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A. Process Simulations

To ensure the sterility of products purporting to be sterile, sterilization, aseptic filling and closing operations must be adequately validated (§ 211.113). The goal of even the most effective sterilization processes can be defeated if the sterilized elements of a product (the drug formulation, the container, and the closure) are brought together under conditions that contaminate any of those elements.

An aseptic processing operation should be validated using a microbiological growth medium in place of the product. This process simulation, also known as a media fill, normally includes exposing the microbiological growth medium to product contact surfaces of equipment, container closure systems, critical environments, and process manipulations to closely simulate the same exposure that the product itself will undergo. The sealed containers filled with the medium are then incubated to detect microbial contamination. Results are then interpreted to assess the potential for a unit of drug product to become contaminated during actual operation (e.g., start-up, sterile ingredient additions, aseptic connections, filling, closing). Environmental monitoring data from the process simulation can also provide useful information for the processing line evaluation.

1. Study Design

A media fill program should incorporate the contamination risk factors that occur on a production line, and accurately assesses the state of process control. Media fill studies should closely simulate aseptic manufacturing operations incorporating, as appropriate, worst-case activities and conditions that provide a challenge to aseptic operation. FDA recommends that the media fill program address applicable issues such as:

- Factors associated with the longest permitted run on the processing line that can pose contamination risk (e.g., operator fatigue)
- Representative number, type, and complexity of normal interventions that occur with each run, as well as nonroutine interventions and events (e.g., maintenance, stoppages, equipment adjustments)
- Lyophilization, when applicable
- Aseptic assembly of equipment (e.g., at start-up, during processing)
- Number of personnel and their activities
- Representative number of aseptic additions (e.g., charging containers and closures as well as sterile ingredients) or transfers
- Shift changes, breaks, and gown changes (when applicable)
- Type of aseptic equipment disconnections/connections
- Aseptic sample collections
- Line speed and configuration

Contains Nonbinding Recommendations

- Weight checks
- Container closure systems (e.g., sizes, type, compatibility with equipment)
- Specific provisions in written procedures relating to aseptic processing (e.g., conditions permitted before line clearance is mandated)

A written batch record, documenting production conditions and simulated activities, should be prepared for each media fill run. The same vigilance should be observed in both media fill and routine production runs. The firm's rationale for the conditions and activities simulated during the media fill should be clearly defined. Media fills should not be used to justify practices that pose unnecessary contamination risks.

2. *Frequency and Number of Runs*

When a processing line is initially qualified, individual media fills should be repeated enough times to ensure that results are consistent and meaningful. This approach is important because a single run can be inconclusive, while multiple runs with divergent results signal a process that is not in control. We recommend that at least three consecutive separate successful runs be performed during initial line qualification. Subsequently, routine semi-annual qualification conducted for each processing line will evaluate the state of control of the aseptic process. Activities and interventions representative of each shift, and shift changeover, should be incorporated into the design of the semi-annual qualification program. For example, the evaluation of a production shift should address unique time-related and operational features.¹³ All personnel who are authorized to enter the aseptic processing room during manufacturing, including technicians and maintenance personnel, should participate in a media fill at least once a year. Participation should be consistent with the nature of each operator's duties during routine production.

Each change to a product or line change should be evaluated using a written change control system. Any changes or events that have the potential to affect the ability of the aseptic process to exclude contamination from the sterilized product should be assessed through additional media fills. For example, facility and equipment modifications, line configuration changes, significant changes in personnel, anomalies in environmental testing results, container closure system changes, extended shutdowns, or end product sterility testing showing contaminated products may be cause for revalidation of the system.

When data from a media fill indicate the process may not be in control, an investigation should be conducted to determine the origin of the contamination and the scope of the problem. Once corrections are instituted, process simulation run(s) should be performed to confirm that deficiencies have been corrected and the process has returned to a state of control. When an investigation fails to reach well-supported, substantive conclusions as to the cause of the media

¹³ One example might be the movement of personnel into and out of the aseptic processing and gowning change rooms during a shift change.

Contains Nonbinding Recommendations

fill failure, three consecutive successful runs in tandem with increased scrutiny of the production process may be warranted.

3. *Duration of Runs*

The duration of aseptic processing operations is a major consideration in media fill design. Although the most accurate simulation model would be the full batch size and duration because it most closely simulates the actual production operations, other appropriate models can be justified. The duration of the media fill run should be determined by the time it takes to incorporate manipulations and interventions, as well as appropriate consideration of the duration of the actual aseptic processing operation. Interventions that commonly occur should be routinely simulated, while those occurring rarely can be simulated periodically.

While conventional manufacturing lines are usually automated, operated at relatively high speeds, and designed to limit operator intervention, some processes still include considerable operator involvement. When aseptic processing employs manual filling or closing, or extensive manual manipulations, the duration of the process simulation should generally be no less than the length of the actual manufacturing process to best simulate contamination risks posed by operators.

For lyophilization operations, FDA recommends that unsealed containers be exposed to partial evacuation of the chamber in a manner that simulates the process. Vials should not be frozen, and precautions should be taken that ensure that the medium remains in an aerobic state to avoid potentially inhibiting the growth of microorganisms.

4. *Size of Runs*

The simulation run sizes should be adequate to mimic commercial production conditions and accurately assess the potential for commercial batch contamination. The number of units filled during the process simulation should be based on contamination risk for a given process and sufficient to accurately simulate activities that are representative of the manufacturing process. A generally acceptable starting point for run size is in the range of 5,000 to 10,000 units. For operations with production sizes under 5,000, the number of media filled units should at least equal the maximum batch size made on the processing line (Ref. 8).

When the possibility of contamination is higher based on the process design (e.g., manually intensive filling lines), a larger number of units, generally at or approaching the full production batch size, should be used. In contrast, a process conducted in an isolator (see Appendix 1) can have a low risk of contamination because of the lack of direct human intervention and can be simulated with a lower number of units as a proportion of the overall operation.

Media fill size is an especially important consideration because some batches are produced over multiple shifts or yield an unusually large number of units. These factors should be carefully evaluated when designing the simulation to adequately encompass conditions and any potential risks associated with the larger operation.

Contains Nonbinding Recommendations

5. *Line Speed*

The media fill program should adequately address the range of line speeds employed during production. Each media fill run should evaluate a single line speed, and the speed chosen should be justified. For example, use of high line speed is often most appropriate in the evaluation of manufacturing processes characterized by frequent interventions or a significant degree of manual manipulation. Use of slow line speed is generally appropriate for evaluating manufacturing processes with prolonged exposure of the sterile drug product and containers/closures in the aseptic area.

6. *Environmental Conditions*

Media fills should be adequately representative of the conditions under which actual manufacturing operations are conducted. An inaccurate assessment (making the process appear cleaner than it actually is) can result from conducting a media fill under extraordinary air particulate and microbial quality, or under production controls and precautions taken in preparation for the media fill. To the extent standard operating procedures permit stressful conditions (e.g., maximum number of personnel present and elevated activity level), it is important that media fills include analogous challenges to support the validity of these studies. Stressful conditions do not include artificially created environmental extremes, such as reconfiguration of HVAC systems to operate at worst-case limits.

7. *Media*

In general, a microbiological growth medium, such as soybean casein digest medium, should be used. Use of anaerobic growth media (e.g., fluid thioglycollate medium) should be considered in special circumstances. The media selected should be demonstrated to promote growth of gram-positive and gram-negative bacteria, and yeast and mold (e.g., USP indicator organisms). The QC laboratory should determine if USP indicator organisms sufficiently represent production-related isolates. Environmental monitoring and sterility test isolates can be substituted (as appropriate) or added to the growth promotion challenge. Growth promotion units should be inoculated with a < 100 CFU challenge. If the growth promotion testing fails, the origin of any contamination found during the simulation should nonetheless be investigated and the media fill promptly repeated.¹⁴

The production process should be accurately simulated using media and conditions that optimize detection of any microbiological contamination. Each unit should be filled with an appropriate quantity and type of microbial growth medium to contact the inner container closure surfaces (when the unit is inverted or thoroughly swirled) and permit visual detection of microbial growth.

Some drug manufacturers have expressed concern over the possible contamination of the facility and equipment with nutrient media during media fill runs. However, if the medium is

¹⁴ The cause of the growth promotion failure should also be investigated.

Contains Nonbinding Recommendations

handled properly and is promptly followed by the cleaning, sanitizing, and, where necessary, sterilization of equipment, subsequently processed products are not likely to be compromised.

8. *Incubation and Examination of Media-Filled Units*

Media units should be incubated under conditions adequate to detect microorganisms that might otherwise be difficult to culture. Incubation conditions should be established in accord with the following general guidelines:

- Incubation temperature should be suitable for recovery of bioburden and environmental isolates and should at no time be outside the range of 20-35°C. Incubation temperature should be maintained within +2.5°C of the target temperature.
- Incubation time should not be less than 14 days. If two temperatures are used for the incubation of the media filled units, the units should be incubated for at least 7 days at each temperature (starting with the lower temperature).

Each media-filled unit should be examined for contamination by personnel with appropriate education, training, and experience in inspecting media fill units for microbiological contamination. If QC personnel do not perform the inspection, there should be QC oversight throughout any such examination. All suspect units identified during examination should be brought to the immediate attention of the QC microbiologist. To allow for visual detection of microbial growth, we recommend substituting clear containers (with otherwise identical physical properties) for amber or other opaque containers. If appropriate, other methods can also be considered to ensure visual detection.

When a firm performs a final product inspection of units immediately following the media fill run, all integral units should proceed to incubation. Units found to have defects not related to integrity (e.g., cosmetic defect) should be incubated; units that lack integrity should be rejected. Erroneously rejected units should be returned promptly for incubation with the media fill lot.

After incubation is underway, any unit found to be damaged should be included in the data for the media fill run, because the units can be representative of drug product released to the market. Any decision to exclude such incubated units (i.e., integral) from the final run tally should be fully justified and the deviation explained in the media fill report. If a correlation emerges between difficult to detect damage and microbial contamination, a thorough investigation should be conducted to determine its cause (see Section VI.B).

Written procedures regarding aseptic interventions should be clear and specific (e.g., intervention type; quantity of units removed), providing for consistent production practices and assessment of these practices during media fills. If written procedures and batch documentation are adequate to describe an associated clearance, the intervention units removed

Contains Nonbinding Recommendations

during media fills do not need to be incubated.¹⁵ Where procedures lack specificity, there would be insufficient justification for exclusion of units removed during an intervention from incubation. For example, if a production procedure requires removal of 10 units after an intervention at the stoppering station infeed, batch records (i.e., for production and media fills) should clearly document conformance with this procedure. In no case should more units be removed during a media fill intervention than would be cleared during a production run.

The ability of a media fill run to detect potential contamination from a given simulated activity should not be compromised by a large-scale line clearance. We recommend incorporating appropriate study provisions to avoid and address a large line clearance that results in the removal of a unit possibly contaminated during an unrelated event or intervention.

Appropriate criteria should be established for yield¹⁶ and accountability (reconciliation of filled units). Media fill record reconciliation documentation should include a full accounting and description of units rejected from a batch.

9. Interpretation of Test Results

The process simulation run should be observed by the QC Unit, and contaminated units should be reconcilable with the approximate time and the activity being simulated during the media fill. Video recording of a media fill may serve as a useful aide in identifying personnel practices that could negatively affect the aseptic process.

Any contaminated unit should be considered objectionable and investigated. The microorganisms should be identified to species level. The investigation should survey the possible causes of contamination. In addition, any future investigation should assess the impact on commercial drugs produced on the line since the last media fill.

Whenever contamination exists in a media fill run, it should be considered indicative of a potential sterility assurance problem, regardless of run size. The number of contaminated units should not be expected to increase in a directly proportional manner with the number of vials in the media fill run. Test results should reliably and reproducibly show that the units produced by an aseptic processing operation are sterile. Modern aseptic processing operations in suitably designed facilities have demonstrated a capability of meeting contamination levels approaching zero (Ref. 8, 9) and should normally yield no media fill contamination. Recommended criteria for assessing state of aseptic line control are as follows:

- When filling fewer than 5000 units, no contaminated units should be detected.
-- One (1) contaminated unit is considered cause for revalidation, following an investigation.

¹⁵ To assess contamination risks during initial aseptic setup (before fill), valuable information can be obtained by incubating all such units that may be normally removed. These units are typically incubated separately, and would not necessarily be included in the acceptance criteria for the media fill.

¹⁶ Total units incubated/total number of units filled.

Contains Nonbinding Recommendations

- When filling from 5,000 to 10,000 units:
 - One (1) contaminated unit should result in an investigation, including consideration of a repeat media fill.
 - Two (2) contaminated units are considered cause for revalidation, following investigation.
- When filling more than 10,000 units:
 - One (1) contaminated unit should result in an investigation.
 - Two (2) contaminated units are considered cause for revalidation, following investigation.

For any run size, intermittent incidents of microbial contamination in media filled runs can be indicative of a persistent low-level contamination problem that should be investigated. Accordingly, recurring incidents of contaminated units in media fills for an individual line, regardless of acceptance criteria, would be a signal of an adverse trend on the aseptic processing line that should lead to problem identification, correction, and revalidation.

A firm's use of media fill acceptance criteria allowing infrequent contamination does not mean that a distributed lot of drug product purporting to be sterile may contain a nonsterile unit. The purpose of an aseptic process is to prevent any contamination. A manufacturer is fully liable for the shipment of any nonsterile unit, an act that is prohibited under the FD&C Act (Section 301(a) 21 U.S.C. 331(a)). FDA also recognizes that there might be some scientific and technical limitations on how precisely and accurately process simulations can characterize a system of controls intended to exclude contamination.

As with any process validation run, it is important to note that *invalidation* of a media fill run should be a rare occurrence. A media fill run should be aborted only under circumstances in which written procedures require commercial lots to be equally handled. Supporting documentation and justification should be provided in such cases.

B. Filtration Efficacy

Filtration is a common method of sterilizing drug product solutions. A sterilizing grade filter should be validated to reproducibly remove viable microorganisms from the process stream, producing a sterile effluent.¹⁷ Currently, such filters usually have a rated pore size of 0.2 μm or smaller.¹⁸ Use of redundant sterilizing filters should be considered in many cases. Whatever filter or combination of filters is used, validation should include microbiological challenges to simulate worst-case production conditions for the material to be filtered and integrity test results of the filters used for the study. Product bioburden should be evaluated when selecting a suitable challenge microorganism to assess which microorganism represents the worst-case challenge to the filter. The microorganism *Brevundimonas diminuta* (ATCC

¹⁷ This document does not address virus removal.

¹⁸ 0.22μ and 0.2μ are considered interchangeable nominal pore size ratings.