

Guidance on the Manufacture of Sterile Pharmaceutical Products Produced by Terminal Sterilization

**Task Force
on**

Sterile Pharmaceutical Products Produced by Terminal Sterilization

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Notice: This English version of the Guidance on Sterile Pharmaceutical Products Produced by Terminal Sterilization is prepared for the convenience of users unfamiliar with the Japanese language. When and if any discrepancy arises between the Japanese original and its English translation, the former is authentic.

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1. Introduction

This guidance document describes the current basic concepts on sterility assurance and associated procedures for manufacturing and controlling sterile pharmaceutical products in order to advise manufacturers of sterile pharmaceutical products and regulatory personnel responsible for pharmaceutical inspections on sterility assurance of the products processed by terminal sterilization procedures.

In principle, the requirements specified in this guidance document are applicable to parenteral drugs; however, there are a number of requirements that may be commonly applied to other forms of sterile dosage forms. The concepts and descriptions contained in this guidance may be superseded by other processes or procedures of manufacture that are justifiably comparable or more stringent (except for the Ministerial Ordinance, “Regulations for Manufacturing Control and Quality Control of Medicinal Products and Quasi-Medicinal Products” [“GMP regulations,” Ministerial Ordinance No. 179, 2004], and other regulatory requirements, notifications, and issues), as long as the quality of pharmaceutical products can be ensured.

2. Glossary

2.1 Action level: Established criteria of microbial or airborne particle level that, when exceeded, should trigger an appropriate investigation and corrective actions based on the investigation.

2.2 Alert level: Established criteria of microbial or airborne particle level (and microbial species, if necessary) providing early warning of potential drift from normal conditions which are not necessarily grounds for definitive corrective action but require follow-up investigation.

2.3 Aseptic processing: A method of producing a sterile product in which sterile bulk drug or sterile raw materials are compounded and assembled with sterile packaging components in a controlled environment, in which the entry or supply of air, materials, equipment, and personnel are regulated to control microbial and particulate contamination to an acceptable level (Grade A or B).

2.4 Bioburden: Population of viable microorganisms on or in raw materials, products, and labeling/packaging materials determined before sterilization.

2.5 Biological indicator (BI): A microbiological test system providing defined resistance to a specified sterilization process under defined conditions, which is used as an indicator of sterilization

cycle efficacy.

2.6 Calibration: A set of operations that establish, under specified conditions, the relationship between values indicated by a measuring instrument or system, or values represented by a material measure or reference material and the corresponding values measured under specified conditions. Predetermined limits for acceptance of measurement results should be established.

2.7 Change control: Formal assessment and determination of the appropriateness of a proposed alteration to product, process, or procedure (see Article 14 of the GMP Regulations).

2.8 Chemical indicator (CI): Test system that reveals fluctuation(s) in one or more predefined process variables based on a chemical or physical change resulting from exposure to a sterilization process.

2.9 Clean area: An area maintained and controlled to prevent contamination with foreign matters and microbes from pharmaceutical products in accordance with defined particle and microbiological cleanliness standards. For the purpose of this document, this term is synonymous with the manufacturing area for aseptic products.

2.10 Cleaning: Removal of contaminants with water/cleaning agents from an item to the extent necessary for further processing or for the intended use.

2.11 Cleanliness level: A quality that indicates the contamination control conditions of a monitored item. The level is classified into Grades A, B, C, and D according to the number of particles larger than 0.5 μm per m^3 in the air.

2.12 Critical area: A designated processing area where sterilized containers, raw materials, intermediate compounds, or the surface of equipment that come into contact with sterilized products may be exposed to the environment. This area is also referred to as the “critical processing area.” The quality of air should be Grade A or higher than Grade C depending on the sterilization conditions to be applied.

2.13 Critical parameter: For the purpose of this guidance, a product quality parameter which is essential for the control of the applied sterility process, and which requires monitoring.

- 2.14 Design qualification (DQ):** Documented verification that the proposed designs of the facilities, equipment, or systems is suitable for the intended uses.
- 2.15 Direct support area:** A background or adjacent area directly supporting the critical area. Air cleanliness level of direct support area should be Grade B (when the critical area is controlled as Grade A) or Grade C.
- 2.16 Disinfection:** A process by which surface bioburden is reduced to a safe level or eliminated.
- 2.17 Dose mapping:** Measurement of the distribution and variability of absorbed dose in material irradiated under defined conditions.
- 2.18 Dosimeter:** An instrument for the precise and reproducible measurement of an absorbed dose of radiation in a given material.
- 2.19 Dosimetric release:** A form of parametric release of irradiated products based on the measurement of absorbed dose by the use of dosimeter rather than sterility test data.
- 2.20 Dosimetry system:** A system consisting of a dosimeter, measurement instrument, associated reference standards, system specifications, and operating procedures for determining the absorbed dose.
- 2.21 Dummy load or reference load:** Specified load that represents a product for use in the confirmation of sterilization.
- 2.22 D value:** A value indicating the extinction rate of microorganisms killed under defined conditions. The *D* value is defined as the time or radiation dose required to inactivate 90% of a population (one tenth of the starting value) of the test microorganism under stated exposure conditions.
- 2.23 Endotoxin:** Lipopolysaccharide contained within the outer membrane of Gram-negative bacteria that may lead to pyrogenic reactions and other biological activities in humans.

2.24 Environmental monitoring program: The planning, organization, and implementation of actions for all subjects to maintain the cleanliness of manufacturing areas and contact surfaces within these areas at a designated level. The intent of the program is to facilitate the manufacture of aseptic pharmaceutical products at a high quality level by foreseeing the deterioration of the environment in manufacturing areas, preventing or eliminating potential risk factors affecting the quality of products, and implementing appropriate measures for cleanliness control.

2.25 Filling and sealing areas: Production areas in which containers, components (e.g. API and excipients), intermediate compounds, and container openings are exposed to environmental conditions. Activities conducted in such areas include equipment setup and aseptic connections.

2.26 F_0 value: The number of equivalent minutes of steam sterilization at a temperature of 121.1°C delivered to a container or unit of product, calculated using a z value of 10K.

2.27 High efficiency particulate (HEPA) air filter: Filters with a minimum efficiency of 99.97% for 0.3 μm particle size, as determined by test.

2.28 Indirect support area: An area where containers, raw materials, and products before sterilization are exposed to the environment and where materials and equipment for aseptic processing are cleaned.

2.29 Installation qualification (IQ): Documented verification that all premises, supporting utilities, or equipment are correctly installed and operate in accordance with the manufacturer's recommended specifications.

2.30 Loading pattern: Specified configurations on fixed chamber parts and the number, type, location, and orientation of products presented for sterilization within the sterilization chamber.

2.31 Maintenance: A combination of all technical and associated administrative actions intended to retain an item in, or restore it to, a state in which it can perform its required function.

2.32 Manufacture: All operations involved in the receipt of materials, production, packaging,

repackaging, labeling, relabeling, quality control, release, storage, and distribution of pharmaceutical ingredients and related controls.

2.33 Manufacturer: A company that carries out at least one step of manufacture.

2.34 Microorganisms: A term used to indicate only bacteria and fungi in this document.

2.35 Operating cycle: A complete combination of manufacturing processes run sequentially in the order determined by automatic control equipment.

2.36 Operational qualification (OQ): Documented verification that installed or improved equipment, systems, or premises operate within predetermined limits when used in accordance with operational procedures.

2.37 Overkill sterilization: A sterilization process providing a sterility assurance level (SAL) of less than 10^{-6} , regardless of the bioburden count in the product being sterilized or the resistance of objective microorganisms to the sterilization. The process generally provides at least a 12-log reduction of indicator microorganism having a minimum *D* value of 1.0 minute.

2.38 Packaging system: A system of immediate containers and other packaging materials that ensure the sterility of a final product is maintained.

2.39 Parametric release: Declaration that a product is sterile based on records, demonstrating that the process parameters are delivered within specified tolerances.

2.40 Performance qualification (PQ): Documented verification that the premises, systems, or equipment operates consistently each other and provides reproducible results within the defined specifications and parameters for prolonged periods of time.

2.41 Process challenge device (PCD): An item used to assess the performance of a sterilization process, as exemplified by the confirmation of heat penetration with steam, by placing a biological indicator (BI) or chemical indicator (CI) inside the item for which steam penetration is difficult to achieve.

2.42 Processing area: An area, including the gowning area, in which actions such as the cultivation, extraction/purification, and weighing of raw materials; washing and drying of containers and stoppers; and preparation of solutions, filling, sealing, and packaging are performed.

2.43 Process parameter: Specified value for a process variable.

2.44 Product: General term used to denote pharmaceutical products including raw materials, intermediate compounds, and finished products.

2.45 Quality system: A system identifying the organizational structure, responsibilities, procedures, processes, and resources for implementing quality management.

2.46 Saturated steam: Water vapor in a state of equilibrium between condensation and evaporation.

2.47 Specifications: A list or statement of authorized particulars, specifying detailed requirements.

2.48 Standard operating procedures (SOPs): Authorized written procedures providing instructions for performing operations not necessarily specific to a given product or material but of a more general nature (e.g. equipment operation, maintenance, and cleaning; validation; cleaning of premises and environmental control; and sampling and inspection).

2.49 Sterile: Free from viable microorganisms.

2.50 Sterility assurance level (SAL): Probability of viable microorganisms being present on a product unit after exposure to the proper sterilization process, normally expressed as 10^{-n} .

2.51 Sterilization: A process that destroys or eliminates all viable microbes to render a product free from viable microorganisms.

2.52 Sterilization cycle: A series of sterilization processes to be performed within a closed sterile chamber, consisting of dehumidification; conditioning; and the injection, product exposure, and

removal of sterilizing agent.

2.53 Sterilizing agent: A combination of physical and/or chemical substances capable of sterilizing a pharmaceutical product under specified conditions.

2.54 Terminal sterilization: A process whereby a product is sterilized in its final container or packaging, which permits the measurement and evaluation of quantifiable microbial lethality. In principle, the SAL should be less than 10^{-6} .

2.55 Validation: A documented act of confirming that the structure and equipment of factory; the procedure, process, and methods of manufacturing; and quality control do in fact lead to expected results.

2.56 z value: Temperature change that results in a ten-fold change of the *D* value.

3. Quality System

The general provision of the quality system to be applied to sterile pharmaceutical products manufactured by terminal sterilization should be structured to satisfy the requirements for the establishment, documentation, implementation, and maintenance of an efficient quality control system and comply with Sections 1 (General Rules) and 3 (Manufacturing Control and Quality Control of Sterile pharmaceutical Products) of Chapter 2 of the current GMP regulations.

3.1 General Requirements

3.1.1 General

The quality system at a factory manufacturing pharmaceutical products by terminal sterilization procedures should comprise an organizational structure and description of operational procedures, manufacturing processes, resources as well as other activities essential for ensuring compliance with the requirements for terminal sterilization of sterile pharmaceutical products as stipulated in this guidance document.

All the activities to be performed in relation to quality assurance, including sterility assurance, should be clarified and documented. The manufacturer who manufactures sterile pharmaceutical products using terminal sterilization should establish and implement standards for efficient quality control as necessary to prevent microbial contamination of the products during processing.

The quality system should include the investigation system for ascertaining any failure in termination sterilization and/or abnormality and deviation in control parameters monitored and the verification system of the corrective and preventive actions and their effectiveness after implementation.

3.1.2 Scope of Application

This guidance is applicable to the quality system governing all processes in manufacturing sterile pharmaceutical products at facilities where pharmaceutical products are manufactured by terminal sterilization procedures. In practice, the scope of application includes environmental control, control of laboratory testing of sterile pharmaceutical products, control of terminal sterilization process as well as processes prior to sterilization, which may affect product quality, and systematized control of manufacturing processes and product quality such as validation, documentation, and change control.

3.1.3 Document Control

The following documents should be prepared in writing, distributed for fulfilling the requirements stipulated in each provision of this guidance, and archived to ensure the sterility of sterilized pharmaceutical products: documents on initial, periodic and change validation; standard operating procedures (SOPs); area maps with cleanliness levels; movement diagrams of raw materials, personnel, intermediate compounds, and finished products; equipment and instrument layout charts; instructions; records of data; deviation control records; change control records; out-of-specification (OOS) test results; calibration records; environmental monitoring records; log books; and records stored on electronic media.

In particular, design control of terminal sterilization process, procedures for sterilization process validation, procedures for sterilization process control, and procedures for product release following sterilization should be documented in detail and maintained. In addition, all probable and possible factors influencing validation, process control procedures, and sterilization processing should be documented and maintained to verify the reproducibility of the functions related to terminal sterilization process.

3.1.4 Quality Risk Management

The concept and procedures for quality risk management should be included in the quality system, and contamination preventive measures implemented to minimize risks of contaminating pharmaceutical products with microorganisms, endotoxins, and foreign matters. The risk management system should be based on risk assessment procedures for analyzing and evaluating

factors affecting product sterility and contamination with endotoxins and foreign matters as well as based on the verification of risk control procedures for demonstrating the reliability and validity of risk avoidance procedures.

3.1.5 Qualification of Processing Equipment

Equipment and instruments used for the manufacture of sterile pharmaceutical products in the processing area as well as other equipment and instruments that may affect the processing should be evaluated for qualification. Based on qualification assessment results, a program for maintaining the equipment and instruments should be established and implemented.

3.1.6 Qualification of Processing Environment

Environmental parameters of the processing area should be identified and verified for qualification. Based on qualification assessment results, a program for heating, ventilating and air conditioning (HVAC) system maintenance and environmental monitoring should be established and implemented.

3.1.7 Prospective Validation and Periodic Review of Process Control

Prospective process validation should be conducted for all processes and operational activities that may affect the sterility of pharmaceutical products. A set of process control programs should be established and evaluated by periodic review or revalidation.

3.1.8 Periodic Revalidation

The periodic revalidation plan should include procedures for testing the worst-case scenario or the worst point where sterilization efficiency has been the poorest, if identified in the prospective validation.

3.1.9 Time Limitation for Aseptic Manufacturing Operations

The maximum allowable time from the preparation of the pharmaceutical solution to the start of the sterilization process should be established by taking into account risks inherent to these processes.

3.1.10 Cleaning and Disinfection of Facilities and Equipment

A program for cleaning and disinfecting facilities and equipment should be established in

relation to the cleanliness requirements of each production area. The disinfection program should be established by taking into account the potential development of drug-resistant microorganisms. The levels of cleanliness and sterility achieved should be assessed by screening and classifying bacterial isolates in each production environment.

3.1.11 Pest Control

An appropriate pest control program should be directed to aseptic manufacturing facilities to prevent contamination of sterile pharmaceutical products with arthropods and other vermin.

3.1.12 Flow of Raw Materials

Flow Diagrams of raw materials to final product into the processing area of sterile pharmaceutical products should be established and, as the situation may require, appropriate disinfection and sterilization procedures should be implemented. Appropriate procedures are also necessary to prevent microbial invasion into working areas during a transfer of raw and other materials.

3.1.13 Gowning and Flow of Personnel

Appropriate procedures should be practiced to prevent microbial invasion into the processing area during the entry or exit of personnel. Gowning procedures and flow of personnel should be standardized.

3.1.14 Change Control

Changes in SOPs and other procedures should be confirmed to have no negative impact on the sterility of pharmaceutical products based on scientific evidences prior to implementation. Changes should also be evaluated by applicable qualification-assessment and validation procedures, and control parameters established by assessing and, wherever possible, controlling potential risks associated with such changes.

3.1.15 Calibration

A calibration program including calibration frequency and accuracy requirements should be established and implemented to calibrate analytical instruments used in laboratory quality testing and measuring, inspection, and control devices used in the manufacturing process. In particular, the calibration program is necessary for all equipment, instruments, and devices to be used in the current guidance-specific terminal sterilization process in a manner suitable to the characteristics of the

sterilization process. If the calibration value of any key instrument or device is found to deviate from the predetermined acceptance limits, the impact of such deviation on the quality of products manufactured with such instrument or device prior to the calibration should be investigated and judged on the acceptability of such products. An example of the investigation is to identify the presence of problems by conducting specification tests with standard or reference materials or products using the products manufactured since the previous calibration and those manufactured separately with a calibrated instrument or device. In the event that any abnormalities are identified, necessary measures should be taken to correct such abnormalities.

3.1.16 Parametric Release

Refer to the information contained in Annex A1 in the case that parametric release is introduced in the terminal sterilization.

3.2 Requirements for Monitoring and Control of Routine Operating Cycles

- 1) Processing areas should be maintained to an appropriate cleanliness standard by controlling the cleanliness of the air supplied and implementing an environmental monitoring program.
- 2) Processing areas should be cleaned and disinfected periodically or as needed and confirmed to meet predetermined manufacturing environment standards.
- 3) Bioburden levels of intermediate products before sterilization should be adequately controlled by establishing acceptance criteria (e.g. control levels) and test procedures.
- 4) Routine maintenance programs should be established and implemented based on results of qualification assessment and validation.
- 5) Process control programs verified by validation experiments should be implemented.
- 6) Process control programs should be routinely verified by periodic review or revalidation.

3.3 Validation

The production of high-quality sterile pharmaceutical products by terminal sterilization procedures can be achieved by compounding hardware configurations such as facilities and equipment and software configurations such as SOPs and control systems and programs. The qualification of processing environments, qualification of manufacturing facilities/equipment, and process validation should be demonstrably adequate for assuring not only the quality of products throughout the manufacturing process but also the maintenance of required cleanliness levels of critical processes such as filtration, filling, sealing, and terminal sterilization processes; scientific verification of the absence of contamination risks in commercial production facilities/equipment and

manufacturing process; and efficient prevention of product contamination. The validation of these critical procedures should include the competency of transportation equipment in protecting materials supplied by vendors and products shipped by the manufacturer from contamination during transport.

The fundamental requirement for quality assurance during manufacture is to control manufacturing processes by using validated operating procedures and process control parameters. When any changes are to be made to the operating procedures and control parameters, proposed changes need to be assessed from risk-management viewpoint for scientific justification and revalidated, as appropriate. Outcomes of the assessment and revalidation of the changes must be documented.

3.4 Continual Improvement

Continual improvements should be made to the factory's effectiveness and efficiency in fulfilling the requirements of the manufacturer by reviewing quality and manufacturing systems based on outcomes of actions taken for claims, deviations, and emergencies; review of SOPs; change control; risk assessments; and various types of monitoring.

4 Personnel

As humans may be a source of contamination with bacteria, endotoxins, insoluble particulate matters, and other foreign matters, it is essential to minimize personnel intervention as a possible source of contamination in a factory manufacturing terminally sterile products in order to eliminate a human source of contamination and non-conforming products. Factory personnel who engage in the manufacture of terminally sterile products should repeatedly receive education and training on the concepts and practical procedures required to perform assigned tasks and maintain morale and skills at high levels.

If personnel are assigned to operate sterilizers, they should be educated on the structure, characteristics, operating procedures, operating condition-supervising procedures, and maintenance and control procedures of the equipment.

4.1 Personnel Training

- 1) Practical procedures that personnel are required to follow during manufacturing operations should be included in the SOPs and their validity properly monitored and assessed. The SOPs should contain the type of procedures with which the personnel are required to comply. Since there are risks of elevated bioburden level and subsequent elevated endotoxin level as well as risks of increasing insoluble particulate matters and foreign matters during manufacture, factory personnel should be trained on the adequate control of intermediate compounds and minimization of their exposure to pharmaceutical solutions filtered for sterilization.
- 2) Personnel who engage in terminal sterilization processing in manufacturing areas should receive adequate education and training from competent personnel with sufficient experience of practical procedures and compliance requirements in the areas. The training program for the personnel responsible for the processes of preparation, filling, sealing, and sterilization should include at least the following topics:
 - Basic microbiology
 - Biochemistry and cell biology of endotoxins released by microorganisms
 - Gowning procedures
 - Personal hygiene practices,
 - Control of contamination risks of insoluble particulate and foreign matters
 - Cleaning and disinfection of processing areas, equipment, and instruments
 - Properties of a pharmaceutical product to be sterilized and effects of bacterial contamination on the product
 - Methods and procedures of sterilization
 - Principles and degree of sterility assurance by the terminal sterilization method employed
 - Structure, characteristics, and operational procedures for manufacturing equipment including sterilization equipment
 - Bioburden control of raw and other materials and pharmaceutical products, including containers before sterilization
 - Procedures for monitoring and supervising operating conditions and those for maintaining and controlling sterilization equipment and other equipment.

The training on these topics should be practiced based on documented training programs and schedules.

- 3) Factory personnel who engage in manufacturing operations in processing areas for manufacturing sterile pharmaceutical products should be trained on at least the following

subjects as basic requirements.

- Personnel should not be allowed to wear make-up, as a rule, except for that to protect the skin and mucosal membrane. Wrist watches and accessories are also prohibited.
 - Personnel should strictly adhere to documented procedures and adopt proper behaviors to minimize contamination of the manufacturing areas for the preparation, filling, and sealing of terminally sterile pharmaceutical solution.
 - Personnel should refrain from speaking after gowning and should not come into direct contact with the cleaned wall, floor, or sanitized surfaces unless necessary.
 - The number of personnel working in the manufacturing areas for the preparation, filling, and sealing of terminally sterile pharmaceutical solution should be minimized as much as possible.
 - In the case that areas of different cleanliness levels (e.g. A and B) are located in a facility, personnel should follow the requirements specified in the guidance, the Manufacturing Methods for Sterile pharmaceutical products Produced by Terminal Sterilization, as a rule.
- 4) Education and training topics should be documented, and the educational effectiveness of the training program should be evaluated against its objectives and goals.
 - 5) Personnel to whom Provisions 2) and 3) above are applicable must receive education and training as listed above and be granted a certificate as qualified. Personnel who have not completed all of the above training topics should nevertheless be allowed to participate in operations to a degree consistent with the amount of training they have completed.
 - 6) Personnel (e.g. manufacturing supervisors, QA/QC personnel, and maintenance/control personnel) who occasionally enter the manufacturing areas for the preparation, filling, and sealing of terminally sterile pharmaceutical solution and areas where sterilized containers and packages are passed through and exposed should be trained on gowning procedures and other topics as listed in Provision 3) above as a minimum to appropriately act and move in the manufacturing areas.
 - 7) As a rule, the entry into the manufacturing areas for the preparation, filling, and sealing of terminally sterile pharmaceutical solution and areas where sterilized containers and packages are passed through and exposed should be restricted to personnel who are qualified to enter those areas. When unqualified personnel need to enter the areas for any reason, such as equipment repair, those personnel should obtain entry permission from the supervisor of the areas and be accompanied by qualified personnel throughout their stay in the areas.

4.2 Education and Training Topics on Gowning

- 1) The training program on gowning should include training on hand washing, gowning, and degowning procedures required before entering and after leaving the manufacturing areas for the preparation, filling, and sealing of terminally sterile pharmaceutical solution or areas where sterilized containers and packages are passed through and exposed.
- 2) The training program should also include procedures to minimize gowning-related contamination risks in the manufacturing areas for the preparation, filling, and sealing of terminally sterile pharmaceutical solution and areas where sterilized containers and packages are passed through and exposed. Gowning training results should be a requirement for permitting entry to and exit from the areas, and supervisory personnel should inform the trainees of their training results.

4.3 Gowning Requirements

- 1) Personnel should wear a gown specifically designed for manufacturing operations and environment before entering the manufacturing areas for the preparation, filling, and sealing of terminally sterile pharmaceutical solution and areas where sterilized containers and packages are passed through and exposed.
- 2) Gowning rooms located before the manufacturing areas for the preparation, filling, and sealing of terminally sterile pharmaceutical solution and areas where sterilized containers and packages are passed through and exposed should be provided with displays of gowning procedures and devices to check for adequacy of gowning.
- 3) Caps, gowns, and gloves to be worn in the manufacturing areas for the preparation, filling, and sealing of terminally sterile pharmaceutical solution should be selected from items that provide highest possible working efficiency and prevention of particulate matters emitted into the environment.
- 4) The frequency of changing gowns and other clothes and their sterilization methods and storage conditions should be examined and established with due care by identifying and eliminating conditions that may adversely affect product quality, such as bioburden and particulate matter levels prior to terminal sterilization processing.
- 5) Procedures for aseptic gowning in the manufacturing areas for the preparation, filling, and sealing of terminally sterile pharmaceutical solution and areas where sterilized containers and packages are passed through and exposed as well as washing procedures for degowned clothing items should be established so as to minimize contamination of clothing items and bringing contaminated materials into these areas.
- 6) Personnel should wear well-fitting caps, gowns, and gloves and check to see if they are torn or

defective. In particular, gowns should show no signs of deterioration due repeated use, washing, and sterilization. If a gown or glove is found to be defective, personnel should promptly exit the area and change the item immediately with care not to contaminate the environment.

- 7) Gowns and other clothing items worn in the manufacturing area should be classified in accordance with cleanliness level, cleaned, and stored separately from clothing items worn in other areas.
- 8) In the case that areas of different cleanliness levels (e.g. A and B) are located in a facility, personnel should follow the requirements specified in the guidance, the Manufacturing Methods for Sterile Pharmaceutical products Produced by Terminal Sterilization, as a rule.

4.4 Personnel Management

- 1) Personnel who engage in operations in the manufacturing areas for the preparation, filling, and sealing of terminally sterile pharmaceutical solution and areas where sterilized containers and packages are passed through and exposed should be subject to personnel management in accordance with an area-specific microbiology monitoring program.
- 2) When agar medium is used for the microbiological testing of gowns and other clothing items, personnel should perform such testing immediately before leaving the working area.
- 3) Environmental monitoring data should be analyzed in individual personnel at appropriate intervals to determine individual trends of increasing risk of contamination. If any personnel show undesirable trends in contamination, education and training of such personnel should be repeated until acceptable microbiological data are obtained.

4.5 Personnel Health Management

- 1) Personnel should report any abnormal physical condition to the supervisory personnel prior to engagement in operations, if they are affected with fever, skin damage, flu, or diarrhea that may affect aseptic processing operations, cleanliness control, and product bioburden control in the working area.
- 2) The supervisor should not permit the entry of the personnel with physical abnormalities into the filling and sealing areas.
- 3) The factory should establish and implement procedures for permitting personnel at risk of acquiring disease, as assumed from, for example, his/her record of visiting an endemic area, prior to the engagement in operations.

5. Buildings and Facilities

5.1 Key Features of Facility Design

Clean areas are generally required to be designed with the following features:

- 1) Clean areas should be clearly separate from toilet facilities, cafeterias, and other areas of lower cleanliness level.
- 2) Clean areas should be clearly designated by individual objectives of processing activities and be of suitable size for such activities.
- 3) Clean areas should be designed so as to achieve efficient flow and control of personnel, products, raw materials, and waste materials within the areas, and equipment should be carefully located in facility to minimize obstructions in flow channels between personnel and materials.
- 4) Individual areas should be separated into sections to prevent mix-up between clean and dirty apparatuses and instruments or sterilized and non-sterilized materials and products.
- 5) Facilities should be designed so as to facilitate the ease of cleaning and maintenance and should be periodically inspected to verify that the facilities are maintained as originally designed. Particular consideration should be given to shields and packing in order to keep processing rooms tightly closed. Quality of heat shield materials should also be closely examined before use for efficient prevention of condensation.
- 6) Ceilings should be effectively air-sealed.
- 7) Designing areas with irregular surfaces and extruding frames around doors and windows, etc. should be avoided, wherever possible, to prevent or minimize the retention of particulate matters and microorganisms and obstruction of airflow. If such designs are unavoidable, the structures should be suitable for easy cleaning.
- 8) Adequate spaces or rooms should be available for gowning, storage of gowns, and disposal of used gowns.
- 9) Operations related to filling and sealing should be controlled, supervised, and recorded. Transparent (e.g. glass) windows or video cameras are preferred to be adequately installed in the processing areas to facilitate observation and supervision of filling and sealing operations inside from outside.
- 10) The processing areas should be designed so as to minimize environmental exposure of open containers or finished products and allow easy access of personnel to these items in the filling and sealing areas during processing or equipment maintenance.
- 11) Equipment not essential for processing in the filling or sealing area should be installed separately in non-critical areas.

- 12) Working areas in other support areas (Grade C or D) should be adequately separated by corridors in order to prevent those areas from being used for routine passage of personnel not directly engaged in processing in the areas.
- 13) Facility design should be structurally suitable for preventing or minimizing a risk of cross contamination if the facility is intended to be used for the processing of physiologically active substances, pathogenic substances, highly toxic substances, radioactive substances, etc.
- 14) Surface materials of walls, floors, and ceilings should be easily cleanable and durable against cleaning agents and disinfectants.
- 15) The cleanliness of the filling and sealing areas should be Grade C or higher. If the risk of product contamination due to environmental factors is high, the grade of these areas should be A and that of direct support areas be Grade C or higher. Examples of high contamination risk include slow filling rate, wide container opening, substantial time lag between filling and sealing processes, frequent personnel interventions, and highly proliferative bacterial activity of the product.
- 16) Drains and sinks should not be installed in areas of Grade B or higher. If drains are placed in other support areas (Grade C or D), certain contamination preventive measures that enable easy cleaning and disinfection should be considered — such as easily cleanable filters to trap foreign matters at the outlet and equipment to block the backflow of drained water. If grooves are to be made on the floor, they should not be deep and their structure be convenient for cleaning.
- 17) Recesses in and surfaces of pipes, ducts, and other utilities in clean areas should be ensured to be easily accessible and cleanable.
- 18) Clean areas should be ventilated with air passed through an appropriate filter, e.g. a high-efficiency particulate air (HEPA) filter, to maintain air quality in the areas and pressure difference between areas at acceptable levels. The pressure difference should be monitored to maintain the difference constant throughout operations.
- 19) Temperature and relative humidity in clean areas should be controlled within the limits compatible with properties of materials and products being handled therein and also should be set at levels suitable for microbiological control.
- 20) Environmental temperature and relative humidity should be controlled within specified limits and, wherever feasible, monitored continuously.
- 21) Air pressure in clean areas should be maintained higher than that in adjacent areas of lower cleanliness levels. Areas provided with the closed ventilation scheme should be designed to be capable of adjusting air pressure effective in preventing proliferation of particulate matters to adjacent areas as well as maintaining the cleanliness at the required level.

- 22) Airflow patterns in filling and sealing areas, if their cleanliness levels are specified as Grade A, should be controlled to meet sterility requirements for the prevention of the surfaces of pharmaceutical products and critical points in the areas from contamination.
- 23) Direct support areas should be separated from adjacent areas by airlocks, if their cleanliness levels are different. If the levels are the same for the two areas, the installation of airlock system should be considered depending on the type of operations to be performed in the areas. Spaces located between direct support areas and adjacent areas should be equipped with pass-through rooms and/or pass-through boxes for the transfer of sterilized materials. Further, pass-through rooms and pass-through boxes should be designed to be capable of decontaminating outer packages of sterilized materials or the materials difficult to sterilize (including measurement instruments), as the situation may require.
- 24) Airlock doors should be equipped with systems that prevent simultaneous opening of both doors (e.g. mechanical and electrical interlocking systems and visual and audible alarm systems).
- 25) The gowning room should be equipped with an airlock system and physically portioned into gowning and degowning areas. Air particulate cleanliness in the gowning room should be maintained at the same level as that set for the working room (in a non-operating state) where gowned personnel are to engage in processing. In order to suppress a temporary increase in particulate matter count due to gowning, air should be introduced through vents at a relatively high position in the room and removed through vents at a lower position, taking into account spatial volume of the room and ventilation frequency (or time required for clean-up). The cleanliness level of air in the pass-through box may be set according to its purpose of use.
- 26) If the cleanliness level of direct support areas is specified as Grade B, gowning rooms should be portioned into entry and exit blocks. As an alternative measure, it is acceptable to control crossing of personnel entering into and exiting from the area by staggering time of entry and exit.
- 27) Gowning rooms should be located in accordance with the need of the rooms as required by the cleanliness level. Even if the cleanliness level is the same between or among areas, additional gowning rooms should preferably be set up by taking into account the level of potential risks of contamination, if there are risks of cross contamination with raw materials or pharmaceutical products.
- 28) Processing rooms for weighing raw materials or washing containers should be carefully designed to secure their seal integrity and maintain appropriate airflow so as not to introduce contaminated air into adjacent rooms.

6. Manufacturing Processing Areas for pharmaceutical Products to be Sterilized by Terminal Sterilization Procedures

6.1 Classification of Manufacturing Areas by Air Cleanliness

Generally, the cleanliness of air in processing areas is defined by the number of airborne particles $\geq 0.5 \mu\text{m}$ in diameter per unit volume of air. The number of particles $\geq 5 \mu\text{m}$ in diameter serves as a reliable parameter for early detection of environmental deterioration when regularly monitored and evaluated by linear trend analysis. Table 1 shows the air cleanliness requirements for classified areas.

Facilities for processing sterile pharmaceutical products by terminal sterilization procedures comprise clean areas controlled based on predefined airborne particle and microbiological standards. The areas are classified as filling and sealing areas, direct support areas, and other areas depending on the nature of the processing conducted.

Table 1. Classification of Clean Areas

Air cleanliness Note 1)	Maximum number of airborne particles (/m ³)			
	Count under non-operating conditions		Count under operating conditions	
	$\geq 0.5 \mu\text{m}$	$\geq 5.0 \mu\text{m}$	$\geq 0.5 \mu\text{m}$	$\geq 5.0 \mu\text{m}$
Grade A (ISO 5)	3,520	20	3,520	20
Grade B (ISO 7)	3,520	29	352,000	2,900
Grade C (ISO 8)	352,000	2,900	3,520,000	29,000
Grade D	3,520,000	29,000	Dependent on process attributes ^{Note 2)}	Dependent on process attributes ^{Note 2)}

Note 1) The ISO class designation in parenthesis refers to the count during operation.

Note 2) There are cases where maximum allowable number may not be specified.

6.1.1 Filling and Sealing Areas

- 1) Filling and sealing areas are areas of processing where materials, products, and container-closure systems are directly exposed to the environment during filling and sealing processes. These processes include installation and connection of container components to the container-closure system.
- 2) The cleanliness of filling and sealing areas should be Grade C or higher, and the grade of each area should be defined by taking into account the characteristics of the pharmaceutical product

manufactured, quality requirements, and potential risk of contamination.

- 3) Personnel entry into filling and sealing areas should be kept to a minimum.
- 4) For the appropriate control of bioburden in products before sterilization, locations having a high risk of product contamination should be monitored by determining the count of airborne particles and microorganisms by appropriate methods at an adequate frequency.

6.1.2 Direct Support Areas

- 1) Direct support area is defined as a supporting area of filling and sealing areas.
- 2) The cleanliness of the direct support area should be Grade C or higher, and the grade of each area should be defined by taking into account the level of potential risk of contamination.
- 3) The count of airborne particles and microorganisms should be regularly monitored by suitable methods in the direct support area. The frequency and method of monitoring should be carefully selected based on evaluation results of product contamination risks in the filling and sealing areas.

6.1.3 Other Support Areas

- 1) Other direct support areas comprise areas for preparing pharmaceutical solutions prior to sterilization and areas for washing and cleaning manufacturing equipment and apparatuses.
- 2) The cleanliness of other direct support areas should be controlled by establishing specifications for acceptable airborne particle count by taking into account the required level of contamination control and type of work performed in the area.
- 3) Weighing and preparation processes of pharmaceutical solution, etc. are usually conducted in Grade C areas. If certain contamination-preventive measures are implemented by, for example, processing in closed systems, the preparation of pharmaceutical solution may be performed in a Grade D area.

6.2 Heating, Ventilating and Air Conditioning Systems

Air in clean areas should be controlled by designing, instituting, and managing suitable heating, ventilation, and air conditioning (HVAC) systems to maintain atmospheric conditions at appropriate levels. The systems should be managed to ensure constant and secure operations against not only temporal variations due to operational activities, such as door opening and closing and facility equipment operation, but also sustained variations due to non-operational activities, such as seasonal changes in outdoor conditions and deterioration of equipment and apparatuses over time.

The HVAC systems and their management programs are comprised of the following basic

elements: temperature, relative humidity, air flow volume, air exchange rate, unidirectional air flow, pressure difference relative to adjacent rooms, integrity of HEPA filter, airborne particle count, and microbial count.

6.2.1 Temperature and Relative Humidity

Temperature and relative humidity can have direct impact on materials and products, comfort of personnel, and potential for microbial contamination in processing areas; therefore, the level of temperature and relative humidity should be appropriately established, controlled, monitored, and maintained throughout processing.

6.2.2 Air

It is critical to secure constant airflow from an area of higher cleanliness level to an area of lower cleanliness level in order to maintain the environmental conditions of clean areas at appropriate levels.

- 1) Pressure difference between areas of different cleanliness levels should be adequately defined, monitored, and controlled.
- 2) When pressure difference is one of the most important factors for controlling bioburden before sterilization, it is recommended to continuously monitor pressure difference between areas and install an alarm system to enable prompt detection of abnormal pressure difference.
- 3) In the case that areas of different cleanliness levels (e.g. Grades A and B) are located in a facility, the air control measure should, as a rule, meet the applicable requirements specified in the Guidance on the Manufacture of Sterile Pharmaceutical Products by Aseptic Processing.

7. Cleaning and Disinfection of Processing Areas for Sterile Pharmaceutical Product Manufacturing

Areas for manufacturing sterile pharmaceutical products should be cleaned and disinfected in accordance with applicable SOPs, and the performance of such activities should be recorded in writing and retained in archive.

7.1 Cleaning Agents and Disinfectants

- 1) Cleaning agents and disinfectants should be evaluated and confirmed by the quality unit prior to use to be suitable and reliable in removing contaminants. The efficacy of disinfectants should be assessed and validated based on the type and count of microorganisms characterized by periodic environmental monitoring.

- 2) SOPs should be established for the application of cleaning agents and disinfectants, cleaning and disinfection schedules, cleaning following disinfection where necessary, precautions for cleaning staff to ensure their safety as well as for caring and storage of cleaning tools. Cleaning agents and disinfectants used in Grade A or B areas should be filtered or treated by some other means before use to ensure their sterility and controlled to prevent internal microbial contamination until use.
- 3) Cleaning agents and disinfectants, when prepared in-house, should be prepared in accordance with applicable SOPs, and records of preparation should be produced and retained. When commercial cleaning agents and disinfectants are used after dilution, details of the dilution procedure—such as diluents, dilution ratio, expiration date, storage conditions, and, if applicable, sterilization methods—should be recorded in writing.
- 4) When cleaned or disinfected, the surfaces of facilities and equipment that may come into direct contact with pharmaceutical products should be verified to be free of cleaning agents or disinfectants by appropriate methods after the completion of cleaning or disinfection procedures.
- 5) Reasonable expiration dates should be established for individual disinfectants, and the agents should be only used before such dates.
- 6) The disinfection of the manufacturing environment should not precede the cleaning procedure, as a rule. If there are any locations in the environment where cleaning agents may reside after cleaning, it should be verified that the cleaning agents do not impair the efficiency of disinfectants.
- 7) Disinfectant containers should not be refilled with disinfectants.
- 8) The following matters should be taken into account in the selection and use of disinfectants:
 - ① The storage and usage of disinfectants should be in accordance with the supplier's instructions.
 - ② The selection of disinfectants and disinfection procedures should be primarily based on the safety of the personnel engaged in disinfection work.
 - ③ If the selected disinfectants are suspected of being ineffective against microorganisms isolated from the environment, the efficacy of the agents should be reevaluated and the replacement or alternate use of different disinfectants should be considered and implemented, as appropriate.
 - ④ If environmental monitoring data indicate or suggest the presence of spore-forming bacteria or fungi, effective sporicides or fungicides should be selected for disinfection, as required.

- ⑤ The directions for use of disinfectants should include the method of disinfection, application sites, and duration of use required for obtaining anticipated effects.
 - ⑥ The chemical properties of cleaning agents and disinfectants, in terms of their effects (e.g. corrosiveness) on facility and equipment surfaces, should be assessed prior to the selection of the agents.
- 9) If sporicides or fungicides (including fumigating agents) are likely to be used in irregular manners in areas for processing sterile pharmaceutical products by terminal sterilization procedures, the type, concentrations, usage, and procedures for efficacy confirmation of the agents should be predetermined and specified in writing.
- 10) Cleaning agents, disinfectants, and cleaning utensils should not be stored in critical areas. Materials necessary for operations in the critical area such as hand sprays to sanitize gloves may be stored in critical areas, if well controlled. If cleaning agents and disinfectants are stored in critical areas, control procedures for their storage in critical areas should be defined in writing.

7.2 Validation of Disinfection Procedures

- 1) The reliability and frequency of disinfection procedures should be established through the environmental monitoring program.
- 2) Disinfectants should be microbiologically assessed prior to use in individual facilities, and appropriate control procedures should also be instituted for such facilities.
- 3) The efficacy of disinfectants should be assessed with respect to ensuring that microorganism counts remain below the count predetermined based on the type and count of isolates collected from various surfaces through the environmental monitoring program.

7.3 Monitoring of Adequacy and Efficacy of Cleaning and Disinfection Procedures

- 1) The adequacy and efficacy of cleaning and disinfection processes should be established through the overall environmental monitoring program.
- 2) Microorganism counts on the surfaces of equipment and instruments should be periodically obtained by environmental monitoring and analyzed to detect trends in occurrence and proliferation. A full investigation is mandatory to determine causes of abnormalities when the microbial count exceeds the action level, when the species ratio of microorganisms is obviously different from that routinely reported, or when abnormalities in the count or species ratio continue for an extended period of time. Corrective and preventive measures should be implemented, as appropriate whenever considered necessary.

- 3) If the established disinfection procedure is not found to be effective for certain types or concentrations of disinfectants used, the reliability of such disinfectants should be reevaluated by, for example, using different disinfectants interchangeably or replacing with other disinfectants, as appropriate.

8. Environmental Monitoring

The primary objectives of environmental monitoring of areas for processing sterile pharmaceutical products by terminal sterilization procedures are to control the levels of microorganisms and airborne particles within specified limits, predict the damage to the environment, and continuously evaluate the efficiency of cleaning, disinfection, and decontamination procedures in the filling and sealing areas as well as in other support areas, where the risk of microbial and particulate contamination is high, in order to maintain the required cleanliness level of the pharmaceutical manufacturing environment. The purpose of environmental monitoring can be classified into two categories: microbiological control and particle control. Microbiological control is intended to allow the scientific identification and characterization of bioburden organisms residing in the manufacturing environment in order to ensure that the manufacture of sterile pharmaceutical products has been conducted in an appropriately controlled environment and institute the measures (e.g. disinfection procedures) necessary to maintain the environment under the predefined conditions.

8.1 General Requirements

- 1) Scope of application

Appropriate environmental monitoring programs should be established and implemented for filling and sealing areas as well as direct support areas. The programs should be applied for other support areas wherever needed.

- 2) Environmental monitoring programs

SOPs for implementing environmental monitoring programs should be established, and the outcome of the implementation should be adequately recorded. The programs should be developed by assessing and examining properties of substances monitored, frequency of monitoring, sampling locations, and action levels in order to appropriately estimate environmental contamination risks.

- 3) Targets for monitoring

Monitoring targets are microorganisms and airborne particles.

- ① Target airborne particles are those $\geq 0.5 \mu\text{m}$ in diameter. Particles of other diameter (e.g.

$\geq 5.0 \mu\text{m}$) should be measured as required by a need of environmental monitoring for better environmental control on an as-needed basis.

- ② Target microorganisms are bacteria and fungi.
- ③ Target microorganisms should include not only airborne microorganisms but also those on the surface of walls, floors, fixtures, equipment, gowns, etc.

4) Preparation of environmental monitoring programs

Environmental monitoring programs should be drawn up prior to performance qualification (PQ). The programs should be reevaluated based on PQ subsequently performed and included in the routine control program for routine practices. Since PQ includes performance testing of the worst-case scenario, the number of sampling locations and frequency of measurement tend to be larger. The number of sampling locations may be reduced by, for example, setting up representative locations for analysis if the monitoring programs are included in the routine control program based on PQ-based reevaluation. Procedures for bacterial monitoring may be simplified by implementing adequate inspection, maintenance, and supervision of facilities and equipment on regular or occasional basis, if the facilities and equipment are provided with isolators, RABS, a blow-fill-seal system, or other devices that prove it sufficiently robust to prevent bacterial contamination. ISO requirements for routine monitoring and control such as the number of samples (e.g. sample locations related to clean room areas) are available in ISO DIS 14644-1 for reference in establishing environmental monitoring programs.

5) Monitoring targets and locations

Environmental monitoring targets should include air that comes in contact with working areas, manufacturing equipment (and process control equipment, where appropriate), and aseptic environments; air for maintaining the aseptic environment clean; and compressed air or gas that comes in contact with the environment and equipment. The monitoring frequency of compressed air and gas supplied for manufacturing equipment or used during manufacturing processes may be separately set, provided the cleanliness level can be maintained by filter integrity test or other suitable tests.

6) Sampling frequency for environmental monitoring

Sampling frequency should be determined in accordance with air cleanliness levels for working areas separately under operating and non-operating conditions. The sampling procedures should include the frequency of sample collection from gown and other clothing of personnel. Frequencies of sampling shown in Table 2 may be helpful for establishing the sampling specifications.

7) Monitoring methods—sampling and testing procedures

Optimal number and locations of monitoring points should be determined for individual

processing areas by taking into account the size of working area, scope of operations, and process flows of materials and products. The monitoring points considered to be necessary for assessing potential product contamination should be added, as appropriate.

- ① Devices for collecting and counting airborne particles should be used only after validated calibration. For the evaluation of particle counts obtained, the counts should be converted to the count per-cubic-meter of atmosphere.
- ② Samples of airborne microorganisms should be collected by one or more suitable procedures including the settle plate, impact, and filtration methods, and microorganisms on the surface should be collected by one or more suitable procedures including the contact plate and swabbing methods. The size of the area to be sampled should be determined based on the shape and surface condition of monitoring targets and locations. In principle, the recommended size of sampling area of equipment and apparatuses is 24 to 30 cm². Air volume to be sampled for airborne microorganism monitoring should be decided by general considerations and upon discussion of factors involved, such as cleanliness of the target area and routine monitoring frequency. If the cleanliness level of a target area is Grade A, air volume to be sampled should be at least 1 m³ each time. Microbial count monitoring should usually use a circular flat plate of 90 cm in diameter and a maximum exposure time of 4 hours.
- ③ Methods and procedures for detecting and counting airborne and surface microorganisms should be established by referring to the “Microbiological Environmental Monitoring Program” contained in the General Information of the JP. Culture medium used for the monitoring should be tested in advance for the absence of cell growth inhibitory substances for selection of a competent medium suitable for the microbial monitoring. The objective of this testing is to ensure that the collection and growth of microorganisms would not be affected by the presence of alcohol, antibiotics, etc. during microorganism collection and culturing processes.
- ④ The incubation temperature of the medium should be suitable for the growth of target microorganisms.

8) Alert and action level specifications

Alert and action levels should be specified for individual target substances and locations to be monitored.

- ① Action level specifications may be established by referring to data shown in Table 3.
- ② Alert level specifications should be established based on results of PQ tests.
- ③ The monitoring program should include the actions and measures to be taken, such as identification of causes of non-compliance and suspension of manufacturing, when alert

or action level specifications are met. In principle, a deviation from the action level specifications during the manufacture of products to be terminally sterilized does not mean termination of product delivery; rather, appropriate counteractions should be taken, such as the investigation of causes of deviations, corrective measures to be implemented, and verification of recovery of acceptable environmental conditions. The verification of the recovery may be readily realized in some instances by, for example, counting particulate matters, but not reproducible in other instances such as bacteria adherence to gowns. If the causes cannot be traced, the recovery should be demonstrated or ensured by implementing general practices and approaches including prohibition of the entry of personnel concerned for a certain period, retraining of personnel, and review of assigned tasks.

8.2 Requirements for Monitoring and Control of Routine Operating Cycles

1) Implementation of the monitoring program

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

2) Microbiological control

The microbiological environmental monitoring program should include periodic investigation of the characteristics of environmental flora and isolates for the assessment of contamination risks to pharmaceutical products.

3) Sample collection

Sampling of surfaces that come in contact, prior to sterilization process, with pharmaceutical products and other materials in filling and sealing areas should be performed immediately after the completion of filling and other processing operations.

4) Gases for manufacturing

Gases that may directly contact pharmaceutical products, primary containers, and surfaces that directly come in contact with pharmaceutical products should be periodically inspected and controlled to ensure the absence of microorganisms. The frequency of monitoring should be separately specified if the sterility of gas supplied needs to be ensured by, for example, the integrity test of filters.

5) Routine analyses

For the adequate maintenance of the manufacturing environment, monitoring data routinely obtained should be analyzed to detect any trends in changes in the environmental conditions and establish specific limits for trend analysis. Even if changes in environmental conditions do

not deviate from the specific limits (alert limits), any trends suggesting variations from normal conditions (trend analysis levels) should be predicted and the causes investigated to maintain the quality of the environment at an appropriate level. Trend analysis results should also be utilized for the maintenance of equipment for environmental control, such as the HVAC system, and for optimization of sterilization and disinfection/cleaning procedures.

8.3 Example Assessment Criteria for Environmental Monitoring

Table 2 shows example frequencies of environmental monitoring classified by cleanliness level, and Table 3 acceptance criteria for airborne particulate-matter and microorganism counts. Environmental monitoring programs may be additionally prepared and implemented by taking into account factors affecting monitoring frequency and acceptance criteria, as appropriate, since the risk of contamination of sterilized products varies depending on the formulation and size or volume of pharmaceutical products, structure/function of manufacturing equipment, automation level, time of retention of closures or containers in processing areas, and availability and performance of equipment for environmental control such as the HVAC system:

- 1) The frequency of microbiological monitoring may be increased or decreased depending on the type and time of processing activities; however, the frequency needs to be adequate for effective monitoring of potential microbiological contamination of pharmaceutical products.
- 2) The monitoring frequency for Grade C and D areas should be determined based on the type of pharmaceutical products, processes, operations, etc. to be monitored in the areas for appropriate quality control and risk management. The frequency may be decreased, as appropriate, if the risk of contamination is low, such as when pharmaceutical products are not directly exposed to the environment.
- 3) Monitoring should be reinforced immediately after the start of facility operation (e.g. at the beginning of PQ) as well as restart following long-term shutdown or partial modifications of operational conditions.
- 4) When personnel enter a Grade A area from a direct support area, surface microbial count on gowns and other clothes should be evaluated against stricter acceptance criteria (i.e. those for Grade A area), depending on the processing type-related level of product contamination risk.
- 5) Samples of particulate matter in Grade A and B areas should be collected preferably through continuous monitoring from equipment assembly until completion of critical operations.
- 6) The monitoring of particulate matters during times when no manufacturing operations are taking place should be conducted on an as-needed basis to maintain the environment at the predetermined cleanliness level by, for example, detecting and correcting malfunction of the air conditioning system.

- 7) Assessment results of particulate matter monitoring may differ depending on the amount of air sampled and air suction capacity of monitoring devices. Air samplers and particle-counting method should be carefully examined and employed so as to be suitable for the particulate matter control system.

Table 2. Frequency of Environmental Monitoring for Microbial Control

Cleanliness grade	Airborne particulate matters	Airborne microorganisms	Surface microorganisms		
			Equipment and walls	Gowns and gloves	
A	Each operating shift	Each operating shift	Sampling after work completion	Sampling after work completion	
B	Each operating shift	Each operating shift	Sampling after work completion	Sampling after work completion	
C, D	Area in which products and containers are exposed to the environment	Once a month	Twice a week	Twice a week	–
	Other areas	Once a month	Once a week	Once a week	–

Table 3. Acceptance Criteria for Environmental Microorganism Count (during Operations)^{note 1}

Cleanliness grade	Airborne microorganisms		Surface microorganisms	
	Airborne	Settle plate ^{Note 2}	Contact plate	Glove
	(CFU/m ³)	(CFU/plate)	(CFU/24–30 cm ²)	(CFU/5 fingers)
A	< 1	< 1	< 1	< 1
B	10	5	5	5
C	100	50	25	–
D	200	100	50	–

Note 1) Acceptance criteria are expressed as mean values.

Note 2) Measurement time per plate is 4 hours at maximum and the measurement is performed during processing operation.

9. Control of Raw Materials, Containers, and Closures

9.1 Control of Raw Materials (API and Additives)

9.1.1 General Requirements

- 1) SOPs should be established for the acceptance, inspection, storage, sampling, and testing of raw materials for control purposes. Acceptability criteria should also be developed and applied for these activities.
- 2) Raw materials should be carefully handled from receipt and storage to consumption by avoiding or minimizing the exposure to poorly-controlled environments that may cause microbial contamination or quality deterioration.
- 3) The sterility of raw materials is recommended to be ensured by periodic monitoring of bioburden level according to the procedure used for establishing the conditions of terminal sterilization.
- 4) Raw materials should be controlled to meet endotoxin specifications.
 - ① If raw materials are not depyrogenated during the manufacturing process, the initial endotoxin level of the materials should be ensured to be below the predetermined level.
 - ② If raw materials are depyrogenated during the manufacturing process, suitable depyrogenation procedures should be instituted by taking into account physicochemical properties of the materials and possible endotoxin levels. It is also necessary to determine

and record the endotoxin level of the materials prior to depyrogenation.

- ③ When raw materials are depyrogenated, the depyrogenation procedure should be validated.

9.2 Control of Containers and Closures

9.2.1 General Requirements

- 1) SOPs should be established for the acceptance, inspection, storage, sampling, and testing of containers and closures for control purposes. Acceptability criteria should also be developed and applied for these activities.
- 2) Containers and closures should be carefully handled from receipt and storage to consumption to avoid contamination with microorganisms and foreign particles.
- 3) Potential microbiological contamination of containers and closures should be adequately monitored to keep the contamination as low as reasonably achievable. It is recommended to periodically monitor bioburden level of containers and closures according to the procedure used for establishing the conditions of terminal sterilization. Bioburden monitoring data of containers and closures should be collected from the suppliers, as required, to confirm the adequacy of their bioburden control during the manufacturing process.
- 4) If washed after acceptance, containers and closures should be washed by appropriate validated procedures. If water is used for washing, final rinsing should be carried out with water for injection.
- 5) Containers and closures should be controlled to meet endotoxin specifications.
 - ① If containers and closures are not depyrogenated in any processes after acceptance, their endotoxin levels should be determined prior to transfer to be below predetermined levels.
 - ② If depyrogenation process is instituted additionally, the procedures for the process should be compatible with physicochemical properties of containers and closures.
- 6) Sterilized containers and closures should be protected from the contamination with particulate matters/foreign particles, microorganisms, or pyrogenic bacteria by appropriate preventive measures.
- 7) Extractability from container/closure components should be assessed in advance of their use.

9.2.2 Validation

If containers and closures are depyrogenated, the depyrogenation procedure should be validated prior to its implementation. Generally, the depyrogenation process is required to achieve at least a 3-log reduction below the endotoxin challenge.

10. Filtration, Filling, and Sealing Processes

10.1 Filtration Process

- 1) Objective of filtration process and bioburden control

The filtration process should be established to achieve sufficient sterilization of materials and products by taking into account relevant control parameters for terminal sterilization procedures. Bioburden levels of pharmaceutical solutions before filtration should be checked, as appropriate, at an appropriate frequency.

- 2) Selection of filters

Filters for filtration should be selected based on their chemical and physical properties, biological safety profile, and extractable profile.

10.2 Filling and Sealing Processes

- 1) Procedures for sterile filling and sealing of liquid drug preparations should be established and documented for the processes from pharmaceutical solution preparation and sterilization to cleaning and washing after filling and sealing. Assignment of responsibility should also be included in the procedures.
- 2) Filling and sealing activities and their environments should be monitored as directed in Chapter 8. "Environmental Monitoring." Environmental monitoring should include the preparation process, and monitoring data duly evaluated.
- 3) Equipment surfaces that come into direct or indirect contact with sterile pharmaceutical products should be controlled to appropriate bioburden levels according to validated (or verified to be efficient) sterilization procedures.
- 4) Equipment and utensils should be handled, maintained, and controlled in a manner that is effective in inhibiting bacterial proliferation to preserve their sterility until use.
- 5) Activities to connect tanks (or containers) of sterile pharmaceutical solutions and sterile filling equipment should be performed only in Grade C or higher environments. Care should be exercised to ensure complete bacterial contamination during connection activities.
- 6) Acceptable upper limits should be established for the time required for preparing sterile pharmaceutical solutions as well as for the time necessary for preparing pharmaceutical solutions until the commencement of sterilization in the final packaging form. The establishment of the limits should also take into account the bioburden level of pharmaceutical solutions immediately before sterilization.
- 7) Operating conditions for sealing equipment should be adequate and optimal for maintaining

tightness of the seal on containers (achieved by terminal sterilization) under the predetermined conditions for a certain period.

- 8) The tightness of the closure after terminal sterilization should be maintained at a level sufficient for controlling water and air content entering into or leaking from the container-closure system as not to affect the quality of pharmaceutical product throughout its shelf-life and adequate in completely preventing the entry of microorganisms from the outside. The validity of the conditions for preventing the entry of microorganisms may be demonstrated using a specified physical measurement method provided that it can be proven to closely correlate with a reliable microbiological method. Such verification of the correlation should include clear evidence-based methods, such as methods available in literature.

11. Moist-Heat Sterilization Process

Moist-heat sterilization including autoclaving is a terminal sterilization process that is the most widely used form of sterilization of pharmaceutical products. The requirements for this process stated in this section are focused on the sterilization by moist heat; however, the basic principles and procedures of the method are common and, therefore, applicable to other sterilization methods.

11.1 Design of Sterilization Process

It is critically important to design the sterilization process based on the information and data collected through research; development; industrialization efforts and experience; and findings accumulated through the manufacturing of other products in such a way that pharmaceutical products are produced and controlled to predetermined quality characteristics and consistently supplied to the market in high quality. In principle, moist-heat sterilization should be performed at 121.1°C for 15 minutes or, if not feasible, at F_0 value of no less than 8 minutes. If sterilization of pharmaceutical products at F_0 8 minutes is not feasible because of low heat tolerance of the formulation or containers, alternative sterilization conditions should be established by identifying process parameters to ensure a SAL of less than 10^{-6} and referring to requirements stated in this section and Annexes A2 and A3. Sterilization conditions thus established should be demonstrated to be scientifically valid.

11.1.1 Desired Properties of Sterilizing Agents

Sterilizing agents used are saturated steam or moist heat (e.g. vapor-air mixture and hot water) and must not contain any substances that may affect the quality of pharmaceutical products. Bactericidal efficiency of each sterilizing agent should be verified and documented before use.

11.1.2 Sterilization Processes and Equipment

11.1.2.1 Process Specifications

Specifications of sterilization processes should be established. The specifications should at least contain the following information:

- ① Details of operating (autoclaving) cycles
- ② Kinds of process parameters to be monitored and their acceptable limits
- ③ Requirements for preconditioning of pharmaceutical products prior to sterilization, if preconditioning is necessary to secure anticipated effects of the sterilization process
- ④ Locations of reference points of measurement
- ⑤ Highest and lowest temperatures in each empty sterilization chamber (including equipment parts fixed inside the chamber)
- ⑥ Rates and limits of pressure increase and decrease in each step of the sterilization process
- ⑦ Quality of liquids, air, gases, and vapors to be supplied into sterilization chamber
- ⑧ Process parameters to be measured and used for verifying proper operations of the sterilization process
- ⑨ Loading patterns of products in vessels for sterilization
- ⑩ Size and/or mass of sterilization load
- ⑪ Sterility assurance of pharmaceutical products

The SAL for the pharmaceutical product should be assured by any one of the following methods a) to d):

- a) Half cycle method
 - b) Overkill sterilization method
 - c) Combined bioburden-BI method
 - d) Absolute bioburden method
- ⑫ In the case that BIs are used to monitor and evaluate the effectiveness of sterilization, BIs used should comply with performance requirements for BIs specified in ISO 11138-1 and ISO 11138-3.
 - ⑬ In the case that CIs are used to monitor and evaluate the effectiveness of sterilization, CIs used should conform to requirements for CIs specified in ISO 11140 series.
 - ⑭ Policy for establishing sterilization cycles

Generally, the sterilization cycle is established by determining adequate operation

parameters and establishing procedures for handling materials and products to be sterilized based on functional tests. It is also possible to establish the cycle efficiently by utilizing the following information based on reasonable grounds:

- a) Information and data on products, direct containers, packaging materials (for products directly packaged in container for sterilization) and sterilization equipment provided by their manufacturers
 - b) Evaluation results of similarities of the planned sterilization cycle with sterilization processes established for groups of similar products already examined for heat permeability, barrier properties, etc.
- ⑮ Procedures for additional processes (e.g. drying) to be implemented after the sterilization cycle, if required and included in the sterilization process itself.

11.1.2.2 Properties of Sterilization Equipment

1) General requirements

- ① Key properties of sterilization equipment including manufacturer's name, type, size, structure, materials of construction, functions, and capacity should be available in writing. The user manual should also be available, outlining the following information: methods of standard operation, default setting, emergency responses, disassembly/reassembly, maintenance control (including calibration), etc.
- ② Sterilization equipment should be capable of fulfilling basic performance requirements for sterilization, such as sterilization conditions and processing capacity.
- ③ The supply of utilities to sterilization equipment, such as electricity and compressed air, should be constant to ensure consistent operation throughout the sterilization process.
- ④ The system that supports or fixes the sterilization load within the chamber should not interfere with the uniform achievement of sterilization conditions or damage pharmaceutical products and their packages.
- ⑤ Process parameters that may impact sterilization efficiency should be able to be freely set within a range suitable for the control of sterilization processing. These parameters should be easily managed with high reproducibility.
- ⑥ An appropriate control system should be established to enable the sterilization cycle to proceed accurately as programmed. The sterilization equipment should be mounted with appropriate sensors and recorders to enable the measurement and control of critical process parameters to achieve the objective of the sterilization processes. The specifications (e.g. type, precision level, and materials used) and location of the sensors

should be appropriate in view of the characteristics and requirements of the sterilization process. The calibration of particularly important measurement loops should be traceable to official standards.

- ⑦ When vacuum is used during the operating cycle, an appropriate method should be established for the measurement of volume of air leaking into the chamber. Separately, acceptable levels of air leakage should be established.
- ⑧ Sterilization equipment should be equipped with a safety function that controls operation conditions of the equipment within permissible ranges at all times during operation. The equipment should also be equipped with safety devices to prevent major accidents in an emergency.
- ⑨ When non-condensed gas may affect sterilization efficiency and when a device for detecting non-condensed gas is available with sterilization equipment, information of such device should be documented in relevant SOPs or operation manual.
- ⑩ The location where sterilization equipment is installed should have a sufficient space for the operator to work and should have environmental conditions suitable for operations of the equipment and its accessories connected.
- ⑪ Sterilization equipment should be designed and structured to facilitate easy manual operations by the operator, such as manipulation of control buttons and transfer of pharmaceutical products into and out of the chamber.
- ⑫ If the computer system of sterilization equipment is connected to and controlled by a higher-level host computer as an integral part of the manufacturing or other process control, specifications for data input/output, system control, and other processing should be precisely documented.

2) Operational procedures for sterilization equipment and its accessories

Operational procedures for the sterilization equipment and its accessories should include provisions on the following matters:

- a) Detailed explanation on routine operational procedures
- b) Measures to be taken in emergency situations
- c) Procedures for detecting abnormalities of sterilization equipment by, for example, an alarm lamp and buzzer
- d) Procedure for calibration and maintenance of the measurement loop including temperature sensors, pressure sensors, control system, and recording system

11.1.2.3 Utilities

All utilities introduced into the sterilization chamber during the sterilization process should be of high quality not affecting the quality, integrity, and specifications of pharmaceutical products. Further, utilities should be provided at constant predetermined flow rates in order to stably attain the anticipated sterilizing effect. Utility functions should be routinely monitored and controlled.

Temperature and pressure control procedures for steam supplied into the jacket of the sterilization equipment should be established and implemented.

11.1.3 Requirements for Pharmaceutical Products to be Sterilized

- 1) Pharmaceutical products to be sterilized should be predetermined.
- 2) Packaging systems should be specified if pharmaceutical products are sterilized in package form.
- 3) Process challenge devices (PCDs) should be individually defined, if they are used representing a challenge to the sterilization process that is equivalent to or greater than the challenge represented by the product load.
- 4) Procedures for verifying the integrity of the container-closure system before and after the exposure to the sterilization process should be established and implemented.

11.2 Validation of Sterilization Procedures

The following guidance is useful in determining the suitability of the sterilization process: Sterilization Validation Standards for Medical Devices (Notification No. 0330/5 issued by the Compliance and Narcotics Division of the Pharmaceutical and Food Safety Bureau, MHLW on March 30, 2011). If there are essential discrepancies in the method of suitability assessment between the Sterilization Validation Standards in this notification and the current guidance, the Sterilization Validation Standards shall take precedence over the current guidance.

11.2.1 Determination of Requirements

It is critical to clearly identify the requirement specifications for sterilization processing prior to designing or purchasing sterilization equipment. The designing and structuring of the sterilization process as well as the identification of requirement specifications should include the determination or prediction of the function of sterility equipment, limits of capacity of the equipment, differences in characteristics of the product to be sterilized, extent of personnel involvement, environmental conditions, variability inherent to the systems for supervising environmental or sterilization conditions. It is also important to estimate potential impact of these factors on the sterilizing

efficiency of the equipment.

Requirement specifications should be clearly identified for individual pharmaceutical products to be sterilized. The following items, although not exhaustive, are examples of product requirements.

- 1) Product requirements after the sterilization process
- 2) Process requirements based on the manufacturing plan
- 3) Procedures for securing the integrity of the container-closure system during sterilization processing
- 4) Detailed information concerning the final packaging for cases when terminal product sterilization is performed using the product in the direct package

11.2.2 Design Qualification (DQ)

It is necessary to select utilities, component materials, operating principles, and capacity characteristics of the sterilization equipment by confirming that they are suitable for the intended purpose before starting designing of the equipment.

Specifications of the sterilization equipment to be delivered should be confirmed and documented to meet the requirement specifications at appropriate stages of designing or purchasing.

- 1) Prior to design qualification (DQ) evaluation, a set of programs should be prepared to address the issues of timing, implementation method, responsible personnel, judgment criteria, etc. for DQ testing.
- 2) Results of DQ tests should be documented and approved by the responsible supervisor, as required.

11.2.3 Facility Qualification (FQ)

Facility qualification (FQ) is generally divided into two major phases: installation qualification (IQ) and PQ. The manufacturer has an option to decide or establish groups of qualification parameters, order of testing parameters, and timing (steps of qualification) of testing; however, any parameters that may affect or influence test results or interpretation of test results of other parameters should be tested prior to the testing of such parameters.

- 1) Installation qualification (IQ)

At the time of installing new sterilization equipment, the equipment should be verified through IQ that it complies with the manufacturer's specifications and has been properly installed at the predetermined location as directed under manufacturer's inspection procedure manual/reports, installation instruction manual/reports, and design drawings. The following items are examples of common IQ tests for sterilization equipment.

Instead, the verification of proper installment may be performed at the site of installation by checking part of manufacturer's records of release testing and on-site work records, if there are reasonable grounds, for example, a range of manuals and reports have been approved by an inhouse qualification certification organization.

- Installation test
- Appearance test and dimension measurement
- Pipeline inspection
- Inspection of materials of equipment components (e.g. sterilization chamber, sanitary pipes, and gasket) that may affect the basic performance of the sterilization equipment
- Welding inspection of pipes for dispensing sterilization media
- Gradient inspection of pipes (e.g. sterilization media and drainage pipes) that may affect basic performance of the sterilization equipment
- Inspection of the chamber and pipes for air tightness
- Inspection of utility connection and supply of liquid, air, or gas to the sterilization equipment
- Instrument loop check including general electrical tests
- Calibration of critical instruments and devices

2) Operational qualification (OQ)

At the time of initiating operation of the new sterilization equipment, the equipment should be verified through OQ that it complies with manufacturer's functional specifications and creates uniform temperature distribution within the equipment in the unloaded condition in a temperature mapping test. The following items are examples of common OQ tests for sterilization equipment.

- Movement inspection of individual parts of the equipment (e.g. door and carrier device)
- Verification of uniform temperature distribution within the sterilization chamber in the unloaded condition
- Verification of software programs including corrective processing sequences
- Chamber leakage test using a software program installed in the sterilization equipment
- Verification of optimal operations in connection with other equipment

In addition to the above information, data on time to attain the targeted degree of

vacuum and other operational data are recommended to be obtained to form the basis of the validation maintenance program.

- ① Prior to the assessment of OQ, the sterilization equipment should be repeatedly run on a trial basis to ensure operational adjustment, and key operational parameters for equipment operation should be identified, established, and documented.
- ② Actions or activities against probable abnormal or erroneous operations should be verified to the extent possible. In particular, corrective measures against an abnormal sterilization temperature should be carefully tested by taking into account the applicable product sterility assurance method.
- ③ The number and location of temperature distribution measurement points should be determined according to the size and shape of the sterilization chamber. In general, the temperature should be measured with thermometers (e.g. thermocouples temporarily affixed to supporting grids adjusted to the inside surface of the chamber).
- ④ Thermometers for the measurement of temperature distribution should be calibrated before and after OQ testing.

11.2.4 Functional Tests

Prior to testing heat permeability, functional tests of the sterilization process should be performed to determine or establish operating conditions and control parameters as well as procedures for handling by the type and loading pattern of individual materials or products to be sterilized. All of these conditions need to be established as highly reliable and reproducible in commercial scale manufacturing.

- 1) The following matters should be evaluated as general requirements for functional tests.
 - Predetermined values of the sterilization cycle, the type of process parameters to be monitored, and their values with acceptable limits;
 - acceptable limits of process parameters should be rationally established by taking into account measurement errors inherent to the particular equipment used and the behavioral characteristics of individual process parameters.
- ① The type and loaded amount (or weight), locations and arrangement within the chamber, and means of support of materials or products to be sterilized
- ② The procedure, if required, for maintaining the sterile condition of the pharmaceutical product itself and/or its primary package after exposure to the sterilization process

- 2) In addition to the matters listed in 1) above, the following matters should be determined for each loading pattern as the specific requirements for the moist-heat sterilization process.
 - ① Maximum and minimum temperatures and their locations within the chamber as well as temperature and pressure profiles in each phase of the sterilization cycle
 - ② Maximum and minimum temperatures and their locations within the pharmaceutical product as well as temperature profile in each phase of the sterilization cycle
 - ③ Identification of cold spot locations in the unit product and entire loading pattern based on the data obtained in ② above. The locations may be identified by determining the lethality factor converted to F_0 value, as required.
 - ④ Detailed information concerning the reference load used to verify the efficiency of the sterilization process
 - ⑤ Information concerning instruments used to monitor the sterilization process and the method for evaluating measurement values
 - ⑥ The level of sterility to be achieved at specified locations when biological monitoring techniques are used to determine the efficiency of the sterilization process
 - ⑦ The method for using product temperature sensors if the sensors are used for the control or management of the sterilization process
- 3) Sterilization conditions should be established by taking into account the heat resistance levels of the pharmaceutical product and container-closure system.

11.2.5 Performance Qualification (PQ)

The PQ of high-pressure steam sterilization equipment comprises the evaluation of heat permeability associated with sterilization load, heat distribution within the sterilization chamber, and the verification of the level of sterilization achieved using BIs. It is recommended that these parameters are simultaneously determined during a single test operation.

- 1) The heat permeability test should in principle be performed with commercial products. The test may be performed using a reference load, except for samples for temperature measurement, provided that the use of the reference load is determined to be

scientifically reasonable based on physical data derived from the reference load.

- 2) The heat permeability test should be performed at least three times for each maximum loading pattern. The test at the minimum loading pattern may be performed where appropriate. Figures and photographs illustrating the loading pattern employed in the test should be retained for record purposes.
- 3) The heat permeability test may be performed for groups of products or loading patterns categorized according to the type and characteristics of the material or pharmaceutical product to be sterilized or batch size for sterilization.
- 4) Thermometers for verification purposes should be placed at cold spots within the pharmaceutical product.
- 5) Predetermined conditions of sterility at cold spots should be confirmed to be satisfactorily met by using thermometers.
- 6) Complete sterilization at cold spots should be verified by using BIs.
- 7) When the sterilization cycle is to be established based on bioburden of the pharmaceutical product, the count, resistance, and assessment method of BIs should be determined by taking into account the predicted or established bioburden levels.
- 8) When sterility test is performed to establish the sterilization process, the testing should be in accordance with the Sterility Test of the JP.
- 9) The sterilization cycle established should be verified to be reliable in securing the integrity of the pharmaceutical product including its container-closure system to be sterilized.
- 10) The time of the sterilization cycle should be verified to be within the exposure time limits permissible under the commercial manufacturing time schedule.
- 11) Thermometers used for temperature distribution measurement should be calibrated before and after testing.

11.3 Monitoring and Control of Routine Operating Cycles

Routine monitoring and control of the sterilization process are essential to continuously ensure that the process is being maintained in a validated condition throughout manufacturing processing. Basic principles, general requirements, and procedures for routine monitoring and control should be consistent with those specified in Chapter 3. In addition, the following matters should be noted as requirements specific to the high-pressure steam sterilization process. Monitoring of raw materials and sterilization equipment should be included in the manufacturing control

policy.

11.3.1 General Requirements for Monitoring and Control

- 1) Utilities including steam, water, and air should be verified to be supplied in compliance with the respective specifications.
- 2) The circulation pump, boiler, and other mechanical components necessary for the moist-heat sterilization process should be verified to remain in a state of control.
- 3) Pipes should be verified to be free of leakage.
- 4) Accessories of the sterilization equipment should be verified to be accurately calibrated and the “use by” date confirmed to remain valid before use.

11.3.2 Operating Procedures for Monitoring and Control

- 1) Data evidencing the achievement of required process parameters should be documented. Recorded data should include readings of the inner pressure and temperature of the sterilization chamber in each sterilization cycle. It is recommended that data useful for trend analysis of sterilization efficiency of equipment and sterilization process (e.g. time to reach vacuum and heatup time) be simultaneously record.
- 2) Variables established as process parameters should be measured and recorded by suitable direct methods to verify that the sterilization process has been completed within the specified limits. If necessary, BIs and CIs should be included in these measurements.
- 3) Profiles of sterilization temperature and pressure should be checked over the sterilization cycle. A leakage test should be periodically performed when the sterilization process incorporates an air elimination process for steam penetration. Any additional performance checks other than sterilization (e.g. dryness), if required in the sterilization process, should be evaluated and recorded according to written procedures.

11.4 Product Release

- 1) Appropriate product release procedures should be established, and the procedures should include requirements for the judgment that the final product has satisfactorily passed through the sterilization process.
- 2) An appropriate system should be established for clearly differentiating materials and products sterilized and those remaining unsterilized.

11.5 Continued Process Verification

The sterilization process must be maintained in a status proven by qualification assessments as long the pharmaceutical product is being placed on the market.

- 1) The qualification should be reevaluated in accordance with predetermined procedures at periodic intervals.
- 2) Any changes to the sterilization process require process qualification following the assessment of potential effects of the changes on product quality, as directed by relevant change control procedures.

Factors to be considered in changing the sterilization process should include the following:

- ① Parts replacement that may cause changes in process parameters
- ② Parts replacement that may induce leakage of fluid or air into the sterilization chamber
- ③ Alterations in load within the sterilization chamber
- ④ Changes to process control procedures and changes or replacement of computer software and hardware associated with the equipment
- ⑤ Changes to packaging systems and/or packaging procedures, materials and components of pharmaceutical product, origin of raw materials, and design of equipment

11.6 Maintenance and Control

The maintenance and control of facilities, utilities, and manufacturing equipment is a critical aspect of verifying the manufacturing processes being appropriately controlled. The status determined by the qualification assessments must be maintained through daily monitoring, maintenance, and calibration.

- 1) Results of qualification assessments of facilities and manufacturing equipment should be periodically evaluated to decide whether or not the assessments should be conducted again.
- 2) Procedures for maintenance and control should be documented and implemented.
- 3) Records of calibration and maintenance should be retained and periodically reviewed by designated personnel.
- 4) Measuring devices for controlling, guiding, and recording the sterilization process should be periodically calibrated.

12. Radiation Sterilization (Gamma-Ray and Electron Beam Irradiation)

Radiation sterilization refers to the irradiation of materials and products with gamma rays emitted from cobalt-60 or cesium-137 radioisotopic source and electron beams emitted from electron accelerator. Currently, there are no international radiation sterilization standards for pharmaceuticals; however, international standards for the radiation sterilization of healthcare products (ISO 11137) were issued in 1994 and revised in 2006. The standards were translated into Japanese (JIS T0806s) and published in 2010. The following basic requirements for management, validation, and maintenance/control for radiation sterilization have been established based on this JIS standard.

12.1 Type of Radiation Sources

The type of radiation sources should be specified for use in terminal sterilization of pharmaceutical products. Gamma rays from cobalt-60 or cesium-137 radioisotopes are not sufficiently potent to radioactivate materials (or pharmaceutical products). If electron beam is irradiated at 10 MeV or higher, pharmaceutical products as well as their packaging materials should be inspected to determine whether the products and materials irradiated have been radioactivated or not.

12.2 Facility and Equipment

Data of radiation equipment and its operation procedures should be available in writing. The documents prepared should include the following information and be appropriately retained in the facility.

- ① Drawings of radiation equipment, indicating the location of the equipment as well
- ② Procedures for classifying and storing radiated and non-radiated materials and products
- ③ Structure, operation procedures, and maintenance procedures of radiation equipment and conveyers
- ④ Routes and speed of conveyors
- ⑤ Dimensions, materials, and structure of radiation exposure vessels
- ⑥ Type, quantity, and location of radioisotopes used (for gamma radiation sterilization)
- ⑦ Beam characteristics (e.g. electron energy, beam current, and scan width) (for electron beam sterilization)
- ⑧ Location of beam sources and method of displaying beam generation
- ⑨ Matters concerning the storage of radiation sources, blockage of radiation beams, and

stoppage of conveyors in case of system failure

Computer software systems employed for process control and monitoring should be validated to function as originally designed prior to use, and outcome of the validation should be maintained in writing.

12.3 Definition of Radiation Sterilization

12.3.1 Maximum Permissible Dose

The maximum permissible dose (MPD) should be established for the sterilization of pharmaceutical products and their packaging materials. The pharmaceutical product tends to show an increase in impurities and development of new molecules due to free radicals formed, quality deterioration of packaging components associated with structural changes, and coloration of the product itself due to time or other causes. Radiation sterilization even at the MPD should be ensured so as not to impair therapeutic efficacies of the product prior to its expiration date.

12.3.2 Sterilization Dose

The dose of radiation sterilization should be the dose required to achieve the sterility assurance level (SAL) of 10^{-6} .

12.4 Method of Sterilization Dose Selection

High dose radiation may cause quality deterioration of pharmaceuticals. It is essential to achieve a SAL of 10^{-6} at the lowest possible radiation sterilization dose, and for the determination of the dose, absolute bioburden method is used. JIS T0806-2 (ISO 11137-2) specifies systematic approaches for determining the radiation dose, as outlined below:

Medical devices are usually sterilized in a dry state; however, the proposed dose determination procedures are applicable to products in liquid form, such as dialyzers, and vacuum blood collection needles.

- 1) Method based on bioburden data (Method 1)

Method 1 is used to verify that the radiation resistance of the bioburden on the pharmaceutical product is equal to or less than the resistance of a microbial population having the standard distribution of resistance.

- 2) Method based on fraction-positive information from incremental dosing in sterility test (Method 2)

In Method 2, a dose incremental test is performed to estimate the D value in Step 1, and the dose at which one in one hundred product units is expected to be non-sterile (i.e. SAL of 10^{-2}) is obtained in Step 2. The estimated D value is extrapolated for SAL below 10^{-2} and the sterilization dose is determined.

3) Method based on the standard distribution of radiation resistance (VD_{\max} method)

The DV_{\max} method is used to verify that the radiation resistance of the product bioburden at the selected minimum sterilization dose is less than that of a microbial population having largest resistance to radiation to give a SAL of 10^{-6} .

12.5 Validation

12.5.1 Installation Qualification (IQ)

The radiation equipment should be confirmed to be installed as directed by the approved design specifications, and the outcome of the confirmation recorded in writing. Written procedures for the confirmation should be available in advance of the installation. The vendor for radiation sterilization is responsible for IQ testing in the case that radiation sterilization is contracted out.

12.5.2 Operational Qualification (OQ)

With respect to the radiation equipment, instruments used for measurement, supervision, control, display, and record of radiation should be calibrated after the completion of IQ. Radiation measurement equipment used should be traceable to international or national reference standards.

In the initial step of OQ testing, the compliance of the equipment with design specifications should be confirmed in a cold run experiment in which radioactive sources are not loaded onto or beams are not generated from the equipment, and the outcome of the confirmation should be documented. In the second step, the equipment is tested whether or not it is capable of irradiating doses as anticipated by design specifications in a hot run experiment in which radioactive sources are loaded onto or beams are generated from the equipment, and the outcome of the confirmation should be documented. In the hot run experiment, radiation-dose mapping should be studied using heaviest dummy set in the design specifications to verify the compliance of processing capacity, penetrating ability of beams, etc. to the specifications. Procedures for assessing the equipment of its compliance with the specification should be available in writing in advance. The vendor for radiation sterilization is responsible for OQ testing in the case that radiation sterilization is contracted out.

12.5.3 Performance Qualification (PQ)

With respect to the radiation equipment, PQ testing should be performed using commercial pharmaceutical products or their dummy to confirm, following the completion of OQ, if terminal sterilization is feasible or not. The objective of PQ is to study radiation-dose mapping to confirm if the radiation is possible at a dose higher than the sterilizing dose and lower than the MPD by using the radiation equipment concerned. The manufacturer is to cooperate with the vendor for radiation sterilization in executing PQ testing in the case that radiation sterilization is contracted out. Results of PQ testing should contain the following information as well as information on the pharmaceutical product, and the results reviewed by the supervisor.

- ① Load size and load density of boxes for sterilization and positioning of the pharmaceutical product in the boxes
- ② Loading configuration of boxes for sterilization in the radiation exposure vessel
- ③ Facilities or conveyer routes used (if multiple routes are available)
- ④ Maximum and minimum radiation doses, locations of radiation sources, and ranges of the doses in dose mapping study
- ⑤ The MPD and sterilization dose for the pharmaceutical product
- ⑥ Product temperature control and time until irradiation completion (if required)
- ⑦ Locations of radiation dose audit, correlation between the doses delivered at the audit points and minimum/maximum doses, and by using these radiation data acceptable limits of doses delivered at the audit points to be established
- ⑧ Adequacy of transportation systems confirmed before and after transportation (in the case that radiation sterilization is contracted out)

In the case that different pharmaceutical products can be regarded as the same products based on dose mapping data (for example, products of different APIs but packaged in boxes for sterilization of the same size and weight), dose mapping data from a pharmaceutical product can be applied to other pharmaceutical product undergoing the same radiation sterilization procedures (“same treatment category”) whenever the rationale for the application is clear.

12.6 Monitoring and Control of Routine Operating Cycles

Irradiation procedures as well as storage conditions of pharmaceutical products before and after irradiation should be specified and be available in writing including the following matters. Radiation sterilization does not require the use of BIs, and the use of CIs is optional.

- ① Appropriate product identification procedures for tracing the sterility status of sterilized products (e.g. sterilization batch numbers)

- ② Procedures checking the quantity of products before and after radiation sterilization
- ③ Procedures for classifying and storing radiated and non-radiated products
- ④ Loading pattern of radiated products in radiation exposure vessels
- ⑤ Locations of radiation-dose audit and application frequency of radiation measurement equipment
- ⑥ Procedures for adjusting conveyer speed according to radioactive decay (for gamma ray sterilization)
- ⑦ Monitoring and recording of the location of radiation sources, conveyer speed, and movement of radiation exposure vessels (for gamma ray sterilization)
- ⑧ Electron beam characteristics and monitoring and recording of conveyer speed (for electron beam sterilization)
- ⑨ Corrective actions for and recording of the interruption and failure of radiation sterilization process, if occurred
- ⑩ Product-temperature control and time until irradiation completion (if required)

12.7 Dosimetric Release

Dosimetric release is defined as the release of product lots by identifying parameters to confirm that all aspects of sterilization are within specifications. The procedures for dosimetric release should be available in writing. Dosimetric parameters should include parameters tested for PQ. When these parameters meet specified values and the radiation dose at the radiation audit location is within specified limits, radiated product lots can be judged to be sterile and therefore the product may be released without sterility testing. As preconditions for this product release procedure, the quality control system must be verified as effectively functioning and that the radiation dose is optimal in sterilizing the pharmaceutical product to control bioburden level within predetermined limits.

12.8 Efficacy Evaluation

12.8.1 Effectiveness of the Sterilization Dose

The procedures and timing for verifying the continued effectiveness of the initially determined sterilization dose should be available in writing. The dose can be judged to be effective if the bioburden and its resistance to the sterilization are equal to or below the respective predetermined levels at a certain designated time after radiation. The efficacy-evaluation method for the radiation dose is specified in the JIS T0806-2 (ISO11137-2), as outlined below.

- 1) Bioburden determinations should be performed every month in the case of products of

low bioburden levels (less than 1.5 counts/product on average) and at least every three months for products of higher bioburden levels.

- 2) The sterilization dose should be promptly audited (radiation resistance test of the bioburden) if the bioburden exceeds the limit specified for the selected sterilization dose.
- 3) Routine sterilization-dose audit should be performed every three months.
- 4) The auditing interval of the sterilization dose may be extended to maximally 12 months if the bioburden levels are within limits for the determinations at every 3 months, if microbial characteristics of bioburden (identification is not required for cell morphology, colony morphology, and staining properties) remain essentially unaffected, and if four consecutive audits provide no major noncompliance issues.

12.8.2 Effectiveness of the Equipment

- 1) The recalibration of instruments used for supervision, control, display, and recording of the sterilization process should be planned, implemented, and recorded.
- 2) The preventative maintenance of equipment should be planned, implemented, and recorded.
- 3) Any changes to the radiation equipment that may affect radiation dose and radiation-dose mapping should be evaluated for their extent and magnitude of influence prior to implementation and approved by the supervisor. IQ, OQ, and/or PQ tests should be repeated in part or in entirety, as appropriate.

13. Bioburden Test

With any type of terminal sterilization procedures, it is essential to verify that the method is effective in achieving a SAL of less than 10^{-6} . The count, type, and properties of microorganisms are needed to demonstrate the scientific rationale for sterilizing conditions employed. This section describes the tests for determining the bioburden on the product before sterilization.

13.1 Bioburden Monitoring

13.1.1 Monitoring Frequency

Bioburden of the product before sterilization should be monitored at predetermined intervals.

13.1.2 Sampling

Bioburden samples should be collected from final-product containers. The timing of

sampling should be selected to account for representative routine manufacturing conditions of product lots (e.g. at beginning, middle, and completion of filling) based on the worst-case scenario. Sample amounts should be decided by assessing potential contamination risks by taking into account relevant factors such as historical data of bioburden, manufacturing process, batch size, frequency of production, materials used, and variation in bioburden. Storage conditions for bioburden samples until assay should be approximated as closely as possible to the commercial manufacturing process.

13.2 Bioburden Test

It is important to collect accurate information concerning the count, type, and properties of bioburden microorganisms in bioburden determination, and, for the assurance of sterility, it is also essential to confirm the absence or presence of heat-resistant microorganisms and, if present, the level of heat resistance should be determined. As the practical procedure for these assessments, either the Microbial Limit Test (Section 13.2.1) or Heat Resistance Test (13.2.2) should be selected and performed depending on the level of contamination risk. Both the Identification of Microorganisms (Section 13.2.3) and Sterilization Resistance Test (Section 13.2.4) should be performed with the isolates of microorganisms detected.

13.2.1 Microbial Limit Test

The Microbial Limit Test should be performed by taking into account an approximate time range from sample collection to sterilization processing of the pharmaceutical product in the entire manufacturing process. The final container-closure system should be tested with the sample amount determined in Section 13.1.2. In the test, the whole volume of the collected sample in specified unit should be taken and passed through a membrane filter under aseptic conditions. If the whole volume of the sample cannot be used or if the membrane-filter method is not feasible, reasons for the inabilities should be clearly stated and another appropriate method should be selected. Incubation conditions should conform to those specified in the Enumeration Methods of the Microbial Limit Test, JP.

13.2.2 Heat Resistance Test

Heat resistance test is a test for screening heat-resistant bacteria (spore-forming) on pharmaceutical products before sterilization and should be performed as the situation may require. The final container-closure system should be tested with the amount determined in Section 13.1.2. In the test, whole volume of the collected sample in specified unit should be taken, heat treated in a hot-water bath at 80°C to 100°C for 10 to 15 minutes, and passed through a membrane filter under

aseptic conditions. If the whole volume of the sample cannot be used or the membrane-filter method is not feasible, reasons for the inabilities should be clearly stated and another appropriate method should be selected. Incubation conditions should conform to those specified in the Enumeration Methods of the Microbial Limit Test, JP.

13.2.3 Identification of Bacterial Species

Bacterial species detected by the Microbial Limit Test or Heat Resistance Test should be identified. Almost all microorganisms resistant to the sterilization process are spore forming. It is critical to accurately identify spore-forming microorganisms. Currently available identification tests include phenotypic identification test, identification test using commercial kits, and identification tests based on molecular structure or gene information (e.g. chemical classification and genetic analyses). At least the family of the bacteria should be identified and its properties examined. Data obtained from the identification tests should be utilized for the conduct of sterilization resistance test, projection of contamination routes, and control of bioburden.

13.2.4 Sterilization Resistance Test

Bacterial spores to be tested should be seeded onto suitable spore-forming medium for growth when bioburden microorganisms identified are spore-forming microorganisms, when heat resistance test has detected heat-resistant microorganisms, or when the Microbial Limit Test detected spore-forming microorganisms that are resistant to heat treatment. Stock suspensions of test spores should be prepared, and *D* value (*z* value, as needed) obtained as an indicator of resistance of microorganisms to the sterilization process in the pharmaceutical product. *D* value should be determined at a product sterilization temperature according to procedures specified in ISO 11138. A solution of microorganisms showing a higher *D* value than the product may be used in the determination of *D* value, if known to be available prior to the test.

If *D*-value determination is difficult, the reason for the difficulty should be clarified, and a suspension of viable cells at a count of more than 10^6 /product should be prepared, heated for half of the sterilization time required to inactivate all of 10^6 counts of microorganisms (on the soybean-casein digest agar medium), and verified to be negative (to achieve 12-log reduction [*12D*] over the predetermined sterilization time) by the Sterility Test, "Membrane Filtration," of the JP to ensure a SAL of 10^{-6} .

13.3 Bioburden Acceptance Criteria

Bioburden acceptance criteria including bioburden count and sterilization resistance (*D* value) should be established for individual pharmaceutical products. The criteria should be dependent on

sterilization conditions to be employed, suitable for achieving a SAL of 10^{-6} , and are established by taking into account the safety rate and bioburden count estimated based on D value of the indicator microorganism set at establishing sterilization conditions. The safety rate should include microbial recovery rate, etc. determined in test method validation. It is also necessary to establish the alert level and action level for the bioburden acceptance criteria.

13.4 Measures to be Taken against Deviations in Bioburden Acceptance Criteria

When bioburden count has deviated from the acceptance criteria in moist-heat sterilization, the count should be assessed by reviewing results of sterilization resistance test together to confirm if the count satisfies the SAL of 10^{-6} (e.g. $\log [\text{bioburden count}] + 6 < \text{sterilization time}/D$ value). In addition, the investigation is necessary for the identification of routes of bioburden contamination and the potential for the presence of bioburden in the product in a larger quantity than that estimated by the bioburden test using samples.

In the case that the D value of bioburden microorganisms is higher than that of the indicator microorganism, complete sterilization at cold spots in the moist-heat sterilization equipment should be verified by using bioburden microorganisms as the indicator microorganism, as directed in Section 11.2.5. Results of the test should be utilized to renew the indicator microorganism. For the renewal, the indicator microorganism should be identified to the species level and preferably deposit the microorganism in a microbial culture collection institute. Alternatively, a microorganism with a higher D value obtainable from a depository center may be designated as the indicator microorganism. If bioburden level can be lowered by practicing applicable techniques to ensure complete elimination of bioburden microorganisms with D value higher than that of the indicator microorganism, the original indicator microorganism may be used as done previously.

13.5 Reflection of Bioburden Test Results to Product Release

If the bioburden determination, except for the test by overkill method, performed before product release has detected spore-forming microorganisms, such microorganisms should be tested and confirmed by a suitable test such as heat resistance test to have lower sterilization resistance as compared to the indicator microorganism used in validation test. If the pharmaceutical product has been found to deviate from the bioburden acceptance criteria, the decision on product release should be made upon reviewing the outcomes of actions taken in accordance with Section 13.4.

Annex

The information contained in this Annex is provided as a supplement to the Guidance on the Manufacture of Sterile Pharmaceutical products by Terminal Sterilization. In cases where A1: Parametric Release is applied, prior approval by the regulatory agency is necessary. A2: Sterility Assurance of Large Volume Preparations Including Parental Infusions is the sterilization method applied to terminally sterile products for which containers and/or formulations are not resistant to heat treatment and not applied to any pharmaceuticals that are resistant to heat treatment.

A1. Parametric Release

The objectives of this section are to provide the general requirements for parametric release of sterile pharmaceutical products processed by terminal sterilization procedures and to present practical requirements for parametric release by moist-heat sterilization. As a rule, concepts represented by such requirements shall be applied to the control of parametric release following moist-heat sterilization. However, it is strongly recommended that the application of parametric release to terminally sterile products should be made after receiving the full approval and continued consultation with the relevant authority.

A1.1 General Requirements for Parametric Release

Sterile pharmaceutical products processed by terminal sterilization procedures are required to achieve a sterility assurance level (SAL) of less than 10^{-6} . The SAL 10^{-6} is the minimum requirement for sterility assurance and, whenever feasible, it is recommended to employ the SAL of 10^{-8} in the validation of sterilization of liquid products. The detection level of microbiological contamination determined by the medial fill test is approximately SAL 10^{-3} and by the sterility test is approximately SAL 10^{-1} . The sterility of products can only be ensured by a validated and comprehensively and consistently-controlled terminal sterilization process, which provides a greater level of sterility than that achieved by the Sterility Test. Thus, it could be considered a scientifically valid rationale to apply the parametric release to sterile pharmaceutical products that have a high level of SAL.

When parametric release is applied to a terminally sterile product, the constant sterility of the pharmaceutical product will be maintained by understanding, implementing, and complying with the requirements set forth in this guidance.

A1.2 Sterilization Methods Controlled by Parametric Release

The appropriate use of a sterilization method controlled by parametric release is dependent on

the compliance with all of the following conditions:

- 1) The mechanism of sterilization has been fully clarified.
- 2) Key parameters for physical control of the sterilization process are established and are measurable for analysis.
- 3) The sterilization process can be validated using appropriate biological indicators (BIs).
- 4) Sterilization processing can be performed in an efficient and reproducible manner.

A1.3 Validation of Sterility Assurance

- 1) An appropriate parameter or parameters that scientifically satisfy the SAL requirement of less than 10^{-6} should be selected based on appropriate validation testing.
- 2) Acceptable process parameter limits for any deviation and/or conditions of correction should be established and, if possible, validated under the assumption that temporary deviations in any of the sterilization conditions may occur during the sterilization cycle due to specific causes such as power failure.
- 3) Sterilization equipment should have an expiration date for each sterilization load and type and be periodically revalidated for sterility assurance. Adopted sterility parameters should also be retested to verify their validity, as appropriate.
- 4) When change control is required, any change that may affect the assurance of product sterility needs to be validated prior to implementation and verified to be capable of achieving a SAL of less than 10^{-6} after implementation. Changes that may affect sterility assurance include, but are not limited to, the composition/volume of the item to be sterilized, container closure system/packaging form of pharmaceuticals, manufacturing process, loading pattern, sterilizer supply conditions, and structure of sterilization equipment.

A1.4 Monitoring and Control of Routine Operating Cycles

A1.4.1 General Requirements for Monitoring and Control

- 1) In handling materials and products to be sterilized, appropriate measures should be initiated to prevent the mix-up of sterilized items with unsterilized items.
- 2) Appropriate measures should be initiated to prevent the contamination of sterilized pharmaceutical products.
- 3) All procedures and control parameters related to sterilization (e.g. process control;

maintenance; supply of gas, air, and water; and verification of sterilization) should be documented appropriately.

- 4) Sterilization procedures should be established, documented appropriately, and implemented based on the results of validation conducted to define conditions of the terminal sterilization process. The SOPs should include, but are not limited to, the following items:
 - ① Process parameters, control parameters, and acceptable limits of the parameters necessary for the routine control of sterilization
 - ② Method and criteria of assessment to determine if the sterilization process meets the necessary requirements
 - ③ Procedures for the recording and storage of terminal sterilization-related records
 - ④ Measures necessary for the correction of deviations
 - ⑤ Loading pattern for each product item in the case that the sterilization equipment is batch-type
- 5) Test items, together with detailed procedures and the frequency, should be established and documented for the periodic revalidation, maintenance, calibration, and functional testing of sterilization equipment.
- 6) Proper procedures should be established and documented for the detection of microorganisms resistant to bioburden testing as well as the sterilization process itself.
- 7) Proper procedures should be established and documented for treating microorganisms found to be resistant to the employed sterilization procedure.
- 8) When a reference load is used for process confirmation, the acceptability of the rationale regarding specifications, effectiveness, and methods of use should be evaluated, verified and documented.

A1.4.2 Procedures for Monitoring and Control

- 1) Monitoring and control of routine operating cycles should be performed on each operating cycle of sterilization according to predetermined procedures.
- 2) All sterilization data should be recorded to verify that the whole sterilization process has been conducted within the specified limits of acceptance. The following records should be reviewed and approved by the supervisor:
 - ① Date of sterilization and processing time from start to finish
 - ② Sterilization equipment used

- ③ Sterilization conditions employed
 - ④ Physical records of sterilization processing
 - ⑤ Assessment criteria for sterilization processes and assessment results
 - ⑥ Identification of sterilized items
 - ⑦ Name of personnel responsible for sterilization processing
- 3) Appropriate corrective measures should be implemented according to established procedures if deviations occur in any of established sterilization processes, alert levels, action levels, or specified limits of parameters.
 - 4) Operations conducted in association with the maintenance and control of the sterilization process and process-support systems should be accurately recorded and managed.
 - 5) Equipment used for the control, measurement, and recording of key parameters of the sterilization cycle should be designated as equipment requiring routine calibration. The frequency and permissible limits of calibration should be established, and calibration conducted accordingly using calibration standards consistent with the relevant public standards. Similarly, calibration should be conducted for control and measurement devices supporting the sterilization process.
 - 6) Storage conditions should not impair the quality of sterilized pharmaceutical products. Location, method, environment, and intended period of storage should be predetermined and controlled accordingly.

A1.4.3 Control of Sterilized Product Release

The sterility of pharmaceutical products processed by terminal sterilization procedures needs to be assured by the inspection of records of sterilization parameters. Therefore, sterilization parameters should be predetermined, the sterilization process confirmed to be sufficiently efficient to satisfy the specified limits, and then the procedure for product release established and documented.

Procedures for assuring sterility in parametric release-based release control should include the following:

- 1) Manufacturing batch records
- 2) Sterilization records should be reviewed and confirmed to ensure that key parameters (e.g., time, temperature, and pressure) are within permissible limits.
- 3) Sterilization records or manufacturing batch records should be reviewed and confirmed to ensure that sterilization processing is conducted by employing the predetermined product loading pattern.

- 4) Confirmation should be obtained that the equipment used for the control, measurement, and recording of key sterilization parameters has been calibrated and that the calibration period has not expired.
- 5) The predetermined revalidation period of the process program should not have yet expired.
- 6) Countermeasures taken for correcting deviations from predetermined procedures, alert levels, action levels, or specified limits of parameters should be reviewed, and outcomes of the countermeasures be acceptable.
- 7) Bioburden data of raw materials and pharmaceutical solutions should be inspected and assessed, as required.
- 8) A bioburden test should be performed for each batch of the product before sterilization.
- 9) Records of microbiological inspection of the manufacturing environment should be reviewed and assessed, as required.
- 10) It should be confirmed that microbial tests are performed with an objective to detect microorganisms resistant to the sterilization procedure concerned, as required.
- 11) In the case that bioburden testing of the pharmaceutical product before sterilization has detected microorganisms more resistant than the sterilization indicator employed in sterilization validation, the *D* value should be obtained and determined according to predefined procedures if it falls within permissible limits.
- 12) In the case that the product-release decision is based on data from the reference load, the data should be within the specified limits. In addition, relevant records should confirm that the configuration and alignment of the reference load conform to the specifications of the test.
- 13) If any key sterilization parameters deviate from predetermined limits, appropriate countermeasures (e.g. re-sterilization and/or destruction of the batch in question) as well as clarification of the reasons for the deviation should be implemented according to established procedures. Once the deviation has occurred, product release by performing an alternative method (e.g. sterility test) is not allowed.

A1.5 Parametric Release by Moist-Heat Sterilization

A1.5.1 Conditions for the Application of Parametric Release

When parametric release by moist-heat sterilization is applied to terminally sterilized products, bioburden test of the products should be performed after the filling and sealing processes but before the sterilization process.

A1.5.2 Points to Consider in Applying Parametric Release to Terminally Sterile Products

The following factors should be taken into account for moist-heat sterilization when parametric release is applied to terminally sterile products.

- 1) Factors to consider when establishing sterilization conditions as process requirements
There are certain process control parameters that may affect sterilization efficiency and sterility assurance such as temperature, pressure, time, heat history, and bioburden in the product before sterilization. As long as the control of these parameters is consistently maintained, the employment of parametric release may be considered. The methods for designing (establishing) the sterilization process include overkill and F_0 control methods. When the F_0 control method is employed, the validity of the established F_0 should be verified using appropriate predetermined parameters.
- 2) Considerations for the validation of sterilization
 - ① In the preparation of sterilization validation, due reference should be made to applicable sections of the Sterility Assurance of Terminally Sterilized Pharmaceutical products, References, JP and the Procedures for Sterilization Validation in this guideline, and the parameters that are able to ensure a SAL of less than 10^{-6} should be determined *via* appropriate validation.
 - ② On the assumption that sterilization temperature may be decreased due to power failure or other reasons during the sterilization cycle, minimum acceptable changes in sterilization conditions (e.g. temperature range and duration of change) and conditions of reheating should be validated in advance, wherever possible.
- 3) Considerations for monitoring and control
 - ① Key operational parameters that may influence the sterilization cycle (e.g. temperature, pressure, and duration) should be identified and their permissible ranges determined and documented.
 - ② Chamber leakage testing should be periodically performed to determine the quantity of leaks, if any, from the chamber.
 - ③ Procedures for the maintenance and control of the sterilization process and its support systems (e.g. pressured air, steam, and water) should be tested, recorded, and managed.
 - ④ In the case of sterilization temperature being temporarily decreased due to power failure or other reasons during the sterilization cycle, the cycle may be continued

only if the chamber is reheated under prevalidated conditions.

4) Considerations for release control of sterilized products

The parametric release-based control of the sterilized pharmaceutical product should include the following information:

- ① A microbial test to detect moist-heat-resistant microorganisms should be conducted, as required. For detail, refer to Section 13. Bioburden Test.
- ② If bioburden testing detects moist-heat-resistant microorganisms at a level higher than that verified to be an acceptable germicidal indicator in sterilization validation, the *D* value of the microorganisms should be obtained to judge whether it falls within permissible limits, and appropriate control procedures should be established for the handling of pharmaceutical products (e.g. rejection of product release and recall of products).

A2. Sterility Assurance of Large Volume Parenterals Including Transfusions

A2.1 Introduction

Pathological conditions and other challenging situations often occur in patients: fluid and electrolyte disturbances, reduced or restricted intake of nutrients essential for maintaining normal bodily functions, surgical insults such as operations, and the application of medical devices. These conditions and situations are often corrected by the use of large volume parenteral solutions including transfusions that are basically formulated with intra- and extracellular body fluids and other body components. As bodily fluids consist of various components, it is impractical to formulate, fill, seal, and supply sterile solutions to meet therapeutic needs on a case-by-case basis in clinical practice. Therefore, various large volume parenteral solutions containing electrolytes, carbohydrates, and amino acids are commercial available as pharmaceuticals in Japan since “the preparation before use” is difficult. Recently, bag-type kit preparations have been introduced to reduce the workload of medical staff with regard to the preparation of infusions.

In Japan, as large volume parenteral solutions need to be administered without the aid of an infusion device, pharmaceutical solutions are usually filled and supplied in flexible bags. Additionally, pharmaceutical solutions that are required to be checked before use are requested to be placed in transparent containers to facilitate visual inspection.

The commercial supply of large volume parenteral solutions that meet the above-mentioned clinical demand requires that the manufacturer assesses the bioburden of raw materials of pharmaceutical products and manufacturing environments and establish moist-heat sterilization conditions for the sterilization of the solutions. In contrast, filling equipment is increasing in size to improve the efficiency of manufacture of large volume parenteral solutions, making the

interaction of personnel with equipment inevitable. Given the need for this interaction of personnel with equipment, the reduction of microbial contamination is a critical issue in controlling the manufacture of large volume parenteral solutions. The basic concept for the manufacture of large volume parenteral solutions is to secure a manufacturing environment that is free from spore-forming bacteria which have higher heat resistance than the indicator microorganism employed for establishing sterilization conditions, and to ensure the sterility of pharmaceutical products by the routine monitoring and control of bioburden.

A2.2 Manufacturing Environment

1) Control of airborne particles in the filling and sealing areas

The non-operating level of cleanliness in the filling and sealing areas should satisfy the cleanliness requirements of ISO Class 5, and these areas need to be protected by circulating Grade A air.

2) Microbiological control of the filling and sealing areas

Airborne and surface microorganisms in the filling and sealing areas should be monitored in the frequency specified in Table 2. Frequency of Environmental Monitoring for Microbial Control and the frequency required for Cleanliness Grade A in Table 3. Acceptance Criteria for Environmental Microorganism Count (during Operations) of Section 8.3 of this document.

The monitoring locations and acceptable limits of environmental microorganisms may be established based on the risk level of product contamination and specified limits of bioburden for pharmaceutical products before sterilization.

If monitoring detected microorganisms, the presence or absence of heat-resistant bacteria (spore-forming) should be determined, and if heat-resistant bacteria are detected, the potential impact of the bacteria on the sterility of the pharmaceutical product should be assessed and, if judged to be positive, appropriate countermeasures should be implemented.

3) Control of airborne particles in the direct support area

The non-operating level of cleanliness in the direct supporting area should satisfy cleanliness requirements of ISO Class 7, and the level during operation should be appropriately adjusted to the risk of product contamination.

4) Microbiological control of the direct support area

Microorganisms (airborne and surface microorganisms) in the direct area should be monitored in the frequency specified in Table 2. Frequency of Environmental Monitoring for Microbial Control and the frequency required for Cleanliness Grade B in Table 3. Acceptance Criteria for Environmental Microorganism Count (during Operations) of Section 8.3 of this document.

The monitoring locations and acceptable limits of environmental microorganisms may be established based on the risk level of product contamination and specified limits of bioburden for pharmaceutical products before sterilization. If monitoring detects microorganisms, the presence or absence of heat-resistant bacteria (spore-forming) should be determined. If heat-resistant bacteria are detected, the potential effect of the bacteria on the sterility of the pharmaceutical product should be evaluated and, if judged to be positive, appropriate countermeasures should be implemented.

5) Gowning requirements in the filling and sealing areas

When engaged in operations in the filling and sealing areas, personnel should wear an adequately sterilized or disinfected gown, shoes, overshoes, gloves, goggles, and mask for cleanliness level-dependent bioburden control.

6) The trapping performance test of the HEPA filter used in the filling, sealing, and direct support areas should be performed by an appropriate method at an adequate frequency.

A2.3 Bioburden Control

1) The targeted value of bioburden for pharmaceutical products before sterilization should be < 1 CFU/product.

2) If bioburden testing reveals the presence of microorganisms exceeding the target value, the presence or absence of heat-resistant (spore-forming) microorganisms should be determined and, if present, the Sterilization Resistance Test should be performed with samples of detected microorganisms to verify that the SAL is less than 10^{-6} .

3) The bioburden test for the pharmaceutical product before sterilization should be conducted as directed in the Microbial Limit Test (Section 13.2.1) of this document and the Enumeration Methods of the Microbial Limit Test, JP.

4) Bioburden for the pharmaceutical product before sterilization should be monitored lot-by-lot, and sampling conducted as directed in Section 13.1. Bioburden Monitoring.

5) A sterilization resistance test of heat-resistant (spore-forming) microorganisms, if present, should be performed as directed in Section 13.2.4 or by the following method:

① Heat resistance screening test

A screening test should be performed with the sterilization indicator microorganism and spore-forming microorganisms detected. A suitable spore-forming medium should be selected to prepare spores and then a spore suspension should be obtained. With this suspension, heat resistance of the spore-forming microorganisms is determined based on the reduction of bacterial count achieved at, for example, the sterilization or

reference temperature at which the *D* value of the sterilization indicator microorganism is one minute.

② Determination of *D* value

In cases where the result of Test ① indicates a higher heat resistance of a heat-resistant microorganism than that of the sterilization indicator microorganism, the *D* value should be determined at three temperature points in accordance with ISO 11138. A hot oil bath may be used for heat exposure.

A2.4 Establishment of Sterilization Conditions

In cases where Half-Cycle Method and Overkill Method cannot be applied, a suitable method should be selected from the following two methods, as appropriate.

1) Combination of BI and bioburden

The selected BI should be a strain with an established sterilization resistance such as *Bacillus subtilis* “5230” (ATCC 35021).

2) Absolute bioburden method

Absolute bioburden method is a method of determining sterilization conditions by using the most heat-resistant microorganism found in the product to be sterilized or the sterilization environment. If a microorganism that is not as heat-resistant as *Bacillus subtilis* “5230,” ATCC 35021 is found, the microorganism detected should be confirmed to be less heat resistant than *Bacillus oleronius*, ATCC 700005, which is then used in place of ATCC 35021.

A2.5 Biological Indicators (BIs)

As a rule, the selected BIs should be strains with well-established sterilization resistance, such as *Bacillus subtilis* “5230,” ATCC 35021 and *Bacillus oleronius*, ATCC 700005.

A3. Establishment of Sterilization Conditions

The SAL of the pharmaceutical product should be ensured by one of the following methods:

- a) Half-cycle method
- b) Overkill method
- c) Combination of BI and bioburden
- d) Absolute bioburden method

a) Half-cycle method

The half-cycle method is a method based on the overkill approach. In this method, a BI is placed within the product, PCD, or sterilization equipment at a location where sterilization conditions are the most difficult to achieve and a sterilization time of twice as long as that required to inactivate all 10^6 CFU of the BI is used. While the sterilization time, D value, for the overkill method is 12, the D value for the half-cycle method is 14 to 16. The probability of survival of microorganisms with the use of a BI having an initial 10^6 count is 10% at the D value of 7 and 1% at the D value of 8, and hence the following equation is obtained: $(7 - 8D) \times 2 = 14 - 16D$. The half-cycle method generally requires a longer sterilization time than the overkill method.

b) Overkill method

The basic concept of the overkill method is “the sterilization process that is demonstrated as delivering at least a 12 Spore Log Reduction (SLR) to a BI having resistance equal to or greater than the product bioburden” as defined in the ISO standards on definitions of terms (ISO/TS 11139) in the fields of health care products (medical devices, pharmaceuticals, and *in vitro* diagnostics). In this method, a sterilization condition giving a SAL of no more than 10^{-6} is used to achieve $12D$ of a known count of BI. The ISO standards also specify the BIs to be used in different sterilization processes, as shown in Table A-1.

Table A-1. Specifications of BIs Recommended for Use in Sterilization Processes

Sterilization method	Ethylene oxide (EO) gas sterilization	Moist-heat sterilization	Dry-heat sterilization	Low-temperature steam and formaldehyde sterilization
Indicator microorganism	<i>Bacillus atrophaeus</i> (ATCC9372), etc.	<i>Geobacillus stearothermophilus</i> (ATCC7953), etc.	<i>Bacillus atrophaeus</i> (ATCC9372), etc.	<i>Geobacillus stearothermophilus</i> (ATCC7953), etc.
Count	$\geq 1.0 \times 10^6$ CFU	$\geq 1.0 \times 10^5$ CFU	$\geq 1.0 \times 10^6$ CFU	$\geq 1.0 \times 10^5$ CFU
D value	≥ 2.5 min (54°C) ≥ 12.5 min (30°C)	≥ 1.5 min (121°C)	≥ 2.5 min (160°C)	≥ 6 min (60°C)
z value	–	$\geq 6^\circ\text{C}$	$\geq 20^\circ\text{C}$	–
ISO code	ISO 11138-1, 2	ISO 11138-1, 3	ISO 11138-1, 4	ISO 11138-1, 5

The ISO standards on moist-heat sterilization specifies the use of *Geobacillus*

stearothermophilus as a BI. In contrast, its annex also lists *Clostridium sporogenes* and *Bacillus coagulans*. Other than these BIs, the BIs for Sterilization <1035> in the USP recommends the use of *Bacillus subtilis*. In principle, the overkill method uses *G. stearothermophilus* as a BI. However, if the sterilization level is changed from 12D, a BI other than *G. stearothermophilus* or the most resistant microorganism found or isolated by each manufacturer from pharmaceutical products or environments should be employed in the test. The USP <1035> specifies that the D_{121} value of *G. stearothermophilus* should be minimally 1.5 minutes and maximally 3.0 minutes. For example, when *G. stearothermophilus* that has D_{121} value of 2.0 minutes is used as a BI to deliver at least a 12 SLR, the sterilization time should be 24 minutes (2.0×12). When samples in the test are commercial products, the D value occasionally becomes larger than the labeled D value of the BI; therefore the sterilization condition of 12D is not recommended to be employed in order to prevent undesirable effects on the product or container-closure system.

On the other hand, because of recent technological improvements in inner temperature measurement and advances in procedures for managing the sterilization process at F_0 , the overkill method is currently required to achieve $F_0 \geq 12$ which is an approach to theoretically provide a minimum of a 12-log reduction in microorganisms at D_{121} for 1 minute (ISO/TS 17665-2).

c) Combination of BI and bioburden

The combination of BI and bioburden generally has a lower thermal load than overkill conditions and is hence employed for the sterilization of pharmaceutical products that can tolerate a greater thermal load than that incurred with the absolute bioburden method. This method is intended to assure the sterility of the product being sterilized by the combined use of the BI and bioburden methods. BI-related information (e.g. bacterial count, D value, and z value) and D value of the most heat-resistant bioburden microorganism identified by bioburden estimation need to be collected in advance.

In this method, the D value of the heat-resistant bioburden microorganism is required to be larger than D value of the BI. The sterilization time is usually calculated according to the following equation:

$$\text{Sterilization time} = D \times \log N_0 / N$$

D : D_{121} value of BI

N : Sterility assurance level (10^{-6})

N_0 : Initial bioburden count

The initial bioburden count is equivalent to the maximum bioburden count which is a count of the mean bioburden plus its standard deviation multiplied by three or a count with 1 to 2 log margin or BI score. In contrast to the overkill method which requires a minimum $12D$ process, the combination of BI and bioburden usually requires only a $6-7D$ thermal load in processing. This method requires the selection of BIs which have a larger D value (generally, *C. sporogenes*, *B. coagulans*, *B. subtilis*, and *G. stearothermophilus*) than the most-resistant microorganism found in the bioburden estimation, and confirmation that the calculated survival probability of the BIs is less than 10^{-6} . Alternatively, a bioburden microorganism may be used as a biological index. Similar to the absolute bioburden method as mentioned below, the resistance of bioburden is determined by the survival curve method or fraction negative method using biological indicator evaluation resistometer (BIER) vessels, as directed in ISO 11138-1. It is not required that a challenging microorganism be eradicated, but the survival probability of the bioburden determined based on the reduction of the challenge BI should be verified to achieve the target SAL level.

For example, if the bioburden investigation shows that the initial bacterial count (estimate) is 10^2 , then if the D value of the most heat-resistant bioburden microorganism obtained by screening test is 0.2 minutes, and if a challenge with a BI with a known D value ($D_{121} = 0.5$) to the actual sterilization cycle provides a 4-log reduction in bacterial count, then the microbial F_0 value indicates the following equation: $F_0 \text{ BIO} = 4 \times D_{121} = 4 \times 0.5 = 2.0$.

The equation $F_0 \text{ BIO} = D_{121} \times (\log_{10} A - \log_{10} B)$ can be modified as $\log_{10} B = \log_{10} A - F_0 \text{ BIO}/D_{121}$. When $A = 10^2$ and $D_{121} = 0.2$ (minute) are assigned to this equation, the following equation is obtained: $\log_{10} B = \log_{10} 10^2 - 2/0.2 = -8$. Because the B value is 10^{-8} , the survival probability of the bioburden is determined to be 10^{-8} . This result indicates that the sterilization process employed is efficient in achieving SAL of 10^{-6} , which is the minimum requirement for sterility assurance.

The combination of BI and bioburden should be periodically performed to routinely monitor bioburden level and to determine the bioburden count and heat resistance of heat-resistant microorganisms in order to ensure the sterility of materials and products being sterilized.

d) Absolute bioburden method

The absolute bioburden method is employed in place of the above-mentioned overkill or bioburden/BI combination method when these methods are not applicable because of potential adverse effects of thermal load on the stability of pharmaceutical products and packages or shape of containers. A screening test (usually a heat shock of 80°C to 100°C for 10 to 15 minutes) should be performed using microorganisms obtained by bioburden testing of

materials and products being sterilized to identify the most heat-resistant microorganisms. Bioburdens should be recovered from products as directed in ISO11737-1. The absolute bioburden method is a procedure for certifying that the survival probability of the most heat-resistant bioburden microorganism is less than 10^{-6} . In this method, BIs are not required in testing.

For example, if the most heat-resistant bioburden microorganism shows a positive relationship between the D value and temperature as illustrated in Fig. A-1, the D_{121} value is obtained from the figure as $D_{121} = 10^{-0.1 \times 121 + 11.5} = 0.25$ (minute). The F_0 value necessary to reduce the count of the microorganism from 10^6 to 10^{-6} can then be obtained from the equation, $F_0 = D_{121} \times (\log_{10} A - \log_{10} B)$.

When $A = 10^6$, $B = 10^{-6}$, and $D_{121} = 0.25$ are assigned to this equation, the following equation is obtained: $F_0 = 0.25 \times (6 + 6) = 3.0$. $F_0 = 3.0$ should be the target value in establishing sterilization conditions, and a heat penetration test should therefore be performed to confirm a F_0 value of less than 3.0 at the location least favorable to sterilization (cold point). The bioburden count in the controlled manufacturing facility is generally far lower than 10^6 . For example, if the maximum bioburden count is determined to be 10^2 , then the F_0 value necessary for the eradication of microorganisms from 10^2 to 10^{-6} is calculated by the equation, $0.25 \times (2 + 6) = 2.0$, and hence the F_0 value needs to be ≥ 2.0 to assure sterility.

In practice, the lethality of sterilization conditions is verified by a challenge test with bioburden microorganisms at 10^6 at the cold point and a sterilization time to achieve a SAL of less than 10^{-6} for the assurance of sterility. In this procedure, the safety factor against the anticipated maximum bioburden count needs to be established by taking account of recovery rate, etc.

When the bioburden method is employed, the bioburden of materials and products should be routinely and frequently monitored after implementation and, if more heat-resistant microorganisms are found, the method should be validated again and the potential effect of the microorganisms on pharmaceutical products already shipped from the factory need to be assessed.

Microbiological test systems such as the estimation of bioburden and sterility testing should be referred to in the ISO 11737 technical report series.

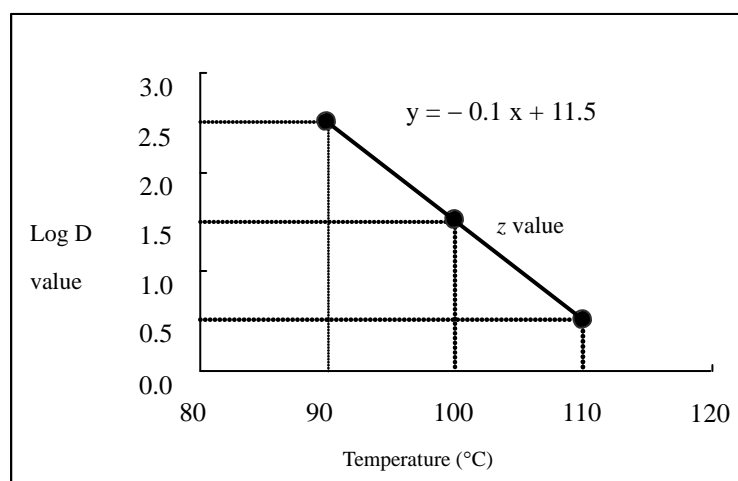


Fig. A-1. Correlation between *D* value and temperature for calculation of *z* value

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