

1 **Annex 1**

2 **Manufacture of Sterile Medicinal Products**

3 **Document map**

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

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6 **1 Scope**

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8 The manufacture of sterile medicinal products covers a wide range of product types, (sterile
9 active substance through to finished dosage form), batch sizes (single unit to multiple units),
10 processes (from highly automated systems to manual processes), primary packaging materials
11 and technologies (e.g. biotechnology, classical small molecule manufacturing and closed
12 systems). This Annex provides general guidance that should be used for all sterile medicinal
13 products and sterile active substances, via adaption, using the principles of Quality Risk
14 Management (QRM), to ensure that microbial, particulate and pyrogen contamination
15 associated with microbes is prevented in the final product.

16

17 **The intent of the Annex is to provide guidance for sterile medicinal products.** However some
18 of the principles and guidance, such as contamination control strategy, room qualification,
19 classification, monitoring and gowning, **may be used to support the manufacture of other**
20 **products that are not intended to be sterile** (such as certain liquids, creams, ointments and low
21 bioburden biological intermediates) but where the control of microbial, particulate and
22 pyrogen contamination, to reduce it as far as possible, is considered important.

23

24 2 Principle

25
26 The manufacture of sterile products is subject to special requirements in order to minimize
27 risks of **microbiological**, **particulate** and **pyrogen** contamination. The following key areas
28 should be considered:

- 29
- 30 a) **Facility**, **equipment** and **process design** must be **optimized**, **qualified** and **validated**
31 according to Annex 11 and Annex15 of EU GMP. The use of appropriate current
32 technologies should be implemented to ensure protection and control of the product
33 from potential extraneous sources of particulate and microbial contamination such as
34 personnel, materials and the surrounding environment.
- 35
- 36 b) **Personnel** must have **appropriate skills**, **training** and **attitudes** with a specific focus
37 on the principles involved in the protection of sterile product during the
38 manufacturing, packaging and distribution processes.
- 39
- 40 c) **Processes** and **monitoring systems** for sterile product manufacture must be **designed**,
41 **commissioned**, **qualified** and **monitored** by personnel with appropriate **process**,
42 **engineering** and **microbiological knowledge**.

43

44 Processes, equipment, facilities and manufacturing activities should be managed in
45 accordance with **QRM principles** that provide a proactive means of identifying, scientifically
46 evaluating and controlling potential risks to quality. **Risk assessments** should be used to
47 justify alternative approaches to those specified in this Annex only if these alternative
48 approaches meet or surpass the intent of this Annex.

49

50 Quality Assurance is particularly important, and manufacture of sterile products must strictly
51 follow carefully established and validated methods of manufacture and control. A
52 **contamination control strategy** should be implemented across the facility in order to assess
53 the effectiveness of all the control and monitoring measures employed. This assessment
54 should lead to corrective and preventative actions being taken as necessary.

55

56 The strategy should consider all aspects of contamination control and its life cycle with
57 ongoing and periodic review and update of the strategy as appropriate.

58

59 Contamination control and steps taken to minimise the risk of contamination from microbial
60 and particulate sources are a series of successively linked events or measures. These are
61 typically assessed, controlled and monitored individually but these many sources should be
62 considered holistically.

63

64 The development of such strategies requires thorough technical and process knowledge.
65 Potential sources of contamination are attributable to microbiological and cellular debris (e.g.
66 pyrogens/endotoxins) as well as particulate matter (glass and other visible and sub-visible
67 particles).

68

69 Elements to be considered within such a documented contamination control strategy should
70 include (but not be limited to):

- 71
- 72 a) **Design** of both the **plant** and **process**.
- 73

- 74 b) Equipment and facilities.
75 c) Personnel.
76
77 d) Utilities.
78
79 e) Raw Materials Control – including in-process controls.
80
81 f) Product containers and closures.
82
83 g) Vendor approval – such as key component suppliers, sterilization of components and
84 single use systems, and services.
85
86 h) For outsourced services, such as sterilization, sufficient evidence should be provided
87 to the contract giver to ensure the process is operating correctly.
88
89 i) Process risk assessment.
90
91 j) Process validation.
92
93 k) Preventative maintenance – maintaining equipment and premises (planned and
94 unplanned maintenance) to a standard that will not add significant risk of
95 contamination.
96
97 l) Cleaning and disinfection.
98
99 m) Monitoring systems - including an assessment of the feasibility of the introduction of
100 scientifically sound, modern methods that optimize the detection of environmental
101 contamination.
102
103 n) Prevention – Trending, investigations, corrective and preventive actions (CAPA),
104 root cause determination and the need for more robust investigational tools.
105
106 o) Continuous improvement based on information from the above systems.
107

108 The manufacturer should take all steps and precautions necessary to assure the sterility of the
109 products manufactured within its facilities. Sole reliance for sterility or other quality aspects
110 must not be placed on any terminal process or finished product test.
111

112 Note 1:

113 This guidance does not lay down detailed methods for determining the microbiological and
114 particulate cleanliness of air, surfaces etc. Reference should be made to other documents such
115 as the EN/ISO Standards and Pharmacopoeial monographs for more detailed guidance.
116

117 Note 2:

118 Where national legislation permits, additional guidance regarding the preparation of
119 unlicensed sterile medicinal products normally performed by healthcare establishments for
120 direct supply to patients, reference may be made to the Annex 1: “Guidelines on the standards
121 required for the sterile preparation of medicinal products” of the PIC/S guide to good
122 practices for the preparation of medicinal products in healthcare establishments, PE 010.
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3 Pharmaceutical Quality System (PQS)

3.1 The manufacture of sterile medicinal products is a complex activity that requires additional controls and measures to ensure the quality of products manufactured. Accordingly, the manufacturer's Pharmaceutical Quality System (PQS) should encompass and address the specific requirements of sterile product manufacture and ensure that all activities are effectively controlled so that all final products are free from microbial and other contamination. In addition to the PQS requirements detailed in chapter 1 of the EU GMPs, the PQS for sterile product manufacturers should also ensure that:

- a) There is an effective **risk management system** integrated into the product life cycle to minimise microbial contamination to ensure the **safety, quality** and **efficacy** of sterile manufactured product, including **assurance of sterility**.
- b) The manufacturer has sufficient knowledge and expertise in relation to the **products** manufactured and the manufacturing **methods** employed.
- c) **Root cause analysis** of procedural, process or equipment failure is key to ensure that the risk to product is correctly understood and suitable corrective and preventative actions are implemented.
- d) **Risk assessment** is performed to **identify, assess, eliminate** (where applicable) and **control** contamination risks to prevent contamination, to monitor and detect contamination, and to establish process requirements and acceptance criteria for all elements of a sterile manufacturing process. The risk assessment should be documented and should include the **rationale** for decisions taken in relation to mitigating risks, discounting of potential risks and residual risk. The risk assessment should be reviewed regularly as part of on-going quality management, during change control and during the periodic product quality review.
- e) Processes associated with the finishing and transport of sterile products should not compromise the finished sterile product in terms of container integrity or pose a risk of contamination and ensure that medicinal products are stored and maintained in accordance with registered storage conditions.
- f) Persons responsible the quality release of sterile medicines should have appropriate access to manufacturing and quality information and possess adequate knowledge and experience in the manufacture of sterile dosage forms and their critical quality attributes in order to be able to ascertain that the medicines have been manufactured in accordance with the registered specification and are of the required safety, quality and efficacy.

3.2 Investigations should be performed into non-conformities, such as sterility test failures or environmental monitoring excursions or deviations from established procedures, with a specific focus regarding the potential impact to sterility, to not only the specific batch concerned but also any other potentially impacted batch. The reasons for including or excluding product from the scope of the investigation should be clearly recorded and justified within the investigation.

174 **4 Personnel**

175 4.1 The manufacturer should ensure that there are sufficient appropriate personnel, suitably
176 qualified and experienced in the manufacture and testing of sterile medicines and any of the
177 specific manufacturing technologies used in the site's manufacturing operations, to ensure
178 compliance with Good Manufacturing Practice applicable to the manufacture of sterile
179 medicinal products.

180
181 4.2 Only the minimum number of personnel required should be present in cleanrooms. The
182 maximum number of operators in critical areas should be determined based on QRM
183 principles, documented in the contamination control strategy, and validated during activities
184 such as initial qualification and aseptic process simulations, so as not to compromise
185 sterility assurance. This is particularly important during aseptic processing. Inspections and
186 controls should be conducted outside the clean areas as far as possible.

187
188 4.3 All personnel (including those performing cleaning and maintenance) employed in such
189 areas should receive regular training, qualification (including sampling of the operators
190 bioburden, using methods such as contact plates, at key locations e.g. hands arms and chest)
191 and assessment in disciplines relevant to the correct manufacture of sterile products. This
192 training should include reference to hygiene, cleanroom practices, contamination control,
193 aseptic techniques, and potential safety implications to the patient of a loss of product
194 sterility and in the basic elements of microbiology.

195
196 4.4 The personnel working in a grade A/B cleanroom should be trained for aseptic gowning
197 and aseptic practices. Compliance with aseptic gowning procedures should be assessed and
198 confirmed and this should be periodically reassessed at least annually and should involve
199 both visual and microbiological assessment (using additional locations such as arms and
200 chest). Only trained personnel who have passed the gowning assessment and have
201 participated in a successful aseptic process simulation (APS) test, during which they
202 performed their normal duties, should be authorized to enter any grade A/B area, in which
203 aseptic operations will be conducted, or are being conducted, whilst unsupervised. The
204 microbial monitoring of personnel in the grade A/B area should be performed to assess their
205 aseptic behaviour. This monitoring should take place immediately after completion of a
206 critical intervention and upon each exit from the cleanroom. It should be noted that there
207 should also be an ongoing continuous monitoring program for personnel including some
208 consideration of periodic monitoring under the supervision of the quality unit.

209
210 4.5 There should be systems in place for disqualification of personnel from entry into
211 cleanrooms, based on aspects including ongoing assessment and/or the identification of an
212 adverse trend from the personnel monitoring program. Once disqualified, retraining and
213 requalification is required before permitting the operator to have any further involvement in
214 aseptic practices. This should include consideration of participation in a successful Aseptic
215 Process Simulation (APS).

216
217 4.6 Manufacturers should establish written procedures outlining the process by which
218 outside staff who have not received such training (e.g. building or maintenance contractors)
219 need to be brought into grade A/B areas. Access by these persons should only be given in
220 exceptional circumstances, evaluated and recorded in accordance with the PQS.

221
222 4.7 High standards of personal hygiene and cleanliness are essential. Personnel involved in

223 the manufacture of sterile preparations should be instructed to report any specific health
224 conditions or ailments which may cause the shedding of abnormal numbers or types of
225 contaminants and therefore preclude clean room access; periodic health checks for such
226 conditions should be performed. Actions to be taken with regard to personnel who
227 could be introducing an undue microbiological hazard should be described in
228 procedures decided by a designated competent person.
229

230 4.8 Staff who have been engaged in the processing of human or animal tissue materials or of
231 cultures of micro-organisms, other than those used in the current manufacturing process, or
232 any activities that may have a negative impact to quality, e.g. microbial contamination,
233 should not enter sterile product areas unless rigorous, clearly defined and effective entry
234 procedures have been followed.
235

236 4.9 Wristwatches, make-up and jewellery and other personal items such as mobile phones
237 should not be allowed in clean areas.
238

239 4.10 Changing and hand washing should follow a written procedure designed to minimize
240 contamination of clean area clothing or carry-through of contaminants to the clean areas.
241 Garments should be visually checked for cleanliness and integrity prior to entry to the clean
242 room. For sterilized garments, particular attention should be taken to ensure that garments
243 and eye coverings have been sterilized and that their packaging is integral before use. Re-
244 usable garments should be replaced based at a set frequency determined by qualification or if
245 damage is identified.
246

247 4.11 The clothing and its quality should be appropriate for the process and the grade of
248 the working area. It should be worn in such a way as to protect the product from
249 contamination.
250

251 4.12 The description of clothing required for each grade is given below:
252

253 a) Grade D: Hair, beards and moustaches should be covered. A general protective suit
254 and appropriately disinfected shoes or overshoes should be worn. Appropriate
255 measures should be taken to avoid any contamination coming from outside the clean
256 area.
257

258 b) Grade C: Hair, beards and moustaches should be covered. A single or two-piece
259 trouser suit gathered at the wrists and with high neck and appropriately disinfected or
260 sterilized shoes or overshoes should be worn. They should shed virtually no
261 fibres or particulate matter.
262

263 c) Grade A/B: Sterile headgear should totally enclose hair and facial hair; it should be
264 tucked into the neck of the sterile suit; a sterile face mask and sterile eye coverings
265 should be worn to cover all facial skin and prevent the shedding of droplets and
266 particles. Appropriate sterilized, non-powdered rubber or plastic gloves and
267 sterilized footwear should be worn. Trouser-legs should be tucked inside the
268 footwear and garment sleeves into the gloves. The protective clothing should shed
269 virtually no fibres or particulate matter and retain particles shed by the body.
270 Garments should be packed and folded in such a way as to allow operators to change
271 into the garments with contact to the outer surfaces of the garment reduced to a
272 minimum.

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Note: This is minimum guidance and higher standards of clothing may be required dependent on the processes performed in the specific area.

4.13 Outdoor clothing should not be brought into changing rooms leading to grade B and C rooms. It is recommended that facility suits, including dedicated socks be worn before entry to change rooms for grade C and B. Where clothing is reused this should be considered as part of the qualification.

4.14 For every worker in a grade A/B area, clean sterilized protective garments (including eye coverings and masks) of an appropriate size should be provided at each work session. Gloves should be regularly disinfected during operations. Garments and gloves should be changed at least for every working session.

4.15 Clean area clothing should be cleaned, handled and worn in such a way that it does not gather additional contaminants which can later be shed. These operations should follow written procedures. Separate laundry facilities for such clothing are desirable. Inappropriate treatment of clothing will damage fibres and may increase the risk of shedding of particles. After washing and before sterilization, garments should be checked for integrity.

4.16 Activities in clean areas, especially when aseptic operations are in progress, should be kept to a minimum and movement of personnel should be controlled and methodical to avoid excessive shedding of particles and organisms due to over-vigorous activity. Operators performing aseptic operations should adhere to strict aseptic technique at all times. To prevent changes in air currents that introduce lower quality air, movement adjacent to the critical area should be restricted and the obstruction of the path of the unidirectional airflow must be avoided. The ambient temperature and humidity should be set to prevent shedding due to operators becoming too cold (leading to excessive movement) or too hot.

5 Premises

5.1 The manufacture of sterile products should be carried out in clean areas, entry to which should be through airlocks for personnel and/or for equipment and materials. Clean areas should be maintained to an appropriate cleanliness standard and supplied with air which has passed through filters of an appropriate efficiency.

5.2 The various operations of component preparation, product preparation and filling should be carried out with appropriate technical and operational separation measures within the clean area.

5.3 For the manufacture of sterile medicinal products 4 grades of clean room can be distinguished.

Grade A: The local zone for high risk operations, e.g. filling zone, stopper bowls, open ampoules and vials, making aseptic connections. Normally, such conditions are provided by a localised air flow protection, such as laminar air flow work stations or isolators. Unidirectional air flow systems should provide a homogeneous air speed in a range of 0.36 – 0.54 m/s (guidance value), the point at which the air speed

323 measurement is taken should be clearly justified in the protocol. During initial
324 qualification and requalification air speeds may be measured either close to the
325 terminal air filter face or at the working height, Where ever the measurement is taken
326 it is important to note that the key objective is to ensure that air visualization studies
327 should correlate with the airspeed measurement to demonstrate air movement that
328 supports protection of the product and open components with unidirectional air at the
329 working height, where high risk operations and product and components are exposed.
330 The maintenance of unidirectional airflow should be demonstrated and validated
331 across the whole of the grade A area. Entry into the grade A area by operators should
332 be minimized by facility, process and procedural design.

334 Grade B: For aseptic preparation and filling, this is the background environment for
335 the grade A zone. In general, only grade C cleanrooms should interface with the grade
336 B aseptic processing area.

337 Lower grades can be considered where isolator technology is used (refer to clause
338 5.19-5.20).

340 Grade C and D: Clean areas for carrying out less critical stages in the manufacture of
341 sterile products.

342
343 5.4 In clean areas, all exposed surfaces should be smooth, impervious and unbroken in
344 order to minimize the shedding or accumulation of particles or micro-organisms and to
345 permit the repeated application of cleaning agents, and disinfectants, where used.

346
347 5.5 To reduce accumulation of dust and to facilitate cleaning there should be no uncleanable
348 recesses and a minimum of projecting ledges, shelves, cupboards and equipment. Doors
349 should be designed to avoid uncleanable recesses.

350
351 5.6 Materials liable to generate fibres should not be permitted in clean areas

352
353 5.7 False ceilings should be designed and sealed to prevent contamination from the space
354 above them.

355
356 5.8 Sinks and drains should be prohibited in grade A/B areas. In other areas air breaks
357 should be fitted between the machine or sink and the drains. Floor drains in lower grade
358 rooms should be fitted with traps or water seals to prevent back flow and should be regularly
359 cleaned and disinfected.

360
361 5.9 Airlocks should be designed and used to provide physical separation and to minimize
362 microbial and particulate contamination of the different areas, and should be present for
363 material and personnel moving from different grades, typically airlocks used for personnel
364 movement are separate to those used for material movement. They should be flushed
365 effectively with filtered air. The final stage of the airlock should, in the at-rest state, be the
366 same grade as the area into which it leads. The use of separate changing rooms for entering
367 and leaving clean areas is generally desirable.

368
369 a) Personnel airlocks. A cascade concept should be followed for personnel (e.g. from
370 grade D to grade C to grade B). In general hand washing facilities should be
371 provided only in the first stage of the changing rooms.

372

- 373 b) Material airlocks (used for materials and equipment).
374
375 i. Pass through hatches without active filtered air supply should be avoided. If
376 necessary, provisions and procedures should be in place to avoid any risk of
377 contamination (e.g. by the incoming material or by entering air).
378
379 ii. For airlocks leading to grade A and B areas, only materials and equipment that
380 have been included as part of the qualification list should be allowed to be
381 transferred into the grade A/B area via the air lock or pass through; the
382 continuity of grade A should be maintained in the aseptic core when the
383 materials have to be transferred from grade B to grade A areas, consideration
384 should be given to listing these items on an authorized list. Any unapproved
385 items that require transfer should be an exception. Appropriate risk evaluation
386 and mitigation strategies should be applied and recorded as per the
387 manufacturer's contamination control strategy and should include a specific
388 sanitisation and monitoring regime approved by quality assurance.
389
390 iii. The movement of material from clean not classified (CNC) to grade C should
391 be based on QRM principles, with cleaning and disinfection commensurate
392 with the risk.
393

394 5.10 Both airlock doors should not be opened simultaneously. The opening of more than
395 one door at a time should be prevented, for airlocks leading to grade A and B an interlocking
396 system should usually be used; for airlocks leading to grade C and D at least a visual and/or
397 audible warning system should be operated. Where required to maintain zone segregation, a
398 time delay between the closing and opening of interlocked doors should be established.
399

400 5.11 A HEPA or ULPA filtered air supply should maintain a positive pressure and an
401 air flow relative to surrounding areas of a lower grade under all operational conditions and
402 should flush the area effectively. Adjacent rooms of different grades should have a pressure
403 differential of 10 - 15 Pascals (guidance values). Particular attention should be paid to the
404 protection of the zone of greatest risk, that is, the immediate environment to which a
405 product and cleaned components which contact the product are exposed. The
406 recommendations regarding air supplies and pressure differentials may need to be
407 modified where it becomes necessary to contain some materials, e.g. pathogenic, highly
408 toxic, radioactive or live viral or bacterial materials or products. Decontamination of
409 facilities, e.g. the clean rooms and HVAC, and the treatment of air leaving a clean area
410 may be necessary for some operations.
411

412 5.12 It should be demonstrated that air-flow patterns do not present a contamination risk,
413 e.g. care should be taken to ensure that air flows do not distribute particles from a particle-
414 generating person, operation or machine to a zone of higher product risk.

415 Air flow patterns should be visualised in grade A/B areas to evaluate if airflow is
416 unidirectional. Where unidirectional air flow is not demonstrated, corrective actions, such as
417 design improvements, should be implemented. In the other areas, the need to demonstrate
418 the air flow patterns should be based on a risk assessment. Air flow pattern studies should be
419 performed under dynamic conditions. Video recordings of the airflow patterns are
420 recommended. The outcome of the air visualisation studies should be considered when
421 establishing the facility's environmental monitoring program.
422

423 5.13 A warning system should be provided to indicate failure in the air supply and reduction
424 of pressure differentials below set limits. Indicators of pressure differences should be fitted
425 between areas, based on QRM principles. These pressure differences should be recorded
426 regularly or otherwise documented.

427
428 5.14 Consideration should be given to designing facilities that permit observation of
429 activities from outside the clean areas, e.g. through the provision of windows or remote
430 camera access with a complete view of the area and processes to allow observation and
431 supervision without entry.

432 433 **Barrier Technologies** 434

435 5.15 Isolator or Restricted Access Barrier System (RABS) technologies, and the associated
436 processes, should be designed so as to provide maximum protection of the grade A
437 environment. The transfer of materials into and out of the RABS or isolator is one of the
438 greatest potential sources of contamination and therefore the entry of additional materials
439 following sterilisation should be minimized. Any activities that potentially compromise the
440 sterility assurance of the critical zone should be assessed and controls applied if they cannot
441 be eliminated.

442
443 5.16 The design of the RABS or isolator shall take into account all critical factors associated
444 with these technologies, including the quality of the air inside and the surrounding area, the
445 materials and component transfer, the decontamination, disinfection or sterilization processes
446 and the risk factors associated with the manufacturing operations and materials, and the
447 operations conducted within the critical zone.

448
449 5.17 The critical zone of the RABS or isolator used for aseptic processes should meet grade
450 A with unidirectional air flow. Under certain circumstances turbulent airflow may be justified
451 in a closed isolator when proven to have no negative impact on the product. The design of the
452 RABS and open isolators should ensure a positive airflow from the critical zones to the
453 surrounding areas; negative pressure isolators should only be used when containment of the
454 product is considered essential.

455
456 5.18 For RABS, the background environment should meet grade B. For open RABS, or
457 where doors may be very rarely opened during processing, and studies should be performed
458 to demonstrate the absence of air ingress.

459
460 5.19 For open, positive pressure isolators or closed isolators with decontamination by a
461 sporicidal agent, the surrounding area should correspond to a minimum of grade D. The
462 disinfection regime should be included as a key consideration when performing the risk
463 assessment to design the contamination control strategy for an isolator.

464
465 5.20 For isolators, the required background environment can vary depending on the design of
466 the isolator, its application and the methods used to achieve bio-decontamination.

467 The decision as to the supporting background environment should be documented in a risk
468 assessment where additional risks are identified, such as for negative pressure isolators.
469 Where items are introduced to the isolator after disinfection then a higher grade of
470 background should be considered.

471

472 5.21 Glove systems, as well as other parts of an isolator, are constructed of various materials
 473 that can be prone to puncture and leakage. The materials used shall be demonstrated to have
 474 good mechanical and chemical resistance. Integrity testing of the barrier systems and leak
 475 testing of the isolator and the glove system should be performed using visual, mechanical and
 476 physical methods. They should be performed at defined periods, at a minimum of the
 477 beginning and end of each batch, and following any intervention that may affect the integrity
 478 of the unit.

479
 480 5.22 Decontamination processes of an isolator or RABS should be validated and controlled in
 481 accordance with defined parameters. Evidence should also be available to demonstrate that
 482 the agent does not affect any process performed in the isolator or RABS, such as having an
 483 adverse impact on product or sterility testing.

484
 485 **Clean room and clean air device qualification**
 486

487 5.23 Clean rooms and clean air devices (clean areas) for the manufacture of products
 488 should be qualified according to the required characteristics of the environment. Each
 489 manufacturing operation requires an appropriate environmental cleanliness level in the
 490 operational state in order to minimize the risks of particulate or microbial contamination
 491 of the product or materials being handled.

492
 493 Note: Classification is a method of assessing the level of air cleanliness against a
 494 specification for a cleanroom or clean area device by measuring the airborne particle
 495 concentration. The classification is part of the qualification of a clean area.

496
 497 5.24 Clean rooms and clean air devices should be qualified in accordance with Annex 15 of
 498 EU GMP. Reference for the classification of the clean rooms and clean air devices can be
 499 found in the ISO 14644 series of standards.

500
 501 5.25 For classification, the airborne particles equal to or greater than 0.5 µm should be
 502 measured. This measurement should be performed both at rest and in operation. The
 503 maximum permitted airborne particle concentration for each grade is given in table 1.
 504

505 Table 1: **Maximum permitted airborne particle concentration during classification**

Grade	Maximum permitted number of particles equal to or greater than 0.5 µm		
	At rest equal to or greater than 0.5 µm per m ³	In operation equal to or greater than 0.5 µm per m ³	ISO classification in operation/at rest
A	3 520	3 520	5/5
B	3 520	352 000	5/7
C	352 000	3 520 000	7/8
D	3 520 000	Not defined ^(a)	8

506

507 (a) For grade D, no “in operation” limits are defined; the company should establish in
508 operation limits based on a risk assessment and on historical data, where applicable.
509

510 5.26 For initial classification the minimum number of sampling locations can be found in ISO
511 14644 Part 1. However, a higher number of samples and sample volume is typically required
512 for the aseptic processing room and the immediately adjacent environment (grade A/B) to
513 include consideration of all critical processing locations such as point of fill stopper bowls.
514 With the exception of the aseptic processing room, the sampling locations should be
515 distributed evenly throughout the area of the clean room. For later stages of qualification and
516 classification, such as performance qualification, locations should be based on a documented
517 risk assessment and knowledge of the process and operations to be performed in the area
518

519 a) The “in operation” and “at rest” states should be defined for each clean room or suite
520 of clean rooms.
521

522 b) The definition of “at rest” is the room complete with all HVAC systems, utilities
523 functioning and with manufacturing equipment installed as specified but without
524 personnel in the facility and the manufacturing equipment is static.
525

526 c) The “in operation” state is the condition where the installation is functioning in the
527 defined operating mode with the specified number of personnel working.
528

529 d) “In operation” classification, qualification and requalification may be performed
530 during normal operations, simulated operations or during aseptic process simulations
531 (where worst case simulation is required).
532

533 e) The particle limits given in Table 1 above for the “at rest” state should be achieved
534 after a “clean up” period on completion of operations. The "clean up" period should
535 be determined during the initial classification of the rooms.
536

537 f) In order to meet “in operation” conditions these areas should be designed to
538 reach certain specified air-cleanliness levels in the “at rest” occupancy state.
539

540 5.27 The microbial load of the clean rooms should be determined as part of the clean room
541 qualification. The recommended maximum limits for microbial contamination during
542 qualification for each grade are given in table 2.
543

544 Table 2: **Recommended limits for microbial contamination in operation**

Grade	air sample cfu/m ³	settle plates (diameter 90 mm) cfu/4 hours ^(a)	contact plates (diameter 55 mm) cfu/plate
A ^(b)	1	1	1
B	10	5	5
C	100	50	25
D	200	100	50

545
546 (a) Individual settle plates may be exposed for less than 4 hours. Where settle plates are
547 exposed for less than 4 hours the limits in the table should still be used, no

548 recalculation is necessary. Settle plates should be exposed for the duration of critical
549 operations and changed as required after 4 hours.

550 (b) It should be noted that for grade A the expected result should be 0 cfu recovered;
551 any recovery of 1 cfu or greater should result in an investigation.

552 Note: For qualification of personnel, the limits given for contact plates and glove
553 prints in table 6 should be applied.

554
555 5.28 Clean room qualification (including classification) should be clearly differentiated from
556 operational process environmental monitoring.

557
558 5.29 Clean rooms should be requalified periodically and after changes to equipment, facility
559 or processes based on the principles of QRM. For grade A and B zones, the maximum time
560 interval for requalification is 6 months. For grades C and D, the maximum time interval for
561 requalification is 12 months.

562
563 5.30 Other characteristics, such as temperature and relative humidity, depend on the product
564 and nature of the operations carried out. These parameters should not interfere with the
565 defined cleanliness standard.

566 **Disinfection**

567
568
569 5.31 The disinfection of clean areas is particularly important. They should be cleaned and
570 disinfected thoroughly in accordance with a written programme (for disinfection to be
571 effective, cleaning to remove surface contamination must be performed first)., More than one
572 type of disinfecting agent should be employed, and should include the periodic use of a
573 sporicidal agent. Disinfectants should be shown to be effective for the duration of their in use
574 shelf-life taking into consideration appropriate contact time and the manner in and surfaces
575 on which they are utilized. Monitoring should be undertaken regularly in order to show the
576 effectiveness of the disinfection program and to detect the development of resistant and/or
577 spore forming strains. Cleaning programs should be effective in the removal of disinfectant
578 residues.

579
580 5.32 Disinfectants and detergents should be monitored for microbial contamination;
581 dilutions should be kept in previously cleaned containers and should only be stored for
582 defined periods. Disinfectants and detergents used in grade A and B areas should be sterile
583 prior to use.

584
585 5.33 Disinfectants should be shown to be effective when used on the specific facilities,
586 equipment and processes that they are used in.

587
588 5.34 Fumigation or vapour disinfection of clean areas such as Vapour Hydrogen Peroxide
589 (VHP) may be useful for reducing microbiological contamination in inaccessible places.

590 **6 Equipment**

591
592
593 6.1 A written, detailed description of the equipment design should be produced (including
594 diagrams as appropriate) and kept up to date. It should describe the product and other critical
595 gas and fluid pathways and controls in place.

596

597 6.2 Equipment monitoring requirements should be determined during qualification. Process
598 alarm events should be reviewed and approved and evaluated for trends.

599
600 6.3 As far as practicable equipment, fittings and services should be designed and installed so
601 that operations, maintenance, and repairs can be carried out outside the clean area, if
602 maintenance has to be performed in the clean area then precautions such as additional
603 disinfection and additional environmental monitoring should be considered. If sterilization is
604 required, it should be carried out, wherever possible, after complete reassembly.

605
606 6.4 When equipment maintenance has been carried out within the clean area, the area
607 should be cleaned, disinfected and/or sterilized where appropriate, before processing
608 recommences if the required standards of cleanliness and/or asepsis have not been
609 maintained during the work.

610
611 6.5 The cleaning process should be validated so that it can be demonstrated that it:

- 612
613 a) Can remove any residues that would otherwise create a barrier between the
614 sterilizing agent and the equipment surfaces.
615
616 b) Prevents chemical and particulate contamination of the product during the process
617 and prior to disinfection.

618
619 6.6 All critical surfaces that come into direct contact with sterile materials should be sterile.

620
621 6.7 All equipment such as sterilizers, air handling and filtration systems, water
622 treatment, generation, storage and distribution systems should be subject to qualification,
623 monitoring and planned maintenance; their return to use should be approved.

624
625 6.8 A conveyor belt should not pass through a partition between a grade A or B area and
626 a processing area of lower air cleanliness, unless the belt itself is continually sterilized (e.g.
627 in a sterilizing tunnel).

628
629 6.9 Particle counters should be qualified (including sampling tubing). Portable particle
630 counters with a short length of sample tubing should be used for qualification purposes.
631 Isokinetic sample heads shall be used in unidirectional airflow systems.

632
633 6.10 Where unplanned maintenance of equipment critical to the sterility of the product is to
634 be carried out, an assessment of the potential impact to the sterility of the product should be
635 performed and recorded.

636
637 **7 Utilities**

638
639 7.1 The nature and amount of controls associated with utilities should be commensurate with
640 the risk associated with the utility determined via risk assessment.

641
642 7.2 In general higher risk utilities are those that:

- 643
644 a) Directly contact product e.g. compressed gases.
645
646 b) Contact materials that ultimately will become part of the product.

647
648 c) Control contamination of surfaces that contact the product.
649
650 d) Or otherwise directly impact the product.
651
652 7.3 Utilities should be installed, operated and maintained in a manner to ensure the utility
653 functions as expected.
654
655 7.4 Results for critical parameters of the high risk utility should be subject to regular trend
656 analysis to ensure that system capabilities remain appropriate.
657
658 7.5 Current drawings should be available that identify critical system attributes such as:
659 pipeline flow, pipeline slopes, pipeline diameter and length, tanks, valves, filters, drains and
660 sampling points.
661
662 7.6 Pipes and ducts and other utilities should be installed so that they do not create
663 recesses, unsealed openings and surfaces which are difficult to clean.
664
665 **Water systems**
666
667 7.7 Water treatment plants and distribution systems should be designed, constructed and
668 maintained to minimize the risk of microbial contamination and proliferation so as to ensure a
669 reliable source of water of an appropriate quality. Water produced should comply with the
670 current monograph of the relevant Pharmacopeia.
671
672 7.8 Water for injections (WFI) should be produced from purified water, stored and distributed
673 in a manner which prevents microbial growth, for example by constant circulation at a
674 temperature above 70°C. Where the WFI is produced by methods other than distillation
675 further techniques post Reverse osmosis (RO) membrane should be considered such as
676 nanofiltration, and ultra-filtration.
677
678 7.9 Water systems should be validated to maintain the appropriate levels of physical,
679 chemical and microbial control, taking seasonal variation into account.
680
681 7.10 Water flow should remain turbulent through the pipes to prevent microbial adhesion.
682
683 7.11 The water system should be configured to prevent the proliferation of microorganisms,
684 e.g. sloping of piping to provide complete drainage and the avoidance of dead legs. Where
685 filters are included in the system, special attention should be taken with regards to the
686 monitoring and maintenance of these filters.
687
688 7.12 Where WFI storage tanks are equipped with hydrophobic bacteria retentive vent filters
689 the filters should be sterilized, and the integrity of the filter tested before and after use.
690
691 7.13 To prevent the formation of biofilms, sterilization or disinfection or regeneration of
692 water systems should be carried out according to a predetermined schedule and also when
693 microbial counts exceed action and alert limits. Disinfection of a water system with
694 chemicals should be followed by a validated rinsing procedure. Water should be analyzed
695 after disinfection/regeneration; results should be approved before the start of use of the
696 water system.

697
698 7.14 A suitable sampling schedule should be in place to ensure that representative water
699 samples are obtained for analysis on a regular basis.
700

701 7.15 Regular ongoing chemical and microbial monitoring of water systems should be
702 performed with alert limits based on the qualification that will identify an adverse trend in
703 the performance of the systems. Sampling should include all outlets and user points at a
704 specified interval. A sample from the worst case sample point, e.g. the end of the
705 distribution loop return, should be included each time the water is used for manufacturing
706 and manufacturing processes. A breach of an alert limit should trigger review and follow-up,
707 which might include investigation and corrective action. Any breach of an action limit
708 should lead to a root cause investigation and risk assessment.
709

710 7.16 WFI systems should include continuous monitoring systems such as Total Organic
711 Carbon (TOC) and conductivity.
712

713 **Steam used for sterilization**

714
715 7.17 Purified water, with a low level of endotoxin, should be used as the minimum quality
716 feed water for the pure steam generator.
717

718 7.18 Steam used for sterilization processes should be of suitable quality and should not
719 contain additives at a level which could cause contamination of product or equipment. The
720 quality of steam used for sterilization of porous loads and for Steam-In-Place (SIP) should
721 be assessed periodically against validated parameters. These parameters should include
722 consideration of the following examples: non-condensable gases, dryness value (dryness
723 fraction), superheat and steam condensate quality.
724

725 **Compressed gases and vacuum systems**

726
727 7.19 Compressed gases that come in direct contact with the product/container primary
728 surfaces should be of appropriate chemical, particulate and microbiological purity, free from
729 oil with the correct dew point specification and, where applicable, comply with appropriate
730 pharmacopoeial monographs. Compressed gases must be filtered through a sterilizing filter
731 (with a nominal pore size of a maximum of 0.22µm) at the point of use. Where used for
732 aseptic manufacturing, confirmation of the integrity of the final sterilization gas filter should
733 be considered as part of the batch release process.
734

735 7.20 There should be prevention of backflow when any vacuum or pressure system is shut
736 off.
737

738 **Cooling systems**

739
740 7.21 Major items of equipment associated with hydraulic and cooling systems should, where
741 possible, be located outside the filling room. Where they are located inside the filling room
742 there should be appropriate controls to contain any spillage and/or cross contamination
743 associated with the hydraulics of cooling system fluids.
744

745 7.22 Any leaks from the cooling system must be detectable (i.e. an indication system for
746 leakage). In addition, there must be adequate cooling flow within the system.

747

748 7.23 The cooling circuit should be subject to leak testing both periodically and following any
749 maintenance.

750

751 7.24 There should be periodic cleaning/disinfection of both the vacuum system and cooling
752 systems.

753

754 **8 Production and Specific Technologies**

755

756 **Terminally sterilized products**

757

758 8.1 Preparation of components and most products should be done in at least a grade
759 D environment in order to give a low risk of microbial, pyrogen and particulate
760 contamination, so that the product is suitable for filtration and sterilization. Where the
761 product is at a high or unusual risk of microbial contamination, (for example, because the
762 product actively supports microbial growth and/or must be held for a long periods before
763 sterilisation and/or is not processed mainly in closed vessels), then preparation should be
764 carried out in a grade C environment.

765

766 8.2 Filling of products for terminal sterilization should be carried out in at least a grade
767 C environment.

768

769 8.3 Where the product is at an unusual risk of contamination from the environment because, for
770 example, the filling operation is slow, the containers are wide necked or are necessarily
771 exposed for more than a few seconds before closing, or the product is held for extended periods
772 prior to terminal sterilization, then the product should be filled in a grade A zone with at least a
773 grade C background. Preparation and filling of ointments, creams, suspensions and
774 emulsions should generally be carried out in a grade C environment before terminal
775 sterilization.

776

777 8.4 Processing of the bulk solution should include a filtration step to reduce bioburden levels
778 and particulates prior to filling into the final product containers.

779

780 8.5 Examples of operations to be carried out in the various grades are given in table 3.

781

782 **Table 3: Examples of operations and grades they should be performed in for**
783 **terminally sterilized products**

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.

784

785 **Aseptic preparation**

786

787 8.6 Aseptic processing is the handling of sterile product, containers and/or devices in a
788 controlled environment, in which the air supply, materials and personnel are regulated to
789 prevent microbial contamination. Additional requirements apply to Restricted Access Barrier
790 Systems (RABS) and isolators (refer clauses 5.15-5.22).

791

792 8.7 The aseptic process should be clearly defined. The risks associated with the aseptic
793 process, and any associated requirements, should be identified, assessed and appropriately
794 controlled. The site's contamination control strategy should clearly define the acceptance
795 criteria for these controls, requirements for monitoring and the review of their effectiveness.
796 Methods and procedures to control these risks should be described and implemented.
797 Residual risks should be justified.

798

799 8.8 Precautions to minimise microbiological, pyrogen and particulate contamination

800 should be taken, as per the site's contamination control strategy, during the preparation of
 801 the aseptic environment, during all processing stages, including the stages before and after
 802 filter sterilization, and until the product is sealed in its final container. Materials liable to
 803 generate fibres should not be permitted in clean areas.

804
 805 8.9 Where possible, the use of equipment such as RABS, isolators or closed systems, should
 806 be considered in order to reduce the need for interventions into the grade A environment and
 807 minimize the risk of contamination. Automation of processes should also be considered to
 808 remove the risk of contamination by interventions (e.g. dry heat tunnel, automated lyophilizer
 809 loading, SIP).

810
 811 8.10 Examples of operations to be carried out in the various environmental grades are given in
 812 the table 4.

813
 814 **Table 4: Examples of operations and which grades they should be performed in**
 815

A	Critical processing zone. Aseptic assembly of filling equipment. Aseptic connections (should be sterilized by steam-in-place whenever feasible). Aseptic compounding and mixing. Replenishment of sterile product, containers and closures. Removal and cooling of items from heat sterilizers. Staging and conveying of sterile primary packaging components. Aseptic filling, sealing, transfer of open or partially stoppered vials, including interventions. Loading and unloading of a lyophilizer
B	Direct support zone for the critical processing (grade A) zone. Transport and preparation of packaged equipment, components and ancillary items for introduction into the grade A zone. Removal of sealed product from the grade A zone.
C	Preparation of solutions to be filtered.
D	Cleaning of equipment. Handling of components, equipment and accessories after washing. Assembly of cleaned equipment to be sterilized.

816
 817 Note: If Isolators are used then a risk assessment should determine the necessary
 818 background environment grade; at least a minimum of grade D should be used. Refer
 819 clauses 5.19-5.20.

820
 821 8.11 Where the product is not subsequently sterile filtered, the preparation of equipment,
 822 components and ancillary items and products should be done in a grade A environment with
 823 a grade B background.

824
 825 8.12 Preparation and filling of sterile products such as ointments, creams, suspensions and
 826 emulsions should be performed in a grade A environment, with a grade B background, when
 827 the product and components are exposed and the product is not subsequently filtered or
 828 sterilized.

829

830 8.13 Unless subsequently sterilized by steam-in-place or conducted with validated intrinsic
831 sterile connection devices, aseptic connections should be performed in a grade A
832 environment with a grade B background (or in an isolator with a suitable background), in a
833 way that minimizes the potential contamination from the immediate environment, e.g. from
834 operators or boundaries with lower grades. Aseptic connections, including those performed to
835 replace equipment, should be appropriately assessed and their effectiveness verified as
836 acceptable by process simulation tests. (For requirements regarding intrinsic sterile
837 connection devices (refer clause 8.115).

838

839 8.14 The transfer of partially closed containers to a lyophilizer, should be done under
840 grade A conditions (e.g. HEPA filtered positive pressure) at all times and, where possible,
841 without operator intervention. Portable transfer systems (e.g. transfer carts, portable Laminar
842 Flow Work Stations, etc.) should ensure that the integrity of transfer system is maintained
843 and the process of transfer should minimize the risk of contamination.

844

845 8.15 Aseptic manipulations (including non-intrinsic aseptic connections) should be
846 minimized using engineering solutions such as the use of preassembled and sterilized
847 equipment. Whenever feasible, product contact piping and equipment should be pre-
848 assembled, then cleaned and sterilized in place. The final sterile filtration should be carried
849 out as close as possible to the filling point and downstream of aseptic connections wherever
850 possible

851

852 8.16 The duration for each aspect of the aseptic manufacturing process should be limited to a
853 defined and validated maximum, including:

854

855 a) Time between equipment, component, and container cleaning, drying and
856 sterilization.

857

858 b) Holding time for sterilized equipment, components, and containers prior to and
859 during filling/assembly.

860

861 c) The time between the start of the preparation of a solution and its sterilization or
862 filtration through a micro-organism-retaining filter. There should be a set maximum
863 permissible time for each product that takes into account its composition and the
864 prescribed method of storage.

865

866 d) Aseptic assembly.

867

868 e) Holding sterile product prior to filling.

869

870 f) Filling.

871

872 g) Maximum exposure time of sterilized containers and closures in the critical
873 processing zone (including filling) prior to closure.

874

875 **Finishing of sterile products**

876

877 8.17 Partially stoppered vials or prefilled syringes should be maintained under grade A
878 conditions (e.g. use of isolator technology, grade A with B background, with physical
879 segregation from operators) or grade A LAF carts (with suitable grade B background

880 environment and physical segregation from operators) at all times until the stopper is fully
881 inserted.

882

883 8.18 Containers should be closed by appropriately validated methods. Containers closed
884 by fusion, e.g. Form-Fill-Seal Small Volume Parenteral (SVP) & Large Volume
885 Parenteral (LVP) bags, glass or plastic ampoules, should be subject to 100% integrity
886 testing. Samples of other containers should be checked for integrity utilising validated
887 methods and in accordance with QRM, the frequency of testing should be based on the
888 knowledge and experience of the container and closure systems being used. A statistically
889 valid sampling plan should be utilized. It should be noted that visual inspection alone is
890 not considered as an acceptable integrity test method.

891

892 8.19 Containers sealed under vacuum should be tested for maintenance of vacuum after an
893 appropriate, pre-determined period and during shelf life.

894

895 8.20 The container closure integrity validation should take into consideration any
896 transportation or shipping requirements.

897

898 8.21 As the equipment used to crimp vial caps can generate large quantities of non-
899 viable particulates, the equipment should be located at a physically separate station
900 equipped with adequate air extraction.

901

902 8.22 Vial capping can be undertaken as an aseptic process using sterilized caps or as a
903 clean process outside the aseptic core. Where this latter approach is adopted, vials
904 should be protected by grade A conditions up to the point of leaving the aseptic
905 processing area, and thereafter stoppered vials should be protected with a grade A air supply
906 until the cap has been crimped. Where capping is a manual process it must be performed in
907 grade A conditions with a grade B background.

908

909 8.23 In the case where capping is conducted as a clean process with grade A air supply
910 protection, vials with missing or displaced stoppers should be rejected prior to capping.
911 Appropriately validated, automated methods for stopper height detection should be in place.
912 Microbial ingress studies (or alternative methods) should be utilized to determine the
913 acceptable stopper height displacement.

914

915 8.24 Where human intervention is required at the capping station, appropriate technology
916 should be used to prevent direct contact with the vials and to minimize microbial
917 contamination.

918

919 8.25 RABS and isolators may be beneficial in assuring the required conditions and
920 minimising direct human interventions into the capping operation.

921

922 8.26 All filled containers of parenteral products should be inspected individually for
923 extraneous contamination or other defects. QRM principles should be used for
924 determination of defect classification and criticality. Factors to consider include, but are not
925 limited, to the potential impact to the patient of the defect and the route of administration.
926 Different defect types should be categorized and batch performance analyzed. Batches with
927 unusual levels of defects, when compared to routine defect levels for the process, should
928 lead to investigation and consideration of partial or the whole rejection of the batch
929 concerned. A defect library should be generated and maintained which captures all known

930 defects. The defect library can be used as a training tool for production and quality
931 assurance personnel. Critical defects should not be identified during any subsequent
932 sampling of acceptable containers as it indicates a failure of the original inspection process.

933
934 8.27 When inspection is done manually, it should be done under suitable and controlled
935 conditions of illumination and background. Inspection rates should be appropriately
936 validated. Operators performing the inspection should undergo robust visual inspection
937 qualification (whilst wearing corrective lenses, if these are normally worn) at least annually.
938 The qualification should be undertaken using appropriate sample sets and taking into
939 consideration worst case scenarios (e.g. inspection time, line speed (where the product is
940 transferred to the operator by a conveyor system), component size or fatigue at the end of
941 shift) and should include consideration of eyesight checks. Operator distractions should be
942 removed and frequent breaks of appropriate duration from inspection should be taken.

943
944 8.28 Where automated methods of inspection are used, the process should be validated to
945 detect known defects with sensitivity equal to or better than manual inspection methods and
946 the performance of the equipment checked prior to start up and at regular intervals.

947
948 8.29 Results of the inspection should be recorded and defect types and levels trended. Reject
949 rates for the various defect types should also be trended. Investigations should be performed
950 as appropriate to address adverse trends or discovery of new defect types. Impact to product
951 on the market should be assessed as part of this investigation.

952 953 **Sterilization**

954
955 8.30 Where possible, finished product should be terminally sterilized using a validated and
956 controlled sterilization process as this provides a greater assurance of sterility than a
957 validated and controlled sterilizing filtration process and/or aseptic processing. Where it is
958 not possible for a product to undergo a sterilisation, consideration should be given to using
959 terminal bioburden reduction steps, such as heat treatments (pasteurization), combined with
960 aseptic processing to give improved sterility assurance.

961
962 8.31 The selection, design and location of the equipment and cycle/programme used for
963 sterilization should be decided using QRM principles. Critical parameters should be defined,
964 controlled, monitored and recorded.

965
966 8.32 There should be mechanisms in place to detect a cycle that does not conform to the
967 validated parameters. Any failed or atypical sterilization cycles must be formally
968 investigated.

969
970 8.33 All sterilization processes should be validated. Particular attention should be given
971 when the adopted sterilization method is not described in the current edition of the
972 Pharmacopoeia, or when it is used for a product which is not a simple aqueous
973 solution. Where possible, heat sterilization is the method of choice. Regardless, the
974 sterilization process must be in accordance with the registered marketing and
975 manufacturing specifications.

976
977 8.34 Before any sterilization process is adopted, its suitability for the product and equipment
978 and its efficacy in achieving the desired sterilizing conditions in all parts of each type of
979 load to be processed should be demonstrated by physical measurements and by biological

980 indicators where appropriate.
981
982 8.35 The validity of the process should be verified at scheduled intervals, with a minimum
983 of at least annually. Revalidation of the sterilization process should be conducted whenever
984 significant modifications have been made to the product, product packaging, sterilization
985 load configuration, sterilizing equipment or sterilization process parameters.
986
987 8.36 For effective sterilization, the whole of the material and equipment must be
988 subjected to the required treatment and the process should be designed to ensure that this is
989 achieved.
990
991 8.37 Routine operating parameters should be established and adhered to for all
992 sterilization processes, e.g. physical parameters and loading patterns, etc.
993
994 8.38 Suitable biological indicators (BIs) placed at appropriate locations may be
995 considered as an additional method for monitoring the sterilization. BIs should be stored
996 and used according to the manufacturer's instructions. Prior to use of a new batch/lot of BIs,
997 the quality of the batch/lot should be verified by confirming the viable spore count and
998 identity. Where BIs are used to validate and/or monitor a sterilization process (e.g. for
999 Ethylene Oxide), positive controls should be tested for each sterilization cycle, with strict
1000 precautions in place to avoid transferring microbial contamination from BIs, including
1001 preventing positive control BIs from contaminating BIs exposed to the sterilization cycle. If
1002 biological indicators are used, strict precautions should be taken to avoid transferring
1003 microbial contamination to the manufacturing or other testing processes.
1004
1005 8.39 There should be a clear means of differentiating products, equipment and components,
1006 which have not been sterilized from those which have. Each basket, tray or other carrier of
1007 products, items of equipment or components should be clearly labelled with the material
1008 name, its batch number and an indication of whether or not it has been sterilized. Indicators
1009 such as autoclave tape, or irradiation indicators may be used, where appropriate, to indicate
1010 whether or not a batch (or sub-batch) has passed through a sterilization process. However,
1011 these indicators show only that the sterilization process has occurred; they do not necessarily
1012 indicate product sterility or achievement of the required sterility assurance level.
1013
1014 8.40 Sterilization records should be available for each sterilization run. They should be
1015 reviewed and approved as part of the batch release procedure.
1016
1017 8.41 Where possible, materials, equipment and components should be sterilized by validated
1018 methods appropriate to the specific material. Suitable protection after sterilization should be
1019 provided to prevent recontamination. If items sterilized "in house" are not used immediately
1020 after sterilization, these should be stored, using appropriately sealed packaging, in at least a
1021 grade B environment, a maximum hold period should also be established. Components that
1022 have been packaged with multiple sterile packaging layers need not be stored in grade B
1023 (where justified) if the integrity and configuration (e.g. multiple sterile coverings that can be
1024 removed at each transfer from lower to higher grade) of the sterile pack allows the items to be
1025 readily disinfected during transfer into the grade A zone. Where protection is achieved by
1026 containment in sealed packaging this process should be undertaken prior to sterilisation.
1027
1028 8.42 Transfer of materials, equipment, and components into an aseptic processing area should
1029 be via a unidirectional process (e.g. through a double-door autoclave, a depyrogenation oven,

1030 effective transfer disinfection, or, for gaseous or liquid materials, a bacteria-retentive filter).

1031

1032 8.43 Where materials, equipment, components and ancillary items are sterilized in sealed
1033 packaging and then transferred into the grade A/B area, this should be done using
1034 appropriate, validated methods (for example, airlocks or pass through hatches) with
1035 accompanying disinfection of the exterior of the sealed packaging. These methods should be
1036 demonstrated to be effective in not posing an unacceptable risk of contamination of the grade
1037 A/B area and, likewise, the disinfection procedure should be demonstrated to be effective in
1038 reducing any contamination on the packaging to acceptable levels for entry of the item into
1039 the grade A/B area. Packaging may be multi-layered to allow removal of a single layer at
1040 each interface to a higher grade.

1041

1042 8.44 Where materials, equipment, components and ancillary items are sterilized in sealed
1043 packaging or containers, the integrity of the sterile protective barrier should be qualified for
1044 the maximum hold time, and the process should include inspection of each sterile item prior
1045 to its use to ensure that the sterile protective measures have remained integral.

1046

1047 8.45 For materials, equipment, components and ancillary items that are necessary for aseptic
1048 processing but cannot be sterilized, an effective and validated disinfection and transfer
1049 process should be in place. These items once disinfected should be protected to prevent
1050 recontamination. These items, and others representing potential routes of contamination,
1051 should be included in the environmental monitoring program.

1052

1053 8.46 When a depyrogenation process is used for any components or product contact
1054 equipment, validation studies should be performed to demonstrate that the process will result
1055 in a minimum 3 log reduction in endotoxin. There is no additional requirement to
1056 demonstrate sterilization in these cases.

1057

1058 **Sterilization by heat**

1059

1060 8.47 Moist heat sterilization utilises clean steam, typically at lower temperatures and shorter
1061 duration than dry heat processes, in order to sterilize a product or article. Moist heat
1062 sterilization is primarily effected by latent heat of condensation and the quality of steam is
1063 therefore important to provide consistent results. The reduced level of moisture in dry heat
1064 sterilization process reduces heat penetration which is primarily effected by conduction. Dry
1065 heat processes may be utilized to sterilize or control bioburden of thermally stable materials
1066 and articles. Dry heat sterilization is of particular use in the removal of thermally robust
1067 contaminants such as pyrogens and is often utilized in the preparation of aseptic filling
1068 components. Moist heat sterilization processes may be utilized to sterilize or control
1069 bioburden (for non-sterile applications) of thermally stable materials, articles or products
1070 and is the preferred method of sterilization, where possible.

1071

1072 8.48 In those cases where parametric release has been authorized, a robust system should be
1073 applied to the product lifecycle validation and the routine monitoring of the manufacturing
1074 process. This system should be periodically reviewed.

1075

1076 8.49 Each heat sterilization cycle should be recorded on a time/temperature chart with
1077 a sufficiently large scale or by other appropriate equipment with suitable accuracy and
1078 precision. Monitoring and recording systems should be independent of the controlling
1079 system.

1080
1081 8.50 The position of the temperature probes used for controlling and/or recording should
1082 have been determined during the validation (which should include heat distribution and
1083 penetration studies), and, where applicable, also checked against a second independent
1084 temperature probe located at the same position.

1085
1086 8.51 Chemical or biological indicators may also be used, but should not take the place
1087 of physical measurements.

1088
1089 8.52 Sufficient time must be allowed for the whole of the load to reach the required
1090 temperature before measurement of the sterilizing time-period is commenced. This time
1091 must be determined for each type of load to be processed.

1092
1093 8.53 After the high temperature phase of a heat sterilization cycle, precautions should be
1094 taken against contamination of a sterilized load during cooling. Any cooling fluid or gas in
1095 contact with the product should be sterilized unless it can be shown that any leaking
1096 container would not be approved for use.

1097
1098 **Moist heat sterilization**

1099
1100 8.54 Time, temperature and pressure should be used to monitor the process. Each item
1101 sterilized should be inspected for damage, seal and packaging material integrity and
1102 moisture on removal from the autoclave. Seal and packaging integrity should also be
1103 inspected immediately prior to use. Any items found not to be fit for purpose should be
1104 removed from the manufacturing area and an investigation performed.

1105
1106 8.55 System and cycle faults should be registered and recorded by the control and
1107 monitoring system and appropriate actions taken prior to release of the process.

1108
1109 8.56 For sterilizers fitted with a drain at the bottom of the chamber, it may also be necessary
1110 to record the temperature at this position throughout the sterilization period. For Steam-In-
1111 Place (SIP) systems, it may also be necessary to record the temperature at condensate drain
1112 locations throughout the sterilization period.

1113
1114 8.57 Validation should include a consideration of equilibration time, exposure time,
1115 correlation of pressure and temperature and maximum temperature range during exposure
1116 for porous cycles and temperature, time and F_0 for fluid cycles. These critical parameters
1117 should be subject to defined limits (including appropriate tolerances) and be confirmed as
1118 part of sterilization validation and routine cycle acceptance criteria. Revalidation should be
1119 performed annually.

1120
1121 8.58 There should be frequent leak tests on the system to be sterilized when a vacuum phase
1122 is part of the cycle or the system is returned, post-sterilization, to a pressure equivalent to or
1123 lower than the environment surrounding the sterilized system. The frequency of testing
1124 should be based on the principles of QRM.

1125
1126 8.59 When the sterilization process includes air purging (e.g. porous autoclave loads,
1127 lyophilizer chambers) there should be adequate assurance of air removal prior to and during
1128 sterilization. Loads to be sterilized should be designed to support effective air removal and
1129 be free draining to prevent the build-up of condensate.

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8.60 The items to be sterilized, other than products in sealed containers, should be dry, wrapped in a material which allows removal of air and penetration of steam but which prevents recontamination after sterilization. All load items should be dry upon removal from the sterilizer. Load dryness should be confirmed as a part of sterilization process acceptance.

8.61 Distortion and damage of flexible containers, such as containers produced by Blow-Fill-Seal and Form-Fill-Seal technology that are terminally sterilized, should be prevented by setting correct counter pressure and loading patterns.

8.62 Care should be taken to ensure that materials or equipment are not contaminated after the sterilization exposure phase of the cycle due to the introduction of non-sterile air into the chamber during subsequent phases; typically only sterile filtered air would be introduced into the chamber during these phases.

8.63 Where Sterilization in place (SIP) systems are used, (for example, for fixed pipework, vessels and lyophilizer chambers), the system should be appropriately designed and validated to assure all parts of the system are subjected to the required treatment. The system should be monitored for temperature, pressure and time at appropriate critical locations during routine use, this is to ensure all areas are effectively and reproducibly sterilized; these critical locations should be demonstrated as being representative, and correlated with, the slowest to heat locations during initial and routine validation. Once a system has been sterilized by SIP it should remain integral prior to use, the maximum duration of the hold time should be qualified.

Dry heat sterilization

8.64 The combination of time and temperature to which product, components and equipment are exposed should produce an adequate and reproducible level of lethality and/or pyrogen (endotoxin) inactivation/removal when operated routinely within the established tolerances.

8.65 Dry heat sterilization or depyrogenation tunnels are typically employed to prepare components for aseptic filling operations but may be used for other processes. Tunnels should be configured to ensure that airflow patterns protect the integrity and performance of the sterilizing zone, by maintaining a stable pressure differential and airflow pattern through the tunnel from the higher grade area to the lower grade area. All air supplied to the tunnel should pass through a HEPA filter; periodic tests should be performed to demonstrate filter integrity. Any tunnel parts that come into contact with sterilized components should be appropriately sterilized or disinfected. Critical process parameters that should be considered during validation and/or routine processing should include, but may not be limited to:

- a) Belt speed or dwell time within sterilising zone.
- b) Temperature – Minimum and maximum temperatures.
- c) Heat penetration of material/article.
- d) Heat distribution/uniformity.
- e) Airflows – correlated with the heat distribution and penetration studies.

1180
1181 8.66 When using endotoxin spiked containers these need to be carefully managed with a full
1182 reconciliation performed. Endotoxin quantification and recovery efficiency should also be
1183 demonstrated.

1184
1185 8.67 Dry heat ovens are typically employed to sterilize or depyrogenate primary packaging
1186 components, finished materials or APIs but may be used for other processes. They should be
1187 maintained at a positive pressure to lower grade areas. All air entering the oven should pass
1188 through a HEPA filter. Critical process parameters that should be considered in validation
1189 qualification and/or routine processing should include, but may not be limited to:

- 1190
- 1191 a) Temperature.
 - 1192
 - 1193 b) Exposure period/time.
 - 1194
 - 1195 c) Chamber pressure.
 - 1196
 - 1197 d) Heat penetration of material/article (slow to heat spots and different loads).
 - 1198
 - 1199 e) Heat distribution/uniformity.
- 1200

1201
1202 8.68 For dry heat sterilization of starting materials and intermediates the same principles
1203 should be applied. Consideration should be given to factors affecting heat penetration such as
1204 the container type, size and packing matrix.

1205
1206 **Sterilization by radiation**

1207
1208 8.69 Guidance regarding ionising radiation sterilization can be found within Annex 12 of the
1209 EU GMP.

1210
1211 8.70 Radiation sterilization is used mainly for the sterilization of heat sensitive materials
1212 and products. Many medicinal products and some packaging materials are radiation-
1213 sensitive, so this method is permissible only when the absence of deleterious effects on
1214 the product has been confirmed. Ultraviolet irradiation is not normally an acceptable
1215 method of sterilization.

1216
1217 8.71 Validation procedures should ensure that the effects of variations in density of the
1218 packages are considered.

1219
1220 **Sterilization with ethylene oxide**

1221
1222 8.72 This method should only be used when no other method is practicable. During
1223 process validation it should be shown that there is no damaging effect on the product
1224 and that the conditions and time allowed for degassing to reduce any residual ethylene
1225 oxide (EO) gas and reaction products to defined acceptable limits for the type of product or
1226 material.

1227
1228 8.73 Direct contact between gas and microbial cells is essential; precautions should be taken
1229 to avoid the presence of organisms likely to be enclosed in material such as crystals or dried

1230 protein. The nature and quantity of packaging materials can significantly affect the process.

1231

1232 8.74 Before exposure to the gas, materials should be brought into equilibrium with
1233 the humidity and temperature required by the process. The time required for this
1234 should be balanced against the opposing need to minimize the time before sterilization.

1235

1236 8.75 Each sterilization cycle should be monitored with suitable biological indicators, using
1237 the appropriate number of test pieces distributed throughout the load unless parametric
1238 release has been authorized by the National Competent Authority.

1239

1240 8.76 Critical process variables that should be considered as part of sterilization process
1241 validation and routine monitoring include, but are not limited to: EO gas concentration,
1242 relative humidity, temperature and EO gas pressure and exposure time.

1243

1244 8.77 After sterilization, the load should be aerated to allow EO gas and/or its reaction
1245 products to desorb from the packaged product to predetermined levels. Aeration can occur
1246 within a sterilizer chamber and/or in a separate aeration chamber or aeration room. The
1247 aeration phase should be validated as part of the overall EO sterilization process validation.

1248

1249 **Filtration of medicinal products which cannot be sterilized in their final container**

1250

1251 8.78 If a liquid product cannot be terminally sterilized by a microbiocidal process, it should
1252 be sterilized by filtration through a sterile, sterilizing grade filter (with nominal pore size of
1253 0.22 micron (or less) or with at least equivalent micro-organism retaining properties), and
1254 subsequently aseptically filled into a previously sterilized container, the selection of the filter
1255 used should ensure that it is compatible with the product, see 8.119.. Suitable bioburden
1256 reduction and/or sterilizing grade filters may be used at multiple points during the
1257 manufacturing process to ensure a low and controlled bioburden of the liquid prior to the
1258 primary sterilizing grade filter. Due to the potential additional risks of a sterilizing filtration
1259 process as compared to other sterilization processes, a second filtration through a sterile,
1260 sterilising grade filter (positioned as per clause 8.15), immediately prior to filling, is
1261 advisable

1262

1263 8.79 The selection of components for the filtration system (including air, gas and vent filters)
1264 and their interconnection and arrangement within the filtration system, including pre-filters,
1265 should be based on the critical quality attributes of the products, documented and justified.
1266 The filtration system should not generate fibres, unacceptable levels of impurities or
1267 otherwise alter the quality and efficacy of the product. Similarly, the filter characteristics
1268 should not be adversely affected by the product to be filtered. Adsorption of product
1269 components and extraction/leaching of filter components should be evaluated (see Single-
1270 Use-Systems, Clauses 8.117-8.119).

1271

1272 8.80 The filtration system should be designed to:

1273

1274 a) Allow operation within validated process parameters.

1275

1276 b) Maintain the sterility of the filtrate.

1277

1278 c) Minimise the number of aseptic connections required between the sterilizing filter
1279 and the final filling of the product.

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- d) Allow cleaning procedures to be conducted as necessary.
- e) Allow sterilization procedures, including SIP, to be conducted as necessary. The sterilization procedures should be validated to ensure achievement of a target sterilization assurance level (SAL) of 10^{-6} or better (e.g. 10^{-7}).
- f) Permit in-place integrity testing, preferably as a closed system, prior to filtration as necessary. In-place integrity testing methods should be selected to avoid any adverse impact on the quality of the product.

8.81 Liquid-sterilizing filtration should be validated during initial process validation. Validation can be grouped by different strengths or variations of a product, but should be done under worst-case conditions. The rationale for grouping fluids should be justified and documented.

8.82 Wherever possible, the product to be filtered should be used for bacterial retention testing. 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.

8.83 Filtration parameters that should be considered in validation and routine processing should include but are not limited to:

- a) If the system is flushed or integrity tested in-situ with a fluid other than the product, then flushing with the product should be part of the process.
- b) The wetting fluid used for filter integrity testing based on filter manufacturer's recommendation or the fluid to be filtered. For the latter, the appropriate integrity test value specification should be established.
- c) Filtration process conditions including:
 - i. Fluid prefiltration holding time and effect on bioburden.
 - ii. Filter conditioning, with fluid if necessary.
 - iii. Maximum filtration time/total time filter is in contact with fluid.
 - iv. Flow rate.
 - v. Filtration volume.
 - vi. Temperature.
 - vii. The time taken to filter a known volume of bulk solution and the pressure difference to be used across the filter. Any significant differences from those validated to those observed during routine manufacturing should be noted and investigated. Results of these checks should be included in the batch record.

1330
1331 8.84 The integrity of the sterilized filter assembly should be verified by testing before use,
1332 in case of damage and loss of integrity caused by processing, and should be verified by on
1333 line testing immediately after use by an appropriate method such as a bubble point,
1334 diffusive flow, water intrusion or pressure hold test. It is recognised that for small batch
1335 sizes, this may not be possible; in these cases an alternative approach may be taken as long as
1336 a formal risk assessment has been performed and compliance is achieved. There should be
1337 written integrity test methods, including acceptance criteria, and failure investigation
1338 procedures and justified conditions under which the filter integrity test can be repeated.
1339 Results of the integrity tests (including failed and repeated tests) should be included in the
1340 batch record.

1341
1342 8.85 The integrity of critical sterile gas and air vent filters in the filter assembly should be
1343 verified by testing after use. The integrity of non-critical air or gas vent filters should be
1344 confirmed and recorded at appropriate intervals.

1345
1346 8.86 For gas filtration, the avoidance of unintended moistening or wetting of the filter or filter
1347 equipment is important. This can be achieved by the use of hydrophobic filters.

1348
1349 8.87 Where serial filtration (one filtration is followed by a subsequent filtration) is a process
1350 requirement the filter train is considered to be a sterilizing unit and all sterilizing-grade filters
1351 within it should satisfactorily pass integrity testing both before use, in case of damage during
1352 processing, and after use.

1353
1354 8.88 Where a redundant sterilizing filter is used, the additional filter does not require post-
1355 integrity testing unless the primary sterilizing filter fails, in which case the redundant filter
1356 must then satisfactorily pass post-use integrity testing. Bioburden samples should be taken
1357 prior to the first filter and the sterilizing filter, systems for taking samples should be designed
1358 so as not to introduce contamination.

1359
1360 8.89 Liquid sterilizing filters should be discarded after the processing of a single lot. The
1361 same filter should not be used for more than one working day unless such use has been
1362 validated.

1363
1364 **Form-Fill-Seal**

1365
1366 8.90 Form-Fill-Seal (FFS) units include blow moulding from thermoplastic granulate and
1367 thermoforming from thermoplastic film typically known as Blow-Fill-Seal (BFS) and
1368 Vertical-Form-Fill-Seal (VFFS) respectively. VFFS process is an automated filling process,
1369 typically for terminally sterilized processes, that may utilize a single or dual web system
1370 which constructs the primary container out of a flat roll of thermoplastic film while
1371 simultaneously filling the formed bags with product and sealing the filled bags in a
1372 continuous process. All such containers are considered to be sealed by fusion and, as such,
1373 fall under the requirement to perform 100% integrity testing.

1374
1375 8.91 Process parameters relating to seal integrity should be validated and appropriately
1376 controlled. Critical parameters include, but are not limited to: seal strength, seal uniformity,
1377 sealing temperatures, pressures, sealing times and dwell time for filling. Seal strength and
1378 uniformity should be monitored routinely.

1379

1380 8.92 Samples of filled containers should be tested for general performance e.g. ease-of-
1381 opening, and seal uniformity. Sample size and frequency should be based on the principles of
1382 QRM.

1383
1384

1385 **Blow-Fill-Seal technology**

1386

1387 8.93 Blow-Fill-Seal (BFS) units are purpose built machines in which, in one continuous
1388 operation, containers are formed from a thermoplastic granulate, filled and then sealed, all by
1389 the one automatic machine, see glossary for full definition.

1390

1391 8.94 Risk management principles should be used to justify the machine's design and
1392 operational controls. These controls should be in alignment with the site's contamination
1393 control strategy. Aspects to be considered should include (but are not limited to):

1394

1395 a) Determination of the "critical zone" that should be protected from contamination,
1396 and its control.

1397

1398 b) Environmental control and monitoring, both of the BFS machine and the background
1399 in which it is placed.

1400

1401 c) Integrity testing of the BFS product pathways.

1402

1403 d) Duration of the batch or filling campaign.

1404

1405 e) Control of polymer starting material.

1406

1407 f) Cleaning-in-place and sterilization-in-place of equipment, and air and product
1408 pathways.

1409

1410 8.95 Shuttle and Rotary-type equipment used for aseptic production which is fitted with an
1411 effective grade A air shower should be installed in at least a grade C environment, provided
1412 that grade A/B clothing is used.

1413

1414 8.96 For Shuttle-type equipment, the environment should comply with the viable and non-
1415 viable limits at rest and the viable limit only when in operation. The shuttle zone should meet
1416 grade A viable limits.

1417

1418 8.97 For Rotary-type equipment the environment should comply with the viable and non-
1419 viable limits "at rest". It is not normally possible to perform environmental monitoring within
1420 the parison during operation" Monitoring of the background environment should be
1421 performed in accordance with risk management principles

1422

1423 8.98 The environmental control and monitoring program should take into consideration the
1424 complex gas flow paths generated by the BFS process and the effect of the high heat outputs
1425 of the process.

1426

1427 8.99 In addition, for Shuttle-type designs, the area between parison cutting and mould sealing
1428 should be covered by a flow of HEPA filtered or sterile air of appropriate quality to provide
1429 grade A at the critical zone.

- 1430
1431 8.100 Blow-Fill-Seal equipment used for the production of products which are terminally
1432 sterilized should be installed in at least a grade D environment.
1433
- 1434 8.101 External particle and microbial contamination of the polymer should be prevented by
1435 appropriate design, control, and maintenance of the polymer storage and distribution systems.
1436
- 1437 8.102 Interventions requiring cessation of filling and/or blowing and sealing and, where
1438 required, re-sterilization of the filling machine should be clearly defined and well described
1439 in the aseptic filling procedure, and included in the aseptic process simulation (refer clause
1440 9.36).
1441
- 1442 8.103 Process validation should take into consideration critical operating parameters and
1443 variables of the equipment that impact on the quality of the product, e.g. filling speed,
1444 extrusion temperature, filling times.
1445
- 1446 8.104 Samples of filled containers should be tested for general performance e.g. ease-of-
1447 opening and wall thickness; sample size and frequency should be based on the principles of
1448 QRM.
1449
- 1450 **Lyophilization**
1451
- 1452 8.105 Lyophilization is a critical process step and all activities that can affect the sterility of
1453 the product or material need to be regarded as extensions of the aseptic processing of that
1454 sterilized product or material. The lyophilization equipment and its processes should be
1455 designed so as to ensure product or material sterility is maintained during lyophilization by
1456 preventing microbiological and particulate contamination between the filling operation and
1457 completion of lyophilization process. All control measures in place should be determined by
1458 the site's contamination control strategy.
1459
- 1460 8.106 The lyophilizer should be sterilized before each load. The lyophilizer should be
1461 protected from contamination after sterilization.
1462
- 1463 8.107 Where there is a closing system for partially closed containers, the surfaces of any
1464 equipment protruding into the chamber to effect sealing should also be sterilized.
1465
- 1466 8.108 Lyophilization trays should be checked to ensure that they are not misshapen and
1467 damaged.
1468
- 1469 8.109 The maximum permitted leakage of air into the lyophilizer should be specified.
1470
- 1471 8.110 The integrity of the system should be monitored periodically along with consideration
1472 of the leak rate test.
1473
- 1474 8.111 With regard to loading and unloading the lyophilizer:
1475
- 1476 a) The loading pattern within the lyophilizer should be specified and documented.
1477
 - 1478 b) Transport to the lyophilizer and loading of filled product, or other equipment into the
1479 lyophilizer should take place under a grade A environment.

- 1480
1481 c) Airflow patterns should not be adversely affected by transport devices and venting
1482 of the loading zone. Unsealed containers should be maintained under grade A
1483 environment.
1484
1485 d) Where seating of the stoppers is not completed prior to opening the lyophilizer
1486 chamber, product removed from the lyophilizer should remain under a grade A
1487 environment during subsequent handling.
1488 e) Utensils used during transfer to, loading and unloading of, the lyophilizer (such as
1489 trays, bags, placing devices, tweezers, etc.) should be subjected to a validated
1490 sterilization process.
1491

1492 **Closed systems**

1493
1494 8.112 Closed systems can be both single use systems (SUS) (i.e. disposable) and fixed
1495 systems (such as vessels with fixed pipework). Guidance in this section is equally applicable
1496 to both systems.
1497

1498 8.113 The use of closed systems can reduce the risk of both microbial and chemical
1499 contamination due to interventions.
1500

1501 8.114 It is critical to ensure the sterility of product contact surfaces of closed systems used for
1502 aseptic processing. The design and selection of any closed system used for aseptic processing
1503 must ensure maintenance of sterility. Tubing/pipework that is not assembled prior to
1504 sterilization should be designed to be connected aseptically, e.g. by intrinsic aseptic
1505 connectors or fusion systems.
1506

1507 8.115 Appropriate systems should be in place to assure the integrity of those components
1508 used. The manner in which this is conducted should be determined based on QRM principles.
1509 Appropriate system integrity tests should be considered when there is a risk of compromising
1510 product sterility.
1511

1512 8.116 The background in which closed systems are located will vary. If there is a high risk
1513 that the system will not remain integral during processing it should be located in a grade A
1514 environment. If the system can be shown to remain integral at every usage then lower grades,
1515 including grade D, can be considered.
1516

1517 **Single use systems**

1518
1519 8.117 Single use systems (SUS) are those technologies used in manufacture of sterile
1520 medicinal products which are designed to replace reusable equipment. SUS are typically
1521 defined systems made up of components such as bags, filters, tubing, connectors, storage
1522 bottles and sensors.
1523

1524 8.118 There are some specific risks associated with SUS which include, but are not limited
1525 to:
1526

- 1527 a) Interaction between the product and product contact surface (adsorption, leachable
1528 and extractables).
1529

- 1530 b) More fragile than fixed reusable systems.
- 1531
- 1532 c) Increase in number and complexity of manual operations and connections made.
- 1533
- 1534 d) Design of the assembly.
- 1535
- 1536 e) Performance of the pre-use integrity testing for sterilizing grade filters. (Refer to
- 1537 clause 8.84.)
- 1538 f) Integrity testing.
- 1539
- 1540 g) Pin-hole and leakage.
- 1541
- 1542 h) The potential for compromising the system at the point of opening the outer
- 1543 packaging.
- 1544
- 1545 i) Assessment of suppliers of disposable systems (including sterilization of these
- 1546 disposable systems.
- 1547
- 1548 j) Risk of particulate contamination.
- 1549

1550 8.119 The compatibility of materials used for product contact surfaces with the products
 1551 should be ensured under the process conditions by evaluating e.g. adsorption and reactivity to
 1552 the product.

1553
 1554 8.120 Extractable profile data obtained from the supplier of the components of SUS may be
 1555 useful to ensure that extractables and leachables from the SUS do not alter the quality of the
 1556 product. A risk assessment should be conducted for each component to evaluate the
 1557 applicability of the extractable profile data. For components considered to be at high risk to
 1558 leachables, including those taking up leachables extensively or those stored for longer
 1559 periods, an assessment of leachable profile studies, including safety concerns, and should be
 1560 taken into consideration, as necessary. If applying simulated processing conditions these
 1561 should accurately reflect the actual processing conditions and be based on a scientific
 1562 rationale.

1563
 1564 8.121 SUS should be designed so as to maintain integrity during the intended operational
 1565 conditions and duration, especially the structural integrity of the single use components under
 1566 extreme process and transport conditions such as during freeze and thaw processes. This
 1567 should include verification that intrinsic aseptic connections (both heat and mechanical)
 1568 remain integral under these conditions.

1569
 1570 8.122 Acceptance procedures should be established and implemented for SUS corresponding
 1571 to the risks or criticality of the products and its processes. On receipt, a visual inspection of
 1572 outer packaging (e.g. appearance of exterior carton, product pouches), label printing, and
 1573 attached documents (e.g. Certificate of Analysis, radiation certificate) should be carried out.
 1574 Prior to use, each piece of SUS should be checked to ensure that they have been
 1575 manufactured and delivered in accordance with the approved specification.

1576
 1577 8.123 Critical manual handling operation of SUS, such as assembling and connecting, should
 1578 be subject to appropriate controls and verified during the aseptic process simulation test.

1579

1580 **9 Viable and non-viable environment & process monitoring**

1581

1582 **General**

1583

1584 9.1 The site's environmental and process monitoring program forms part of the overall
1585 contamination control strategy designed to minimise the risk of microbial and particulate
1586 contamination.

1587

1588 9.2 This program is typically comprised of the following elements:

1589 a) Environmental monitoring – non viable.

1590 b) Environmental monitoring – viable.

1591

1592 c) Aseptic process simulation (aseptically manufactured product only).

1593

1594 9.3 These key elements provide information with regards to the process and facility
1595 capabilities with respect to the maintenance of sterility assurance. The information from these
1596 systems should be used for routine batch release and for periodic assessment during process
1597 review or investigations.

1598

1599 **Environmental monitoring**

1600

1601 9.4 In order to establish a robust environmental monitoring program, i.e. locations,
1602 frequency of monitoring and incubation conditions (e.g. time, temperature(s) and aerobic
1603 and or anaerobic), appropriate risk assessments should be conducted based on detailed
1604 knowledge of the process inputs, the facility, equipment, specific processes, operations
1605 involved and knowledge of the typical microbial flora found, consideration of other aspects
1606 such as air visualization studies should also be included. These risk assessments should be
1607 re-evaluated at defined intervals in order to confirm the effectiveness of the site's
1608 environmental monitoring program, and they should be considered in the overall context of
1609 the trend analysis and the contamination control strategy for the site.

1610

1611 9.5 Routine monitoring for clean rooms, clean air devices and personnel should be performed
1612 "in operation" throughout all critical stages, including equipment set up. The locations,
1613 frequency, volume and duration of monitoring should be determined based on the risk
1614 assessment and the results obtained during the qualification.

1615 9.6 Monitoring should also be performed outside of operations within the area, e.g. pre
1616 disinfection, post disinfection, prior to start of manufacturing and after a shutdown period
1617 etc., in order to detect potential incidents of contamination which may affect the controls
1618 within the areas. The number of samples and frequency of monitoring should be considered
1619 in the context of the risk assessments and contamination control strategy.

1620

1621 9.7 For grade A monitoring, it is important that sampling should be performed at locations
1622 posing the highest risk of contamination to the sterile equipment surfaces, container-closures
1623 and product in order to evaluate maintenance of aseptic conditions during critical operations.

1624

1625 9.8 Appropriate alert and action limits should be set for the results of particulate and
1626 microbiological monitoring. Alert levels should be established based on results of
1627 Performance Qualification (PQ) tests or trend data and should be subject to periodic review.

1628

1629 9.9 The alert limits for grade B, c and D should be set based on the area performance, with
1630 the aim to have limits lower than those specified as action limits, in order to minimise risks
1631 associated and identify potential changes that may be detrimental to the process.
1632

1633 9.10 If action limits are exceeded operating procedures should prescribe a root-cause
1634 investigation followed by corrective and preventive action. If alert limits are exceeded,
1635 operating procedures should prescribe scrutiny and follow-up, which might include
1636 investigation and corrective action.

1637 9.11 Surfaces and personnel should be monitored after critical operations. Results from
1638 monitoring should be considered when reviewing batch documentation for finished product
1639 release.
1640

1641
1642 **Non-viable monitoring**
1643

1644 9.12 Non-viable particle monitoring systems should be established to obtain data for
1645 assessing potential contamination risks and to maintain the environment for sterile operations
1646 in the qualified state.
1647

1648 9.13 The recommended limits for airborne particle concentration in monitoring for each
1649 grade are given in Table 5.
1650

1651 **Table 5: Recommended limits for airborne particle concentration for the monitoring of**
1652 **non-viable contamination**
1653

Grade	Recommended maximum limits for particles $\geq 0.5 \mu\text{m}^3$		Recommended maximum limits for particles $\geq 5 \mu\text{m}^3$	
	in operation	at rest	in operation	at rest
A	3 520	3 520	20	20
B	352 000	3 520	2 900	29
C	3 520 000	352 000	29 000	2 900
D	Set a limit based on the risk assessment	3 520 000	Set a limit based on the risk assessment	29 000

1654
1655 Note 1: The particle limits given in the table for the “at rest” state should be achieved
1656 after a short “clean up” period defined during qualification in an unmanned state after
1657 the completion of operations (see 5.26e).
1658

1659 Note 2: With regards to the monitoring of $5.0 \mu\text{m}$, the limit of 20 is selected due to the
1660 limitations of monitoring equipment. It should be noted that alert limits should also be
1661 set based on historical and qualification data, such that frequent sustained recoveries
1662 below the action limit should also trigger an investigation.
1663

1664 9.14 For grade A zones, particle monitoring should be undertaken for the full duration of
1665 critical processing, including equipment assembly.
1666

1667 9.15 The grade A zone should be monitored continuously and with a suitable sample size (at

1668 least 28 litres (a cubic foot) per minute) so that all interventions, transient events and any
1669 system deterioration would be captured and alarms triggered if alert limits are exceeded.
1670

1671 9.16 It is recommended that a similar system be used for grade B zones although the sample
1672 frequency may be decreased. The design of the monitoring system should be based on risk
1673 assessment and be commensurate with the risk of the process to the product sterility
1674 assurance. The grade B zone should be monitored at such a frequency and with suitable
1675 sample sizes that the programme captures any change in levels of contamination and system
1676 deterioration. If alert limits are exceeded, alarms should be triggered.
1677

1678 9.17 The monitoring of grade C and D areas in operation should be performed in
1679 accordance with the principles of QRM to provide sufficient data to allow effective trend
1680 analysis. The requirements and alert/action limits will depend on the nature of the
1681 operations carried out.
1682

1683 9.18 The selection of the monitoring system should take account of any risk presented
1684 by the materials used in the manufacturing operation, for example those involving live
1685 organisms or radiopharmaceuticals that may give rise to biological or chemical hazards.
1686

1687 9.19 In the case where contaminants present due to the processes involved would damage the
1688 particle counter or present a hazard, e.g. live organisms and radiological hazards, the
1689 frequency and strategy employed should be such as to assure the environment classification
1690 both prior to and post exposure to the risk. Additionally, monitoring should be performed
1691 during simulated operations. Such operations should be performed at appropriately defined
1692 intervals. The approach should be defined in the contamination control strategy.
1693

1694 9.20 Where powdery products are manufactured, monitoring of particles may have to take
1695 into consideration an alternative monitoring scheme and frequency, e.g. monitoring for
1696 particle levels prior to and after the manufacturing process step.
1697

1698 9.21 The sample sizes taken for monitoring purposes using automated systems will usually
1699 be a function of the sampling rate of the system used. It is not necessary for the sample
1700 volume to be the same as that used for formal qualification of clean rooms and clean air
1701 devices.
1702

1703 9.22 Although monitoring of $\geq 5.0 \mu\text{m}$ particles are not required for room qualification and
1704 classification purposes, it is required for routine monitoring purposes as they are an important
1705 diagnostic tool for early detection of machine, equipment and HVAC failure.
1706

1707 9.23 The occasional indication of macro particle counts, especially $\geq 5.0 \mu\text{m}$, may be
1708 considered false counts due to electronic noise, stray light, coincidence, etc. However,
1709 consecutive or regular counting of low levels may be indicative of a possible contamination
1710 event and should be investigated. Such events may indicate early failure of the room air
1711 supply filtration (HVAC) system, filling equipment failure, or may also be diagnostic of poor
1712 practices during machine set-up and routine operation.
1713

1714 9.24 Monitoring conditions such as frequency, sampling volume or duration, alert and
1715 action limits and corrective action including investigation should be established in each
1716 manufacturing area based on risk assessment.
1717

1718 **Viable monitoring**

1719

1720 9.25 Where aseptic operations are performed, microbiological monitoring should be
1721 frequent using a combination of methods such as settle plates, volumetric air, glove print
1722 and surface sampling (e.g. swabs and contact plates).

1723

1724 9.26 Monitoring should include sampling of personnel at periodic intervals during the
1725 process. Particular consideration should be given to monitoring personnel following
1726 involvement in critical interventions and on exit from the grade A/B processing area.

1727

1728 9.27 Continuous monitoring in grade A and B areas should be undertaken for the full duration
1729 of critical processing, including equipment (aseptic set up) assembly and filling operations
1730 (i.e., an understanding of function and interactions of each clean area). The monitoring
1731 should be performed in such a way that all interventions, transient events and any system
1732 deterioration would be captured and any risk caused by interventions of the monitoring
1733 operations is avoided.

1734

1735 9.28 Rapid microbial monitoring methods may be adopted after validation as long as they are
1736 demonstrated to be at least equivalent to the established methodology.

1737

1738 9.29 Sampling methods should not pose a risk of contamination to the manufacturing
1739 operations.

1740

1741 9.30 Additional microbiological monitoring should also be performed outside production
1742 operations, e.g. after validation of systems, cleaning and disinfection.

1743

1744 9.31 Recommended action limits for microbial contamination are shown in Table 6

1745

1746 **Table 6: Recommended maximum limits for microbial contamination**

1747

Grade	Air sample cfu/m³	Settle plates (diam. 90 mm) cfu/4 hours^(a)	Contact plates (diam. 55mm), cfu/ plate	Glove print 5 fingers on both hands cfu/ glove
A ^(b)	1	1	1	1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

1748

1749 ^(a) Individual settle plates may be exposed for less than 4 hours. Where settle plates are
1750 exposed for less than 4 hours the limits in the table should still be used. Settle plates
1751 should be exposed for the duration of critical operations and changed as required after
1752 4 hours.

1753 ^(b) It should be noted that for grade A the expected result should be 0 cfu recovered;
1754 any recovery of 1 cfu or greater should result in an investigation.

1755

1756 9.32 Monitoring procedures should define the approach to trending. Trends can include but
1757 are not limited to:

1758

- 1759 a) Increasing numbers of action or alert limit breaches.
1760
1761 b) Consecutive breaches or alert limits.
1762
1763 c) Regular but isolated breaches of limits that may have a common cause, for example
1764 single excursions that always follow planned preventative maintenance.
1765
1766 d) Changes in flora type and numbers.
1767

1768 9.33 If microorganisms are detected in a grade A or B zone, they should be identified to
1769 species level and the impact of such microorganisms on product quality (for each batch
1770 implicated) and state of control should be evaluated. Consideration may also be given to the
1771 identification of grade C and D contaminants and the requirements should be defined in the
1772 contamination control strategy.
1773

1774 **Aseptic process simulation (APS)¹** 1775

1776 9.34 Periodic verification of the effectiveness of the controls in place for aseptic
1777 processing should include a process simulation test using a sterile nutrient media and/or
1778 placebo. Selection of an appropriate nutrient media should be made based on the ability of
1779 the media to imitate product characteristics at all processing stages. Where processing stages
1780 may indirectly impact the viability of any introduced microbial contamination, (e.g. sterile
1781 aseptically produced semi-solids, powders, solid materials, microspheres, liposomes and
1782 other formulations where product is cooled or heated or lyophilized, etc.), alternative
1783 surrogate procedures that represent the operations as closely as possible can be developed and
1784 justified. Where surrogate materials, such as buffers, are used in parts of the process
1785 simulation, the surrogate material should not inhibit the growth of any potential
1786 contamination.
1787

1788 9.35 The process simulation test should imitate as closely as possible the routine
1789 aseptic manufacturing process and include all the critical manufacturing steps. Specifically:
1790

- 1791 a) Process simulation tests should assess all aseptic operations performed subsequent to
1792 the sterilisation of materials utilised in the process.
1793
1794 b) For non-filterable formulations any additional aseptic steps should be assessed.
1795
1796 c) Aseptic manufacturing performed in a strict anaerobic environment should be
1797 evaluated with an anaerobic media in addition to aerobic evaluation.
1798
1799 d) Processes requiring the addition of sterile powders should employ an acceptable
1800 surrogate material in containers identical to those utilised in the process being
1801 evaluated.
1802
1803 e) Processes involving blending, milling and subdivision of a sterile powder require
1804 similar attention.
1805

¹ For further details on the validation of aseptic processing, please refer to the PIC/S Recommendation on the Validation of Aseptic Processing (PI 007) **For PICS version only**

1806 f) The process simulation test for lyophilized products should include the entire aseptic
1807 processing chain, including filling, transport, loading, chamber dwell, unloading and
1808 sealing. The process simulation should duplicate the lyophilization process, with the
1809 exception of freezing and sublimation, including partial vacuum and cycle duration
1810 and parameters as appropriate for the media. Boiling over or actual freezing of the
1811 solution should be avoided.

1812

1813 9.36 The process simulation testing should take into account various aseptic manipulations
1814 and interventions known to occur during normal production as well as worst-case situations,
1815 including:

1816

1817 a) Inherent interventions at the maximum accepted frequency per number of filled
1818 units.

1819 b) Corrective interventions in representative number and with the highest degree of
1820 intrusion acceptable.

1821

1822 9.37 There should be an approved list of allowed interventions, both inherent and corrective,
1823 which may occur during production and in the APS. The procedures listing the types of
1824 inherent and corrective interventions, and how to perform them, should be updated, as
1825 necessary, to ensure consistency with the actual manufacturing activities.

1826

1827 9.38 In developing the process simulation test plan, risk management principles should be
1828 used and consideration should be given to the following:

1829

1830 a) Identification of worst case conditions covering the relevant variables and their
1831 microbiological impact on the process. The outcome of the assessment should justify
1832 the variables selected.

1833

1834 b) Determining the representative sizes of container/closure combinations to be used
1835 for validation. Bracketing or a matrix approach can be considered for initial
1836 validation of the same container/closure configuration.

1837

1838 c) The volume filled per container, which should be sufficient to ensure that the media
1839 contacts all equipment and component surfaces that may directly contaminate the
1840 sterile product.

1841

1842 d) Maximum permitted holding times for sterile product and associated sterile
1843 components exposed during the aseptic process.

1844

1845 e) Ensuring that any contamination is detectable.

1846

1847 f) The requirement for substitution of any inert gas used in the routine aseptic
1848 manufacturing process by air, unless anaerobic simulation is intended.

1849

1850 g) The duration of the process simulation filling run to ensure it is conducted over the
1851 maximum permitted filling time. If this is not possible, then the run should be of
1852 sufficient duration to challenge the process, the operators that perform interventions,
1853 and the capability of the processing environment to provide appropriate conditions
1854 for the manufacture of a sterile product.

1855

- 1856 h) Simulating normal aseptic manufacturing interruptions where the process is idle. In
1857 these cases, environmental monitoring should be conducted to ensure that grade A
1858 conditions have been maintained.
1859
- 1860 i) The special requirements and considerations for manually intensive operations.
1861
- 1862 j) Where campaign manufacturing occurs, such as in the use of barrier technologies or
1863 manufacture of sterile active substances, consideration should be given to designing
1864 and performing the process simulation so that it simulates the risks associated with
1865 both the beginning and the end of the campaign and demonstrating that the campaign
1866 duration does not pose any risk. If end of production campaign APS are used, then it
1867 should be demonstrated that any residual product does not negatively impact the
1868 recovery of any potential microbiological contamination.
1869
- 1870 k) Where barrier technologies (RABS, isolators, BFS, etc.) are used in the routine
1871 aseptic manufacturing process, the relative risk and unique aspects of these
1872 technologies should be taken into consideration when assessing the design of aseptic
1873 process simulation tests.
1874

1875 9.39 For sterile active substances, batch sizes should be large enough to represent routine
1876 operation, simulate intervention operation at the worst case, and cover potential contact
1877 surfaces. In addition, all the simulated materials (surrogates of growth medium) should be
1878 subjected to microbiological evaluation. The recovery rate from simulation materials should
1879 be sufficient to satisfy the evaluation of the process being simulated and should not
1880 compromise the recovery of micro-organisms.
1881

1882 9.40 Process simulation tests should be performed as initial validation, generally with
1883 three consecutive satisfactory simulation tests per shift, and after any significant
1884 modification to the HVAC system, equipment, major facility shut down, process and
1885 number of shifts, etc. Normally process simulation tests (periodic revalidation) should be
1886 repeated twice a year (approximately every six months) for each aseptic process and filling line,
1887 and at least annually for each operator. Consideration should be given to performing an APS after
1888 the last batch prior to shut down, before long periods of inactivity or before decommissioning or
1889 relocation of a line.
1890

1891 9.41 Where manual filling occurs, each product, container closure, equipment train and
1892 operator should be revalidated approximately every 6 months. The APS batch size should
1893 mimic that used in the routine aseptic manufacturing process. An aseptic process or filling
1894 should be subject to a repeat of the initial validation when:
1895

- 1896 a) Revalidation of the unique process has failed and corrective actions have been taken.
1897
- 1898 b) The specific aseptic process has not been in operation for an extended period of
1899 time..
1900
- 1901 c) A change to the process, equipment, personnel, procedures or environment that has
1902 potential to affect the aseptic process or the addition of new product containers or
1903 container-closure combinations.
1904

1905 9.42 The number of units processed (filled) for process simulation tests should be

1906 sufficient to effectively simulate all activities that are representative of the aseptic
1907 manufacturing process; justification for the number of units to be filled should be clearly
1908 captured in the PQS. For small batches, e.g. those under 5,000 units filled, the number of
1909 containers for media fills should at least equal the size of the production batch.

1910
1911 9.43 The target should be zero growth and any contaminated unit should result in an
1912 investigation (refer to clause 9.47) to determine the root cause (if possible) and to identify
1913 appropriate CAPA. Following implementation of CAPA, a repeat APS will be required to
1914 validate the effectiveness of the CAPA. The number of APS to be repeated should be
1915 determined using QRM principles taking into consideration the number and type of CAPA
1916 and the level of contamination found in the failed APS. Typically 3 successful consecutive
1917 repeat APS would be expected; any differences to this expectation should be clearly justified
1918 prior to repeat performance.

1919
1920 9.44 Filled APS units should be agitated, swirled or inverted before incubation to ensure
1921 contact of the media with all interior surfaces in the container. Cosmetic defects, non-
1922 destructive weight checks and all other units should be identified and incubated with the other
1923 units. Units discarded during the process simulation and not incubated should be comparable
1924 to units discarded during a routine fill.

1925 9.45 Filled APS units should be incubated in a clear container to ensure visual detection of
1926 microbial growth. Microorganisms isolated from contaminated units should be identified to at
1927 least the genus, and to the species level when practical, to assist in the determination of the
1928 likely source of the contaminant. The selection of the incubation duration and temperature
1929 should be justified and appropriate for the process being simulated and the selected growth
1930 medium.

1931
1932 9.46 All products that have been manufactured on a line subsequent to the process simulation
1933 should be quarantined until a successful resolution of the process simulation has occurred.

1934
1935 9.47 In the case of a failed process simulation there should be a prompt review of all
1936 appropriate records relating to aseptic production since the last successful process simulation.
1937 The outcome of the review should include a risk assessment of the non-sterility for batches
1938 manufactured since the last successful process simulation, and the justification for the
1939 disposition of batches of product affected. Subsequent to a failed APS, in addition to a full
1940 investigation, production should resume only upon further successful APS unless adequately
1941 justified. The number of repeat successful APS prior to resuming production should also be
1942 justified.

1943
1944 9.48 Where results indicate that an operator may have failed qualification, actions to restrict
1945 entry of the operator to the aseptic processing areas should be taken.

1946
1947 9.49 All process simulation runs should be fully documented and include a reconciliation of
1948 units processed and changes in the custody of the APS batch. All interventions performed
1949 during the process simulations should be recorded, including the start and end of each
1950 intervention.

1951

1952 **10 Quality Control (QC)**

1953

1954 10.1 Microbiological contamination of starting materials should be minimal.
1955 Specifications should include requirements for microbiological quality when the need for
1956 this has been indicated by monitoring and/or by the contamination control strategy.
1957

1958 10.2 The bioburden assay should be performed on each batch for both aseptically filled
1959 product and terminally sterilized products and the results considered as part of the final
1960 batch review. There should be working limits on contamination immediately before
1961 sterilization, which are related to the efficiency of the method to be used.
1962

1963 10.3 Where overkill sterilization parameters are set for terminally sterilized products,
1964 bioburden should be monitored at suitable scheduled intervals.
1965

1966 10.4 For parametric release systems, the bioburden assay should be performed on each batch
1967 and considered as an in-process test. Where appropriate, the level of endotoxins should
1968 be monitored.
1969

1970 10.5 The sterility test applied to the finished product should only be regarded as the last in
1971 a series of control measures by which sterility is assured. The test should be validated for
1972 the product(s) concerned.
1973

1974 10.6 The sterility test should be performed under aseptic conditions, which are at least
1975 consistent with the standard of clean room required for the aseptic manufacture of
1976 pharmaceutical products.
1977

1978 10.7 Samples taken for sterility testing should be representative of the whole of the batch,
1979 but should in particular include samples taken from parts of the batch considered to be
1980 most at risk of contamination, for example:
1981

1982 a) Products which have been filled aseptically, samples should include containers
1983 filled at the beginning and end of the batch and after any significant intervention.
1984

1985 b) Products which have been heat sterilized in their final containers, consideration
1986 should be given to taking samples from the potentially coolest part of the load.
1987

1988 c) Each sterilized load should be considered as different batches and require a separate
1989 sterility test.
1990

1991 d) Products that have been lyophilized in different lyophilization loads..
1992

1993 Note: Where sterilization or lyophilization leads to separate sterility tests, consideration of
1994 performing separate testing for other finished product tests should also be given.
1995

1996 10.8 Any process (e.g. VHP) used to decontaminate sterility samples prior to testing should
1997 not negatively impact the sensitivity of the test method.
1998

1999 10.9 Media used for environmental monitoring and APS should be tested for its growth
2000 promotion capability, in accordance with a formal written program.
2001

2002 10.10 Environmental monitoring data generated in grade A and B areas should be reviewed
2003 as part of product batch release. A written plan should be available that describes the actions

2004 to be taken when data from environmental monitoring are found out of trend or out of
2005 specification.
2006
2007 10.11 The use of rapid microbial methods can also be considered. These methods should be
2008 validated for the product(s) or processes concerned and be approved in the registered product
2009 testing specification.
2010

2011 **11 Glossary**

2012

2013 Air lock - A small room with interlocked doors, constructed to maintain air pressure control
2014 between adjoining rooms (generally with different air cleanliness standards). The intent of an
2015 aseptic processing airlock is to preclude ingress of particulate matter and microorganism
2016 contamination from a lesser controlled area.

2017

2018 Alert Level - An established microbial or airborne particle level giving early warning of
2019 potential drift from normal operating conditions and triggers appropriate scrutiny and follow-
2020 up to address the potential problem. Alert levels are always lower than action levels and are
2021 established based on historical and qualification trend data and periodically reviewed.

2022

2023 Action Level - An established microbial or airborne particle level that, when exceeded,
2024 should trigger appropriate investigation and corrective action based on the investigation.

2025

2026 Aseptic Manufacturing Area - The classified part of a facility that includes the aseptic
2027 processing room and ancillary cleanrooms. For purposes of this document, this term is
2028 synonymous with “aseptic processing facility”.

2029

2030 Aseptic Processing Facility - A building, or segregated segment of it, containing cleanrooms
2031 in which air supply, materials, and equipment are regulated to control microbial and particle
2032 contamination.

2033

2034 Aseptic Processing Room - A room in which one or more aseptic activities or processes are
2035 performed.

2036

2037 Asepsis - A state of control attained by using an aseptic work area and performing activities
2038 in a manner that precludes microbiological contamination of the exposed sterile product.

2039

2040 Bacterial retention testing – This test is performed to validate that a filter can remove bacteria
2041 from a gas or solution. The test is usually performed using a standard organism, such as
2042 *Brevundimonas diminuta* at a minimum concentration of 10⁷ Colony Forming Units/ml.

2043

2044

2045 Bioburden - The total number of microorganisms associated with a specific item prior to
2046 sterilization.

2047

2048 Barrier - A physical partition that affords aseptic processing area (grade A) protection by
2049 partially separating it from the surrounding area such as RABS or isolators.

2050

2051 Biological Indicator (BI) - A population of microorganisms inoculated onto a suitable
2052 medium (e.g. solution, container or closure) and placed within appropriate sterilizer load
2053 locations to determine the sterilization cycle efficacy of a physical or chemical process. The
2054 challenge microorganism is selected based upon its resistance to the given process. Incoming
2055 lot D-value and microbiological count define the quality of the BI.

2056

2057 Blow-Fill-Seal - Blow-Fill-Seal (BFS) technology is a pharmaceutical filling process in
2058 which containers are formed from a thermoplastic granulate, filled with product, and then
2059 sealed in a continuous, integrated, automatic operation. The two most common types of BFS
2060 machines are the Shuttling machine (with Parison cut) and the Rotary machine (Closed

2061 Parison) types. The equipment design, operation, and therefore controls for these differ. For
2062 Shuttling systems the processes of container extrusion and filling occur at two separate
2063 locations within the machine. The extrusion of the container parison occurs adjacent to the
2064 filling zone, the extruded plastic is collected from underneath the extruder head, is cut and
2065 formed and automatically transferred (usually by horizontal shuttling) to the filling and
2066 sealing zone. For Rotary design machines the filling needles are enclosed within the extruded
2067 parison and therefore there is limited exposure of the inner surfaces of the container to the
2068 external environment.

2069
2070 Clean Area - An area with defined particle and microbiological cleanliness standards.

2071
2072 Cleanroom - A room designed, maintained, and controlled to prevent particle and
2073 microbiological contamination of drug products. Such a room is assigned and reproducibly
2074 meets an appropriate air cleanliness classification.

2075
2076 Clean Non Classified (CNC) area - An area that does not meet any of the formal pre-
2077 determined grades of cleanliness included in the Annex, i.e. grades A to D, but where a
2078 manufacturer defined level of microbial control is still required. The area should be subject to
2079 a formal cleaning/disinfection regime and formal environmental monitoring program to
2080 achieve the defined level of control. The level, type and frequency of both the cleaning
2081 program and the environmental monitoring program (including contamination limits) should
2082 be based on a formal risk assessment (captured within the wider contamination control
2083 strategy) and should be commensurate with the specific risks to the processes and product
2084 performed manufactured within each CNC area.

2085
2086 It is possible that different CNC areas within the same facility may have different approaches
2087 to control and monitoring, based on differing risks to processes and products.

2088
2089 Clean Zone - See Clean Area.

2090
2091 Closed system – A system in which the sterile product is not exposed to the surrounding
2092 environment.

2093
2094 Colony Forming Unit (cfu) - A microbiological term that describes the formation of a single
2095 macroscopic colony after the introduction of one or more microorganisms to microbiological
2096 growth media. One colony forming unit is expressed as 1 cfu.

2097
2098 Commissioning – Activities to verify that equipment and systems are installed according to
2099 specification

2100
2101 Component - Any ingredient intended for use in the manufacture of a drug product, including
2102 those that may not appear in the final drug product.

2103
2104 Critical Area - An area designed to maintain sterility of sterile materials. Sterilized product,
2105 containers, closures, and equipment may be exposed in critical areas such as the grade A area
2106 or a closed system.

2107
2108 Critical surfaces - Surfaces that may come into contact with, or directly affect, a sterilized
2109 product or its containers or closures. Critical surfaces are rendered sterile prior to the start of
2110 the manufacturing operation, and sterility is maintained throughout processing.

2111
2112 Critical zone – See critical area
2113
2114 D value - The time (in minutes) of exposure at a given temperature that causes a one-log or
2115 90 per cent reduction in the population of a specific microorganism.
2116
2117 Deadleg – length of pipe that is not part of the circuit that is greater than 3 internal pipe
2118 diameters
2119
2120 Decontamination - A process that eliminates viable bioburden via use of chemical agents.
2121
2122 Depyrogenation - A process used to destroy or remove pyrogens (e.g. endotoxin).
2123
2124 Disinfection – The process by which surface bioburden is reduced to a safe level or
2125 eliminated. Some disinfection agents are effective only against vegetative microbes, while
2126 others possess additional capability to effectively kill bacterial and fungal spores.
2127
2128 Dynamic - Conditions relating to clean area classification under normal production
2129 operations.
2130
2131 Endotoxin - A pyrogenic product (e.g. lipopolysaccharide) present in the bacterial cell wall.
2132 Endotoxin can lead to reactions in patients receiving injections ranging from fever to death.
2133
2134 Extractables - Chemical entities that migrate from the surface of the process equipment
2135 contacting with model solvents under appropriate testing conditions (e.g. kind of solvent,
2136 temperature) that exceed “worst case” process conditions.
2137
2138 Form Fill seal – Similar to Blow fill Seal, this involves the formation of a large tube formed
2139 from a flexible packaging material, in the filling machine, the tube is then filled to form large
2140 volume bags.
2141
2142 Gowning Qualification - A program that establishes, both initially and on a periodic basis, the
2143 capability of an individual to don the complete sterile gown in an aseptic manner.
2144
2145 Grade A air – Air which is passed through a filter qualified as capable of producing grade A
2146 non-viable quality air, but where there is no requirement to continuously perform non-viable
2147 monitoring or meet grade A viable monitoring limits.
2148
2149 HEPA filter - High efficiency particulate air filter with minimum 0.3 µm particle retaining
2150 efficiency of 99.97 percent.
2151
2152 HVAC - Heating, ventilation, and air conditioning.
2153
2154 Intervention - An aseptic manipulation or activity that occurs at the critical area.
2155
2156 Intrinsic sterile connection device - A device that removes the risk of contamination during
2157 the connection process; these can be mechanical or fusion devices.
2158

2159 Isokinetic sampling head – A sampling head designed to disturb the air as little as possible so
2160 that the same particles go into the nozzle as would have passed the area of the nozzle had it
2161 not been there.

2162
2163 Isolator - A decontaminated unit supplied with grade A (ISO 5) or higher air quality that
2164 provides uncompromised, continuous isolation of its interior from the external environment
2165 (e.g., surrounding cleanroom air and personnel). There are two major types of isolators:

2166
2167 *Closed isolator systems* exclude external contamination from the isolator’s interior by
2168 accomplishing material transfer via aseptic connection to auxiliary equipment, rather
2169 than use of openings to the surrounding environment. Closed systems remain sealed
2170 throughout operations.

2171
2172 *Open isolator systems* are designed to allow for the continuous or semi-continuous
2173 ingress and/or egress of materials during operations through one or more openings.
2174 Openings are engineered (e.g., using continuous overpressure) to exclude the entry of
2175 external contamination into the isolator.

2176
2177 Laminar flow - An airflow moving in a single direction and in parallel layers at constant
2178 velocity from the beginning to the end of a straight line vector.

2179
2180 Leachables - Chemical entities that migrate into medicinal products from the product contact
2181 surface of the process equipment under actual product and process conditions.

2182
2183 Lyophilization A physical-chemical drying process designed to remove solvents from both
2184 aqueous and non-aqueous systems, primarily to achieve product or material stability.
2185 Lyophilization is synonymous to the term freeze-drying.

2186
2187 Manual Filling –Where the product is transferred into the final container by systems where
2188 operator intervention is required to complete the filling of each container e.g. pipetting
2189 liquids.

2190
2191 Operator - Any individual participating in the aseptic processing operation, including line set-
2192 up, filler, maintenance, or other personnel associated with aseptic line activities.

2193
2194 Overkill sterilization process - A process that is sufficient to provide at least a 12 log
2195 reduction of microorganisms having a minimum D value of 1 minute.

2196
2197 Pass through hatch – refer to airlock.

2198
2199 Pyrogen - A substance that induces a febrile reaction in a patient.

2200
2201 Qualification - Establishing documented evidence that provides a high degree of assurance
2202 that equipment or facilities will perform to the required specification detailed in the user
2203 requirement specification and the design qualification.

2204
2205 Restricted Access Barrier System (RABS) - A restricted access barrier system (RABS)
2206 provides an enclosed, but not closed, environment meeting defined cleanroom conditions
2207 using a rigid-wall enclosure and air overspill to separate its interior from the surrounding
2208 environment.

2209
2210 Active RABS: integral HEPA-filtered air supply
2211
2212 Passive RABS: air supply by ceiling mounted HEPA-filters.
2213
2214 Open RABS. Where there are vents in the barrier that allow air to move from the grade A
2215 to the grade B area.
2216
2217 Sterile Product - For purposes of this guidance, sterile product refers to one or more of the
2218 elements exposed to aseptic conditions and ultimately making up the sterile finished drug
2219 product. These elements include the containers, closures, and components of the finished
2220 drug product.
2221
2222 Sterilizing grade filter - A filter that, when appropriately validated, will remove a defined
2223 microbial challenge from a fluid stream, producing a sterile effluent.
2224
2225 Single Use Systems (SUS) - Systems in which some product contact components are used
2226 only once (i.e. single use components) to replace reusable equipment such as stainless steel
2227 transfer lines or bulk containers. SUS covered in this document are those that are used in
2228 manufacturing processes of sterile medicinal products (e.g. sterile API, sterile bio bulk, sterile
2229 finish dosage), and are typically made up of components such as bags, filters, tubing,
2230 connectors, storage bottles and sensors.
2231
2232 Terminal sterilization - The application of a lethal sterilizing agent to finished product within
2233 a sealed container to achieve a predetermined sterility assurance level (SAL) of 10^{-6} or better
2234 (i.e. the theoretical probability of there being a single viable microorganism present on or in a
2235 sterilized unit is equal to or less than 1×10^{-6} (one in a million)).
2236
2237 ULPA filter - Ultra-low penetration air filter with minimum 0.3 μm particle retaining
2238 efficiency of 99.999 per cent.
2239
2240 Unidirectional flow - An airflow moving in a single direction, in a robust and uniform
2241 manner, and at sufficient speed, to reproducibly sweep particles away from the critical
2242 processing or testing area.
2243
2244 Validation - Establishing documented evidence that provides a high degree of assurance that
2245 a specific process will consistently produce a product meeting its predetermined
2246 specifications and quality attributes.
2247
2248 Worst case - A set of conditions encompassing upper and lower processing limits and
2249 circumstances, including those within standard operating procedures, that pose the greatest
2250 chance of process or product failure (when compared to ideal conditions). Such conditions do
2251 not necessarily induce product or process failure.