

1 **Annex 1 : Manufacture of Sterile Products**

2

3 **Document map**

Section Number	General overview
1. Scope	Includes additional areas (other than sterile products) where the general principles of the annex can be applied.
2. Principle	General principles as applied to the manufacture of sterile products.
3. Pharmaceutical Quality System (PQS)	Highlights the specific requirements of the PQS when applied to sterile products.
4. Premises	General guidance regarding the specific needs for premises design and also guidance on the qualification of premises including the use of Barrier Technology.
5. Equipment	General guidance on the design and operation of equipment.
6. Utilities	Guidance with regards to the special requirements of utilities such as water, gas and vacuum.
7. Personnel	Guidance on the requirements for specific training, knowledge and skills. Also gives guidance to the qualification of personnel.
8. Production and specific technologies	Discusses the approaches to be taken with regards to aseptic and terminal sterilization processes. Discusses approaches to sterilization of products, equipment and packaging components. Also discusses different technologies such as lyophilization and Form-Fill-Seal where specific requirements apply.
9. Viable and non-viable environmental and process monitoring	<p>This section differs from guidance given in section 4 in that the guidance here applies to ongoing routine monitoring with regards to the design of systems and setting of action limits alert levels and reviewing trend data.</p> <p>The section also gives guidance on the requirements of Aseptic Process Simulation (APS).</p>
10. Quality control (QC)	Gives guidance on some of the specific Quality Control requirements relating to sterile products.
11. Glossary	Explanation of specific terminology.

4

5

6 1 Scope

7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58

The manufacture of sterile products covers a wide range of sterile product types (active substance, sterile excipient, primary packaging material and finished dosage form), packed sizes (single unit to multiple units), processes (from highly automated systems to manual processes) and technologies (e.g. biotechnology, classical small molecule manufacturing and closed systems). This Annex provides general guidance that should be used for the manufacture of all sterile products using the principles of Quality Risk Management (QRM), to ensure that microbial, particulate and pyrogen contamination is prevented in the final product.

QRM applies to this document in its entirety and will not be referred to in specific paragraphs. Where specific limits or frequencies are written, these should be considered as a minimum requirement. They are stated due to regulatory historical experience of issues that have previously been identified and have impacted the safety of patients.

The intent of the Annex is to provide guidance for the manufacture of sterile products. However, some of the principles and guidance, such as contamination control strategy, design of premises, cleanroom classification, qualification, monitoring and personnel gowning, may be used to support the manufacture of other products that are not intended to be sterile such as certain liquids, creams, ointments and low bioburden biological intermediates but where the control and reduction of microbial, particulate and pyrogen contamination is considered important. Where a manufacturer elects to apply guidance herein to non-sterile products, the manufacturer should clearly document which principles have been applied and acknowledge that compliance with those principles should be demonstrated.

2 Principle

2.1 The manufacture of sterile products is subject to special requirements in order to minimize risks of microbial, particulate and pyrogen contamination. The following key areas should be considered:

- i. Facility, equipment and process design should be optimized, qualified and validated according to the relevant sections of the Good Manufacturing Practices (GMP) guide. The use of appropriate technologies (e.g. Restricted Access Barriers Systems (RABS), isolators, robotic systems, rapid microbial testing and monitoring systems) should be considered to increase the protection of the product from potential extraneous sources of particulate and microbial contamination such as personnel, materials and the surrounding environment, and assist in the rapid detection of potential contaminants in the environment and product.
- ii. Personnel should have adequate qualifications and experience, training and attitude with a specific focus on the principles involved in the protection of sterile product during the manufacturing, packaging and distribution processes.
- iii. Processes and monitoring systems for sterile product manufacture should be designed, commissioned, qualified and monitored by personnel with appropriate process, engineering and microbiological knowledge.

2.2 Processes, equipment, facilities and manufacturing activities should be managed in accordance with QRM principles to provide a proactive means of identifying, scientifically evaluating and controlling potential risks to quality. Where alternative approaches are used, these should be supported by appropriate rationales and risk assessment and should meet the intent of this Annex.

QRM priorities should include good design of the facility, equipment and process in the first instance, then implementation of well-designed procedures, with monitoring systems as the final element that

59 demonstrate that the design and procedures have been correctly implemented and continue to perform
60 in line with expectations. Exclusively monitoring or testing does not give assurance of sterility.

61
62 2.3 Quality Assurance is particularly important, and manufacture of sterile products must strictly
63 follow carefully established and validated methods of manufacture and control. A Contamination
64 Control Strategy (CCS) should be implemented across the facility in order to define all critical control
65 points and assess the effectiveness of all the controls (design, procedural, technical and
66 organisational) and monitoring measures employed to manage risks associated with contamination.
67 The CCS should be actively updated and should drive continuous improvement of the manufacturing
68 and control methods.

69
70 2.4 Contamination control and steps taken to minimize the risk of contamination from microbial and
71 particulate sources are a series of successively linked events and measures. These are typically
72 assessed, controlled and monitored individually but their collective effectiveness should be considered
73 altogether.

74
75 2.5 The development of the CCS requires thorough technical and process knowledge. Potential
76 sources of contamination are attributable to microbial and cellular debris (e.g. pyrogen, endotoxins) as
77 well as particulate matter (e.g. glass and other visible and sub-visible particulates).

78 Elements to be considered within a documented CCS should include (but are not limited to):

- 79
80 i. Design of both the plant and processes.
- 81
82 ii. Premises and equipment.
- 83
84 iv. Personnel.
- 85
86 v. Utilities.
- 87
88 vi. Raw material controls – including in-process controls.
- 89
90 vii. Product containers and closures.
- 91
92 viii. Vendor approval – such as key component suppliers, sterilization of components and single
93 use systems (SUS), and services.
- 94
95 ix. For outsourced services, such as sterilization, sufficient evidence should be provided to the
96 contract giver to ensure the process is operating correctly.
- 97
98 x. Process risk assessment.
- 99
100 xi. Process validation.
- 101
102 xii. Preventative maintenance – maintaining equipment, utilities and premises (planned and
103 unplanned maintenance) to a standard that will not add significant risk of contamination.
- 104
105 xiii. Cleaning and disinfection.
- 106
107 xiv. Monitoring systems - including an assessment of the feasibility of the introduction of
108 scientifically sound, modern methods that optimize the detection of environmental
109 contamination.
- 110
111 xv. Prevention – trending, investigation, corrective and preventive actions (CAPA), root cause
112 determination and the need for more comprehensive investigational tools.
- 113

114 xvi. Continuous improvement based on information derived from the above.
115

116 2.6 The CCS should consider all aspects of contamination control and its life cycle with ongoing and
117 periodic review resulting in updates within the quality system as appropriate.
118

119 2.7 The manufacturer should take all steps and precautions necessary to assure the sterility of the
120 products manufactured within its facilities. Sole reliance for sterility or other quality aspects should
121 not be placed on any terminal process or finished product test.
122

123 **3 Pharmaceutical Quality System (PQS)**

124 3.1 The manufacture of sterile products is a complex activity that requires specific controls and
125 measures to ensure the quality of products manufactured. Accordingly, the manufacturer's PQS
126 should encompass and address the specific requirements of sterile product manufacture and ensure
127 that all activities are effectively controlled so that microbial, particulate and pyrogen contamination is
128 minimized in sterile products. In addition to the PQS requirements detailed in Chapter 1 of the GMPs,
129 the PQS for sterile product manufacture should also ensure that:
130

131 i. An effective risk management system is integrated into all areas of the product life cycle
132 with the aim to minimize microbial contamination and to ensure the quality of sterile
133 products manufactured.
134

135 ii. The manufacturer has sufficient knowledge and expertise in relation to the products
136 manufactured and the equipment, engineering and manufacturing methods employed that
137 have an impact on product quality.
138

139 iii. Root cause analysis of procedural, process or equipment failure is performed in such a
140 way that the risk to product is correctly understood and suitable corrective and
141 preventative actions (CAPA) are implemented.
142

143 iv. Risk management is applied in the development and maintenance of the CCS, to identify,
144 assess, reduce/eliminate (where applicable) and control contamination risks. Risk
145 management should be documented and should include the rationale for decisions taken
146 in relation to risk reduction and acceptance of residual risk.
147

148 v. The risk management outcome should be reviewed regularly as part of on-going quality
149 management, during change control and during the periodic product quality review.
150

151 vi. Processes associated with the finishing and transport of sterile products should not
152 compromise the sterile product. Aspects that should be considered include: container
153 integrity, risks of contamination and avoidance of degradation by ensuring that products
154 are stored and maintained in accordance with the registered storage conditions.
155

156 vii. Persons responsible for the quality release of sterile products have appropriate access to
157 manufacturing and quality information and possess adequate knowledge and experience
158 in the manufacture of sterile products and their critical quality attributes. This is in order
159 to allow such persons to ascertain that the sterile products have been manufactured in
160 accordance with the registered specifications and are of the required quality.
161

162 3.2 All non-conformities, such as sterility test failures, environmental monitoring excursions or
163 deviations from established procedures should be investigated. The investigation should determine the
164 potential impact upon process and product quality and whether any other processes or batches are
165 potentially impacted. The reason for including or excluding a product or batch from the scope of the
166 investigation should be clearly justified and recorded.
167

168 4 Premises

169

170 4.1 The manufacture of sterile products should be carried out in appropriate cleanrooms, entry to
171 which should be through changing rooms that act as airlocks for personnel and airlocks for
172 equipment and materials. Cleanrooms should be maintained to an appropriate cleanliness standard
173 and supplied with air which has passed through filters of an appropriate efficiency. Controls and
174 monitoring should be scientifically justified and capable of evaluating the state of environmental
175 conditions for cleanrooms, airlocks and pass-throughs used for material and equipment transfer.

176

177 4.2 The various operations of component preparation, product preparation and filling should be
178 carried out with appropriate technical and operational separation measures within the cleanroom or
179 facility to prevent mix up and contamination.

180

181 4.3 Restricted Access Barrier Systems (RABS) and isolators are beneficial in assuring the required
182 conditions and minimizing the microbial contamination associated with direct human interventions
183 in the critical zone. Their use should be considered in the CCS. Any alternative approaches to the use
184 of RABS or isolators should be justified.

185

186 4.4 For the manufacture of sterile products there are four grades of cleanroom.

187

188 Grade A zone: The critical zone for high risk operations or for making aseptic connections by
189 ensuring protection by first air (e.g. aseptic processing line, filling zone, stopper bowl, open
190 ampoules and vials). Normally, such conditions are provided by a localised airflow protection,
191 such as unidirectional airflow work stations, RABS or isolators. The maintenance of
192 unidirectional airflow should be demonstrated and qualified across the whole of the Grade A
193 zone. Direct intervention (e.g. without the protection of barrier and glove port technology) into
194 the Grade A zone by operators should be minimized by premises, equipment, process and
195 procedural design.

196

197 Grade B area: For aseptic preparation and filling, this is the background cleanroom for the
198 Grade A zone (where it is not an isolator). When transfer holes are used to transfer filled,
199 closed products to an adjacent cleanrooms of a lower grade, airflow visualization studies should
200 demonstrate that air does not ingress from the lower grade cleanrooms to the Grade B. Pressure
201 differentials should be continuously monitored. Cleanrooms of lower grade than Grade B can
202 be considered where isolator technology is used (refer to paragraph 4.22).

203

204 Grade C and D area: These are cleanrooms used for carrying out less critical stages in the
205 manufacture of aseptically filled sterile products but can be used for the preparation /filling
206 of terminally sterilized products. (See section 8 for the specific details on terminal sterilization
207 activities).

208

209 4.5 In cleanrooms, all exposed surfaces should be smooth, impervious and unbroken in order to
210 minimize the shedding or accumulation of particulates or micro-organisms and to permit the
211 repeated application of cleaning, disinfectant and sporicidal agents where used.

212

213 4.6 To reduce accumulation of dust and to facilitate cleaning there should be no recesses that are
214 difficult to clean effectively therefore projecting ledges, shelves, cupboards and equipment should be
215 kept to a minimum. Doors should be designed to avoid recesses that cannot be cleaned.

216

217 4.7 Materials used in cleanrooms should be selected to minimize generation of particles.

218

219 4.8 Ceilings should be designed and sealed to prevent contamination from the space above them.

220

221 4.9 Sinks and drains are prohibited in Grade A zone and Grade B area. In other cleanrooms, air
222 breaks should be fitted between the machine or sink and the drains. Floor drains in lower grade

223 cleanrooms should be fitted with traps or water seals designed to prevent back flow and should be
224 regularly cleaned, disinfected and maintained.

225

226 4.10 The transfer of equipment and materials into and out of the cleanrooms and critical zones is one
227 of the greatest potential sources of contamination. Any activities with the potential to compromise
228 the cleanliness of cleanrooms or the critical zone should be assessed and if they cannot be
229 eliminated, appropriate controls should be implemented.

230

231 4.11 The transfer of materials, equipment, and components into an aseptic processing area should be
232 carried out via a unidirectional process. Where possible, items should be sterilized and passed into
233 the area through double-ended sterilizers (e.g. through a double-door autoclave or depyrogenation
234 oven/tunnel) sealed into the wall. Where sterilization on transfer of the items is not possible, a
235 procedure which achieves the same objective of not introducing contaminant should be validated and
236 implemented, (e.g. using an effective transfer disinfection, rapid transfer systems for isolators or, for
237 gaseous or liquid materials, a bacteria-retentive filter).

238

239 4.12 Airlocks should be designed and used to provide physical separation and to minimize microbial
240 and particulate contamination of the different areas, and should be present for material and personnel
241 moving between different grades. Wherever possible, airlocks used for personnel movement should
242 be separated from those used for material movement. Where this is not practical, time-based
243 separation of movement (personnel /material) by procedure should be considered. Airlocks should be
244 flushed effectively with filtered air to ensure that the grade of the cleanroom is maintained. The final
245 stage of the airlock should, in the “at rest” state, be of the same cleanliness grade (viable and non-
246 viable) as the cleanroom into which it leads. The use of separate changing rooms for entering and
247 leaving Grade B cleanrooms is desirable. Where this is not practical, time-based separation of
248 activities (ingress/egress) by procedure should be considered. Where the CCS indicates that the risk
249 of cross-contamination is high, separate changing rooms for entering and leaving production areas
250 should be considered. Airlocks should be designed as follow:

251

252 i. Personnel airlocks: Areas of increasing cleanliness used for entry of personnel (e.g. from
253 Grade D to Grade C to Grade B). In general hand washing facilities should be provided
254 only in the first stage of the changing room and not be present in changing rooms directly
255 accessing Grade B cleanrooms.

256

257 ii. Material airlocks: used for materials and equipment transfer.

258

259 • Only materials and equipment that have been included on an approved list, developed
260 during validation of the transfer process, should be allowed to be transferred into the
261 Grade A zone or Grade B cleanroom via an airlock or pass-through hatch. Equipment
262 and materials (intended for use in the Grade A zone) should be protected when
263 transiting through the Grade B cleanroom. Any unapproved items that require transfer
264 should be pre-approved as an exception. Appropriate risk assessment and mitigation
265 measures should be applied and recorded as per the manufacturer's CCS and should
266 include a specific disinfection and monitoring programme approved by quality
267 assurance.

268

269 • Pass-through hatches should be designed to protect the higher grade environment, for
270 example by effective flushing with an active filtered air supply.

271

272 • The movement of material or equipment from lower grade or unclassified area to
273 higher grade clean areas should be subject to cleaning and disinfection commensurate
274 with the risk and in line with the CCS.

275

276 4.13 Both sets of doors for pass-throughs and airlocks (for material and personnel) should not be

277 opened simultaneously. For airlocks leading to a Grade A zone and Grade B areas, an interlocking
278 system should be used. For airlocks leading to Grade C and D cleanrooms, a visual and/or audible
279 warning system should be operated as a minimum. Where required to maintain zone segregation, a
280 time delay between the closing and opening of interlocked doors should be established.

281
282 4.14 Cleanrooms should be supplied with a filtered air supply that maintains a positive pressure
283 and/or an airflow relative to the background environment of a lower grade under all operational
284 conditions and should flush the area effectively. Adjacent rooms of different grades should have
285 pressure differentials of a minimum of 10 pascals (guidance value). Particular attention should be
286 paid to the protection of the critical zone. The recommendations regarding air supplies and pressures
287 may need to be modified where it is necessary to contain certain materials (e.g. pathogenic, highly
288 toxic or radioactive products or live viral or bacterial materials). The modification may include
289 positively or negatively pressurized airlocks that prevent the hazardous material from contaminating
290 surrounding areas. Decontamination of facilities (e.g. the cleanrooms and the heating, ventilation,
291 and air conditioning (HVAC) systems) and the treatment of air leaving a clean area, may be
292 necessary for some operations. Where containment requires air to flow into a critical zone, the
293 source of the air should be from an area of the same grade.

294
295 4.15 Airflow patterns within cleanrooms and zones should be visualised to demonstrate that there is
296 no ingress from lower grade to higher grade areas and that air does not travel from less clean areas
297 (such as the floor) or over operators or equipment that may transfer contaminant to the higher grade
298 areas. Where air movement is shown to be a risk to the clean area or critical zone, corrective actions,
299 such as design improvement, should be implemented. Airflow pattern studies should be performed
300 both at rest and in operation (e.g. simulating operator interventions). Video recordings of the airflow
301 patterns should be retained. The outcome of the air visualisation studies should be considered when
302 establishing the facility's environmental monitoring program.

303
304 4.16 Indicators of pressure differences should be fitted between cleanrooms and/or isolators. Set-
305 points and the criticality of pressure differentials should be documented within the CCS. Pressure
306 differentials identified as critical should be continuously monitored and recorded. A warning system
307 should be in place to instantly indicate and warn operators of any failure in the air supply or
308 reduction of pressure differentials (below set limits for those identified as critical). The warning
309 signal should not be overridden without assessment and a procedure should be available to outline
310 the steps to be taken when a warning signal is given. Where alarm delays are set, these should be
311 assessed and justified within the CCS. Other pressure differentials should be monitored and recorded
312 at regular intervals.

313
314 4.17 Facilities should be designed to permit observation of production activities from outside the
315 Grade A zone and Grade B area (e.g. through the provision of windows or remote cameras with a
316 full view of the area and processes to allow observation and supervision without entry). This
317 requirement should be considered when designing new facilities or during refurbishment of existing
318 facilities.

319 **Barrier Technologies**

320
321
322 4.18 Isolator or RABS technologies, and the associated processes, should be designed to provide
323 protection of the Grade A environment. The entry of materials during processing (and after
324 decontamination) should be minimized and preferably supported by rapid transfer technologies or
325 transfer isolators.

326
327 4.19 The design of the RABS or isolator should take into account all critical factors associated with
328 these technologies including the quality of the air inside and the background environment, the
329 materials and component transfer, the decontamination and/or sterilization processes, the risk factors
330 associated with the manufacturing operations and the operations conducted within the critical zone.

331

332 4.20 The critical zone of the RABS or open isolator used for aseptic processes should meet Grade A
333 requirements with unidirectional airflow. In closed isolator systems where airflow may not be
334 unidirectional, it should provide Grade A conditions and be demonstrated to provide adequate
335 protection for exposed products during processing. The design of the RABS and open isolators should
336 ensure a positive airflow from the critical zones to the supporting background environment; (unless
337 containment is required in which case localized air extraction is required to prevent contamination
338 transfer to the surrounding room). Negative pressure isolators should only be used when containment
339 of the product is considered essential and risk control measures are applied to ensure the critical zone
340 is not compromised.

341
342 4.21 For RABS used for aseptic processing, the background environment should meet at least Grade
343 B. The background environment for open isolators should meet Grade C or D, based on a risk
344 assessment. Airflow studies should be performed to demonstrate the absence of air ingress during
345 interventions, such as door openings.

346
347 4.22 The background environment of a closed isolator should correspond to a minimum of Grade D.
348 The disinfection/decontamination programme should be included as a key consideration when
349 performing the risk assessment for the CCS of an isolator. Where additional process risks are
350 identified, a higher grade of background should be considered. The decision as to the supporting
351 background environment should be documented in the CCS.

352
353 4.23 The materials used for glove systems (for both RABS and isolators), as well as other parts of an
354 isolator, should be demonstrated to have good mechanical and chemical resistance. Integrity testing of
355 the barrier systems, and leak testing of the glove system and the isolator should be performed using a
356 methodology demonstrated to be suitable for the task and criticality. The testing should be performed
357 at defined periods, at a minimum at the beginning and end of each batch, and should include a visual
358 inspection following any intervention that may affect the integrity of the system. For single unit batch
359 sizes, integrity may be verified based on other criteria, such as the beginning and end of each
360 manufacturing session. RABS gloves used in Grade A zone should be sterilized before installation
361 and sterilized (or effectively decontaminated by a validated method which achieves the same
362 objective) prior to each manufacturing campaign. The frequency of glove replacement should be
363 defined within the CCS.

364
365 4.24 For RABS and isolator systems, decontamination methods should be validated and controlled
366 within defined cycle parameters. The cleaning process prior to the disinfection step is essential; any
367 residues that remain may inhibit the effectiveness of the decontamination process:

368
369 i. For isolators, the decontamination process should be automated and should include a
370 sporicidal agent in a suitable form (e.g. gaseous, aerosolized or vaporized form) to ensure
371 thorough microbial decontamination of its interior. Decontamination methods (cleaning and
372 sporicidal disinfection) should render the interior surfaces and critical zone of the isolator free
373 of viable microorganisms.

374
375 ii. For RABS systems, the disinfection should include the routine application of a sporicidal
376 agent using a method that has been validated and demonstrated to robustly disinfect the
377 interior and ensure a suitable environment for aseptic processing.

378
379 Evidence should also be available to demonstrate that the agent used does not have adverse impact on
380 the product produced within the RABS or isolator. The holding time before use of these systems
381 should be validated.

382
383 **Cleanroom and clean air equipment qualification**

384
385 4.25 Cleanrooms and clean air equipment such as unidirectional airflow units (UDAFs),
386 RABS and isolators, used for the manufacture of sterile products, should be qualified and

387 classified according to the required characteristics of the environment. Each manufacturing
388 operation requires an appropriate environmental cleanliness level in the operational state in
389 order to minimize the risk of particulate or microbial contamination of the product or materials
390 being handled.

391
392 4.26 Cleanrooms and clean air equipment should be qualified using methodology in accordance with
393 the requirements of Annex 15. Cleanroom qualification (including classification) should be clearly
394 differentiated from operational environmental monitoring.

395
396 4.27 Cleanroom Qualification is the overall process of assessing the level of compliance of a
397 classified cleanroom or clean air equipment with its intended use. As part of the qualification
398 requirements of Annex 15, the qualification of cleanrooms and clean air equipment should include
399 (where relevant to the design/operation of the installation):

- 400 i. Installed filter leakage and integrity testing.
- 401
- 402 ii. Airflow measurement - Volume and velocity.
- 403
- 404 iii. Air pressure difference measurement.
- 405
- 406 iv. Airflow direction and visualisation.
- 407
- 408 v. Microbial airborne and surface contamination.
- 409
- 410 vi. Temperature measurement.
- 411
- 412 vii. Relative humidity measurement.
- 413
- 414 viii. Recovery testing.
- 415
- 416 ix. Containment leak testing.
- 417

418
419 4.28 Cleanroom classification is part of a cleanroom qualification and is a method of assessing the
420 level of air cleanliness against a specification for a cleanroom or clean air equipment by measuring
421 the non-viable airborne particulate concentration. Reference for the classification of the cleanrooms
422 and clean air equipment can be found in the ISO 14644 series of standards.

423
424 4.29 For cleanroom classification, the airborne particulates equal to or greater than 0.5 and 5 μm
425 should be measured. For Grade A zone and Grade B at rest, classification should include
426 measurement of particles equal to or greater than 0.5 μm ; however, measurement using a second,
427 larger particle size, e.g. 1 μm in accordance with ISO 14644 may be considered. This measurement
428 should be performed both at rest and in operation. The maximum permitted airborne particulate
429 concentration for each grade is given in Table 1.

430
431

432 **Table 1: Maximum permitted airborne particulate concentration during classification**

433

Grade	Maximum limits for particulates $\geq 0.5 \mu\text{m}/\text{m}^3$		Maximum limits for particulates $\geq 5 \mu\text{m}/\text{m}^3$	
	at rest	in operation	at rest	in operation
A	3 520	3 520	Not applicable	Not applicable
B	3 520	352 000	Not applicable	2 900
C	352 000	3 520 000	2 900	29 000
D	3 520 000	Not defined ^(a)	29 000	Not defined ^(a)

434

435

436 ^(a) For Grade D, in operation limits are not defined. The company should establish in operation
437 limits based on a risk assessment and historical data where applicable.

438

439 4.30 For classification of the cleanroom, the minimum number of sampling locations and their
440 positioning can be found in ISO 14644 Part 1. In addition, for the aseptic processing room and the
441 background environment (Grade A zone and Grade B area, respectively), sample locations should also
442 consider all critical processing zones such as the point of fill and stopper bowls. Critical processing
443 locations should be based on a documented risk assessment and knowledge of the process and
444 operations to be performed in the area.

445

446 4.31 Clean room classification should be carried out in the “at rest” and “in operation” states.

447

448 i. The definition of “at rest” state is the condition whereby the installation of all the utilities is
449 complete including any functioning HVAC, with the main manufacturing equipment installed
450 as specified and standing by for operation, without personnel in the room.

451

452 ii. The definition of “in operation” state is the condition where the installation of the cleanroom
453 is complete, the HVAC system fully operational, equipment installed and functioning in the
454 manufacturer’s defined operating mode with the maximum number of personnel present
455 performing or simulating routine operational work. In operation classification may be
456 performed during simulated operations or during aseptic process simulations (where worst
457 case simulation is required).

458

459 iii. The particulate limits given in Table 1 above for the “at rest” state should be achieved after
460 a “clean up” period on completion of operations. The "clean up" period should be
461 determined during the classification of the rooms (guidance value of 15 to 20 minutes).

462

463 4.32 The speed of air supplied by unidirectional airflow systems should be clearly justified in the
464 qualification protocol including the location for air speed measurement. Air speed should be designed,
465 measured and maintained to ensure that appropriate unidirectional air movement provides protection
466 of the product and open components at the working height (e.g. where high risk operations and
467 product and/or components are exposed). Unidirectional airflow systems should provide a
468 homogeneous air speed in a range of 0.36 – 0.54 m/s (guidance value) at the working position, unless
469 otherwise scientifically justified in the CCS. Airflow visualization studies should correlate with the air
470 speed measurement.

471 4.33 The microbial concentration of the cleanrooms should be determined as part of the cleanroom
 472 qualification. The number of sampling locations should be based on a documented risk assessment,
 473 including the results of the classification, air visualization studies and knowledge of the process and
 474 operations to be performed in the area. The maximum limits for microbial contamination during
 475 qualification for each grade are given in Table 2. Qualification should include both at rest and in
 476 operation states.

477
 478 **Table 2: Limits for microbial contamination during qualification**

Grade	Air sample cfu/m ³	Settle plates (diameter 90 mm) cfu/4 hours ^(a)	Contact plates (diameter 55 mm) cfu/plate
A ^(b)	No growth ^(b)		
B	10	5	5
C	100	50	25
D	200	100	50

479 (a) Settle plates should be exposed for the duration of operations and changed as required after 4
 480 hours. Exposure time should be based on recovery studies and should not allow desiccation of the
 481 media used.

482
 483 (b) It should be noted that for Grade A, the expected result should be no growth.

484 Note 1: All methods indicated for a specific Grade in the table should be used for qualifying the
 485 area of that specific Grade. If one of the methods is not used, or alternative methods are used, the
 486 approach taken should be appropriately justified.

487 Note 2: Limits are applied using cfu throughout the document. If different or new technologies
 488 are used that present results in a manner different from cfu, the manufacturer should scientifically
 489 justify the limits applied and where possible correlate them to cfu.

490 Note 3: For qualification of personnel gowning, the limits given for contact plates and glove prints in
 491 Table 7 should apply.

492 Note 4: Sampling methods should not pose a risk of contamination to the manufacturing operations.

493
 494 4.34 The requalification of cleanrooms and clean air equipment should be carried out periodically
 495 following defined procedures. The requirement for requalification of cleanroom areas is as follows:
 496

497 **Table 3: Minimum test requirements for the requalification of cleanrooms**

Grade	Determination of the concentration of airborne viable and non- viable particles	Integrity Test of Terminal Filters	Airflow volume measurement	Verification of air pressure difference between rooms	Air Velocity test
A	Yes	Yes	Yes	Yes	Yes
B	Yes	Yes	Yes	Yes	*
C	Yes	Yes	Yes	Yes	*
D	Yes	Yes	Yes	Yes	*

498 * performed according to a risk assessment documented as part of the CCS. However, required
499 for filling zones (e.g. when filling terminally sterilised products) and background to Grade A
500 RABS.

501 For Grade A & B areas, the maximum time interval for requalification is 6 months.

502 For Grade C & D areas, the maximum time interval for requalification is 12 months.

503 Appropriate requalification consisting of at least the above tests should also be carried out following
504 completion of remedial action implemented to rectify an out-of-compliance equipment or facility
505 condition or after changes to equipment, facility or processes. The significance of a change should be
506 determined through the change management process. Examples of changes to be considered include
507 but are not limited to the following:

508

509 i. Change in the operational use of the cleanroom, or of the operational setting parameters of
510 the HVAC system.

511 ii. Interruption of air movement which affects the operation of the installation.

512 iii. Special maintenance which affects the operation of the installation (e.g. change of final
513 filters).

514 4.35 Other characteristics, such as temperature and relative humidity, should be controlled within
515 ranges that align with product/processing requirements and support maintenance of defined
516 cleanliness standards (e.g. Grade A or B).

517

518 **Disinfection**

519

520 4.36 The disinfection of cleanrooms is particularly important. They should be cleaned and disinfected
521 thoroughly in accordance with a written programme. For disinfection to be effective, prior cleaning to
522 remove surface contamination should be performed. More than one type of disinfecting agent should
523 be employed to ensure that where they have different modes of action and their combined usage is
524 effective against all bacteria and fungi. Disinfection should include the periodic use of a sporicidal
525 agent. Monitoring should be undertaken regularly in order to assess the effectiveness of the
526 disinfection program and to detect changes in types of microbial flora (e.g. organisms resistant to the
527 disinfection regime currently in use). Cleaning programs should effectively remove disinfectant
528 residues.

529

530 4.37 The disinfection process should be validated. Validation studies should demonstrate the
531 suitability and effectiveness of disinfectants in the specific manner in which they are used and should
532 support the in-use expiry periods of prepared solutions.

533

534 4.38 Disinfectants and detergents used in Grade A zone and Grade B areas should be sterile prior to
535 use (disinfectants used in Grade C and D may also be required to be sterile). Where the disinfectants
536 and detergents are made up by the sterile product manufacturer, they should be monitored for
537 microbial contamination. Dilutions should be kept in previously cleaned containers and should only
538 be stored for defined periods. If the disinfectants and detergents are supplied “ready-made” then results
539 from certificates of analysis or conformance can be accepted subject to successful completion of the
540 appropriate vendor qualification.

541

542 4.39 Fumigation or vapour disinfection (e.g. Vapour-phased Hydrogen Peroxide) of cleanrooms and
543 associated surfaces may be useful for reducing microbial contamination in inaccessible places.

544

545 **5 Equipment**

546

547 5.1 A written, detailed description of the equipment design should be available (including process and
548 instrumentation diagrams as appropriate). This should form part of the initial qualification package
549 and be kept up to date as part of the ongoing review of the CCS.

550
551 5.2 Equipment monitoring requirements should be defined in “user requirements specifications” and
552 during early stages of development, and confirmed during qualification. Process and equipment alarm
553 events should be reviewed and approved and evaluated for trends. The frequency at which alarms are
554 assessed should be based on their criticality (with critical alarms reviewed immediately).
555

556 5.3 As far as practicable, equipment, fittings and services should be designed and installed so that
557 operations, maintenance, and repairs can be performed outside the cleanroom. If maintenance has to
558 be performed in the cleanroom, and the required standards of cleanliness and/or asepsis cannot be
559 maintained, then precautions such as restricting access to the work area to specified personnel,
560 generation of clearly defined work protocols and maintenance procedures should be considered.
561 Cleaning, additional disinfection and additional environmental monitoring should also be considered.
562 If sterilization of equipment is required, it should be carried out, wherever possible, after complete
563 reassembly.
564

565 5.4 The cleaning process should be validated to:

- 566
- 567 i. Remove any residue or debris that would detrimentally impact the effectiveness of the
 - 568 disinfecting agent used.
 - 569 ii. Minimize chemical, microbial and particulate contamination of the product during the process
 - 570 and prior to disinfection.
- 571

572 5.5 Direct and indirect contact parts should be sterilized. Direct contact parts are those that the
573 product passes through, such as filling needles or pumps. Indirect product contact parts are
574 equipment parts that come into contact with sterilized critical items and components.
575

576 5.6 All equipment such as sterilizers, air handling systems (including air filtration) and water
577 systems should be subject to qualification, monitoring and planned maintenance. Upon completion
578 of maintenance, their return to use should be approved.
579

580 5.7 Where unplanned maintenance of equipment critical to the sterility of the product is to be carried
581 out, an assessment of the potential impact to the sterility of the product should be performed and
582 recorded.
583

584 5.8 A conveyor belt should not pass through a partition between a Grade A or B area and a
585 processing area of lower air cleanliness, unless the belt itself is continually sterilized (e.g. in a
586 sterilizing tunnel).
587

588 5.9 Particle counters, including sampling tubing, should be qualified. The tubing length should be no
589 greater than 1 meter with a minimum number of bends and bend radius should be greater than 15 cm.
590 Portable particle counters with a short length of sample tubing should be used for classification
591 purpose. Isokinetic sampling heads should be used in unidirectional airflow systems and should be
592 positioned as close as possible to sample air representative of the critical location.
593

594 **6 Utilities**

595

596 6.1 The nature and extent of controls applied to utility systems should be commensurate with the risk
597 to product quality associated with the utility. The impact should be determined via a risk assessment
598 documented as part of the CCS.
599

600 6.2 In general higher risk utilities are those that:

- 601
- 602 i. Directly contact product e.g. water for washing and rinsing, gases and steam for
 - 603 sterilization.
- 604

605 ii. Contact materials that will ultimately become part of the product.

606

607 iii. Contact surfaces that come into contact with the product.

608

609 iv. Otherwise directly impact the product.

610

611 6.3 Utilities should be designed, installed, operated, maintained and monitored in a manner to ensure
612 that the utility functions as expected.

613

614 6.4 Results for critical parameters and critical quality attributes of high risk utilities should be subject
615 to regular trend analysis to ensure that system capabilities remain appropriate.

616

617 6.5 Records of utility installation should be maintained throughout the system's life-cycle. Such
618 records should include current drawings and schematic diagrams, construction material lists and
619 specifications. Typically, important information includes attributes such as:

620

621 i. Pipeline flow direction, slopes, diameter and length.

622

623 ii. Tank and vessel details.

624

625 iii. Valves, filters, drains, sampling and user points.

626

627 6.6 Pipes, ducts and other utilities should not be present in cleanrooms. If unavoidable, then they
628 should be installed so that they do not create recesses, unsealed openings and surfaces which are
629 difficult to clean. Installation should allow cleaning and disinfection of outer surface of the pipes.

630

631 **Water systems**

632

633 6.7 Water treatment plant and distribution systems should be designed, constructed and maintained to
634 minimize the risk of particulates, microbial contamination/proliferation and pyrogens (e.g. sloping of
635 piping to provide complete drainage and the avoidance of dead legs), and prevent the formation of
636 biofilms to ensure a reliable source of water of an appropriate quality. Where filters are included in
637 the system, special attention should be given to the monitoring and maintenance of these filters. Water
638 produced should comply with the current monograph of the relevant Pharmacopeia.

639

640 6.8 Water systems should be qualified to maintain the appropriate levels of physical, chemical and
641 microbial control, taking seasonal variation into account.

642

643 6.9 Water flow should remain turbulent through the pipes to minimize the risk of microbial adhesion,
644 and subsequent biofilm formation.

645

646 6.10 Water for injections (WFI) should be produced from water meeting specifications that have been
647 defined during the qualification process, stored and distributed in a manner which minimizes the risk
648 of microbial growth (for example by constant circulation at a temperature above 70°C). Where the
649 WFI is produced by methods other than distillation, further techniques such as nanofiltration and
650 ultra-filtration as well as electrodeionization (EDI) should be considered in conjunction with reverse
651 osmosis (RO) membranes.

652

653 6.11 Where WFI storage tanks are equipped with hydrophobic bacteria retentive vent filters, the
654 filters should be sterilized and the integrity of the filter tested before installation and after removal
655 following use.

656

657 6.12 To minimize the risk of biofilm formation, sterilization or disinfection or regeneration of water
658 systems should be carried out according to a predetermined schedule and when microbial counts
659 exceed action limits. Disinfection of a water system with chemicals should be followed by a

660 validated rinsing/flushing procedure. Water should be tested after disinfection/regeneration. The
661 results should be approved before the water system is returned to use.
662

663 6.13 Regular ongoing chemical and microbial monitoring of water systems should be performed.
664 Alert levels should be based on the qualification or a review of ongoing monitoring data that will
665 identify an adverse trend in system performance. Sampling programs should reflect the requirements
666 of the CCS and include:
667

668 i. All points of use, at a specified interval, to ensure that representative water samples are
669 obtained for analysis on a regular basis.
670

671 ii. Potential worst case sampling locations.
672

673 iii. A sample from the point at the end of the distribution loop each day that the water is used.
674

675 6.14 Breaches of alert levels should be documented and reviewed, and include investigation of
676 system trends to determine whether the breach is a single (isolated) event or if results are indicative
677 of loss of control or system deterioration. Each breach of action limits should be investigated to
678 determine the root cause of the issue and any impact on the quality of products and manufacturing
679 processes as a result of the potential use of the water.
680

681 6.15 WFI systems should include continuous monitoring systems such as Total Organic Carbon
682 (TOC) and conductivity, (unless justified otherwise) as these may give a better indication of overall
683 system performance than discrete sampling. Sensor locations should be based on risk and the
684 outcome of qualification.
685

686 **Steam used as a direct sterilizing agent** 687

688 6.16 Feed water to a pure steam (clean steam) generator should be appropriately purified. Pure steam
689 generators should be designed, qualified and operated in a manner to ensure that the quality of steam
690 produced meets defined chemical and endotoxin levels.
691

692 6.17 Steam used as a direct sterilizing agent should be of suitable quality and should not contain
693 additives at a level which could cause contamination of product or equipment. For a pure steam
694 generator supplying pure steam used for the direct sterilization of materials or product-contact
695 surfaces (e.g. porous hard-goods autoclave loads), steam condensate should meet the current
696 monograph for WFI of the relevant Pharmacopoeia. A suitable sampling schedule should be in place
697 to ensure that representative pure steam samples are obtained for analysis on a regular basis. Other
698 aspects of the quality of pure steam used for sterilization should be assessed periodically against
699 validated parameters. These parameters should include the following: non-condensable gases,
700 dryness value (dryness fraction) and superheat.
701

702 **Gases and vacuum systems** 703

704 6.18 Gases that come in direct contact with the product/primary container surfaces should be of
705 appropriate chemical, particulate and microbial quality. All relevant parameters, including oil and
706 water content, should be specified, taking into account the use and type of the gas, the design of the
707 gas generation system and, where applicable, comply with the appropriate Pharmacopoeia
708 monographs.
709

710 6.19 Gases used in aseptic processes should be filtered through a sterilizing filter (with a nominal pore
711 size of a maximum of 0.22 µm) at the point of use. Where the filter is used on a batch basis (e.g. for
712 filtration of gas used for overlay of aseptically filled products) or as product vessel vent filter, then the
713 filter should be integrity tested and the results included as part of the batch certification process. Any
714 transfer pipework or tubing that is located after the final sterilizing filter should be sterilized. When

715 gases are used in the process, microbial monitoring of the gas should be performed periodically at the
716 point of use.

717

718 6.20 Where backflow from vacuum or pressure systems poses a potential risk to the product, there
719 should be mechanism(s) to prevent backflow when the vacuum or pressure system is shut off.

720

721 **Heating and cooling and hydraulic systems**

722

723 6.21 Major items of equipment associated with hydraulic, heating and cooling systems, e.g. such as
724 those associated with Blow-Fill-Seal equipment should, where possible, be located outside the filling
725 room. Where they are located inside the filling room there should be appropriate controls to contain
726 any spillage and/or cross contamination associated with the hydraulic system fluids. Where possible,
727 the system should be at a lower pressure than the processed fluid.

728

729 6.22 Any leaks from these systems that would present a risk to the product should be detectable (i.e.
730 an indication system for leakage).

731

732 6.23 For both vacuum and cooling systems there should be periodic cleaning/disinfection as
733 determined in the CCS.

734

735 **7 Personnel**

736 7.1 The manufacturer should ensure that there are sufficient appropriate personnel, suitably qualified,
737 trained and experienced in the manufacture and testing of sterile products, and any of the specific
738 manufacturing technologies used in the site's manufacturing operations, to ensure compliance with
739 GMP applicable to the manufacture and handling of sterile products.

740

741 7.2 Only the minimum number of personnel required should be present in cleanrooms. The
742 maximum number of operators in cleanrooms should be determined, documented and validated
743 during activities such as initial qualification and aseptic process simulations, so as not to
744 compromise sterility assurance. This is particularly important during aseptic processing.

745

746 7.3 Non-essential processes such as product inspection and in process testing should be conducted
747 outside the clean areas wherever possible.

748

749 7.4 All personnel including those performing cleaning, maintenance, monitoring and those that
750 access cleanrooms should receive regular training, gowning qualification and assessment in
751 disciplines relevant to the correct manufacture of sterile products. This training should include the
752 basic elements of microbiology, hygiene, with a specific focus on cleanroom practices,
753 contamination control, aseptic techniques and the protection of sterile products (for those operators
754 entering the Grade B cleanrooms and/or intervening into the Grade A zone) and the potential safety
755 implications to the patient if product is not sterile. The level of training should be based on the
756 criticality of the function and area in which the personnel are working.

757

758 7.5 The personnel working in a Grade A zone and Grade B areas should be trained for aseptic
759 gowning and aseptic practices. Compliance with aseptic gowning procedures should be assessed and
760 confirmed, periodically reassessed at least annually and should involve both visual and microbial
761 assessment (using monitoring locations such as hands, arms, chest and forehead. Refer to paragraph
762 9.30 for the expected limits). The unsupervised access to Grade A zone and Grade B areas where
763 aseptic operations are or will be conducted should be restricted to appropriately qualified personnel,
764 who have passed the gowning assessment and have participated in a successful aseptic process
765 simulation (APS).

766

767 7.6 Unqualified personnel (e.g. building and maintenance contractors and regulatory inspectors)

768 should not enter Grade B cleanrooms or Grade A zones in operation. If needed in exceptional cases,
769 manufacturers should establish written procedures outlining the process by which unqualified
770 personnel are brought into the Grade B and A areas. Access by these persons should be assessed and
771 recorded in accordance with the PQS. An authorized person from the manufacturer should supervise
772 the unqualified personnel during their activities and should assess the impact of these activities on
773 the cleanliness of the area.

774
775 7.7 There should be systems in place for disqualification of personnel from entry into cleanrooms
776 based on aspects including ongoing assessment and/or identification of an adverse trend from the
777 personnel monitoring program and/or after participation in a failed APS. Once disqualified,
778 retraining and requalification should be completed before permitting the operator to have any further
779 involvement in aseptic practices. For operators entering Grade B cleanrooms or performing
780 intervention into Grade A zone, this requalification should include consideration of participation in a
781 successful APS.

782
783 7.8 High standards of personal hygiene and cleanliness are essential to prevent excessive shedding or
784 increased risk of introduction of microbial contamination. Personnel involved in the manufacture of
785 sterile products should be instructed to report any specific health conditions or ailments which may
786 cause the shedding of abnormal numbers or types of contaminants and therefore preclude cleanroom
787 access. Health conditions and actions to be taken with regard to personnel who could be introducing
788 an undue microbial hazard should be provided by the designated competent person and described in
789 procedures.

790
791 7.9 Staff who have been engaged in the processing of human or animal tissue materials or of cultures
792 of micro-organisms, other than those used in the current manufacturing process, or any activities that
793 may have a negative impact to quality (e.g. microbial contamination), should not enter clean areas
794 unless clearly defined and effective decontamination and entry procedures have been followed.

795
796 7.10 Wristwatches, make-up, jewellery, other personal items such as mobile phones and any other
797 non-essential items should not be allowed in clean areas. Electronic devices used in cleanrooms, e.g.
798 mobile phones and tablets, that are supplied by the company solely for use in the cleanrooms, may
799 be acceptable if suitably designed to permit cleaning and disinfection commensurate with the Grade
800 in which they are used. The use and disinfection of such equipment should be included in the CCS.

801
802 7.11 Cleanroom gowning and hand washing should follow a written procedure designed to minimize
803 contamination of cleanroom clothing and/or the transfer of contaminants to the clean areas.

804
805 7.12 The clothing and its quality should be appropriate for the process and the grade of the
806 working area. It should be worn in such a way as to protect the product from contamination. When the
807 type of clothing chosen needs to provide the operator protection from the product, it should not
808 compromise the protection of the product from contamination. Garments should be visually checked
809 for cleanliness and integrity immediately prior to gowning and prior to entry to the cleanroom. Gown
810 integrity should also be checked upon exit. For sterilized or effectively decontaminated garments and
811 eye coverings, particular attention should be taken to ensure they have been processed, are within
812 their specified hold time and that the packaging is visually inspected to ensure it is integral before use.
813 Reusable garments (including eye coverings) should be replaced if damage is identified or at a set
814 frequency that is determined during qualification studies. . Damage to garments may not be identified
815 by visual inspection alone, so the qualification should consider any necessary garment testing
816 requirements.

817
818 7.13 Clothing should be chosen to prevent shedding due to operators moving excessively (when
819 cold) or sweating (when hot).

820
821 7.14 The description of clothing required for each grade is given below:
822

- 823 i. Grade A / B: Dedicated garments to be worn under a sterilized suit. Sterile headgear should
824 enclose all hair (including facial hair) and where separate from the rest of the gown, it
825 should be tucked into the neck of the sterile suit. A sterile face mask and sterile eye
826 coverings (e.g. goggles) should be worn to cover and enclose all facial skin and prevent the
827 shedding of droplets and particulates. Appropriate sterilized, non-powdered, rubber or
828 plastic gloves and sterilized footwear (such as overboots) should be worn. Trouser-legs
829 should be tucked inside the footwear and garment sleeves into the gloves. The protective
830 clothing should minimize shedding of fibres or particulate matter and retain particulates
831 shed by the body. Garments should be packed and folded in such a way as to allow operators
832 to gown without contacting the outer surface of the garment.
833
- 834 ii. Grade C: Hair, beards and moustaches should be covered. A single or two-piece trouser suit
835 gathered at the wrists and with high neck and appropriately disinfected shoes or overshoes
836 should be worn. They should minimize the shedding of fibres and particulate matter.
837
- 838 iii. Grade D: Hair, beards and moustaches should be covered. A general protective suit and
839 appropriately disinfected shoes or overshoes should be worn. Appropriate measures should
840 be taken to avoid any ingress of contaminants from outside the clean area.
841
- 842 iv. Gloves should be worn in Grade C and D areas when performing activities considered to be a
843 contamination risk as defined by the CCS.
844
- 845 7.15 Outdoor clothing (other than personal underwear) should not be brought into changing rooms
846 leading directly to Grade B and C cleanrooms. Facility suits, covering the full length of the arms
847 and the legs, and socks covering the feet, should be worn before entry to change rooms for Grades B
848 and C. Facility suits and socks should not present a risk of contamination to the gowning area or
849 processes.
850
- 851 7.16 Every operator entering Grade B or A areas should gown into clean, sterilized protective
852 garments (including eye coverings and masks) of an appropriate size at each entry. The maximum
853 duration of each garment use should be defined as part of the garment qualification.
854
- 855 7.17 Garments and gloves should be changed immediately if they become damaged and present any
856 risk of product contamination. Gloves should be regularly disinfected during operations.
857
- 858 7.18 Clean area clothing should be cleaned in a dedicated laundry facility using a qualified process
859 ensuring that the clothing is not damaged and/or contaminated by fibres and particles during the
860 laundry process. Inappropriate handling and use of clothing will damage fibres and may increase the
861 risk of shedding of particles. After washing and before packing, garments should be visually
862 inspected for damage. The garment management processes should be evaluated and determined as
863 part of the garment qualification program.
864
- 865 7.19 Activities in clean areas that are not critical to the production processes should be kept to a
866 minimum, especially when aseptic operations are in progress. Movement of personnel should be
867 slow, controlled and methodical to avoid excessive shedding of particulates and organisms due to
868 over-vigorous activity. Operators performing aseptic operations should adhere to aseptic technique
869 at all times to prevent changes in air currents that introduce air of lower quality into the critical zone.
870 Movement adjacent to the critical zone should be restricted and the obstruction of the path of the
871 unidirectional (first air) airflow should be avoided. Airflow visualisation studies should be
872 considered as part of the operator's training programme.
873
874

875 **8 Production and Specific Technologies**

876

877 **Terminally sterilized products**

878

879 8.1 Preparation of components and materials should be performed in at least a Grade D
880 cleanroom in order to limit the risk of microbial, pyrogen and particulate contamination, so that the
881 product is suitable for sterilization. Where the product is at a high or unusual risk of microbial
882 contamination (e.g. the product actively supports microbial growth, the product must be held for
883 long periods before filling or the product is not processed mostly in closed vessels), then
884 preparation should be carried out in a Grade C environment. Preparation of ointments, creams,
885 suspensions and emulsions should be carried out in a Grade C environment before terminal
886 sterilization.

887

888 8.2 Primary packaging containers and components should be cleaned using validated processes to
889 ensure that particulate, pyrogen and bioburden contamination is appropriately controlled.

890

891 8.3 Filling of products for terminal sterilization should be carried out in at least a Grade C
892 environment.

893

894 8.4 Where the product is at an unusual risk of contamination from the environment because, for
895 example, the filling operation is slow, the containers are wide necked or are necessarily exposed for
896 more than a few seconds before closing, then the product should be filled in a Grade A zone with at least
897 a Grade C background.

898

899 8.5 Processing of the bulk solution should include a filtration step with a microorganism retaining
900 filter, where possible, to reduce bioburden levels and particulates prior to filling into the final
901 product containers and there should be a maximum permissible time between preparation and filling.

902

903 8.6 Examples of operations to be carried out in the various grades are given in Table 4.

904

905 **Table 4: Examples of operations and grades for terminally sterilized preparation and**
906 **processing operations**

A	Filling of products, when unusually at risk.
C	Preparation of solutions, when unusually at risk. Filling of products.
D	Preparation of solutions and components for subsequent filling.

907

908 **Aseptic preparation and processing**

909

910 8.7 Aseptic preparation and processing is the handling of sterile product, containers and/or devices in
911 a controlled environment in which the air supply, materials and personnel are regulated to prevent
912 microbial, pyrogenic and particulate contamination.

913

914 8.8 The aseptic process should be clearly defined. The risks associated with the aseptic process, and
915 any associated requirements, should be identified, assessed and appropriately controlled. The site's
916 CCS should clearly define the acceptance criteria for these controls, requirements for monitoring and
917 the review of their effectiveness. Methods and procedures to control these risks should be described
918 and implemented. Accepted residual risks should be formally documented.

919

920 8.9 Precautions to minimize microbial, pyrogenic and particulate contamination should be taken,
921 as per the site's CCS, during the preparation of the aseptic environment, during all processing stages
922 (including the stages before and after bulk product sterilization), and until the product is sealed in its
923 final container. The presence of materials liable to generate particulates and fibres should be minimized
924 in cleanrooms.

925
926
927
928
929
930
931
932
933
934
935
936

8.10 Where possible, the use of equipment such as RABS, isolators or other systems, should be considered in order to reduce the need for critical interventions into the Grade A zone and to minimize the risk of contamination. Robotics and automation of processes can also be considered to eliminate direct human critical interventions (e.g. dry heat tunnel, automated lyophilizer loading, sterilization in place).

8.11 Examples of operations to be carried out in the various environmental grades are given in the Table 5.

Table 5: Examples of operations and grades for aseptic preparation and processing operations

Grade A	<p>Critical zone for</p> <ul style="list-style-type: none"> - Aseptic assembly of filling equipment. - Connections made under aseptic conditions (where sterilized product contact surfaces are exposed) that are post the final sterilizing filter. These connections should be sterilized by steam-in-place whenever feasible. - Aseptic compounding and mixing. - Replenishment of sterile bulk product, containers and closures. - Removal and cooling of unprotected (e.g. with no packaging) items from sterilizers. - Staging and conveying of sterile primary packaging components. - Aseptic filling, sealing of containers such as ampoules, vial closure, transfer of open or partially stoppered vials. - Loading of a lyophilizer.
Grade B	<p>Background support for the Grade A zone (when not in an isolator).</p> <ul style="list-style-type: none"> - Transport, while protected from the surrounding environment, of equipment, components and ancillary items for introduction into the Grade A zone.
Grade C	<ul style="list-style-type: none"> - Preparation of solutions to be filtered including weighing.
Grade D	<ul style="list-style-type: none"> - Cleaning of equipment. - Handling of components, equipment and accessories after washing. - Assembly of cleaned components, equipment and accessories prior to sterilization. - Assembly of closed and sterilized SUS using intrinsic aseptic connectors.

937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952

8.12 For sterile products that cannot be filtered, the following should be considered:

- i. All product and component contact equipment should be sterilized prior to use.
- ii. All raw materials should be sterilized and aseptically added or subsequently sterilized by filtration.
- iii. Bulk solutions should be sterilized by a validated process, e.g. by heat, chemical sterilization or via sterile filtration.
- iv. All materials added to the sterile bulk product should be sterilized prior to addition.

8.13 The unwrapping, assembly and preparation of sterilized equipment, components and ancillary items and the preparation and filling of the sterile product should be treated as an aseptic process and performed in a Grade A zone with a Grade B background. Where an isolator or RABS is used, the

953 background should be in accordance with paragraphs 4.21 & 4.22.
954
955 8.14 Preparation and filling of sterile products such as ointments, creams, suspensions and
956 emulsions should be performed in a Grade A zone with a Grade B background when the product and
957 components are exposed to the environment and the product is not subsequently filtered (via a
958 sterilizing filter) or terminally sterilized. Where an isolator or RABS is used, the background should
959 be in accordance with paragraphs 4.21 & 4.22.
960
961 8.15 Aseptic connections should be performed in a Grade A zone with a Grade B background unless
962 subsequently sterilized in place or conducted with validated intrinsic sterile connection devices that
963 minimize any potential contamination from the immediate environment. Where an isolator or RABS
964 is used, the background should be in accordance with paragraphs 4.21 & 4.22. Aseptic connections
965 should be appropriately assessed and their effectiveness verified. For requirements regarding intrinsic
966 sterile connection devices refer to paragraph 8.120.
967
968 8.16 Aseptic manipulations (including non-intrinsic aseptic connections) should be minimized
969 through the use of engineering design solutions such as preassembled and sterilized equipment.
970 Whenever feasible, product contact piping and equipment should be pre-assembled, and sterilized in
971 place.
972
973 8.17 There should be an authorized list of allowed interventions, both inherent and corrective, that
974 may occur during production. The procedures listing the types of inherent and corrective
975 interventions, and how to perform them, should be updated, as necessary to ensure consistency with
976 the actual manufacturing activities. In the event that an unauthorized intervention is required, details
977 of the intervention conducted should be recorded and fully assessed under the manufacturer's PQS.
978
979 8.18 The duration of each aspect of aseptic preparation and processing should be limited to a defined
980 and validated maximum time, including:
981
982 i. The holding time between equipment, component, and container cleaning, drying and
983 sterilization.
984
985 ii. The holding time for sterilized equipment, components, and containers before use and
986 during filling/assembly.
987
988 iii. The holding time for a decontaminated environment, such as the RABS and isolator before
989 and during filling /assembly.
990
991 iv. The time between the start of the preparation of a product and its sterilization or filtration
992 through a microorganism-retaining filter (if applicable), through to the end of the aseptic
993 filling process. There should be a maximum permissible time for each product that takes
994 into account its composition and the prescribed method of storage.
995
996 v. The holding time for sterilized product prior to filling.
997
998 vi. The aseptic processing time.
999
1000 vii. The filling time.
1001
1002 viii. The maximum exposure time of sterilized containers and closures in the critical processing
1003 zone (including filling) prior to closure.
1004
1005 8.19 Aseptic operations (including APS) should be observed at a regular basis by personnel with
1006 specific expertise in aseptic processing to verify the correct performance of operations and address
1007 inappropriate practices if detected.

1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062

Finishing of sterile products

8.20 Open primary packaging containers (including partially stoppered vials or prefilled syringes) should be maintained under Grade A conditions with Grade B background (e.g. Barrier Technology), or under Grade A conditions with physical segregation from operators (e.g. UDAF carts) until the stopper is fully inserted.

8.21 Containers should be closed by appropriately validated methods. Containers closed by fusion, e.g. Blow-fill-seal (BFS), Form-Fill-Seal (FFS), Small and Large Volume Parenteral (SVP & LVP) bags, glass or plastic ampoules, should be subject to 100% integrity testing. Samples of containers closed by other methods should be taken and checked for integrity using validated methods. The frequency of testing should be based on the knowledge and experience of the container and closure systems being used. A scientifically valid sampling plan should be utilized. The sample size should be based on information such as supplier approval, packaging component specifications and process knowledge. It should be noted that visual inspection alone is not considered as an acceptable integrity test method.

8.22 Containers sealed under vacuum (where the vacuum is necessary for the product stability) should be tested for maintenance of vacuum after an appropriate pre-determined period and during shelf life.

8.23 The container closure integrity validation should take into consideration any transportation or shipping requirements that may negatively impact the integrity of the container (e.g. by decompression or temperature extremes).

8.24 Where the equipment used to crimp vial caps can generate large quantities of non-viable particulates, measures to prevent particulate contamination such as locating the equipment at a physically separate station equipped with adequate air extraction should be taken.

8.25 Vial capping can be undertaken as an aseptic process using sterilized caps or as a clean process outside the aseptic core. Where the latter approach is adopted, vials should be protected by Grade A conditions up to the point of leaving the aseptic processing area, and thereafter stoppered vials should be protected with a Grade A air supply until the cap has been crimped. Where capping is a manual process it should be performed under Grade A conditions either in an appropriately designed isolator or into Grade A zone with a Grade B background.

8.26 Where capping of aseptically filled sterile product is conducted as a clean process with Grade A air supply protection, vials with missing or displaced stoppers should be rejected prior to capping. Appropriately qualified, automated methods for stopper height detection should be in place.

8.27 Where human intervention is required at the capping station, appropriate technological and organizational measures should be used to prevent direct contact with the vials and to minimize microbial contamination.

8.28 RABS and isolators may be beneficial in assuring the required conditions and minimizing the microbial contamination associated with direct human interventions into the capping operation.

8.29 All filled containers of parenteral products should be inspected individually for extraneous contamination or other defects. Defect classification and criticality should be determined during qualification and based on risk and historical knowledge. Factors to consider include, but are not limited to, the potential impact of the defect to the patient and the route of administration. Different defect types should be categorized and batch performance analysed. Batches with unusual levels of defects, when compared with routine defect numbers for the process (based on historical and trend data), should lead to an investigation. A defect library should be generated and maintained which

1063 captures all known classes of defects. The defect library should be used for the training of production
1064 and quality assurance personnel. Critical defects should not be identified during any subsequent
1065 sampling and inspection of acceptable containers. Any critical defect identified should trigger an
1066 investigation as it indicates a possible failure of the original inspection process.

1067
1068 8.30 When inspection is done manually, it should be performed under suitable and controlled
1069 conditions of illumination and background. Inspection rates should be appropriately controlled and
1070 qualified. Operators performing the inspection should undergo visual inspection qualification (whilst
1071 wearing corrective lenses, if these are normally worn) at least annually. The qualification should be
1072 undertaken using appropriate samples from the manufacturer's defect library sets and taking into
1073 consideration worst case scenarios (e.g. inspection time, line speed where the product is transferred to
1074 the operator by a conveyor system, container size or fatigue at the end of shift) and should include
1075 consideration of eyesight checks. Operator distractions should be minimized and frequent breaks, of
1076 an appropriate duration, from inspection should be taken.

1077
1078 8.31 Where automated methods of inspection are used, the process should be validated to detect
1079 known defects (which may impact the product quality, safety or efficacy) and be equal to, or better
1080 than, manual inspection methods. The performance of the equipment should be challenged using
1081 representative defects prior to start up and at regular intervals.

1082
1083 8.32 Results of the inspection should be recorded and defect types and numbers trended. Reject levels
1084 for the various defect types should also be trended based on statistical principles. Impact to product on
1085 the market should be assessed as part of the investigation when adverse trends are observed.

1086 **Sterilization**

1087
1088
1089 8.33 Where possible, finished product should be terminally sterilized, using a validated and controlled
1090 sterilization process, as this provides a greater assurance of sterility than a validated and controlled
1091 sterile filtration process and/or aseptic processing. Where it is not possible for a product to undergo
1092 terminal sterilization, consideration should be given to using terminal bioburden reduction steps, such
1093 as heat treatments (e.g. pasteurization), combined with aseptic process to give improved sterility
1094 assurance.

1095
1096 8.34 The selection, design and location of the equipment and cycle/programme used for sterilization
1097 should be based on scientific principles and data which demonstrate repeatability and reliability of the
1098 sterilization process. Critical parameters should be defined, controlled, monitored and recorded.

1099
1100 8.35 All sterilization processes should be validated. Validation studies should take into account the
1101 product composition, storage conditions and maximum time between the start of the preparation of a
1102 product or material to be sterilized and its sterilization. Before any sterilization process is adopted, its
1103 suitability for the product and equipment, and its efficacy in consistently achieving the desired
1104 sterilizing conditions in all parts of each type of load to be processed should be validated notably by
1105 physical measurements and where appropriate by biological indicators (BI). For effective sterilization,
1106 the whole of the product, and surfaces of equipment and components should be subject to the required
1107 treatment and the process should be designed to ensure that this is achieved.

1108
1109 8.36 Particular attention should be given when the adopted sterilization method is not described in the
1110 current edition of the Pharmacopoeia, or when it is used for a product which is not a simple aqueous
1111 solution. Where possible, heat sterilization is the method of choice.

1112
1113 8.37 Validated loading patterns should be established for all sterilization processes and should be
1114 subject to periodic revalidation. Maximum and minimum loads should also be considered as part of
1115 the overall load validation strategy.

1116

1117 8.38 The validity of the sterilizing process should be reviewed and verified at scheduled intervals
1118 based on risk. Heat sterilization cycles should be revalidated with a minimum frequency of at least
1119 annually.
1120

1121 8.39 Routine operating parameters should be established and adhered to for all sterilization
1122 processes, e.g. physical parameters and loading patterns.
1123

1124 8.40 There should be mechanisms in place to detect a sterilization cycle that does not conform to the
1125 validated parameters. Any failed sterilization or sterilization that deviated from the validated process
1126 (e.g. have longer or shorter phases such as heating cycles) should be investigated.
1127

1128 8.41 Suitable BIs placed at appropriate locations may be considered as an additional method to
1129 support the validation of the sterilization process. BIs should be stored and used according to the
1130 manufacturer's instructions. Where BIs are used to support validation and/or to monitor a
1131 sterilization process (e.g. for ethylene oxide), positive controls should be tested for each sterilization
1132 cycle. If BIs are used, strict precautions should be taken to avoid transferring microbial contamination to
1133 the manufacturing or other testing processes. BI results in isolation do not give assurance of
1134 sterilization and should not be used to override other critical parameters and process design
1135 elements.
1136

1137 8.42 The reliability of BIs is important. Suppliers should be qualified and transportation and storage
1138 conditions should be controlled in order that BI quality is not compromised. Prior to use of a new
1139 batch/lot of BIs, the population and identity of the indicator organism of the batch/lot should be
1140 verified. For other critical parameters, e.g. D-value, Z- value, the batch certificate provided by the
1141 qualified supplier can normally be used.
1142

1143 8.43 There should be a clear means of differentiating products, equipment and components, which
1144 have not been subjected to the sterilization process from those which have. Containers used to carry
1145 products such as baskets or trays, items of equipment and/or components should be clearly labelled
1146 (or electronically tracked) with the material name, product batch number and an indication of
1147 whether or not it has been sterilized. Indicators such as autoclave tape, or irradiation indicators may
1148 be used, where appropriate, to indicate whether or not a batch (or sub-batch) has passed through a
1149 sterilization process. However, these indicators show only that the sterilization process has occurred,
1150 they do not indicate product sterility or achievement of the required sterility assurance level.
1151

1152 8.44 Sterilization records should be available for each sterilization run. Each cycle should have a
1153 unique identifier. They should be reviewed and approved as part of the batch certification procedure.
1154

1155 8.45 Where possible, materials, equipment and components should be sterilized by validated methods
1156 appropriate to the specific material. Suitable protection after sterilization should be provided to
1157 prevent recontamination. If sterilized items are not used immediately after sterilization, these should
1158 be stored using appropriately sealed packaging. A maximum hold time should also be established.
1159 Where justified, components that have been packaged with multiple sterile packaging layers need not
1160 be stored in a cleanroom if the integrity and configuration of the sterile pack allows the items to be
1161 readily disinfected during transfer by operators into the Grade A zone, (e.g. by the use of multiple
1162 sterile coverings that can be removed at each transfer from lower to higher grade). Where protection is
1163 achieved by containment in sealed packaging, this packaging process should be undertaken prior to
1164 sterilization.
1165

1166 8.46 Where materials, equipment, components and ancillary items are sterilized in sealed packaging
1167 and then transferred into the Grade A zone, this should be done using appropriate, validated methods
1168 (for example, airlocks or pass-through hatches) with accompanying disinfection of the exterior of the
1169 sealed packaging. The use of rapid transfer port technology should also be considered. These methods
1170 should be demonstrated to effectively control the potential risk of contamination of the Grade A zone
1171 and Grade B area and, likewise, the disinfection procedure should be demonstrated to be effective in

1172 reducing any contamination on the packaging to acceptable levels for entry of the item into the Grade
1173 B and Grade A areas.

1174

1175 8.47 Where materials, equipment, components and ancillary items are sterilized in sealed packaging
1176 or containers, the packaging sealing process should be validated. The validation should consider the
1177 integrity of the sterile protective barrier system and the maximum hold time before sterilization and
1178 maximum shelf life assigned to the sterilized items. The integrity of the sterile protective barrier
1179 system for each of the sterilized items should be confirmed prior to use.

1180

1181 8.48 For materials, equipment, components and ancillary items that are necessary for aseptic
1182 processing but cannot be sterilized, an effective and validated disinfection and transfer process should
1183 be in place. These items, once disinfected, should be protected to prevent recontamination. These
1184 items, and others representing potential routes of contamination, should be included in the
1185 environmental monitoring program.

1186

1187

1188 **Sterilization by heat**

1189

1190 8.49 Each heat sterilization cycle should be recorded either electronically or by hardcopy, on
1191 equipment with suitable accuracy and precision. Monitoring and recording systems should be
1192 independent of the controlling system (e.g. by the use of duplex/double probes).

1193

1194 8.50 The position of the temperature probes used for controlling and/or recording should be
1195 determined during the validation which should include heat distribution and penetration studies
1196 and, where applicable, also checked against a second independent temperature probe located at the
1197 same position.

1198

1199 8.51 Sufficient time should be allowed for the whole of the load to reach the required temperature
1200 before measurement of the sterilizing time-period starts. For sterilization cycles controlled by
1201 using a reference probe within the load, specific consideration should be given to ensuring the
1202 load probe temperature is controlled within defined temperature range prior to cycle
1203 commencement.

1204

1205 8.52 After completion of the high temperature phase of a heat sterilization cycle, precautions should
1206 be taken against contamination of a sterilized load during cooling. Any cooling liquid or gas that
1207 comes in contact with the product or sterilized material should be sterilized.

1208

1209 8.53 In those cases where parametric release has been authorized, a robust system should be applied
1210 to the product lifecycle validation and the routine monitoring of the manufacturing process. This
1211 system should be periodically reviewed. Further guidance regarding parametric release is provided in
1212 Annex 17.

1213

1214 **Moist heat sterilization**

1215

1216 8.54 Moist heat sterilization utilises steam or superheated water, typically at lower temperatures and
1217 shorter duration than dry heat processes, in order to sterilize a product or article. Moist heat
1218 sterilization of hard goods or porous loads is primarily effected by latent heat of condensation of
1219 clean steam and the quality of steam is therefore important to provide consistent results. For aqueous
1220 liquid-filled containers, energy from moist heat is transferred through conduction and/or convection
1221 to the content of the container without direct contact with the autoclave steam. In these cases, time
1222 and temperature are the key parameters and steam quality does not have the same impact to the
1223 process. Moist heat sterilization processes may be utilized to sterilize or control bioburden (for non-
1224 sterile applications) of thermally stable materials, articles or products and is the preferred method of
1225 sterilization, where possible. Moist heat sterilization can be achieved using steam, (direct or indirect
1226 contact), but also includes other systems such as superheated water systems. Superheated systems

1227 are typically used for the terminal sterilization of product in flexible containers where the pressure
1228 differentials associated with the steam would cause damage to the primary container.
1229

1230 8.55 For porous cycles (hard goods) time, temperature and pressure should be used to monitor the
1231 process. Each item sterilized should be inspected for damage, packaging material integrity and
1232 moisture on removal from the autoclave. Any item found not to be fit for purpose should be
1233 removed from the manufacturing area and an investigation performed.
1234

1235 8.56 For autoclaves fitted with a drain at the bottom of the chamber, the temperature should be
1236 recorded at this position throughout the sterilization period. For steam in place systems, the
1237 temperature should be recorded at condensate drain locations throughout the sterilization period.
1238

1239 8.57 Validation of porous cycles should include a calculation of equilibration time, exposure time,
1240 correlation of pressure and temperature and maximum temperature range during exposure.
1241 Validation of fluid cycles should include temperature, time and/or F_0 . These critical processing
1242 parameters should be subject to defined limits (including appropriate tolerances) and be confirmed as
1243 part of the sterilization validation and routine cycle acceptance criteria.
1244

1245 8.58 Leak tests on the sterilizing system should be carried out periodically (normally weekly) when a
1246 vacuum phase is part of the cycle or the system is returned, post-sterilization, to a pressure lower
1247 than the environment surrounding the sterilized system.
1248

1249 8.59 There should be adequate assurance of air removal prior to and during sterilization when the
1250 sterilization process includes air purging (e.g. porous autoclave loads, lyophilizer chambers). For
1251 autoclaves, this should include an air removal test cycle (normally performed on a daily basis) or an
1252 air detector system. Loads to be sterilized should be designed to support effective air removal and be
1253 free draining to prevent the build-up of condensate.
1254

1255 8.60 The items to be sterilized, other than products in sealed containers, should be dry, wrapped in a
1256 material which allows removal of air and penetration of steam and prevents recontamination after
1257 sterilization. All loaded items should be dry upon removal from the sterilizer. Load dryness should
1258 be confirmed by visual inspection as a part of the sterilization process acceptance.
1259

1260 8.61 If it is necessary to wet equipment using WFI (e.g. ultrafiltration membrane) prior to the
1261 sterilization process, then a risk-based assessment should be carried out to demonstrate the
1262 acceptable dryness level that will not impact the sterility of the equipment sterilized and the product
1263 sterility assurance level. The hold time between the wetting phase and sterilization should be
1264 justified and validated.
1265

1266 8.62 Distortion and damage of non-rigid containers that are terminally sterilized, such as containers
1267 produced by Blow-Fill-Seal or Form-Fill-Seal technologies, should be prevented by appropriate cycle
1268 design and control (for instance setting correct pressure, heating and cooling rates and loading
1269 patterns).
1270

1271 8.63 Where steam in place systems are used (e.g. for fixed pipework, vessels and lyophilizer
1272 chambers), the system should be appropriately designed and validated to assure all parts of the
1273 system are subjected to the required treatment. The system should be monitored for temperature,
1274 pressure and time at appropriate locations during routine use to ensure all areas are effectively and
1275 reproducibly sterilized. These locations should be demonstrated as being representative of, and
1276 correlated with, the slowest to heat locations during initial and routine validation. Once a system has
1277 been sterilized by steam in place it should remain integral and held under positive pressure prior to
1278 use.
1279

1280 8.64 For systems using superheated water rather than steam, as the sterilizing agent, the heated water
1281 should consistently reach all of the required contact points. Initial qualification studies should

1282 include temperature mapping of the entire load. There should be routine checks on the equipment to
1283 ensure that nozzles (where the water is introduced) are not blocked and drains remain free from
1284 debris.

1285
1286 8.65 For the qualification of superheated systems it should be demonstrated that all parts of the load
1287 meet the minimum required temperature and that routine monitoring probes are located in the worst
1288 case positions identified during the qualification process.

1289
1290 **Dry heat sterilization**

1291
1292 8.66 Dry heat sterilization is of particular use in the removal of thermally robust contaminants such as
1293 pyrogens and is often used in the preparation of components for aseptic filling. The combination of
1294 time and temperature to which product, components and equipment are exposed should produce an
1295 adequate and reproducible level of lethality and/or pyrogen (endotoxin) inactivation/removal when
1296 operated routinely within the established limits.

1297
1298 8.67 Dry heat sterilization/depyrogenation tunnels should be configured to ensure that airflow protects
1299 the integrity and performance of the Grade A sterilizing zone by maintaining pressure differentials
1300 and airflow through the tunnel from the higher grade area to the lower grade area. Airflow patterns
1301 should be visualised and correlated with temperature studies. The impact of any airflow change
1302 should be assessed to ensure the heating profile is maintained. All air supplied to the tunnel should
1303 pass through at least a HEPA filter and periodic tests should be performed to demonstrate air filter
1304 integrity (at least biannually). Any tunnel parts that come into contact with sterilized components
1305 should be appropriately sterilized or disinfected. Critical process parameters that should be considered
1306 during validation and/or routine processing should include, but may not be limited to:

- 1307
1308 i. Belt speed or dwell time within the sterilizing zone.
1309
1310 ii. Temperature – minimum and maximum temperatures.
1311
1312 iii. Heat penetration of the material/article.
1313
1314 iv. Heat distribution/uniformity.
1315
1316 v. Airflows – correlated with the heat distribution and penetration studies.

1317
1318 8.68 When a thermal depyrogenation process is used for any component or product contact
1319 equipment, validation studies should be performed to demonstrate that the process provides a suitable
1320 F_h value and results in a minimum 3 log reduction in endotoxins concentration.

1321
1322 8.69 Containers inoculated with endotoxin should be used during validation and should be carefully
1323 managed with a full reconciliation performed. Containers should be representative of the materials
1324 normally processed. Endotoxin quantification and recovery efficiency should also be demonstrated
1325 through biological measurement.

1326
1327 8.70 Dry heat ovens are typically employed to sterilize or depyrogenate primary packaging
1328 components, finished materials or active substances but may be used for other processes. They should
1329 be maintained at a positive pressure relative to lower grade areas throughout the sterilization and post
1330 sterilization hold process. All air entering the oven should pass through a sterilizing filter. Critical
1331 process parameters that should be considered in qualification and/or routine processing should
1332 include, but may not be limited to:

- 1333
1334 i. Temperature.
1335
1336 ii. Exposure period/time.

- 1337
1338 iii. Chamber pressure (for maintenance of over pressure).
1339
1340 iv. Air speed.
1341
1342 v. Air quality within the oven.
1343
1344 vi. Heat penetration of material/article (slow to heat spots).
1345
1346 vii. Heat distribution/uniformity.
1347

1348 8.71 For dry heat sterilization of starting materials and intermediates, the same principles should be
1349 applied. Consideration should also be given to factors affecting heat penetration such as the container
1350 type, size and packing matrix.

1351
1352 **Sterilization by radiation**

1353
1354 8.72 Guidance regarding ionising radiation sterilization can be found within Annex 12.
1355

1356 8.73 Sterilization by radiation is used mainly for the sterilization of heat sensitive materials and
1357 products. Ultraviolet irradiation is not an acceptable method of sterilization.
1358

1359 8.74 Validation procedures should ensure that the effects of variations in density of the product and
1360 packages are considered.
1361

1362 **Sterilization with ethylene oxide**

1363
1364 8.75 This method should only be used when no other method is practicable. During process
1365 validation, it should be shown that there is no damaging effect on the product and that the
1366 conditions and time allowed for degassing result in the reduction of any residual ethylene oxide
1367 (EO) gas and reaction products to defined acceptable limits for the given product or material.
1368

1369 8.76 Direct contact between gas and microbial cells is essential, precautions should be taken to avoid
1370 the presence of organisms likely to be enclosed in material such as crystals or dried protein. The
1371 nature, porosity and quantity of packaging materials can significantly affect the process.
1372

1373 8.77 Before exposure to the gas, materials should be brought into equilibrium with the
1374 humidity and temperature required by the process. The time required for this should be
1375 balanced against the opposing need to minimize the time before sterilization.
1376

1377 8.78 Each sterilization cycle should be monitored with suitable BIs, using the appropriate number of
1378 test units distributed throughout the load at defined locations that have been shown to be worst case
1379 during validation.
1380

1381 8.79 Critical process variables that could be considered as part of the sterilization process validation
1382 and routine monitoring include, but are not limited to:

- 1383
1384 i. EO gas concentration.
1385
1386 ii. EO gas pressure.
1387
1388 iii. Amount of EO gas used.
1389
1390 iv. Relative humidity.
1391

1392 v. Temperature.

1393

1394 vi. Exposure time.

1395

1396 8.80 After sterilization, the load should be aerated to allow EO gas and/or its reaction products to
1397 desorb from the packaged product to predetermined levels. Aeration can occur within a sterilizer
1398 chamber and/or in a separate aeration chamber or aeration room. The aeration phase should be
1399 validated as part of the overall EO sterilization process validation.

1400

1401 **Filter sterilization of products which cannot be sterilized in their final container**

1402

1403 8.81 If the product cannot be sterilized in the final container, solutions or liquids should be sterilized
1404 by filtration through a sterile sterilizing grade filter (with a nominal pore size of 0.22 µm (or less)
1405 that has been appropriately validated to obtain a sterile filtrate) and subsequently aseptically filled
1406 into a previously sterilized container. The selection of the filter used should ensure that it is
1407 compatible with the product and as described in the marketing authorization (refer to paragraph
1408 8.125).

1409

1410 8.82 Suitable bioburden reduction prefilters and/or sterilizing grade filters may be used at multiple
1411 points during the manufacturing process to ensure a low and controlled bioburden of the liquid prior
1412 to the primary sterilizing grade filter. Due to the potential additional risks of a sterile filtration
1413 process, as compared with other sterilization processes, a second filtration through a sterile sterilizing
1414 grade filter, immediately prior to filling, should be considered as part of an overall CCS.

1415

1416 8.83 The selection of components for the filtration system and their interconnection and arrangement
1417 within the filtration system, including pre-filters, should be based on the critical quality attributes of
1418 the product, justified and documented. The filtration system should minimize the generation of fibres
1419 and particulates, not cause or contribute to unacceptable levels of impurities, or possess characteristics
1420 that otherwise alter the quality and efficacy of the product. Similarly, the filter characteristics should
1421 be compatible with the fluid and not be adversely affected by the product to be filtered. Adsorption of
1422 product components and extraction/leaching of filter components should be evaluated (refer to
1423 paragraph 8.125).

1424

1425 8.84 The filtration system should be designed to:

1426

1427 i. Allow operation within validated process parameters.

1428

1429 ii. Maintain the sterility of the filtrate.

1430

1431 iii. Minimize the number of aseptic connections required between the sterilizing filter and the
1432 final filling of the product.

1433

1434 iv. Allow cleaning procedures to be conducted as necessary.

1435

1436 v. Allow sterilization procedures, including sterilization in place, to be conducted as necessary.

1437

1438 vi. Permit in-place integrity testing, of the 0.22 µm sterilizing filter, preferably as a closed
1439 system, prior to filtration as necessary. In-place integrity testing methods should be selected
1440 to avoid any adverse impact on the quality of the product.

1441

1442 8.85 Sterile filtration of liquids should be validated in accordance with European (or other relevant)
1443 Pharmacopeia requirements. Validation can be grouped by different strengths or variations of a
1444 product but should be done under worst case conditions. The rationale for grouping should be
1445 justified and documented.

1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499

8.86 During filter validation, wherever possible, the product to be filtered should be used for bacterial retention testing of the sterilizing filter. Where the product to be filtered is not suitable for use in bacterial retention testing, a suitable surrogate product should be justified for use in the test. The challenge organism used in the bacterial retention test should be justified.

8.87 Filtration parameters that should be considered and established in validation and monitored in routine processing should include, but are not limited to:

- i. The wetting fluid used for filter integrity testing should be based on the filter manufacturer's recommendation or the fluid to be filtered. The appropriate integrity test value specification should be established.
- ii. If the system is flushed or integrity tested in-situ with a fluid other than the product, appropriate actions are taken to avoid any deleterious effect on product quality.
- iii. Filtration process conditions including:
 - Fluid pre-filtration holding time and effect on bioburden.
 - Filter conditioning, with fluid if necessary.
 - Maximum filtration time/total time filter is in contact with fluid.
 - Maximum operating pressure.
 - Flow rate.
 - Maximum filtration volume.
 - Temperature.
 - The time taken to filter a known volume of bulk solution and the pressure difference to be used across the filter.

Note: Results of these checks should be included in the batch record. Any significant difference in parameters from those validated to those observed during routine manufacturing should be noted and investigated.

8.88 The integrity of the sterilized filter assembly should be verified by integrity testing before use, to check for damage and loss of integrity caused by the filter preparation prior to use. A sterilizing grade filter that is used to sterilize a fluid should be subject to a non-destructive integrity test post-use prior to removal of the filter from its housing. Test results should correlate to the microbial retention capability of the filter established during validation. Examples of tests that are used include bubble point, diffusive flow, water intrusion or pressure hold test. It is recognized that pre-use post sterilization integrity testing (PUPSIT) may not always be possible after sterilization due to process constraints (e.g. the filtration of very small volumes of solution). In these cases, an alternative approach may be taken providing that a thorough risk assessment has been performed and compliance is achieved by the implementation of appropriate controls to mitigate any risk of non-sterility. Points to consider in such a risk assessment should include but are not be limited to:

- i. In depth knowledge and control of the sterilization process to ensure that the potential for damage to the filter is minimized.

- 1500
1501 ii. In depth knowledge and control of the supply chain to include:
1502 • Contract sterilization facilities.
1503 • Defined transport mechanisms.
1504 • Packaging of the sterilized filter, to prevent damage to the filter during transportation
1505 and storage.
1506 iii. In depth process knowledge such as:
1507 • The specific product type, including particulate burden and whether there exists any
1508 risk of impact on filter integrity values, such as the potential to alter integrity testing
1509 values and therefore prevent the detection of a non-integral filter during a post-use
1510 filter integrity test.
1511 • Pre-filtration and processing steps, prior to the sterilizing filter, which would remove
1512 particulate burden and clarify the product prior to the sterile filtration.
1513
1514 8.89 The integrity of critical sterile gas and air vent filters (that are directly linked to the sterility of
1515 the product) should be verified by testing after use, with the filter remaining in the filter assembly.
1516
1517 8.90 The integrity of non-critical air or gas vent filters should be confirmed and recorded at
1518 appropriate intervals. Where gas filters are in place for extended periods such as vent filters, integrity
1519 testing should be carried out pre and post-use. The maximum duration of use should be specified and
1520 monitored based on risk (e.g. considering the maximum number of uses and sterilization cycles
1521 permitted).
1522
1523 8.91 For gas filtration, attention should be paid to avoiding unintended moistening or wetting of the
1524 filter or filter equipment. This can be achieved by the use of hydrophobic filters.
1525
1526 8.92 If the sterilizing filtration process has been validated as a system consisting of multiple filters to
1527 achieve the sterility for a given fluid, the filtration system is considered to be a single sterilizing unit
1528 and all filters within the system should satisfactorily pass integrity testing after use.
1529
1530 8.93 In a redundant filtration system (where a second filter is present as a backup but the sterilizing
1531 process is validated as only requiring one filter), post-use integrity test of the primary sterilizing filter
1532 should be performed and if demonstrated to be integral, then a post-use integrity test of the secondary
1533 filter is not necessary. However, in the event of a failure of the post-use integrity test on the primary
1534 filter, a risk assessment should be carried out to determine the acceptability of performing a post-use
1535 integrity test on the secondary (redundant) filter.
1536
1537 8.94 Bioburden samples should be taken from the bulk product and immediately prior to the final
1538 sterile filtration. Systems for taking samples should be designed so as not to introduce contamination.
1539
1540 8.95 Liquid sterilizing filters should be discarded after the processing of a single lot and the same
1541 filter should not be used for more than one working day unless such use has been validated.
1542
1543 8.96 Where campaign manufacture of a product has been appropriately justified in the CCS and
1544 validated, the filter user should:
1545 i. Assess and document the risks associated with the duration of filter use for the sterile
1546 filtration process for a given fluid.
1547 ii. Conduct and document effective validation and qualification studies to demonstrate that the
1548 duration of filter use for a given sterile filtration process and for a given fluid does not
1549 compromise performance of the sterilizing filter or filtrate quality.

1549 iii. Document the maximum validated duration of use for the filter and implement controls to
1550 ensure that filters are not used beyond the validated maximum duration. Records of these
1551 controls should be maintained.

1552 iv. Implement controls to ensure that filters contaminated with fluid or cleaning agent residues,
1553 or considered defective in any other way, are removed from use.

1554 **Form-Fill-Seal**

1555
1556 8.97 Form-Fill-Seal (FFS) units include blow moulding from thermoplastic granulate and
1557 thermoforming from thermoplastic film, typically known as Blow-Fill-Seal (BFS) and Vertical-Form-
1558 Fill-Seal (VFFS), respectively. VFFS process is an automated filling process, typically for terminally
1559 sterilized products, that may utilize a single or dual web system which constructs the primary
1560 container out of a flat roll of thermoplastic film while simultaneously filling the formed bags with
1561 product and sealing the filled bags in a continuous process. All such containers are considered to be
1562 closed through sealing by fusion and, as such, fall under the requirement to perform 100% integrity
1563 testing (refer to paragraph 8.21).

1564
1565 8.98 Process parameters relating to seal integrity should be qualified and appropriately controlled.

1566
1567 8.99 Critical parameters include, but are not limited to:

1568
1569 i. Seal strength.

1570
1571 ii. Seal uniformity.

1572
1573 iii. Sealing temperatures.

1574
1575 iv. Sealing pressures.

1576
1577 v. Sealing times.

1578
1579 vi. Dwell time for filling.

1580
1581 8.100 Seal strength and uniformity should be monitored routinely.

1582
1583 8.101 The controls identified during qualification should be in alignment with the site's CCS. Aspects
1584 to be considered include but are not limited to:

1585
1586 i. Determination of the boundaries of the critical zone.

1587
1588 ii. Environmental control and monitoring, both of the machine and the background in which it
1589 is placed.

1590
1591 iii. Integrity testing of the product filling lines.

1592
1593 iv. Integrity testing of the cooling system.

1594
1595 v. Duration of the batch or filling campaign.

1596
1597 vi. Control of polymer starting material (including resin pellets).

1598
1599 vii. Cleaning-in-place and sterilization-in-place of equipment in direct contact to the formulation
1600 (product filling lines); sterilization-in-place of sterile air pathways.

1601

1602 **Blow-Fill-Seal**

1603

1604 8.102 Blow-Fill-Seal (BFS) units are purpose built machines in which, in one continuous operation,
1605 containers are formed from a thermoplastic granulate, filled and then sealed by one automatic
1606 machine. Air that makes contact with critical surfaces of the container during extrusion, formation or
1607 sealing of the moulded container should undergo appropriate filtration.

1608

1609 8.103 For shuttle type equipment used for aseptic filling, the area between parison cutting and mould
1610 sealing should be covered by a flow of filtered air to provide Grade A conditions at the critical zone.
1611 The equipment should be installed in at least a Grade C environment, provided that Grade A/B
1612 clothing is used. The filling environment should meet Grade A for viable and non-viable limits at rest
1613 and the viable limit only when in operation.

1614

1615 8.104 For rotary-type equipment, used for aseptic filling, the filling environment should be designed
1616 to meet Grade A conditions. Other sterility assurance controls such as monitoring of critical
1617 parameters and alarms during each batch and parison support filter integrity testing should be
1618 considered.

1619

1620 8.105 The environmental control and monitoring program should take into consideration the moving
1621 parts and complex airflow paths generated by the BFS process and the effect of the high heat outputs
1622 of the process, e.g. by performing smoke studies and/or other equivalent studies. Environmental
1623 monitoring should be applied taking into consideration elements such as air-filter configuration, air-
1624 filter integrity, cooling systems integrity, equipment design and installation.

1625

1626 8.106 Blow-Fill-Seal equipment used for the manufacture of products which are terminally sterilized
1627 should be installed in at least a Grade D environment. The conditions at the point of fill should
1628 comply with the environmental requirements of paragraphs 8.3 and 8.4.

1629

1630 8.107 External particulate and microbial contamination of the polymer should be prevented by
1631 appropriate design, control, and maintenance of the polymer storage, sampling and distribution
1632 systems. The capability of the extrusion system to provide appropriate sterility assurance for the
1633 moulded container should be fully understood and validated. The sampling frequency, the bioburden
1634 and, where applicable, endotoxins levels of the raw polymer should be defined and controlled within
1635 the CCS.

1636

1637 8.108 Interventions requiring cessation of filling and/or extrusion, moulding and sealing and, where
1638 required, re-sterilization of the filling machine should be clearly defined and well described in the
1639 aseptic filling procedure, and included in the APS (refer to paragraphs 9.36, 9.37 and 9.38).

1640

1641 8.109 The moulds used to form containers are considered critical equipment and any changes or
1642 modification to moulds should result in an assessment of finished product container integrity, and
1643 should be supported by validation.

1644

1645 **Lyophilization**

1646

1647 8.110 Lyophilization is a critical process step and all activities that can affect the sterility of the
1648 product or material need to be regarded as extensions of the aseptic processing of the sterilized
1649 product. The lyophilization equipment and its processes should be designed to ensure that product or
1650 material sterility is maintained during lyophilization by preventing microbial and particulate
1651 contamination between the filling of products for lyophilization, and completion of lyophilization
1652 process. All control measures in place should be determined by the site's CCS.

1653

1654 8.111 The sterilization of lyophilizers and associated equipment, (e.g. trays, vial support rings) should
1655 be validated and holding times between sterilization cycles appropriately challenged during aseptic
1656 process simulations. The lyophilizer should be sterilized regularly, based on system design. Re-

1657 sterilization should be performed following maintenance or cleaning. Sterilized lyophilizers and
1658 associated equipment should be protected from contamination after sterilization.

1659
1660 8.112 Lyophilizers that are manually loaded or unloaded should normally be sterilized before each
1661 load. For lyophilizers loaded by automated closed systems or located within systems that exclude
1662 operator intervention, the frequency of sterilization should be justified and documented as part of the
1663 CCS.

1664
1665 8.113 The integrity of the lyophilizer system should be maintained following sterilization and during
1666 use. The filter used to maintain lyophilizer integrity should be sterilized before each use of the system
1667 and its integrity testing results should be part of the batch certification. The frequency of vacuum/leak
1668 integrity testing of the chamber should be documented and the maximum permitted leakage of air into
1669 the lyophilizer should be specified and checked at the start of every cycle.

1670
1671 8.114 Lyophilization trays should be checked regularly to ensure that they are not misshapen or
1672 damaged.

1673
1674 8.115 Points to consider for the design of loading (and unloading, where the lyophilised material is
1675 not in a sealed container (e.g. open tray dried materials), include but are not limited to:

- 1676
- 1677 i. The loading pattern within the lyophilizer should be specified and documented.
 - 1678
 - 1679 ii. The transfer of partially closed containers to a lyophilizer should be undertaken under Grade
1680 A conditions at all times and handled in a manner designed to minimize direct operator
1681 intervention. Technologies such as conveyor systems, portable transfer systems (e.g. clean air
1682 transfer carts, portable unidirectional airflow workstations) should be used to ensure that the
1683 cleanliness of the system used to transfer the partially closed containers is maintained).
1684 Alternatively, where supported by validation, containers closed in the Grade A zone and not
1685 reopened whilst in the Grade B may be used to protect partially stoppered vials (e.g. sealed
1686 sterilized trays).
 - 1687
 - 1688 iii. Airflow patterns should not be adversely affected by transport devices and venting of the
1689 loading zone.
 - 1690
 - 1691 iv. Unsealed containers (such as partially stoppered vials) should be maintained under Grade A
1692 conditions and should normally be separated from operators by physical barrier technology or
1693 any other appropriate measures.
 - 1694
 - 1695 v. Where seating of the stoppers is not completed prior to opening the lyophilizer chamber,
1696 product removed from the lyophilizer should remain under Grade A conditions during
1697 subsequent handling.
 - 1698
 - 1699 vi. Utensils used during transfer to and loading and unloading of the lyophilizer (such as trays,
1700 bags, placing devices, tweezers, etc.) should be subject to a validated sterilization process.

1701
1702 **Closed systems**

1703
1704 8.116 Closed systems can be single use systems (i.e. disposable systems) and fixed systems (such as
1705 vessels with fixed pipework). Guidance in this section is equally applicable to both systems.

1706
1707 8.117 The use of closed systems can reduce the risk of extraneous contamination such as microbial,
1708 particulate and chemical from the adjacent environment. Closed systems should always be designed to
1709 reduce the need for, and complexity of manual interventions.

1710
1711 8.118 It is critical to ensure the sterility of all product contact surfaces of closed systems used for

1712 aseptic processing. The design and selection of any closed system used for aseptic processing should
1713 ensure maintenance of sterility. Connection of sterile equipment (e.g. tubing / pipework) to the
1714 sterilized product pathway after the final sterilizing filter should be designed to be connected
1715 aseptically (e.g. by intrinsic aseptic connectors or fusion systems).

1716
1717 8.119 Appropriate measures should be in place to ensure the integrity of components used in aseptic
1718 connections. The means by which this is achieved should be determined and captured in the CCS.
1719 Appropriate system integrity tests should be considered when there is a risk of compromising product
1720 sterility. Supplier assessment should include the collation of data in relation to potential failure modes
1721 that may lead to a loss of system sterility.

1722
1723 8.120 The background in which closed systems are located should be based on their design and the
1724 processes undertaken. For aseptic processing and where there are any risks that system integrity may
1725 be compromised, the system should be located in a Grade A zone. If the system can be shown to
1726 remain integral at every usage (e.g. via pressure testing and/or monitoring) then a lower classified area
1727 may be used. If the closed system is opened (e.g. for maintenance of a bulk manufacturing line) then
1728 this should be performed in a classified area appropriate to the materials (e.g. Grade C for terminally
1729 sterilization processes, or Grade A for aseptic processing) or be subject to further cleaning and
1730 disinfection (and sterilization in case of aseptic processes).

1731 1732 **Single use systems (SUS)** 1733

1734 8.121 SUS are those technologies used in manufacture of sterile products which are used as an
1735 alternative to reusable equipment. SUS can be individual components or made up of multiple
1736 components such as bags, filters, tubing, connectors, valves, storage bottles and sensors.

1737
1738 8.122 There are some specific risks associated with SUS which should be assessed as part of the CCS.
1739 These risks include but are not limited to:

- 1740
- 1741 i. The interaction between the product and product contact surface (such as adsorption, or the
1742 formation of leachables and extractables).
 - 1743
 - 1744 ii. The fragile nature of the system compared to fixed reusable systems.
 - 1745
 - 1746 iii. The increase in the number and complexity of manual operations (including inspection and
1747 handling of the system) and connections made.
 - 1748
 - 1749 iv. The complexity of the assembly.
 - 1750
 - 1751 v. The performance of the pre-use integrity test for sterilizing grade filters (refer to paragraph
1752 8.88).
 - 1753
 - 1754 vi. The risk of holes and leakage.
 - 1755
 - 1756 vii. The potential for compromising the system at the point of opening the outer packaging.
 - 1757
 - 1758 viii. The risk of particulate contamination.
 - 1759

1760 8.123 Sterilization processes for SUS should be validated and shown to have no adverse impact on
1761 system performance.

1762
1763 8.124 Assessment of suppliers of disposable systems including sterilization is critical to the selection
1764 and use of these systems. For sterile SUS, verification of sterility should be performed as part of the
1765 supplier qualification and on receipt and use of each unit.

1766

1767 8.125 The adsorption and reactivity of the product with product contact surfaces should be evaluated
1768 under process conditions.

1769
1770 8.126 The extractable and leachable profile of the SUS and any impact on the quality of the product
1771 especially where the system is made from polymer-based materials should be evaluated. An
1772 assessment should be carried out for each component to evaluate the applicability of the extractable
1773 profile data. For components considered to be at high risk from leachables, including those that may
1774 absorb processed materials or those with extended material contact times, an assessment of leachable
1775 profile studies, including safety concerns, should be taken into consideration. If applying simulated
1776 processing conditions, these should accurately reflect the actual processing conditions and be based
1777 on a scientific rationale.

1778
1779 8.127 SUS should be designed to maintain integrity throughout processing under the intended
1780 operational conditions. Attention to the structural integrity of the single use components is necessary
1781 where these may be exposed to more extreme conditions (e.g. freezing and thawing processes) either
1782 during routine processing or transportation. This should include verification that intrinsic aseptic
1783 connections (both heat sealed and mechanically sealed) remain integral under these conditions.

1784
1785 8.128 Acceptance criteria should be established and implemented for SUS corresponding to the risks
1786 or criticality of the products and its processes. On receipt, each piece of SUS should be checked to
1787 ensure that they have been manufactured, supplied and delivered in accordance with the approved
1788 specification. A visual inspection of the outer packaging (e.g. appearance of exterior carton, product
1789 pouches), label printing, and review of attached documents (e.g. certificate of conformance and proof
1790 of sterilization) should be carried out and documented prior to use.

1791
1792 8.129 Critical manual handling operations of SUS such as assembly and connections should be
1793 subject to appropriate controls and verified during the APS.

1794
1795 **9 Viable and non-viable environmental & process monitoring**

1796
1797 **General**

1798
1799 9.1 The site's environmental and process monitoring program forms part of the overall CCS and is
1800 used to monitor the controls designed to minimize the risk of microbial and particulate contamination.
1801 It should be noted that the reliability of each of the elements of the monitoring system (viable, non-
1802 viable and APS) when taken in isolation is limited and should not be considered individually to be an
1803 indicator of asepsis. When considered together, their reliability is dependent on the design, validation
1804 and operation of the system that they are monitoring.

1805
1806 9.2 This program is typically comprised of the following elements:

- 1807 i. Environnemental monitoring – non-viable particles.
1808
1809 ii. Environmental and personnel monitoring – viable particles.
1810
1811 iii. Aseptic process simulation (aseptically manufactured product only).
1812

1813 9.3 The information from these systems should be used for routine batch certification and for periodic
1814 assessment during process review or investigation. This applies for both terminal sterilization and
1815 aseptic processes, however, the criticality of the impact may differ depending upon the product and
1816 process type.

1817
1818 **Environmental monitoring**

1819
1820 9.4 Risk assessments should be performed in order to establish a comprehensive environmental

1821 monitoring program, i.e. sampling locations, frequency of monitoring, monitoring method used and
1822 incubation conditions (e.g. time, temperature(s), aerobic and/or anaerobic conditions). These risk
1823 assessments should be conducted based on detailed knowledge of; the process inputs and final
1824 product, the facility, equipment, specific processes, the operations involved, historical monitoring
1825 data, monitoring data obtained during qualification and knowledge of typical microbial flora isolated
1826 from the environment. Consideration of other information such as air visualization studies should
1827 also be included. These risk assessments should be reviewed regularly in order to confirm the
1828 effectiveness of the site's environmental monitoring program. The monitoring program should be
1829 considered in the overall context of the trend analysis and the CCS for the site.
1830

1831 9.5 Routine monitoring of cleanrooms, clean air equipment and personnel should be performed in
1832 operation throughout all critical stages, including equipment set-up.
1833

1834 9.6 The monitoring of Grade A zones should demonstrate the maintenance of aseptic processing
1835 conditions during critical operations. Monitoring should be performed at locations posing the highest
1836 risk of contamination to the sterile equipment surfaces, container, closures and product. The selection
1837 of monitoring locations and the orientation and positioning of sampling devices should be justified
1838 and appropriate to obtain reliable data from the critical zones.
1839

1840 9.7 Sampling methods should not pose a risk of contamination to the manufacturing operations.
1841

1842 9.8 Appropriate alert levels and action limits should be set for the results of viable and non-viable
1843 particle monitoring. Alert levels should be established based on results of cleanroom qualification
1844 tests or trend data and should be subject to periodic review.
1845

1846 9.9 Alert levels for Grade A (non-viable particles only) Grade B, Grade C and Grade D should be set
1847 such that adverse trends (e.g. a numbers of events or individual events that indicate a deterioration of
1848 cleanliness) are detected and addressed.
1849

1850 9.10 Monitoring procedures should define the approach to trending. Trends can include, but are not
1851 limited to:

1852

- 1853 i. Increasing numbers of action limit or alert level breaches.
- 1854
- 1855 ii. Consecutive breaches of alert levels.
- 1856
- 1857 iii. Regular but isolated breaches of action limits that may have a common cause, for example
1858 single excursions that always follow planned preventative maintenance.
1859
- 1860 iv. Changes in microbial flora type and numbers and predominance of specific organisms.
1861 Particular attention should be given to objectionable organisms or those that can be difficult
1862 to control such as spore-forming microorganisms.
1863

1864 9.11 The monitoring of Grade C and D cleanrooms in operation should be performed based on data
1865 collected during qualification and historical data to allow effective trend analysis. The requirements
1866 of alert levels and action limits will depend on the nature of the operations carried out. Action limits
1867 may be more stringent than those listed in Table 6 and Table 7.
1868

1869 9.12 If action limits are exceeded, operating procedures should prescribe a root cause investigation,
1870 an assessment of the potential impact to product and requirements for corrective and preventive
1871 actions. If alert levels are exceeded, operating procedures should prescribe assessment and follow-
1872 up, which should include consideration of an investigation and/or corrective actions to avoid any
1873 further deterioration of the environment.
1874

1875 9.13 Results from environmental monitoring should be considered when reviewing batch

1876 documentation for finished product batch certification.

1877

1878 **Environmental monitoring- non-viable particles**

1879

1880 9.14 Non-viable particulate monitoring systems should be established to obtain data for assessing
1881 potential contamination risks and to ensure the maintenance of the environment for sterile operations
1882 in a qualified state.

1883

1884 9.15 The limits for environmental monitoring of airborne particulate concentrations for each graded
1885 area are given in Table 6.

1886

1887 **Table 6: Limits for airborne particulate concentration for the monitoring of non-viable**
1888 **contamination.**

1889

Grade	Maximum limits for particulates $\geq 0.5 \mu\text{m}/\text{m}^3$		Maximum limits for particulates $\geq 5 \mu\text{m}/\text{m}^3$	
	at rest	in operation	at rest	in operation
A	3 520	3 520	29	29
B	3 520	352 000	29	2 900
C	352 000	3 520 000	2 900	29 000
D	3 520 000	Not defined ^(a)	29 000	Not defined ^(a)

1890

1891

1892

1893 ^(a) For Grade D, in operation limits are not defined. The company should establish in operation
1894 limits based on a risk assessment and on historical data, where applicable.

1895

1896 Note 1: The particulate limits given in the table for the “at rest” state should be achieved after
1897 a short “clean up” period (defined during qualification with a guidance value of 15 to 20
1898 minutes) in an unmanned state, after the completion of operations (refer to paragraph 4.30 and
1899 4.31).

1900

1901 Note 2: With regards to the monitoring of airborne particulates $\geq 5 \mu\text{m}$ particulate
1902 concentration, the limit of 29 (Grade A) is selected due to the limitations of monitoring
1903 equipment. Alert levels should be set based on historical data, such that frequent sustained
1904 counts below the action limit which may be indicative of system contamination or
1905 deterioration should trigger an investigation. For the Grade A zone and Grade B area the
1906 importance of monitoring the $\geq 5 \mu\text{m}$ particulates is to identify negative trends as defined in the
1907 manufacturer's CCS.

1908

1909 9.16 For the Grade A zone, particulate monitoring should be undertaken for the full duration of
1910 critical processing, including equipment assembly.

1911

1912 9.17 The Grade A zone should be monitored continuously (for particulates ≥ 0.5 and $\geq 5 \mu\text{m}$) and
1913 with a suitable sample flow rate (at least 28 litres (1ft³) per minute) so that all interventions, transient
1914 events and any system deterioration is captured. The system should frequently correlate each
1915 individual sample result with the limits in Table 6 at such a frequency that any potential excursion
1916 can be identified and responded to in a timely manner. Alarms should be triggered if alert levels are
1917 exceeded. Procedures should define the actions to be taken in response to alarms including the
1918 consideration of additional microbial monitoring.

1919
1920 9.18 It is recommended that a similar system be used for Grade B area although the sample frequency
1921 may be decreased. The Grade B zone should be monitored at such a frequency and with suitable
1922 sample size that the programme captures any increase in levels of contamination and system
1923 deterioration. If alert or action levels are exceeded, alarms should be triggered.

1924
1925 9.19 The selection of the monitoring system should take into account any risk presented by
1926 the materials used in the manufacturing operation (for example, those involving live organisms,
1927 powdery products or radiopharmaceuticals) that may give rise to biological or chemical hazards.

1928
1929 9.20 In the case where contaminants are present due to the processes involved and would potentially
1930 damage the particle counter or present a hazard (e.g. live organisms, powdery products and radiation
1931 hazards), the frequency and strategy employed should be such as to assure the environmental
1932 classification both prior to and post exposure to the risk. An increase in viable particle monitoring
1933 should be considered to ensure comprehensive monitoring of the process. Additionally, monitoring
1934 should be performed during simulated operations. Such operations should be performed at appropriate
1935 intervals. The approach should be defined in the CCS.

1936
1937 9.21 The size of monitoring samples taken using automated systems will usually be a function of the
1938 sampling rate of the system used. It is not necessary for the sample volume to be the same as that
1939 used for formal classification of cleanrooms and clean air equipment. Monitoring sample volumes
1940 should be justified.

1941
1942 9.22 The occasional indication of macro particulate counts, especially $\geq 5 \mu\text{m}$, may be considered to
1943 be false counts due to electronic noise, stray light, coincidence, etc. However, consecutive or regular
1944 counting of low levels may be indicative of a possible contamination event and should be
1945 investigated. Such events may indicate early failure of the room air supply filtration system, filling
1946 equipment failure, or may also be diagnostic of poor practices during machine set-up and routine
1947 operation.

1948
1949 9.23 Monitoring conditions such as frequency, sampling volume or duration, alert levels and action
1950 limits and corrective actions (including an investigation) should be established in each
1951 manufacturing area based on data generated during the initial qualification process, ongoing routine
1952 monitoring and periodic review of data.

1953 1954 **Environmental and personnel monitoring-viable particles**

1955
1956 9.24 Where aseptic operations are performed, microbial monitoring should be frequent using a
1957 combination of methods such as settle plates, volumetric air sampling, glove, gown and surface
1958 sampling (e.g. swabs and contact plates). The method of sampling used should be justified within
1959 the CCS and should be demonstrated not to have a detrimental impact on Grade A and B airflow
1960 patterns.

1961
1962 9.25 Monitoring should include sampling of personnel at periodic intervals during the process.
1963 Sampling of personnel should be performed in such a way that it will not compromise the process.
1964 Particular consideration should be given to monitoring personnel following involvement in critical
1965 interventions and on each exit from the Grade B cleanroom.

1966
1967 9.26 Viable particle monitoring should also be performed within the cleanrooms when normal
1968 manufacturing operations are not occurring (e.g. post disinfection, prior to start of manufacturing,
1969 on completion of the batch and after a shutdown period), and in associated rooms that have not
1970 been used, in order to detect potential incidents of contamination which may affect the controls
1971 within the cleanrooms. In case of an incident, additional sample locations may be used as a
1972 verification of the effectiveness of a corrective action (i.e. cleaning and disinfection).

1973

1974 9.27 Continuous viable air monitoring in the Grade A zone (e.g. air sampling or settle plates) should
 1975 be undertaken for the full duration of critical processing, including equipment (aseptic set-up)
 1976 assembly and filling operations. A similar approach should be considered for Grade B cleanrooms
 1977 based on the risk of impact on the aseptic processing. The monitoring should be performed in such a
 1978 way that all interventions, transient events and any system deterioration would be captured and any
 1979 risk caused by interventions of the monitoring operations is avoided.

1980
 1981 9.28 The adoption of suitable rapid or automated monitoring systems should be considered by
 1982 manufacturers in order to expedite the detection of microbiological contamination issues and to
 1983 reduce the risk to product. These rapid and automated microbial monitoring methods may be adopted
 1984 after validation has demonstrated their equivalency or superiority to the established methodology.

1985
 1986 9.29 Sampling methods and equipment used should be fully understood and procedures should be in
 1987 place for the correct operation and interpretation of results obtained. The recovery efficiency of the
 1988 sampling methods chosen should be qualified.

1989
 1990 9.30 Action limits for viable particle contamination are shown in Table 7

1991 **Table 7: Maximum action limits for viable particle contamination**

Grade	Air sample cfu/m ³	Settle plates (diam. 90 mm) cfu/4 hours ^(a)	Contact plates (diam. 55mm), cfu/ plate ^(c)	Glove print, Including 5 fingers on both hands cfu/ glove
A	No growth ^(b)			
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

1994
 1995 ^(a) Settle plates should be exposed for the duration of operations and changed as required after
 1996 4 hours (exposure time should be based on validation including recovery studies and it should
 1997 not have any negative effect on the suitability of the media used). Individual settle plates may
 1998 be exposed for less than 4 hours.

1999 ^(b) It should be noted that for Grade A, any growth should result in an investigation.

2000
 2001 ^(c) Contact plate limits apply to equipment room and gown surfaces within the Grade A zone
 2002 and Grade B area. Routine gown monitoring is not normally required for Grade C and D areas,
 2003 depending on their function.

2004
 2005 Note 1: It should be noted that the types of monitoring methods listed in the table above are
 2006 examples and other methods can be used provided they meet the intent of providing
 2007 information across the whole of the critical process where product may be contaminated (e.g.
 2008 aseptic line set-up, filling and lyophilizer loading).

2009
 2010 Note 2: Limits are applied using cfu throughout the document. If different or new technologies
 2011 are used that present results in a manner different from cfu, the manufacturer should
 2012 scientifically justify the limits applied and where possible correlate them to cfu.

2013
 2014 9.31 Microorganisms detected in Grade A zone and Grade B area should be identified to species level
 2015 and the potential impact of such microorganisms on product quality (for each batch implicated) and
 2016 overall state of control should be evaluated. Consideration should also be given to the identification of
 2017 microorganisms detected in Grade C and D areas (for example where action limits or alert levels are

2018 exceeded or where atypical or potentially objectionable microorganisms are recovered). The approach
2019 to organism identification and investigation should be documented.

2020

2021 9.32 Personnel gloves (and any part of the gown that may potentially have direct impact on the
2022 product sterility (e.g. the sleeves if these enter a critical zone) should be monitored for viable
2023 contamination after critical operations and on exit from the cleanroom. Other surfaces should be
2024 monitored at the end of an operation.

2025

2026 9.33 Microbial monitoring of personnel in the Grade A zone and Grade B area should be performed to
2027 assess their aseptic behaviour. Where filling operations are manual in nature e.g. hand filling, the
2028 process in its entirety may be considered as one critical intervention. In these cases, the frequency of
2029 microbial monitoring of gowning should be based on scientific principles and justified as part of the
2030 CCS. Where monitoring is routinely performed by manufacturing personnel, consideration should be
2031 given to periodic monitoring under the supervision of the quality unit.

2032

2033 **Aseptic process simulation (APS) (also known as media fill)**

2034

2035 9.34 Periodic verification of the effectiveness of the controls in place for aseptic processing should
2036 include a process simulation test using a sterile nutrient media and/or surrogate in place of the
2037 product. Selection of an appropriate nutrient media should be made based on the ability of the media
2038 and/or surrogate to imitate product characteristics at all processing stages. Where processing stages
2039 may indirectly impact the viability of any introduced microbial contamination, (e.g. sterile aseptically
2040 produced semi-solids, powders, solid materials, microspheres, liposomes and other formulations
2041 where product is cooled or heated or lyophilized), alternative procedures that represent the operations
2042 as closely as possible can be developed and justified. Where surrogate materials, such as buffers, are
2043 used in parts of the process simulation, the surrogate material should not inhibit the growth of any
2044 potential contamination.

2045

2046 9.35 The process simulation test should imitate as closely as possible the routine aseptic
2047 manufacturing process and include all the critical manufacturing steps, specifically:

2048

2049 i. Process simulation tests should assess all aseptic operations performed subsequent to the
2050 sterilization and decontamination cycles of materials utilised in the process to the point
2051 where the container is sealed.

2052

2053 ii. For non-filterable formulations, any additional aseptic steps should be assessed.

2054

2055 iii. Where aseptic manufacturing is performed under an inert atmosphere, the inert gas should
2056 be substituted with air in the process simulation unless anaerobic simulation is intended.

2057

2058 iv. Processes requiring the addition of sterile powders should use an acceptable surrogate
2059 material in containers identical to those used in the process under evaluation.

2060

2061 v. Separate simulations of individual unit operations (e.g. processes involving drying,
2062 blending, milling and subdivision of a sterile powder) should generally be avoided. Any use
2063 of individual simulations should be supported by a documented justification and ensure that
2064 the sum total of the individual simulations continues to fully cover the whole process.

2065

2066 vi. The process simulation procedure for lyophilized products should represent the entire
2067 aseptic processing chain including filling, transport, loading, chamber dwell, unloading and
2068 sealing under specified, documented and justified conditions representing worst case
2069 operating parameters.

2070

2071 vii. The lyophilization process simulation should duplicate all aspects of the process, except
2072 those that may affect the viability or recovery of contaminants. For instance, boiling-over or
2073 actual freezing of the solution should be avoided. Factors to consider in determining APS

2074 design include, where applicable:

2075

2076 • The use of air to break vacuum instead of nitrogen.

2077

2078 • Replicating the maximum interval between sterilization of the lyophilizer and its
2079 use.

2080

2081 • Replicating the maximum period of time between sterilization and lyophilization.

2082

2083 • Quantitative aspects of worst case situations, e.g. loading the largest number of
2084 trays, replicating the longest duration of loading where the chamber is open to the
2085 environment.

2086

2087 9.36 The process simulation testing should take into account various aseptic manipulations and
2088 interventions known to occur during normal production as well as worst case situations, including:

2089

2090 i. Inherent interventions representative of the routine process at the maximum accepted
2091 frequency per number of filled units (e.g. loading of vials into a lyophilizer).

2092

2093 ii. Corrective interventions, that occur frequently during routine production, in a representative
2094 number and with the highest degree of acceptable intrusion (e.g. correcting jammed
2095 stoppers).

2096

2097 9.37 Interventions should not be designed or selected to justify poor process or facility design or to
2098 assess unacceptable interventions that rarely occur and which should lead to a thorough investigation
2099 and product assessment when they do occur.

2100

2101 9.38 In developing the process simulation test plan, consideration should be given to the following:

2102

2103 i. Identification of worst case conditions covering the relevant variables, such as container size
2104 and line speed, and their impact on the process. The outcome of the assessment should
2105 justify the variables selected.

2106

2107 ii. Determining the representative sizes of container/closure combinations to be used for
2108 validation. Bracketing or matrix approach may be considered for validation of the same
2109 container/closure configuration for different products where process equivalence is
2110 scientifically justified.

2111

2112 iii. The volume filled per container, which should be sufficient to ensure that the media contacts
2113 all equipment and component surfaces that may directly contaminate the sterile product. The
2114 volume used should provide sufficient headspace to support potential microbial growth and
2115 ensure that turbidity can be detected during inspection.

2116

2117 iv. Maximum permitted holding times for sterile product and associated sterile components and
2118 equipment exposed during the aseptic process.

2119

2120 v. The method of detection of microbial contamination should be scientifically justified to
2121 ensure that any contamination is detectable.

2122

2123 vi. The selected nutrient media should be capable of growing a designated group of reference
2124 microorganisms as described by the relevant pharmacopeia and suitably representative local
2125 isolates and supporting recovery of low numbers of these microorganisms.

2126

2127 vii. The requirement for substitution of any inert gas used in the routine aseptic manufacturing
2128 process by air unless anaerobic simulation is intended. In these situations, inclusion of

2129 occasional anaerobic simulations as part of the overall validation strategy should be
2130 considered (refer to paragraph 9.35 point iii).

2131
2132 viii. The process simulation should be of sufficient duration to challenge the process, the
2133 operators that perform interventions, shift changes and the capability of the processing
2134 environment to provide appropriate conditions for the manufacture of a sterile product.

2135
2136 ix. Where the manufacturer operates different shifts then the APS should be designed to capture
2137 specific factors (e.g. for those manufacturing during a night or extended shift, fatigue should
2138 be considered).

2139
2140 x. Simulating normal aseptic manufacturing interruptions where the process is idle (e.g. shift
2141 changeovers, recharging dispensing vessels, introduction of additional equipment, etc.).

2142
2143 xi. Ensuring that environmental monitoring is conducted as required for routine production, and
2144 throughout the entire duration of the process simulation.

2145
2146 xii. Where campaign manufacturing occurs, such as in the use of Barrier Technologies or
2147 manufacture of sterile active substances, consideration should be given to designing and
2148 performing the process simulation so that it simulates the risks associated with both the
2149 beginning and the end of the campaign and demonstrating that the campaign duration does
2150 not pose any risk. The performance of "end of production or campaign APS" may be used as
2151 additional assurance or investigative purposes; however, their use should be justified in the
2152 CCS and should not replace routine APS. If used, it should be demonstrated that any
2153 residual product does not negatively impact the recovery of any potential microbial
2154 contamination.

2155
2156 9.39 For sterile active substances, batch size should be large enough to represent routine operation,
2157 simulate intervention operation at the worst case, and cover potential contact surfaces. In addition, all
2158 the simulated materials (surrogates or growth medium) should be subjected to microbial evaluation.
2159 The simulation materials should be sufficient to satisfy the evaluation of the process being simulated
2160 and should not compromise the recovery of micro-organisms.

2161
2162 9.40 Process simulation tests should be performed as part of the initial validation, with at least three
2163 consecutive satisfactory simulation tests that cover all working shifts that the aseptic process may
2164 occur in, and after any significant modification to operational practices, facilities, services or
2165 equipment (e.g. modification to the HVAC system, equipment, major facility shut down, changes to
2166 process, number of shifts and numbers of personnel etc.). Normally, process simulation tests (periodic
2167 revalidation) should be repeated twice a year (approximately every six months) for each aseptic
2168 process, each filling line and each shift. Each operator should participate in at least one successful
2169 APS annually. Consideration should be given to performing an APS after the last batch prior to shut
2170 down, before long periods of inactivity or before decommissioning or relocation of a line.

2171
2172 9.41 Where manual operation (e.g. aseptic compounding or filling) occurs, each type of container,
2173 container closure and equipment train should be initially validated with each operator participating in
2174 at least 3 consecutive successful APS and revalidated with one APS approximately every 6 months for
2175 each shift. The APS batch size should mimic that used in the routine aseptic manufacturing process.

2176
2177 9.42 The number of units processed (filled) for APS tests should be sufficient to effectively simulate
2178 all activities that are representative of the aseptic manufacturing process. Justification for the number
2179 of units to be filled should be clearly captured in the PQS. Typically, a minimum of 5000 to 10000
2180 units are filled. For small batches (e.g. those under 5000 units), the number of containers for media fill
2181 should at least equal the size of the production batch.

2182
2183 9.43 Filled APS units should be agitated, swirled or inverted before incubation to ensure contact of
2184 the media with all interior surfaces in the container. Units with cosmetic defects or those who have

2185 gone through non-destructive in process control checks should be identified and incubated. Units
2186 discarded during the process simulation and not incubated should be comparable with units discarded
2187 during a routine fill. Examples may include those normally discarded after the set-up process or due to
2188 an intervention or where the integrity of the unit is compromised as would be identified by the routine
2189 inspection process for the product.

2190
2191 9.44 Where processes have materials that contact the product contact surfaces but are then discarded,
2192 the discarded material should be simulated with nutrient media and be incubated as part of the APS,
2193 unless it can be clearly demonstrated that this waste process would not impact the sterility of the
2194 product.

2195
2196 9.45 Filled APS units should be incubated in a clear container to ensure visual detection of microbial
2197 growth. Where the product container is not clear (i.e. amber glass, opaque plastic), clear containers of
2198 identical configuration may be substituted to aid in the detection of contamination. When a clear
2199 container of identical configuration cannot be substituted, a suitable method for the detection of
2200 microbial growth should be developed and validated. Microorganisms isolated from contaminated
2201 units should be identified to at least genus, and to the species level when practical, to assist in the
2202 determination of the likely source of the contaminant. The selection of the incubation conditions and
2203 duration should be scientifically justified and validated to provide an appropriate level of sensitivity
2204 of detection of microbial contamination.

2205
2206 9.46 Filled APS units should be incubated without unnecessary delay to achieve the best possible
2207 recovery of potential contamination.

2208
2209 9.47 On completion of incubation:

- 2210
2211 i. Filled APS units should be inspected by staff, who have been trained and qualified in the
2212 visual inspection procedures, under conditions similar to those for visual inspection, that
2213 facilitate the identification of any microbial contamination.
2214
2215 ii. Samples of these units should undergo positive control by inoculation with a suitable range of
2216 reference organisms and local isolates.

2217
2218 9.48 The target should be zero growth. Any contaminated unit should result in a failed process
2219 simulation and the following actions should occur:

- 2220
2221 i. An investigation to determine the most probable root causes.
2222
2223 ii. Determination and implementation of appropriate corrective measures.
2224
2225 iii. A sufficient number of successful, consecutive repeat media fills (normally a minimum of 3)
2226 should be conducted in order to demonstrate that the process has been returned to a state of
2227 control.
2228
2229 iv. A prompt review of all appropriate records relating to aseptic production since the last
2230 successful APS. The outcome of the review should include a risk assessment of potential
2231 sterile breaches in batches manufactured since the last successful process simulation. All other
2232 batches not released to the market should be included in the scope of the investigation. Any
2233 decision regarding their release status should consider the investigation outcome.
2234
2235 v. All products that have been manufactured on a line subsequent to a process simulation failure
2236 should be quarantined until a successful resolution of the process simulation failure has
2237 occurred.
2238
2239 vi. Production should resume only after completion of successful revalidation.

2240
2241 9.49 APS should be carefully observed by personnel with specific expertise in aseptic processing to
2242 assess the correct performance of operations and address inappropriate practices if detected.

2243 9.50 Where results indicate that an operator may have failed qualification, actions to limit the
2244 operator's activities, until retrained and requalified, should be taken.

2245
2246 9.51 An aseptic process or filling should be subject to a repeat of the initial validation when:
2247
2248 i. The specific aseptic process has not been in operation for an extended period of time.
2249
2250 ii. There is a change to the process, equipment, procedures or environment that has the
2251 potential to affect the aseptic process or an addition of new product containers or container-
2252 closure combinations.
2253

2254 9.52 All process simulation runs should be fully documented and include a reconciliation of units
2255 processed (e.g. units filled, incubated, not incubated, and rejected). All interventions performed
2256 during the process simulations should be recorded, including the start and end of each intervention.
2257 All microbial monitoring data as well as other testing data should be recorded in the APS batch
2258 record.
2259

2260 **10 Quality Control (QC)**
2261

2262 10.1 It is important that there are personnel with appropriate training and experience in microbiology
2263 and knowledge of the process to support the design of the manufacturing process, environmental
2264 monitoring regime and any investigation assessing the impact of microbiologically linked events to
2265 the safety of the sterile product.
2266

2267 10.2 Specifications for raw materials, components and products should include requirements for
2268 microbial quality when the need for this has been indicated by monitoring and/or by the CCS.
2269

2270 10.3 The bioburden assay should be performed on each batch for both aseptically filled product and
2271 terminally sterilized products and the results considered as part of the final batch review. There should
2272 be defined limits for bioburden immediately before the sterilizing filter or the terminal sterilization
2273 process, which are related to the efficiency of the method to be used. Samples should be taken to be
2274 representative of the worst case scenario (e.g. at the end of hold time). Where overkill sterilization
2275 parameters are set for terminally sterilized products, bioburden should be monitored at suitable
2276 scheduled intervals.
2277

2278 10.4 A pre-sterilization bioburden monitoring program for the product and components should be
2279 developed to support parametric release. The bioburden should be performed for each batch. The
2280 sampling locations of filled units before sterilization should be based on a worst case scenario and be
2281 representative of the batch. Any organisms found during bioburden testing should be identified and
2282 their impact on the effectiveness of the sterilizing process determined. Where appropriate, the level of
2283 pyrogen (endotoxins) should be monitored.
2284

2285 10.5 The sterility test applied to the finished product should only be regarded as the last in a series of
2286 control measures by which sterility is assured. It cannot be used to assure sterility of a product that
2287 does not meet its design, procedural or qualification parameters. The test should be validated for the
2288 product concerned.
2289

2290 10.6 The sterility test should be performed under aseptic conditions. Samples taken for sterility
2291 testing should be representative of the whole of the batch but should in particular include samples

2292 taken from parts of the batch considered to be most at risk of contamination, for example:
2293

2294 i. For products which have been filled aseptically, samples should include containers filled at
2295 the beginning, middle and end of the batch and after any significant intervention (e.g.
2296 interventions where the integrity of a barrier is breached (open door)) or an operator
2297 intervention into critical zones.
2298

2299 ii. For products which have been heat sterilized in their final containers, samples taken
2300 should be representative of the worst case locations (e.g. the potentially coolest or slowest to
2301 heat part of each load).
2302

2303 iii. For products that are lyophilized, samples taken from different lyophilization loads.
2304

2305 Note: Where the manufacturing process results in sub-batches (e.g. for terminally sterilized
2306 products) then sterility samples from each sub-batch should be taken and a sterility test for each sub-
2307 batch performed. Consideration should also be given to performing separate testing for other
2308 finished product tests.
2309

2310 10.7 For some products it may not be possible to perform a sterility test prior to release because the
2311 shelf life of the product is too short to allow completion of a sterility test. In these cases, the CCS
2312 should clearly capture the identified risks, the additional considerations of design of the process and
2313 additional monitoring required to mitigate the identified risks.
2314

2315 10.8 Any process (e.g. Vaporized Hydrogen Peroxide or VH202, Ultra Violet) used to decontaminate
2316 the external surfaces of sterility samples prior to testing should not negatively impact the sensitivity of
2317 the test method.
2318

2319 10.9 Media used for environmental monitoring and APS should be tested for its growth promotion
2320 capability, in accordance with a formal written program.
2321

2322 10.10 Environmental monitoring data and trend data generated for classified areas should be reviewed
2323 as part of product batch certification. A written plan should be available that describes the actions to
2324 be taken when data from environmental monitoring are found out of trend or exceeding the
2325 established limits. For products with short shelf life, the environmental data for the time of
2326 manufacture may not be available; in these cases, the certification should include a review of the most
2327 recent available data. Manufacturers of these products should consider the use of rapid monitoring
2328 systems.
2329

2330 10.11 Where rapid and automated microbial methods are used for general manufacturing purposes,
2331 these methods should be validated for the product(s) or processes concerned.
2332

2333 Glossary

2334

2335 Airlock – An enclosed space with interlocked doors, constructed to maintain air pressure control
2336 between adjoining rooms (generally with different air cleanliness standards). The intent of an airlock
2337 is to preclude ingress of particulate matter and microorganism contamination from a lesser controlled
2338 area.

2339

2340 Action limit – An established relevant measure (e.g. microbial, or airborne particulate limits) that,
2341 when exceeded, should trigger appropriate investigation and corrective action based on the
2342 investigation.

2343

2344 Alert level – An established relevant measure (e.g. microbial, or airborne particulate levels) giving
2345 early warning of potential drift from normal operating conditions and validated state, which does not
2346 necessarily give grounds for corrective action but triggers appropriate scrutiny and follow-up to
2347 address the potential problem. Alert levels are established based on historical and qualification trend
2348 data and periodically reviewed. The alert level can be based on a number of parameters including
2349 adverse trends, individual excursions above a set limit and repeat events.

2350

2351 Aseptic processing room – A room in which one or more aseptic activities or processes are performed.

2352

2353 Aseptic Process Simulation (APS) –A simulation of the entire aseptic formulation and filling process
2354 in order to determine the capability of the process to assure product sterility.

2355

2356 Asepsis – A state of control attained by using an aseptic work area and performing activities in a
2357 manner that precludes microbial contamination of the exposed sterile product.

2358

2359 Bacterial retention testing – This test is performed to validate that a filter can remove bacteria from a
2360 gas or liquid. The test is usually performed using a standard organism, such as *Brevundimonas*
2361 *diminuta* at a minimum concentration of 10^7 Colony Forming Units/cm².

2362

2363 Barrier – A physical partition that affords aseptic processing area (usually Grade A) protection by
2364 separating it from the background environment. Such systems frequently use in part or totally the
2365 Barrier Technologies known as RABS or isolators.

2366

2367 Bioburden – The total number of microorganisms associated with a specific item such as personnel,
2368 manufacturing environments (air and surfaces), equipment, product packaging, raw materials
2369 (including water), in-process materials, or finished products.

2370

2371 Biological Indicator (BI) – A population of microorganisms inoculated onto a suitable medium (e.g.
2372 solution, container or closure) and placed within a sterilizer or load or room locations to determine the
2373 sterilization or disinfection cycle efficacy of a physical or chemical process. The challenge
2374 microorganism is selected and validated based upon its resistance to the given process. Incoming lot
2375 D value, microbiological count and purity define the quality of the BI.

2376

2377 Blow-Fill-Seal (BFS) – A technology in which containers are formed from a thermoplastic granulate,
2378 filled with product, and then sealed in a continuous, integrated, automatic operation. The two most
2379 common types of BFS machines are the Shuttle type (with Parison cut) and the Rotary type (Closed
2380 Parison) types.

2381

2382 Classified area – An area that contains a number of cleanrooms (see cleanroom definition).

2383

2384 Cleaning – A process for removing contamination e.g. product residues and disinfectant residues.

2385

2386 Clean area – An area with defined particulate and microbiological cleanliness standards usually
2387 containing a number of joined cleanrooms.

2388
2389 Cleanroom – A room designed, maintained, and controlled to prevent particulate and microbial
2390 contamination of drug products. Such a room is assigned and reproducibly meets an appropriate air
2391 cleanliness level. Grade A will be referred to as Grade A zone.
2392
2393 Cleanroom classification – A method of assessing the level of air cleanliness against a specification
2394 for a cleanroom or clean air equipment by measuring the non-viable airborne particulate
2395 concentration.
2396
2397 Cleanroom qualification – A method of assessing the level of compliance of a classified cleanroom or
2398 clean air equipment with its intended use.
2399
2400 Closed system – A system in which the sterile product is not exposed to the surrounding environment.
2401 For example, this can be achieved by the use of bulk products holders (such as tanks or bags) that are
2402 connected to each other by pipes or tubes as a system, with the system being sterilized after the
2403 connections are made. Examples of these can be (but are not limited to) large scale reusable systems,
2404 such as those seen in active substance manufacturing, or disposable bag and manifold systems, such
2405 as those seen in the manufacture of biological products. Closed systems, when used in this document,
2406 does not refer to systems such as RABS or isolator systems which are referred to as Barrier
2407 Technologies.
2408
2409 Colony Forming Unit (CFU) – A microbiological term that describes a single detectable colony that
2410 originates from one or more microorganisms. Colony forming units are typically expressed as cfu per
2411 ml for liquid samples, and cfu per cm² for samples captured on solid medium such as settle or contact
2412 plates.
2413
2414 Contamination – The undesired introduction of impurities of a microbiological nature (quantity and
2415 type of microorganisms, pyrogens), or of foreign particulate matter, into or onto a raw material,
2416 intermediate, active substance or drug product during production, sampling, packaging or
2417 repackaging, storage or transport with the potential to adversely impact product quality.
2418
2419 Contamination Control Strategy (CCS) – A planned set of controls for microorganisms, pyrogens and
2420 particulates, derived from current product and process understanding that assures process performance
2421 and product quality. The controls can include parameters and attributes related to active substance,
2422 excipient and drug product materials and components, facility and equipment operating conditions, in-
2423 process controls, finished product specifications, and the associated methods and frequency of
2424 monitoring and control.
2425
2426 Corrective intervention – An intervention that is performed to correct or adjust an aseptic process
2427 during its execution. These may not occur with the same frequency in the routine aseptic process.
2428 Examples include such as clearing component jams, stopping leaks, adjusting sensors, and replacing
2429 equipment components. Corrective measures should be taken to reduce their extent and frequency.
2430
2431 Critical surfaces – Surfaces that may come directly into contact with, or directly affect, a sterile
2432 product or its containers or closures. Critical surfaces are rendered sterile prior to the start of the
2433 manufacturing operation, and sterility is maintained throughout processing.
2434
2435 Critical zone – A location within the aseptic processing area in which product and critical surfaces are
2436 exposed to the environment.
2437
2438 Critical intervention – An intervention (corrective or inherent) into the critical zone.
2439
2440 D value – The value of a parameter of sterilization (duration or absorbed dose) required to reduce the
2441 number of viable organisms to 10 per cent of the original number.
2442

2443 Dead leg – Length of non-circulating pipe (where fluid may remain static) that is greater than 3
2444 internal pipe diameters.
2445
2446 Decommission – When a process, equipment or cleanroom are closed where they will not be used
2447 again.
2448
2449 Decontamination – The overall process of removal or reduction of any contaminants (chemical, waste,
2450 residue or microorganisms) from an area, object, or person. The method of decontamination used (e.g.
2451 cleaning, disinfection, sterilization) should be chosen and validated to achieve a level of cleanliness
2452 appropriate to the intended use of the item decontaminated.
2453
2454 Depyrogenation – A process designed to remove or inactivate pyrogenic material (e.g. endotoxins) to
2455 a specified minimum quantity.
2456
2457 Disinfection – The process by which the reduction of the number of microorganisms is achieved by
2458 the irreversible action of a product on their structure or metabolism, to a level judged to be
2459 appropriate for a defined purpose.
2460
2461 Endotoxin – A pyrogenic product (e.g. lipopolysaccharide) present in the bacterial cell wall.
2462 Endotoxin can lead to reactions in patients receiving injections ranging from fever to death.
2463
2464 Extractables - Chemical entities that migrate from the surface of the process equipment, exposed to an
2465 appropriate solvent at extreme conditions, into the product or material being processed.
2466
2467 First Air – Refers to filtered air that has not been interrupted by items (such as operators) with the
2468 potential to add contamination to the air prior to reaching the critical zone.
2469
2470 Form-Fill-Seal (FFS) – Similar to Blow fill Seal, this involves the formation of a large tube formed
2471 from a flexible packaging material, in the filling machine, and generally the tube is filled to form the
2472 bags.
2473
2474 Gowning qualification – A program that establishes, both initially and on a periodic basis, the
2475 capability of an individual to don the complete sterile gown in an aseptic manner.
2476
2477 Grade A air supply – Air which is passed through a filter qualified as capable of producing Grade A
2478 non-viable quality air, but where there is no requirement to perform continuous non-viable monitoring
2479 or meet Grade A viable monitoring limits and the area itself is not classified. Specifically used for the
2480 protection of fully stoppered vials where the cap has not been crimped and the equipment and
2481 engineering systems that have a direct impact on product quality.
2482
2483 HEPA filter - High efficiency particulate air filter with 0.3 µm particulate retaining efficiency of no
2484 less than 99.95 percent according to the relevant norms (e.g. EN 1822)..
2485
2486 Inherent interventions – An intervention that is an integral part of the aseptic process and is required
2487 for either set-up, routine operation and/or monitoring (e.g. aseptic assembly, container replenishment,
2488 environmental sampling, etc.). Inherent interventions are required by procedure or work instruction
2489 for the execution of the aseptic process.
2490
2491 Integrity test - A test to confirm that a filter (product, gas or HVAC filter) retain their retentive
2492 properties and have not been damaged during handling, installation or processing.
2493
2494 Intrinsic Sterile Connection device – A device that reduces the risk of contamination during the
2495 connection process; these can be mechanical or fusion sealing.
2496

2497 Isokinetic sampling head – A sampling head designed to disturb the air as little as possible so that the
2498 same particulates go into the nozzle as would have passed the area if the nozzle had it not been there
2499 i.e. the sampling condition in which the mean velocity of the air entering the sample probe inlet is
2500 nearly the same (± 20 percent) as the mean velocity of the airflow at that location.

2501
2502 Isolator – A decontaminated unit, with an internal work zone meeting Grade A conditions that
2503 provides uncompromised, continuous isolation of its interior from the external environment (e.g.
2504 surrounding cleanroom air and personnel). There are two major types of isolators

2505
2506 i. Closed isolator systems exclude external contamination of the isolator's interior by
2507 accomplishing material transfer via aseptic connection to auxiliary equipment, rather than
2508 use of openings to the surrounding environment. Closed systems remain sealed throughout
2509 operations.

2510 ii. Open isolator systems are designed to allow for the continuous or semi-continuous ingress
2511 and/or egress of materials during operations through one or more openings. Openings are
2512 engineered (e.g. using continuous overpressure) to exclude the entry of external contaminant
2513 into the isolator.

2514
2515 Leachables – Chemical entities that migrate into products from the product contact surface of the
2516 process equipment or containers under normal condition of use and/or storage.

2517
2518 Local Isolates – Suitably representative microorganisms of the site that are frequently recovered
2519 through environmental monitoring within the classified zone/areas especially Grade A zone and
2520 Grade B area, personnel monitoring or positive sterility test results.

2521
2522 Lyophilization – A physical-chemical drying process designed to remove solvents, by way of
2523 sublimation, from both aqueous and non-aqueous systems, primarily to achieve product or material
2524 stability. Lyophilization is synonymous to the term freeze-drying.

2525
2526 Manual Filling – A filling process where operator intervention is required to complete the filling of
2527 each container (e.g. as occurs during aseptic compounding operations).

2528
2529 Operator - Any individual participating in the processing operation, including line set-up, filler,
2530 maintenance, or other personnel associated with manufacturing activities.

2531
2532 Overkill sterilization – A process that is sufficient to provide at least a 12 log reduction of
2533 microorganisms having a minimum D value of 1 minute.

2534
2535 Pass-through hatch – Synonymous with airlock (refer to airlock definition) but typically smaller in
2536 size.

2537
2538 Pyrogen – A substance that induces a febrile reaction in a patient.

2539
2540 Rapid transfer system (RTP) – A System used for the transfer of items into RABS and isolators that
2541 minimize the risk to the critical zone. An example would be a rapid transfer container with an
2542 alpha/beta port.

2543
2544 Raw material – Any ingredient intended for use in the manufacture of a sterile product, including
2545 those that may not appear in the final drug product.

2546
2547 Restricted Access Barrier System (RABS) – System that provides an enclosed, but not sealed,
2548 environment meeting defined cleanroom conditions (for aseptic processing Grade A, (but where used
2549 for non-sterile applications can be lesser grade) and using a rigid-wall enclosure and air overspill to
2550 separate its interior from the surrounding environment. The inner surfaces of the RABS are
2551 disinfected and decontaminated with a sporicidal agent. Operators use gloves, half suits, rapid transfer

2552 systems (RTPs) and other integrated transfer ports to perform manipulations or convey materials to
2553 the interior of the RABS. Depending on the design, doors are rarely or never opened:

- 2554
- 2555 i. Active RABS: integral HEPA-filtered air supply.
 - 2556
 - 2557 ii. Passive RABS: air supply by ceiling mounted HEPA-filters.
 - 2558
 - 2559 iii. Closed RABS: where the air is vented in return ducts within the cabinet.
 - 2560
 - 2561 iv. Open RABS: Where there are vents in the barrier that allow air to move from the Grade A
2562 zone to the Grade B area.
 - 2563

2564 Single Use Systems (SUS) – Systems in which product contact components are used only once (i.e.
2565 single use components) to replace reusable equipment such as stainless steel transfer lines or bulk
2566 containers. SUS covered in this document are those that are used in manufacturing processes of sterile
2567 products (e.g. sterile active substance, sterile bio bulk, sterile finished dosage), and are typically made
2568 up of disposable components such as bags, filters, tubing, connectors, storage bottles and sensors.

2569

2570 Sporicidal agent – An agent that destroys bacterial and fungal spores when used in sufficient
2571 concentration for specified contact time. It is expected to kill all vegetative microorganisms.

2572

2573 Sterile Product – For purpose of this guidance, sterile product refers to one or more of the sterilized
2574 elements exposed to aseptic conditions and ultimately making up the sterile active substance or
2575 finished sterile product. These elements include the containers, closures, and components of the
2576 finished drug product. Or, a product that is rendered sterile by a terminal sterilization process.

2577

2578 Sterilizing grade filter – A filter that, when appropriately validated, will remove a defined microbial
2579 challenge from a fluid or gas producing a sterile effluent. Usually such filters have a pore size equal or
2580 less than 0.22 µm (for the purposes of this document 0.2 µm and 0.22 µm are used interchangeably
2581 and deemed equivalent).

2582

2583 Terminal Sterilization – The application of a lethal sterilizing agent or conditions to a product within a
2584 sealed container to achieve a predetermined sterility assurance level (SAL) of 10^{-6} or better (i.e. the
2585 theoretical probability of there being a single viable microorganism present on or in a sterilized unit is
2586 equal to or less than 1×10^{-6} (one in a million)).

2587

2588 Turbulent airflow – Air that is not unidirectional. Turbulent air in cleanrooms should flush the
2589 cleanroom via mixed flow dilution and ensure maintenance of acceptable air quality.

2590

2591 Unidirectional airflow – An airflow moving in a single direction, in a robust and uniform manner, and
2592 at sufficient speed, to reproducibly sweep particulates away from the critical processing or testing
2593 area.

2594

2595 Unidirectional Airflow Unit (UDAF) – A cabinet supplied with filtered unidirectional airflow
2596 (previously referred to as a Laminar Airflow Unit or LAF).

2597

2598 Vertical-Form-Fill-Seal (VFFS) – An automated filling process, typically for terminally sterilized
2599 products, that may utilize a single or dual web system which constructs the primary container out of a
2600 flat roll of thermoplastic film while simultaneously filling the formed bags with product and sealing
2601 the filled bags in a continuous process.

2602

2603 Worst case – A set of conditions encompassing processing limits and circumstances, including those
2604 within standard operating procedures, that pose the greatest chance of process or product failure
2605 (when compared with ideal conditions). Such conditions have the highest potential to, but do not
2606 necessarily always induce, product or process failure.

2607
2608
2609
2610

Water system – A system for producing, storing and distributing water, usually compliant to a specific pharmacopeia grade e.g. purified and water for injection (WFI).