Category II: The viable count of bacteria or yeasts and moulds in mixed samples at the same level or less than the inoculum at 14 and 28 days after inoculation.

The above interpretation criteria are summarized in Table 1. In addition, the bacterial count and the yeast and mould counts for uninoculated controls at 14 and 28 days should be at the same level or less than the count at the initial inoculation.

Culture Media

Culture media and buffer solution used for Preservatives-Effectiveness Tests are described below. Other media may be used if they have similar nutritive ingredients and selective and growth-promoting properties for the microorganisms to be tested.

Soybean Casein Digest Agar Medium

Casein peptone	15.0 g
Soybean peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water	1,000 mL

Mix all of the components and sterilize at 121 °C for 15 - 20 minutes in an autoclave.

pH after sterilization: 7.1 - 7.5.

Sabouraud Glucose Agar Medium

Peptone (animal tissue and casein)	10.0 g
lucose	40.0 g
Agar	15.0 g
Water	1,000 mL

Mix all of the components and sterilize at 121 °C for 15 - 20 minutes in an autoclave.

pH after sterilization: 5.4 - 5.8. Addition of antibiotics not required.

Glucose Peptone (GP) Agar Medium

Glucose	20.0 g
Yeast extract	2.0 g
Magnesium sulfate	0.5 g
Peptone	5.0 g
Monobasic potassium phosphate	1.0 g
Agar	15.0 g
Water	1,000 mL

Mix all of the components and sterilize at 121 °C for 15 - 20 minutes in an autoclave.

pH after sterilization: 5.6 - 5.8. Addition of antibiotics not required.

Potato Dextrose Agar Medium

Potato extract	4.0 g
Glucose	20.0 g
Agar	15.0 g
Water	1,000 mL

Mix all of the components and sterilize at 121 °C for 15 - 20 minutes in an autoclave.

pH after sterilization: 5.4 - 5.8. Addition antibiotics not required.

0.1% Peptone Water

Peptone	1.0 g
Sodium chloride	8.9 g
Water	1,000 mL

Mix all of the components and sterilize at 121 °C for 15 - 20 minutes in an autoclave.

pH after sterilization: 7.2 - 7.4.

13. Sterility Assurance for Terminally Sterilized Pharmaceutical Products

As indicated in the "Terminal Sterilization and Sterilization Indicators", the pharmaceuticals to which terminal sterilization can be applied, generally must be sterilized so that a sterility assurance level of 10^{-6} or less is obtained. The sterility assurance level of 10^{-6} or less can be proven by using a sterilization process validation based on physical and microbiological methods, but cannot be proven by sterility tests of the sterilized products. This chapter deals with the necessary requirements for the appropriate management of the important control points of the sterilization process for the parametric release of products, without performing sterility tests on products which have been subjected to terminal sterilization (in the case of radiation sterilization, called dosimetric release). Parametric release is a method that can be applied in cases where the sterilization system is clearly defined, important control points are clearly specified, and the sterilization system process can be validated by microbiological methods using appropriate biological indicators.

1. Definitions

The definitions of the terminology used in this chapter are provided below.

1.1 Terminal sterilization

A process whereby a product is sterilized in its final container or packaging, and which permits the measurement and evaluation of quantifiable microbial lethality.

1.2 Validation

A documented procedure for obtaining, recording and interpreting the results needed to show that a process will consistently yield a product complying with predetermined specifications.

1.3 Periodic re-validation

Validation that is regularly performed to reconfirm that a process is consistently yielding a product complying with predetermined specifications. It should confirm that variables and the acceptable ranges are permissible to yield a product consistently of the required quality.

1.4 Facility/equipment qualification

This is to provide evidence that the manufacturing facilities/equipment, measuring equipment, and manufacturing environment control facilities, etc. have been properly selected, correctly installed, and are operated in conformity with the specifications at the time of installation and during operation.

1.5 Operation qualification

This is to provide evidence to confirm physically, chemically and microbiologically that equipment, operated in accordance with its operational instructions, operates as specified and affords a product meeting the specifications.

1.6 Support system for sterilization process

This refers to the facility/equipment that is associated with the sterilization devices, such as the preconditioning and aeration for ethylene oxide sterilization, the steam supply equipment for moist heat sterilization, and the loading devices for radiation sterilization.

1.7 Quality system

The procedures, resources and organizational structure of a manufacturer (responsibilities, authorities and relationships between these) required to implement quality management.

1.8 Change control system

A system designed to evaluate all of the changes that may affect the quality of the pharmaceutical product, in order to ensure that the process is continuously controlled.

1.9 F_0 value

Assume a value of 10°C for the Z value defined as the number of degrees of temperature required for a 10-fold change in the D value. The F_0 value indicates the time (minutes) required to give the equivalent lethality at T_b of the sterilization heat obtained by integrating the lethality rate (L) over an entire heating cycle.

$$L = \log^{-1}\frac{T_0 - T_b}{Z} = 10^{\frac{T_0 - T_b}{Z}}$$

T_0 = Temperature inside the chamber or inside the product to be sterilized

T_b = Reference temperature (121°C)

$$F_0 = \int_{t_0}^{t_1} L\,dt$$

$t_1 - t_0$ = Processing time (minutes)

1.10 Control device

A general term for the devices and measurement equipment, including the equipment for controlling, measuring and recording the physical parameters that can be measured (temperature, humidity, pressure, time, radiation dose, etc.).

1.11 Parametric release

A release procedure based on an evaluation of the production records and critical parameters of the sterilization process (temperature, humidity, pressure, time, radiation dose, etc.) based on the results of validation, in lieu of release based on testing results of the final product.

2. Sterilization Validation

2.1 Subject of the Implementation

A manufacturer of sterile pharmaceuticals (hereafter, "manufacturer") must establish a quality system, implement product sterilization validation for the categories below as a general rule, and continuously control the sterilization process based on the results of the sterilization validation.

a. Sterilization process

b. Sterilization process support system

2.2 Documenting Sterilization Validation Procedure

2.2.1 The manufacturer must prepare a "Sterilization Validation Procedure" defining the items listed below regarding the procedures for managing the sterilization process.

a. Details related to the range of duties of the persons responsible for the validation, as well as the extent of their authority

b. Details related to the implementation period for the sterilization validation

c. Details related to the creation, modification, and approval of the sterilization validation plan documents

d. Details related to the reporting, evaluation, and approval of the sterilization validation implementation results

e. Details related to the storage of documentation concerning the sterilization validation

f. Other required matters

2.2.2 The sterilization validation procedure must list the names of the enactors, the date of enactment, and when there are revisions, must also list the revisers, date of revisions, revised sections and reasons for the revisions.

2.2.3 The manufacturer must properly store and maintain the sterilization validation procedure after clarifying the procedures related to alterations and deletions of the contents of the sterilization validation procedure.

2.3 Persons Responsible for the Validation

The manufacturer must assign persons to be responsible for the sterilization validation. The responsible parties must perform each of the duties listed below according to the sterilization validation procedure.

2.3.1 For products that are to be produced according to the sterilization validation procedure, a written sterilization validation implementation plan must be prepared. The implementation plan will specify the following points based on a consideration of the implementation details of the sterilization validation.

a. Subject pharmaceutical name (product name)

b. Purpose of the applicable sterilization validation

c. Expected results

d. Verification methods (including inspection results and evaluation methods)

e. Period of verification implementation

f. Names of persons performing the sterilization validation (persons-in-charge)

g. Names of the persons who created the plan, creation date, and in the event of revisions, the names of the revisers, date of the revisions, revised sections, and reasons for revision.

h. Technical requirements for the applicable sterilization validation

i. Other required matters for the implementation of the applicable sterilization validation

2.3.2 The following sterilization validation is implemented according to the plan defining the items above.

a. When the manufacturing license and additional (modification) licenses for product production are obtained, implementation items for the sterilization validation to be executed

1 Product qualification

2 Facility/equipment qualification

1) Installation qualification

2) Operation qualification

3 Performance qualification

1) Physical performance qualification

2) Microbiological performance qualification

b. Sterilization validation to be executed until it is time to renew the manufacturing license

1 Re-validation when there are changes

2 Periodic re-validation (The items implemented, etc. must be determined based on a consideration of relevant factors such as the sterilization method.)

2.3.3 Evaluate the results of the sterilization validation and verify that sterility is assured.

2.3.4 Make a written report of the results of the sterilization validation to the manufacturer's authorized person.

2.3.5 Perform the day-to-day management of the sterilization process.

3. Microorganism Control Program

When parametric release is adopted, it is important to control the bioburden in the raw materials of the product, the containers and stoppers, and in the product before sterilization. The bioburden is measured with a previously specified method and frequency, and when required, surveys of the characteristics of the isolated microorganisms are made to investigate their resistance to the applicable sterilization method. Refer to the "Microbiological Evaluation of Process Areas for Sterile Pharmaceutical Products" regarding the method for evaluating the environmental microorganisms in the processing areas of pharmaceutical products.

4. Sterilization Indicators

Biological indicators (BI), chemical indicators (CI), and dosimeters are among the means used to monitor a sterilization process and as indices of sterility (refer to Terminal Sterilization and Sterilization Indicators). When using sterilization indicators it is important to consider environmental and human safety, and to take all necessary precautions. The BI used for sterilization validation and daily process control must be defined in the specification, and recorded in writing. When BI are used for daily process control it must be verified that the loading pattern on the form, product, or simulated product has a resistance equal to or greater than that used for the microbiological performance qualification.

5. Establishment of a Change Control System

Changes which have a large effect on the sterile quality, such as changes in sterilization equipment, loading pattern, and sterilization conditions, correspond to changes of the parametric release conditions for the relevant pharmaceutical product. A change control system must be defined in the sterilization validation procedure; and when there are changes in the causes of variation that have been previously specified, there must be an investigation of the causes of variation and of acceptable conditions to verify that the pharmaceutical product is guaranteed always to conform to the quality standards. Furthermore, before modifications are made to a sterilization process that has been validated, it is mandatory to obtain approval for the implementation of the modifications in question from the appropriate authorized person.

6. Release Procedure

A release procedure must be created to clarify the conditions required for shipment based on parametric release of terminally sterilized products. The following points must be evaluated and recorded when a product is released.

Depending on the sterilization method, some of these items may be omitted or modified.

a) Batch record

b) Microorganism evaluation data of production environment

c) Bioburden data for the raw materials and product before sterilization

d) Data related to the sterilization indicators

e) Data on the maintenance management of the sterilization process and sterilization process support systems

f) Data on the management of sterilization parameters

g) Data on the calibrations of measurement equipment

h) Re-validation data

i) Other

7. Critical Control Points

The important control points for each sterilization method are presented.

7.1 Moist heat sterilization

Moist heat sterilization is a method for killing microorganisms in which saturated water vapor is generated or introduced into a sterilization chamber at the appropriate temperature and pressure, and the chamber is then heated for a certain period of time. It is roughly classified into saturated vapor sterilization, in which the target microorganisms are directly exposed to the saturated vapor, and unsaturated vapor sterilization, in which the fluid inside a container, such as an ampule, is subjected to moist heat energy or highfrequency energy from the outside.

7.1.1 Important control points

A process control procedure must be created, specifying the process parameters that affect the sterile quality of the pharmaceutical product, and the permissible range of variation for each parameter. The important control points for the moist heat sterilization are indicated below.

a) Heating history (usually indicated by F_0 value)

b) Temperature

c) Pressure

d) Time

e) Product loading format/loading density

f) Other necessary matters

7.1.2 Utilities

The utilities and control devices required for moist heat sterilization determine the quality and precision.

a) Quality of the vapor used

b) Quality of the air introduced into the sterilization chamber to restore pressure, etc.

c) Quality of the water used for cooling

d) Precision of the temperature control devices

e) Precision of the pressure control devices

f) Precision of the time control devices

g) Other

7.2 Ethylene oxide gas sterilization

Ethylene oxide gas allows sterilization at low temperatures, so there is typically little injury to the substance being sterilized; however, since the gas is toxic it must be handled with extreme caution. The sterilization process consists of preconditioning, a sterilization cycle, and aeration. The preconditioning is performed before the sterilization cycle to process the product so that temperature and relative humidity in the room or container are within the range in the specifications. The sterilization cycle indicates the stage at which the actual sterilization is performed, and consists of removal of the air, conditioning (when used), injection of the sterilization gas, maintenance of the sterilization conditions, removal of the sterilization gas, and replacement of the air. The aeration is the process of eliminating the residual ethylene oxide gas from the product, either inside the sterilization chamber or in a separate location.

7.2.1 Important control points

The important control points for the ethylene oxide gas sterilization are indicated below.

7.2.1.1 Preconditioning (when performed)

a) Time, temperature, humidity

b) Product loading pattern/loading density

c) Sterilization loading temperature and/or humidity

d) Time from the end of preconditioning until the start of the sterilization

e) Other necessary matters

7.2.1.2 Conditioning

a) If pressure reduction is performed, the pressure achieved and required time

b) Reduced pressure maintenance period

c) Time, temperature, pressure, humidity

d) Sterilization loading temperature and humidity

e) Other necessary matters

7.2.1.3 Sterilization cycle

a) Pressure increase, injection time, and final pressure for the injection of the sterilization gas

b) Concentration of the ethylene oxide gas (it is desirable to analyze directly the gas concentration inside the sterilization chamber, but the following alternatives are acceptable if direct analysis is difficult)

i) Mass of gas used

ii) Volume of gas used

iii) Conversion calculation using the initial low pressure level and the gas injection pressure

c) Temperature within the sterilization chamber

d) Temperature of the loaded products to be sterilized

e) Effect time (exposure time)

f) Product loading pattern/loading density

g) BI placement points and cultivation results

h) Other necessary matters

7.2.1.4 Aeration

a) Time, temperature

b) Loaded sterilized substance temperature

c) Pressure variation in the sterilization chamber and/or the aeration room

d) Rate of change of the air or other gases in the aeration room

e) Other necessary matters

7.2.2 Utilities

The utilities and control devices required for ethylene oxide sterilization determine the quality and precision.

a) Quality of the ethylene oxide gas

b) Quality of the injected vapor or water

c) Quality of the replacement air after the completion of sterilization

d) Quality of the BI

e) Precision of the temperature control devices

f) Precision of the pressure control devices

g) Precision of the humidity control devices

h) Precision of the time control devices

i) Other

7.3 Irradiation Sterilization

Irradiation sterilization refers to methods of killing microorganisms through exposure to ionizing radiation. The types of ionizing radiation used are gamma-rays (γ-rays) emitted from a radioisotope such as ^{60}Co or ^{137}Cs, or electron beams and bremsstrahling (X-ray) generated from an electron accelerator. In the case of γ-rays, the cells are killed by secondarily generated electrons, while in the case of the electron beam, the cells are killed by the electrons generated directly from the electron accelerator. For this reason, the processing time for electron beam sterilization is generally shorter than that for γ-ray sterilization; but, since the penetration of the γ-rays is better than that of the electron beam, there must be appropriate consideration of the density and thickness of the substance being sterilized when choosing between these methods. For an irradiation sterilization process, the control procedures primarily make use of dosimeters and measure the absorbed dose in the substance being sterilized. This is called dosimetric release.

7.3.1 Important control points

The important control points for the irradiation sterilization are indicated below.

7.3.1.1 γ-ray radiation

a) Irradiation time (timer setting or conveyor speed)

b) Absorbed dose

c) Product loading pattern

d) Other necessary matters

7.3.1.2 Electron beam and X-ray radiation

a) Electron beam characteristics (average electron beam current, electron energy, scan width)

b) Conveyor speed

c) Absorbed dose

d) Product loading pattern

e) Other necessary matters

7.3.2 Utilities

A traceable calibration, performed according to national standards, must be performed for the radiation devices and dose measurement systems. This calibration must be performed as specified in a written plan in order to verify that the equipment is kept within the required range of accuracy.

7.3.2.1 Required calibration items for gamma-radiation equipment

a) Cycle time or conveyor speed

b) Weighing device

c) Dose measurement system

d) Other

7.3.2.2 Required calibration items for electron-beam and X-ray radiation equipment

a) Electron beam characteristics

b) Conveyor speed

c) Weighing device

d) Dose measurement system

e) Other

References

1) Validation Standards, PAB Notification No.158, Ministry of Health and Welfare 1995

2) Sterilization Validation Standards, PMSB/IGD Notification No.1, Ministry of Health and Welfare 1997

3) Quality Assurance Standards for Medical Devices, PAB Notification No.1128, Ministry of Health and Welfare 1994

4) ISO 9000 series, International Standards for Quality Assurance

5) ISO 11134 Industrial moist heat sterilization

6) ISO 11135 Ethylene oxide sterilization

7) ISO 11137 Radiation sterilization

8) ISO 11138 Biological indicators

9) ISO 11140 Chemical indicators

10) ISO 11737-1 Microbiological Methods Part 1: Estimation of population of microorganisms on products

11) USP 〈1222〉 Terminally Sterilized Pharmaceutical Products - Parametric Release

14. Tablet Friability Test

The Tablet Friability Test is a method to determine the