental monitoring qualification programs (EMPQ) and cleanroom qualifications. Equipment should also be qualified for use in the cleanrooms. There should be cleaning validations that include clean and dirty hold times of equipment.

Also, in the realm of validations and qualifications is the topic of process validations (i.e., media fills). Media fills help to demonstrate that the manufacturing process can produce a sterile final product. The manufacturing process should include interventions and aseptic connections. “An appropriate microbiological media will be treated as if it were product, and it will be put through an entire manufacturing process simulation. This study will show contamination control effectiveness throughout the manufacturing process.”[4]

DECONTAMINATION, CLEANING, AND DISINFECTION PROGRAMS

Decontamination, cleaning, and disinfection programs must be established. The programs must be governed by SOPs and should describe what gets cleaned, how the cleaning is performed, how often the cleaning is performed, what cleaning agents are utilized, and the validation of the cleaning, decontamination,

or disinfection process. Room cleanings should include items like the walls, floors, ceilings, and equipment. Again, there should be established clean and dirty hold times for equipment and the cleanrooms.

When utilizing disinfectants, consider items like disinfectant efficacy date, wet contact times, and the method of application of the disinfectants. Cleaning, disinfection, and/or decontamination concepts should be considered for both product contact and non-product contact surfaces.

PRODUCT AND MATERIALS

Products and materials must also be controlled to prevent contamination and increase sterility assurance. Raw materials, components, active pharmaceutical ingredients, container closures, and product contact surfaces should all be monitored and controlled. "Sterility must be assured for cleaning solutions, tools and equipment, raw materials, container closures, and any other materials that will be introduced into the area."[4]

MANUFACTURING PRACTICES

As previously mentioned with media fills, the manufacturing process must be monitored and controlled. This includes interventions and aseptic connections. Proper aseptic technique and behaviors must be utilized to prevent cross contamination of product during manufacturing.

LABORATORY TESTING

Laboratory testing to consider when thinking of sterility assurance programs include items like sterility testing, endotoxin testing, bioburden testing, raw material testing, in-process testing, and container closure integrity testing. "A sterile product is to undergo analysis for microbial endotoxins and sterility testing to assure the absence of contamination."[4] This is not an all-inclusive list of laboratory tests, but these quality control assays help boost the confidence in sterile products by providing a secondary layer to sterility assurance practices.

ENVIRONMENTAL MONITORING PROGRAM

Environmental monitoring assesses the microbial contamination level in the cleanrooms and adjacent areas. This data may highlight areas that need extra cleaning, monitoring, and/or maintenance. Air and surfaces are routinely monitored within the cleanrooms to make sure the environment continuously meets specifications.[4]

UTILITY AND WATER SYSTEMS

Monitoring of utility systems includes water systems, compressed air, compressed nitrogen, compressed oxygen, or similar systems. Depending on the system, testing could include bioburden, total organic carbon (TOC), conductivity, or non-viable particulate sampling. When thinking of utilities, it is important to design facilities so that there are no drains or sinks in the cleanrooms as these can be a source of microbial contamination.

STORAGE CONDITIONS

When considering storage conditions, it is important to think of warehouse cleanliness, order, and quarantine areas. Temperature and humidity should be monitored and controlled when required. Conditions should be maintained to ensure the sterility of the final product. In addition, container closure integrity should be established to ensure the product remains sterile in its packaging.

CONCLUSION

The components described above of a sterility assurance program for an aseptic manufactured product do not comprise an all-inclusive list. Rather, this list demonstrates the breadth of the items that could impact the sterility of a product or device that is purported to be sterile. When designing a sterility assurance program, consider all the items that could impact sterility and develop procedures and methods that will increase the assurance of the sterility of the product or device and minimize risks to consumers.

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ESTABLISHING BEST PRACTICES FOR RISK-BASED ENVIRONMENTAL MONITORING OF MODERN DRUG PRODUCT FACILITIES

Dawn Watson

Director - Sterile Technology & Commercialization, Merck

EM programs are one of the most effective tools in monitoring the state of control of classified manufacturing areas.

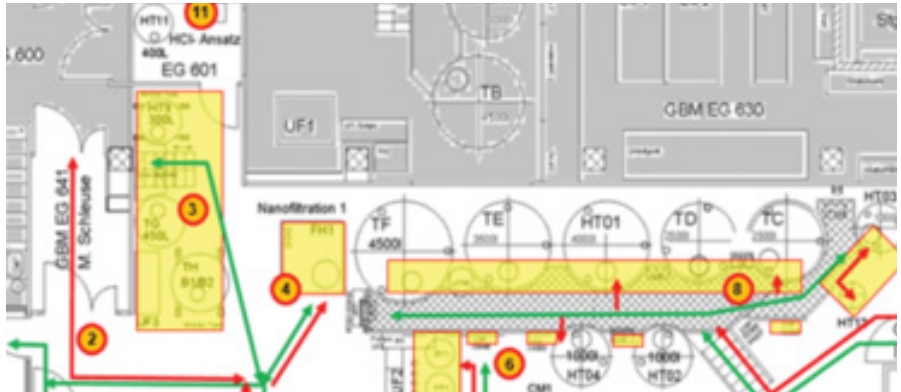
Environmental monitoring (EM) data provide assurance that medicines made in classified manufacturing areas are safe for patients and supply is not interrupted due to contamination issues. An EM program is thus a vital part of the quality system in all modern drug production facilities and is the subject of scrutiny by regulatory agencies.

An EM program should provide verification that a particular area is capable of being maintained within the appropriate microbiological contamination limits required by the respective classification of that area. Data from an EM program provide information about aspects of contamination prevention such as facility design, heating, ventilation, and air conditioning (HVAC) performance, high efficiency particulate air (HEPA) filter system function, the cleaning and sanitization program, effectiveness of aseptic techniques used during interventions, personnel behavior, gowning practices, material and personnel flow, equipment used, and ongoing verification of the bio-decontamination cycles in isolators. An EM program is only reliable, however, if an appropriate number of sampling points are located in the right positions in concordance with risk levels using appropriate sampling methods.

Contamination risks in biopharmaceutical manufacturing vary depending on the type of room and the manufacturing processes used. A risk-based approach to the design of EM programs is expected by regulatory agencies (see references). It is standard practice in the industry to perform a risk assessment in the facility and to design an EM program to match the risk profile. It is a well understood regulatory expectation that facilities use scientifically justified rationales for sample locations and inspectors review supporting documentation. Key legislation and guidances are referenced below.

A STANDARD IS REQUIRED TO ESTABLISH BEST PRACTICES

Designing an EM program is a relatively complex task; facilities vary considerably and there is inadequate specific guidance on how to design EM programs and no single standard risk



assessment methodology exists. It is left to each facility and regulatory agency inspector to determine the risk factors for microbiologic contamination and the adequacy of monitoring.

Whilst there are many different tools available, there is no consensus on best practice for a risk assessment (RA). RA tools used to date have been originally developed for assessing process or product risks, such as failure modes and effects analysis (FMEA) and, in the food industry, hazard analysis and critical control points (HACCP). These RA tools are useful to a degree but are of limited use for risk-based EM as they do not include guidance on how to perform an RA systematically, which risk factors to consider, how to assess the risk factors for relative risk, and how to design an EM program according to risk level.

Historically, EM programs have evolved following a "more is better" approach, largely due to corrective actions to microbial excursions that have occurred during batch manufacturing and regulatory agency inspector observations, which often differ from one inspector to another. Without best practice guidance, existing EM programs can be difficult to explain or justify and may not be assessing the highest-risk locations with most probable microbial recoveries. Sustaining best practice over time is also more difficult without an accepted standard. Moreover, interventions to conduct EM in themselves may not be evaluated as part of the overall risk assessment. EM programs are typically reflective of traditional aseptic processing paradigms. When we compared EM programs amongst different companies and within companies, there was a substantial variation with respect to sample location, sampling frequency, and sampling method. In fact, without a framework and common language, it was even difficult to discuss and compare EM programs. This wide variation leads to further uncertainty about whether the EM program is fit for purpose. The rationale of an EM program should be easy to follow and compare with best practice.

An industry standard would reduce the need for individual judgement, ensure minimum sampling requirements for all higher risk locations, and enable the adoption of best practices across the industry. Moreover, as facilities modernize, a standard provides a framework for developing EM programs in line with technological improvements.

A PROPOSED BEST PRACTICE GUIDANCE DOCUMENT

The authors took on the challenge of developing a harmonized risk-based methodology. Collaborating for four years and facilitated by BioPhorum, we have drawn on many years of collective experience designing EM programs for a wide range of facilities across the biopharmaceutical manufacturing industry. As far as we are aware, this is the first attempt to comprehensively document and propose best practices.

The first version of this guidance document, *Environmental monitoring (EM): harmonized risk-based approach to selecting monitoring points and defining monitoring plan*, was published in early 2019. It comprises a methodology that addresses the four key questions for the design of an EM performance qualification (EMPQ) program and subsequent routine EM program:

1. What are the risk factors to consider?
2. How to systematically assess a room with these risk factors?
3. How to define risk levels?
4. What are the minimum standards for monitoring for different risk levels?

Covered in the guidance document are grade A-D rooms, including conventional aseptic filling lines, RABS, isolators, background rooms, and support rooms *(see Fig. 1 page 38)*

The risk assessment tool systematically evaluates locations within the facility/filling line against standard environmental control elements to determine which areas have the most potential for re-contamination of isolators or contamination risk of rooms during routine manufacturing processes. The six factors identified are:

1. Amenability of equipment and surfaces to cleaning and sanitization.
2. Personnel presence and flow.
3. Material flow.
4. Proximity of open product or product contact material.
5. The need for interventions/operations by personnel and their complexity.
6. The frequency of interventions (only applicable for grade A). *(see Fig. 2 page 39)*

The guidance document describes how to use these six factors to systematically assess all areas of a room and ranks them according to probability of contamination. A set of principles and minimum standards is then proposed to determine where to locate sampling points and select sampling methods to match risk levels. *(see Fig. 3 page 39)*

NEXT STEPS TOWARD HARMONIZATION OF BEST PRACTICES

The guidance has now been updated with a second version. The update to the approach includes revisions in response to feedback and incorporates context from a series of case studies, which we believe adds significant value for all stakeholders in adopting a global standard.

The toolkit is available for free download from the BioPhorum website.

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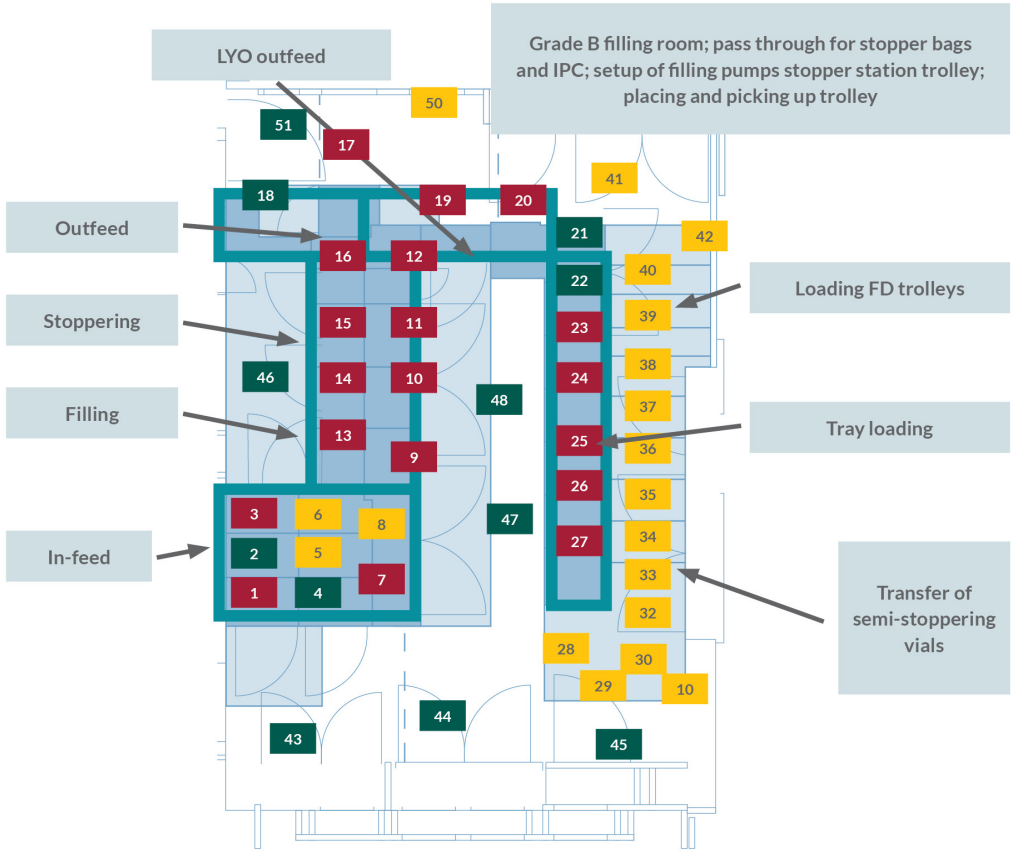

Figure 1

	Factor 1: Amenability of equipment and surfaces to cleaning and sanitization	Factor 2: Personnel presence and flow	Factor 3: Material flow	Factor 4: Proximity to open product or exposed direct product-contact material	Factor 5: Interventions/ operations by personnel and their complexity	Factor 6: Frequency of interventions/ process operations	Risk score (product of six ratings)
Low 1	1	1	1	1	1	1	1
Medium 2	2	2	2	4	2	2	128
High 4	4	4	4	8	4	4	8192

Figure 2

Lower-risk grid	For EMPQ and routine EM sampling: sampling is not mandatory in these grids because of the lower-risk categorization (but it is optional in the context of an overall EMPQ monitoring plan).
Intermediate-risk grid	For EMPQ sampling: the minimum sampling in this grid is required. Place in this grid at least one from the list below: • Non-viable • Viable – active • Viable – passive • Viable surface For routine EM sampling: this depends on the results from the EMPQ (in practice, there would usually be less monitoring than that performed during the EMPQ). Compare the number of excursion results during respective EMPQ and, if above the pre-set limit, then routine monitoring is recommended in this grid along with investigation and corrective/preventive actions.
Higher-risk grid	For EMPQ and routine EM sampling: the minimum sampling in this grid is required. Place in this grid at least one from the list below: • Non-viable • Viable – active • Viable – passive • Viable surface Unless the minimum required is covered by sampling in an adjacent grid, which is in the same functional section, the sampling location can be between the two adjacent grids. It is recommended that sections that have grids with higher scores have viable and non-viable monitoring defined for initial qualification. They should also be considered for inclusion in the routine EM program if the identified risk factors are not minimized through the introduction of control measures. The type of sampling selected depends on the nature of the activity in this grid and/or the line/process configuration.

Figure 3

AN INTRODUCTION TO TRENDING IN ENVIRONMENTAL MONITORING PROGRAMS

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Trending environmental monitoring (EM) data is a regulatory requirement. However, it is useful in obtaining significant information about the facility. Trends can help determine if a facility is in a state of microbial control and relay the environmental monitoring data to facility management in a meaningful format. In this two-part article exploring environmental monitoring trending, I look first at the regulations and guidelines around EM. In part two, I will discuss tools and best practices for using the trends to ensure that an efficient environmental monitoring program is established.

EM is a required, essential component of current good manufacturing practices (cGMP). It is used to measure and monitor the microbial bioburden levels in a facility and to determine if the facility is in a state of microbial control. EM consists of many different data points that culminate in a single program. Some of the data points in EM include:

- Non-viable particulates
- Active viable air samples
- Passive viable air samples (also known as settle plates or fall-out plates)
- Surface samples (e.g., contact plates and/or swabs)
- Personnel monitoring
- Microorganism identifications
- Some companies include water system monitoring and/or compressed gas system monitoring in their EM trending program as well.

Analyzing the data generated from EM one data point at a time can be difficult to decipher and the overall health of the facility environment may be misinterpreted. EM trending helps to overcome this difficulty. EM trends examine data over time to look for changes or movements in a general direction. The Parenteral Drug Association Technical Report 13 (PDA TR13) describes trend analysis as "a review performed in response to an alert or action condition. This review provides an analysis of specific environmental monitoring data to identify adverse trends."[1]

EM trending can be performed for many reasons, including:

- Regulatory compliance
- Ensuring a state of control of the facility
- The ability to be proactive before a problem gets out of hand
- To provide a graphical representation of the data
- To determine any problem areas in the facility
- To determine if the cleaning and disinfection program is working as expected
- Monitoring the microbial flora of the facility and seasonal trends
- Providing a simpler means of communication of the EM data to management
- Identifying sources of microbial contamination.
- Establishing alert and action levels

There are a multitude of acceptable configurations in which the data can be presented in EM trend reports due to the various components of the EM program. The importance of performing EM trending has been demonstrated repeatedly and this importance has not been lost on regulators. The Pharmaceutical Microbiology Manual published for the FDA states that each laboratory is required to monitor and trend data to ensure compliance and detect any abnormalities.[2]

REGULATIONS AND GUIDANCE DOCUMENTS

Regarding regulations and guidelines, there are many sources that mention the need for EM and trending. The following excerpts from various sources is not intended to be an all-inclusive list.

- 21 CFR 211.113(a): "Appropriate written procedures, designed to prevent objectionable microorganisms in drug products not required to be sterile, shall be established and followed."[3]
- 21 CFR 211.113(b): "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of all aseptic and sterilization processes."[3]
- FDA Guidance for Industry, Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice (2004).[4]
  - "The quality control unit should provide routine oversight of near-term (e.g., daily, weekly, monthly, quarterly) and long-term trends in environmental and personnel monitoring data. Trend reports should include data generated by location, shift, room, operator, or other parameters. The quality control unit should be responsible for producing specialized data reports (e.g., a search on a particular isolate over a year period) with the goal of investigating results beyond established levels and identifying any appropriate follow-up actions. Significant changes in microbial flora should be considered in the review of the ongoing environmental monitoring data."[4]
  - "In aseptic processing, one of the most important laboratory controls is the environmental monitoring program. This program provides meaningful information on the quality of the aseptic processing environment (e.g., when a given batch is being manufactured) as well as environmental trends of ancillary clean areas. Environmental monitoring should promptly identify potential routes of contamination, allowing for implementation of corrections before product contamination occurs (21 CFR 211.42 and 21 CFR 211.113)."[4]
- European Commission EudraLex (2014), The Rules Governing Medicinal Products in the European Union, Volume 4 "EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use," Part 1, Chapter 6: Quality Control, 6.9 "Some kinds of data (e.g., tests results, yields, environmental controls) should be recorded in a manner permitting trend evaluation. Any out of trend or out of specification data should be addressed and subject to investigation."[5]
- PIC/S (2007) Document PI 012-3, "Recommendation on Sterility Testing." 10.6 "Records should be maintained of the numbers and type of organisms isolated and results presented in a format that facilitates early detection of trends. Routine identification of environmental microorganisms to at least the genus level should assist in detecting trends. Sensitive techniques such as molecular typing techniques will be required for identification of microorganisms if equivalence of identity of environmental and test isolates is the sole rationale used to invalidate

the original sterility test (refer to clause 13.1)."[6]

- United States Pharmacopeia (USP) <1116> Microbiological Control and Monitoring of Aseptic Processing Environments.[7]
    - "The analysis of contamination trends in an aseptic environment has long been a component of the environmental control program."[7]
    - "Data from a routine microbial environmental monitoring program that can be related to time, shift, facility, etc. This information is periodically evaluated to establish the status or pattern of that program to ascertain whether it is under adequate control. A trend analysis is used to facilitate decision-making for requalification of a controlled environment or for maintenance and sanitization schedules."[7]
- FDA (2020) Pharmaceutical Microbiology Manual (PMM), Document Number ORA.007, Revision 02. "Each laboratory is required to monitor and trend data to ensure compliance and detect any abnormalities."[2]
- Parenteral Drug Association Technical Report 13 (PDA TR13) Fundamentals of an Environmental Monitoring Plan (2014). "To effectively execute microbiological, surveillance support systems, a documented system should be in place for identifying excursions and adverse trends; in addition, a feedback mechanism should be implemented for verification of effectiveness of any action taken in response to data. All data should be documented and trended."[1]

A multitude of warning letters and regulatory observations regarding trending have been written. An FDA 483 observation dated May 2, 2014 stated that "[REDACTED] Water trending records are not always accurate or supported by raw data. Specifically, the [REDACTED] Water System Microbial Analysis Results tables for the [REDACTED] of [REDACTED] included test results for Total Combined Mold & Yeast counts, and Total Coliform Count. None of the [REDACTED] water samples submitted for microbiological analysis during this time were analyzed for these two specific tests. The [REDACTED] Water System Microbial Analysis Results tables were approved by Quality Assurance. Note: Total Combined Mold & Yeast counts, and Total Coliform count are not required tests for [REDACTED] Water."[8]

This observation demonstrates that regulators will ask to review environmental monitoring trending reports and raw data. Be prepared and ensure that trending reports are accurate, properly reviewed, and performed in a timely manner.

Another FDA 483 observation dated Oct. 2, 2014 stated "observation #s IA, 9 and 10 cited during the previous inspection of 2012 on inadequate Quality Unit Oversight since 2007 to identify adverse trend of molds, investigate adverse trends, and implement corrective actions to prevent reoccurrence of molds in the manufacturing environment were again noted during this inspection in the firm's [REDACTED] vaccines manufacturing buildings' Grades [REDACTED] environmental classified areas. The firm currently has implementation corrective actions completion date of Q4 2017 for the adverse molds contamination in [REDACTED] of the manufacturing buildings."[8]

This observation demonstrates that regulators will follow up on past inspection action items as well as ask to review environmental monitoring trending reports and raw data. Be sure to have any past commitment items from previous inspections properly closed out and documented.

An FDA warning letter dated May 13, 2020 discusses environmental monitoring and trending by stating "your firm failed to establish an adequate system for monitoring environmental conditions in aseptic processing areas (21 CFR 211.42(c)(10)(iv)) …In response to this letter, provide a comprehensive, independent and retrospective review of personnel and environmental monitoring data since 2018. This review should include your assessment and corrective action and preventive action (CAPA) for your environmental monitoring program (including personnel monitoring) to ensure the CAPA supports robust environmental control of your aseptic processing facility. The assessment and CAPA, including any recommendations from the independent review, should include justification of sampling locations, frequency of sampling, alert and action limits, adequacy of sampling techniques, and the trending program. See FDA's guidance document, Sterile Drug Products Produces by Aseptic Processing – current Good Manufacturing Practice, to help you meet the cGMP requirements when manufacturing sterile drugs using aseptic processing, at https://www.fda.gov/media/71026/download."[8]

The second part of this series will look at tools and best practices for using EM trends to ensure that an efficient and successful environmental monitoring program is established.

I would like to thank my PSC Biotech colleagues AyCee Carter and J Alexander Thompson for their review of this article.

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TOOLS AND BEST PRACTICES FOR TRENDING ENVIRONMENTAL MONITORING DATA

Crystal M. Booth, M.M.
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Environmental monitoring (EM) trending is an essential component of the EM program and can be used to evaluate the overall health of the facility in terms of microbial control. EM trending should be clearly defined within the company to ensure that the data is evaluated consistently. Part one of this series looked at the regulations and guidelines around EM. This part will discuss tools and best practices for using the trends to ensure that an efficient environmental monitoring program is established.

There are many different methods and tools for performing EM trending. However, different does not mean that it is wrong. For example, trending personnel data can be done by sorting the data either by the individual operator or across all operators performing similar operations or functions. The determination of which data set to use will depend on what information is anticipated to be gained.[1]

Regarding tools, trending can be performed manually with the use of applications like Microsoft Excel or with electronic systems such as MODA, NOVATEK EM, or laboratory information management systems (LIMS), to name a few. Before implementation, all database applications used should be validated or qualified for specific software applications.[2] Graphs, such as histograms, can be generated from these applications in order to provide the data in a pictorial format. Histograms or tables characterized by a number of data points that fall within a common frequency are valuable tools.[2] These graphs can be used to easily explain complex EM data to management. The graphs can be useful in proactively identifying potential problematic areas, proactively correcting microbial contamination, and determining if the cleaning and disinfection program is working effectively.

For the EM data to be useful, the data should be grouped and sorted in strategic ways to obtain a clear picture of the microbial state of the facility. Each area (or area type) and accompanying data set must be viewed as distinct.[2] For instance, all ISO 7 areas can be grouped together for trending if all the ISO 7 areas are used in a similar fashion for manufacturing.[1] When deciding on how to collect and trend EM data, keep in mind that the EM program and subsequent trending of the data should be geared toward the needs of the facility and the information that is sought after in the EM trending.[3] Trending

data may also be used to monitor the microbial flora of the facility, seasonal trends, and sources of contamination.

ENVIRONMENTAL MONITORING BEST PRACTICES

Firms must establish alert and action limits with their environmental monitoring data. There are many sources that provide action limits for environmental monitoring. Some of these sources include the Annex 1 of the European Union's GMP guide, ISO 14464-1, and USP <1116>. Alert levels for new companies are typically established at 50% of the action level until enough data is generated to statistically calculate the alert limits. PDA TR13 provides many different options to statistically calculate alert limits. One popular method is to use a 95% confidence interval where 95% of the samples taken would be expected to pass the established alert limit.

It is important to use historical data to establish the alert levels of the environmental monitoring program so that the limits and excursions obtained will provide a better view of the control of the program and what the system can accomplish. Both alert levels and action limits should be reviewed minimally on an annual basis to ensure that the established limits still reflect the normal operating levels of the facility.[1]

USP <1116> describes the use of contamination recovery rates (CRRs). The USP states that when optimum operational conditions are achieved within a facility, contamination recovery rate levels typically become relatively stable within a normal range of variability.[4] A change in the CRR may be a signal of a change to the state of control of the facility and this must be investigated. USP <1116> provides recommended contamination recovery rates for aseptic environments in which all operators are aseptically gowned. In areas where operators are not aseptically gowned, it may be more appropriate to establish realistic CRRs based on historical EM data.

"Once alert and action levels have been established, the limits should be periodically reviewed, as part of routine trend analysis. The alert and action levels may be revised to reflect improvements, advances in technology, changes in use patterns, or other changes."[2] Meetings should be held with quality management, also known as a quality management review, to evaluate the environmental monitoring trends along with other quality metrics and key performance indicators (KPIs). Any decisions or changes that may need to be made to the EM program or associated programs, such as the cleaning and disinfection program, should be made by experienced and qualified personnel.

Reports should be clearly written and documented in a concise logical manner to describe the EM trends. These reports should list and describe items such as applicable procedures (e.g., EM, material and personnel flow, cleaning, and disinfection), facility maps and room classifications, the EM program and samples that are taken, summary of alert and action levels, contamination recovery rates (if applicable), investigations, facility maintenance, applicable procedural changes, any graphs that were created, and comparisons to previous trends. The quality unit should review the monitoring and trend reports.[2] The data needs to be analyzed and any identified discrepancies and adverse trends must be investigated.

Routine review and analysis of environmental monitoring data for trends must occur at appropriate intervals to assess the control of the facility. Management must keep up with trends and the state of operations within the facility and review the quarterly and yearly monitoring reports.[2] However, the appropriate interval for trending must allow for a proper statistical evaluation to be performed. Many companies perform trending monthly, quarterly, or yearly. Whether these frequencies are appropriate depends on the amount of collected data.[1]

As previously mentioned, trends should be clearly defined. In general, they may include a gradual increase or decrease in the overall counts observed over time, or a change in flora or counts on several plates of a particular area on a given day. Three or more consecutive points or drifts may be a pattern or cluster formation that, if above the alert level, signals a trend requires investigation.[2]

SUMMARY

Understanding the potential impact of the results generated during EM is critical to a successful environmental monitoring program.[2] EM trending assists in this critical understanding and proper EM program establishment. Properly trended data helps to confirm the following:

- Regulatory compliance
- A state of microbial control

- The ability to be proactive before a problem gets out of hand.
- A graphical representation of the data that is created.
- Any problem areas in the facility can be identified.
- That the cleaning and disinfection program are working as expected.
- The monitoring of the microbial flora of the facility and seasonal trends are performed.
- A simpler means of communication of the EM data to management.
- That the sources of microbial contamination can be identified.
- Alert and action limits are properly established.

There are numerous regulatory guidance documents that describe EM trending. Warning letters and observations have been written regarding EM and trending programs. Regulators want to ensure that the facility is operating in a state of control and will ask to see the trending reports. It is imperative that management is kept informed of environmental trends and that proper decisions are made to keep the facility in a healthy controlled state. Properly established EM programs and trending reports are essential in keeping management informed of the EM data, what the EM data means, if there are any contamination issues, and of the routine microbial flora that is present in the facility.

I would like to thank my PSC Biotech colleagues AyCee Carter and J Alexander Thompson for their review of this article.

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