3) Wherever separation by pressure differential is an essential part of sterility assurance, it is recommended that pressure differentials between areas or rooms be monitored continuously and alarm systems be installed to enable prompt detection of problems.

4) Airflow in critical areas (Grade A) should be unidirectional during operations and supplied at a velocity and uniformity sufficient to swiftly remove particles away from the critical area. Also, airflow should be supplied with care so as not to create reverse currents from adjacent areas (direct supporting areas, Grade B) into critical areas.

5) Upon installation of airflow equipment, appropriate airflow direction and strength, as described above, should be validated by smoke studies or other qualification studies. Similar validation is also necessary when airflow patterns are changed or when alterations are suspected.

6) Where unidirectional airflow is specified, as changes in velocity can affect the unidirectional pattern of the airflow, velocities should be evaluated at predetermined intervals for each HEPA filter.

7) An appropriate air change rate should be established for individual processing rooms and gowning rooms in APAs in order to maintain specified air cleanliness levels. The rate should be monitored at regular intervals to verify that the rate is being maintained as specified.

8) The differential pressure and airflow patterns that are to be in place during manufacturing operations should be specified in writing, and *in situ* differential pressure and air patterns for clean areas during manufacturing times should be verified as suitable and appropriate. The impact of turbulence created by the movement of personnel on the cleanliness of the manufacturing environment should be evaluated, and the results should be reflected in the relevant SOPs.

7.3. Integrity of HEPA Filters

7.3.1. Certification of Quality

1) HEPA filters should be accompanied by a vendor's certificate of quality that verifies that the filter is efficient enough to eliminate at least 99.97% of particles 0.3 μm or larger in size.

2) HEPA filters to be used in critical areas (Grade A) and direct supporting areas (Grade B) should be tested to detect any breaches in integrity using appropriate leak testing aerosols, e.g., poly-alpha-olefin (PAO) and dioctylphthalate (DOP)*. When alternate aerosols are used, such aerosols should be evaluated to ensure that they do not promote microbial growth.

* Formally known as diethylhexylphthalate (DEHP)

7.3.2 Testing of HEPA Filters at Installation and at Regular Intervals

1) HEPA filters should be tested for leaks at installation and thereafter at suitable time intervals. The testing procedure and frequency should be tailored to the environment, in which the filters are installed, and their intended purpose.

2) HEPA filters installed in critical areas (Grade A) should be tested for uniformity of air velocity across the filter at installation and thereafter at suitable time intervals.

3) Pressure differentials created by HEPA filters should be tested at installation and thereafter at suitable time intervals.
4) Airflow patterns should be reevaluated when the pattern has been intentionally altered or is suspected of having been altered.
5) HEPA filters should be tested according to relevant SOPs whenever any events or circumstances that may damage filter integrity occur, or when air quality is judged to have deteriorated.

8. Cleaning and Disinfection of Processing Areas

APAs for the manufacture of sterile drug products should be cleaned and disinfected according to relevant SOPs. Such cleaning and disinfection should be recorded and retained.

8.1. Disinfectants and Detergents

1) Only disinfecting agents and/or detergents that have been evaluated and deemed to be effective in removing potential contaminants should be used for disinfection and/or cleaning purposes. The levels of cleanliness and disinfection should be periodically assessed.
2) Disinfecting agents and detergents to be used in APAs should be filtered or treated by some other means before use to ensure their sterility. These solutions should be stored in an adequate microbial contamination-resistant system until use. Sterile commercial products may be used as they are.
3) Disinfectants and detergents, when prepared in-house, should be prepared according to applicable SOPs, and each preparation should be recorded and retained.
4) SOPs for the preparation of disinfectants and detergents should address the following elements: use of authorized disinfectants and detergents, cleaning and disinfection schedules, directions for the use of disinfectants and detergents, and, where necessary, need for cleaning following disinfection, precautions for operators to ensure their safety, and procedures for the cleaning, sterilization, and storage of cleaning tools.
5) Product contact surfaces of equipment, when cleaned or disinfected, should be tested by appropriate methods to verify that the disinfecting agents and/or detergents used have been removed from the surfaces to less than or equal to the pre-defined level.
6) All disinfectants should have an expiration date. The use of the disinfectant should not be permitted after this date.
7) Disinfectants should be used after cleaning, as a rule, when used to disinfect the manufacturing environment.
8) Used disinfectant containers should not be refilled with disinfectants.
9) When selecting and using disinfectants, the following should be taken into account.
   (A) The storage and use of disinfectants should be in accordance with the supplier's recommendations.
   (B) Disinfectants and the method of their use should be selected with due consideration for the safety of personnel engaged in operations.
   (C) In view of possible variations in species and species ratio of microorganisms isolated from the environment, different disinfectants should be used interchangeably or replaced with other disinfectants as appropriate to avoid the development of microbial resistance to the disinfectants.

(D) Suitable sporicides or fungicides should be selected for disinfection if environmental monitoring data suggest the presence of spore-forming bacteria or fungi.

10) The type or brand, strength, directions for use, and other information related to the use of sporicides or fungicides should be specified in writing if the frequency of use in the APA is irregular.

11) The items described above should also be applied to the selection and use of fumigating agents (including those of aerosol formulation), as required. The properties of fumigating agents should be examined well before selection.

8.2. Validation of Disinfection Procedures

1) The reliability and frequency of disinfection procedures should be established through an environmental monitoring program.

2) Microbiological assessment of disinfecting agents should be performed in each facility, and appropriate control procedures should be also established for each facility.

3) The efficacy of each disinfectant should be assessed by evaluating its ability to control the microbial counts to within predetermined limits. These limits will be based on the type and count of isolates collected from various surfaces via the environmental monitoring program.

8.3. Monitoring of Adequacy and Efficacy of Cleaning and Disinfection Processes

1) Methods for determining the adequacy and efficacy of the cleaning and disinfection processes should be established as part of the overall environmental monitoring program.

2) When environmental monitoring indicates that the microbial count exceeds action limits, when the species or species ratio of microorganisms usually present apparent changes, or when any atypical occurrences are found successively, the possible causes should be investigated, and corrective or preventative actions should be implemented as necessary.

3) If a disinfection procedure is found to be unreliable, the efficacy of the disinfection procedure should be reevaluated with regard to the disinfectant type and concentration. One way to accomplish this is to compare the species and counts of microorganisms obtained before and after disinfection.

9. Pest Control of Processing Areas

9.1. General Requirements

Pest control in both the sterile and non-sterile drug product-processing areas is critical in maintaining a clean manufacturing environment. Identification of these pests is also important for maintaining cleanliness as insects can serve as an indicator of overall biological cleanliness (i.e., unseen fungi may present as a food source; insects may also be spreading the microbes or spores they carry on their bodies).

In this document, the term "insects" means arthropod pests that may inhabit the processing areas of pharmaceutical plants. These arthropods may include the following classes, in addition to others: Insecta, Arachnida (spiders, mites), Chilopoda (centipedes), and Isopoda (sow bugs).

Insects can be found even in APAs. Since the population density of insects may be very low and their body sizes so small, a customized sampling program is necessary to detect insects in clean

areas. Separately, a suitable insect control program needs to be implemented to detect and remove insects (especially fungivorous insects) emerging from inside the facility, given that insects are rarely carried into or invade from outside the area.

9.2. Pest Control Program

1) A pest control program suitable for clean areas should be established and implemented, and records of control practices should be produced and retained.
2) The pest control program should be effective, safe, and in writing.
3) The effectiveness of the program should be verified by on-site inspection for the presence of insect activity.

9.3. Standard Operating Procedures (SOPs)

It is recommended that the documented procedures for pest control meet the following requirements:

1) All procedures, from monitoring to corrective actions, are clearly defined.
2) Procedures for preventing and correcting deviations from pest control criteria are available.
3) Procedures for follow-up inspection of deviations are available.
4) If fungivorous insects are detected, the source of fungal contamination must be located.
5) Cleaning procedures must be reevaluated if insects that feed on the organic matter present in dust are detected.

9.4. Monitoring

1) Monitoring objectives

The manufacturing environment should be monitored to assess the possible sources of insects, fungal contamination, or foreign matter that may contaminate drug products.

2) Extent of monitoring

It is recommended that environmental monitoring for pest control be performed primarily in "direct supporting areas" and "other supporting areas," and not directly in the APA itself. This will aid in the evaluation of the influence the pests have on Grade A areas and the quality of drug products. It is also recommended that environmental monitoring be performed in the APA whenever buildings or facilities are newly constructed or remodeled.

3) Sampling procedures and sample sizes
   (A) Monitoring equipment should be carried into the APA only after taking suitable precautions to avoid contamination.
   (B) Sampling procedures should be established taking into consideration the ecology of insects that may inhabit the processing areas.
4) The frequency of environmental monitoring for pest control and geographical points of sampling should be based on previous monitoring data (i.e., the ecology of the insects previously found in a given location).
5) The reliability of monitoring procedures should be verified.
6) Identification of insects

Insects detected in the processing areas should be identified and classified in such detail that their food sources and general behavior may be determined. This information should be used to facilitate the development of suitable pest control programs.

9.5. Control Criteria

1) It is recommended that alert levels and action levels be established.
2) Most of the spatial distribution patterns for insects depend on clumped distribution, not on normal distribution. Therefore, the criteria for pest control should be based on the maximum number of insects, not on the mean number of insects.
3) It is recommended that insects breeding inside the processing areas and those invading from the outside be counted and evaluated separately.
4) It is necessary to evaluate not only population but also behavior and distribution.

9.6. Corrective and Preventive Actions

1) Corrective action should be taken immediately upon finding evidence of insect infestation. Suitable preventive action should be implemented based on this evidence and the effectiveness of corrective and preventive actions should be verified under normal operating conditions.
2) Trends in insect breeding from the inside or invasion from the outside should be analyzed and appropriate preventive actions should be developed and implemented.

9.7. Record Keeping

1) Records of deviations from pest control criteria, corrective and preventive actions taken, and the effectiveness of the actions should be recorded and retained.
2) A record of captured insects categorized by area of monitoring and species should be maintained.
3) Insect monitoring data should be retained.
4) The records mentioned above should be accessible upon request.

9.8. Preventive Measures against Insects

1) An appropriate and effective preventive measure should be implemented for the species of insects that are identified through the monitoring program.

   (A) Target specific insect control

   Different species of insects have different food habits and behavioral patterns. The pest control program should be tailored for the control of individual target species. For example, cleaning procedures are key for controlling insects that consume organic matter found in dust, and fungal eradication should be performed to eliminate fungivorous species.

   (B) Fungus control

   The breeding of most insects in clean areas is due to the presence of fungus. Fungivorous insects proliferate because this food source is commonly present in structurally defective facilities. Fungus control including reevaluation of facility design and structure is necessary for pest control.

   (C) Supervision of facility and its design for the prevention against insects

If there are insects invading from outside or the population of insects breeding inside, facility and its design should be reevaluated to prevent the insects.

9.9. Notes

1) Use of insecticides
   (A) In principle, insecticides are not recommended for use in clean areas. When insecticides are used to control insect populations that have grown significantly compared to the numbers usually present, appropriate measures should be instituted to prevent contamination of the clean area environment. When insecticides are used in non-clean areas, appropriate measures should be instituted to prevent the insecticides from contaminating non-treated areas.
   (B) When insecticides are used in clean areas, the surfaces of the areas should be cleaned by a suitable method to remove insecticide residue. The area should be sampled to check the presence or absence of the residues on cleaned surfaces.
   (C) When insecticides are used in clean areas, their Material Safety Data Sheets (MSDS) should be retained, and information on the chemicals used should be recorded and retained.

10. Control of Raw Materials, Containers, and Closures

10.1. Control of Raw Materials

10.1.1. General Requirements

1) SOPs should be established for receiving, identifying, holding, sampling, and testing raw materials for control purposes. Acceptance criteria should be also established.
2) Raw materials should be received, stored, and used in a manner that prevents contamination.
3) When raw materials are required to be sterile, sterility should be confirmed by reviewing existing sterility assurance data and, if necessary, additional sterility testing should be performed prior to use.
4) When non-sterile raw materials are to be used, bioburden data should be collected and an appropriate sterilization procedure should be established. When deciding upon the sterilization procedure, acceptable microbial limits and the characteristics of the raw materials should be taken into account. Bioburden should be periodically monitored either by the supplier or during raw material manufacture to ensure that the microorganisms are in a controlled state.
5) Proof of endotoxin content for raw materials that the level is below the predetermined level must be provided when they are to be used for the manufacture of injectable drug products that will not be subjected to depyrogenation during the manufacturing process. When it is necessary, the materials should be tested prior to use.
6) When a raw material is not subject to the processing of endotoxin reduction during its manufacturing processes, but is depyrogenated subsequently, the endotoxin content should be measured during that process. A suitable procedure for depyrogenation should then be established based on the endotoxin content as well as the characteristics of the raw material. Raw material endotoxin content should be measured periodically by the supplier or during manufacture to ensure control.

7) When several raw materials are combined, endotoxin levels should be measured before and after depyrogenation.

10.1.2. Validation

1) When sterility is required, all sterilization processes should be validated to assure the sterility of raw materials.
2) When non-sterile raw materials are supplied, a suitable sterilization process should be developed and validated in accordance with accepted bioburden limits and the characteristics of the raw materials.
3) The sterilization process for bulk liquid products, as well as individual raw materials, should be validated when multiple raw materials are sterilized separately.
4) When the release of raw materials is dependent on sterilization using steam or irradiation, the parametric or dosimetric methods used should be validated.
5) The depyrogenation procedure should be validated, when raw materials are subjected to the reduction of endotoxins.

10.2. Control of Containers/Closures

10.2.1. Washing and Sterilization of Containers/Closures

10.2.1.1. Washing Procedure

Washing and rinsing procedures for containers/closures should be validated to ensure that at least a 3-log reduction (99.9%) of spiked endotoxin is achieved.

1) Washing with water

The initial washing of containers/closures should employ purified water, water for injection, or other appropriate high-purity water, followed by washing with rapidly flowing water. Final rinses should be with water for injection.

2) Washing with chemical solutions

It is recommended that containers/closures, if heavily contaminated with microorganisms or chemical agents, be washed using circulating high-purity water containing a cleaning agent at a concentration of 2-5% (e.g., surfactant, ammonium nitrate, or an organic acid).

10.2.1.2. Sterilization Procedure

Containers/closures should be appropriately sterilized as stated in Section 14 of this document.

10.2.2. Sterility Tests of Containers/Closures

Sterility of containers/closures should be in accordance with the Japanese Pharmacopoeia Sterility Test.

11. Storage and Transportation of Sterile Intermediate Products

The sterile intermediate products referred to in this document are active pharmaceutical ingredients (API) or intermediate solutions or powders that are stored or transported in a sterile state after preparation under sterile conditions. This section does not describe the requirements for sterilization of intermediate products, but rather the requirements for containers/closures suitable for maintaining the sterility of intermediate products.

11.1. General Requirements

1) The containers/closures suitable for the storage and transportation of sterile intermediate products (i.e., cargo transporters, drums, bags, and tanks) named in this document should be capable of isolating said products from the surrounding non-sterile environment and maintaining the sterility of intermediate products. These containers should also be durable enough to withstand the handling and environmental conditions encountered during storage and transportation.
2) The containers should be cleaned and sterilized before being filled and before storing or transporting sterile intermediate products.
3) SOPs should be established for filling and discharging from containers for storage as well as for transferring between containers. These SOPs should emphasize that intervention by personnel should be minimized.
4) Sterile intermediate products should be exposed to the air only in critical areas (Grade A) that pose little risk of contamination.
5) The hermetic condition of sealed containers for the aseptic storage and/or transportation of sterile intermediate products should be verified, as appropriate.

11.2. Containers/Closures for Storage and Transportation

11.2.1. Design of Containers/Closures

The following points should be considered before selecting containers for the storage and transportation of sterile intermediate products.

1) Isolation from non-aseptic areas

In order to meet this requirement, the following points should be taken into consideration when designing or selecting containers.

(A) Container structure ensures hermetic sealing

(B) Capability of maintaining a state of positive pressure of the inside of the container (using sterile gas) if the container cannot be hermetically sealed

(C) Installation of sterile-grade vent filters as a safeguard against a change in pressure should the air-tightness of the container be compromised due to environmental pressure changes. Suitable vent filters should be selected and their integrity should be tested at appropriate intervals.

(D) Dual-structure design should be used as appropriate

2) If the containers are to be transported into APAs (Grades A and B), their size should be suitable for transport through pass boxes and air lock rooms.

3) Container/closure surfaces should be able to withstand cleaning and disinfection if the surfaces are cleaned and sterilized prior to transport into APAs.
4) Casters and other movable parts of transport devices should be protected from the generation of dust and particulate matter if such devices are used in APAs.

11.2.2. Verification of Hermetic Sealing

It is critical to ensure that containers are hermetically sealed. Listed below are several procedures for verifying the seal, which should be conducted on container/closure systems stored or transported under routine conditions.

1) Positive-pressure leak test
2) Negative-pressure leak test
3) Microbial aerosol challenge test, as appropriate
4) Leak test using a gas leakage detector

11.3. Storage and Transportation

When charging and discharging sterile intermediate products in and out of containers, the following should be taken into consideration:

1) Automatization

   The filling (e.g., divided charging) and discharge system should be designed to be automatic, wherever feasible, to minimize personnel intervention.

2) Minimization of risks due to personnel intervention

   When automatization is not feasible or is limited, the following points should be addressed by SOPs in order to manage the risk involved:

   (A) Operators should not block or disrupt the direction or intensity of airflow at the filling or discharge sites.

   (B) Filling and discharge operations should be performed in a Grade A area (e.g., in a clean booth).

   (C) Operators should move slowly while filling and discharge operations are going on.

   (D) Detailed written SOPs should be established for manual operations. Personnel should be trained on these procedures.

3) Media fill

   The capability of the aseptic process for discharging intermediate products should be evaluated by media fill process simulation tests.

4) Time limitations

   Time is always a critical factor for maintaining sterile conditions; the longer the filling and discharge times, the greater the risk of contamination. It is recommended that time limits be specified for these operations and, if more than one container or tank is used per shift, that these vessels be marked with numbers to facilitate a first-in first-out order of operations.

11.4. Storage and Transportation Conditions

Any risk of contamination involved in the storage or transportation of aseptic products (e.g., temperature, environment, container integrity, or vibration) should be specified and addressed by

appropriate SOPs. For verification of the suitability of established conditions, control parameters should be determined either by simulation or by monitoring actual operations. The results should be recorded in writing.

12. Environmental and Personnel Monitoring

The primary objectives of environmental monitoring are to control the levels of microorganisms and airborne particles within specified limits for individual APAs and other supporting areas. The goals are to maintain the cleanliness of these APAs for the manufacture of sterile drug products, to prevent product contamination by predicting the deterioration of the environment, and to continuously evaluate the efficiency of the cleaning, disinfection, and decontamination procedures. Environmental monitoring can be classified into two categories, microbiological control and particle control. The former is intended primarily to allow the scientific identification and characterization of bioburden organisms, not for the purpose of identifying all microorganisms present in the environment, but rather to ensure that the manufacture of sterile drug products is conducted in an appropriately controlled environment. Identification and quantification of the microorganisms present also allows the manufacturer to institute the measures (e.g., disinfection) necessary to maintain the environment at the required cleanliness levels.

12.1. General Requirements

1) Scope

   Environmental monitoring should be performed in critical processing areas (Grade A), direct supporting areas (Grade B), and, when necessary, in other supporting areas (Grade C or D) adjacent to APAs.

2) Monitoring programs

   An environmental monitoring program, and SOPs for implementing the program, should be established. Monitoring record forms should also be created. The monitoring program should be adequate to assess environmental contamination risks.

3) Monitoring targets

   Microorganisms and airborne particles should be monitored.

   (A) Target airborne particles should be 0.5 μm or larger in size.

   Particles smaller than 5 μm should be measured if appropriate.

   (B) Target microorganisms should be bacteria and fungi.

   (C) Target microorganisms should include those in the air as well as those on the surfaces of walls, floors, fixtures, equipment, and personnel attire.

4) Qualification of monitoring programs during operations

   The environmental monitoring program should be prepared prior to performance qualification (PQ). The program should be finalized after the completion of PQ performed under the operating conditions specified in the program. PQ usually involve frequent sampling at many sites assuming the worst-case scenario. However, the sampling locations and frequency for routine monitoring and control may be reduced once the program has been qualified and put in place.

5) Monitoring locations

Environmental monitoring data should include information on the quality of the processing areas or rooms (including corridors and fixtures), manufacturing equipment (and process-monitoring apparatuses, where appropriate), air that comes in contact with or maintains the aseptic environment, and compressed air/gas that comes in contact with the environment.

6) Frequency of monitoring

Sampling frequency should be specified according to the cleanliness required in the processing areas or rooms under both operational and non-operational conditions. The sampling program should also include specifications for sampling the operator's attire. The frequencies in Tables 2 and 3 may be helpful for establishing specifications.

7) Monitoring methods: sampling and testing procedures

Devices to be used for sampling should be suitable for the quantitative measurement of microorganisms and particulate matter.

(A) Devices to be used for collecting and counting airborne particulate matter and microorganisms should be validated and calibrated.

(B) Sampling should include one or more suitable procedures for collecting airborne microorganisms (settling plates, impact sampling, and filtration methods), and for collecting microorganisms on surfaces (contact plates and swabs). The area to be surface-sampled should be designated according to the size and shape of the area as well as the characteristics of the microorganisms to be monitored. In principle, the recommended sampling area for equipment or apparatuses is 24-30 cm^2. The volume of air to be sampled for airborne microorganism monitoring should be at least one cubic meter at each sampling point. The air volume may be increased or decreased in consideration of the condition of the areas to be monitored, sampling frequency, etc.

(C) The microbiological culture media to be used for the detection and enumeration of airborne or surface microorganisms should be suitable for the growth of microorganisms such as aerobic bacteria, fungi (i.e., yeasts, molds), and anaerobic bacteria.

(D) The incubation temperature of the media should be suitable for the growth of target microorganisms.

8) Alert and action level specifications

Alert and action levels should be established depending on target organisms or substances and the locations to be monitored.

(A) Action level specifications may be established by referring to Table 4.

(B) Alert level specifications should be established based on results from qualification tests performed during operations.

(C) Precise descriptions of actions to be taken when specifications are not met, i.e., statement of the reason for specification non-compliance or suspension of manufacturing, should be included in the monitoring program. In principle, deviations from the alert level do not require the suspension of manufacturing, but appropriate actions or measures should be taken. Deviations from the action level should be counteracted by immediate suspension of the release of products manufactured during the time when the deviations occurred, or related processes, clarification of reasons for the deviations, institution of countermeasures, and verification of the recovery of acceptable operating conditions.

12.2. Routine Monitoring and Control

1) Implementation of the monitoring program

Microorganisms and particulate matter should be routinely monitored in accordance with the monitoring program.

2) Microbiological control

Potential microbiological contamination should be monitored routinely. The microbiological environmental monitoring program should include periodic characterization of environmental flora and isolates to assess the risk to the products.

3) Sampling

Sampling at the surfaces that come in contact with drug products or other materials in critical areas should be performed immediately after the completion of filling and aseptic processing operations.

4) Gases for manufacturing

Gases that may directly or indirectly come into contact with the drug products themselves, their containers, or any manufacturing surfaces should be periodically monitored and controlled to ensure the absence of microorganisms.

5) Data analysis

For the adequate maintenance of the manufacturing environment, data obtained on a routine basis should be analyzed to detect trends in the environment and establish monitoring limits for trend analysis. Even if changes in the environment do not deviate from the specified limits (at the alert level), any trends suggesting deviation from normal conditions (trend analysis levels) should be identified and the cause(s) of the changes investigated to allow the quality of the environment to be maintained at appropriate levels. Trend analysis should be also utilized for the maintenance of equipment for environmental control, e.g., heating/air-conditioning units, and for adjustment of sterilization and disinfection procedures.

12.3. Example Assessment Criteria for Environmental Monitoring

Tables 2 and 3 show examples of environmental monitoring frequencies, and table 4 summarizes the evaluation criteria. However, the environmental monitoring program should be customized for the area in question since the risk of contamination varies. The formulation and volume of drug products produced and the presence and performance of the heating/air-conditioning units and other equipment for environmental control should also be taken into consideration.

The definition of "throughout the entire aseptic procedure" and "shift of manufacturing operation" may vary among manufacturers depending on, for example, the type and duration of operations and preparatory activities, operations by robotic manipulation, or partial human intervention in an aseptic manufacturing process performed in the area in question. The definition should be defined in order to meet individual differences in operational procedures and in the operation of equipment at each manufacturing site.

Table 2. Frequency of Microbiological Monitoring

Area	Airborne microorganisms	Microorganisms on surfaces (e.g., equipment)	Microorganisms on surfaces (operators)
Grade A	For each shift	At the completion of each processing	For each shift

Grade B	For each shift	At the completion of each processing	Once per day of operation
Grade C	As appropriate	As appropriate	As appropriate
Grade D	As appropriate	As appropriate	As appropriate

1) The frequency of microbiological sampling may be increased or decreased according to processing activities or time; however, the frequency needs to be adequate for monitoring the potential microbiological contamination of drug products.
2) The frequency of microbiological sampling for personnel should be commensurate with the level of ability and experience of the individual personnel. It is recommended that sampling be more frequent for operators with less aseptic processing experience. The ability and experience of individual personnel should be collectively evaluated based on, for example, the frequency of engagement in works, microorganism monitoring data, frequency of participation in media fill tests, etc.
3) The monitoring frequency in Grade C and D areas should be established based on the type of processing and activities to be performed in the areas.
4) The monitoring rate for microorganisms and particulate matter should be increased immediately following the start of operations in a facility (beginning of the operational qualification procedure), at the start of a long-term shutdown, and after partial changes in operation are made.
5) The surface microbiological count for personnel entering into an area of Grade B air cleanliness shall be controlled according to a microbiological monitoring program specific to the Grade B area. When personnel enter into Grade A and B air cleanliness levels alternatively or during a shift, the airborne particle count shall be monitored and controlled according to a stricter monitoring program.

Table 3. Frequency of Testing for Particulate Matter Monitoring

Area	Monitoring frequency
Grade A	Throughout the entire aseptic procedure
Grade B	Throughout the entire aseptic procedure
Grade C	Once a month
Grade D	As appropriate

The sampling of particulate matter in Grade A and B areas needs to be conducted with appropriate frequency. Continuous monitoring is not required, but the frequency of monitoring, i.e. "as appropriate," "frequently," or "continuously," needs to be determined and programmed based on the overall consideration of the quality of the aseptic manufacturing environment and its influence on the quality of the drug products. Continuous sampling is recommended from the viewpoint of the maintenance of the environment at predetermined cleanliness levels. However,

continuous sampling is not always required since sampling frequency and location can be established or modified as needed.

1) When manufacturing equipment is not in operation, the monitoring of particulate matter should be adequately and appropriately performed from the viewpoint of early identification and correction of impaired facilities and equipment, including environmental control equipment, i.e., air-conditioners, and implementation of environment maintenance procedures, i.e., entry restriction for personnel.

2) The assessment criteria for monitoring particulate matter should be based on the amount of air sampled and the air suction capability of the monitoring devices. Air samplers and methods of assessment should be appropriate for the area and type of assessment.

Table 4. Example Alert* and Action Levels for Microbiological Monitoring

Test	Cleanliness grade	Sampling location	Limit for action
Airborn microorganisms	A		<1 (cfu/m^3)
	B		≤10 (cfu/m^3)
	C		≤100 (cfu/m^3)
Microorganisms on surfaces	A	Equipment	<1 (cfu/ plate)
		Walls	≤1 (cfu/plate)
		Floors	≤5 (cfu/plate)
	B	Walls	≤5 (cfu/plate)
		Floors	≤10 (cfu/plate)
	C	Floors	≤30 (cfu/plate)
Microorganisms on hands	A		≤1 (cfu/5 fingers
	B		≤5 (cfu/5 fingers
	C		As appropriate
Microorganisms on operator's gown	A	Both arms, chest, head, shoulders, etc.	≤5 (cfu/plate)
	B	Both arms, chest, head, shoulders, etc.	≤20 (cfu/plate)
	C		As appropriate

Note 1: Alert levels should be established by applicable limits for qualification or based on mean+2σ (σ: SD) of past measurements.

Note 2: The surface area of the plate should be 24-30 cm^2.

Note 3: The action level listed in the table indicates the maximum bacterial count allowable. When action is taken based on the mean values, potential contamination risks should be taken into account.

Note 4: The locations on a gown that are to be sampled for microorganisms should be selected and justified in relation to the type of activities the operators have been engaged in and the potential influence their movements have on aseptic processing.

13. Qualification of Equipment and Utilities

13.1. General Requirements

1) In this document, the term "equipment" means equipment used for sterilizing, filtering, filling, stoppering, freeze-drying, and sealing for sterile drug products manufacturing in APAs as well as incubators/fermenters and cleaning equipment to be installed, as required, in supporting areas.
2) In this document, the term "utilities" means systems for supplying different qualities of water, pure steam, compressed air, and different kinds of gases to be used in the manufacture of sterile drug products.
3) For equipment and utilities qualification, qualification protocols and reports, including responsibility assignment for necessary procedures, should be documented.
4) Equipment and utilities to be used for the manufacture of sterile drug products should be designed to minimize their potential influence on the sterility of the drug products. When designing equipment and utilities, the following qualification requirements should be carefully followed:
   (A) Equipment shape and material of construction should allow for easy cleaning, disinfection/sterilization, and maintenance. This requirement should be particularly applicable to surfaces of equipment that may come into direct contact with sterile drug products, sterile raw materials, or sterile water/steam/gases.
   (B) The flow lines for sterile drug products and sterile raw materials should be optimal.
   (C) Personnel movement and intervention in critical areas should be minimized.
   (D) Turbulence and particulate generations should be minimized in critical areas. The flow of clean air from the air supply inlet to the air return or exhaust should be designed to be optimal in direct and indirect supporting areas.
   (E) Equipments should be laid out to minimize the burden on operators.
5) During installation qualification, it should be verified that the equipment or utilities have been installed as indicated in the design drawings.
6) During operational qualification, equipment and utilities should be evaluated and it should be confirmed that they are of a capacity necessary to meet design specifications.
7) All processes conducted in the APAs that may influence the sterility of sterile drug products should be evaluated scientifically and validated appropriately.
8) SOPs for equipment and utilities should describe the procedures for operating all key equipment as well as relevant parameters in an adequate manner.
9) During process validation, sterility assurance levels should be evaluated to ensure that drug products are indeed sterile. All equipment should be evaluated during the process validation, including those for cleaning, sterilizing, incubating/fermenting, filtering, filling, stoppering, freeze-drying, and sealing. It is acceptable to validate sterility assurance levels for multiple processes together when the manufacturing processes utilize the same pieces of equipment in a continuous manner.

10) The sterilization of equipment surfaces that may come into direct or indirect contact with sterile drug products should be validated.

11) Qualification studies should be conducted for utility systems, including those that supply purified water, water for injection, compressed air/other gases, and pure steam as well as CIP/SIP systems.

13.2. Equipment Maintenance

1) Written schedules and procedures, including the assignment of responsibility, should be prepared for the preventative maintenance of equipment and utilities.

2) Written procedures for the cleaning, disinfection, and sterilization of equipment and utilities should be established, and in such a way that they will serve as a permission for the subsequent use of the equipment the next time sterile drug product is manufactured. The procedures should be as detailed as possible so that operators can clean, disinfect, and sterilize equipment in an efficient and reproducible manner. These procedures should address the following:

   (A) Assignment of responsibility for the cleaning, disinfection, and sterilization of equipment and utilities

   (B) Cleaning, disinfection, and sterilization schedules

   (C) A complete description of the procedures (including the procedure for diluting cleaning agents), equipment, and agents to be used for cleaning, disinfection, and sterilization

   (D) When appropriate, instructions for the disassembly and reassembly of each piece of equipment should be included so that they may be cleaned, disinfected, and sterilized properly

   (E) Instructions for the removal or obliteration of previous batch identification

   (F) Instructions for keeping clean equipment from becoming contaminated

   (G) Inspection of equipment for cleanliness and sterility immediately before use, if possible

   (H) Establishment of the maximum time that equipment may remain in an uncleaned state before it is cleaned, disinfected, and sterilized, where appropriate

3) Equipment and utensils should be cleaned, washed, dried, stored, and, when necessary, disinfected or sterilized to minimize any potential compromise of drug product sterility.

4) When equipment is assigned for continuous or campaign production of successive lots of the same sterile drug product, the equipment should be cleaned, disinfected, and sterilized at appropriate intervals that have been validated as effective for the prevention of microbiological contamination.

5) The cleaning, disinfection, and sterilization processes for equipment used to manufacture sterile drug products such as live vaccines should be evaluated for their inactivation/sterilization efficacy on potentially infectious drug product residue. The evaluation of sterilization efficacy may be omitted when the microorganisms in question have been documented to be less resistant to these processes than microorganisms routinely used in the various studies of the Japanese Pharmacopoeia and other official compendia.

6) The choice of cleaning procedures and cleaning/disinfecting agents should be specified and justified.

7) The specifications and state of cleanliness of utensils and each piece of equipment should be identified by appropriate means.

13.3. Calibration

1) Written schedules and procedures, including the assignment of responsibility, should be established for the key equipment used for the control, measurement, monitoring, and calibration of equipment and utilities. The procedures should be performed according to the written schedule to ensure the sterility of the drug products.
2) The calibration of equipment should be performed using measurements derived from certified standards, if such exist.
3) Records of these calibrations should be maintained.
4) The current calibration status of critical equipment should be known and verifiable.
5) Instruments that do not meet calibration criteria should not be used.
6) Deviations from approved standard calibration values for instruments that are critical for assuring the sterility of sterile drug products should be investigated. Lots produced during the time period in question should be evaluated to determine if their sterility could have been compromised by these deviations.

13.4. Change Control

1) Documented procedures should be established concerning the identification, documentation, appropriate review, and approval of changes in equipment and utilities, (including parameters), and procedures that may affect the sterility of sterile drug products. Assignment of responsibility should be included in these documented procedures.
2) Any proposed changes to the items listed above should be drafted, reviewed, and approved by the appropriate organizational units followed by review and approval by the quality unit.
3) The potential impact that the proposed changes would have on the sterility of the drug products should be evaluated by taking the following into consideration:
   (A) The impact on cleaning, disinfection/sterilization, and maintenance: particular attention should be paid to the impact that the changes will have on the sterility of surfaces that come into direct contact with sterile drug products, sterile raw materials, or sterile water/steam/gases.
   (B) The impact that flow lines will have on sterile drug products and sterile raw materials
   (C) The impact that personnel movement and operator intervention will have on sterile drug production
   (D) The impact that the following will have on sterile drug production:
      i) Turbulence and particulate generation in critical areas
      ii) The path of air flow from the clean air supply inlets to the return or exhaust ducts in direct and indirect supporting areas
   (E) Impact on operator burden
4) Written procedures should be established to ensure that, when approved changes are implemented, all documents to be affected by the approved changes are reviewed without fail.
5) After an approved change has been implemented, the first several lots produced should be evaluated to determine what impact the changes have had on the sterility of the drug products.
6) The potential impact of critical process changes on the valid time period for use of sterilized manufacturing equipment should be evaluated.

14. Sterilization Processes

14.1. General Requirements

1) Containers and closures that may come into direct contact with drug products should be sterilized by appropriate methods suitable for maintaining product sterility.
2) Where appropriate, surfaces of equipment that may come into direct contact with the above-mentioned containers and closures should also be sterilized.
3) Appropriate measures should be instituted to avoid mixing-up sterile and non-sterile materials.
4) Appropriate measures should be employed to prevent sterilized materials from being re-contaminated. In principal, the procedures for preventing recontamination should be based on the aseptic processing procedures described in this guidance.
5) Each sterilization process for equipment and utensils to be used in critical areas should be validated. It is recommended that the sterilization process be revalidated at least once a year.
6) Procedures and control parameters for process control, routine monitoring and control, maintenance, supplies, and confirmation of sterility related to the sterilization process should be fully documented.

14.2. Autoclaving

1) The quality of steam for sterilization should be sufficient to allow for the proper sterilization of materials or equipment. Quality of the steam and feed water for sterilization should be analyzed periodically and, whenever deterioration of quality is suspected, the cause of deterioration should be investigated and proper measures should be taken to correct the situation. Pure steam is usually used for autoclaving.
2) Materials that are repeatedly steam-sterilized (e.g., filters, utensils, and aseptic gowns) should be able to withstand repeated exposure to steam at its maximum intensity while maintaining their specifications, safety, and ability to function as intended.

14.2.1. Sterilization Process

1) Acceptable limits of sterilization-related process parameters should be established and documented.
2) When a vacuum process is requested during the sterilization process, the maximum acceptable limits of leakage rate and volume should be established. Procedures for checking steam penetration within the chamber should also be established.
3) If air or water come into direct contact with materials during the sterilization process, their purity and physical characteristics (e.g., pressure, temperature) should not adversely impact the intended function or safety of the materials.
4) It is recommended that the biological indicators (BI) and chemical indicators (CI) to be used in the verification of the sterilization process conform to international standards or other official criteria.
5) When the validity of a given sterilization process is tested by simulation using dummy materials, the acceptability, efficacy, and limits of use for the set conditions and parameters should be verified and documented.

6) When a process or processes other than sterilization is integrated into the sterilization process (e.g., drying), the assessment method for the process or processes should be established, documented, and implemented as one of the critical control parameters.

7) The need for pre-treatment procedures (such as cleaning) should be established and controlled so as not to impair the validity of the sterilization process.

14.2.2. Sterilization Equipments

1) The main specifications of the sterilization equipment, e.g., the manufacturer's name, type, size, structure, materials of construction, performance capability, and capacity should be documented. The manual for operations should include procedures for setting default values, emergency operation, disassembly and reassembly, and maintenance (including calibration) as well as procedures for routine operation.

2) The sterilization equipment should be sufficient to fulfill the requirements for sterilization (e.g., sufficient and appropriate operational parameters and processing capacity).

3) Sterilization equipment surfaces (e.g., inner walls, sensors, or pipes) that will be exposed to the stress of sterilization should be made of materials that can withstand exposure to such conditions. It should be verified that none of the materials of construction release any substance that may impair the quality of the sterilization process or compromise the integrity of the drug products.

4) The stability of the supply of utilities such as electricity and compressed air should be sufficient to maintain consistent operation of the sterilization equipment throughout the sterilization process.

5) When the materials to be sterilized are not hermetically sealed, gases to be used for aeration and pressure recovery should be sterile. The filters to be used for gas sterilization should be suitable for sterilization and ready for their integrity test.

6) Process parameters that may impact sterilization efficiency should be able to be freely set within a range suitable for the sterilization process. These parameters should be easily reproducible (e.g., easily reprogrammed into the machine), rendering the management of the process easy. The process parameters should be based on the physical characteristics and the physical state (solid/liquid/gas) of the materials to be sterilized.

7) There should be a mechanism (e.g., computerized control system) in place that enables the sterilization process to proceed automatically according to the programmed parameters. If the sterilization equipment is of the continuous type, there should be a mechanism that enables the correct transfer of products into and/or from the sterilizer chamber.

8) Critical process parameters should be measured and monitored by appropriate sensors and recorders so as to verify that the required level of sterilization for critical processes was attained. The specifications (e.g., type, precision level, and materials) and the location of the sensors should be suitable to adequately monitor the sterilization process.

9) Safety mechanisms should be designed to keep the operating conditions of the sterilization process within acceptable limits. In addition, safety devices (e.g., safety valves) should be in place as emergency measures to prevent major accidents in the case of abnormal or unexpected operations.

10) The facility where the sterilization equipment is installed should be big enough to allow operators and maintenance personnel access. The facility should also be sufficiently clean to protect the quality of the drug products.

11) The equipment area should be designed to facilitate the activities necessary for the sterilization process, such as operation of the control panel and transfer of the drug products into and out of the chamber.

12) When the sterilization process is managed electronically as one part of a larger computer network, the input/output data, control specifications, etc. should be documented in detail.

13) Physical changes made to the sterilization equipment and any changes in the sterilization process should be reflected in the relevant specifications.

14.2.3. Routine Monitoring and Control

1) The creation of the process parameters and controls necessary for the routine monitoring and control of the sterilization process should be based on validation data. These parameters and controls should be verified as reproducible for every sterilization load and cycle type.

2) Concrete operating procedures, including frequency, should be documented for the following: routine check-ups, maintenance, calibration, and check lists.

3) Routine management and control of the sterilization process should be performed on a cycle-by-cycle basis.

4) Evidence documenting that the required process parameters were achieved should be kept. The recorded data should include readings of the inner pressure and temperature of the sterilizing chamber and of the materials to be sterilized during each sterilization cycle.

5) Variables set as process parameters should be monitored and recorded by direct methods to verify that the sterilization process has been completed within specified limits. If necessary, BI and CI may be used for these measurements.

6) Leak tests should be performed periodically when the sterilization process incorporates an air elimination process for steam penetration. Any additional performance check other than sterilization (e.g., dryness), if required in the sterilization process, should be evaluated and recorded according to written procedures.

14.2.4. Handling of Sterilized Materials

1) SOPs for handling of sterilized materials should be prepared and implemented. The SOPs should include methods and criteria for assessing the adequacy of the sterilization process. When the testing of any additional parameters (e.g., BI, CI) is required to determine if sterility has been achieved, the necessary methods/materials should be included in the assessment criteria.

2) The SOPs for sterilization should specify the type of records relevant to the supply and processing of materials and the retention of such records. The sterilization records should include the items listed below. The records should be reviewed and approved by appropriate supervisory personnel.

   (A) Time started and ended, and date of processing

   (B) Equipment used

   (C) Sterilization conditions employed

   (D) Criteria and results of assessment

   (E) Records of physical process parameters (temperature, pressure, etc.)

   (F) Identification of materials sterilized and their traceability