> Section 211.94 (drug product containers and closures) states that "drug product containers and closures shall be clean and, where indicated by the nature of the drug, sterilized and processed to remove pyrogenic properties to assure that they are suitable for their intended use." It also states that "Standards or specifications, methods of testing, and, where indicated, methods of cleaning, sterilizing and processing to remove pyrogenic properties shall be written and followed for drug product containers and closures."
>
> Section 211.113(b) requires "validation of any sterilization process" as part of designing procedures "to prevent microbiological contamination of drug products purporting to be sterile."

54
55 *1. Preparation*
56
57 Containers and closures should be rendered sterile and, for parenteral drug products, pyrogen-
58 free. The type of processes used will depend primarily on the nature of the material comprising
59 the container and/or closure. The validation study for any such process should be adequate to
60 demonstrate its ability to render materials sterile and pyrogen-free. Written procedures should
61 specify the frequency of revalidation of these processes as well as time limits for holding sterile,
62 depyrogenated containers and closures.
63
64 Presterilization preparation of glass containers usually involves a series of wash and rinse cycles.
65 These cycles serve an important role in removing foreign matter. Rinse water should be of high
66 purity so as not to contaminate containers. For parenteral products, final rinse water should meet
67 the specifications of Water for Injection, USP.
68
69 The adequacy of the depyrogenation process can be assessed by spiking containers or closures
70 with known quantities of endotoxin, followed by measuring endotoxin content after
71 depyrogenation. The challenge studies should be performed with a reconstituted endotoxin
72 solution applied directly onto the surface being tested and air-dried. Positive controls should be
73 used to measure the percentage of endotoxin recovery by the test method. Validation study data
74 should demonstrate that the process reduces the endotoxin content by at least 99.9% (3 logs).
75
76 Glass containers are generally subjected to dry heat for sterilization and depyrogenation.
77 Validation of dry heat sterilization/depyrogenation should include appropriate heat distribution
78 and penetration studies as well as the use of worst-case process cycles, container characteristics
79 (e.g., mass), and specific loading configurations to represent actual production runs. See Section
80 IX.C.
81
82 Pyrogen on plastic containers can be generally removed by multiple WFI rinses. Plastic
83 containers can be sterilized with an appropriate gas, irradiation or other suitable means. For
84 gases such as EtO, the parameters and limits of the EtO sterilization cycle (e.g. temperature,
85 pressure, humidity, gas concentration, exposure time, degassing, aeration, and determination of
86 residuals) should be specified and monitored closely. Biological indicators are of special
87 importance in demonstrating the effectiveness of EtO and other gas sterilization processes.
88
89 Rubber closures (e.g., stoppers and syringe plungers) are cleaned by multiple cycles of washing
90 and rinsing prior to final steam or irradiation sterilization. At minimum, the initial rinses for the
91 washing process should employ Purified Water USP of minimal endotoxin content, followed by
92 final rinse(s) with WFI for parenteral products. Normally, depyrogenation is achieved by

493 multiple rinses of hot WFI. The time between washing and sterilizing should be minimized
494 because moisture on the stoppers can support microbial growth and the generation of endotoxins.
495 Because rubber is a poor conductor of heat, extra attention should be given to the validation of
496 processes that use heat to sterilize rubber stoppers. Validation data should also demonstrate
497 successful endotoxin removal from rubber materials.
498
499 A potential source of contamination is the siliconization of rubber stoppers. Silicone used in the
500 preparation of rubber stoppers should be rendered sterile and should not have an adverse effect
501 on the safety, quality, or purity of the drug product.
502
503 See Section VIII for discussion of the need to establish production time limits for the holding of
504 sterilized containers and closures.
505
506 Contract facilities that perform sterilization and depyrogenation of containers and closures are
507 subject to the same CGMP requirements as those established for in-house processing. The
508 finished dosage form manufacturer is responsible for the review and approval of the contractor's
509 validation protocol and final validation report.
510
511 *2. Inspection of Container-Closure System*
512
513 A container-closure system that permits penetration of air, or microorganisms, is unsuitable for a
514 sterile product. Any damaged or defective units should be detected, and removed, during
515 inspection of the final sealed product. Safeguards should be implemented to strictly preclude
516 shipment of product that may lack container-closure integrity and lead to non-sterility.
517 Equipment suitability problems or incoming container or closure deficiencies have caused loss of
518 container-closure system integrity. As examples, failure to detect vials fractured by faulty
519 machinery, or by mishandling of bulk finished stock, has led to drug recalls. If damage that is
520 not readily detected leads to loss of container-closure integrity, improved procedures should be
521 rapidly implemented to prevent and detect such defects.
522
523 Functional defects in delivery devices (e.g., syringe device defects, delivery volume) can also
524 result in product quality problems, and should be monitored by appropriate in-process testing.
525
526 Any defects or results outside the specifications established for in-process and final inspection
527 should be investigated in accord with Section 211.192.
528
529 **VII. ENDOTOXIN CONTROL**
530

Section 211.63, equipment design, size, and location, states that equipment "shall be of appropriate design, adequate size, and suitably located to facilitate operations for its intended use and for its cleaning and maintenance."

Section 211.65, equipment construction requires, in part, that "equipment shall be constructed so that surfaces that contact the components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality or purity of the drug product beyond the official or other established requirements."

Section 211.67, equipment cleaning and maintenance requires, states that "equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety,

> identity, strength, quality, or purity of the drug product beyond the official or other established requirements."
>
> Section 211.94 states that "drug product containers and closures shall be clean, and where indicated by the nature of the drug, sterilized and processed to remove pyrogenic properties to assure that they are suitable for their intended use."
>
> Section 211.167 states: "For each batch of drug product purporting to be sterile and/or pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements. The test procedures shall be in writing and shall be followed."

31
32 Endotoxin contamination of an injectable product can be a result of poor CGMP controls.
33 Certain patient populations (e.g., neonates), those receiving other injections concomitantly, or
34 those administered a parenteral in atypically large volumes or doses can be at greater risk for
35 pyrogenic reaction than anticipated by the established limits based on body weight of a normal
36 healthy adult (Ref. 6,7). Such clinical concerns reinforce the need for appropriate CGMP
37 controls to prevent generation of endotoxin. Drug product components, container-closures,
38 equipment, and storage time limitations are among the concerns to address in establishing
39 endotoxin control.
40
41 Adequate cleaning, drying, and storage of equipment provides for control of bioburden and
42 prevents contribution of endotoxin load. Equipment should be designed to be easily assembled
43 and disassembled, cleaned, sanitized, and/or sterilized. Endotoxin control should be exercised
44 for all product contact surfaces both prior to and after sterile filtration.
45
46 Endotoxin on equipment surfaces is inactivated by high temperature dry heat, or removed from
47 equipment surfaces by validated cleaning procedures. Some clean-in-place procedures employ
48 initial rinses with appropriate high purity water and/or a cleaning agent (e.g., acid, base,
49 surfactant), followed by final rinses with heated WFI. Equipment should be dried following
50 cleaning. Sterilizing filters and moist heat sterilization have not been shown to be effective in
51 removing endotoxins. Processes that are designed to achieve depyrogenation should demonstrate
52 a 3-log reduction of endotoxin.
53

54 VIII. TIME LIMITATIONS

55

> Section 211.111 (time limitations on production) states: "When appropriate, time limits for the completion of each phase of production shall be established to assure the quality of the drug product."

56
57 Time limits should be established for each phase of aseptic processing. Time limits should
58 include, for example, the period between the start of bulk product compounding and its filtration,
59 filtration processes, product exposure while on the processing line, and storage of sterilized
60 equipment, containers and closures. Maintenance of in-process quality at different production
61 phases should be supported by data. Bioburden and endotoxin load should be assessed when
62 establishing time limits for stages such as the formulation processing stage.
63
64 The total time for product filtration should be limited to an established maximum in order to
65 prevent microorganisms from penetrating the filter. Such a time limit should also prevent a

566 significant increase in upstream bioburden and endotoxin load. Sterilizing filters should
567 generally be replaced following each manufactured lot. Because they can provide a substrate for
568 microbial attachment, maximum use times for those filters used upstream for solution
569 clarification or particle removal should also be established and justified.
570
571 **IX. PROCESS VALIDATION AND EQUIPMENT QUALIFICATION**
572

> Section 211.113(b) (control of microbiological contamination) states: "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 any sterilization process."
>
> Sections 211.63, 211.65, and 211.67 address, respectively, "Equipment, design, size, and location," "Equipment construction," and "Equipment cleaning and maintenance."
>
> Section 211.84(c)(3) states that "sterile equipment and aseptic sampling techniques shall be used when necessary."

573
574 The following sections primarily discuss routine qualification and validation study expectations.
575 Change control procedures are only briefly addressed, but are an important part of the quality
576 systems established by a firm. A change in equipment, process, test method, or systems requires
577 evaluation through the written change control program, and should trigger an evaluation of the
578 need for revalidation or requalification.
579
580 **A. Process Simulations**
581
582 In order to ensure the sterility of products purporting to be sterile, both sterilization and aseptic
583 filling/closing operations must be adequately validated (211.113). The goal of even the most
584 effective sterilization processes can be defeated if the sterilized elements of a product (the drug,
585 the container and the closure) are brought together under conditions that contaminate those
586 elements. Similarly, product sterility is compromised when the product elements are non-sterile
587 at the time they are assembled.
588
589 The validation of an aseptic processing operation should include the use of a microbiological
590 growth nutrient medium in place of product. This has been termed a "media fill" or "process
591 simulation." The nutrient medium is exposed to product contact surfaces of equipment,
592 container systems, critical environments, and process manipulations to closely simulate the same
593 exposure that the product itself will undergo. The sealed containers filled with the media are
594 then incubated to detect microbial contamination. The results are interpreted to determine the
595 potential for any given unit of drug product to become contaminated during actual operations
596 (e.g., start-up, sterile ingredient additions, aseptic connections, filling, closing). Environmental
597 monitoring data is integral to the validation of an aseptic processing operation.
598
599 *1. Study Design*
600
601 A validation protocol should detail the overall strategy, testing requirements, and acceptance
602 criteria for the media fill. Media fill studies should simulate aseptic manufacturing operations as

closely as possible, incorporating a "worst-case" approach. A media fill study should address applicable issues such as:

a) factors associated with the longest permitted run on the processing line
b) ability to produce sterile units when environmental conditions impart a greater risk to the product
c) number and type of normal interventions, atypical interventions, unexpected events (e.g., maintenance), stoppages, equipment adjustments or transfers
d) lyophilization, when applicable
e) aseptic assembly of equipment (e.g., at start-up, during processing)
f) number of personnel and their activities
g) number of aseptic additions (e.g., charging containers and closures as well as sterile ingredients)
h) shift changes, breaks, and gown changes (when applicable)
i) number and type of aseptic equipment disconnections/connections
j) aseptic sample collections
k) line speed and configurations
l) manual weight checks
m) operator fatigue
n) container-closure systems (e.g., sizes, type, compatibility with equipment)
o) consideration of temperature and humidity set point extremes
p) specific provisions of aseptic processing related Standard Operating Procedures (conditions permitted before line clearance is mandated, etc.).

A written batch record, documenting conditions and activity simulated, should be prepared for each media fill run. The same vigilance should be observed in both media fill and routine production runs. Media fills cannot be used to "validate" an unacceptable practice.

2. Frequency and number of runs

When a processing line is initially validated, separate 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. A minimum of three consecutive separate successful runs should be performed during initial line qualification. Subsequently, routine semi-annual revalidation runs should be conducted for each shift and processing line to evaluate the state of control of the aseptic process. All personnel who enter the aseptic processing area, including technicians and maintenance personnel, should participate in a media fill at least once a year

Each change to a product or line change should be evaluated using a written change control system. Any changes or events that appear 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 modification, line configuration change, significant changes in personnel, anomalies in environmental testing results, container-closure system changes or, end product sterility testing showing contaminated products may be cause for revalidation of the system.

649
650 Where a media fill's data indicates the process may not be in control, a comprehensive
651 documented investigation should be conducted to determine the origin of the contamination and
652 the scope of the problem. Once corrections are instituted, multiple repeat process simulation
653 runs should be performed to confirm that deficiencies in practices and procedures have been
654 corrected and the process has returned to a state of control. However, when an investigation fails
655 to reach well-supported, substantive conclusions as to the cause of the media fill failure, three
656 consecutive successful runs and increased scrutiny (i.e., extra supervision, monitoring) of the
657 production process should be implemented.
658
659 *3. Size and Duration of runs*
660
661 The duration of aseptic processing operations is a major consideration in determining the size of
662 the media fill run. Although the most accurate simulation model would be the full batch size and
663 duration because it most closely simulates the actual production run, other appropriate models
664 can be justified. In any study protocol, the duration of the run and the overall study design
665 should adequately mimic worst-case operating conditions and cover all manipulations that are
666 performed in the actual processing operation. Adequate batch sizes are needed to simulate
667 commercial production conditions and accurately assess the potential for commercial batch
668 contamination. The number of units filled should be sufficient to reflect the effects of potential
669 operator fatigue, as well as the maximum number of interventions and stoppages. The run
670 should be large enough to accurately simulate production conditions and sensitive enough to
671 detect a low incidence of contaminated units. For batches produced over multiple shifts or
672 yielding an unusually large number of units, the media fill protocol should adequately encompass
673 conditions and any potential risks associated with the larger operation.
674
675 While conventional manufacturing lines are highly automated, often operate at relatively high
676 speeds, and are designed to limit operator intervention, there are some processes that include
677 considerable operator involvement. When aseptic processing employs manual filling or closing,
678 or extensive manual manipulations, the duration of the process simulation should generally be no
679 less than the length of the actual manufacturing process in order to best simulate operator fatigue.
680
681 For simulation of lyophilization operations, unsealed containers should be exposed to
682 pressurization and partial evacuation of the chamber in a manner that is representative of process
683 stresses. Vials should not be frozen, as this may inhibit the growth of microorganisms.
684
685 *4. Line Speed*
686
687 The media fill program should adequately address the range of line speeds (e.g., by bracketing all
688 vial sizes and fill volumes) employed during production. In some cases, more than one line speed
689 should be evaluated in the course of a study.
690
691 Each individual media fill run should evaluate a single worst-case line speed and the speed
692 chosen for each batch during a study should be justified. For example, use of high line speed is
693 justified for manufacturing processes characterized by frequent interventions or a significant

degree of manual manipulation. Use of slow line speed is justified for manufacturing processes characterized by prolonged exposure of sterile components in the aseptic area

5. Environmental Conditions

Media fills should be conducted under environmental conditions that simulate normal as well as "worst case" conditions of production. 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, it is crucial that media fills should include rigorous challenges in order to support the validity of these studies.

6. Media

In general, a microbiological growth medium such as soybean casein digest medium should be used. Use of anaerobic growth media (such as Fluid Thioglycollate Medium) is appropriate in special circumstances. Media selected should be demonstrated to promote growth of USP <71> indicator microorganisms as well as isolates that have been identified by environmental monitoring, personnel monitoring, and positive sterility test results. Positive control units should be inoculated with a <100 CFU challenge and incubated. For those instances in which the growth promotion testing fails, the origin of any contamination found during the simulation should nonetheless be investigated and the media fill should be promptly repeated.

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 and swirled) and permit visual detection of microbial growth.

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

7. Incubation and Examination of Media Filled Units

Media units should be incubated for a sufficient time (a period of not less than 14 days) at a temperature adequate to enhance detection of organisms that can otherwise be difficult to culture.

Each media filled unit should be examined for contamination by personnel with appropriate education, training and experience in microbiological techniques. There should be direct quality control unit oversight throughout any such examination. Clear containers with otherwise identical physical properties should be used as a substitute for amber or other opaque containers to allow visual detection of microbial growth.

739 When a firm performs a final product inspection of units immediately following the media fill
740 run, all integral units should proceed to incubation. Units found to have defects not related to
741 integrity (e.g., cosmetic defect) should be incubated; units that lack integrity should be rejected.[7]
742 Erroneously rejected units should be returned promptly for incubation with the media fill lot.
743
744 After incubation is underway, any unit found to be damaged should be included in the data for
745 the media fill batch, because the incubation of the units simulates release to the market. Any
746 decision to exclude such incubated units (i.e., non-integral) from the final batch tally should be
747 fully justified, and the deviation explained in the media fill report. If a correlation emerges
748 between difficult to detect damage and microbial contamination, a thorough investigation should
749 be conducted to determine its cause (See Section VI.B).
750
751 Written procedures regarding aseptic interventions should be clear and specific (e.g., intervention
752 type; quantity of units removed), providing for consistent production practices and assessment of
753 these practices during media fills. If written procedures and batch documentation are adequate,
754 these intervention units do not need to be incubated during media fills. Where procedures lack
755 specificity, there would be insufficient justification for exclusion of units removed during an
756 intervention from incubation. As an example, if a production procedure requires removal of ten
757 units after an intervention at the stoppering station infeed, batch records (i.e., for production and
758 media fills) should clearly document conformance with this procedure. In no case should more
759 units be removed during a media fill intervention than would be cleared during a production run.
760 The ability of a media fill run to detect potential contamination from a given simulated activity
761 should not be compromised by a large scale line clearance, which can result in removal of a
762 positive unit caused by an unrelated event or intervention. If unavoidable, appropriate study
763 provisions should be made to compensate in such instances.
764
765 Appropriate criteria should be established for yield and accountability. Batch record
766 reconciliation documentation should include an accurate accounting and description of units
767 rejected from a batch.
768
769 *8. Interpretation of Test Results*
770
771 The process simulation run should be observed, and contaminated units should be reconcilable
772 with the approximate time and the activity being simulated during the media fill. Videotaping of
773 a media fill has been found to be useful in identifying personnel practices which could negatively
774 impact on the aseptic process.
775
776 Any contaminated unit should be considered as objectionable and fully investigated. The
777 microorganisms should be identified to species level. In the case of a media fill failure, a
778 comprehensive investigation should be conducted, surveying all possible causes of the
779 contamination. The impact on commercial drugs produced on the line since the last successful
780 media fill should also be assessed.
781

[7] Separate incubation of certain categories of rejected units may nonetheless provide valuable information with respect to contamination that may arise from container/closure integrity deficiencies.

Whenever contamination exists in a media fill batch, it should be considered as indicative of a potential production problem. The use of statistics has limitations for media fill evaluation in that 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 show, with a high degree of confidence, 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) and should normally yield no media fill contamination. For example, a single contaminated unit in a 10,000 unit media fill batch should be fully investigated, but is normally not considered on its own to be sufficient cause for line revalidation. However, intermittent incidents at this media fill contamination level can be indicative of a persistent low level contamination problem. Accordingly, any pattern of media fill batches with such low level contamination should be comprehensively investigated and would be cause for line 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 non-sterile unit. The purpose of an aseptic process is to prevent any contamination. A manufacturer is fully liable for the shipment of any non-sterile unit, an act that is prohibited under the FD&C Act. FDA also recognizes that there might be some scientific and technical limitations on how precisely and accurately validation can characterize a system of controls intended to exclude contamination.

As with any validation batch, it is important to note that "invalidation" of a media fill run should be a rare occurrence. A media fill lot 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. An appropriate sterilizing grade filter is one which reproducibly removes all microorganisms from the process stream, producing a sterile effluent. Such filters usually have a rated porosity of 0.2 micron or smaller. Whatever filter or combination of filters is used, validation should include microbiological challenges to simulate "worst case" production conditions regarding the size of microorganisms in the material to be filtered and integrity test results of the filters used for the study. The microorganisms should be small enough to both challenge the nominal porosity of the filter and simulate the smallest microorganism that may occur in production. The microorganism *Brevundimonas diminuta* (ATCC 19146) when properly grown, harvested and used, can be satisfactory in this regard because it is one of the smallest bacteria (0.3 micron mean diameter). Bioburden of unsterilized bulk solutions should be determined, in order to trend the characteristics of potentially contaminating organisms. In certain cases, when justified as equivalent or better than use of *Brevundimonas diminuta*, it may be appropriate to conduct bacterial retention studies with a bioburden isolate. The number of microorganisms in the challenge is important because a filter can contain a number of pores larger than the nominal rating which have potential to allow passage of microorganisms (Ref. 9). The probability of such passage is considered to increase as the number of organisms (bioburden) in the material to be filtered increases (Ref. 10). A challenge concentration of at least 10^7 organisms per cm^2 of

828 effective filtration area of *B. diminuta* is generally used. A commercial lot's actual influent
829 bioburden should not include microorganisms of a size and/or concentration that would present a
830 challenge beyond that considered by the validation study.
831
832 Direct inoculation into the drug formulation provides an assessment of the effect of drug product
833 on the filter matrix and on the challenge organism. However, directly inoculating *B. diminuta*
834 into products with inherent bactericidal activity or into oil-based formulations can lead to
835 erroneous conclusions. When sufficiently justified, the effects of the product formulation on the
836 membrane's integrity can be assessed using an appropriate alternate method. For example, the
837 drug product could be filtered in a manner in which the worst-case combination of process
838 specifications and conditions are simulated. This step could be followed by filtration of the
839 challenge organism for a significant period of time, under the same conditions, using an
840 appropriately modified product (e.g., lacking an antimicrobial preservative or other antimicrobial
841 component) as the vehicle. Any divergence from a simulation using the actual product and
842 conditions of processing should be justified. Factors which can affect filter performance
843 normally include: (1) viscosity of the material to be filtered; (2) pH; (3) compatibility of the
844 material or formulation components with the filter itself; (4) pressures; (5) flow rates; (6)
845 maximum use time; (7) temperature; (8) osmolality; (9) and the effects of hydraulic shock.
846 When designing the validation protocol, it is important to address the effect of the extremes of
847 processing factors on the filter capability to produce sterile effluent. Filter validation should be
848 conducted using the worst case conditions, such as maximum filter use time and pressure (Ref.
849 11). Filter validation experiments, including microbial challenges, need not be conducted in the
850 actual manufacturing areas. However, it is essential that laboratory experiments simulate actual
851 production conditions. The specific type of filter used in commercial production should be
852 evaluated in filter validation studies. When the more complex filter validation tests go beyond
853 the capabilities of the filter user, tests are often conducted by outside laboratories or by filter
854 manufacturers. However, it is the responsibility of the filter user to review the validation data on
855 the efficacy of the filter in producing a sterile effluent. The data should be applicable to the
856 user's products and conditions of use because filter performance may differ significantly for
857 various conditions and products.
858
859 After a filtration process is properly validated for a given product, process and filter, it is
860 important to ensure that identical filter replacements (membrane or cartridge) used in production
861 runs will perform in the same manner. Sterilizing filters should be routinely discarded after
862 processing of a single batch. Normally, integrity testing of the filter is performed after the filter
863 unit is assembled and sterilized prior to use. It is important that the integrity testing be
864 conducted after filtration in order to detect any filter leaks or perforations that might have
865 occurred during the filtration. "Forward flow" and "bubble point" tests, when appropriately
866 employed, are two acceptable integrity tests. A production filter's integrity test specification
867 should be consistent with data generated during filtration efficacy studies.
868
869 **C. Sterilization of Equipment and Container/Closures**
870
871 In order to maintain sterility, equipment surfaces that contact sterilized drug product or sterilized
872 container/closure surfaces must be sterile so as not to alter purity of the drug (211.63 and
873 211.113). Those surfaces that are in the vicinity of sterile product or container-closures, but do

74 not directly contact product should also be rendered sterile where reasonable contamination
75 potential exists. It is as important in aseptic processing to properly validate the processes used to
76 sterilize such critical equipment as it is to validate processes used to sterilize the drug product
77 and its container/closure. Moist heat and dry heat sterilization are most widely used and the
78 primary processes discussed in this document. It should be noted that many of the heat
79 sterilization principles discussed in this document are also applicable to other sterilization
80 methods.
81
82 Sterility of aseptic processing equipment (e.g., stopper hoppers) should be maintained by batch-
83 by-batch sterilization. Following sterilization of equipment, containers, or closures, any
84 transportation or assembly needs to be performed in a manner in which its sterile state is
85 protected and sustained, with adherence to strict aseptic methods.
86
87 *1. Sterilizer Qualification and Validation*
88
89 Validation studies should be conducted demonstrating the efficacy of the sterilization cycle.
90 Requalification studies should also be performed on a periodic basis. For both the validation
91 studies and routine production, use of a specified load configuration should be documented in the
92 batch records.
93
94 Unevacuated air's insulating properties prevent moist heat from penetrating or heating up
95 materials, and achieving the lethality associated with saturated steam. Consequently, there is a
96 far slower thermal energy transfer and rate of kill from the dry heat in insulated locations in the
97 load. It is important to remove all of the air from the autoclave chamber during the sterilization
98 cycle. Special attention should be given to the nature or type of the materials to be sterilized and
99 the placement of biological indicator within the sterilization load. D-value of the biological
00 indicator can vary widely depending on the material (e.g., glass versus Teflon) to be sterilized.
01 Difficult to reach locations within the sterilizer load and specific materials should be an
02 important part of the evaluation of sterilization cycle efficacy. Thereafter,
03 requalification/revalidation should continue to focus on load areas identified as the most difficult
04 to penetrate or heat (e.g., worst-case locations of tightly wrapped or densely packed supplies,
05 securely fastened load articles, lengthy tubing, the sterile filter apparatus, hydrophobic filters,
06 stopper load).
07
08 The formal program providing for regular (i.e., semiannual, annual) revalidation should consider
09 the age of the sterilizer and its past performance. Change control procedures should adequately
10 address issues such as a load configuration change or a modification of the sterilizer.
11
12 a) Qualification: Empty Chamber
13
14 Temperature distribution studies evaluate numerous locations throughout an empty
15 sterilizing unit (e.g., steam autoclave, dry heat oven) or equipment train (e.g., large tanks,
16 immobile piping). It is important that these studies assess temperature uniformity at
17 various locations throughout the sterilizer to identify potential "cold spots" where there
18 can be insufficient heat to attain sterility. These heat uniformity or "temperature
19 mapping" studies should be conducted by placing calibrated temperature measurement

920 devices in numerous locations throughout the chamber.
921
922 b) Validation: Loaded Chamber
923
924 Heat penetration studies should be performed using the established sterilizer load(s).
925 Validation of the sterilization process with a loaded chamber demonstrates the effects of
926 loading on thermal input to the items being sterilized, and may identify "cold spots"
927 where there is insufficient heat to attain sterility. The placement of biological indicators
928 (BI) at numerous positions in the load, including the most difficult to sterilize places, is a
929 direct means of demonstrating the efficacy of any sterilization procedure. In general, the
930 thermocouple (TC) is placed adjacent to the BI so as to assess the correlation between
931 microbial lethality and thermal input. Validation of sterilization can be performed using
932 a partial or half-cycle approach. In some cases, the "bioburden" based cycle is used for
933 sterilization validation. For further information on validation using moist heat
934 sterilization, please refer to FDA guidance, "Guideline for the Submission of
935 Documentation for Sterilization Process Validation in Applications for Human and
936 Veterinary Drug Products" (November, 1994).
937
938 Sterilization cycle specifications are based upon the delivery of adequate thermal input to
939 the slowest to heat locations. When determining which articles are most difficult to
940 sterilize, special attention should be given to the sterilization of filters. For example,
941 some filter installations in piping cause a significant pressure differential across the filter,
942 resulting in a significant temperature drop on the downstream side. Biological indicators
943 should be placed at appropriate downstream locations of this equipment to determine if
944 the drop in temperature affects the thermal input at these sites. Established load
945 configuration should be part of batch record documentation. A sterility assurance level of
946 10^{-6} or better should be demonstrated for the sterilization process.
947
948 *2. Equipment Controls and Instrument Calibration*
949
950 For both validation and routine process control, the reliability of the data generated by
951 sterilization cycle monitoring devices should be considered to be of the utmost importance.
952 Devices that measure cycle parameters should be routinely calibrated. Written procedures
953 should be established to ensure these devices are maintained in a calibrated state. For example:
954 • Temperature monitoring devices for heat sterilization should be calibrated at suitable
955 intervals, as well as before and after validation runs.
956 • Devices used to monitor dwell time in the sterilizer should be periodically calibrated.
957 • The microbial count and D-value of a biological indicator should be confirmed before
958 a validation study.
959 • Instruments used to determine the purity of steam should be calibrated.
960 • For dry heat depyrogenation tunnels, devices (e.g. sensors and transmitters) used to
961 measure belt speed should be routinely calibrated.
962
963 Sterilizing equipment should be properly maintained to allow for consistently satisfactory
964 function. Evaluation of sterilizer performance attributes such as equilibrium ("come up") time
965 studies should be helpful in assessing if the unit continues to operate properly.

66
67 **X. LABORATORY CONTROLS**
68

Section 211.160 (General Requirements) states "Laboratory controls shall include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to assure that components, drug product containers, closures, in-process materials, labeling, and drug products conform to appropriate standards of identity, strength, quality, and purity." Sections 211.165 and 211.194 require that validation of test methods be established and documented. Sections 211.22 (c) states that "the quality control unit shall have the responsibility for approving or rejecting all procedures and specifications impacting on the identity, strength, quality, and purity of the drug product." Section 211.42 requires, for aseptic processes, the establishment of a "system for monitoring environmental conditions." Section 211.56 requires, "written procedures assigning responsibility for sanitation and describing in sufficient detail the cleaning schedules, methods, equipment, and materials to be used in cleaning the buildings and facilities." The "written procedures shall be designed to prevent the contamination of equipment, components, drug product containers, closures, packaging, labeling materials, or drug products and shall be followed." Section 211.113 (b) requires that "appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed." Section 211.192 states that "all drug product production and control records, including those for packaging and labeling, shall be reviewed and approved by the quality control unit to determine compliance with all established, approved, written procedures before a batch is released or distributed."

'69
'70 **A. Environmental Monitoring**
'71
'72 *1. General Written Program*
'73
'74 In aseptic processing, one of the most important laboratory controls is the establishment of an
'75 environmental monitoring program. This monitoring provides meaningful information on the
'76 quality of the aseptic processing environment when a given batch is being manufactured as well
'77 as environmental trends of the manufacturing area. An adequate program identifies potential
'78 routes of contamination, allowing for implementation of corrections before product
'79 contamination occurs (211.42 and 211.113).
'80
'81 Evaluating the quality of air and surfaces in the cleanroom environment should start with a well-
'82 defined written program and validated methods. The monitoring program should cover all
'83 production shifts and include air, floors, walls, and equipment surfaces, including the critical
'84 surfaces in contact with product and container/closures. Written procedures should include a list
'85 of locations to be sampled. Sample timing, frequency, and location should be carefully selected
'86 based upon its relationship to the operation performed. Samples should be taken throughout the
'87 aseptic processing facility (e.g., aseptic corridors; gowning rooms) using appropriate,
'88 scientifically sound sampling procedures, standards, and test limits.
'89

990 Locations posing the most microbiological risk to the product are a critical part of the program.
991 It is especially important to monitor the microbiological quality of the aseptic processing clean
992 zone to determine whether or not aseptic conditions are maintained during filling/closing
993 activities. Critical surfaces which contact sterile product should be sterile. Critical surface
994 sampling should be performed at the conclusion of the aseptic processing operation to avoid
995 direct contact with sterile surfaces during processing. Air and surface samples should be taken at
996 the actual working site and at locations where significant activity or product exposure occurs
997 during production.
998
999 Environmental monitoring methods do not always recover microorganisms present in the
000 sampled area. In particular, low level contamination can be particularly difficult to detect.
001 Because of the likelihood of false negatives, consecutive growth results are only one type of
002 adverse trend. Increased incidence of contamination over a given period in comparison to that
003 normally detected is an equally significant trend to be tracked.
004
005 All environmental monitoring locations should be described in SOPs with sufficient detail to
006 allow for reproducible sampling of a given location surveyed. Written SOPs should also address
007 areas such as: (1) frequency of sampling; (2) when the samples are taken (i.e., during or at the
008 conclusion of operations); (3) duration of sampling; (4) sample size (e.g., surface area, air
009 volume); (5) specific sampling equipment and techniques; (6) alert and action limits; and (7)
010 appropriate response to deviations from alert or action limits.
011
012 *2. Establishing Limits and a Trending Program*
013
014 Microbiological monitoring limits should be established based on the relationship of the sampled
015 location to the operation. The limits should be based on the need to maintain adequate
016 microbiological control throughout the entire sterile manufacturing facility. One should also
017 consider environmental monitoring data from historical databases, media fills, cleanroom
018 qualification, and sanitization procedure studies in developing monitoring limits.
019
020 Microbiological environmental monitoring should include both alert and action limits. Each
021 individual sample result should be evaluated for its significance by comparing to the alert or
022 action limits. Averaging of results can mask unacceptable localized conditions. A result at the
023 alert limit urges attention to the approaching action conditions. A result at the action level
024 should prompt a more thorough investigation. Written procedures should be established,
025 detailing data review frequency, identification of contaminants, and actions to be taken. The
026 quality control unit should provide routine oversight of near term (e.g., daily, weekly, monthly,
027 quarterly) and long term trends in environmental and personnel monitoring data.
028
029 Trend reports should include data generated by location, shift, lot, room, operator, or other
030 search parameters. The quality control unit is responsible for producing specialized data reports
031 (e.g., a search on a particular atypical isolate over a year period) in order to investigate results
032 beyond established limits and identify any appropriate follow-up actions. In addition to
033 microbial counts beyond alert and action limits, the presence of any atypical microorganisms in
034 the cleanroom environment should be investigated, with any appropriate corrective action
035 promptly implemented.

36
37 Written procedures should define the system whereby the most responsible managers are
38 regularly informed and updated on trends and investigations.
39
40 *3. Sanitization Efficacy*
41
42 The suitability, efficacy, and limitations of sanitization agents should be assessed with their
43 implementation for use in clean areas. The effectiveness of these sanitization procedures
44 should be measured by their ability to ensure that potential contaminants are adequately removed
45 from surfaces (i.e., via obtaining samples before and after sanitization).
46
47 Upon preparation, disinfectants should be rendered sterile, and used for a limited time, as
48 specified by written procedures. Disinfectants should retain efficacy against the normal
49 microbial flora and be effective against spore-forming microorganisms. Many common sanitizers
50 are ineffective against spores, for example, 70% isopropyl alcohol is not effective against
51 *Bacillus,* spp. spores. A sporicidal agent should be used regularly to prevent contamination of
52 the manufacturing environment with otherwise difficult to eradicate spore forming bacteria or
53 fungi.
54
55 After the initial assessment of sanitization procedures, ongoing sanitization efficacy should be
56 frequently monitored through specific provisionsin the environmental monitoring program, with
57 a defined course of action in the event samples are found to exceed limits.
58
59 *4. Monitoring Methods*
60
61 Acceptable methods of monitoring the microbiological quality of the environment include:
62
63 a. Surface Monitoring-
64 Environmental monitoring should include testing of various surfaces for microbiological
65 quality. For example, product contact surfaces, floors, walls, ceilings, and equipment
66 should be tested on a regular basis. Routinely used for such tests are touch plates, swabs,
67 and contact plates. Other surfaces in controlled areas should be tested to show the
68 adequacy of cleaning and sanitizing procedures.
69
70 b. Active Air Monitoring-
71 The method of assessing the microbial quality of air should involve the use of "active"
72 devices such as slit to agar samplers, , those using liquid impingement and membrane
73 filtration, or centrifugal samplers. Each device has certain advantages and disadvantages,
74 although all allow a quantitative testing of the number of organisms per volume of air
75 sampled. The use of such devices in aseptic areas is considered an essential part of
76 evaluating the environment during each production shift, at carefully chosen critical
77 locations. Manufacturers should be aware of a device's air monitoring capabilities, and
78 should determine suitability of any new or current devices with respect to sensitivity and
79 limit of quantification.
80
81 c. Passive Air Monitoring (Settling Plates)-

082 Another method is the use of passive air samplers such as settling plates (petri dishes
083 containing nutrient growth medium exposed to the environment). These settling plates
084 lack value as quantitative air monitors because only microorganisms that settle onto the
085 agar surface will be detected. Their value as qualitative indicators in critical areas is
086 enhanced by positioning plates in locations posing the greatest risk of product
087 contamination. As part of methods validation, the quality control laboratory should
088 evaluate what media exposure conditions optimize recovery of low levels of
089 environmental isolates. Exposure conditions should preclude desiccation (e.g., caused by
090 lengthy sampling periods and/or high airflows), which inhibits recovery of
091 microorganisms. The data generated by passive air sampling can be useful when
092 considered in combination with results from other types of air samples.
093
094 **B. Microbiological Media and Identification**
095
096 The environmental monitoring program should include routine characterization of recovered
097 microorganisms. Monitoring of critical and immediately surrounding areas as well as personnel
098 should include routine identification of microorganisms to the species (or, where appropriate,
099 genus) level.
100
101 In some cases, environmental trending data has revealed migration of microorganisms into the
102 aseptic processing room from either uncontrolled or lesser-controlled areas. To detect such
103 trends, an adequate program of differentiating microorganisms in lesser-controlled environments
104 (e.g., Class 100,000) should be in place. At minimum, the program should require species (or,
105 where appropriate, genus) identification of microorganisms in ancillary environments at frequent
106 intervals to establish a valid, current database of contaminants present in the facility during
107 processing (and to demonstrate that cleaning and sanitization procedures continue to be
108 effective). Environmental isolates often correlate with the contaminants found in a media fill or
109 product sterility testing failure, and the overall environmental picture provides valuable
110 information for the associated investigation.
111
112 The goal of microbiological monitoring is to reproducibly detect microorganisms for purposes of
113 monitoring the state of environmental control. Consistent methods will yield a database that
114 allows for sound data comparisons and interpretations. The microbiological culture media used
115 in environmental monitoring should be validated as capable of detecting fungi (i.e., yeasts and
116 molds) as well as bacteria, and incubated at appropriate conditions of time and temperature.
117 Total aerobic bacterial count can be obtained by incubating at 30 to 35°C for 48 to 72 hours.
118 Total combined yeast and mold count is generally obtained by incubating at 20 to 25°C for 5 to 7
119 days.
120
121 Incoming lots of environmental monitoring media should include positive and negative controls.
122 Growth promotion testing should be performed on all lots of prepared media. Where
123 appropriate, inactivating agents should be used to prevent inhibition of growth by clean room
124 disinfectants.
125
126 **C. Pre-filtration Bioburden**
127

28 For any parenteral manufacturing process, pre-filtration bioburden should be minimal. In
29 addition to increasing the challenge to the sterilizing filter, high bioburden can contribute
30 endotoxin or other impurities to the drug formulation. An in-process limit for bioburden level
31 for each formulated product (generally sampled immediately preceding sterile filtration) should
32 be established.
33
34 **D. Particulate Monitoring**
35
36 Routine particle monitoring is useful in detecting significant deviations in air cleanliness from
37 qualified processing norms (e.g., clean area classification). A result outside the established
38 specifications at a given location should be investigated consistent with the severity of the
39 "excursion." Appropriate corrective action should be implemented to prevent future deviations.
40
41 See Section IV.A for additional guidance on particulate monitoring.
42
43 **XI. STERILITY TESTING**
44

Section 211.167 (Special Testing Requirements) states: "For each batch of drug product purporting to be sterile and/or pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements. The test procedures shall be in writing and shall be followed."

Section 211.165 states "For each batch of drug product, there shall be appropriate laboratory determination of satisfactory conformance to final specifications for the drug product ...prior to release."

Section 211.165(e) requires methods for testing to be validated as reliable and reproducible (e.g., bacteriostasis/fungistasis, method robustness, etc.), stating: "The accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented. Such validation and documentation may be accomplished in accordance with Sec. 211.194(a)(2)."

Section 211.110 requires, in part, that sampling procedures are established in order to ensure batch uniformity: The "control procedures shall be established to monitor the output and to validate the performance of those manufacturing processes that may be responsible for causing variability in the characteristics of in-process material and the drug product."

Section 211.160 requires the establishment of sound and appropriate sampling plans which are representative of the batch.

Section 210 defines "representative sample" as one based on rational criteria that provide an "accurate portrayal" of the material or batch being sampled.

Section 211.180 states a review of, "at least annually, the quality standards of each drug product to determine the need for changes in drug product specifications or manufacturing or control procedures." Investigations conducted under Section 211.192 for each drug product are required to be addressed within this annual review.

45

146 Certain aspects of sterility testing are of particular importance, including control of the testing
147 environment, understanding the test limitations, and the investigation of manufacturing systems
148 following a positive test.
149
150 The testing laboratory environment should employ facilities and controls comparable to those
151 used for filling/closing operations. Poor or deficient sterility test facilities or controls can result
152 in a high rate of test failures. If production facilities and controls are significantly better than
153 those for sterility testing there is the danger of attributing the cause of a positive sterility test
154 result to the faulty laboratory even when the product tested could have, in fact, been non-sterile.
155 Therefore, some manufacturing deficiency may go undetected. The use of isolators to perform
156 sterility testing is a well-established means for minimizing false positives.
157
158 **A. Choice of Methods**
159
160 Sterility testing methodologies are required to be accurate and reproducible, in accord with
161 211.194 and 211.165. The methodology selected should present the lowest potential for yielding
162 a false positive. The USP specifies membrane filtration as the method of choice, when feasible.
163
164 As a part of methods validation, appropriate bacteriostasis/fungistasis testing should be
165 conducted. Such testing should demonstrate reproducibility of the method in recovering each of
166 a panel of representative microorganisms. Study documentation should include evaluation of
167 whether microbial recovery from inoculated controls and product samples is comparable
168 throughout the incubation period. If growth is inhibited, modifications (e.g., increased dilution,
169 additional membrane filter washes, addition of inactivating agents) in the methodology should be
170 implemented to optimize recovery. Ultimately, methods validation studies should demonstrate
171 that the methodology does not provide an opportunity for "false negatives."
172
173 **B. Media**
174
175 It is essential that the media used to perform sterility testing be rendered sterile and demonstrated
176 as growth promoting.
177
178
179 **C. Personnel**
180
181 Personnel performing sterility testing should be qualified and trained for the task. A written
182 program should be in place to regularly update training of personnel and confirm acceptable
183 sterility testing practices.
184
185 **D. Sampling and Incubation**
186
187 Sterility tests are limited in their ability to detect low levels of contamination. For example,
188 statistical evaluations indicate that the USP sterility test sampling plan has been described by
189 USP as "only enabling the detection of contamination in a lot in which 10% of the units are
190 contaminated about nine times out of ten in making the test" (Ref. 12). To further illustrate, if a

10,000 unit lot with a 0.1% contamination level was sterility tested using 20 units, there is a 98% chance that the batch would pass the test.

This limited sensitivity makes it necessary to ensure that for batch release purposes an appropriate number of units are tested and that the samples uniformly represent the:

(1) Entire batch-
Samples should be taken at the beginning, middle, and end of the aseptic processing operation;
(2) Batch processing circumstances-
Samples should be taken in conjunction with processing interventions or excursions.

Because of the limited sensitivity of the test, any positive result is considered a serious CGMP issue and should be thoroughly investigated.

E. Investigation of Sterility Positives

Care should be taken in the performance of the sterility test to preclude any activity that allows for possible sample contamination. When microbial growth is observed, the lot should be considered to be non-sterile. It is inappropriate to attribute a positive result to laboratory error on the basis of a retest that exhibits no growth.[8] The evaluation of a positive sterility test result should include an investigation to determine whether the growth observed in the test arose from product contamination or from laboratory error.

Although it is recognized that such a determination may not be reached with absolute certainty, it is usually possible to acquire persuasive evidence showing that causative laboratory error is absent. When available evidence is inconclusive, batches should be rejected as not conforming to sterility requirements.

It would be difficult to support invalidation of a positive sterility test. Only if conclusive and documented evidence clearly shows that the contamination occurred as part of testing should a new test be performed.

After considering all relevant factors concerning the manufacture of the product and testing of the samples, the comprehensive written investigation should include specific conclusions, and identify corrective actions. The investigation's persuasive evidence of the origin of the contamination should be based upon at least the following:

1. Identification (speciation) of the organism in the sterility test. Identification of the sterility test isolate(s) should be to the species level. Microbiological monitoring data should be reviewed to determine if the organism is also found in laboratory and production environments, personnel, or product bioburden.

[8] Underscoring this regulatory standard, USP XXV, section <71>, states that an initial positive test is invalid only in an instance in which "microbial growth can be without a doubt ascribed to" laboratory error (as described in the monograph).

234 2. Record of laboratory tests and deviations. Review of trends in laboratory findings can help
235 to eliminate or implicate the laboratory as the source of contamination. If the organism is
236 seldom found in the laboratory environment, then product contamination is likely. If the
237 organism is found in laboratory and production environments, it can indicate product
238 contamination.
239
240 The proper handling of deviations is an essential aspect of laboratory control. When a
241 deviation occurs during sterility testing, it should be documented, investigated, and remedied.
242 If any deviation is considered to have compromised the integrity of the sterility test, the test
243 should be invalidated immediately without incubation.
244
245 Deviation and sterility test positive trends should be evaluated periodically (e.g., quarterly,
246 annually) to provide an overview of operations. A sterility positive result can be viewed as
247 indicative of production or laboratory problems and should be investigated globally since
248 such problems often can extend beyond a single batch.
249
250 In order to more accurately monitor potential contamination sources, it is useful to keep
251 separate trends by product, container type, filling line, and personnel. Where the degree of
252 sterility test sample manipulation is similar for a terminally sterilized product and an
253 aseptically processed product, a higher rate of initial sterility failures for the latter should be
254 taken as indicative of aseptic processing production problems. See Section IX.A, Process
255 Simulations, which includes similar issues that are investigated as part of a media fill failure
256 investigation.
257
258 Microbial monitoring of the laboratory environment and personnel over time can also reveal
259 trends that are informative. Upward trends in the microbial load in the laboratory should be
260 promptly investigated as to cause, and corrected. In some instances, such trends can appear
261 to be more indicative of laboratory error as a possible source of a sterility test failure.
262
263 Where a laboratory has a good track record with respect to errors, this history can help
264 remove the lab as a source of contamination since chances are higher that the contamination
265 arose from production. However, the converse is not true. Specifically, where the laboratory
266 has a poor track record, firms should not assume that the contamination is automatically
267 more attributable to the error in laboratory and consequently overlook a genuine production
268 problem. Accordingly, all sterility positives should be thoroughly investigated.
269
270 3. Monitoring of production area environment. Of particular importance is trend analysis of
271 microorganisms in the critical and immediately adjacent area. Trends are an important tool
272 in investigating the product as the possible source of a sterility failure. Consideration of
273 environmental microbial loads should not be limited to results of monitoring the production
274 environment for the lot, day, or shift associated with the suspect lot. For example, results
275 showing little or no recovery of microorganisms can be misleading, especially when
276 preceded or followed by a finding of an adverse trend or atypically high microbial counts. It
277 is therefore important to look at both short and long term trend analysis.
278