Guidance for Industry

Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice

DRAFT GUIDANCE

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Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)
Office of Regulatory Affairs (ORA)

August 2003
Pharmaceutical CGMPs

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Guidance for Industry[1]
Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This draft guidance is intended to help manufacturers meet the requirements in the Agency's current good manufacturing practice (CGMP) regulations (2l CFR parts 210 and 211) when manufacturing sterile drug and biological products using aseptic processing. This guidance, when finalized, will replace the 1987 *Industry Guideline on Sterile Drug Products Produced by Aseptic Processing*. This revision updates and clarifies the 1987 guidance.

For sterile drug products subject to a new or abbreviated drug application (NDA or ANDA), this guidance document should be read in conjunction with the 1994 guidance on the content of sterile drug applications, entitled *Guideline for the Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products*. The 1994 submission guidance describes the types of information and data that should be included in drug applications to demonstrate the efficacy of a manufacturer's sterilization process. This draft guidance compliments the 1994 guidance by describing procedures and practices that will help enable a sterile drug manufacturing facility to meet CGMP requirements relating, for example, to facility design, equipment suitability, process validation, and quality control.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are

[1] This guidance was developed by the Office of Compliance in the Center for Drug Evaluation and Research (CDER) in cooperation with the Center for Biologics Evaluation and Research (CBER) and the Office of Regulatory Affairs (ORA).

cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

The text boxes included in this guidance discuss specific sections of parts 210 and 211 of the Code of Federal Regulations (CFR), which address current good manufacturing practice for drugs. The intent of including the citations in the text boxes is to aid the reader by providing a portion of an applicable regulation being addressed in the guidance. The citations included in the text boxes are not intended to be exhaustive. Readers of this document should reference the complete CFR to ensure that they have complied, in full, with all relevant sections of the regulations.

II. BACKGROUND

This sections describes briefly both the regulatory and technical reasons why the Agency is developing this guidance document.

A. Regulatory Framework

This draft guidance pertains to current good manufacturing practice (CGMP) regulations (21 CFR parts 210 and 211) when manufacturing sterile drug and biological products using aseptic processing. For biological products regulated under 21 CFR parts 600 through 680, sections 210.2(a) and 211.1(b) provide that where it is impossible to comply with the applicable regulations in both parts 600 through 680 and parts 210 and 211, the regulation specifically applicable to the drug product in question shall apply. In the event that it is impossible to comply with all applicable regulations in these parts, the regulations specifically applicable to the drug in question shall supersede the more general.

B. Technical Framework

There are basic differences between the production of sterile drug products using aseptic processing and production using terminal sterilization.

Terminal sterilization usually involves filling and sealing product containers under high-quality environmental conditions. Products are filled and sealed in this type of environment to minimize the microbial content of the in-process product and to help ensure that the subsequent sterilization process is successful. In most cases, the product, container, and closure have low bioburden, but they are not sterile. The product in its final container is then subjected to a sterilization process such as heat or irradiation.

In an aseptic process, the drug product, container, and closure are first subjected to sterilization methods separately, as appropriate, and then brought together.[2] Because there is no process to

[2] Due to their nature, certain products are aseptically processed at an earlier stage in the process, or in their entirety. Cell-based therapy products are an example. All components and excipients for these products are rendered sterile, and release of the final product is contingent on determination of sterility. See Appendix III.

82 sterilize the product in its final container, it is critical that containers be filled and sealed in an
83 extremely high-quality environment. Aseptic processing involves more variables than terminal
84 sterilization. Before aseptic assembly into a final product, the individual parts of the final
85 product are generally subjected to several sterilization processes. For example, glass containers
86 are subjected to dry heat sterilization; rubber closures are subjected to moist heat sterilization;
87 and liquid dosage forms are subjected to sterile filtration. Each of these aseptic manufacturing
88 processes requires thorough validation and control. Each process also could introduce an error
89 that ultimately could lead to the distribution of a contaminated product. Any manual or
90 mechanical manipulation of the sterilized drug, components, containers, or closures prior to or
91 during aseptic assembly poses the risk of contamination and thus necessitates careful control. A
92 terminally sterilized drug product, on the other hand, undergoes a single sterilization process in a
93 sealed container, thus limiting the possibilities for error.[3]
94
95 Manufacturers should have a keen awareness of the public health implications of distributing a
96 nonsterile product. Poor CGMP conditions at a manufacturing facility can ultimately pose a life-
97 threatening health risk to a patient.
98
99
100 **III. SCOPE**
101
102 This guidance document discusses selected issues and does not address all aspects of aseptic
103 processing. For example, the guidance addresses primarily finished drug product CGMP issues
104 while only limited information is provided regarding upstream bulk processing steps. This
105 guidance updates the 1987 guidance primarily with respect to personnel qualification, cleanroom
106 design, process design, quality control, environmental monitoring, and review of production
107 records. The use of isolators for aseptic processing is also discussed.
108
109 Although this guidance document discusses CGMP issues relating to the sterilization of
110 components, containers, and closures, terminal sterilization of drug products is not addressed. It
111 is a well-accepted principle that sterile drugs should be manufactured using aseptic processing
112 only when terminal sterilization is infeasible. However, some final packaging may afford some
113 unique and substantial advantage (e.g., some dual-chamber syringes) that would not be possible
114 if terminal sterilization were employed. In such cases, a manufacturer can explore the option of
115 adding adjunct processing steps to increase the level of sterility confidence.
116
117 A list of references that may be of value to the reader is included at the conclusion of this
118 document.

[3] Nearly all drugs recalled due to nonsterility or lack of sterility assurance in the period spanning 1980-2000 were produced via aseptic processing.

119
120 **IV. BUILDINGS AND FACILITIES**
121
122

> 21 CFR 211.42(c) states, in part, that "Operations shall be performed within specifically defined areas of adequate size. There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mixups during the course of the following procedures: *** (10) Aseptic processing, which includes as appropriate: *** (iii) An air supply filtered through high-efficiency particulate air filters under positive pressure ***; (iv) A system for monitoring environmental conditions; *** (vi) A system for maintaining any equipment used to control the aseptic conditions."
>
> 21 CFR 211.46(b) states that "Equipment for adequate control over air pressure, micro-organisms, dust, humidity, and temperature shall be provided when appropriate for the manufacture, processing, packing, or holding of a drug product."
>
> 21 CFR 211.46(c) states, in part, that "Air filtration systems, including prefilters and particulate matter air filters, shall be used when appropriate on air supplies to production areas."

123
124
125 As provided for in the regulations, aseptic processing facilities must have separate areas of
126 operation that are appropriately controlled to attain different degrees of air quality depending on
127 the nature of the operation. Design of a given area should be based on satisfying microbiological
128 and particle standards defined by the equipment, components, and products exposed, as well as
129 the particular operation conducted in the area.
130
131 Critical areas and support areas of the aseptic processing operation should be classified and
132 supported by microbiological and particle data obtained during qualification studies. Although
133 initial cleanroom qualification should include some assessment of air quality under as-built and
134 static conditions, the final room or area classification should be derived from data generated
135 under dynamic conditions (i.e., with personnel present, equipment in place, and operations
136 ongoing). The aseptic processing facility monitoring program should also assess conformance
137 with specified clean area classifications under dynamic conditions on a routine basis.
138
139 The following table summarizes clean area air classifications (Ref. 1).

140
141
142 **TABLE 1- Air Classifications**[a]
143

Clean Area Classification (0.5 um particles/ft^3)	ISO Designation[b]	≥ 0.5 um particles/m^3	Microbiological Active Air Action Levels[c] (cfu/m^3)	Microbiological Settling Plates Action Levels[c,d] (diam. 90mm; cfu/4 hours)
100	5	3,520	1[e]	1[e]
1000	6	35,200	7	3
10,000	7	352,000	10	5
100,000	8	3,520,000	100	50

144
145
146 a- All classifications based on data measured in the vicinity of exposed materials/articles during periods of activity.
147 b- ISO 14644-1 designations provide uniform particle concentration values for cleanrooms in multiple industries. An ISO 5 particle
148 concentration is equal to Class 100 and approximately equals EU Grade A.
149 c- Values represent recommended levels of environmental quality. You may find it appropriate to establish alternate microbiological levels
150 due to the nature of the operation.
151 d- The additional use of settling plates is optional.
152 e- Samples from Class 100 (ISO 5) environments should normally yield no microbiological contaminants.
153
154 Two clean areas are of particular importance to sterile drug product quality: the critical area and
155 the supporting clean areas associated with it.
156

157 ## A. Critical Area – Class 100 (ISO 5)

158
159 A critical area is one in which the sterilized drug product, containers, and closures are exposed to
160 environmental conditions designed to preserve sterility. Activities conducted in this area include
161 manipulations (e.g., aseptic connections, sterile ingredient additions) of sterile materials prior to
162 and during filling and closing operations.
163
164 This area is critical because the product is not processed further in its immediate container and is
165 vulnerable to contamination. To maintain product sterility, the environment in which aseptic
166 operations (e.g., equipment setup, filling) are conducted should be of appropriate quality. One
167 aspect of environmental quality is the particle content of the air. Particles are significant because
168 they can enter a product and contaminate it physically or, by acting as a vehicle for
169 microorganisms, biologically (Ref. 2). Particle content in critical areas should be minimized by
170 appropriately designed air handling systems.
171
172 Air in the immediate proximity of exposed sterilized containers/closures and filling/closing
173 operations would be of appropriate particle quality when it has a per-cubic-meter particle count
174 of no more than 3520 in a size range of 0.5 micron and larger when counted at representative
175 locations normally not more than 1 foot away from the work site, within the airflow, and during
176 filling/closing operations. This level of air cleanliness is also known as Class 100 (ISO 5).
177 Deviations from this critical area monitoring parameter should be documented as to cause and
178 significance.
179
180 Measurements to confirm air cleanliness in aseptic processing zones should be taken with the
181 particle counting probe oriented in the direction of oncoming airflow and at the sites where there
182 is most potential risk to the exposed sterilized product and container-closures. Regular
183 monitoring should be performed during each shift. Nonviable particle monitoring with a remote

184 counting system is generally less invasive than the use of portable particle counting units and
185 provides the most comprehensive data. See Section X.D. Particle Monitoring.
186
187 Some powder filling operations can generate high levels of powder particles that, by their nature,
188 do not pose a risk of product contamination. It may not, in these cases, be feasible to measure air
189 quality within the one-foot distance and still differentiate background levels of powder particles
190 from air contaminants. In these instances, air should be sampled in a manner that, to the extent
191 possible, characterizes the true level of extrinsic particle contamination to which the product is
192 exposed. Initial certification of the area under dynamic conditions without the actual powder
193 filling function should provide some baseline information on the nonproduct particle generation
194 of the operation.
195
196 Air in critical areas should be supplied at the point of use as HEPA-filtered laminar flow air at a
197 velocity sufficient to sweep particles away from the filling/closing area and maintain
198 unidirectional airflow during operations. The velocity parameters established for each
199 processing line should be justified and appropriate to maintain unidirectional airflow and air
200 quality under dynamic conditions within a defined space (Ref. 3).[4]
201
202 Proper design and control should prevent turbulence or stagnant air in the aseptic processing line
203 or clean area. Once relevant parameters are established, airflow patterns should be evaluated for
204 turbulence or eddy currents that can act as a channel or reservoir for the accumulation of air
205 contaminants (e.g., from an adjoining lower classified area). Air pattern analysis or smoke
206 studies should be conducted that demonstrate unidirectional airflow and sweeping action over
207 and away from the product under dynamic conditions. The studies should be well documented
208 with written conclusions, including an evaluation of the impact of aseptic manipulations.
209 Videotape or other recording mechanisms have been found to be useful in assessing airflow
210 initially as well as facilitating evaluation of subsequent equipment configuration changes.
211 However, even successfully qualified systems can be compromised by poor operational,
212 maintenance or personnel practices.
213
214 Air monitoring of critical areas should normally yield no microbiological contaminants.
215 Contamination in this environment should receive investigative attention.
216
217 **B. Supporting Clean Areas**
218
219 Supporting clean areas can have various classifications and functions. Many support areas
220 function as zones in which nonsterile components, formulated products, in-process materials,
221 equipment, and container/closures are prepared, held, or transferred. These environments should
222 be designed to minimize the level of particle contaminants in the final product and control the
223 microbiological content (bioburden) of articles and components that are subsequently sterilized.
224
225 The nature of the activities conducted in a supporting clean area should determine its
226 classification. An area classified at Class 100,000 (ISO 8) would be used for less critical

[4] A velocity from 0.45 to 0.51 meters/second (90 to 100 feet per minute) is generally established, with a range of plus or minus 20 percent around the setpoint. Higher velocities may be appropriate in operations generating high levels of particulates.

227 activities (such as initial equipment preparation). The area immediately adjacent to the aseptic
228 processing line should, at a minimum, meet Class l0,000 (ISO 7) standards (see Table 1) under
229 dynamic conditions. Depending on the operation, manufacturers can also classify this area as
230 Class 1,000 (ISO 6) or maintain the entire aseptic filling room at Class 100 (ISO 5).
231
232 **C. Clean Area Separation**
233
234 Adequately separating areas of operation is an important part of contamination prevention. To
235 maintain air quality in areas of higher cleanliness, it is important to achieve a proper airflow and
236 a positive pressure differential relative to adjacent less clean areas. Rooms of higher air
237 cleanliness should have a substantial positive pressure differential relative to adjacent rooms of
238 lower air cleanliness. For example, a positive pressure differential of at least 12.5 Pascals (Pa)[5]
239 should be maintained at the interface between classified and unclassified areas. This same
240 overpressure should be maintained between the aseptic processing room and adjacent rooms
241 (with doors closed). When doors are open, outward airflow should be sufficient to minimize
242 ingress of contamination, and the time that a door can remain ajar should be strictly controlled
243 (Ref. 4). Pressure differentials between cleanrooms should be monitored continuously
244 throughout each shift and frequently recorded, and deviations from established limits should be
245 investigated.
246
247 An adequate air change rate should be established for a cleanroom. For Class 100,000 (ISO 8)
248 supporting rooms, airflow sufficient to achieve at least 20 air changes per hour would be
249 typically acceptable. For areas of higher air cleanliness, significantly higher air change rates will
250 provide an increased level of air purification.
251
252 Facility monitoring systems should be established to rapidly detect atypical changes that can
253 compromise the facility's environment. Operating conditions should be restored to established,
254 qualified levels before reaching action levels. For example, pressure differential specifications
255 should enable prompt detection (i.e., alarms) of an emerging low pressure problem to preclude
256 ingress of unclassified air into a classified room.
257
258 **D. Air Filtration**
259
260 *1. Membrane*
261
262 A compressed gas should be of appropriate purity (e.g., free from oil and water vapor) and its
263 microbiological and particle quality should be equal to or better than air in the environment into
264 which the gas is introduced. Compressed gases such as air, nitrogen, and carbon dioxide are
265 often used in cleanrooms and are frequently employed in operations involving purging or
266 overlaying.
267
268 Membrane filters allow the filtering of compressed gases to meet an appropriate high-quality
269 standard. Membrane filters can be used to produce a sterile compressed gas to conduct
270 operations involving sterile materials, such as components and equipment. For example, sterile
271 membrane filters should be used for autoclave air lines, lyophilizer vacuum breaks, and tanks

[5] Equal to 0.05 inches of water gauge.

272 containing sterilized materials. Sterilized holding tanks and any contained liquids should be held
273 under continuous overpressure to prevent microbial contamination. Safeguards should be in
274 place to prevent a pressure change that can result in contamination due to back flow of nonsterile
275 air or liquid.
276
277 Gas filters (including vent filters) should be dry. Condensate in a gas filter can cause blockage
278 or microbial contamination. Use of hydrophobic filters, as well as application of heat to these
279 filters where appropriate, prevents problematic moisture residues. Filters also should be integrity
280 tested upon installation and periodically thereafter (e.g., including at end of use). Integrity test
281 failures should be investigated, and filters should be replaced at appropriate intervals.
282
283 *2. High-Efficiency Particulate Air (HEPA)*[6]
284
285 An essential element in ensuring aseptic conditions is the maintenance of HEPA filter integrity.
286 Leak testing should be performed at installation to detect integrity breaches around the sealing
287 gaskets, through the frames, or through various points on the filter media. Thereafter, leak tests
288 should be performed at suitable time intervals for HEPA filters in the aseptic processing facility.
289 For example, such testing should be performed twice a year for the aseptic processing room.
290 Additional testing may be appropriate when air quality is found to be unacceptable, facility
291 renovations might be the cause of disturbances to ceiling or wall structures, or as part of an
292 investigation into a media fill or drug product sterility failure. Among the filters that should be
293 leak tested are those installed in dry heat depyrogenation tunnels commonly used to
294 depyrogenate glass vials.
295
296 Any aerosol used for challenging a HEPA filter should meet specifications for critical
297 physicochemical attributes such as viscosity. Dioctylphthalate (DOP) and Poly-alpha-olefin
298 (PAO) are examples of appropriate leak testing aerosols. Some alternative aerosols are
299 problematic because they pose the risk of microbial contamination of the environment being
300 tested. Firms should ensure that any alternative used does not promote microbial growth.
301
302 There is a major difference between *filter leak testing* and *efficiency testing*. An efficiency test is
303 a general test used to determine only the rating of the filter.[7] An intact HEPA filter should be
304 capable of retaining at least 99.97 percent of particulates greater than 0.3 micron in diameter.
305
306 The purpose of performing regularly scheduled leak tests, on the other hand, is to detect leaks
307 from the filter media, filter frame, or seal. The challenge should be conducted using a
308 polydispersed aerosol usually composed of particles with a light-scattering mean droplet
309 diameter in the submicron size range, including a sufficient number of particles at approximately
310 0.3 microns. Performing a leak test without introducing a sufficient upstream challenge of
311 particles of known size upstream of the filter is ineffective for detecting leaks. For example,

[6] The same broad principles can be applied to ULPA filters.

[7] The efficiency test uses a monodispersed aerosol of 0.3 micron size particles and assesses filter media. Downstream readings represent an average over the entire filter surface. Efficiency tests are not intended to test for filter leaks.

312 depending on the accuracy of the photometer, a DOP challenge should introduce the aerosol
313 upstream of the filter in a concentration ranging from approximately 25 to l00 micrograms/liter
314 of air at the filter's designed airflow rating. The leak test should be done in place, and the filter
315 face scanned on the downstream side with an appropriate photometer probe, at a sampling rate of
316 at least one cubic foot per minute. The downstream leakage measured by the probe should then
317 be calculated as a percent of the upstream challenge. Scanning should be conducted on the entire
318 filter face and frame at a position about one to two inches from the face of the filter. This
319 comprehensive scanning of HEPA filters should be fully documented.
320
321 A single probe reading equivalent to 0.01 percent of the upstream challenge should be
322 considered as indicative of a significant leak and should result in replacement of the HEPA filter
323 or, when appropriate, repair in a limited area. A subsequent confirmatory re-test should be
324 performed in the area of any repair.
325
326 HEPA filter leak testing alone is not sufficient to monitor filter performance. This testing is
327 usually done only on a semi-annual basis. It is important to conduct periodic monitoring of filter
328 attributes such as uniformity of velocity across the filter (and relative to adjacent filters).
329 Variations in velocity generally increase the possibility of contamination, as these changes (e.g.,
330 velocity reduction) can have an effect on unidirectional airflow. Airflow velocities are measured
331 6 inches from the filter face and at a defined distance proximal to the work surface for HEPA
332 filters in the critical area. Regular velocity monitoring can provide useful data on the clean area
333 in which aseptic processing is performed. HEPA filters should be replaced when nonuniformity
334 of air velocity across an area of the filter is detected or airflow patterns may be adversely
335 affected.
336
337 Although vendors often provide these services, drug manufacturers are responsible for ensuring
338 that these essential certification activities are conducted satisfactorily.

339 **E. Design**
340
341

21 CFR 211.42(b) states, in part, that "The flow of components, drug product containers, closures, labeling, in-process materials, and drug products through the building or buildings shall be designed to prevent contamination."

21 CFR 211.42(c) states, in part, that "There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mixups during the course of the following procedures: *** (10) Aseptic processing, which includes as appropriate: (i) Floors, walls, and ceilings of smooth, hard surfaces that are easily cleanable; *** (iii) An air supply filtered through high-efficiency particulate air filters under positive pressure *** (iv) A system for monitoring environmental conditions; (v) A system for cleaning and disinfecting the room and equipment to produce aseptic conditions; (vi) A system for maintaining any equipment used to control the aseptic conditions."

21 CFR 211.46(b) states that "Equipment for adequate control over air pressure, micro-organisms, dust, humidity, and temperature shall be provided when appropriate for the manufacture, processing, packing, or holding of a drug product."

21 CFR 211.46(c) states, in part, that "Air filtration systems, including pre-filters and particulate matter air filters, shall be used when appropriate on air supplies to production areas."

21 CFR 211.63 states that "Equipment used in the manufacture, processing, packing, or holding of a drug product shall be of appropriate design, adequate size, and suitably located to facilitate operations for its intended use and for its cleaning and maintenance."

21 CFR 211.65(a) states that "Equipment shall be constructed so that surfaces that contact 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."

21 CFR 211.67(a) states that "Equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identify, strength, quality, or purity of the drug product beyond the official or other established requirements."

21 CFR 211.113(b) states that "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."

342
343
344 Aseptic processes are designed to minimize exposure of sterile articles to the potential
345 contamination hazards of the manufacturing operation. Limiting the duration of exposure of sterile
346 product elements, providing the highest possible environmental control, optimizing process flow,
347 and designing equipment to prevent entrainment of lower quality air into the Class 100 (ISO 5)
348 clean area are essential to achieving high assurance of sterilty (Ref. 4).
349
350 Both personnel and material flow should be optimized to prevent unnecessary activities that
351 could increase the potential for introducing contaminants to exposed product, container-closures,
352 or the surrounding environment. The layout of equipment should provide for ergonomics that
353 optimize comfort and movement of operators. The number of personnel in an aseptic processing
354 room should be minimized. The flow of personnel should be designed to limit the frequency

355 with which entries and exits are made to and from an aseptic processing room and, most
356 significantly, its critical area. Regarding the latter, the number of transfers into an isolator, or
357 into the critical area of a traditional clean room, should be minimized. To prevent changes in air
358 currents that introduce lower quality air, movement adjacent to the critical area should be
359 appropriately restricted.
360
361 Any intervention or stoppage during an aseptic process can increase the risk of contamination.
362 The design of equipment used in aseptic processing should limit the number and complexity of
363 aseptic interventions by personnel. For example, personnel intervention can be reduced by
364 integrating an on-line weight check device, thus eliminating a repeated manual activity within
365 the critical area. Rather than performing an aseptic connection, sterilizing the prefastened
366 connection using sterilize-in-place (SIP) technology also can eliminate a significant aseptic
367 manipulation. Automation of other process steps, including the use of technologies such as
368 robotics, can further reduce risk to the product.
369
370 Transfer of products should be performed under appropriate cleanroom conditions. For example,
371 lyophilization processes include transfer of aseptically filled product in partially sealed
372 containers. To prevent contamination, partially closed sterile product should be transferred only
373 in critical areas. Facility design should ensure that the area between a filling line and the
374 lyophilizer and the transport and loading procedures provide Class 100 (ISO 5)
375 protection.
376
377 The sterile drug product and container closures should be protected by equipment of suitable
378 design. Carefully designed curtains, rigid plastic shields, or other barriers should be used in
379 appropriate locations to achieve significant segregation of the aseptic processing line. Use of an
380 isolator system further enhances product protection (see Appendix 1).
381
382 Due to the interdependence of the various rooms that make up an aseptic processing facility, it is
383 essential to carefully define and control the dynamic interactions permitted between cleanrooms.
384 Use of a double-door or integrated sterilizer is valuable in ensuring direct product flow, often
385 from a lower to a higher classified area. Airlocks and interlocking doors facilitate better control
386 of air balance throughout the aseptic processing facility. Airlocks should be installed between
387 the aseptic processing area entrance and the adjoining uncontrolled area. Other interfaces such
388 as personnel transitions or material staging areas are appropriate locations for air locks. It is
389 critical to adequately control material (e.g., in-process supplies, equipment, utensils) as it
390 transfers from lesser to higher controlled clean areas to prevent the influx of contaminants. For
391 example, written procedures should address how materials should be introduced into the aseptic
392 processing room to ensure that room conditions are not compromised. In this regard, materials
393 should be disinfected in accord with appropriate procedures.
394
395 Cleanrooms are normally designed as functional units with specific purposes. A well-designed
396 cleanroom is constructed with materials that allow for ease of cleaning and sanitizing. Examples
397 of adequate design features include seamless and rounded floor to wall junctions as well as
398 readily accessible corners. Floors, walls, and ceilings are constructed of smooth, hard surfaces
399 that can be easily cleaned (211.42). Ceilings and associated HEPA filter banks should be

400 designed to protect sterile materials from contamination. Cleanrooms also should not contain
401 unnecessary equipment, fixtures, or materials.
402
403 Processing equipment and systems should be equipped with sanitary fittings and valves. With
404 rare exceptions, drains are not considered appropriate for classified areas of the aseptic
405 processing facility.
406
407 When applicable, equipment should be suitably designed for ease of sterilization (211.63). Ease
408 of installation to facilitate aseptic setup is also an important consideration. The effect of
409 equipment design on the cleanroom environment should be addressed. Flat surfaces or ledges
410 that accumulate particles should be avoided. Equipment should not obstruct airflow and, in
411 critical areas, its design should not perturb airflow.
412
413 Deviation or change control systems should address atypical conditions posed by shutdown of air
414 handling systems or other utilities, and the impact of construction activities on facility control.
415

416
417 ## V. PERSONNEL TRAINING, QUALIFICATION, & MONITORING
418

21 CFR 211.22(c) states that "The quality control unit shall have the responsibility for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the drug product."

21 CFR 211.25(a) states that "Each person engaged in the manufacture, processing, packing, or holding of a drug product shall have education, training, and experience, or any combination thereof, to enable that person to perform the assigned functions. Training shall be in the particular operations that the employee performs and in current good manufacturing practice (including the current good manufacturing practice regulations in this chapter and written procedures required by these regulations) as they relate to the employee's functions. Training in current good manufacturing practice shall be conducted by qualified individuals on a continuing basis and with sufficient frequency to assure that employees remain familiar with CGMP requirements applicable to them."

21 CFR 211.25(b) states that "Each person responsible for supervising the manufacture, processing, packing, or holding of a drug product shall have the education, training, and experience, or any combination thereof, to perform assigned functions in such a manner as to provide assurance that the drug product has the safety, identity, strength, quality, and purity that it purports or is represented to possess."

21 CFR 211.25(c) states that "There shall be an adequate number of qualified personnel to perform and supervise the manufacture, processing, packing, or holding of each drug product."

21 CFR 211.28(a) states that "Personnel engaged in the manufacture, processing, packing, or holding of a drug product shall wear clean clothing appropriate for the duties they perform. Protective apparel, such as head, face, hand, and arm coverings, shall be worn as necessary to protect drug products from contamination."

21 CFR 211.28(b) states that "Personnel shall practice good sanitation and health habits."

21 CFR 211.28(c) states that "Only personnel authorized by supervisory personnel shall enter those areas of the buildings and facilities designated as limited-access areas."

21 CFR 211.28(d) states that "Any person shown at any time (either by medical examination or supervisory observation) to have an apparent illness or open lesions that may adversely affect the safety or quality of drug products shall be excluded from direct contact with components, drug product containers, closures, in-process materials, and drug products until the condition is corrected or determined by competent medical personnel not to jeopardize the safety or quality of drug products. All personnel shall be instructed to report to supervisory personnel any health conditions that may have an adverse effect on drug products."

21 CFR 211.42(c) states, in part, that "There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mixups during the course of the following procedures: *** (10) Aseptic processing, which includes as appropriate: *** (iv) A system for monitoring environmental conditions***."

21 CFR 211.113(b) states that "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."

419
420 ### A. Personnel
421

422 A well-designed aseptic process minimizes personnel intervention. As operator activities
423 increase in an aseptic processing operation, the risk to finished product sterility also increases.

To ensure maintenance of product sterility, operators involved in aseptic manipulations should adhere to the basic principles of aseptic technique at all times.

Appropriate training should be conducted before an individual is permitted to enter the aseptic processing area and perform operations. For example, such training should include aseptic technique, cleanroom behavior, microbiology, hygiene, gowning, patient safety hazards posed by a nonsterile drug product, and the specific written procedures covering aseptic processing area operations. After initial training, personnel should be updated regularly by an ongoing training program. Supervisory personnel should routinely evaluate each operator's conformance to written procedures during actual operations. Similarly, the quality control unit should provide regular oversight of adherence to established, written procedures and basic aseptic techniques during manufacturing operations.

Some of these techniques aimed at maintaining sterility of sterile items and surfaces include:

- Contacting sterile materials only with sterile instruments

  Sterile instruments (e.g., forceps) should always be used in the handling of sterilized materials. Between uses, instruments should be placed only in sterilized containers. Instruments should be replaced as necessary throughout an operation.

  After initial gowning, sterile gloves should be regularly sanitized to minimize the risk of contamination. Personnel should not directly contact sterile products, containers, closures, or critical surfaces.

- Moving slowly and deliberately

  Rapid movements can create unacceptable turbulence in the critical zone. Such movements disrupt the sterile field, presenting a challenge beyond intended cleanroom design and control parameters. The principle of slow, careful movement should be followed throughout the cleanroom.

- Keeping the entire body out of the path of unidirectional air

  Unidirectional airflow design is used to protect sterile equipment surfaces, container-closures, and product. Personnel should not disrupt the path of unidirectional flow air in the aseptic processing zone.

- Approaching a necessary manipulation in a manner that does not compromise sterility of the product

  To maintain sterility of nearby sterile materials, a proper aseptic manipulation should be approached from the side and not above the product (in vertical unidirectional flow operations). Also, an operator should refrain from speaking when in direct proximity to an aseptic processing line.

470 • Maintaining Proper Gown Control
471
472 Prior to and throughout aseptic operations, an operator should not engage in any activity
473 that poses an unreasonable contamination risk to the gown.
474
475 Only personnel who have been qualified and appropriately gowned should be permitted access to
476 the aseptic processing area. An aseptic processing area gown should provide a barrier between
477 the body and exposed sterilized materials and prevent contamination from particles generated by,
478 and microorganisms shed from, the body. Gowns should be sterile and nonshedding and should
479 cover the skin and hair (face-masks, hoods, beard/moustache covers, protective goggles, elastic
480 gloves, cleanroom boots, and shoe overcovers are examples of common elements of gowns).
481 Written procedures should detail the methods used to don each gown component in an aseptic
482 manner. An adequate barrier should be created by the overlapping of gown components (e.g.,
483 gloves overlapping sleeves). If an element of a gown is found to be torn or defective, it should
484 be changed immediately.
485
486 There should be an established program to regularly assess or audit conformance of personnel to
487 relevant aseptic manufacturing requirements. An aseptic gowning qualification program should
488 assess the ability of a cleanroom operator to maintain the quality of the gown after performance
489 of gowning procedures. Gowning qualification should include microbiological surface sampling
490 of several locations on a gown (e.g., glove fingers, facemask, forearm, chest, other sites).
491 Following an initial assessment of gowning, periodic requalification should monitor various
492 gowning locations over a suitable period to ensure the consistent acceptability of aseptic
493 gowning techniques. Semi-annual or yearly requalification is sufficient for automated operations
494 where personnel involvement is minimized.
495
496 To protect exposed sterilized product, personnel should be expected to maintain gown quality
497 and strictly adhere to appropriate aseptic method. Written procedures should adequately address
498 circumstances under which personnel should be retrained, requalified, or reassigned to other
499 areas.
500
501 **B. Laboratory Personnel**
502
503 The basic principles of training, aseptic technique, and personnel qualification in aseptic
504 manufacturing also are applicable to those performing aseptic sampling and microbiological
505 laboratory analyses. Processes and systems cannot be considered to be in control and
506 reproducible if the validity of data produced by the laboratory is in question.
507
508 **C. Monitoring Program**
509
510 Personnel can significantly affect the quality of the environment in which the sterile product is
511 processed. A vigilant and responsive personnel monitoring program should be established.
512 Monitoring should be accomplished by obtaining surface samples of each operator's gloves on a
513 daily basis, or in association with each batch. This sampling should be accompanied by an
514 appropriate sampling frequency for other strategically selected locations of the gown (Ref. 5).
515 The quality control unit should establish a more comprehensive monitoring program for

516 operators involved in operations which are especially labor intensive (i.e., those requiring
517 repeated or complex aseptic manipulations).
518
519 Asepsis is fundamental to an aseptic processing operation. An ongoing goal for manufacturing
520 personnel in the aseptic processing room is to maintain contamination-free gloves throughout
521 operations. Sanitizing gloves just prior to sampling is inappropriate because it can prevent
522 recovery of microorganisms that were present during an aseptic manipulation. When operators
523 exceed established levels or show an adverse trend, an investigation should be conducted
524 promptly. Follow-up actions can include increased sampling, increased observation, retraining,
525 gowning requalification, and in certain instances, reassignment of the individual to operations
526 outside of the aseptic processing area. Microbiological trending systems, and assessment of the
527 impact of atypical trends, are discussed in more detail under Section XI. Laboratory Controls.
528
529

530 VI. COMPONENTS AND CONTAINER/CLOSURES

531

532 A. Components

533

> 21 CFR 210.3(b)(3) states that "*Component* means any ingredient intended for use in the manufacture of a drug product, including those that may not appear in such drug product."
>
> 21 CFR 211.80(a) states that "There shall be written procedures describing in sufficient detail the receipt, identification, storage, handling, sampling, testing, and approval or rejection of components and drug product containers and closures; such written procedures shall be followed."
>
> 21 CFR 211.80(b) states that "Components and drug product containers and closures shall at all times be handled and stored in a manner to prevent contamination."
>
> 21 CFR 211.84(d)(6) states that "Each lot of a component, drug product container, or closure that is liable to microbiological contamination that is objectionable in view of its intended use shall be subjected to microbiological tests before use."

534
535 A drug product produced by aseptic processing can become contaminated through the use of one
536 or more components (e.g., active ingredients, excipients, Water for Injection) that are
537 contaminated with microorganisms or endotoxins. It is important to characterize the microbial
538 content of each component that could be contaminated and establish appropriate acceptance
539 limits based on information on bioburden. Knowledge of bioburden is critical in assessing
540 whether the sterilization process is adequate.
541
542 In aseptic processing, each component is individually sterilized or several components are
543 combined, with the resulting mixture sterilized.[8] There are several methods for sterilizing
544 components (see relevant discussion in Section IX). A widely used method is filtration of a
545 solution formed by dissolving the component(s) in a solvent such as USP Water for Injection
546 (WFI). The solution is passed through a sterilizing membrane or cartridge filter. Filter

[8] See Appendix III for discussion of certain biologic components that are aseptically handled from the start of the process.

547 sterilization is used where the component is soluble and is likely to be adversely affected by heat.
548 A variation of this method involves subjecting the filtered solution to aseptic crystallization and
549 precipitation (or lyophilization) of the component as a sterile powder. However, this method
550 involves more handling and manipulation and therefore has a higher potential for contamination
551 during processing. If a component is not adversely affected by heat, and is soluble, it can be
552 made into a solution and subjected to steam sterilization, typically in an autoclave or a fixed
553 pressurized sterilize-in-place (SIP) vessel.
554
555 Dry heat sterilization is a suitable method for components that are heat stable and insoluble.
556 However, carefully designed heat penetration and distribution studies should be performed for
557 powder sterilization because of the insulating effects of the powder.
558
559 Ethylene oxide (EtO) exposure is often used for surface sterilization, and for sterilizing certain
560 packages with porous overwrapping. Such methods should be carefully controlled and validated
561 if used for powders to evaluate whether consistent penetration of the sterilant can be achieved
562 and to minimize residual ethylene oxide and by-products.
563
564 Parenteral products are intended to be nonpyrogenic. There should be written procedures and
565 appropriate specifications for acceptance or rejection of each lot of components that might
566 contain endotoxins. Any components failing to meet defined endotoxin limits should be
567 rejected.

568
569 **B. Containers/Closures**
570

> 21 CFR 211.94(c) 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."
>
> 21 CFR 211.94(d) 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."
>
> 21 CFR 211.113(b) states that "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."

571
572 *1. Preparation*
573
574 Containers and closures should be rendered sterile and, for parenteral drug products, pyrogen-
575 free. The type of processes used will depend primarily on the nature of the container and/or
576 closure materials. The validation study for such a process should be adequate to demonstrate its
577 ability to render materials sterile and pyrogen-free. Written procedures should specify the
578 frequency of revalidation of these processes as well as time limits for holding sterile,
579 depyrogenated containers and closures.
580
581 Presterilization preparation of glass containers usually involves a series of wash and rinse cycles.
582 These cycles serve an important role in removing foreign matter. Rinse water should be of high
583 purity so as not to contaminate containers. For parenteral products, final rinse water should meet
584 the specifications of Water for Injection, USP.
585
586 The adequacy of the depyrogenation process can be assessed by spiking containers or closures
587 with known quantities of endotoxin, followed by measuring endotoxin content after
588 depyrogenation. The challenge studies should be performed with a reconstituted endotoxin
589 solution applied directly onto the surface being tested and air-dried. Positive controls should be
590 used to measure the percentage of endotoxin recovery by the test method. Validation study data
591 should demonstrate that the process reduces the endotoxin content by at least 99.9 percent (3
592 logs) (see Section VII).
593
594 Glass containers are generally subjected to dry heat for sterilization and depyrogenation.
595 Validation of dry heat sterilization and depyrogenation should include appropriate heat
596 distribution and penetration studies as well as the use of worst-case process cycles, container
597 characteristics (e.g., mass), and specific loading configurations to represent actual production
598 runs. See Section IX.C.
599
600 Pyrogen on plastic containers can be generally removed by multiple WFI rinses. Plastic
601 containers can be sterilized with an appropriate gas, irradiation, or other suitable means. For
602 gases such as EtO, the parameters and limits of the EtO sterilization cycle (e.g. temperature,
603 pressure, humidity, gas concentration, exposure time, degassing, aeration, and determination of

604 residuals) should be specified and monitored closely. Biological indicators are of special
605 importance in demonstrating the effectiveness of EtO and other gas sterilization processes.
606
607 Rubber closures (e.g., stoppers and syringe plungers) can be cleaned by multiple cycles of
608 washing and rinsing prior to final steam or irradiation sterilization. At minimum, the initial
609 rinses for the washing process should employ Purified Water, USP, of minimal endotoxin
610 content, followed by final rinse(s) with WFI for parenteral products. Normally, depyrogenation
611 is achieved by multiple rinses of hot WFI. The time between washing, drying (where
612 appropriate), and sterilizing should be minimized because residual moisture on the stoppers can
613 support microbial growth and the generation of endotoxins. Because rubber is a poor conductor
614 of heat, extra attention should be given to the validation of processes that use heat with respect to
615 its penetration into the rubber stopper load (See Section XI.C). Validation data from the washing
616 procedure should demonstrate successful endotoxin removal from rubber materials.
617
618 A potential source of contamination is the siliconization of rubber stoppers. Silicone used in the
619 preparation of rubber stoppers should meet appropriate quality control criteria and not have an
620 adverse effect on the safety, quality, or purity of the drug product.
621
622 Contract facilities that perform sterilization and/or depyrogenation of containers and closures are
623 subject to the same CGMP requirements as those established for in-house processing. The
624 finished dosage form manufacturer is responsible for the review and approval of the contractor's
625 validation protocol and final validation report.
626
627 *2. Inspection of Container Closure System*
628
629 A container closure system that permits penetration of air, or microorganisms, is unsuitable for a
630 sterile product. Any damaged or defective units should be detected, and removed, during
631 inspection of the final sealed product. Safeguards should be implemented to strictly preclude
632 shipment of product that may lack container closure integrity and lead to nonsterility.
633 Equipment suitability problems or incoming container or closure deficiencies have caused loss of
634 container closure system integrity. As examples, failure to detect vials fractured by faulty
635 machinery, or by mishandling of bulk finished stock, has led to drug recalls. If damage that is
636 not readily detected leads to loss of container closure integrity, improved procedures should be
637 rapidly implemented to prevent and detect such defects.
638
639 Functional defects in delivery devices (e.g., syringe device defects, delivery volume) can also
640 result in product quality problems and should be monitored by appropriate in-process testing.
641
642 Any defects or results outside the specifications established for in-process and final inspection
643 should be investigated in accord with Section 211.192.
644
645
646 **VII. ENDOTOXIN CONTROL**
647

21 CFR 211.63 states that "Equipment used in the manufacture, processing, packing, or holding of a drug product shall be of appropriate design, adequate size, and suitably located to facilitate operations for its intended use and for its cleaning and maintenance."

21 CFR 211.65(a) states that "Equipment shall be constructed so that surfaces that contact 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."

21 CFR 211.67(a) states that "Equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identify, strength, quality, or purity of the drug product beyond the official or other established requirements."

21 CFR 211.94(c) 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."

21 CFR 211.167(a) states that "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."

648
649 Endotoxin contamination of an injectable product can be a result of poor CGMP controls.
650 Certain patient populations (e.g., neonates), those receiving other injections concomitantly, or
651 those administered a parenteral in atypically large volumes or doses can be at greater risk for
652 pyrogenic reaction than anticipated by the established limits based on body weight of a normal
653 healthy adult (Ref. 6, 7). Such clinical concerns reinforce the need for appropriate CGMP
654 controls to prevent generation of endotoxin. Drug product components, container closures,
655 equipment, and storage time limitations are among the areas to address in establishing endotoxin
656 control.
657
658 Adequate cleaning, drying, and storage of equipment provides for control of bioburden and
659 prevents contribution of endotoxin load. Equipment should be designed to be easily assembled
660 and disassembled, cleaned, sanitized, and/or sterilized. Endotoxin control should be exercised
661 for all product contact surfaces both prior to and after sterile filtration.
662
663 Endotoxin on equipment surfaces is inactivated by high-temperature dry heat, or removed from
664 equipment surfaces by validated cleaning procedures. Some clean-in-place procedures employ
665 initial rinses with appropriate high purity water and/or a cleaning agent (e.g., acid, base,
666 surfactant), followed by final rinses with heated WFI. Equipment should be dried following
667 cleaning. Sterilizing-grade filters and moist heat sterilization have not been shown to be
668 effective in removing endotoxins. Processes that are designed to achieve depyrogenation should
669 demonstrate a 3-log reduction of endotoxin.
670
671
672 **VIII. TIME LIMITATIONS**
673

> 21 CFR 211.111 states, in part, that "When appropriate, time limits for the completion of each phase of production shall be established to assure the quality of the drug product."

674
675 Time limits should be established for each phase of aseptic processing. Time limits should
676 include, for example, the period between the start of bulk product compounding and its filtration,
677 filtration processes, product exposure while on the processing line, and storage of sterilized
678 equipment, containers and closures. Maintenance of in-process quality at different production
679 phases should be supported by data. Bioburden and endotoxin load should be assessed when
680 establishing time limits for stages such as the formulation processing stage.
681
682 The total time for product filtration should be limited to an established maximum to prevent
683 microorganisms from penetrating the filter. Such a time limit should also prevent a significant
684 increase in upstream bioburden and endotoxin load. Sterilizing-grade filters should generally be
685 replaced following each manufactured lot. Because they can provide a substrate for microbial
686 attachment, maximum use times for those filters used upstream for solution clarification or
687 particle removal should also be established and justified.
688
689
690

IX. VALIDATION OF ASEPTIC PROCESSING AND STERILIZATION

691

> 21 CFR 211.63, 211.65, and 211.67 address, respectively, "Equipment design, size, and location," "Equipment construction," and "Equipment cleaning and maintenance."
>
> 21 CFR 211.84(c)(3) states that "Sterile equipment and aseptic sampling techniques shall be used when necessary."
>
> 21 CFR 211.113(b) states that "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."

692
693 This section primarily discusses routine qualification and validation study recommendations.
694 Change control procedures are addressed only briefly, but are an important part of the quality
695 systems established by a firm. As noted above, a change in equipment, process, test method, or
696 systems should be evaluated through the written change control program and should trigger an
697 evaluation of the need for revalidation or requalification.
698
699

A. Process Simulations

700
701 To ensure the sterility of products purporting to be sterile, both sterilization and aseptic filling
702 and closing operations must be adequately validated (211.113). The goal of even the most
703 effective sterilization processes can be defeated if the sterilized elements of a product (the drug,
704 the container, and the closure) are brought together under conditions that contaminate any of
705 those elements. Similarly, product sterility will be compromised if product elements are not
706 sterile when they are assembled.

707
708 The validation of an aseptic processing operation should include the use of a microbiological
709 growth nutrient medium in place of the product. This has been termed a *media fill or process*
710 *simulation*. In the normal media fill simulation, the nutrient medium should be exposed to
711 product contact surfaces of equipment, container closure systems, critical environments, and
712 process manipulations to closely simulate the same exposure that the product itself will undergo.
713 The sealed containers filled with the media are then incubated to detect microbial contamination.
714 The results should be interpreted to determine the potential for a unit of drug product to become
715 contaminated during actual operations (e.g., start-up, sterile ingredient additions, aseptic
716 connections, filling, closing). Environmental monitoring data from the process simulation can
717 also provide useful information for the processing line evaluation.
718
719 *1. Study Design*
720
721 A recommended media fill program incorporates the contamination risk factors that occur on a
722 production line, and accurately assesses the state of process control. Media fill studies should
723 simulate aseptic manufacturing operations as closely as possible, incorporating a worst-case
724 approach. The media fill program should address applicable issues such as:
725

726 - factors associated with the longest permitted run on the processing line
727 - number and type of normal interventions, atypical interventions, unexpected events
728 (e.g., maintenance), stoppages, equipment adjustments or transfers
729 - lyophilization, when applicable
730 - aseptic assembly of equipment (e.g., at start-up, during processing)
731 - number of personnel and their activities
732 - number of aseptic additions (e.g., charging containers and closures as well as sterile
733 ingredients)
734 - shift changes, breaks, and gown changes (when applicable)
735 - number and type of aseptic equipment disconnections/connections
736 - aseptic sample collections
737 - line speed and configurations
738 - manual weight checks
739 - operator fatigue
740 - container closure systems (e.g., sizes, type, compatibility with equipment)
741 - specific provisions of aseptic processing related Standard Operating Procedures (e.g.,
742 conditions permitted before line clearance is mandated)

743

744 A written batch record, documenting production conditions and simulated activities, should be
745 prepared for each media fill run. The same vigilance should be observed in both media fill and
746 routine production runs. Media fills should not be used to justify an unacceptable practice.
747
748 *2. Frequency and Number of Runs*
749
750 When a processing line is initially qualified, separate media fills should be repeated enough
751 times to ensure that results are consistent and meaningful. This approach is important because a
752 single run can be inconclusive, while multiple runs with divergent results signal a process that is
753 not in control. At least three consecutive separate successful runs should be performed during
754 initial line qualification. Subsequently, routine semi-annual qualification should be conducted
755 for each processing line to evaluate the state of control of the aseptic process. Activities and
756 interventions representative of each shift, and shift changeover, should be incorporated into the
757 design of the semi-annual qualification. For example, the evaluation of a shift should address its
758 unique time-related and operational features. All personnel who enter the aseptic processing
759 area, including technicians and maintenance personnel, should participate in a media fill at least
760 once a year. Participation should be consistent with the nature of each operator's duties during
761 routine production. Each change to a product or line change should be evaluated using a written
762 change control system. Any changes or events that have the potential to affect the ability of the
763 aseptic process to exclude contamination from the sterilized product should be assessed through
764 additional media fills. For example, facility and equipment modifications, line configuration
765 changes, significant changes in personnel, anomalies in environmental testing results, container
766 closure system changes or, end product sterility testing showing contaminated products may be
767 cause for revalidation of the system.
768
769 Where data from a media fill indicate the process may not be in control, a comprehensive
770 documented investigation should be conducted to determine the origin of the contamination and
771 the scope of the problem. Once corrections are instituted, repeat process simulation runs should
772 be performed to confirm that deficiencies in practices and procedures have been corrected and
773 the process has returned to a state of control. When an investigation fails to reach well-
774 supported, substantive conclusions as to the cause of the media fill failure, three consecutive
775 successful runs and increased scrutiny (e.g., extra supervision, monitoring) of the production
776 process should be implemented.
777
778 *3. Duration of Runs*
779
780 The duration of aseptic processing operations is a major consideration in determining the size of
781 the media fill run. Although the most accurate simulation model would be the full batch size and
782 duration because it most closely simulates the actual production run, other appropriate models
783 can be justified. In any study protocol, the duration of the run and the overall study design
784 should adequately mimic worst-case operating conditions and cover all manipulations that are
785 performed in the actual processing operation. In this regard, interventions that commonly occur
786 should be routinely simulated, while those occurring rarely can be simulated periodically.
787
788 While conventional manufacturing lines are highly automated, often operate at relatively high
789 speeds, and are designed to limit operator intervention, there are some processes that include

790 considerable operator involvement. When aseptic processing employs manual filling or closing,
791 or extensive manual manipulations, the duration of the process simulation should generally be no
792 less than the length of the actual manufacturing process to best simulate contamination risks
793 posed by operators.
794
795 For lyophilization operations, unsealed containers should be exposed to pressurization and partial
796 evacuation of the chamber in a manner that simulates the process. Vials should not be frozen, as
797 this may inhibit the growth of microorganisms.
798
799 *4. Size of Runs*
800
801 The simulation run sizes should be adequate to mimic commercial production conditions and
802 accurately assess the potential for commercial batch contamination. The number of units filled
803 during the process simulation should be based on contamination risk for a given process and
804 sufficient to accurately simulate activities that are representative of the manufacturing process.
805 A generally acceptable starting point for run size is in the range of 5,000 to 10,000 units. For
806 operations with production sizes under 5,000, the number of media filled units should equal the
807 maximum batch size made on the processing line (Ref. 8).
808
809 When the possibility of contamination is higher based on the process design (e.g., manually
810 intensive filling lines), a larger number of units, generally at or approaching the full production
811 batch size, should be used. In contrast, a process conducted in an isolator (see Appendix 1) can
812 have a low risk of contamination because of the lack of direct human intervention and can be
813 simulated with a lower number of units as a proportion of the overall operation.
814
815 Some batches are produced over multiple shifts or yield an unusually large number of units, and
816 media fill size and duration are especially important considerations in the media fill protocol.
817 These factors should be carefully considered when designing the simulation to adequately
818 encompass conditions and any potential risks associated with the larger operation.
819
820 *5. Line Speed*
821
822 The media fill program should adequately address the range of line speeds (e.g., by bracketing all
823 vial sizes and fill volumes) employed during production. Each individual media fill run should
824 evaluate a single worst-case line speed, and the speed chosen for each run during a study should
825 be justified. For example, use of high line speed is often most appropriate in the evaluation of
826 manufacturing processes characterized by frequent interventions or a significant degree of
827 manual manipulation. Use of slow line speed is generally appropriate for evaluating
828 manufacturing processes characterized by prolonged exposure of the sterile drug product and
829 container closures in the aseptic area.
830
831 *6. Environmental Conditions*
832
833 Media fills should be adequately representative of the range of conditions under which actual
834 manufacturing operations are conducted. An inaccurate assessment (making the process appear
835 cleaner than it actually is) can result from conducting a media fill under extraordinary air

836 particulate and microbial quality, or under production controls and precautions taken in
837 preparation for the media fill. To the extent standard operating procedures permit stressful
838 conditions, it is important that media fills include analogous challenges to support the validity of
839 these studies.
840
841 *7. Media*
842
843 In general, a microbiological growth medium, such as soybean casein digest medium, should be
844 used. Use of anaerobic growth media (e.g., fluid thioglycollate medium) would be appropriate in
845 special circumstances. The media selected should be demonstrated to promote growth of USP
846 <71> indicator microorganisms as well as representative isolates identified by environmental
847 monitoring, personnel monitoring, and positive sterility test results. Positive control units should
848 be inoculated with a <100 CFU challenge and incubated. For those instances in which the
849 growth promotion testing fails, the origin of any contamination found during the simulation
850 should nonetheless be investigated, and the media fill should be promptly repeated.
851
852 The production process should be accurately simulated using media and conditions that optimize
853 detection of any microbiological contamination. Each unit should be filled with an appropriate
854 quantity and type of microbial growth medium to contact the inner container closure surfaces
855 (when the unit is inverted or thoroughly swirled) and permit visual detection of microbial
856 growth.
857
858 Some drug manufacturers have expressed concern over the possible contamination of the facility
859 and equipment with the nutrient media during media fill runs. However, if the medium is
860 handled properly and is promptly followed by the cleaning, sanitizing, and, where necessary,
861 sterilization of equipment, subsequently processed products are not likely to be compromised.
862
863 *8. Incubation and Examination of Media-Filled Units*
864
865 Media units should be incubated under conditions adequate to detect organisms that can
866 otherwise be difficult to culture. Incubation conditions should be established in accord with the
867 following general guidelines:
868
869 - Incubation temperature should be suitable for recovery of bioburden and environmental
870 isolates and should at no time be outside the range of 20-35°C. Incubation temperature
871 should be maintained within 2.5^{o}C of the target temperature.
872
873 - Incubation time should not be less than 14 days. If two temperatures are used for the
874 incubation of the media filled samples, the samples should be incubated for at least 7
875 days at each temperature.
876
877 Each media-filled unit should be examined for contamination by personnel with appropriate
878 education, training, and experience in microbiological techniques. There should be direct quality
879 control unit oversight throughout any such examination. Clear containers with otherwise
880 identical physical properties should be used as a substitute for amber or other opaque containers
881 to allow visual detection of microbial growth.

882
883 When a firm performs a final product inspection of units immediately following the media fill
884 run, all integral units should proceed to incubation. Units found to have defects not related to
885 integrity (e.g., cosmetic defect) should be incubated; units that lack integrity should be rejected.
886 Erroneously rejected units should be returned promptly for incubation with the media fill lot.
887
888 After incubation is underway, any unit found to be damaged should be included in the data for
889 the media fill run, because the incubation of the units simulates release to the market. Any
890 decision to exclude such incubated units (i.e., nonintegral) from the final run tally should be fully
891 justified and the deviation explained in the media fill report. If a correlation emerges between
892 difficult to detect damage and microbial contamination, a thorough investigation should be
893 conducted to determine its cause (see Section VI.B).
894
895 Written procedures regarding aseptic interventions should be clear and specific (e.g., intervention
896 type; quantity of units removed), providing for consistent production practices and assessment of
897 these practices during media fills. If written procedures and batch documentation are adequate,
898 these intervention units do not need to be incubated during media fills.[9] Where procedures lack
899 specificity, there would be insufficient justification for exclusion of units removed during an
900 intervention from incubation. As an example, if a production procedure requires removal of 10
901 units after an intervention at the stoppering station infeed, batch records (i.e., for production and
902 media fills) should clearly document conformance with this procedure. In no case should more
903 units be removed during a media fill intervention than would be cleared during a production run.
904 The ability of a media fill run to detect potential contamination from a given simulated activity
905 should not be compromised by a large-scale line clearance, which can result in removal of a
906 positive unit caused by an unrelated event or intervention. If unavoidable, appropriate study
907 provisions should be made to compensate in such instances.
908
909 Appropriate criteria should be established for yield and accountability. Media fill record
910 reconciliation documentation should include a full accounting and description of units rejected
911 from a batch.
912
913 *9. Interpretation of Test Results*
914
915 The process simulation run should be observed, and contaminated units should be reconcilable
916 with the approximate time and the activity being simulated during the media fill. Video
917 recording of a media fill has been found to be useful in identifying personnel practices that could
918 negatively impact the aseptic process.
919
920 Any contaminated unit should be considered as objectionable and fully investigated. The
921 microorganisms should be identified to species level. In the case of a media fill failure, a
922 comprehensive investigation should be conducted, surveying all possible causes of the
923 contamination. The effects on commercial drugs produced on the line since the last successful
924 media fill should also be assessed.
925

[9] To assess contamination risk during initial aseptic setup (before fill), valuable information can be obtained by incubating all such units that may be normally removed.

926 Whenever contamination exists in a media fill run, it should be considered indicative of a
927 potential sterility assurance problem, regardless of run size. The number of contaminated units
928 should not be expected to increase in a directly proportional manner with the number of vials in
929 the media fill run. Test results should reliably and reproducibly show that the units produced by
930 an aseptic processing operation are sterile. Modern aseptic processing operations in suitably
931 designed facilities have demonstrated a capability of meeting contamination levels approaching
932 zero (Ref. 8, 9) and should normally yield no media fill contamination. Recommended criteria
933 for assessing state of aseptic line control are as follows:
934
935 • When filling fewer than 5000 units, no contaminated units should be detected.
936
937 • When filling from 5,000 to 10,000 units:
938 -- 1 contaminated unit should result in an investigation, including consideration of a
939 repeat media fill.
940 -- 2 contaminated units are considered cause for revalidation, following investigation.
941
942 • When filling more than 10,000 units:
943 -- 1 contaminated unit should result in an investigation.
944 -- 2 contaminated units are considered cause for revalidation, following investigation.
945
946 For any run size, intermittent incidents of microbial contamination in media filled runs can be
947 indicative of a persistent low-level contamination problem that should be investigated.
948 Accordingly, recurring incidents of contaminated units in media fills for an individual line,
949 regardless of acceptance criteria, would be a signal of an adverse trend on the aseptic processing
950 line that should lead to problem identification, correction, and revalidation.
951
952 A firm's use of media fill acceptance criteria allowing infrequent contamination does not mean
953 that a distributed lot of drug product purporting to be sterile may contain a nonsterile unit. The
954 purpose of an aseptic process is to prevent any contamination. A manufacturer is fully liable for
955 the shipment of any nonsterile unit, an act that is prohibited under the FD&C Act (§ 301(a) 21
956 U.S.C. 331(a)). FDA also recognizes that there might be some scientific and technical
957 limitations on how precisely and accurately validation can characterize a system of controls
958 intended to exclude contamination.
959
960 As with any validation run, it is important to note that *invalidation* of a media fill run should be a
961 rare occurrence. A media fill run should be aborted only under circumstances in which written
962 procedures require commercial lots to be equally handled. Supporting documentation and
963 justification should be provided in such cases.
964
965 **B. Filtration Efficacy**
966
967 Filtration is a common method of sterilizing drug product solutions. An appropriate sterilizing
968 grade filter is one that reproducibly removes all microorganisms from the process stream,
969 producing a sterile effluent. Such filters usually have a rated porosity of 0.2 micron or smaller.
970 Whatever filter or combination of filters is used, validation should include microbiological
971 challenges to simulate worst-case production conditions regarding the size of microorganisms in

972 the material to be filtered and integrity test results of the filters used for the study. The
973 microorganisms should be small enough to both challenge the nominal porosity of the filter and
974 simulate the smallest microorganism that may occur in production. The microorganism
975 *Brevundimonas diminuta* (ATCC 19146) when properly grown, harvested and used, can be
976 satisfactory in this regard because it is one of the smallest bacteria (0.3 micron mean diameter).
977 Bioburden of unsterilized bulk solutions should be determined to trend the characteristics of
978 potentially contaminating organisms. In certain cases, when justified as equivalent or better than
979 use of *Brevundimonas diminuta*, it may be appropriate to conduct bacterial retention studies with
980 a bioburden isolate. The number of microorganisms in the challenge is important because a filter
981 can contain a number of pores larger than the nominal rating, which has the potential to allow
982 passage of microorganisms. The probability of such passage is considered to increase as the
983 number of organisms (bioburden) in the material to be filtered increases. A challenge
984 concentration of at least 10^7 organisms per cm^2 of effective filtration area of *B. diminuta* should
985 generally be used. A commercial lot's actual influent bioburden should not include
986 microorganisms of a size and/or concentration that would present a challenge beyond that
987 considered by the validation study (Refs. 10, 11, 12).
988
989 Direct inoculation into the drug formulation provides an assessment of the effect of drug product
990 on the filter matrix and on the challenge organism. However, directly inoculating *B. diminuta*
991 into products with inherent bactericidal activity or into oil-based formulations can lead to
992 erroneous conclusions. When sufficiently justified, the effects of the product formulation on the
993 membrane's integrity can be assessed using an appropriate alternate method. For example, the
994 drug product could be filtered in a manner in which the worst-case combination of process
995 specifications and conditions are simulated. This step could be followed by filtration of the
996 challenge organism for a significant period of time, under the same conditions, using an
997 appropriately modified product (e.g., lacking an antimicrobial preservative or other antimicrobial
998 component) as the vehicle. Any divergence from a simulation using the actual product and
999 conditions of processing should be justified.
1000
1001 Factors that can affect filter performance normally include (1) viscosity of the material to be
1002 filtered, (2) pH, (3) compatibility of the material or formulation components with the filter itself,
1003 (4) pressures, (5) flow rates, (6) maximum use time, (7) temperature, (8) osmolality, (9) and the
1004 effects of hydraulic shock. When designing the validation protocol, it is important to address the
1005 effect of the extremes of processing factors on the filter capability to produce sterile effluent.
1006 Filter validation should be conducted using the worst-case conditions, such as maximum filter
1007 use time and pressure (Ref. 12). Filter validation experiments, including microbial challenges,
1008 need not be conducted in the actual manufacturing areas. However, it is essential that laboratory
1009 experiments simulate actual production conditions. The specific type of filter used in
1010 commercial production should be evaluated in filter validation studies. When the more complex
1011 filter validation tests go beyond the capabilities of the filter user, tests are often conducted by
1012 outside laboratories or by filter manufacturers. However, it is the responsibility of the filter user
1013 to review the validation data on the efficacy of the filter in producing a sterile effluent. The data
1014 should be applicable to the user's products and conditions of use because filter performance may
1015 differ significantly for various conditions and products.
1016

1017 After a filtration process is properly validated for a given product, process, and filter, it is
1018 important to ensure that identical filter replacements (membrane or cartridge) used in production
1019 runs will perform in the same manner. Sterilizing filters should be routinely discarded after
1020 processing of a single batch. Normally, integrity testing of the filter is performed prior to
1021 processing, after the filter apparatus has already been assembled and sterilized. It is important
1022 that integrity testing be conducted after filtration to detect any filter leaks or perforations that
1023 might have occurred during the filtration. *Forward flow and bubble point* tests, when
1024 appropriately employed, are two integrity tests that can be used. A production filter's integrity
1025 test specification should be consistent with data generated during filtration efficacy studies.
1026
1027 We recommend you consider use of sterilizing-grade filters in series; this is a common practice.
1028
1029 **C. Sterilization of Equipment and Container and Closures**
1030
1031 To maintain sterility, equipment surfaces that contact a sterilized drug product or sterilized
1032 container or closure surfaces must be sterile so as not to alter purity of the drug (211.63 and
1033 211.113). Those surfaces that are in the vicinity of sterile product or container closures, but do
1034 not directly contact the product should also be rendered sterile where reasonable contamination
1035 potential exists. It is as important in aseptic processing to properly validate the processes used to
1036 sterilize such critical equipment as it is to validate processes used to sterilize the drug product
1037 and its container and closure. Moist heat and dry heat sterilization are most widely used and the
1038 primary processes discussed in this document. It should be noted that many of the heat
1039 sterilization principles discussed in this document are also applicable to other sterilization
1040 methods.
1041
1042 Sterility of aseptic processing equipment should be maintained by batch-by-batch sterilization.
1043 Following sterilization of equipment, containers, or closures, transportation or assembly should
1044 be performed with adherence to strict aseptic methods in a manner that protects and sustains the
1045 product's sterile state.
1046
1047 *1. Sterilizer Qualification and Validation*
1048
1049 Validation studies should be conducted demonstrating the efficacy of the sterilization cycle.
1050 Requalification studies should also be performed on a periodic basis. For both the validation
1051 studies and routine production, use of a specified load configuration should be documented in the
1052 batch records.
1053
1054 The insulating properties of unevacuated air prevent moist heat under pressure from penetrating
1055 or heating up materials and achieving the lethality associated with saturated steam.
1056 Consequently, for such processes, there is a far slower thermal energy transfer and rate of kill
1057 from the dry heat in insulated locations in the load. It is important to remove air from the
1058 autoclave chamber as part of a moist heat under pressure sterilization cycle.
1059
1060 For the various methods of sterilization, special attention should be given to the nature or type of
1061 the materials to be sterilized and the placement of biological indicators within the sterilization
1062 load. D-value of the biological indicator can vary widely depending on the material to be

1063 sterilized. Potentially difficult to reach locations within the sterilizer load or equipment train (for
1064 SIP applications) should be evaluated in initial studies. For example, filter installations in piping
1065 can cause a substantial pressure differential across the filter, resulting in a significant temperature
1066 drop on the downstream side. Biological indicators should be placed at appropriate downstream
1067 locations of this equipment to determine if the drop in temperature affects the thermal input at
1068 these sites. Requalification and/or revalidation should continue to focus on the load areas
1069 identified as most difficult to penetrate or heat (e.g., worst-case locations of tightly wrapped or
1070 densely packed supplies, securely fastened load articles, lengthy tubing, the sterile filter
1071 apparatus, hydrophobic filters, stopper load).
1072
1073 The formal program providing for regular revalidation should consider the age of the sterilizer
1074 and its past performance. Change control procedures should adequately address issues such as a
1075 load configuration change or a modification of the sterilizer.
1076
1077 a. Qualification: Empty Chamber
1078
1079 Temperature distribution studies evaluate numerous locations throughout an empty
1080 sterilizing unit (e.g., steam autoclave, dry heat oven) or equipment train (e.g., large tanks,
1081 immobile piping). It is important that these studies assess temperature uniformity at
1082 various locations throughout the sterilizer to identify potential *cold spots* where there can
1083 be insufficient heat to attain sterility. These heat uniformity or *temperature mapping*
1084 studies should be conducted by placing calibrated temperature measurement devices in
1085 numerous locations throughout the chamber.
1086
1087 b. Validation: Loaded Chamber
1088
1089 Heat penetration studies should be performed using the established sterilizer load(s).
1090 Validation of the sterilization process with a loaded chamber demonstrates the effects of
1091 loading on thermal input to the items being sterilized, and may identify *cold spots* where
1092 there is insufficient heat to attain sterility. The placement of biological indicators (BI) at
1093 numerous positions in the load, including the most difficult to sterilize places, is a direct
1094 means of demonstrating the efficacy of any sterilization procedure. In general, the
1095 thermocouple (TC) is placed adjacent to the BI so as to assess the correlation between
1096 microbial lethality and thermal input. When determining which articles are most difficult
1097 to sterilize, special attention should be given to the sterilization of filters.
1098
1099 Ultimately, cycle specifications for such sterilization methods are based on the delivery
1100 of adequate thermal input to the slowest to heat locations. A sterility assurance level of
1101 10^{-6} or better should be demonstrated for a sterilization process. For more information,
1102 please also refer to the FDA guidance entitled *Guideline for the Submission of*
1103 *Documentation for Sterilization Process Validation in Applications for Human and*
1104 *Veterinary Drug Products*.
1105
1106 *2. Equipment Controls and Instrument Calibration*
1107

1108 For both validation and routine process control, the reliability of the data generated by
1109 sterilization cycle monitoring devices should be considered to be of the utmost importance.
1110 Devices that measure cycle parameters should be routinely calibrated. Written procedures
1111 should be established to ensure that these devices are maintained in a calibrated state. For
1112 example:
1113
1114 • Temperature monitoring devices for heat sterilization should be calibrated at suitable
1115 intervals, as well as before and after validation runs.
1116 • Devices used to monitor dwell time in the sterilizer should be periodically calibrated.
1117 • The microbial count and D-value of a biological indicator should be confirmed before
1118 a validation study.
1119 • Bacterial endotoxin challenges should be appropriately prepared and measured by the
1120 laboratory.
1121 • Instruments used to determine the purity of steam should be calibrated as appropriate.
1122 • For dry heat depyrogenation tunnels, devices (e.g. sensors and transmitters) used to
1123 measure belt speed should be routinely calibrated.
1124
1125 To ensure robust process control, sterilizing equipment should be properly designed with
1126 attention to features such as accessibility to sterilant, piping slope, and proper condensate
1127 removal (as applicable). Equipment control should be ensured through placement of measuring
1128 devices at those risk-based control points that are most likely to rapidly detect unexpected
1129 process variability. Where manual manipulations of valves are required for sterilizer operations,
1130 these steps should be documented in manufacturing procedures. Sterilizing equipment should be
1131 properly maintained to allow for consistently satisfactory function. Evaluation of sterilizer
1132 performance attributes such as equilibrium (come up) time studies should be helpful in assessing
1133 if the unit continues to operate properly.
1134

1135
1136
X. LABORATORY CONTROLS
1137
1138

21 CFR 211.22(c) states that "The quality control unit shall have the responsibility for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the drug product."

21 CFR 211.42(c) states, in part, that "There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mixups during the course of the following procedures: *** (10) Aseptic processing, which includes as appropriate: *** (iv) A system for monitoring environmental conditions***."

21 CFR 211.56(b) states that "There shall be 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; such written procedures shall be followed."

21 CFR 211.56(c) states, in part, that "There shall be written procedures for use of suitable rodenticides, insecticides, fungicides, fumigating agents, and cleaning and sanitizing agents. Such 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."

21 CFR 211.113(b) states that "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."

21 CFR 211.160(b) states that "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. Laboratory controls shall include: (1) Determination of conformance to appropriate written specifications for the acceptance of each lot within each shipment of components, drug product containers, closures, and labeling used in the manufacture, processing, packing, or holding of drug products. The specifications shall include a description of the sampling and testing procedures used. Samples shall be representative and adequately identified. Such procedures shall also require appropriate retesting of any component, drug product container, or closure that is subject to deterioration. (2) Determination of conformance to written specifications and a description of sampling and testing procedures for in-process materials. Such samples shall be representative and properly identified. (3) Determination of conformance to written descriptions of sampling procedures and appropriate specifications for drug products. Such samples shall be representative and properly identified. (4) The calibration of instruments, apparatus, gauges, and recording devices at suitable intervals in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for remedial action in the event accuracy and/or precision limits are not met. Instruments, apparatus, gauges, and recording devices not meeting established specifications shall not be used."

21 CFR 211.165(e) states that "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 211.194(a)(2)."

21 CFR 211.192 states, in part, 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."

1139

1140 **A. Environmental Monitoring**
1141
1142 *1. General Written Program*
1143
1144 In aseptic processing, one of the most important laboratory controls is the establishment of an
1145 environmental monitoring program. This monitoring provides meaningful information on the
1146 quality of the aseptic processing environment (when a given batch is being manufactured) as
1147 well as environmental trends of the manufacturing area. An adequate program identifies
1148 potential routes of contamination, allowing for implementation of corrections before product
1149 contamination occurs (211.42 and 211.113).
1150
1151 Evaluating the quality of air and surfaces in the cleanroom environment should start with a well-
1152 defined written program and scientifically sound methods. The monitoring program should
1153 cover all production shifts and include air, floors, walls, and equipment surfaces, including the
1154 critical surfaces that come in contact with the product, container, and closures. Written
1155 procedures should include a list of locations to be sampled. Sample timing, frequency, and
1156 location should be carefully selected based upon their relationship to the operation performed.
1157 Samples should be taken throughout the aseptic processing facility (e.g., aseptic corridors,
1158 gowning rooms) using scientifically sound sampling procedures. Sampling sizes should be
1159 sufficient to optimize detection of environmental contaminants at levels that might be expected
1160 in a given clean area.
1161
1162 Locations posing the most microbiological risk to the product are a critical part of the program. It
1163 is especially important to monitor the microbiological quality of the aseptic processing clean area
1164 to determine whether or not aseptic conditions are maintained during filling and closing
1165 activities. Air and surface samples should be taken at the actual working site and at locations
1166 where significant activity or product exposure occurs during production. Critical surfaces that
1167 come in contact with the sterile product should be sterile. When identifying critical sites to be
1168 sampled, consideration should be given to the points of contamination risk in a process,
1169 including factors such as difficulty of setup, length of processing time, impact of interventions.
1170 Critical surface sampling should be performed at the conclusion of the aseptic processing
1171 operation to avoid direct contact with sterile surfaces during processing. Detection of microbial
1172 contamination on a critical site should not necessarily result in batch rejection. The contaminated
1173 critical site sample should be investigated with an awareness of the potential for a low incidence
1174 of false positives and should include an assessment of operational information and data.
1175
1176 Environmental monitoring methods do not always recover microorganisms present in the
1177 sampled area. In particular, low-level contamination can be particularly difficult to detect.
1178 Because of the likelihood of false negatives, consecutive growth results are only one type of
1179 adverse trend. Increased incidence of contamination over a given period is an equal or more
1180 significant trend to be tracked.
1181
1182 In the absence of any adverse trend, a single result above an action level should trigger an
1183 evaluation and a determination about whether remedial measures may be appropriate. In all
1184 room classes, remedial measures should be taken in response to unfavorable trends.
1185

1186 All environmental monitoring locations should be described in SOPs with sufficient detail to
1187 allow for reproducible sampling of a given location surveyed. Written SOPs should also address
1188 areas such as (1) frequency of sampling, (2) when the samples are taken (i.e., during or at the
1189 conclusion of operations), (3) duration of sampling, (4) sample size (e.g., surface area, air
1190 volume), (5) specific sampling equipment and techniques, (6) alert and action levels, and (7)
1191 appropriate response to deviations from alert or action levels.
1192
1193 *2. Establishing Levels and a Trending Program*
1194
1195 Microbiological monitoring levels should be established based on the relationship of the sampled
1196 location to the operation. The levels should be based on the need to maintain adequate
1197 microbiological control throughout the entire sterile manufacturing facility. One should also
1198 consider environmental monitoring data from historical databases, media fills, cleanroom
1199 qualification, and sanitization studies, in developing monitoring levels. Published data from
1200 similar operations can also be helpful in setting action and alert levels, especially for a new
1201 operation.
1202
1203 Monitoring the microbiological quality of the environment should include both alert and action
1204 levels. Each individual sample result should be evaluated for its significance by comparison to
1205 the alert or action levels. Averaging of results can mask unacceptable localized conditions. A
1206 result at the alert level urges attention to the approaching action conditions. A result at the action
1207 level should prompt a more thorough investigation. Written procedures should be established,
1208 detailing data review frequency, identification of contaminants, and actions to be taken. The
1209 quality control unit should provide routine oversight of near-term (e.g., daily, weekly, monthly,
1210 quarterly) and long-term trends in environmental and personnel monitoring data.
1211
1212 Trend reports should include data generated by location, shift, lot, room, operator, or other
1213 search parameters. The quality control unit should be responsible for producing specialized data
1214 reports (e.g., a search on a particular isolate over a year period) with the goal of investigating
1215 results beyond established levels and identifying any appropriate follow-up actions. Significant
1216 changes in microbial flora should be considered in the review of the ongoing environmental
1217 monitoring data.
1218
1219 Written procedures should define the system whereby the most responsible managers are
1220 regularly informed and updated on trends and investigations.
1221
1222 *3. Sanitization Efficacy*
1223
1224 The suitability, efficacy, and limitations of sanitization agents and procedures should be
1225 assessed. The effectiveness of these sanitization agents and procedures should be measured by
1226 their ability to ensure that potential contaminants are adequately removed from surfaces (i.e., via
1227 obtaining samples before and after sanitization).
1228
1229 Upon preparation, disinfectants should be rendered sterile, and used for a limited time, as
1230 specified by written procedures. Routinely used disinfectants should be effective against the
1231 normal microbial vegetative flora recovered from the facility. Many common sanitizers are

1232 ineffective against spores, for example, 70 percent isopropyl alcohol is ineffective against
1233 *Bacillus,* spp. spores. Therefore a sound disinfectant program also includes a sporicidal agent,
1234 used according to a written schedule and when environmental data suggest the presence of
1235 sporeforming organisms.
1236
1237 Sanitization procedures should be described in sufficient detail (e.g., preparation, work sequence,
1238 contact time) to enable reproducibility. Once the procedures are established, their adequacy
1239 should be evaluated using a routine environmental monitoring program.
1240
1241 *4. Monitoring Methods*
1242
1243 Acceptable methods for monitoring the microbiological quality of the environment include:
1244
1245 a. Surface Monitoring
1246
1247 Environmental monitoring should include testing of various surfaces for microbiological
1248 quality. For example, product contact surfaces, floors, walls, ceilings, and equipment
1249 should be tested on a regular basis. Routinely used for such tests are touch plates, swabs,
1250 and contact plates.
1251
1252 b. Active Air Monitoring
1253
1254 The method for assessing the microbial quality of air should involve the use of *active*
1255 devices such as slit to agar samplers, those using liquid impingement and membrane (or
1256 gelatin) filtration, and centrifugal samplers. Each device has certain advantages and
1257 disadvantages, although all allow a quantitative testing of the number of organisms per
1258 volume of air sampled. The use of such devices in aseptic areas is considered an
1259 essential part of evaluating the environment during each production shift, at carefully
1260 chosen critical locations. Manufacturers should be aware of a device's air monitoring
1261 capabilities, and the air sampler should be evaluated for its suitability for use in an
1262 aseptic environment based on cleanability, ability to be sterilized, and disruption of
1263 unidirectional airflow. Manufacturers should ensure that such devices are calibrated and
1264 used according to appropriate procedures.[10] Because devices vary, the user should assess
1265 the suitability of all monitoring devices before they are placed into service.
1266
1267 c. Passive Air Monitoring (Settling Plates)
1268
1269 Another method is the use of passive air samplers, such as settling plates (petri dishes
1270 containing nutrient growth medium exposed to the environment). Settling plates lack
1271 value as quantitative air monitors because only microorganisms that settle onto the agar
1272 surface will be detected. Their value as qualitative indicators in critical areas is enhanced
1273 by positioning plates in locations posing the greatest risk of product contamination. As
1274 part of methods validation, the quality control laboratory should evaluate what media
1275 exposure conditions optimize recovery of low levels of environmental isolates. Exposure

[10] For example, the volume of air sampled should be sufficient to yield meaningful measurements of air quality in a given environment.

1276 conditions should preclude desiccation (e.g., caused by lengthy sampling periods and/or
1277 high airflows), which inhibits recovery of microorganisms. The data generated by
1278 passive air sampling can be useful when considered in combination with results from
1279 other types of air samples.
1280
1281 **B. Microbiological Media and Identification**
1282
1283 Characterization of recovered microorganisms is an important aspect of the environmental
1284 monitoring program. Environmental isolates often correlate with the contaminants found in a
1285 media fill or product sterility testing failure, and the overall environmental picture provides
1286 valuable information for an investigation. Monitoring of critical and immediately surrounding
1287 clean areas as well as personnel should include routine identification of microorganisms to the
1288 species (or, where appropriate, genus) level. In some cases, environmental trending data have
1289 revealed migration of microorganisms into the aseptic processing room from either uncontrolled
1290 or lesser-controlled areas. Establishing an adequate program for differentiating microorganisms
1291 in the lesser-controlled environments, such as Class 100,000 (ISO 8), is instrumental in detecting
1292 such trends. At minimum, the program should require species (or, where appropriate, genus)
1293 identification of microorganisms in these ancillary environments at frequent intervals to establish
1294 a valid, current database of contaminants present in the facility during processing (and to
1295 demonstrate that cleaning and sanitization procedures continue to be effective).
1296
1297 Rapid genotypic methods are recommended for purposes of identification, as these methods have
1298 been shown to be more accurate and precise than biochemical and phenotypic techniques.
1299
1300 The goal of microbiological monitoring is to reproducibly detect microorganisms for purposes of
1301 monitoring the state of environmental control. Consistent methods will yield a database that
1302 allows for sound data comparisons and interpretations. The microbiological culture media used
1303 in environmental monitoring should be validated as capable of detecting fungi (i.e., yeasts and
1304 molds) as well as bacteria and incubated at appropriate conditions of time and temperature.
1305 Total aerobic bacterial count can be obtained by incubating at 30 to 35°C for 48 to 72 hours.
1306 Total combined yeast and mold count is generally obtained by incubating at 20 to 25°C for 5 to 7
1307 days.
1308
1309 Incoming lots of environmental monitoring media should include positive and negative controls.
1310 Growth promotion testing should be performed on all lots of prepared media. Where
1311 appropriate, inactivating agents should be used to prevent inhibition of growth by cleanroom
1312 disinfectants or product residuals (e.g., antibiotics).
1313
1314 **C. Prefiltration Bioburden**
1315
1316 For any parenteral manufacturing process, prefiltration bioburden should be minimal. In
1317 addition to increasing the challenge to the sterilizing filter, high bioburden can contribute
1318 endotoxin or other impurities to the drug formulation. An in-process limit for bioburden level
1319 for each formulated product (generally sampled immediately preceding sterile filtration) should
1320 be established.
1321

1322 **D. Alternate Microbiological Test Methods**
1323
1324 Other suitable microbiological test methods (e.g., rapid test methods) can be considered for in-
1325 process control testing and finished product release testing. We recommend the use of test
1326 methods that, upon evaluation, demonstrate increased accuracy, sensitivity, and reproducibility.
1327
1328 **E. Particle Monitoring**
1329
1330 Routine particle monitoring is useful in rapidly detecting significant deviations in air cleanliness
1331 from qualified processing norms (e.g., clean area classification). A result outside the established
1332 specifications at a given location should be investigated. The extent of investigation should be
1333 consistent with the severity of the *excursion* and include an evaluation of trending data.
1334 Appropriate corrective action should be implemented to prevent future deviations.
1335
1336 See Section IV.A for additional guidance on particle monitoring.
1337

1338
1339
XI. STERILITY TESTING
1340

21 CFR 210.3(b)(21) states that "*Representative sample* means a sample that consists of a number of units that are drawn based on rational criteria such as random sampling and intended to assure that the sample accurately portrays the material being sampled."

21 CFR 211.110(a) states, in part, that "To assure batch uniformity and integrity of drug products, written procedures shall be established and followed that describe the in-process controls, and tests, or examinations to be conducted on appropriate samples of in-process materials of each batch. Such 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."

21 CFR 211.160(b) states that "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. Laboratory controls shall include: (1) Determination of conformance to appropriate written specifications for the acceptance of each lot within each shipment of components, drug product containers, closures, and labeling used in the manufacture, processing, packing, or holding of drug products. The specifications shall include a description of the sampling and testing procedures used. Samples shall be representative and adequately identified. Such procedures shall also require appropriate retesting of any component, drug product container, or closure that is subject to deterioration. (2) Determination of conformance to written specifications and a description of sampling and testing procedures for in-process materials. Such samples shall be representative and properly identified. (3) Determination of conformance to written descriptions of sampling procedures and appropriate specifications for drug products. Such samples shall be representative and properly identified. (4) The calibration of instruments, apparatus, gauges, and recording devices at suitable intervals in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for remedial action in the event accuracy and/or precision limits are not met. Instruments, apparatus, gauges, and recording devices not meeting established specifications shall not be used."

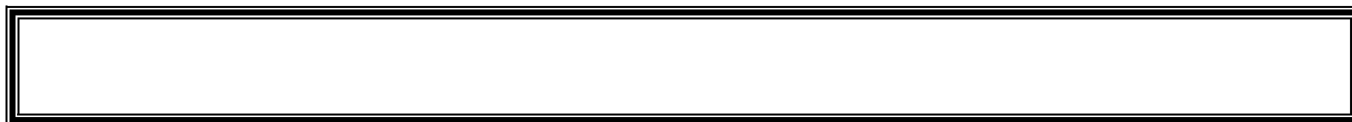
21 CFR 211.165(a) states that "For each batch of drug product, there shall be appropriate laboratory determination of satisfactory conformance to final specifications for the drug product, including the identity and strength of each active ingredient, prior to release."

21 CFR 211.165(e) states that "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 211.194(a)(2)."

21 CFR 211.167(a) states that "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."

21 CFR 211.180(e) states, in part, that "Written records required by this part shall be maintained so that data therein can be used for evaluating, 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."

21 CFR 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. Any unexplained discrepancy (including a percentage of theoretical yield exceeding the maximum or minimum percentages established in master production and control records) or the failure of a batch or any of its components to meet any of its specifications shall be thoroughly investigated, whether or not the batch has already been distributed. The investigation shall extend to other batches of the same drug product and other drug products that may have been associated with the specific failure or discrepancy. A written record of the investigation shall be made and shall include the conclusions and followup."



1341
1342 Certain aspects of sterility testing are of particular importance, including control of the testing
1343 environment, understanding the test limitations, and investigating manufacturing systems
1344 following a positive test.
1345
1346 The testing laboratory environment should employ facilities and controls comparable to those
1347 used for filling and closing operations. Poor or deficient sterility test facilities or controls can
1348 result in a high rate of test failures. If production facilities and controls are significantly better
1349 than those for sterility testing, the danger exists of mistakenly attributing a positive sterility test
1350 result to a faulty laboratory even when the product tested could have, in fact, been nonsterile.
1351 Therefore, some manufacturing deficiency may go undetected. We recommend the use of
1352 isolators to perform sterility testing. This is a well-established means for minimizing false
1353 positives.
1354
1355 **A. Choice of Methods**
1356
1357 Sterility testing methodologies are required to be accurate and reproducible, in accord with
1358 211.194 and 211.165. The methodology selected should present the lowest potential for yielding
1359 a false positive. The USP specifies membrane filtration as the method of choice, when feasible.
1360
1361 As a part of methods validation, appropriate bacteriostasis/fungistasis testing should be
1362 conducted. Such testing should demonstrate reproducibility of the method in recovering each of
1363 a panel of representative microorganisms. Study documentation should include evaluation of
1364 whether microbial recovery from inoculated controls and product samples is comparable
1365 throughout the incubation period. If growth is inhibited, modifications (e.g., increased dilution,
1366 additional membrane filter washes, addition of inactivating agents) in the methodology should be
1367 implemented to optimize recovery. Ultimately, methods validation studies should demonstrate
1368 that the methodology does not provide an opportunity for false negatives.
1369
1370 **B. Media**
1371
1372 It is essential that the media used to perform sterility testing be rendered sterile and demonstrated
1373 as growth promoting.
1374
1375 **C. Personnel**
1376
1377 Personnel performing sterility testing should be qualified and trained for the task. A written
1378 program should be in place to regularly update training of personnel and confirm acceptable
1379 sterility testing practices.
1380
1381 **D. Sampling and Incubation**
1382

1383 Sterility tests are limited in their ability to detect low incidences of contamination. For example,
1384 statistical evaluations indicate that the USP sterility test sampling plan has been described by
1385 USP as "only enabling the detection of contamination in a lot in which 10% of the units are
1386 contaminated about nine times out of ten in making the test" (Ref. 13). To further illustrate, if a
1387 10,000-unit lot with a 0.1 percent contamination level was sterility tested using 20 units, there is
1388 a 98 percent chance that the batch would pass the test.
1389
1390 This limited sensitivity is why, for batch release purposes, it is important that an appropriate
1391 number of units are tested,[11] and that the samples uniformly represent:
1392
1393 - the entire batch – samples should be taken at the beginning, middle, and end of the
1394 aseptic processing operation
1395 - the batch processing circumstances – samples should be taken in conjunction with
1396 processing interventions or excursions
1397
1398 Because of the limited sensitivity of the test, any positive result is considered a serious CGMP
1399 issue that should be thoroughly investigated.
1400
1401 **E. Investigation of Sterility Positives**
1402
1403 Care should be taken in the performance of the sterility test to preclude any activity that allows
1404 for possible sample contamination. When microbial growth is observed, the lot should be
1405 considered to be nonsterile and an investigation conducted. It is inappropriate to attribute a
1406 positive result to laboratory error on the basis of a retest that exhibits no growth.[12]
1407
1408 Although it is recognized that a determination of whether growth arose from product
1409 contamination or laboratory error may not be reached with absolute certainty, it is usually
1410 possible to acquire persuasive evidence showing that causative laboratory error is absent.
1411 It is difficult to support invalidation of a positive sterility test. Only if conclusive and
1412 documented evidence clearly shows that the contamination occurred as part of testing should a
1413 new test be performed. When available evidence is inconclusive, batches should be rejected as
1414 not conforming to sterility requirements.
1415
1416 After considering all relevant factors concerning the manufacture of the product and testing of
1417 the samples, the comprehensive written investigation should include specific conclusions and
1418 identify corrective actions. The investigation's persuasive evidence of the origin of the
1419 contamination should be based on at least the following:
1420
1421 1. Identification (speciation) of the organism in the sterility test
1422
1423 Identification of the sterility test isolate(s) should be to the species level. Microbiological
1424 monitoring data should be reviewed to determine if the organism is also found in laboratory and

[11] USP <71> includes standards for the minimum quantity of units to be analyzed in a valid sterility test.
[12] 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).

1425 production environments, personnel, or product bioburden. Nucleic acid-based methods are
1426 recommended for microbial identification purposes.
1427
1428 2. Record of laboratory tests and deviations
1429
1430 Review of trends in laboratory findings can help to eliminate or implicate the laboratory as the
1431 source of contamination. For example, if the organism is seldom found in the laboratory
1432 environment, product contamination is likely. If the organism is found in laboratory and
1433 production environments, it can still indicate product contamination.
1434
1435 The proper handling of deviations is an essential aspect of laboratory control. When a deviation
1436 occurs during sterility testing, it should be documented, investigated, and remedied. If any
1437 deviation is considered to have compromised the integrity of the sterility test, the test should be
1438 invalidated immediately without incubation.
1439
1440 Deviation and sterility test positive trends should be evaluated periodically (e.g., quarterly,
1441 annually) to provide an overview of operations. A sterility positive result can be viewed as
1442 indicative of production or laboratory problems and should be investigated globally since such
1443 problems often can extend beyond a single batch.
1444
1445 To more accurately monitor potential contamination sources, we recommend you keep separate
1446 trends by product, container type, filling line, and personnel. Where the degree of sterility test
1447 sample manipulation is similar for a terminally sterilized product and an aseptically processed
1448 product, a higher rate of initial sterility failures for the latter should be taken as indicative of
1449 aseptic processing production problems.
1450
1451 Microbial monitoring of the laboratory environment and personnel over time can also reveal
1452 trends that are informative. Upward trends in the microbial load in the laboratory should be
1453 promptly investigated as to cause, and corrected. In some instances, such trends can appear to be
1454 more indicative of laboratory error as a possible source of a sterility test failure.
1455
1456 Where a laboratory has a good track record with respect to errors, this history can help remove
1457 the lab as a source of contamination since chances are higher that the contamination arose from
1458 production. However, the converse is not true. Specifically, where a laboratory has a poor track
1459 record, firms should not assume that the contamination is automatically more attributable to the
1460 laboratory and consequently overlook a genuine production problem. Accordingly, all sterility
1461 positives should be thoroughly investigated.
1462
1463 3. Monitoring of production area environment
1464
1465 Of particular importance is trend analysis of microorganisms in the critical and immediately
1466 adjacent areas. Trends are an important tool in the investigation of a sterility failure.
1467 Consideration of environmental microbial data should not be limited to results of monitoring the
1468 production environment for the lot, day, or shift associated with the suspect lot. For example,
1469 results showing little or no recovery of microorganisms can be misleading, especially when

1470 preceded or followed by a finding of an adverse trend or atypically high microbial counts. It is
1471 therefore important to look at both short- and long-term trend analysis.
1472
1473 4. Monitoring Personnel
1474
1475 Data and associated trends from daily monitoring of personnel should be reviewed and can in
1476 some cases strongly indicate a route of contamination. The adequacy of personnel practices and
1477 training should also be considered.
1478
1479 5. Product Presterilization Bioburden
1480
1481 Trends in product bioburden should be reviewed (counts and identity). Adverse bioburden
1482 trends occurring during the time period of the test failure should be considered during the
1483 investigation.
1484
1485 6. Production record review
1486
1487 Complete batch and production control records should be reviewed to detect any signs of failures
1488 or anomalies that could have a bearing on product sterility. For example, the investigation
1489 should evaluate batch and trending data that indicate whether utility and/or support systems (e.g.,
1490 HVAC, WFI) are functioning properly. Records of air quality monitoring for filling lines could
1491 reveal or show a time at which there was improper air balance or an unusually high particle
1492 count.
1493
1494 7. Manufacturing history
1495
1496 The manufacturing history of a product or similar products should be reviewed as part of the
1497 investigation. Past deviations, problems, or changes (e.g., process, components, equipment) are
1498 among the factors that can provide an indication of the origin of the problem.

XII. BATCH RECORD REVIEW: PROCESS CONTROL DOCUMENTATION

> 21 CFR 211.100(a) states that "There shall be written procedures for production and process control designed to assure that the drug products have the identity, strength, quality, and purity they purport or are represented to possess. Such procedures shall include all requirements in this subpart. These written procedures, including any changes, shall be drafted, reviewed, and approved by the appropriate organizational units and reviewed and approved by the quality control unit."
>
> 21 CFR 211.100(b) states that "Written production and process control procedures shall be followed in the execution of the various production and process control functions and shall be documented at the time of performance. Any deviation from the written procedures shall be recorded and justified."
>
> 21 CFR 211.186 and 211.188 address, respectively, "Master production and control records" and "Batch production and control records."
>
> 21 CFR 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. Any unexplained discrepancy (including a percentage of theoretical yield exceeding the maximum or minimum percentages established in master production and control records) or the failure of a batch or any of its components to meet any of its specifications shall be thoroughly investigated, whether or not the batch has already been distributed. The investigation shall extend to other batches of the same drug product and other drug products that may have been associated with the specific failure or discrepancy. A written record of the investigation shall be made and shall include the conclusions and followup."

Manufacturers should build process and environmental control activities into their aseptic processing operation. It is critical that these activities be maintained and strictly implemented on a daily basis. The requirement for review of all batch records and data for conformance with written procedures, operating parameters, and product specifications prior to arriving at the final release decision for an aseptically processed batch calls for an overall review of process and system performance for that given cycle of manufacture. All in-process data must be included with the batch record documentation in accordance with section 211.188. Review of environmental and personnel monitoring data, as well as other data relating to acceptability of output from support systems (e.g., HEPA / HVAC, WFI, steam generator) and proper functioning of equipment (e.g., batch alarms report; integrity of various filters), should be viewed as essential elements of the batch release decision.

While interventions and/or stoppages are normally recorded in the batch record, the manner of documenting these occurrences varies. In particular, line stoppages and any unplanned interventions should be sufficiently documented in batch records with the associated time and duration of the event. In addition to dwell time of sterile product elements in the critical area, an extensive intervention can increase contamination risk. Sterility failures can be attributed to atypical or extensive interventions that have occurred as a response to an undesirable event during the aseptic process. Written procedures describing the need for line clearances in the event of certain interventions, such as machine adjustments and any repairs, should be established. Such interventions should be documented with more detail than minor events. Interventions that result in substantial activity near exposed product or container closures or that

1526 last beyond a reasonable exposure time should, where appropriate, result in a local or full line
1527 clearance.
1528
1529 Any disruption in power supply, however momentary, during aseptic processing is a
1530 manufacturing deviation and must be included in batch records (211.100, 211.192).

1531
1532 ## APPENDIX 1: ASEPTIC PROCESSING ISOLATORS
1533
1534 Aseptic processing using isolation systems minimizes the extent of personnel involvement and
1535 separates the external cleanroom environment from the aseptic processing line. A well-designed
1536 positive pressure isolator, supported by adequate procedures for its maintenance, monitoring, and
1537 control, offers tangible advantages over classical aseptic processing, including fewer
1538 opportunities for microbial contamination during processing. However, users should not adopt a
1539 false sense of security with these systems. Manufacturers should also be aware of the need to
1540 establish new procedures addressing issues unique to isolators.
1541
1542 ### A. Maintenance
1543
1544 #### 1. General
1545
1546 Isolator systems have a number of special maintenance issues. Although no isolator unit forms
1547 an absolute seal, very high integrity can be achieved in a well-designed unit. However, a leak in
1548 any of certain components of the system can constitute a significant breach of integrity. The
1549 integrity of gloves, half-suits, seams, gaskets, and seals should receive daily attention as well as a
1550 comprehensive preventative maintenance program. Replacement frequencies should be
1551 established in written procedures that ensure parts will be changed before they breakdown or
1552 degrade.
1553
1554 #### 2. Glove Integrity
1555
1556 A faulty glove or sleeve (gauntlet) assembly represents a route of contamination and a critical
1557 breach of isolator integrity. The choice of durable glove materials, coupled with a well-justified
1558 replacement frequency, are two aspects of good manufacturing practice that should be addressed.
1559 With every use, gloves should be visually evaluated for any macroscopic physical defect.
1560 Mechanical integrity tests should also be performed routinely. An attentive preventive
1561 maintenance program can identify and eliminate gloves lacking integrity and will minimize the
1562 possibility of placing a sterile product at risk. Such a breach can be of serious consequence.
1563
1564 Due to the potential for microbial migration through microscopic holes in gloves and the lack of
1565 a highly sensitive glove integrity test, the inner part of the installed glove should be sanitized
1566 regularly and the operator should also wear a second pair of thin gloves.
1567
1568 ### B. Design
1569
1570 #### 1. Airflow
1571
1572 There are two types of aseptic processing isolators: *open* and *closed*. Closed isolators employ
1573 connections with auxiliary equipment for material transfer. Open isolators have openings to the
1574 surrounding environment that are carefully engineered to segregate the inner isolator
1575 environment from the surrounding room via overpressure.
1576

1577 Turbulent flow is normally acceptable within closed isolators, which are generally compact in
1578 size and do not house large processing lines. Other aseptic processing isolators, employ
1579 unidirectional airflow that sweeps over and away from exposed sterile materials, avoiding any
1580 turbulence or stagnant airflow in the area of exposed sterilized materials, product, and container
1581 closures. In most sound designs, air showers over the critical zone once, and then is
1582 systematically exhausted. The air handling system should be capable of maintaining the requisite
1583 environmental conditions within the isolator.
1584
1585 2. Materials of Construction
1586
1587 As in any aseptic processing design, suitable materials should be chosen based on durability, as
1588 well as ease of cleaning and sterilization. For example, rigid wall construction incorporating
1589 stainless steel and glass materials is widely used.
1590
1591 3. Pressure Differential
1592
1593 Isolators that include an open exit portal represent a potential compromise in achieving complete
1594 physical separation from the external environment. A positive air pressure differential adequate
1595 to achieve this full separation should be employed and supported by qualification studies.
1596 Positive air pressure differentials from the isolator to the surrounding environment have largely
1597 ranged from approximately 0.07" to 0.2" water gauge. The appropriate minimum pressure
1598 differential specification established by a firm will depend on the system's design and, when
1599 applicable, its exit port. Air balance between the isolator and other direct interfaces (e.g., dry
1600 heat tunnel) should also be qualified.
1601
1602 The positive pressure differential should be coupled with appropriate protection at the product
1603 egress point(s) to overcome the potential for ingress of any airborne particles from the
1604 external environment by induction. Induction can result from local turbulent flow causing air
1605 swirls or pressure waves that can push extraneous particles into the isolator. Local Class 100
1606 (ISO 5) protection at an opening can provide a further barrier to induction of surrounding room
1607 air into the isolator.
1608
1609 4. Clean Area Classifications
1610
1611 The interior of the isolator should, at minimum, meet Class 100 (ISO 5) standards. The
1612 classification of the environment surrounding the isolator should be based on the design of its
1613 interfaces (e.g., transfer ports), as well as the number of transfers into and out of the isolator. A
1614 Class 100,000 (ISO 8) background can be appropriate depending on isolator design and
1615 manufacturing situations. An aseptic processing isolator should not be located in an unclassified
1616 room.
1617
1618 C. Transfer of Materials/Supplies
1619
1620 The ability to maintain integrity and sterility of an isolator is impacted by the design of transfer
1621 ports. Various adaptations, of differing capabilities, allow for the transfer of supplies into and
1622 out of the isolator.

1623
1624 1. General:
1625
1626 Multiple material transfers are generally made during the processing of a batch. Frequently,
1627 transfers are performed via direct interface with manufacturing equipment. Properly maintained
1628 and operated rapid transfer ports (RTPs) are an effective transfer mechanism for aseptic transfer
1629 of materials into and out of isolators. Some transfer ports can have significant limitations,
1630 including marginal decontaminating capability (e.g., ultraviolet) or a design that has the potential
1631 to compromise isolation by allowing ingress of air from the surrounding room. In the latter case,
1632 localized HEPA-filtered unidirectional airflow cover in the area of such a port should be
1633 implemented.
1634
1635 2. Discharge
1636
1637 Isolators often include a *mousehole* or other exit port through which product is discharged,
1638 opening the isolator to the outside environment. The mousehole represents a potential route of
1639 contamination. Sufficient overpressure should be supplied and monitored on a continuous basis
1640 at this location to ensure that isolation is maintained.
1641
1642 D. Decontamination
1643
1644 1. Surface Exposure
1645
1646 Written procedures for decontamination of the isolator should be developed. A decontamination
1647 process should be developed that provides full exposure of all isolator surfaces to the chemical
1648 agent. For example, to facilitate contact with the sterilant, the glove apparatus should be fully
1649 extended with glove fingers separated during the decontamination cycle. The interior of the
1650 isolator should also be cleaned per appropriate procedures to allow for robust decontamination.
1651
1652 2. Efficacy
1653
1654 A decontamination method should be developed that renders the inner surfaces of the isolator free of
1655 viable microorganisms. Decontamination can be accomplished using a number of vaporized agents,
1656 although these agents possess limited capability to penetrate obstructed or covered surfaces. Process
1657 development and validation studies should include a thorough determination of cycle capability. The
1658 characteristics of these agents generally preclude the reliable use of statistical methods (e.g., fraction
1659 negative) to determine process lethality (Ref. 14). An appropriate, quantified BI challenge should be
1660 placed on various materials[13] and in many locations throughout the isolator, including difficult to reach
1661 areas. Cycles should be developed with an appropriate margin of extra kill to provide confidence in
1662 robustness of the decontamination processes. Normally, a four- to six-log reduction can be justified
1663 depending on the application. The specific BI spore titer used and the selection of BI placement sites
1664 should be justified. For example, demonstration of a four-log reduction should be sufficient for

[13] If the various isolator materials are thoroughly evaluated during cycle development, a firm might consider placing more focus on material texture and porosity.

1665 introduction of controlled, very low bioburden materials into an aseptic processing isolator, including
1666 wrapped sterile supplies that are briefly exposed to the surrounding cleanroom environment.
1667
1668 The uniform distribution of the defined concentration of decontaminating agent should also be
1669 evaluated concurrent with these studies (Ref. 15). Chemical indicators may also be useful as a
1670 qualitative tool to show that the decontaminating agent reached a given location.
1671
1672 3. Frequency
1673
1674 While isolators vary widely in design, their interior and content should be designed to be
1675 frequently decontaminated. When an isolator is used for multiple days between decontamination
1676 cycles, the frequency adopted should include a built-in safety margin and be well justified. This
1677 frequency, established during validation studies, should be reevaluated and increased if
1678 production data indicate any deterioration of the microbiological quality of the isolator
1679 environment.
1680
1681 A breach of isolator integrity should lead to a decontamination cycle. Integrity can be impacted
1682 by power failures, valve failure, inadequate overpressure, holes in gloves and seams or other
1683 leaks. Breaches of integrity should be investigated and any product that may have been impacted
1684 by the breach rejected.
1685
1686 E. Filling Line Sterilization
1687
1688 To ensure sterility of product contact surfaces from the start of each operation, the entire path of
1689 the sterile liquid stream should be sterilized. In addition, loose materials or aseptic processing
1690 equipment to be used within the isolator should be chosen based on their ability to withstand
1691 steam sterilization (or equivalent method). It is expected that materials that permit heat
1692 sterilization (e.g., SIP) will be rendered sterile by such methods. Where decontamination
1693 methods are used to render certain product contact surfaces free of viable organisms, a minimum
1694 of a six-log reduction should be demonstrated using a suitable biological indicator.
1695
1696 F. Environmental Monitoring
1697
1698 An appropriate environmental monitoring program should be established that routinely ensures
1699 acceptable microbiological quality of air, surfaces, and gloves (or half-suits) as well as particle
1700 levels, within the isolator. Air quality should be monitored periodically during each shift. For
1701 example, the exit port should be monitored for particles to detect any unusual results.
1702
1703 G. Personnel
1704
1705 While cleanroom apparel requirements are generally reduced in an isolator operation, the
1706 contamination risk contributed by manual factors should not be overlooked. Isolation processes
1707 generally include periodic or even frequent use of one or more gloves for aseptic manipulations
1708 and handling of material transfers into and out of the isolator. One should be aware that
1709 locations on gloves, sleeves, or half suits can be among the more difficult to reach places during
1710 surface sterilization, and glove integrity defects may not be promptly detected. Traditional

1711 aseptic processing vigilance is appropriate, with an understanding that contaminated isolator
1712 gloves can lead to product nonsterility. Accordingly, meticulous aseptic technique standards
1713 must be observed (211.113), including appropriate use of sterile tools for manipulations.

1714
1715 **APPENDIX 2: BLOW-FILL- SEAL TECHNOLOGY**
1716
1717 Blow-fill-seal (BFS) technology is an automated process by which containers are formed, filled,
1718 and sealed in a continuous operation. This manufacturing technology includes economies in
1719 container closure processing and reduced human intervention, and is often used for filling and
1720 packaging ophthalmics and, less frequently, injectables. This appendix discusses some of the
1721 critical control points of this technology. Except where otherwise noted below, the aseptic
1722 processing standards discussed elsewhere in this document should apply to blow fill seal
1723 technology.
1724
1725 A. Equipment Design and Air Quality
1726
1727 Most BFS machines operate using the following steps.
1728
1729 • Heat a plastic polymer resin
1730 • Extrude it to form a parison (a tubular form of the hot resin)
1731 • Cut the parison with a high-temperature knife
1732 • Move the parison under the blow-fill needle (mandrel)
1733 • Inflate it to the shape of the mold walls
1734 • Fill the formed container with the liquid product
1735 • Remove the mandrel
1736 • Seal
1737
1738 Throughout this operation, sterile-air is used, for example, to form the parison and inflate it prior
1739 to filling. In most operations, the three steps with the greatest potential for exposure to particle
1740 contamination and/or surrounding air are those in which (1) the parison is cut, (2) the parison is
1741 moved under the blow-fill mandrel, and (3) the mandrel is removed (just prior to sealing).
1742
1743 BFS machinery and its surrounding barriers should be designed to prevent potential for
1744 extraneous contamination. As with any aseptic processing operation, it is critical that contact
1745 surfaces be sterile. A validated steam-in-place cycle should be used to sterilize the equipment
1746 path through which the product is conveyed. In addition, any other surface with the potential to
1747 contaminate the sterile product should be sterile.
1748
1749 The classified environment surrounding BFS machinery should generally meet Class 10,000
1750 (ISO 7) standards, but special design provisions (e.g., isolation technology) can justify an
1751 alternate classification. HEPA-filtered or sterile air provided by membrane filters should be used
1752 during the steps when sterile products or materials are exposed (e.g., parison formation,
1753 container molding or filling steps). Air in the critical area should meet Class 100 (ISO 5)
1754 microbiological standards. A well-designed BFS system should also normally achieve Class 100
1755 (ISO 5) airborne particle levels.
1756
1757 Equipment design should incorporate specialized measures to reduce particle levels. In contrast
1758 to nonpharmaceutical applications using BFS machinery, control of air quality (i.e., particles) is
1759 critical for sterile drug product manufacture. Particles generated during the plastic extrusion,

1760 cutting, and sealing processes should be controlled. Provisions for carefully controlled airflow
1761 can protect the product by forcing generated particles outward while preventing any ingress from
1762 the adjacent environment. Furthermore, designs separating the filling zone from the surrounding
1763 environment are important to ensure product protection. Barriers, pressure vacuums,
1764 microenvironments, and appropriately directed high velocities of sterile air have been found
1765 useful in preventing contamination (Ref. 16). Smoke studies and multi-location particle data can
1766 provide valuable information when performing qualification studies to assess whether proper
1767 particle control dynamics have been achieved throughout the critical area.
1768
1769 In addition to suitable design, an adequate preventative maintenance program should be
1770 established. For example, because of its potential to contaminate the sterile drug product, the
1771 integrity of the cooling or boiling system (e.g., mold plates, gaskets) should be carefully
1772 monitored and maintained.
1773
1774 B. Validation/Qualification
1775
1776 Advantages of BFS processing are known to include rapid container closure processing and
1777 minimized aseptic interventions. However, only a properly functioning process can realize these
1778 advantages. Setup, troubleshooting of equipment, and related aseptic personnel procedures
1779 should be given special attention. Equipment sterilization, media fills, polymer sterilization,
1780 endotoxin removal, product-plastic compatibility, forming and sealing integrity, and unit weight
1781 variation are among the key issues that should be covered by validation and qualification studies.
1782
1783
1784 Appropriate data should ensure that BFS containers are sterile and, if used for parenteral drugs,
1785 nonpyrogenic. This can generally be achieved by validating that time temperature conditions of
1786 the extrusion process are effective against endotoxin or spore challenges in the polymeric
1787 material.
1788
1789 The plastic polymer material chosen should be pharmaceutical grade, safe, pure, and pass
1790 appropriate criteria (Ref. 17) for plastics. Polymer suppliers should be qualified and monitored
1791 for raw material quality.
1792
1793 C. Batch Monitoring and Control
1794
1795 In-process monitoring should include various control parameters (e.g., container weight
1796 variation, fill weight, leakers, air pressure) to ensure ongoing process control. Microbial air
1797 quality is particularly important. Samples should be taken per a comprehensive sampling plan
1798 that provides data representative of the entire filing operation. Continuous monitoring of
1799 particles can provide valuable data relative to the control of a blow-fill-seal operation.
1800
1801
1802 Container closure defects can be a major problem in control of a BFS operation. It is critical that
1803 the operation be designed and set-up to uniformly manufacture leak-proof units. As a final
1804 measure, the inspection of each unit of a batch should include a reliable, sensitive, final product
1805 examination that is capable of identifying defective units (e.g., *leakers*). Significant defects due

1806 to heat or mechanical problems, such as mold thickness, container or closure interface
1807 deficiencies, poorly formed closures, or other deviations should be investigated in accord with §§
1808 211.100 and 211.192.
1809

1810 **APPENDIX 3: PROCESSING PRIOR TO FILLING AND SEALING OPERATIONS**
1811
1812 The purpose of this appendix is to supplement the guidance provided in this document with
1813 information on products regulated by CBER or CDER that are subject to aseptic processing at
1814 points early in the manufacturing process, or that require aseptic processing through the entire
1815 manufacturing process because it is impossible to filter sterilyze the final drug product. The
1816 scope of this appendix includes aseptic processing activities that take place prior to the filling
1817 and sealing of the finished drug product. Special considerations include those for:
1818
1819 A. Aseptic processing from early manufacturing steps
1820
1821 Some products should undergo aseptic processing at some or all manufacturing steps preceding
1822 the final product closing step. With some products, there is a point in the process after which a
1823 product can no longer be rendered sterile by filtration. In such cases, the product would be
1824 handled aseptically at all steps subsequent to filter sterilization. In other instances, the final drug
1825 product cannot be filter sterilyzed, and, therefore, each component in the formulation would be
1826 rendered sterile and mixed aseptically. For example, products containing aluminum adjuvant are
1827 formulated aseptically because once they are alum adsorbed, they cannot be sterile-filtered.
1828
1829 When a product is processed aseptically from the early stages, the product and all components or
1830 other additions are rendered sterile prior to entering the manufacturing process. It is critical that
1831 all transfers, transports, and storage stages be carefully controlled at each step of the process to
1832 maintain sterility of the product.
1833
1834 Procedures (e.g., aseptic connection) that expose a product or product contact surfaces should be
1835 performed under unidirectional airflow in a Class 100 (ISO 5) environment. The environment of
1836 the room surrounding the Class 100 (ISO 5) environment should be Class 10,000 (ISO 7) or
1837 better. Microbiological and airborne particle monitoring should be performed during operations.
1838 Microbial surface monitoring should be performed at the end of operations, but prior to cleaning.
1839 Personnel monitoring should be performed in association with operations.
1840
1841 Process simulation studies should be designed to incorporate all conditions, product
1842 manipulations, and interventions that could impact on the sterility of the product during
1843 manufacturing. The process simulation, from the early process steps, should demonstrate that
1844 process controls are adequate to protect the product during manufacturing. These studies should
1845 incorporate all product manipulations, additions, and procedures involving exposure of product
1846 contact surfaces to the environment. The studies should include worst-case conditions such as
1847 maximum duration of open operations and maximum number of participating operators.
1848 However, process simulations do not need to mimic total manufacturing time if the
1849 manipulations that occur during manufacturing are adequately represented.
1850
1851 It is also important that process simulations incorporate storage of product or transport to other
1852 manufacturing areas. For instance, there should be assurance of bulk vessel integrity for
1853 specified holding times. The transport of bulk tanks or other containers should be simulated as
1854 part of the media fill. Please refer to Section IX.A for more guidance on media simulation

1855 studies. Process simulation studies for the formulation stage should be performed at least twice
1856 per year.
1857
1858 B. Aseptic processing of cell-based therapy products (or of products intended for use as cell
1859 based therapies)
1860
1861 Cell-based therapy products represent a subset of the products for which aseptic manipulations
1862 are used throughout the process. Where possible, closed systems should be used during
1863 manufacturing. Cell-based therapy products often have short processing times at each
1864 manufacturing stage, even for the final product. Often, these products are administered to
1865 patients before final product sterility testing results are available. In situations where results of
1866 final sterility testing are not available before the product is administered, additional controls and
1867 testing should be considered. For example, additional sterility tests can be performed at
1868 intermediate stages of manufacture, especially after the last manipulation of the product prior to
1869 administration. Other tests that may indicate microbial contamination, such as microscopic
1870 examination, gram stains, and endotoxin testing should be performed prior to product release.

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1873

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1910 **RELEVANT GUIDANCE DOCUMENTS**
1911
1912 Some relevant FDA guidance documents include:
1913
1914 - Guidance for the Submission of Documentation for Sterilization Process Validation in
1915 Applications for Human and Veterinary Drug Products
1916 - Guideline for Validation of Limulus Amebocyte Lysate Test as an End Product Endotoxin
1917 Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices
1918 - Guide to Inspections of Lyophilization of Parenterals
1919 - Guide to Inspections of High Purity Water Systems
1920 - Guide To Inspections of Microbiological Pharmaceutical Quality Control Laboratories
1921 - Guide To Inspections of Sterile Drug Substance Manufacturers
1922 - Pyrogens: Still a Danger; (Inspection Technical Guide)
1923 - Bacterial Endotoxins/Pyrogens; (Inspection Technical Guide)
1924 - Heat Exchangers to Avoid Contamination; (Inspection Technical Guide)
1925 - See also the draft guidance *Container and Closure Integrity Testing in Lieu of Sterility*
1926 *Testing as a Component of the Stability Protocol for Sterile Products*, which was issued in
1927 1998. Once final, it will represent the Agency's thinking on this topic.

1928
1929 **GLOSSARY**
1930

1931 Air lock- A small room with interlocked doors, constructed to maintain air pressure control
1932 between adjoining rooms (generally with different air cleanliness standards). The intent of an
1933 aseptic processing airlock is to preclude ingress of particulate matter and microorganism
1934 contamination from a lesser controlled area.
1935

1936 Alert Level- An established microbial or airborne particle level giving early warning of potential
1937 drift from normal operating conditions and triggers appropriate scrutiny and follow-up to address
1938 the potential problem. Alert levels are always lower than action levels.
1939

1940 Action Level- An established microbial or airborne particle level that, when exceeded, should
1941 trigger appropriate investigation and corrective action based on the investigation.
1942

1943 Aseptic Processing Facility- A building containing cleanrooms in which air supply, materials,
1944 and equipment are regulated to control microbial and particle contamination.
1945

1946 Aseptic Processing Room- A room in which one or more aseptic activities or processes is
1947 performed.
1948

1949 Asepsis- A state of control attained by using an aseptic work area and performing activities in a
1950 manner that precludes microbiological contamination of the exposed sterile product.
1951

1952 Bioburden- The total number of microorganisms associated with a specific item prior to
1953 sterilization.
1954

1955 Barrier- A physical partition that affords aseptic manufacturing zone protection by partially
1956 separating it from the surrounding area.
1957

1958 Biological Indicator (BI)- A population of microorganisms inoculated onto a suitable medium
1959 (e.g., solution, container or closure) and placed within appropriate sterilizer load locations to
1960 determine the sterilization cycle efficacy of a physical or chemical process. The *challenge*
1961 *microorganism* is selected based upon its resistance to the given process. Incoming lot D-value
1962 and microbiological count define the quality of the BI.
1963

1964 Clean Area- An area with defined particle and microbiological cleanliness standards.
1965

1966 Cleanroom- A room designed, maintained, and controlled to prevent particle and microbiological
1967 contamination of drug products. Such a room is assigned and reproducibly meets an appropriate
1968 air cleanliness classification.

1969 Component- Any ingredient intended for use in the manufacture of a drug product, including
1970 those that may not appear in the final drug product.
1971

1972 Colony Forming Unit (CFU)- A microbiological term that describes the formation of a single
1973 macroscopic colony after the introduction of one or more microorganisms to microbiological
1974 growth media. One colony forming unit is expressed as 1 CFU.
1975
1976 Critical Area - An area designed to maintain sterility of sterile materials. Sterilized product,
1977 containers or closures, and equipment may be exposed in critical areas.
1978
1979 Clean Zone- See Clean Area.
1980
1981 Critical surfaces- Surfaces that may come into contact with or directly affect a sterilized product
1982 or its containers or closures. Critical surfaces are rendered sterile prior to the start of the
1983 manufacturing operation, and sterility is maintained throughout processing.
1984
1985 Decontamination- A process that eliminates viable bioburden via use of sporicidal chemical
1986 agents.
1987
1988 Depyrogenation- A process used to destroy or remove pyrogens (e.g., endotoxin).
1989
1990 D value- The time (in minutes) of exposure at a given temperature that causes a one-log or 90
1991 percent reduction in the population of a specific microorganism.
1992
1993 Dynamic- Conditions relating to clean area classification under conditions of normal production.
1994
1995 Endotoxin- A pyrogenic product (e.g., lipopolysaccharide) present in the bacterial cell wall.
1996 Endotoxin can lead to reactions in patients receiving injections ranging from fever to death.
1997
1998 Gowning Qualification- A program that establishes, both initially and on a periodic basis, the
1999 capability of an individual to don the complete sterile gown in an aseptic manner.
2000
2001 HEPA filter- High efficiency particulate air filter with minimum 0.3 micron particle retaining
2002 efficiency of 99.97 percent.
2003
2004 HVAC- Heating, ventilation, and air conditioning.
2005
2006 Intervention- An aseptic manipulation or activity that occurs at the critical zone.
2007
2008 Isolator- A decontaminated unit, supplied with Class 100 (ISO 5) or higher air quality, that
2009 provides uncompromised, continuous isolation of its interior from the external environment (e.g.,
2010 surrounding clean room air and personnel). There are two major types of isolators:
2011
2012 *Closed isolator systems* exclude external contamination from the isolator's critical zone
2013 by accomplishing material transfer via aseptic connection to auxiliary equipment, rather
2014 than use of openings to the surrounding environment. Closed systems remain sealed
2015 throughout operations.
2016

2017 *Open isolator systems* are designed to allow for the continuous or semi-continuous
2018 ingress and/or egress of materials during operations through one or more openings.
2019 Openings are engineered (e.g., using continuous overpressure) to exclude the entry of
2020 external contamination into the isolator.
2021
2022 Laminar flow- An airflow moving in a single direction and in parallel layers at constant velocity
2023 from the beginning to the end of a straight line vector.
2024
2025 Operator- Any individual participating in the aseptic processing operation, including line set-up,
2026 filler, maintenance, or other personnel associated with aseptic line activities.
2027
2028 Overkill sterilization process- A process that is sufficient to provide at least a 12 log reduction of
2029 microorganisms having a minimum D value of 1 minute.
2030
2031 Pyrogen- A substance that induces a febrile reaction in a patient.
2032
2033 Sterile Product- For purposes of this guidance, *sterile product* refers to one or more of the
2034 elements exposed to aseptic conditions and ultimately making up the sterile finished drug
2035 product. These elements include the containers, closures, and components of the finished drug
2036 product.
2037
2038 Sterilizing grade filter- A filter that, when appropriately validated, will remove all
2039 microorganisms from a fluid stream, producing a sterile effluent.
2040
2041 Unidirectional flow- An airflow moving in a single direction, in a robust and uniform manner,
2042 and at sufficient speed to reproducibly sweep particles away from the critical processing or
2043 testing area.
2044
2045 Terminal sterilization- The application of a lethal agent to sealed, finished drug products for the
2046 purpose of achieving a predetermined sterility assurance level (SAL) of usually less than 10^{-6}
2047 (i.e., a probability of a nonsterile unit of greater than one in a million).
2048
2049 ULPA filter- Ultra-low penetration air filter with minimum 0.3 micron particle retaining
2050 efficiency of 99.999 percent.
2051
2052 Validation- Establishing documented evidence that provides a high degree of assurance that a
2053 specific process will consistently produce a product meeting its predetermined specifications and
2054 quality attributes.
2055
2056 Worst case- A set of conditions encompassing upper and lower processing limits and
2057 circumstances, including those within standard operating procedures, that pose the greatest
2058 chance of process or product failure (when compared to ideal conditions). Such conditions do
2059 not necessarily induce product or process failure.