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Sterile Drug Products Produced by Aseptic Processing Draft

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III. SCOPE

This document discusses only selected issues and thus does not address all aspects of aseptic processing. Finished drug product CGMP issues are primarily addressed, with only limited guidance regarding upstream bulk processing steps. Updates relative to the 1987 document include guidance on: personnel qualification, clean room classifications under dynamic conditions, room design, quality control, environmental monitoring, and review of production records. The aseptic processing isolator is also discussed.

Although this document discusses CGMP issues relating to the sterilization of components, containers, and closures, terminal sterilization of the drug product is not addressed. It is a well-accepted principle that sterile drugs should be manufactured by aseptic processing only when terminal sterilization is not feasible. However, unacceptable degradation of the product can occur as a result of terminal sterilization, or the market presentation can afford some unique and substantial clinical advantage not possible if terminal sterilization were employed. In such cases, adjunct processing steps (e.g., heat exposure conditions which provide some F₀) to increase the level of sterility confidence should be considered.

A list of references, which may be of value to the reader, is included at the conclusion of this document.

IV. BUILDINGS AND FACILITIES

Section 211.42 (design and construction features) requires, in part, that aseptic processing operations be “performed within specifically defined areas of adequate size. There shall be separate or defined areas for the firm’s operations to prevent contamination or mixups.” Aseptic processing operations must also “include, as appropriate, an air supply filtered through high efficiency particulate air (HEPA) filters under positive pressure,” as well as systems for “monitoring environmental conditions...” and “maintaining any equipment used to control aseptic conditions.”

Section 211.46 (ventilation, air filtration, air heating and cooling) states, in part, that “equipment for adequate control over air pressure, microorganisms, dust, humidity, and temperature shall be provided when appropriate for the manufacture, processing, packing or holding of a drug product.” This regulation also states that “air filtration systems, including pre-filters and particulate matter air filters, shall be used when appropriate on air supplies to production areas.”

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In aseptic processing, there are various areas of operation which require separation and control, with each area having different degrees of air quality depending on the nature of the operation. Area design is based upon satisfying microbiological and particulate standards defined by the equipment, components, and products exposed, as well as the particular operation conducted, in the given area.

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72 Critical and support areas of the aseptic processing operation should be classified and
73 supported by microbiological and particulate data obtained during qualification studies. While
74 initial clean room qualification includes some assessment of air quality under as-built and static
75 conditions, the final room or area classification should be derived from data generated under
76 dynamic conditions, i.e., with personnel present, equipment in place, and operations ongoing.
77 The aseptic processing facility monitoring program should assess conformance with specified
78 clean area classifications under dynamic conditions, on a routine basis.

79

80 The following table summarizes clean area air classifications (Ref. 1).

81

82 **TABLE 1- Air Classifications^a**

83

Clean Area Classification	$\geq 0.5 \mu\text{m}$ particles/ft ³	$\geq 0.5 \mu\text{m}$ particles/m ³	Microbiological Limit ^b	
			cfu/10 ft ³	cfu/m ³
100	100	3,500	< 1 ^c	< 3 ^c
1000	1000	35,000	< 2	< 7
10,000	10,000	350,000	< 5	< 18
100,000	100,000	3,500,000	< 25	< 88

84

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89

a- All classifications based on data measured in the vicinity of exposed articles during periods of activity.

b- Alternate microbiological standards may be established where justified by the nature of the operation. c- Samples from class 100 environments should normally yield no microbiological contaminants.

90 Two clean areas are of particular importance to sterile drug product quality: the critical area
91 and the supporting clean areas associated with it.

92

93 **A. Critical Area (Class 100)**

94

95 A critical area is one in which the sterilized drug product, containers, and closures are exposed
96 to environmental conditions designed to preserve sterility. Activities conducted in this area
97 include manipulations (e.g., aseptic connections, sterile ingredient additions) of sterile
98 materials prior to and during filling and closing operations.

99

100 This area is critical because the product is not processed further in its immediate container and
101 is vulnerable to contamination. In order to maintain product sterility, the environment in which
102 aseptic operations are conducted should be of appropriate quality throughout operations. One
103 aspect of environmental quality is the particulate content of the air. Particulates are significant
104 because they can enter a product and contaminate it physically or, by acting as a vehicle for
105 microorganisms, biologically. Particle content in critical areas should be minimized by
106 effective air systems.

107

108 Air in the immediate proximity of exposed sterilized containers/closures and filling/closing
109 operations is of acceptable particulate quality when it has a per-cubic-foot particle count of no
110 more than 100 in a size range of 0.5 micron and larger (Class 100) when counted at

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111 representative locations normally not more than one foot away from the work site, within the
112 airflow, and during filling/closing operations. Deviations from this critical area monitoring
113 parameter should be documented as to origin and significance.

114
115 Measurements to confirm air cleanliness in aseptic processing zones should be taken with the
116 particle counting probe oriented in the direction of oncoming airflow and at specified sites
117 where sterilized product and container-closure are exposed. Regular monitoring should be
118 performed during each shift. Nonviable particulate monitoring with a remote counting system
119 is generally less invasive than the use of portable particle counting units and provides the most
120 comprehensive data. See Section X.D, "Particulate Monitoring."

121
122 Some powder filling operations can generate high levels of powder particulates that, by their
123 nature, do not pose a risk of product contamination. It may not, in these cases, be feasible to
124 measure air quality within the one foot distance and still differentiate "background noise"
125 levels of powder particles from air contaminants. In these instances, air should be sampled in
126 a manner that, to the extent possible, characterizes the true level of extrinsic particulate
127 contamination to which the product is exposed. Initial certification of the area under dynamic
128 conditions without the actual powder filling function should provide some baseline information
129 on the non-product particle generation of the operation.

130
131 Air in critical areas should be supplied at the point of use as HEPA filtered laminar flow air at
132 a velocity sufficient to sweep particulate matter away from the filling/closing area and maintain
133 laminarity during operations. The velocity parameters established for each processing line
134 should be justified, and appropriate to maintain laminarity and air quality under dynamic
135 conditions within a defined space (Ref. 2).³

136
137 Proper design and control should prevent turbulence or stagnant air in the aseptic processing
138 line or clean zone. Once relevant parameters are established, airflow patterns should be
139 evaluated for turbulence. Air pattern or "smoke" studies demonstrating laminarity and
140 sweeping action over and away from the product under dynamic conditions should be
141 conducted. The studies should be well-documented with written conclusions. Videotape or
142 other recording mechanisms have been found to be useful in assessing airflow initially as well
143 as facilitating evaluation of subsequent equipment configuration changes. However, even
144 successfully qualified systems can be compromised by poor personnel, operational, or
145 maintenance practices.

146
147 Active air monitoring of critical areas should normally yield no microbiological contaminants.
148 Contamination in this environment should receive investigative attention.

149
150 **B. Supporting Clean Areas**

151

³ A velocity from 90 to 100 feet per minute is generally established, with a range of plus or minus 20% around the setpoint. Higher velocities may be appropriate in operations generating high levels of particulates.

152 Supporting clean areas include various classifications and functions. Many support areas
153 function as zones in which non-sterile components, formulated product, in-process materials,
154 equipment, and container/closures are prepared, held, or transferred. These environments
155 should be designed to minimize the level of particulate contaminants in the final product and
156 control the microbiological content (bioburden) of articles and components that are
157 subsequently sterilized.

158
159 The nature of the activities conducted in a supporting clean area should determine its
160 classification. An area classified at Class 100,000 would be used for less critical activities
161 (such as initial equipment preparation). The area immediately adjacent to the aseptic
162 processing line should, at a minimum, meet Class 10,000 standards (see Table 1) under
163 dynamic conditions. Depending on the operation, manufacturers can also classify this area as
164 Class 1000 or maintain the entire aseptic filling room at Class 100.

165

166 **C. Clean Area Separation**

167

168 Adequate separation is necessary between areas of operation to prevent contamination
169 (211.42). In order to maintain air quality in areas of higher cleanliness, it is important to
170 achieve a proper airflow and a positive pressure differential relative to adjacent less clean
171 areas. Rooms of higher classification should have a positive pressure differential relative to
172 adjacent lower classified areas of generally at least 0.05 inch of water (with doors closed).
173 When doors are open, outward airflow should be sufficient to minimize ingress of
174 contamination (Ref. 3). Pressure differentials between clean rooms should be monitored
175 continuously throughout each shift and frequently recorded, and deviations from established
176 limits investigated.

177

178 An adequate air change rate should be established for a cleanroom. For Class 100,000
179 supporting rooms, airflow sufficient to achieve at least 20 air changes per hour is typically
180 acceptable.

181

182 Facility monitoring systems should be established to rapidly detect atypical changes that can
183 compromise the facility's environment. Operating conditions should be restored to established,
184 qualified levels before reaching action levels. For example, pressure differential specifications
185 should enable prompt detection (i.e., alarms) of any emerging low pressure problem in order
186 to preclude ingress of unclassified air into a classified room.

187

188 **D. Air Filtration**

189

190 *1. Membrane (Compressed Gases)*

191

192 A compressed gas should be of appropriate purity (e.g., free from oil and water vapor) and its
193 microbiological and particulate quality should be equal to or better than air in the environment
194 into which the gas is introduced. Compressed gases such as air, nitrogen, and carbon dioxide

195 are often used in clean rooms and are frequently employed in operations involving purging or
196 overlaying.

197
198 Membrane filters allow for the filtration of compressed gases to meet an appropriate high
199 quality standard, and can be used to produce a sterile compressed gas. A sterile-filtered gas is
200 used when the gas contacts a sterilized material. Certain equipment also should be supplied
201 with a sterile-filtered gas. For example, sterile bacterial retentive membrane filters should be
202 used for autoclave air lines, lyophilizer vacuum breaks, vessels containing sterilized materials,
203 and hot air sterilizer vents. Sterilized tanks or liquids should be held under continuous
204 overpressure to prevent microbial contamination. Safeguards should be in place to prevent a
205 pressure change that can result in contamination due to back flow of non-sterile air or liquid.

206
207 Gas filters (including vent filters) should be dry. Condensate in a gas filter can cause blockage
208 or microbial contamination. Frequent replacement, heating, and use of hydrophobic filters
209 prevent moisture residues in a gas supply system. These filters also should be integrity tested
210 upon installation, and periodically thereafter (e.g., including at end of use). Integrity test
211 failures should be investigated.

212

213 2. *High Efficiency Particulate Air (HEPA)*⁴

214

215 An essential element in ensuring aseptic conditions is the maintenance of HEPA filter integrity.
216 Integrity testing should be performed at installation to detect leaks around the sealing gaskets,
217 through the frames or through various points on the filter media. Thereafter, integrity tests
218 should be performed at suitable time intervals for HEPA filters in the aseptic processing
219 facility. For example, such testing should be performed twice a year for the aseptic processing
220 room. Additional testing may be needed when air quality is found to be unacceptable, or as
221 part of an investigation into a media fill or drug product sterility failure. Among the filters
222 that should be integrity tested are those installed in dry heat depyrogenation tunnels commonly
223 used to depyrogenate glass vials.

224

225 One recognized method of testing the integrity of HEPA filters is use of a dioctylphthalate
226 (DOP) aerosol challenge. However, alternative aerosols may be acceptable. Poly-alpha-olefin
227 can also be used, provided it meets specifications for critical physicochemical attributes such as
228 viscosity. Some alternative aerosols are problematic because they pose a risk of microbial
229 contamination of the environment being tested. Firms should ensure that any alternative does
230 not promote microbial growth.

231

232 An intact HEPA filter is capable of retaining at least 99.97 percent of particulates greater than
233 0.3 micron in diameter. It is important to ensure that the aerosol used for the challenge has a
234 sufficient number of particles of this size range. Performing an integrity test without
235 introducing particles of known size upstream of the filter is ineffective for detecting leaks. The
236 DOP challenge should introduce the aerosol upstream of the filter in a concentration of 80 to

⁴ The same broad principles can be applied to ULPA filters.

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237 100 micrograms/liter of air at the filter's designed airflow rating. The downstream side of the
238 filter is then scanned with an appropriate photometer probe at a sampling rate of at least one
239 cubic foot per minute. Scanning should be conducted on the entire filter face and frame at a
240 position about one to two inches from the face of the filter. This comprehensive scanning of
241 HEPA filters should be fully documented. While vendors often provide these services, the
242 drug manufacturer is responsible for ensuring that these essential certification activities are
243 conducted satisfactorily. A single probe reading equivalent to 0.01 percent of the upstream
244 challenge should be considered as indicative of a significant leak and should result in
245 replacement of the HEPA filter or perhaps repair in a limited area. A subsequent confirmatory
246 re-test should be performed in the area of any repair.

247
248 There is a major difference between filter integrity testing and efficiency testing. The purpose
249 of regularly scheduled integrity testing is to detect leaks from the filter media, filter frame and
250 seal. The challenge is a polydispersed aerosol usually composed of particles ranging in size
251 from one to three microns. The test is done in place and the filter face is scanned with a
252 probe; the measured downstream leakage is taken as a percent of the upstream challenge. The
253 efficiency test, on the other hand, is a test used only to determine the rating of the filter.⁵

254
255 HEPA filter integrity testing alone is not sufficient to monitor filter performance. This testing
256 is usually done only on a semi-annual basis. It is important to conduct periodic monitoring of
257 filter attributes such as uniformity of velocity across the filter (and relative to adjacent filters).
258 Variations in velocity generally increase the possibility of contamination, as these changes
259 (e.g., velocity reduction) can have an effect on the laminarity of the airflow. Airflow
260 velocities are measured six inches from the filter face or at a defined distance proximal to the
261 work surface for each HEPA filter. For example, velocity monitoring as frequently as weekly
262 may be appropriate for the clean zone in which aseptic processing is performed. HEPA filters
263 should be replaced when inadequate airflow (e.g., due to blockage) or non-uniformity of air
264 velocity across an area of the filter is detected.

265
266 **E. Design**

267

⁵ The efficiency test uses a monodispersed aerosol of 0.3 micron size particles, relates to filter media, and usually requires specialized testing equipment. Downstream readings represent an average over the entire filter surface. Therefore, the efficiency test is not intended to test for leakage in a filter.

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Section 211.42 requires that aseptic processing operations be “performed within specifically defined areas of adequate size. There shall be separate or defined areas for the firm’s operations to prevent contamination or mixups.”

Section 211.42 states that “flow of components, drug products containers, closures, labeling, in-process materials, and drug products through the building or building shall be designed to prevent contamination.” HEPA filtered air as appropriate, as well as “floors, walls and ceilings of smooth, hard surfaces that are easily cleanable” are some additional requirements of this section.

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

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

Section 211.68 includes requirements for “automatic, mechanical and electronic equipment.”

Section 211.113 states that “appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed.”

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An aseptic process is designed to minimize exposure of sterile articles to dynamic conditions and potential contamination hazards presented by the operation. Limiting the duration of open container exposure, providing the highest possible environmental control, and designing equipment to prevent entrainment of lower quality air into the Class 100 zone are essential to this goal (Ref. 3).

Any intervention or stoppage during an aseptic process can increase the risk of contamination. Personnel and material flow should be optimized to prevent unnecessary activities that increase the potential for introducing contaminants to exposed product, container-closures, or the surrounding environment. The layout of equipment should provide for ergonomics that optimize comfort and movement of operators. The flow of personnel should be designed to limit the frequency with which entries and exits are made to and from the aseptic processing room and, more significantly, its critical area. In order to prevent changes in air currents that introduce lower quality air, movement adjacent to the critical area should be limited. For example, personnel intervention can be reduced by integrating an on-line weight check device, thus eliminating a repeated manual activity within the critical zone. It is also important to minimize the number of personnel in the aseptic processing room.

Transfer of products should be performed under appropriate clean room conditions. For example, lyophilization processes include transfer of aseptically filled product in partially-sealed containers. To prevent contamination, partially-closed sterile product should be staged and transferred only in critical areas. Facility design should assure that the area between a filling line and the lyophilizer, and the transport and loading procedures, provide Class 100 protection.

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294 The sterile product and container-closures should also be protected from activities occurring
295 adjacent to the line. Carefully designed curtains, rigid plastic shields, or other barriers should
296 be used in appropriate locations to partially segregate the aseptic processing line.

297
298 Airlocks and interlocking doors facilitate better control of air balance throughout the aseptic
299 processing area. Airlocks should be installed between the aseptic processing area entrance and
300 the adjoining uncontrolled area. Other interfaces such as personnel entries, or the juncture of
301 the aseptic processing room and its adjacent room, are also appropriate locations for air locks.

302
303 Clean rooms are normally designed as functional units with specific purposes. A well-
304 designed clean room is constructed with material that allows for ease of cleaning and
305 sanitizing. Examples of adequate design features include seamless and rounded floor to wall
306 junctions as well as readily accessible corners. Floors, walls, and ceilings are constructed of
307 smooth, hard surfaces that can be easily cleaned (211.42). Ceilings and associated HEPA filter
308 banks should be designed to protect sterile materials from contamination. Clean rooms also
309 should not contain unnecessary equipment, fixtures, or materials.

310
311 Processing equipment and systems should be equipped with sanitary fittings and valves.
312 Drains are not considered appropriate for rooms in classified areas of the aseptic processing
313 facility.

314
315 When applicable, equipment must be suitably designed for ease of sterilization (211.63). The
316 effect of equipment layout and design on the clean room environment should be addressed.
317 Flat surfaces or ledges that accumulate dust and debris should be avoided. Equipment should
318 not obstruct airflow and, in critical zones, its design should not perturb airflow.

319
320 **V. PERSONNEL TRAINING, QUALIFICATION, & MONITORING**

321

Sections 211.22 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.”

Section 211.113(b) addresses the procedures designed to prevent microbiological contamination, stating that “appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed.”

Section 211.25, Personnel Qualifications requires that “each person engaged in 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...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.” This section also requires “an adequate number of qualified personnel to perform and supervise the manufacture, processing, packing or holding of each drug product.” Section 211.25 also requires that continuing training in CGMP “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.” The training “shall be in the particular operations that the employee performs and in current good manufacturing practice (including the

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current good manufacturing practice regulations in this chapter and written procedures required by these regulations), as they relate to the employee's functions.”

Section 211.28, Personnel Responsibilities 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.” It also states that “personnel shall practice good sanitization and health habits” and specifies that “protective apparel, such as head, face, hand, and arm coverings, shall be worn as necessary to protect drug products from contamination.” It also states that “any person shown at any time (either by medical examination or supervisory examination) 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.”

This section also addresses restrictions on entry into limited access areas: “Only personnel authorized by supervisory personnel shall enter those areas of the buildings and facilities designated as limited-access areas.”

Section 211.42 requires the establishment of a “system for monitoring environmental conditions.”

322

323 **A. Manufacturing Personnel**

324

325 A well-designed aseptic process minimizes personnel intervention. As operator activities
326 increase in an aseptic processing operation, the risk to finished product sterility also increases.
327 It is essential that operators involved in aseptic manipulations adhere to the basic principles of
328 aseptic technique at all times to assure maintenance of product sterility.

329

330 Appropriate training should be conducted before an individual is permitted to enter the aseptic
331 processing area and perform operations. For example, such training should include aseptic
332 technique, clean room behavior, microbiology, hygiene, gowning, and patient safety hazard
333 posed by a non-sterile drug product, and the specific written procedures covering aseptic
334 processing area operations. After initial training, personnel should be updated regularly by an
335 ongoing training program. Supervisory personnel should routinely evaluate each operator’s
336 conformance to written procedures during actual operations. Similarly, the quality control unit
337 should provide regular oversight of adherence to established, written procedures and basic
338 aseptic techniques during manufacturing operations.

339

340 Adherence to basic aseptic technique is a continuous requirement for operators in an aseptic
341 processing operation. Some of these techniques aimed at maintaining sterility of sterile items
342 and surfaces include:

343

344 1. *Contacting sterile materials only with sterile instruments.* Sterile instruments (e.g.,
345 forceps) are should always be used in the handling of sterilized materials. Between
346 uses, instruments should be placed only in sterilized containers. These instruments
347 should be replaced as necessary throughout the operation.

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

352
353 2. *Moving slowly and deliberately.* Rapid movements can create unacceptable
354 turbulence in the critical zone. Such movements disrupt the sterile field, presenting a
355 challenge beyond intended cleanroom design and control parameters. The principle of
356 slow, careful movement should be followed throughout the cleanroom.

357
358 3. *Keeping the entire body out of the path of laminar air.* Laminar airflow design is
359 used to protect sterile equipment surfaces, container-closures, and product. Personnel
360 should not disrupt the path of laminar flow air in the aseptic processing zone.

361
362 4. *Approaching a necessary manipulation in a manner that does not compromise*
363 *sterility of the product.* In order to maintain sterility of nearby sterile materials, a
364 proper aseptic manipulation should be approached from the side and not above the
365 product (in vertical laminar flow operations). Also, speaking when in direct proximity
366 to an aseptic processing line is not an acceptable practice.

367
368 Personnel who have been qualified and permitted access to the aseptic processing area should
369 be appropriately gowned. An aseptic processing area gown should provide a barrier between
370 the body and exposed sterilized materials, and prevent contamination from particles generated
371 by, and microorganisms shed from, the body. Gowns need to be sterile and non-shedding, and
372 should cover the skin and hair. Face masks, hoods, beard/moustache covers, protective
373 goggles, elastic gloves, clean room boots, and shoe overcovers are examples of common
374 elements of gowns. An adequate barrier should be created by the overlapping of gown
375 components (e.g., gloves overlapping sleeves). If an element of the gown is found to be torn
376 or defective, it should be changed immediately.

377
378 There should be an established program to regularly assess or audit conformance of personnel
379 to relevant aseptic manufacturing requirements. An aseptic gowning qualification program
380 should assess the ability of a cleanroom operator to maintain the sterile quality of the gown
381 after performance of gowning procedures. Gowning qualification should include
382 microbiological surface sampling of several locations on a gown (e.g., glove fingers,
383 facemask, forearm, chest, other sites). Following an initial assessment of gowning, periodic
384 requalification should monitor various gowning locations over a suitable period to ensure the
385 consistent acceptability of aseptic gowning techniques. Semi-annual or yearly requalification is
386 acceptable for automated operations where personnel involvement is minimized.

387
388 To protect exposed sterilized product, personnel are expected to maintain sterile gown quality
389 and aseptic method standards in a consistent manner. Written procedures should adequately
390 address circumstances under which personnel should be retrained, requalified, or reassigned to
391 other areas.

392

393 **B. Laboratory Personnel**

394

395 The basic principles of training, aseptic technique, and personnel qualification in aseptic
396 manufacturing are equally applicable to those performing aseptic sampling and microbiological
397 laboratory analyses. Processes and systems cannot be considered to be in control and
398 reproducible if there is any question regarding the validity of data produced by the laboratory.
399

400 **C. Monitoring Program**

401

402 Personnel can have substantial impact on the quality of the environment in which the sterile
403 product is processed. A vigilant and responsive personnel monitoring program should be
404 established. Monitoring should be accomplished by obtaining surface samples of each aseptic
405 processing operator's gloves on an at least a daily basis, or in association with each batch.
406 This sampling should be accompanied by an appropriate sampling frequency for other
407 strategically selected locations of the gown (Ref. 7). The quality control unit should establish a
408 more comprehensive monitoring program for operators involved in operations which are
409 especially labor intensive, i.e. those requiring repeated or complex aseptic manipulations.
410

411 Asepsis is fundamental to an aseptic processing operation. An ongoing goal for manufacturing
412 personnel in the aseptic processing room is to maintain contamination-free gloves throughout
413 operations. Sanitizing gloves just prior to sampling is inappropriate because it can prevent
414 recovery of microorganisms that were present during an aseptic manipulation. When operators
415 exceed established levels or show an adverse trend, an investigation should be conducted
416 promptly. Follow-up actions may include increased sampling, increased observation,
417 retraining, gowning requalification, and in certain instances, reassignment of the individual to
418 operations outside of the aseptic processing area. Microbiological trending systems, and
419 assessment of the impact of atypical trends, are discussed in more detail under Section XI.,
420 Laboratory Controls.

421

422 **VI. COMPONENTS AND CONTAINER/CLOSURES**

423

424 **A. Components**

425

Section 210.3(b)(3) defines a "component" as "any ingredient intended for use in the manufacture of a drug product, including those that may not appear in such drug product."

Section 211.80, General Requirements, requires, in part, the establishment of 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...Components and drug product containers and closures shall at all times be handled and stored in a manner to prevent contamination."

Section 211.84, Testing and approval or rejection of components, drug product containers, and closures, requires 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."

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427 A drug product produced by aseptic processing can become contaminated by use of one or
428 more components (e.g., active ingredients, excipients, Water for Injection) that are
429 contaminated with microorganisms or endotoxins. It is important to characterize the microbial
430 content of each component liable to contamination and establish appropriate
431 acceptance/rejection limits based on information on bioburden. Knowledge of bioburden is
432 critical in assessing whether the sterilization process is adequate.

433
434 In aseptic processing, each component is individually sterilized or several components are
435 combined, with the resulting mixture sterilized.⁶ There are several methods to sterilize
436 components (see relevant discussion in Section IX). A widely used method is filtration of a
437 solution formed by dissolving the component(s) in a solvent such as USP Water For Injection
438 (WFI). The solution is passed through a sterilizing membrane or cartridge filter. Filter
439 sterilization is used where the component is soluble and is likely to be adversely affected by
440 heat. A variation of this method involves subjecting the filtered solution to aseptic
441 crystallization and precipitation of the component as a sterile powder. However, this method
442 involves more handling and manipulation and therefore has a higher potential for
443 contamination during processing. If a component is not adversely affected by heat, and is
444 soluble, it may be made into a solution and subjected to steam sterilization, typically in an
445 autoclave or a pressurized vessel.

446
447 Dry heat sterilization is a suitable method for components that are heat stable and insoluble.
448 However, carefully designed heat penetration and distribution studies should be performed for
449 powder sterilization because of the insulating effects of the powder.

450
451 Ethylene oxide (EtO) exposure is often used for surface sterilization. Such methods should be
452 carefully controlled and validated if used for powders to evaluate whether consistent
453 penetration of the sterilant is achieved and to minimize residual ethylene oxide and by-
454 products.

455
456 Parenteral products are intended to be non-pyrogenic. There should be written procedures and
457 appropriate specifications for acceptance or rejection of each lot of components that might
458 contain endotoxins. Any components failing to meet endotoxin specifications should be
459 rejected.

460 **B. Containers/Closures**

462

⁶ See Appendix III for discussion of certain biologic components that are aseptically handled from the start of the process.

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Section 211.94 (drug product containers and closures) states that “drug product containers and closures shall be clean and, where indicated by the nature of the drug, sterilized and processed to remove pyrogenic properties to assure that they are suitable for their intended use.” It also states that “Standards or specifications, methods of testing, and, where indicated, methods of cleaning, sterilizing and processing to remove pyrogenic properties shall be written and followed for drug product containers and closures.”

Section 211.113(b) requires “validation of any sterilization process” as part of designing procedures “to prevent microbiological contamination of drug products purporting to be sterile.”

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1. Preparation

Containers and closures should be rendered sterile and, for parenteral drug products, pyrogen-free. The type of processes used will depend primarily on the nature of the material comprising the container and/or closure. The validation study for any such process should be adequate to demonstrate its ability to render materials sterile and pyrogen-free. Written procedures should specify the frequency of revalidation of these processes as well as time limits for holding sterile, depyrogenated containers and closures.

Presterilization preparation of glass containers usually involves a series of wash and rinse cycles. These cycles serve an important role in removing foreign matter. Rinse water should be of high purity so as not to contaminate containers. For parenteral products, final rinse water should meet the specifications of Water for Injection, USP.

The adequacy of the depyrogenation process can be assessed by spiking containers or closures with known quantities of endotoxin, followed by measuring endotoxin content after depyrogenation. The challenge studies should be performed with a reconstituted endotoxin solution applied directly onto the surface being tested and air-dried. Positive controls should be used to measure the percentage of endotoxin recovery by the test method. Validation study data should demonstrate that the process reduces the endotoxin content by at least 99.9% (3 logs).

Glass containers are generally subjected to dry heat for sterilization and depyrogenation. Validation of dry heat sterilization/depyrogenation should include appropriate heat distribution and penetration studies as well as the use of worst-case process cycles, container characteristics (e.g., mass), and specific loading configurations to represent actual production runs. See Section IX.C.

Pyrogen on plastic containers can be generally removed by multiple WFI rinses. Plastic containers can be sterilized with an appropriate gas, irradiation or other suitable means. For gases such as EtO, the parameters and limits of the EtO sterilization cycle (e.g. temperature, pressure, humidity, gas concentration, exposure time, degassing, aeration, and determination of residuals) should be specified and monitored closely. Biological indicators are of special importance in demonstrating the effectiveness of EtO and other gas sterilization processes.

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499 Rubber closures (e.g., stoppers and syringe plungers) are cleaned by multiple cycles of
500 washing and rinsing prior to final steam or irradiation sterilization. At minimum, the initial
501 rinses for the washing process should employ Purified Water USP of minimal endotoxin
502 content, followed by final rinse(s) with WFI for parenteral products. Normally,
503 depyrogenation is achieved by multiple rinses of hot WFI. The time between washing and
504 sterilizing should be minimized because moisture on the stoppers can support microbial growth
505 and the generation of endotoxins. Because rubber is a poor conductor of heat, extra attention
506 should be given to the validation of processes that use heat to sterilize rubber stoppers.
507 Validation data should also demonstrate successful endotoxin removal from rubber materials.
508

509 A potential source of contamination is the siliconization of rubber stoppers. Silicone used in
510 the preparation of rubber stoppers should be rendered sterile and should not have an adverse
511 effect on the safety, quality, or purity of the drug product.
512

513 See Section VIII for discussion of the need to establish production time limits for the holding
514 of sterilized containers and closures.
515

516 Contract facilities that perform sterilization and depyrogenation of containers and closures are
517 subject to the same CGMP requirements as those established for in-house processing. The
518 finished dosage form manufacturer is responsible for the review and approval of the
519 contractor's validation protocol and final validation report.
520

521 *2. Inspection of Container-Closure System*
522

523 A container-closure system that permits penetration of air, or microorganisms, is unsuitable for
524 a sterile product. Any damaged or defective units should be detected, and removed, during
525 inspection of the final sealed product. Safeguards should be implemented to strictly preclude
526 shipment of product that may lack container-closure integrity and lead to non-sterility.
527 Equipment suitability problems or incoming container or closure deficiencies have caused loss
528 of container-closure system integrity. As examples, failure to detect vials fractured by faulty
529 machinery, or by mishandling of bulk finished stock, has led to drug recalls. If damage that is
530 not readily detected leads to loss of container-closure integrity, improved procedures should be
531 rapidly implemented to prevent and detect such defects.
532

533 Functional defects in delivery devices (e.g., syringe device defects, delivery volume) can also
534 result in product quality problems, and should be monitored by appropriate in-process testing.
535

536 Any defects or results outside the specifications established for in-process and final inspection
537 should be investigated in accord with Section 211.192.
538

539 **VII. ENDOTOXIN CONTROL**
540

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

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

Section 211.67, equipment cleaning and maintenance requires, states that “equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements.”

Section 211.94 states that “drug product containers and closures shall be clean, and where indicated by the nature of the drug, sterilized and processed to remove pyrogenic properties to assure that they are suitable for their intended use.”

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

541
542 Endotoxin contamination of an injectable product can be a result of poor CGMP controls.
543 Certain patient populations (e.g., neonates), those receiving other injections concomitantly, or
544 those administered a parenteral in atypically large volumes or doses can be at greater risk for
545 pyrogenic reaction than anticipated by the established limits based on body weight of a normal
546 healthy adult (Ref. 6,7). Such clinical concerns reinforce the need for appropriate CGMP
547 controls to prevent generation of endotoxin. Drug product components, container-closures,
548 equipment, and storage time limitations are among the concerns to address in establishing
549 endotoxin control.

550
551 Adequate cleaning, drying, and storage of equipment provides for control of bioburden and
552 prevents contribution of endotoxin load. Equipment should be designed to be easily assembled
553 and disassembled, cleaned, sanitized, and/or sterilized. Endotoxin control should be exercised
554 for all product contact surfaces both prior to and after sterile filtration.

555
556 Endotoxin on equipment surfaces is inactivated by high temperature dry heat, or removed from
557 equipment surfaces by validated cleaning procedures. Some clean-in-place procedures employ
558 initial rinses with appropriate high purity water and/or a cleaning agent (e.g., acid, base,
559 surfactant), followed by final rinses with heated WFI. Equipment should be dried following
560 cleaning. Sterilizing filters and moist heat sterilization have not been shown to be effective in
561 removing endotoxins. Processes that are designed to achieve depyrogenation should
562 demonstrate a 3-log reduction of endotoxin.

563
564 **VIII. TIME LIMITATIONS**
565

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

566

567 Time limits should be established for each phase of aseptic processing. Time limits should
568 include, for example, the period between the start of bulk product compounding and its
569 filtration, filtration processes, product exposure while on the processing line, and storage of
570 sterilized equipment, containers and closures. Maintenance of in-process quality at different
571 production phases should be supported by data. Bioburden and endotoxin load should be
572 assessed when establishing time limits for stages such as the formulation processing stage.

573
574 The total time for product filtration should be limited to an established maximum in order to
575 prevent microorganisms from penetrating the filter. Such a time limit should also prevent a
576 significant increase in upstream bioburden and endotoxin load. Sterilizing filters should
577 generally be replaced following each manufactured lot. Because they can provide a substrate
578 for microbial attachment, maximum use times for those filters used upstream for solution
579 clarification or particle removal should also be established and justified.

580

581 **IX. PROCESS VALIDATION AND EQUIPMENT QUALIFICATION**

582

Section 211.113(b) (control of microbiological contamination) states: “Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilization process.”

Sections 211.63, 211.65, and 211.67 address, respectively, “Equipment, design, size, and location,” “Equipment construction,” and “Equipment cleaning and maintenance.”

Section 211.84(c)(3) states that “sterile equipment and aseptic sampling techniques shall be used when necessary.”

583

584 The following sections primarily discuss routine qualification and validation study
585 expectations. Change control procedures are only briefly addressed, but are an important part
586 of the quality systems established by a firm. A change in equipment, process, test method, or
587 systems requires evaluation through the written change control program, and should trigger an
588 evaluation of the need for revalidation or requalification.

589

590 **A. Process Simulations**

591

592 In order to ensure the sterility of products purporting to be sterile, both sterilization and aseptic
593 filling/closing operations must be adequately validated (211.113). The goal of even the most
594 effective sterilization processes can be defeated if the sterilized elements of a product (the
595 drug, the container and the closure) are brought together under conditions that contaminate
596 those elements. Similarly, product sterility is compromised when the product elements are
597 non-sterile at the time they are assembled.

598

599 The validation of an aseptic processing operation should include the use of a microbiological
600 growth nutrient medium in place of product. This has been termed a “media fill” or “process
601 simulation.” The nutrient medium is exposed to product contact surfaces of equipment,

602 container systems, critical environments, and process manipulations to closely simulate the
603 same exposure that the product itself will undergo. The sealed containers filled with the media
604 are then incubated to detect microbial contamination. The results are interpreted to determine
605 the potential for any given unit of drug product to become contaminated during actual
606 operations (e.g., start-up, sterile ingredient additions, aseptic connections, filling, closing).
607 Environmental monitoring data is integral to the validation of an aseptic processing operation.

608

609 *1. Study Design*

610

611 A validation protocol should detail the overall strategy, testing requirements, and acceptance
612 criteria for the media fill. Media fill studies should simulate aseptic manufacturing operations
613 as closely as possible, incorporating a "worst-case" approach. A media fill study should
614 address applicable issues such as:

615

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

636

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

640

641 *2. Frequency and number of runs*

642

643 When a processing line is initially validated, separate media fills should be repeated enough
644 times to ensure that results are consistent and meaningful. This approach is important because
645 a single run can be inconclusive, while multiple runs with divergent results signal a process

646 that is not in control. A minimum of three consecutive separate successful runs should be
647 performed during initial line qualification. Subsequently, routine semi-annual revalidation runs
648 should be conducted for each shift and processing line to evaluate the state of control of the
649 aseptic process. All personnel who enter the aseptic processing area, including technicians and
650 maintenance personnel, should participate in a media fill at least once a year

651
652 Each change to a product or line change should be evaluated using a written change control
653 system. Any changes or events that appear to affect the ability of the aseptic process to
654 exclude contamination from the sterilized product should be assessed through additional media
655 fills. For example, facility and equipment modification, line configuration change, significant
656 changes in personnel, anomalies in environmental testing results, container-closure system
657 changes or, end product sterility testing showing contaminated products may be cause for
658 revalidation of the system.

659
660 Where a media fill's data indicates the process may not be in control, a comprehensive
661 documented investigation should be conducted to determine the origin of the contamination and
662 the scope of the problem. Once corrections are instituted, multiple repeat process simulation
663 runs should be performed to confirm that deficiencies in practices and procedures have been
664 corrected and the process has returned to a state of control. However, when an investigation
665 fails to reach well-supported, substantive conclusions as to the cause of the media fill failure,
666 three consecutive successful runs and increased scrutiny (i.e., extra supervision, monitoring) of
667 the production process should be implemented.

668 669 *3. Size and Duration of runs*

670
671 The duration of aseptic processing operations is a major consideration in determining the size
672 of the media fill run. Although the most accurate simulation model would be the full batch
673 size and duration because it most closely simulates the actual production run, other appropriate
674 models can be justified. In any study protocol, the duration of the run and the overall study
675 design should adequately mimic worst-case operating conditions and cover all manipulations
676 that are performed in the actual processing operation. Adequate batch sizes are needed to
677 simulate commercial production conditions and accurately assess the potential for commercial
678 batch contamination. The number of units filled should be sufficient to reflect the effects of
679 potential operator fatigue, as well as the maximum number of interventions and stoppages.
680 The run should be large enough to accurately simulate production conditions and sensitive
681 enough to detect a low incidence of contaminated units. For batches produced over multiple
682 shifts or yielding an unusually large number of units, the media fill protocol should adequately
683 encompass conditions and any potential risks associated with the larger operation.

684
685 While conventional manufacturing lines are highly automated, often operate at relatively high
686 speeds, and are designed to limit operator intervention, there are some processes that include
687 considerable operator involvement. When aseptic processing employs manual filling or
688 closing, or extensive manual manipulations, the duration of the process simulation should

689 generally be no less than the length of the actual manufacturing process in order to best
690 simulate operator fatigue.

691

692 For simulation of lyophilization operations, unsealed containers should be exposed to
693 pressurization and partial evacuation of the chamber in a manner that is representative of
694 process stresses. Vials should not be frozen, as this may inhibit the growth of
695 microorganisms.

696

697 *4. Line Speed*

698

699 The media fill program should adequately address the range of line speeds (e.g., by bracketing
700 all vial sizes and fill volumes) employed during production. In some cases, more than one line
701 speed should be evaluated in the course of a study.

702

703 Each individual media fill run should evaluate a single worst-case line speed and the speed
704 chosen for each batch during a study should be justified. For example, use of high line speed
705 is justified for manufacturing processes characterized by frequent interventions or a significant
706 degree of manual manipulation. Use of slow line speed is justified for manufacturing
707 processes characterized by prolonged exposure of sterile components in the aseptic area

708

709 *5. Environmental Conditions*

710

711 Media fills should be conducted under environmental conditions that simulate normal as well as
712 "worst case" conditions of production. An inaccurate assessment (making the process appear
713 "cleaner" than it actually is) can result from conducting a media fill under extraordinary air
714 particulate and microbial quality, or under production controls and precautions taken in
715 preparation for the media fill. To the extent standard operating procedures permit stressful
716 conditions, it is crucial that media fills should include rigorous challenges in order to support
717 the validity of these studies.

718

719 *6. Media*

720

721 In general, a microbiological growth medium such as soybean casein digest medium should be
722 used. Use of anaerobic growth media (such as Fluid Thioglycollate Medium) is appropriate in
723 special circumstances. Media selected should be demonstrated to promote growth of USP
724 <71> indicator microorganisms as well as isolates that have been identified by environmental
725 monitoring, personnel monitoring, and positive sterility test results. Positive control units
726 should be inoculated with a <100 CFU challenge and incubated. For those instances in which
727 the growth promotion testing fails, the origin of any contamination found during the simulation
728 should nonetheless be investigated and the media fill should be promptly repeated.

729

730 The production process should be accurately simulated using media and conditions that
731 optimize detection of any microbiological contamination. Each unit should be filled with an
732 appropriate quantity and type of microbial growth medium to contact the inner container-

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733 closure surfaces (when the unit is inverted and swirled) and permit visual detection of
734 microbial growth.

735
736 Some drug manufacturers have expressed concern over the possible contamination of the
737 facility and equipment with the nutrient media during media fill runs. However, if the medium
738 is handled properly and is promptly followed by the cleaning, sanitizing, and, where
739 necessary, sterilization of equipment, subsequently processed products are not likely to be
740 compromised.

741
742 *7. Incubation and Examination of Media Filled Units*

743
744 Media units should be incubated for a sufficient time (a period of not less than 14 days) at a
745 temperature adequate to enhance detection of organisms that can otherwise be difficult to
746 culture.

747
748 Each media filled unit should be examined for contamination by personnel with appropriate
749 education, training and experience in microbiological techniques. There should be direct
750 quality control unit oversight throughout any such examination. Clear containers with
751 otherwise identical physical properties should be used as a substitute for amber or other opaque
752 containers to allow visual detection of microbial growth.

753
754 When a firm performs a final product inspection of units immediately following the media fill
755 run, all integral units should proceed to incubation. Units found to have defects not related to
756 integrity (e.g., cosmetic defect) should be incubated; units that lack integrity should be
757 rejected.⁷ Erroneously rejected units should be returned promptly for incubation with the
758 media fill lot.

759
760 After incubation is underway, any unit found to be damaged should be included in the data for
761 the media fill batch, because the incubation of the units simulates release to the market. Any
762 decision to exclude such incubated units (i.e., non-integral) from the final batch tally should be
763 fully justified, and the deviation explained in the media fill report. If a correlation emerges
764 between difficult to detect damage and microbial contamination, a thorough investigation
765 should be conducted to determine its cause (See Section VI.B).

766
767 Written procedures regarding aseptic interventions should be clear and specific (e.g.,
768 intervention type; quantity of units removed), providing for consistent production practices and
769 assessment of these practices during media fills. If written procedures and batch
770 documentation are adequate, these intervention units do not need to be incubated during media
771 fills. Where procedures lack specificity, there would be insufficient justification for exclusion
772 of units removed during an intervention from incubation. As an example, if a production
773 procedure requires removal of ten units after an intervention at the stoppering station infeed,
774 batch records (i.e., for production and media fills) should clearly document conformance with

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

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775 this procedure. In no case should more units be removed during a media fill intervention than
776 would be cleared during a production run. The ability of a media fill run to detect potential
777 contamination from a given simulated activity should not be compromised by a large scale line
778 clearance, which can result in removal of a positive unit caused by an unrelated event or
779 intervention. If unavoidable, appropriate study provisions should be made to compensate in
780 such instances.

781
782 Appropriate criteria should be established for yield and accountability. Batch record
783 reconciliation documentation should include an accurate accounting and description of units
784 rejected from a batch.

785
786 *8. Interpretation of Test Results*

787
788 The process simulation run should be observed, and contaminated units should be reconcilable
789 with the approximate time and the activity being simulated during the media fill. Videotaping
790 of a media fill has been found to be useful in identifying personnel practices which could
791 negatively impact on the aseptic process.

792
793 Any contaminated unit should be considered as objectionable and fully investigated. The
794 microorganisms should be identified to species level. In the case of a media fill failure, a
795 comprehensive investigation should be conducted, surveying all possible causes of the
796 contamination. The impact on commercial drugs produced on the line since the last successful
797 media fill should also be assessed.

798
799 Whenever contamination exists in a media fill batch, it should be considered as indicative of a
800 potential production problem. The use of statistics has limitations for media fill evaluation in
801 that the number of contaminated units should not be expected to increase in a directly
802 proportional manner with the number of vials in the media fill run. Test results should show,
803 with a high degree of confidence, that the units produced by an aseptic processing operation
804 are sterile. Modern aseptic processing operations in suitably designed facilities have
805 demonstrated a capability of meeting contamination levels approaching zero (Ref.8) and should
806 normally yield no media fill contamination. For example, a single contaminated unit in a
807 10,000 unit media fill batch should be fully investigated, but is normally not considered on its
808 own to be sufficient cause for line revalidation. However, intermittent incidents at this media
809 fill contamination level can be indicative of a persistent low level contamination problem.
810 Accordingly, any pattern of media fill batches with such low level contamination should be
811 comprehensively investigated and would be cause for line revalidation.

812
813 A firm's use of media fill acceptance criteria allowing infrequent contamination does not mean
814 that a distributed lot of drug product purporting to be sterile may contain a non-sterile unit.
815 The purpose of an aseptic process is to prevent any contamination. A manufacturer is fully
816 liable for the shipment of any non-sterile unit, an act that is prohibited under the FD&C Act.
817 FDA also recognizes that there might be some scientific and technical limitations on how
818 precisely and accurately validation can characterize a system of controls intended to exclude

819 contamination.

820

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

825

826 **B. Filtration Efficacy**

827

828 Filtration is a common method of sterilizing drug product solutions. An appropriate sterilizing
829 grade filter is one which reproducibly removes all microorganisms from the process stream,
830 producing a sterile effluent. Such filters usually have a rated porosity of 0.2 micron or
831 smaller. Whatever filter or combination of filters is used, validation should include
832 microbiological challenges to simulate "worst case" production conditions regarding the size of
833 microorganisms in the material to be filtered and integrity test results of the filters used for the
834 study. The microorganisms should be small enough to both challenge the nominal porosity of
835 the filter and simulate the smallest microorganism that may occur in production. The
836 microorganism *Brevundimonas diminuta* (ATCC 19146) when properly grown, harvested and
837 used, can be satisfactory in this regard because it is one of the smallest bacteria (0.3 micron
838 mean diameter). Bioburden of unsterilized bulk solutions should be determined, in order to
839 trend the characteristics of potentially contaminating organisms. In certain cases, when
840 justified as equivalent or better than use of *Brevundimonas diminuta*, it may be appropriate to
841 conduct bacterial retention studies with a bioburden isolate. The number of microorganisms in
842 the challenge is important because a filter can contain a number of pores larger than the
843 nominal rating which have potential to allow passage of microorganisms (Ref. 9). The
844 probability of such passage is considered to increase as the number of organisms (bioburden) in
845 the material to be filtered increases (Ref. 10). A challenge concentration of at least 10^7
846 organisms per cm^2 of effective filtration area of *B. diminuta* is generally used. A commercial
847 lot's actual influent bioburden should not include microorganisms of a size and/or
848 concentration that would present a challenge beyond that considered by the validation study.

849

850 Direct inoculation into the drug formulation provides an assessment of the effect of drug
851 product on the filter matrix and on the challenge organism. However, directly inoculating *B.*
852 *diminuta* into products with inherent bactericidal activity or into oil-based formulations can
853 lead to erroneous conclusions. When sufficiently justified, the effects of the product
854 formulation on the membrane's integrity can be assessed using an appropriate alternate
855 method. For example, the drug product could be filtered in a manner in which the worst-case
856 combination of process specifications and conditions are simulated. This step could be
857 followed by filtration of the challenge organism for a significant period of time, under the
858 same conditions, using an appropriately modified product (e.g., lacking an antimicrobial
859 preservative or other antimicrobial component) as the vehicle. Any divergence from a
860 simulation using the actual product and conditions of processing should be justified. Factors
861 which can affect filter performance normally include: (1) viscosity of the material to be
862 filtered; (2) pH; (3) compatibility of the material or formulation components with the filter

863 itself; (4) pressures; (5) flow rates; (6) maximum use time; (7) temperature; (8) osmolality; (9)
864 and the effects of hydraulic shock. When designing the validation protocol, it is important to
865 address the effect of the extremes of processing factors on the filter capability to produce
866 sterile effluent. Filter validation should be conducted using the worst case conditions, such as
867 maximum filter use time and pressure (Ref. 11). Filter validation experiments, including
868 microbial challenges, need not be conducted in the actual manufacturing areas. However, it is
869 essential that laboratory experiments simulate actual production conditions. The specific type
870 of filter used in commercial production should be evaluated in filter validation studies. When
871 the more complex filter validation tests go beyond the capabilities of the filter user, tests are
872 often conducted by outside laboratories or by filter manufacturers. However, it is the
873 responsibility of the filter user to review the validation data on the efficacy of the filter in
874 producing a sterile effluent. The data should be applicable to the user's products and
875 conditions of use because filter performance may differ significantly for various conditions and
876 products.

877
878 After a filtration process is properly validated for a given product, process and filter, it is
879 important to ensure that identical filter replacements (membrane or cartridge) used in
880 production runs will perform in the same manner. Sterilizing filters should be routinely
881 discarded after processing of a single batch. Normally, integrity testing of the filter is
882 performed after the filter unit is assembled and sterilized prior to use. It is important that the
883 integrity testing be conducted after filtration in order to detect any filter leaks or perforations
884 that might have occurred during the filtration. "Forward flow" and "bubble point" tests, when
885 appropriately employed, are two acceptable integrity tests. A production filter's integrity test
886 specification should be consistent with data generated during filtration efficacy studies.

887 888 **C. Sterilization of Equipment and Container/Closures**

889
890 In order to maintain sterility, equipment surfaces that contact sterilized drug product or
891 sterilized container/closure surfaces must be sterile so as not to alter purity of the drug (211.63
892 and 211.113). Those surfaces that are in the vicinity of sterile product or container-closures,
893 but do not directly contact product should also be rendered sterile where reasonable
894 contamination potential exists. It is as important in aseptic processing to properly validate the
895 processes used to sterilize such critical equipment as it is to validate processes used to sterilize
896 the drug product and its container/closure. Moist heat and dry heat sterilization are most
897 widely used and the primary processes discussed in this document. It should be noted that
898 many of the heat sterilization principles discussed in this document are also applicable to other
899 sterilization methods.

900
901 Sterility of aseptic processing equipment (e.g., stopper hoppers) should be maintained by
902 batch-by-batch sterilization. Following sterilization of equipment, containers, or closures, any
903 transportation or assembly needs to be performed in a manner in which its sterile state is
904 protected and sustained, with adherence to strict aseptic methods.

905

906 *1. Sterilizer Qualification and Validation*

907
908 Validation studies should be conducted demonstrating the efficacy of the sterilization cycle.
909 Requalification studies should also be performed on a periodic basis. For both the validation
910 studies and routine production, use of a specified load configuration should be documented in
911 the batch records.

912
913 Unevacuated air's insulating properties prevent moist heat from penetrating or heating up
914 materials, and achieving the lethality associated with saturated steam. Consequently, there is a
915 far slower thermal energy transfer and rate of kill from the dry heat in insulated locations in
916 the load. It is important to remove all of the air from the autoclave chamber during the
917 sterilization cycle. Special attention should be given to the nature or type of the materials to be
918 sterilized and the placement of biological indicator within the sterilization load. D-value of the
919 biological indicator can vary widely depending on the material (e.g., glass versus Teflon) to be
920 sterilized. Difficult to reach locations within the sterilizer load and specific materials should
921 be an important part of the evaluation of sterilization cycle efficacy. Thereafter,
922 requalification/revalidation should continue to focus on load areas identified as the most
923 difficult to penetrate or heat (e.g., worst-case locations of tightly wrapped or densely packed
924 supplies, securely fastened load articles, lengthy tubing, the sterile filter apparatus,
925 hydrophobic filters, stopper load).

926
927 The formal program providing for regular (i.e., semiannual, annual) revalidation should
928 consider the age of the sterilizer and its past performance. Change control procedures should
929 adequately address issues such as a load configuration change or a modification of the
930 sterilizer.

931
932 a) Qualification: Empty Chamber

933
934 Temperature distribution studies evaluate numerous locations throughout an empty
935 sterilizing unit (e.g., steam autoclave, dry heat oven) or equipment train (e.g., large
936 tanks, immobile piping). It is important that these studies assess temperature
937 uniformity at various locations throughout the sterilizer to identify potential "cold
938 spots" where there can be insufficient heat to attain sterility. These heat uniformity or
939 "temperature mapping" studies should be conducted by placing calibrated temperature
940 measurement devices in numerous locations throughout the chamber.

941
942 b) Validation: Loaded Chamber

943
944 Heat penetration studies should be performed using the established sterilizer load(s).
945 Validation of the sterilization process with a loaded chamber demonstrates the effects of
946 loading on thermal input to the items being sterilized, and may identify "cold spots"
947 where there is insufficient heat to attain sterility. The placement of biological
948 indicators (BI) at numerous positions in the load, including the most difficult to sterilize
949 places, is a direct means of demonstrating the efficacy of any sterilization procedure.

950 In general, the thermocouple (TC) is placed adjacent to the BI so as to assess the
951 correlation between microbial lethality and thermal input. Validation of sterilization
952 can be performed using a partial or half-cycle approach. In some cases, the
953 “bioburden” based cycle is used for sterilization validation. For further information on
954 validation using moist heat sterilization, please refer to FDA guidance, “Guideline for
955 the Submission of Documentation for Sterilization Process Validation in Applications
956 for Human and Veterinary Drug Products” (November, 1994).

957
958 Sterilization cycle specifications are based upon the delivery of adequate thermal input
959 to the slowest to heat locations. When determining which articles are most difficult to
960 sterilize, special attention should be given to the sterilization of filters. For example,
961 some filter installations in piping cause a significant pressure differential across the
962 filter, resulting in a significant temperature drop on the downstream side. Biological
963 indicators should be placed at appropriate downstream locations of this equipment to
964 determine if the drop in temperature affects the thermal input at these sites. Established
965 load configuration should be part of batch record documentation. A sterility assurance
966 level of 10^{-6} or better should be demonstrated for the sterilization process.

968 *2. Equipment Controls and Instrument Calibration*

969
970 For both validation and routine process control, the reliability of the data generated by
971 sterilization cycle monitoring devices should be considered to be of the utmost importance.
972 Devices that measure cycle parameters should be routinely calibrated. Written procedures
973 should be established to ensure these devices are maintained in a calibrated state. For
974 example:

- 975 • Temperature monitoring devices for heat sterilization should be calibrated at
976 suitable intervals, as well as before and after validation runs.
- 977 • Devices used to monitor dwell time in the sterilizer should be periodically
978 calibrated.
- 979 • The microbial count and D-value of a biological indicator should be confirmed
980 before a validation study.
- 981 • Instruments used to determine the purity of steam should be calibrated.
- 982 • For dry heat depyrogenation tunnels, devices (e.g. sensors and transmitters) used to
983 measure belt speed should be routinely calibrated.

984
985 Sterilizing equipment should be properly maintained to allow for consistently satisfactory
986 function. Evaluation of sterilizer performance attributes such as equilibrium (“come up”) time
987 studies should be helpful in assessing if the unit continues to operate properly.

988
989
990

X. LABORATORY CONTROLS

Section 211.160 (General Requirements) states “Laboratory controls shall include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to assure that components, drug product containers, closures, in-process materials, labeling, and drug products conform to appropriate standards of identity, strength, quality, and purity.”

Sections 211.165 and 211.194 require that validation of test methods be established and documented.

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

Section 211.42 requires, for aseptic processes, the establishment of a “system for monitoring environmental conditions.”

Section 211.56 requires, “written procedures assigning responsibility for sanitation and describing in sufficient detail the cleaning schedules, methods, equipment, and materials to be used in cleaning the buildings and facilities.” The “written procedures shall be designed to prevent the contamination of equipment, components, drug product containers, closures, packaging, labeling materials, or drug products and shall be followed.” Section 211.113 (b) requires that “appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed.”

Section 211.192 states that “all drug product production and control records, including those for packaging and labeling, shall be reviewed and approved by the quality control unit to determine compliance with all established, approved, written procedures before a batch is released or distributed.”

991

A. Environmental Monitoring

992

993

994

1. General Written Program

995

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999

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1001

1002

In aseptic processing, one of the most important laboratory controls is the establishment of an environmental monitoring program. This monitoring provides meaningful information on the quality of the aseptic processing environment when a given batch is being manufactured as well as environmental trends of the manufacturing area. An adequate program identifies potential routes of contamination, allowing for implementation of corrections before product contamination occurs (211.42 and 211.113).

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Evaluating the quality of air and surfaces in the cleanroom environment should start with a well-defined written program and validated methods. The monitoring program should cover all production shifts and include air, floors, walls, and equipment surfaces, including the critical surfaces in contact with product and container/closures. Written procedures should include a list of locations to be sampled. Sample timing, frequency, and location should be carefully selected based upon its relationship to the operation performed. Samples should be taken throughout the aseptic processing facility (e.g., aseptic corridors; gowning rooms) using appropriate, scientifically sound sampling procedures, standards, and test limits.

1011
1012 Locations posing the most microbiological risk to the product are a critical part of the
1013 program. It is especially important to monitor the microbiological quality of the aseptic
1014 processing clean zone to determine whether or not aseptic conditions are maintained during
1015 filling/closing activities. Critical surfaces which contact sterile product should be sterile.
1016 Critical surface sampling should be performed at the conclusion of the aseptic processing
1017 operation to avoid direct contact with sterile surfaces during processing. Air and surface
1018 samples should be taken at the actual working site and at locations where significant activity or
1019 product exposure occurs during production.

1020
1021 Environmental monitoring methods do not always recover microorganisms present in the
1022 sampled area. In particular, low level contamination can be particularly difficult to detect.
1023 Because of the likelihood of false negatives, consecutive growth results are only one type of
1024 adverse trend. Increased incidence of contamination over a given period in comparison to that
1025 normally detected is an equally significant trend to be tracked.

1026
1027 All environmental monitoring locations should be described in SOPs with sufficient detail to
1028 allow for reproducible sampling of a given location surveyed. Written SOPs should also
1029 address areas such as: (1) frequency of sampling; (2) when the samples are taken (i.e., during
1030 or at the conclusion of operations); (3) duration of sampling; (4) sample size (e.g., surface
1031 area, air volume); (5) specific sampling equipment and techniques; (6) alert and action limits;
1032 and (7) appropriate response to deviations from alert or action limits.

1033
1034 *2. Establishing Limits and a Trending Program*

1035
1036 Microbiological monitoring limits should be established based on the relationship of the
1037 sampled location to the operation. The limits should be based on the need to maintain adequate
1038 microbiological control throughout the entire sterile manufacturing facility. One should also
1039 consider environmental monitoring data from historical databases, media fills, cleanroom
1040 qualification, and sanitization procedure studies in developing monitoring limits.

1041
1042 Microbiological environmental monitoring should include both alert and action limits. Each
1043 individual sample result should be evaluated for its significance by comparing to the alert or
1044 action limits. Averaging of results can mask unacceptable localized conditions. A result at the
1045 alert limit urges attention to the approaching action conditions. A result at the action level
1046 should prompt a more thorough investigation. Written procedures should be established,
1047 detailing data review frequency, identification of contaminants, and actions to be taken. The
1048 quality control unit should provide routine oversight of near term (e.g., daily, weekly,
1049 monthly, quarterly) and long term trends in environmental and personnel monitoring data.

1050
1051 Trend reports should include data generated by location, shift, lot, room, operator, or other
1052 search parameters. The quality control unit is responsible for producing specialized data
1053 reports (e.g., a search on a particular atypical isolate over a year period) in order to investigate
1054 results beyond established limits and identify any appropriate follow-up actions. In addition to

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1055 microbial counts beyond alert and action limits, the presence of any atypical microorganisms in
1056 the cleanroom environment should be investigated, with any appropriate corrective action
1057 promptly implemented.

1058
1059 Written procedures should define the system whereby the most responsible managers are
1060 regularly informed and updated on trends and investigations.

1061
1062 *3. Sanitization Efficacy*

1063
1064 The suitability, efficacy, and limitations of sanitization agents should be assessed with their
1065 implementation for use in clean areas. The effectiveness of these sanitization procedures
1066 should be measured by their ability to ensure that potential contaminants are adequately
1067 removed from surfaces (i.e., via obtaining samples before and after sanitization).

1068
1069 Upon preparation, disinfectants should be rendered sterile, and used for a limited time, as
1070 specified by written procedures. Disinfectants should retain efficacy against the normal
1071 microbial flora and be effective against spore-forming microorganisms. Many common
1072 sanitizers are ineffective against spores, for example, 70% isopropyl alcohol is not effective
1073 against *Bacillus*, spp. spores. A sporicidal agent should be used regularly to prevent
1074 contamination of the manufacturing environment with otherwise difficult to eradicate spore
1075 forming bacteria or fungi.

1076
1077 After the initial assessment of sanitization procedures, ongoing sanitization efficacy should be
1078 frequently monitored through specific provisions in the environmental monitoring program,
1079 with a defined course of action in the event samples are found to exceed limits.

1080
1081 *4. Monitoring Methods*

1082
1083 Acceptable methods of monitoring the microbiological quality of the environment include:

1084
1085 a. Surface Monitoring-
1086 Environmental monitoring should include testing of various surfaces for microbiological
1087 quality. For example, product contact surfaces, floors, walls, ceilings, and equipment
1088 should be tested on a regular basis. Routinely used for such tests are touch plates,
1089 swabs, and contact plates. Other surfaces in controlled areas should be tested to show
1090 the adequacy of cleaning and sanitizing procedures.

1091
1092 b. Active Air Monitoring-
1093 The method of assessing the microbial quality of air should involve the use of "active"
1094 devices such as slit to agar samplers, , those using liquid impingement and membrane
1095 filtration, or centrifugal samplers. Each device has certain advantages and
1096 disadvantages, although all allow a quantitative testing of the number of organisms per
1097 volume of air sampled. The use of such devices in aseptic areas is considered an
1098 essential part of evaluating the environment during each production shift, at carefully

1099 chosen critical locations. Manufacturers should be aware of a device's air monitoring
1100 capabilities, and should determine suitability of any new or current devices with respect
1101 to sensitivity and limit of quantification.

1102

1103 c. Passive Air Monitoring (Settling Plates)-

1104 Another method is the use of passive air samplers such as settling plates (petri dishes
1105 containing nutrient growth medium exposed to the environment). These settling plates
1106 lack value as quantitative air monitors because only microorganisms that settle onto the
1107 agar surface will be detected. Their value as qualitative indicators in critical areas is
1108 enhanced by positioning plates in locations posing the greatest risk of product
1109 contamination. As part of methods validation, the quality control laboratory should
1110 evaluate what media exposure conditions optimize recovery of low levels of
1111 environmental isolates. Exposure conditions should preclude desiccation (e.g., caused
1112 by lengthy sampling periods and/or high airflows), which inhibits recovery of
1113 microorganisms. The data generated by passive air sampling can be useful when
1114 considered in combination with results from other types of air samples.

1115

1116 **B. Microbiological Media and Identification**

1117

1118 The environmental monitoring program should include routine characterization of recovered
1119 microorganisms. Monitoring of critical and immediately surrounding areas as well as
1120 personnel should include routine identification of microorganisms to the species (or, where
1121 appropriate, genus) level.

1122

1123 In some cases, environmental trending data has revealed migration of microorganisms into the
1124 aseptic processing room from either uncontrolled or lesser-controlled areas. To detect such
1125 trends, an adequate program of differentiating microorganisms in lesser-controlled
1126 environments (e.g., Class 100,000) should be in place. At minimum, the program should
1127 require species (or, where appropriate, genus) identification of microorganisms in ancillary
1128 environments at frequent intervals to establish a valid, current database of contaminants present
1129 in the facility during processing (and to demonstrate that cleaning and sanitization procedures
1130 continue to be effective). Environmental isolates often correlate with the contaminants found
1131 in a media fill or product sterility testing failure, and the overall environmental picture
1132 provides valuable information for the associated investigation.

1133

1134 The goal of microbiological monitoring is to reproducibly detect microorganisms for purposes
1135 of monitoring the state of environmental control. Consistent methods will yield a database that
1136 allows for sound data comparisons and interpretations. The microbiological culture media
1137 used in environmental monitoring should be validated as capable of detecting fungi (i.e., yeasts
1138 and molds) as well as bacteria, and incubated at appropriate conditions of time and
1139 temperature. Total aerobic bacterial count can be obtained by incubating at 30 to 35°C for 48
1140 to 72 hours. Total combined yeast and mold count is generally obtained by incubating at 20 to
1141 25°C for 5 to 7 days.

1142

1143 Incoming lots of environmental monitoring media should include positive and negative
1144 controls. Growth promotion testing should be performed on all lots of prepared media.
1145 Where appropriate, inactivating agents should be used to prevent inhibition of growth by clean
1146 room disinfectants.

1147

1148 **C. Pre-filtration Bioburden**

1149

1150 For any parenteral manufacturing process, pre-filtration bioburden should be minimal. In
1151 addition to increasing the challenge to the sterilizing filter, high bioburden can contribute
1152 endotoxin or other impurities to the drug formulation. An in-process limit for bioburden level
1153 for each formulated product (generally sampled immediately preceding sterile filtration) should
1154 be established.

1155

1156 **D. Particulate Monitoring**

1157

1158 Routine particle monitoring is useful in detecting significant deviations in air cleanliness from
1159 qualified processing norms (e.g., clean area classification). A result outside the established
1160 specifications at a given location should be investigated consistent with the severity of the
1161 "excursion." Appropriate corrective action should be implemented to prevent future
1162 deviations.

1163

1164 See Section IV.A for additional guidance on particulate monitoring.

1165

1166 **XI. STERILITY TESTING**

1167

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

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

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

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

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

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

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

1168
1169 Certain aspects of sterility testing are of particular importance, including control of the testing
1170 environment, understanding the test limitations, and the investigation of manufacturing systems
1171 following a positive test.

1172
1173 The testing laboratory environment should employ facilities and controls comparable to those
1174 used for filling/closing operations. Poor or deficient sterility test facilities or controls can
1175 result in a high rate of test failures. If production facilities and controls are significantly better
1176 than those for sterility testing there is the danger of attributing the cause of a positive sterility
1177 test result to the faulty laboratory even when the product tested could have, in fact, been non-
1178 sterile. Therefore, some manufacturing deficiency may go undetected. The use of isolators to
1179 perform sterility testing is a well-established means for minimizing false positives.

1180
1181 **A. Choice of Methods**

1182
1183 Sterility testing methodologies are required to be accurate and reproducible, in accord with
1184 211.194 and 211.165. The methodology selected should present the lowest potential for
1185 yielding a false positive. The USP specifies membrane filtration as the method of choice,
1186 when feasible.

1187
1188 As a part of methods validation, appropriate bacteriostasis/fungistasis testing should be
1189 conducted. Such testing should demonstrate reproducibility of the method in recovering each
1190 of a panel of representative microorganisms. Study documentation should include evaluation
1191 of whether microbial recovery from inoculated controls and product samples is comparable
1192 throughout the incubation period. If growth is inhibited, modifications (e.g., increased
1193 dilution, additional membrane filter washes, addition of inactivating agents) in the methodology
1194 should be implemented to optimize recovery. Ultimately, methods validation studies should
1195 demonstrate that the methodology does not provide an opportunity for "false negatives."

1196
1197 **B. Media**

1198
1199 It is essential that the media used to perform sterility testing be rendered sterile and
1200 demonstrated as growth promoting.

1201
1202

1203 **C. Personnel**

1204
1205 Personnel performing sterility testing should be qualified and trained for the task. A written
1206 program should be in place to regularly update training of personnel and confirm acceptable
1207 sterility testing practices.

1208
1209 **D. Sampling and Incubation**

1210
1211 Sterility tests are limited in their ability to detect low levels of contamination. For example,
1212 statistical evaluations indicate that the USP sterility test sampling plan has been described by
1213 USP as "only enabling the detection of contamination in a lot in which 10% of the units are
1214 contaminated about nine times out of ten in making the test" (Ref. 12). To further illustrate, if
1215 a 10,000 unit lot with a 0.1% contamination level was sterility tested using 20 units, there is a
1216 98% chance that the batch would pass the test.

1217
1218 This limited sensitivity makes it necessary to ensure that for batch release purposes an
1219 appropriate number of units are tested and that the samples uniformly represent the:

- 1220
- 1221 (1) Entire batch-
 - 1222 Samples should be taken at the beginning, middle, and end of the aseptic processing
 - 1223 operation;
 - 1224 (2) Batch processing circumstances-
 - 1225 Samples should be taken in conjunction with processing interventions or excursions.
- 1226

1227 Because of the limited sensitivity of the test, any positive result is considered a serious CGMP
1228 issue and should be thoroughly investigated.

1229
1230 **E. Investigation of Sterility Positives**

1231
1232 Care should be taken in the performance of the sterility test to preclude any activity that allows
1233 for possible sample contamination. When microbial growth is observed, the lot should be
1234 considered to be non-sterile. It is inappropriate to attribute a positive result to laboratory error
1235 on the basis of a retest that exhibits no growth.⁸ The evaluation of a positive sterility test result
1236 should include an investigation to determine whether the growth observed in the test arose
1237 from product contamination or from laboratory error.

1238
1239 Although it is recognized that such a determination may not be reached with absolute certainty,
1240 it is usually possible to acquire persuasive evidence showing that causative laboratory error is
1241 absent. When available evidence is inconclusive, batches should be rejected as not conforming
1242 to sterility requirements.

1243

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

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1244 It would be difficult to support invalidation of a positive sterility test. Only if conclusive and
1245 documented evidence clearly shows that the contamination occurred as part of testing should a
1246 new test be performed.

1247
1248 After considering all relevant factors concerning the manufacture of the product and testing of
1249 the samples, the comprehensive written investigation should include specific conclusions, and
1250 identify corrective actions. The investigation's persuasive evidence of the origin of the
1251 contamination should be based upon at least the following:

- 1252
- 1253 1. Identification (speciation) of the organism in the sterility test. Identification of the sterility
1254 test isolate(s) should be to the species level. Microbiological monitoring data should be
1255 reviewed to determine if the organism is also found in laboratory and production
1256 environments, personnel, or product bioburden.
 - 1257
 - 1258 2. Record of laboratory tests and deviations. Review of trends in laboratory findings can help
1259 to eliminate or implicate the laboratory as the source of contamination. If the organism is
1260 seldom found in the laboratory environment, then product contamination is likely. If the
1261 organism is found in laboratory and production environments, it can indicate product
1262 contamination.

1263
1264 The proper handling of deviations is an essential aspect of laboratory control. When a
1265 deviation occurs during sterility testing, it should be documented, investigated, and
1266 remedied. If any deviation is considered to have compromised the integrity of the sterility
1267 test, the test should be invalidated immediately without incubation.

1268
1269 Deviation and sterility test positive trends should be evaluated periodically (e.g., quarterly,
1270 annually) to provide an overview of operations. A sterility positive result can be viewed as
1271 indicative of production or laboratory problems and should be investigated globally since
1272 such problems often can extend beyond a single batch.

1273
1274 In order to more accurately monitor potential contamination sources, it is useful to keep
1275 separate trends by product, container type, filling line, and personnel. Where the degree of
1276 sterility test sample manipulation is similar for a terminally sterilized product and an
1277 aseptically processed product, a higher rate of initial sterility failures for the latter should
1278 be taken as indicative of aseptic processing production problems. See Section IX.A,
1279 Process Simulations, which includes similar issues that are investigated as part of a media
1280 fill failure investigation.

1281
1282 Microbial monitoring of the laboratory environment and personnel over time can also
1283 reveal trends that are informative. Upward trends in the microbial load in the laboratory
1284 should be promptly investigated as to cause, and corrected. In some instances, such trends
1285 can appear to be more indicative of laboratory error as a possible source of a sterility test
1286 failure.

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1288 Where a laboratory has a good track record with respect to errors, this history can help
1289 remove the lab as a source of contamination since chances are higher that the contamination
1290 arose from production. However, the converse is not true. Specifically, where the
1291 laboratory has a poor track record, firms should not assume that the contamination is
1292 automatically more attributable to the error in laboratory and consequently overlook a
1293 genuine production problem. Accordingly, all sterility positives should be thoroughly
1294 investigated.

1295
1296 3. Monitoring of production area environment. Of particular importance is trend analysis of
1297 microorganisms in the critical and immediately adjacent area. Trends are an important tool
1298 in investigating the product as the possible source of a sterility failure. Consideration of
1299 environmental microbial loads should not be limited to results of monitoring the production
1300 environment for the lot, day, or shift associated with the suspect lot. For example, results
1301 showing little or no recovery of microorganisms can be misleading, especially when
1302 preceded or followed by a finding of an adverse trend or atypically high microbial counts.
1303 It is therefore important to look at both short and long term trend analysis.

1304
1305 4. Monitoring of Personnel. Daily personnel monitoring data and associated trends should be
1306 reviewed and can in some cases strongly indicate a route of contamination. The adequacy
1307 of personnel practices and training should also be considered.

1308
1309 5. Product pre-sterilization bioburden. Trends in product bioburden should be reviewed
1310 (counts and identity). Adverse bioburden trends occurring during the time period of the
1311 test failure should be considered in the investigation.

1312
1313 6. Production record review. Complete batch and production control records should be
1314 reviewed to detect any signs of failures or anomalies which could have a bearing on
1315 product sterility. For example, the investigation should evaluate batch and trending data
1316 that indicate whether utility/support systems (e.g., HVAC, WFI) are functioning properly.
1317 Records of air quality monitoring for filling lines should show a time at which there was
1318 improper air balance, an unusual high particulate count, etc.

1319
1320 7. Manufacturing history. The manufacturing history of the product or similar products
1321 should be reviewed as part of the investigation. Past deviations, problems, or changes
1322 (e.g., process, components, equipment) are among the factors that can provide an
1323 indication of the origin of the problem.

1324
1325
1326

XII. BATCH RECORD REVIEW: PROCESS CONTROL DOCUMENTATION

Sections 211.100, 211.186, and 211.188 address documentation of production and control of a batch, including recording various production and process control activities at the time of performance. Section 211.100 (b) requires a documented record and evaluation of any deviation from written procedures.

Section 211.192 states that "All drug product production and control records, including those for packaging and labeling, shall be reviewed and approved by the quality control unit to determine compliance with all established, approved written procedures before a batch is released or distributed. 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."

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Maintaining process and environmental control is a daily necessity for an aseptic processing operation. 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 per Section 211.188. Review of environmental monitoring data as well as other data relating to the 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 general, there is a correlation between product (or container-closure) dwell time in the aseptic processing zone and the probability of contamination. 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/container-closures or that last beyond a reasonable exposure time should, where appropriate, result in a local or full line clearance

Any disruption in power supply, however momentary, during aseptic processing is a manufacturing deviation and must be included in batch records (211.100, 211.192).

1355 **APPENDIX 1: ASEPTIC PROCESSING ISOLATORS**

1356
1357 An emerging aseptic processing technology uses isolation systems to minimize the extent of
1358 personnel involvement and to separate the external cleanroom environment from the aseptic
1359 processing line. A well designed positive pressure barrier isolator, supported by adequate
1360 procedures for its maintenance, monitoring, and control, appears to offer an advantage over
1361 classical aseptic processing, including fewer opportunities for microbial contamination during
1362 processing. However, users should not adopt a “false sense of security” with these systems.
1363 Manufacturers should be also aware of the need to establish new procedures addressing issues
1364 unique to these systems.

1365
1366 **A) Maintenance**

1367
1368 *1. General*

1369
1370 Isolator systems have a number of special maintenance requirements. While no isolator unit
1371 forms an absolute seal, very high integrity can be achieved in a well-designed unit. However,
1372 a leak in any of certain components of the system can constitute a significant breach of
1373 integrity. The integrity of gloves, half-suits, seams, gaskets, and seals require daily attention
1374 and a comprehensive preventative maintenance program. Replacement frequencies should be
1375 established in written procedures that require changing parts before they breakdown or
1376 degrade.

1377
1378 *2. Glove Integrity*

1379
1380 A faulty glove or sleeve (gauntlet) assembly represents a route of contamination and a critical
1381 breach of isolator integrity. The choice of durable glove materials, coupled with a well-
1382 justified replacement frequency, are two aspects of good manufacturing practice that should be
1383 addressed. With every use, gloves should be visually evaluated for any macroscopic physical
1384 defect. Mechanical integrity tests should also be performed routinely. This attentive
1385 preventative maintenance program is necessary to prevent use of gloves lacking integrity that
1386 would place the sterile product at risk. When such a breach is discovered, the operation
1387 should be terminated.

1388
1389 Due to the potential for microbial migration through microscopic holes in gloves and the lack
1390 of a highly sensitive glove integrity test, the inner part of the installed glove should be
1391 sanitized regularly and the operator should also wear a second pair of thin gloves.

1392
1393 **B) Design**

1394
1395 *1. Airflow*

1396
1397 The design of an aseptic processing isolator normally employs unidirectional airflow that
1398 sweeps over and away from exposed sterile materials, avoiding any turbulence or stagnant

1399 airflow in the area of exposed sterilized materials, product, and container-closures. In most
1400 sound designs, air showers over the critical zone once, and then is systematically exhausted.
1401 Air handling systems should employ HEPA and/or ULPA filters in series.

1402

1403 *2. Materials of Construction*

1404

1405 As in any aseptic processing design, suitable materials should be chosen based on durability, as
1406 well as ease of cleaning and sterilization. For example, rigid wall construction incorporating
1407 stainless steel and glass materials is widely used.

1408

1409 *3. Pressure Differential*

1410

1411 Isolators that include an open exit portal represent a potential compromise in achieving
1412 complete physical separation from the external environment. A positive air pressure
1413 differential adequate to achieve this full separation should be employed and supported by
1414 qualification studies. Positive air pressure differentials from the isolator to the surrounding
1415 environment have largely ranged from approximately 0.07" to 0.2" water gauge. The
1416 appropriate minimum pressure differential specification established by a firm will be dependent
1417 on the system's design and, when applicable, its exit port. Air balance between the isolator
1418 and other direct interfaces (e.g., dry heat tunnel) should also be qualified.

1419

1420 The positive pressure differential should be coupled with appropriate protection at the product
1421 egress point(s) in order to overcome the potential for ingress of any airborne particles from the

1422

1423 external environment by induction. Induction can result from local turbulent flow causing air
1424 swirls or pressure waves that can push extraneous particles into the isolator. Local Class 100
1425 protection at an opening can provide a further barrier to induction of outside air into the
1426 isolator.

1427

1428 *4. Clean Area Classifications*

1429

1430 The interior of the isolator should, at minimum, meet Class 100 standards. The classification
1431 of the environment surrounding the isolator should be based on the design of the product
1432 interfaces, such as transfer ports and discharge points, as well as the number of transfers into
1433 and out of the isolator. A Class 10,000 or Class 100,000 background is appropriate depending
1434 on isolator design and manufacturing situations. The area surrounding the isolator should be
1435 justified. An isolator should not be located in an unclassified room.

1436

1437 **C) Transfer of Materials/Supplies**

1438

1439 The ability to maintain integrity and sterility of an isolator is impacted by the design of transfer
1440 ports. Various adaptations, of differing capabilities, allow for the transfer of supplies into and
1441 out of the isolator.

1442

1443 1. *Introduction:*

1444

1445 Multiple material transfers are generally made during the processing of a batch.
1446 Frequently, transfers are performed via direct interface with a decontaminating transfer
1447 isolator or dry heat depyrogenation tunnel with balanced airflow. Such provisions, if
1448 well designed, help ensure that microbiological ingress does not result from the
1449 introduction of supplies. Properly operated RTPs (rapid transfer ports) are also
1450 generally considered to be an effective transfer mechanism. The number of transfers
1451 should be kept to a minimum because the risk of ingress of contaminants increases with
1452 each successive material transfer.

1453

1454 Some transfer ports can have significant limitations, including marginal
1455 decontaminating capability (e.g., ultraviolet) or a design that would compromise
1456 isolation by allowing ingress of air from the surrounding room. In the latter case,
1457 localized HEPA-filtered laminar airflow cover in the area of such a port should be
1458 implemented.

1459

1460 2. *Discharge:*

1461

1462 Isolators often include a "mousehole" or other exit port through which product is
1463 discharged, opening the isolator to the outside environment. The mousehole represents
1464 a potential route of contamination. Sufficient overpressure should be supplied and
1465 monitored on a continuous basis at this location to ensure that isolation is maintained.

1466

1467 **D) Decontamination**

1468

1469 1. *Surface Exposure*

1470

1471 Written procedures for decontamination of the isolator should be established. The
1472 decontamination process should provide full exposure of all isolator surfaces to the
1473 chemical agent. For example, in order to facilitate contact with the sterilant, the glove
1474 apparatus should be fully extended with glove fingers separated during the
1475 decontamination cycle.

1476

1477 2. *Efficacy*

1478

1479 A decontamination method should be developed which renders the inner surfaces of the
1480 isolator free of viable microorganisms. Decontamination can be accomplished using a
1481 number of vaporized agents, although these agents possess limited capability to
1482 penetrate obstructed or covered surfaces. Process development and validation studies
1483 should include a thorough determination of cycle capability. The characteristics of
1484 these agents generally preclude the reliable use of statistical methods (e.g., fraction
1485 negative) to determine process lethality. An appropriate, quantified BI challenge should
1486 be placed on various materials and in many locations throughout the isolator, including

1487 difficult to reach areas. Cycles should be developed with an appropriate margin of
1488 extra kill to provide confidence in robustness of the decontamination processes. For
1489 most production applications, demonstration of a six-log reduction of the challenge BI
1490 is recommended.

1491
1492 The uniform distribution of the defined concentration of decontaminating agent should
1493 also be evaluated concurrent with these studies. Chemical indicators may also be useful
1494 as a qualitative tool to show that the decontaminating agent reached a given location.

1495 1496 *3. Frequency*

1497
1498 While isolators vary widely in design, their interior and content should be designed to
1499 be frequently decontaminated. If an isolator is to be used for multiple days between
1500 decontamination cycles, the frequency adopted should include a built-in safety margin
1501 and be well justified. This frequency, established during validation studies, should be
1502 reevaluated and increased if production data indicate any deterioration of the
1503 microbiological quality of the isolator environment.

1504
1505 A breach of isolator integrity (e.g., power failure, glove/seam tear, other air leaks,
1506 valve failure, out of specification pressure) should lead to a decontamination cycle.
1507 Breaches of integrity should be investigated and any product that may have been
1508 impacted by the breach rejected.

1509 1510 **E) Filling Line Sterilization**

1511
1512 In order to ensure sterility of product contact surfaces from the start of each operation, the
1513 entire path of the sterile liquid stream should be sterilized. In addition, loose materials or
1514 equipment to be used within the isolator should be chosen based on their ability to withstand
1515 steam sterilization (or equivalent method). It is expected that any materials that can be
1516 subjected to a steam sterilization cycle will, in fact, be autoclaved.

1517 1518 **F) Environmental Monitoring**

1519
1520 An appropriate environmental monitoring program should be established which routinely
1521 ensures acceptable microbiological quality of air, surfaces, and gloves (or half-suits) as well as
1522 particulate levels, within the isolator. Air quality should be monitored periodically during each
1523 shift. As an example, the exit port should be monitored for particulates to detect any unusual
1524 results.

1525 1526 **G) Personnel**

1527
1528 While cleanroom apparel requirements are generally reduced, the contribution of human factor
1529 to contamination should not be overlooked. Isolation processes generally include periodic or
1530 even frequent use of one or more gloves for aseptic manipulations and handling of component

1531 transfers into and out of the isolator. Contaminated gloves can lead to product non-sterility.
1532 This concern is heightened because locations on gloves, sleeves, or half suits can be among the
1533 more difficult to reach places during surface sterilization. Meticulous aseptic technique
1534 standards must be observed (211.113).

1535

1536 **APPENDIX 2: BLOW-FILL- SEAL TECHNOLOGY**

1537

1538 Blow-fill-seal (BFS) technology is an automated process by which containers are formed,
1539 filled, and sealed in a continuous operation. This manufacturing technology includes
1540 economies in container-closure processing and reduced human intervention, and is often used
1541 for filling and packaging of ophthalmics and, less frequently, for injectables. This section
1542 discusses some of the critical control points of this technology. Except where otherwise noted
1543 below, the aseptic processing standards discussed elsewhere in this document should be applied
1544 to Blow Fill Seal technology.

1545

1546 **A) Equipment Design and Air Quality**

1547

1548 A BFS machine operates by 1) heating a plastic polymer resin; 2) extruding it to form a
1549 parison (a tubular form of the hot resin); 3) cutting the parison with a high temperature knife;
1550 4) moving the parison under the blow-fill needle (mandrel); 5) inflating it to the shape of the
1551 mold walls; 5) filling the formed container with the liquid product; 6) removing the mandrel;
1552 7) sealing. Throughout this operation sterile-air is used, for example, to form the parison and
1553 inflate it prior to filling. In most operations, the three steps which pose greatest potential for
1554 exposure to particle contamination and/or surrounding air are those in which: the parison is
1555 cut; the parison is moved under the blow-fill mandrel; and the mandrel is removed (just prior
1556 to sealing).

1557

1558 BFS machinery and its surrounding barriers should be designed to prevent potential for
1559 extraneous contamination. As with any aseptic processing operation, it is critical that contact
1560 surfaces be sterile. A validated steam-in-place cycle should be used to sterilize the equipment
1561 path through which the product is conveyed. In addition, any other surface (e.g., above or
1562 nearby) that has potential to contaminate the sterile product needs to be sterile.

1563

1564 The classified environment surrounding BFS machinery should generally meet Class 10,000
1565 standards, but special design provisions (e.g., isolation technology) can justify an alternate
1566 classification. HEPA-filtered or sterile air provided by membrane filters is necessary in the
1567 critical zone in which sterile product or materials are exposed (e.g., parison formation,
1568 container molding/filling steps). Air in the critical zone should meet Class 100 microbiological
1569 standards. A well-designed BFS system should also normally achieve Class 100 particulate
1570 levels.

1571

1572 Equipment design should incorporate specialized measures to reduce particulate levels. In
1573 contrast to non-pharmaceutical applications using BFS machinery, control of air quality (i.e.,
1574 particulates) is critical for sterile drug product manufacture. Particles generated during the

1575 plastic extrusion, cutting, and sealing processes provide a potential means of transport for
1576 microorganisms into open containers prior to sealing. Provisions for carefully controlled
1577 airflow could protect the product by forcing generated particles outward while preventing any
1578 ingress from the adjacent environment. Furthermore, designs separating the filling zone from
1579 the surrounding environment are important in ensuring product protection. Barriers, pressure
1580 vacuums, microenvironments, and appropriately directed high velocities of sterile air have
1581 been found useful in preventing contamination (Ref. 13). Smoke studies and multi-location
1582 particulate data are vital when performing qualification studies to assess whether proper
1583 particulate control dynamics have been achieved throughout the critical area.

1584
1585 In addition to suitable design, an adequate preventative maintenance program should be
1586 established. For example, because of its potential to contaminate the sterile drug product, the
1587 integrity of the boiling system (e.g., mold plates, gaskets) should be carefully monitored and
1588 maintained.

1589 **B) Validation/Qualification**

1591
1592 Advantages of BFS processing are known to include rapid container/closure processing and
1593 minimized interventions. However, a properly functioning process is necessary to realize these
1594 advantages. Equipment qualification/requalification and personnel practices should be given
1595 special attention. Equipment sterilization, media fills, polymer sterilization, endotoxin
1596 removal, product-plastic compatibility, forming/sealing integrity, and unit weight variation are
1597 among the key issues that should be covered by validation/qualification studies.

1598
1599 Appropriate data should ensure that BFS containers are sterile and non-pyrogenic. This can
1600 generally be achieved by validating that time-temperature conditions of the extrusion process
1601 destroy the worst-case endotoxin load on the polymeric material.

1602
1603 The plastic polymer material chosen should be pharmaceutical grade, safe, pure, and pass USP
1604 criteria for plastics. Polymer suppliers should be qualified and monitored for raw material
1605 quality.

1606 **C) Batch Monitoring and Control**

1608
1609 In-process monitoring should include various control parameters (e.g., container weight
1610 variation, fill weight, leakers, air pressure, etc.) to ensure ongoing process control.
1611 Environmental monitoring is particularly important. Samples should be taken during each shift
1612 at specified locations under dynamic conditions. Due to the generation of high levels of
1613 particles near the exposed drug product, continuous monitoring of particles can provide
1614 valuable data relative to the control of a blow-fill-seal operation.

1615
1616 Container-closure defects can be a major problem in control of a BFS operation. It is
1617 necessary for the operation to be designed and set-up to uniformly manufacture leak-proof
1618 units. As a final measure, inspection of each unit of a batch should employ a reliable, sensitive

1619 final product examination capable of detecting a defective unit (e.g., “leakers”). Significant
1620 defects due to heat or mechanical problems, such as mold thickness, container/closure interface
1621 deficiencies, poorly formed closure, or other deviations should be investigated in accord with
1622 Sections 211.100 and 211.192.

1623

1624 **APPENDIX 3: PROCESSING PRIOR TO FILLING/SEALING OPERATIONS**

1625

1626 The purpose of this appendix is to supplement the guidance provided in this document with
1627 information on products regulated by CBER or CDER that are subject to aseptic processing
1628 from early in the manufacturing process, or that require aseptic processing through the entire
1629 manufacturing process, due to their inability to be sterilized. The scope of this appendix
1630 includes aseptic processing activities that take place prior to the filling and sealing of the
1631 finished drug product. Special considerations include those for:

1632

1633 **A) Aseptic processing from early manufacturing steps**

1634

1635 Due to their nature, some products undergo aseptic processing at some or all manufacturing
1636 steps preceding the final product closing step. There is a point in the process after which a
1637 product can no longer be rendered sterile by filtration, and the product is handled aseptically in
1638 all subsequent steps. Some products are formulated aseptically because the formulated product
1639 cannot be sterilized by filtration. For example, products containing aluminum adjuvant are
1640 formulated aseptically because once they are alum adsorbed, they cannot be sterile filtered.

1641

1642 When a product is processed aseptically from early steps, the product and all components or
1643 other additions are rendered sterile prior to entering the manufacturing process. It is critical
1644 that all transfers, transports, and storage stages are carefully controlled at each step of the
1645 process to maintain sterility of the product.

1646

1647 Procedures that expose the product or product contact equipment surfaces to the environment,
1648 such as aseptic connections, should be performed under unidirectional airflow in a Class 100
1649 environment. The environment of the room surrounding the Class 100 environment should be
1650 class 10,000 or better. Microbiological and particulate monitoring should be performed during
1651 operations. Microbial surface monitoring should be performed at the end of operations, but
1652 prior to cleaning. Personnel monitoring should be performed in association with operations.

1653

1654 Process simulation studies should be designed to incorporate all conditions, product
1655 manipulations, and interventions that could impact on the sterility of the product during
1656 manufacturing. The process simulation, from early process steps, should demonstrate that
1657 controls over the process are adequate to protect the product during manufacturing. These
1658 studies should incorporate all product manipulations, additions, and procedures involving
1659 exposure of product contact surfaces to the environment. The studies should include worst-
1660 case conditions such as maximum duration of open operations and maximum number of
1661 participating operators. However, process simulations do not need to mimic total

1662 manufacturing time if the manipulations that occur during manufacturing are adequately
1663 represented.

1664
1665 It is also important that process simulations incorporate storage of product or transport to other
1666 manufacturing areas. For instance, there should be assurance of bulk vessel integrity for
1667 specified holding times. The transport of bulk tanks or other containers should be simulated as
1668 part of the media fill. Please refer to Section IX.A for more guidance on media simulation
1669 studies. Process simulation studies for the formulation stage should be performed at least
1670 twice per year.

1671
1672 **B) Aseptic processing of cell-based therapy products (or of products intended for use as**
1673 **cell based therapies)**

1674
1675 Cell-based therapy products represent a subset of the products for which aseptic manipulations
1676 are used throughout the process. Where possible, closed systems should be used during
1677 production. Cell-based therapy products often have short processing times at each
1678 manufacturing stage, even for the final product. Often, it is appropriate for these products to
1679 be administered to patients before final product sterility testing results are available. In
1680 situations where results of final sterility testing are not available before the product is
1681 administered, additional controls and testing should be considered. For example, additional
1682 sterility tests can be performed at intermediate stages of manufacture, especially after the last
1683 manipulation of the product prior to administration. Other tests that may indicate microbial
1684 contamination, such as microscopic examination, gram stains, and endotoxin testing should be
1685 performed prior to product release.

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RELEVANT GUIDANCE DOCUMENTS

1718
1719
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Some relevant FDA guidances include:

- Guidance for the Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Product, 1994
- Guideline for Validation of Limulus Amebocyte Lysate Test as an End Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices, 1987
- Guide to Inspections of Lyophilization of Parenterals, 1993
- Guide to Inspections of High Purity Water Systems, 1993
- Guide To Inspections of Microbiological Pharmaceutical Quality Control Laboratories, 1993
- Guide To Inspections of Sterile Drug Substance Manufacturers, 1994
- Pyrogens: Still a Danger; 1979 (Inspection Technical Guide)
- Bacterial Endotoxins/Pyrogens; 1985 (Inspection Technical Guide)
- Heat Exchangers to Avoid Contamination; 1979 (Inspection Technical Guide)

For more information on FDA guidance, see our website at www.fda.gov.

DRAFT GLOSSARY

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

Alert Limit- An established microbial or particulate level giving early warning of potential drift from normal operating conditions and which trigger appropriate scrutiny and follow-up to address the potential problem. Alert Limits are always lower than Action Limits.

Action Limit- An established microbial or particulate level which when exceeded should trigger appropriate investigation and corrective action based on the investigation.

Aseptic Processing Facility- Building containing cleanrooms in which air supply, materials, and equipment are regulated to control microbial and particulate contamination.

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

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

Bioburden- Total number of microorganisms associated with a specific item prior to sterilization.

Barrier- Physical partition that affords aseptic manufacturing zone protection by partially separating it from the surrounding area.

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

Clean Area- An area with defined particulate and microbiological cleanliness standards (e.g., Class 100, Class 10,000 or Class 100,000).

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

*Preliminary Concept Paper
Not for Implementation*

1779 Clean Zone- See Clean Area.

1780

1781 Component- Any ingredient intended for use in the manufacture of a drug product, including
1782 those that may not appear in the final drug product.

1783

1784 Colony Forming Unit (CFU)- A microbiological term which describes the formation of a
1785 single macroscopic colony after the introduction of one (or more) microorganism(s) to
1786 microbiological growth media. One colony forming unit is expressed as 1 CFU.

1787

1788 Critical areas - Areas designed to maintain sterility of sterile materials. Sterilized product,
1789 container/closures, and equipment may be exposed in critical areas.

1790

1791 Critical surfaces - Surfaces which may come into contact with or directly impact on sterilized
1792 product or containers/closures. Critical surfaces are rendered sterile prior to the start of the
1793 manufacturing operation and sterility is maintained throughout processing.

1794

1795 Decontamination- A process which eliminates viable bioburden via use of sporicidal chemical
1796 agents.

1797

1798 Depyrogenation- A process used to destroy or remove pyrogens (e.g., endotoxin).

1799

1800 D value - The time (in minutes) of exposure to a given temperature that causes a one-log or
1801 90% reduction in the population of a specific microorganism.

1802

1803 Dynamic- Conditions relating to clean area classification under conditions of normal
1804 production.

1805

1806 Endotoxin- A pyrogenic product (e.g., lipopolysaccharide) present in the bacterial cell wall.
1807 Endotoxin can lead to reactions in patients receiving injections ranging from fever to death.

1808

1809 Gowning Qualification- Program which establishes, both initially and on a periodic basis, the
1810 capability of an individual to don the complete sterile gown in an aseptic manner.

1811

1812 HEPA filter- High Efficiency Particulate Air filter with minimum 0.3 micron particle retaining
1813 efficiency of 99.97%.

1814

1815 HVAC- Heating, Ventilation, and Air Conditioning.

1816

1817 Intervention- An aseptic manipulation or activity that occurs at the critical zone.

1818

1819 Isolator - A decontaminated unit, supplied with HEPA or ULPA filtered air, which provides
1820 uncompromised, continuous isolation of its interior from the external environment (e.g.,
1821 surrounding clean room air and personnel).

1822

*Preliminary Concept Paper
Not for Implementation*

1823 Laminarity- Unidirectional air flow at a velocity sufficient to uniformly sweep particulate
1824 matter away from a critical processing or testing area.

1825

1826 Operator- Any individual participating in the aseptic processing operation, including line set-
1827 up, filler, maintenance, or other personnel associated with aseptic line activities.

1828

1829 Overkill sterilization process - A process that is sufficient to provide at least a 12 log reduction
1830 of microorganisms having a minimum D value of 1 minute.

1831

1832 Pyrogen- Substance which induces a febrile reaction in a patient.

1833

1834 Sterilizing grade filter- A filter which, when appropriately validated, will remove all
1835 microorganisms from a fluid stream, producing a sterile effluent.

1836

1837 Terminal sterilization- The application of a lethal agent to sealed, finished drug products for
1838 the purpose of achieving a predetermined sterility assurance level (SAL) of usually less than
1839 10^{-6} (i.e., a probability of a non-sterile unit of greater than one in a million).

1840

1841 ULPA filter- Ultra-Low Penetration Air filter with minimum 0.3 micron particle retaining
1842 efficiency of 99.999 %.

1843

1844 Validation- Establishing documented evidence which provides a high degree of assurance that a
1845 specific process will consistently produce a product meeting its predetermined specifications
1846 and quality attributes.

1847

1848 Worst case- A set of conditions encompassing upper and lower processing limits and
1849 circumstances, including those within standard operating procedures, which pose the greatest
1850 chance of process or product failure (when compared to ideal conditions). Such conditions do
1851 not necessarily induce product or process failure.