



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

Section 1.01 Principle

The manufacture of sterile products is subject to special requirements in order to minimize risks of microbiological contamination, and of particulate and pyrogen contamination. Much depends on the skill, training and attitudes of the personnel involved. Quality Assurance is particularly important and this type of manufacture must strictly follow carefully established and validated methods of preparation and procedure. Sole reliance for sterility or other quality aspects must not be placed on any terminal process or finished product test.

Note: This guidance does not lay down detailed methods for determining the microbiological and particulate cleanliness of air, surfaces, etc.

Section 1.02 General

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.
2. The various operations of component preparation, product preparation and filling should be carried out in separate areas within the clean area. Manufacturing operations are divided into two categories; firstly, those where the product is terminally sterilized, and secondly those which are conducted aseptically at some or all stages.
3. Clean areas for the manufacture of sterile products are classified according to the required characteristics of the environment. Each manufacturing operation requires an appropriate environmental cleanliness level in the operational state in order to minimize the risks of particulate or microbial contamination of the product or materials being handled.

In order to meet “in operation” conditions these areas should be designed to reach certain specified air-cleanliness levels in the “at rest” occupancy state. The “at rest” state is the condition where the installation is installed and operating, complete with production equipment but with no operating personnel present. The “in



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

operation” state is the condition where the installation is functioning in the defined operating mode with the specified number of personnel working. The “in operation” and “at rest” states should be defined for each clean room or suite of clean rooms.

For the manufacture of sterile medicinal products 4 grades 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 laminar air flow work station. Laminar air flow systems should provide a homogeneous air speed in a range of 0.36 – 0.54 m/s (guidance value) at the working position in open clean room applications. The maintenance of laminarity should be demonstrated and validated. A uni-directional air flow and lower velocities may be used in closed isolators and glove boxes.

Grade B: For aseptic preparation and filling, this is the background environment for grade A zone.

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

The airborne particulate classification for these grades is given in the following table.

Grade	At rest ^(b)		In operation ^(b)	
	Maximum permitted number of particles/m ³ above ^(a)		Maximum permitted number of particles/m ³ equal to or above ^(a)	
	0.5µm ^(d)	5µm	0.5µm ^(d)	5µm
A	3,500	1 ^(e)	3,500	1 ^(e)
B ^(c)	3,500	1 ^(e)	350,000	2,000



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

C ^(c)	350,000	2,000	3,500,000	20,000
D ^(c)	3,500,000	20,000	not defined ^(f)	not defined ^(f)

Notes:

- (a) Particle measurement based on the use of a discrete airborne particle counter to measure the concentration of particles at designated sizes equal to or greater than the threshold stated. A continuous measurement system should be used for monitoring the concentration of particles in the grade A zone, and is recommended for the surrounding grade B areas. For routine testing the total sample volume should not be less than 1 m³ for grade A and B areas and preferably also in grade C areas.
- (b) The particulate conditions given in the table for the “at rest” state should be achieved after a short “clean up” period of 15-20 minutes (guidance value) in an unmanned state after completion of operations. The particulate conditions for grade A “in operation” given in the table should be maintained in the zone immediately surrounding the product whenever the product or open container is exposed to the environment. It is accepted that it may not always be possible to demonstrate conformity with particulate standard at the point of fill when filling is in progress, due to the generation of particles or droplets from the product itself.
- (c) In order to reach the B, C and D air grades, the number of air changes should be related to the size of the room and the equipment and personnel present in the room. The air system should be provided with appropriate terminal filters such as HEPA for grades A, B and C.
- (d) The guidance given for the maximum permitted number of particles in the “at rest” and “in operation” conditions correspond approximately to the cleanliness classes in the EN/ISO 14644-1 at a particle size of 0.5 µm.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

- (e) These areas are expected to be completely free from particles of size greater than 5 micrometer. As it is impossible to demonstrate the absence of particles with any statistical significance, the limits are set to 1 particle /m³. During the clean room qualification it should be shown that the areas can be maintained within the defined limits.
- (f) The requirements and limits will depend on the nature of the operations carried out.

Examples of operations to be carried out in the various grades are given in the table below (see also para. 11 and 12):

Grade	Examples of operations for terminally sterilised products (see para. 11)
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

Grade	Examples of operations for terminally sterilized products (see para. 12)
A	Aseptic preparation and filling
C	Preparation of solutions to be filtered
D	Handling of components after washing



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

4. The areas should be monitored during operation in order to control the particulate cleanliness of the various grades.
5. Where aseptic operations are performed monitoring should be frequent using methods such as settle plates, volumetric air and surface sampling (e.g. swabs and contact plates). Sampling methods used in operation should not interfere with zone protection. Results from monitoring should be considered when reviewing batch documentation for finished product release. Surfaces and personnel should be monitored after critical operations.

Additional microbiological monitoring is also required outside production operations, e.g. after validation of systems, cleaning and sanitization.

Recommended limits for microbiological monitoring of clean areas during operation:

Recommended limits for microbial contamination ^(a)				
Grade	Air sample cfu/m ³	Settle plates (diam. 90 mm), cfu/4 hours ^(b)	Contact plates (diam. 55 mm), cfu/plate	Glove print 5 fingers cfu/glove
A	< 1	< 1	< 1	< 1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

Notes:

- (a) These are average values.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

- (b) Individual settle plates may be exposed for less than 4 hours.
6. Appropriate alert and action limits should be set for the results of particulate and microbiological monitoring. If these limits are exceeded operating procedures should prescribe corrective action.

Section 1.03 Isolator Technology

7. The utilization of isolator technology to minimize human interventions in processing areas may result in a significant decrease in the risk of microbiological contamination of aseptically manufactured products from the environment. There are many possible designs of isolators and transfer devices. The isolator and the background environment should be designed so that the required air quality for the respective zones can be realized. Isolators are constructed of various materials more or less prone to puncture and leakage. Transfer devices may vary from a single door to double door designs to fully sealed systems incorporating sterilization mechanisms.

The transfer of materials into and out of the unit is one of the greatest potential sources of contamination. In general the area inside the isolator is the local zone for high risk manipulations, although it is recognized that laminar air flow may not exist in the working zone of all such devices. The air classification required for the background environment depends on the design of the isolator and its application. It should be controlled and for aseptic processing be at least grade D.

8. Isolators should be introduced only after appropriate validation. Validation should take into account all critical factors of isolator technology, for example the quality of the air inside and outside (background) the isolator, sanitation of the isolator, the transfer process and isolator integrity.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

9. Monitoring should be carried out routinely and include frequent leak testing of the isolator and glove/sleeve system.

Section 1.04 Blow/Fill/Seal Technology

10. Blow/fill/seal units are purpose-built machines in which, in one continuous operation, containers are formed from a thermoplastic granulate, filled and then sealed, all by the one automatic machine. Blow/fill/seal equipment used for aseptic production which is fitted with an effective grade A air shower may be installed in at least a grade C environment, provided that grade A/B clothing is used. The environment should comply with the viable and non-viable limits “at rest” and the viable limit only when in operation. Blow/fill/seal equipment used for the production of products for terminal sterilization should be installed in at least a grade D environment.

Because of this special technology particular attention should be paid to at least the following: equipment design and qualification, validation and reproducibility of cleaning-in-place and sterilization-in-place, background clean room environment in which the equipment is located, operator training and clothing, and interventions in the critical zone of the equipment including any aseptic assembly prior to the commencement of filling.

Section 1.05 Terminally Sterilized Products

11. Preparation of components and most products should be done in at least a grade D environment in order to give low risk of microbial and particulate contamination, suitable for filtration and sterilization. Where there is unusual risk to the product because of microbial contamination, for example, because the product actively supports microbial growth or must be held for a long period before sterilization or is necessarily processed not mainly in closed vessels, preparation should be done in a grade C environment.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

Filling of products for terminal sterilization should be done in at least a grade C environment. Where the product is at unusual risk of contamination from the environment, for example because the filling operation is slow or the containers are wide-necked or are necessarily exposed for more than a few seconds before sealing, the filling should be done in a grade A zone with at least a grade C background. Preparation and filling of ointments, creams, suspensions and emulsions should generally be done in a grade C environment before terminal sterilization.

Section 1.06 Aseptic Preparation

12. Components after washing should be handled in at least a grade D environment. Handling of sterile starting materials and components, unless subjected to sterilization or filtration through a micro-organism-retaining filter later in the process, should be done in a grade A environment with grade B background.

Preparation of solutions which are to be sterile filtered during the process should be done in a grade C environment; if not filtered, the preparation of materials and products should be done in a grade A environment with a grade B background.

Handling and filling of aseptically prepared products should be done in a grade A environment with a grade B background.

Transfer of partially closed containers, as used in freeze drying, should, prior to the completion of stoppering, be done either in a grade A environment with grade B background or in sealed transfer trays in a grade B environment.

Preparation and filling of sterile ointments, creams, suspensions and emulsions should be done in a grade A environment, with a grade B background, when the product is exposed and is not subsequently filtered.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

Section 1.07 Personnel

13. Only the minimum number of personnel required should be present in clean areas; this is particularly important during aseptic processing. Inspections and controls should be conducted outside the clean areas as far as possible.
14. All personnel (including those concerned with cleaning and maintenance) employed in such areas should receive regular training in disciplines relevant to the correct manufacture of sterile products, including reference to hygiene and to the basic elements of microbiology. When outside staff who have not received such training (e.g. building or maintenance contractors) need to be brought in, particular care should be taken over their instruction and supervision.
15. Staff who have been engaged in the processing of animal tissue materials or of cultures of micro-organisms other than those used in the current manufacturing process should not enter sterile-product areas unless rigorous and clearly defined entry procedures have been followed.
16. High standards of personnel hygiene and cleanliness are essential. Personnel involved in the manufacture of sterile preparations should be instructed to report any condition which may cause the shedding of abnormal numbers or types of contaminants; periodic health checks for such conditions are desirable. Actions to be taken about personnel who could be introducing undue microbiological hazard should be decided by a designated competent person
17. Changing and washing should follow a written procedure designed to minimize contamination of clean area clothing or carry-through of contaminants to the clean areas.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

18. Wristwatches, make-up and jewellery should not be worn in clean areas.
19. The clothing and its quality should be appropriate for the process and the grade of the working area. It should be worn in such a way as to protect the product from contamination.

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

Grade D: Hair and, where relevant, beard should be covered. A general protective suit and appropriate shoes or overshoes should be worn. Appropriate measures should be taken to avoid any contamination coming from outside the clean area.

Grade C: Hair and, where relevant, beard and moustache should be covered. A single or two-piece trouser suit, gathered at the wrists and with high neck and appropriate shoes or overshoes should be worn. They should shed virtually no fibres or particulate matter.

Grade A/B: Headgear should totally enclose hair and, where relevant, beard and moustache; it should be tucked into the neck of the suit; a face mask should be worn to prevent the shedding of droplets. Appropriate sterilized, non-powdered rubber or plastic gloves and sterilized or disinfected footwear should be worn. Trouser-bottoms should be tucked inside the footwear and garment sleeves into the gloves. The protective clothing should shed virtually no fibres or particulate matter and retain particles shed by the body.

20. Outdoor clothing should not be brought into changing rooms leading to grade B and C rooms. For every worker in a grade A/B area, clean sterile (sterilized or adequately sanitized) protective garments should be provided at each work session. Gloves should be regularly disinfected during operations. Masks and gloves should be changed at least at every working session.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

21. Clean area clothing should be cleaned and handled 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.

Section 1.08 Premises

22. In clean areas, all exposed surfaces should be smooth, impervious and unbroken in order to minimize the shedding or accumulation of particles or micro-organisms and to permit the repeated application of cleaning agents, and disinfectants where used.
23. To reduce accumulation of dust and to facilitate cleaning there should be no uncleanable recesses and a minimum of projecting ledges, shelves, cupboards and equipment. Doors should be designed to avoid those uncleanable recesses; sliding doors may be undesirable for this reason.
24. False ceilings should be sealed to prevent contamination from the space above them.
25. Pipes and ducts and other utilities should be installed so that they do not create recesses, unsealed openings and surfaces which are difficult to clean.
26. Sinks and drains should be prohibited in grade A/B areas used for aseptic manufacture. In other areas air breaks should be fitted between the machine or sink and the drains. Floor drains in lower grade clean rooms should be fitted with traps or water seals to prevent back-flow.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

27. Changing rooms should be designed as airlocks and used to provide physical separation of the different stages of changing and so minimize microbial and particulate contamination of protective clothing. They should be flushed effectively with filtered air. The final stage of the changing room should, in the “at rest” state, be the same grade as the area into which it leads. The use of separate changing rooms for entering and leaving clean areas is sometimes desirable. In general hand washing facilities should be provided only in the first stage of the changing rooms.
28. Both airlock doors should not be opened simultaneously. An interlocking system or a visual and/or audible warning system should be operated to prevent the opening of more than one door at a time.
29. A filtered air supply should maintain a positive pressure and an air flow relative to surrounding areas of a lower grade under all operational conditions and should flush the area effectively. Adjacent rooms of different grades should have a pressure differential of 10-15 pascals (guidance values). Particular attention should be paid to the protection of the zone of greatest risk, that is, the immediate environment to which a product and cleaned components which contact the product are exposed. The various recommendations regarding air supplies and pressure differentials may need to be modified where it becomes necessary to contain some materials, e.g. pathogenic, highly toxic, radioactive or live viral or bacterial materials or products. Decontamination of facilities and treatment of air leaving a clean area may be necessary for some operations.
30. It should be demonstrated that air-flow patterns do not present a contamination risk, e.g. care should be taken to ensure that air flows do not distribute particles from a particle-generating person, operation or machine to a zone of higher product risk.
31. A warning system should be provided to indicate failure in the air supply. Indicators of pressure differences should be fitted between areas where these differences are



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

important. These pressure differences should be recorded regularly or otherwise documented.

Section 1.09 Equipment

32. A conveyor belt should not pass through a partition between a grade A or B area and a processing area of lower air cleanliness, unless the belt itself is continually sterilized (e.g. in a sterilizing tunnel).
33. As far as practicable, equipment, fittings and services should be designed and installed so that operations, maintenance and repairs can be carried out outside the clean area. If sterilization is required, it should be carried out after complete reassembly wherever possible.
34. When equipment maintenance has been carried out within the clean area, the area should be cleaned, disinfected and/or sterilized where appropriate, before processing recommences if the required standards of cleanliness and/or asepsis have not been maintained during the work.
35. Water treatment plants and distribution systems should be designed, constructed and maintained so as to ensure a reliable source of water of an appropriate quality. They should not be operated beyond their designed capacity. Water for injections should be produced, stored and distributed in a manner which prevents microbial growth, for example by constant circulation at a temperature above 70°C.
36. All equipment such as sterilizers, air handling and filtration systems, air vent and gas filters, water treatment, generation, storage and distribution systems should be subject to validation and planned maintenance; their return to use should be approved.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

Section 1.10 Sanitation

37. The sanitation of clean areas is particularly important. They should be cleaned thoroughly in accordance with a written programme. Where disinfectants are used, more than one type should be employed. Monitoring should be undertaken regularly in order to detect the development of resistant strains.
38. Disinfectants and detergents should be monitored for microbial contamination; dilutions should be kept in previously cleaned containers and should only be stored for defined periods unless sterilised. Disinfectants and detergents used in Grades A and B areas should be sterile prior to use.
39. Fumigation of clean areas may be useful for reducing microbiological contamination in inaccessible places.

Section 1.11 Processing

40. Precautions to minimize contamination should be taken during all processing stages including the stages before sterilization.
41. Preparations of microbiological origin should not be made or filled in areas used for the processing of other medicinal products; however, vaccines of dead organisms or of bacterial extracts may be filled, after inactivation, in the same premises as other sterile medicinal products.
42. Validation of aseptic processing should include a process simulation test using a nutrient medium (media fill). Selection of the nutrient medium should be made based on dosage form of the product and selectivity, clarity, concentration and suitability for sterilization of the nutrient medium. The process simulation test should imitate as closely as possible the routine aseptic manufacturing process



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

and include all the critical subsequent manufacturing steps. It should also take into account various interventions known to occur during normal production as well as worst case situations. Process simulation tests should be performed as initial validation with three consecutive satisfactory simulation tests per shift and repeated at defined intervals and after any significant modification to the HVAC system, equipment, process and number of shifts. Normally process simulation tests should be repeated twice a year per shift and process. The number of containers used for media fills should be sufficient to enable a valid evaluation. For small batches, the number of containers for media fills should at least equal the size of the product batch. The target should be zero growth but a contamination rate of less than 0.1% with 95% confidence limit is acceptable. The manufacturer should establish alert and action limits. Any contamination should be investigated.

43. Care should be taken that any validation does not compromise the processes.
44. Water sources, water treatment equipment and treated water should be monitored regularly for chemical and biological contamination and, as appropriate, for endotoxins. Records should be maintained of the results of the monitoring and of any action taken.
45. Activities in clean areas and 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. The ambient temperature and humidity should not be uncomfortably high because of the nature of the garments worn.
46. Microbiological contamination of starting materials should be minimal. Specifications should include requirements for microbiological quality when the need for this has been indicated by monitoring.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

47. Containers and materials liable to generate fibres should be minimized in clean areas.
48. Where appropriate, measures should be taken to minimize the particulate contamination of the end product.
49. Components, containers and equipment should be handled after the final cleaning process in such a way that they are not re-contaminated.
50. The interval between the washing and drying and the sterilization of components, containers and equipment as well as between their sterilization and use should be minimized and subject to a time-limit appropriate to the storage conditions.
51. The time between the start of the preparation of a solution and its sterilization or filtration through a micro-organism-retaining filter should be minimized. There should be a set maximum permissible time for each product that takes into account its composition and the prescribed method of storage.
52. The bioburden should be monitored before sterilization. There should be working limits on contamination immediately before sterilization which are related to the efficiency of the method to be used. Where appropriate the absence of pyrogens should be monitored. All solutions, in particular large volume infusion fluids, should be passed through a micro-organism-retaining filter, if possible sited immediately before filling.
53. Components, containers, equipment and any other article required in a clean area where aseptic work takes place should be sterilized and passed into the area through double-ended sterilizers sealed into the wall, or by a procedure which achieves the same objective of not introducing contamination. Noncombustible gases should be passed through micro-organism retentive filters.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

54. The efficacy of any new procedure should be validated, and the validation verified at scheduled intervals based on performance history or when any significant change is made in the process or equipment.

Section 1.12 Sterilization

55. All sterilization processes should be validated. Particular attention should be given when the adopted sterilization method is not described in the current edition of the European Pharmacopoeia, or when it is used for a product which is not a simple aqueous or oily solution. Where possible, heat sterilization is the method of choice. In any case, the sterilization process must be in accordance with the marketing and manufacturing authorizations.
56. Before any sterilization process is adopted its suitability for the product and its efficacy in achieving the desired sterilizing conditions in all parts of each type of load to be processed should be demonstrated by physical measurements and by biological indicators where appropriate. The validity of the process should be verified at scheduled intervals, at least annually, and whenever significant modifications have been made to the equipment. Records should be kept of the results.
57. For effective sterilization the whole of the material must be subjected to the required treatment and the process should be designed to ensure that this is achieved.
58. Validated loading patterns should be established for all sterilization processes.
59. Biological indicators should be considered as an additional method for monitoring the sterilization. They should be stored and used according to the manufacturer's instructions, and their quality checked by positive controls.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

If biological indicators are used, strict precautions should be taken to avoid transferring microbial contamination from them.

60. There should be a clear means of differentiating products which have not been sterilized from those which have. Each basket, tray or other carrier of products or components should be clearly labelled with the material name, its batch number and an indication of whether or not it has been sterilized. Indicators such as autoclave tape may be used, where appropriate, to indicate whether or not a batch (or sub-batch) has passed through a sterilization process, but they do not give a reliable indication that the lot is, in fact, sterile.
61. Sterilization records should be available for each sterilization run. They should be approved as part of the batch release procedure.

Section 1.13 Sterilization by Heat

62. Each heat sterilization cycle should be recorded on a time/temperature chart with a suitably large scale or by other appropriate equipment with suitable accuracy and precision. The position of the temperature probes used for controlling and/or recording should have been determined during the validation and, where applicable, also checked against a second independent temperature probe located at the same position.
63. Chemical or biological indicators may also be used, but should not take the place of physical measurements.
64. Sufficient time must be allowed for the whole of the load to reach the required temperature before measurement of the sterilizing time-period is commenced. This time must be determined for each type of load to be processed.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

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

Section 1.14 Moist Heat

66. Both temperature and pressure should be used to monitor the process. Control instrumentation should normally be independent of monitoring instrumentation and recording charts. Where automated control and monitoring systems are used for these applications, they should be validated to ensure that critical process requirements are met. System and cycle faults should be registered by the system and observed by the operator. The reading of the independent temperature indicator should be routinely checked against the chart recorder during the sterilization period. For sterilizers fitted with a drain at the bottom of the chamber, it may also be necessary to record the temperature at this position, throughout the sterilization period. There should be frequent leak tests on the chamber when a vacuum phase is part of the cycle.
67. The items to be sterilized, other than products in sealed containers, should be wrapped in a material which allows removal of air and penetration of steam but which prevents recontamination after sterilization. All parts of the load should be in contact with the sterilizing agent at the required temperature for the required time.
68. Care should be taken to ensure that steam used for sterilization is of suitable quality and does not contain additives at a level which could cause contamination of product or equipment.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

Section 1.15 Dry Heat

69. The process used should include air circulation within the chamber and the maintenance of a positive pressure to prevent the entry of non-sterile air. Any air admitted should be passed through a HEPA filter. Where this process is also intended to remove pyrogens, challenge tests using endotoxins should be used as part of the validation.

Section 1.16 Sterilization by Radiation

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

71. During the sterilization procedure the radiation dose should be measured. For this purpose, dosimetry indicators which are independent of dose rate should be used, giving a quantitative measurement of the dose received by the product itself. Dosimeters should be inserted in the load in sufficient number and close enough together to ensure that there is always a dosimeter in the irradiator. Where plastic dosimeters are used they should be used within the time-limit of their calibration. Dosimeter absorbances should be read within a short period after exposure to radiation.

72. Biological indicators may be used as an additional control.

73. Validation procedures should ensure that the effects of variations in density of the packages are considered.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

74. Materials handling procedures should prevent mix-up between irradiated and non-irradiated materials. Radiation-sensitive colour disks should also be used on each package to differentiate between packages which have been subjected to a irradiation and those which have not.
75. The total radiation dose should be administered within a predetermined time span.

Section 1.17 Sterilization with Ethylene Oxide

76. This method should only be used when no other method is practicable. During process validation it should be shown that there is no damaging effect on the product and that the conditions and time allowed for degassing are such as to reduce any residual gas and reaction products to defined acceptable limits for the type of product or material.
77. Direct contact between gas and microbial cells is essential; precautions should be taken to avoid the presence of organisms likely to be enclosed in material such as crystals or dried protein. The nature and quantity of packaging materials can significantly affect the process.
78. Before exposure to the gas, materials should be brought into equilibrium with the humidity and temperature required by the process. The time required for this should be balanced against the opposing need to minimize the time before sterilization.
79. Each sterilization cycle should be monitored with suitable biological indicators, using the appropriate number of test pieces distributed throughout the load. The information so obtained should form part of the batch record.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

80. For each sterilization cycle, records should be made of the time taken to complete the cycle, of the pressure, temperature and humidity within the chamber during the process and of the gas concentration and of the total amount of gas used. The pressure and temperature should be recorded throughout the cycle on a chart. The record(s) should form part of the batch record.
81. After sterilization, the load should be stored in a controlled manner under ventilated conditions to allow residual gas and reaction products to reduce to the defined level. This process should be validated.

Section 1.18 Filtration of Medicinal Products Which Cannot Be Sterilized In Their Final Container

82. Filtration alone is not considered sufficient when sterilization in the final container is possible. With regard to methods currently available, steam sterilization is to be preferred. If the product cannot be sterilized in the final container, solutions or liquids can be filtered through a sterile filter of nominal pore size of 0.22 micron (or less), or with at least equivalent micro-organism retaining properties, into a previously sterilized container. Such filters can remove most bacteria and moulds, but not all viruses or mycoplasma's. Consideration should be given to complementing the filtration process with some degree of heat treatment.
83. Due to the potential additional risks of the filtration method as compared with other sterilization processes, a second filtration via a further sterilized microorganism retaining filter, immediately prior to filling, may be advisable. The final sterile filtration should be carried out as close as possible to the filling point.
84. Fibre shedding characteristics of filters should be minimal.



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

85. The integrity of the sterilized filter should be verified before use and should be confirmed immediately after use by an appropriate method such as a bubble point, diffusive flow or pressure hold test. The time taken to filter a known volume of bulk solution and the pressure difference to be used across the filter should be determined during validation and any significant differences during routine manufacturing from this should be noted and investigated. Results of these checks should be included in the batch record. The integrity of critical gas and air vent filters should be confirmed after use. The integrity of other filters should be confirmed at appropriate intervals.
86. The same filter should not be used for more than one working day unless such use has been validated.
87. The filter should not affect the product by removal of ingredients from it or by release of substances into it.

Section 1.19 Finishing Of Sterile Products

88. Containers should be closed by appropriately validated methods. Containers closed by fusion, e.g. glass or plastic ampoules should be subject to 100% integrity testing. Samples of other containers should be checked for integrity according to appropriate procedures.
89. Containers sealed under vacuum should be tested for maintenance of that vacuum after an appropriate, pre-determined period.
90. Filled containers of parenteral products should be inspected individually for extraneous contamination or other defects. When inspection is done visually, it should be done under suitable and controlled conditions of illumination and background. Operators doing the inspection should pass regular eye-sight checks,



ANNEX 1: MANUFACTURE OF STERILE MEDICINAL PRODUCTS

with spectacles if worn, and be allowed frequent breaks from inspection. Where other methods of inspection are used, the process should be validated and the performance of the equipment checked at intervals. Results should be recorded.

Section 1.20 Quality Control

91. The sterility test applied to the finished product should only be regarded as the last in a series of control measures by which sterility is assured. The test should be validated for the product(s) concerned.
92. In those cases where parametric release has been authorized, special attention should be paid to the validation and the monitoring of the entire manufacturing process.
93. Samples taken for sterility testing should be representative of the whole of the batch, but should in particular include samples taken from parts of the batch considered to be most at risk of contamination, e.g.:
 - a) for products which have been filled aseptically, samples should include containers filled at the beginning and end of the batch and after any significant intervention;
 - b) for products which have been heat sterilized in their final containers, consideration should be given to taking samples from the potentially coolest part of the load.