matography. If necessary use a guard column prepared by coating the inside wall of a fused silica tube, 0.53 mm in inside diameter and 5 m in length, to 5 μm thickness with 5% phenyl-methyl silicon polymer for gas chromatography.

Column temperature: Maintain at 35°C for 5 minutes, then increase to 175°C at 8°C per minute, further increase to 260°C at 35°C per minute if necessary, and keep at 260°C for 16 minutes.

Injection port temperature: A constant temperature of about 70°C

Detector temperature: A constant temperature of about 260°C

Carrier gas: Helium

Flow rate: 35 cm/second

Split ratio: Splitless

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, the resolution between the peaks is not less than 1.0. (Note: In the case that the number of substances to be tested is two or more.)

System repeatability: When the test is repeated 3 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of the substance to be tested is not more than 15%.

Test conditions (3)

Operating conditions—

Detector: Hydrogen flame-ionization detector

Column: Coat the inside wall of a fused silica tube, 0.32 mm in inside diameter and 30 m in length, to 0.25 μm thickness with polyethylene glycol 20M for gas chromatography. Use a guard column if necessary.

Column temperature: Maintain at 50°C for 20 minutes, then increase to 165°C at 6°C per minute if necessary, and keep at 165°C for 20 minutes.

Injection port temperature: A constant temperature of about 140°C

Detector temperature: A constant temperature of about 250°C

Carrier gas: Helium

Flow rate: 35 cm/second

Split ratio: 1:5

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, the resolution between the peaks is not less than 1.0. (Note: In the case that the number of substances to be tested is two or more.)

System repeatability: When the test is repeated 3 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of the substance to be tested is not more than 15%.

5. International Harmonization Implemented in the Japanese Pharmacopoeia Fourteenth Edition

Items for which harmonization has been agreed among the European Pharmacopoeia, the United States Pharmacopeia and the Japanese Pharmacopoeia are implemented in the Japanese Pharmacopoeia Fourteenth Edition (JP 14). They are shown in the table below. The column headed Harmonized items shows the harmonized items written in the Pharmacopoeial Harmonization Agreement Document, and the column headed JP 14 shows the items as they appear in JP 14. In the Remarks column, notes on any differences between JP 14 and the agreement are shown as occasion demands.

Harmonized items	JP 14	Remarks
Bacterial Endotoxin Test	Bacterial Endotoxin Test	
Apparatus	Apparatus	
Preparation of Standard Endotoxin Stock solution	Preparation of Standard Endotoxin Stock solution	
Preparation of Standard Endotoxin solution	Preparation of Standard Endotoxin solution	
Preparation of sample solutions	Preparation of sample solutions	
Determination of Maximum Valid Dilution	Determination of Maximum Valid Dilution	
Gel-clot technique	Gel-clot technique	
(1) Preparatory testing	(1) Preparatory testing	
(2) Limit test	(2) Limit test	
(3) Assay	(3) Assay	
Photometric techniques	Photometric techniques	
(1) Turbidimetric technique	(1) Turbidimetric technique	
(2) Chromogenic technique	(2) Chromogenic technique	
(3) Preparatory testing	(3) Preparatory testing	
(4) Assay	(4) Assay	
Reagents, Test Solutions	Reagents, Test Solutions	
Amebocyte lysate	Lysate reagent	
Lysate TS	Lysate TS	
Water for bacterial endotoxins test (BET)	Water for bacterial endotoxins test	

Note: The method for decision of the limit for bacterial endotoxins was agreed between the three pharmacopoeias, but in the Decision of Limit for Bacterial Endotoxins under the General Information in JP 14, the maximum adult dose is calculated based on an average body mass of an adult of 60 kg.

6. Media Fill Test

The media fill test (MFT) is one of the processing validations employed to evaluate the propriety of the aseptic processing of pharmaceutical products using sterile media, etc. instead of actual products. Therefore, media fill tests should be conducted with the manipulations normally performed in actual processing, e.g. filling and closing operation, operating environment, processing operation, number of personnel involved, etc., and conducted under processing conditions that include "worst case" conditions. Refer to GMP (1), WHO/GMP for pharmaceutical products (2), and ISO 13408 (3), etc. for necessary information to conduct this test.

1. Frequency of media fills

1.1 Initial performance qualification

Initial performance qualification should be conducted for each new facility, item of equipment, filling line, and container design (except for multiple sizes of the same container design), etc. For production batch sizes exceeding 3,000 units, a minimum of three media fill runs should be conducted on separate days. For production batch sizes of less than 3,000 units, see Table 3.

1.2 Periodic performance requalification

1) Conduct media fill requalifications periodically on each working shift for the filling line. Employees working in the aseptic processing area should be trained for aseptic processing operations and take part in media fills.

2) When filling lines have not been used for over six months, conduct appropriate numbers of media fill runs in the same way as for the initial performance qualification prior to resumption of use of the filling lines.

3) In cases of facility and equipment modification (interchanging parts may not require requalification), changes in personnel working in critical aseptic processing (e.g. new crews), anomalies in environmental testing results, or a product sterility test showing contaminated products, conduct appropriate numbers of media fill runs in the same way as for the initial performance qualification prior to the scheduled media fills.

2. Acceptance criteria of media fills

A large number of media filled units is required to detect 0.1% contamination rate. The alert level is more than 0.05% but less than 0.1%, and the action level is more than 0.1% contamination rate at the upper 95% confidence level. Table 1 shows alert and action levels for media filled units. Table 2 is to be used for the calculation of contamination rate of contaminated units found in media filled units. The contamination rate of 0.05% in media fills is the minimum acceptable level, and manufacturers should make efforts to achieve lower contamination rate than this. Table 3 shows the alert and action levels for initial performance qualification of an aseptic processing line, and actions required for each level. Table 4 shows the alert and action levels for requalification of an aseptic processing line, and actions required for each level. The alert and action levels for production batch sizes of less than 3,000 units take semiautomatic or manual operation into consideration.

2.1 Actions required for each level

2.1.1 Initial performance qualification

1) When the result of the media fill run is less than the alert level, the media fill run meets the requirement of the MFT.

2) When the results of any media fill run done with at least three replicate runs reach the alert or the action level, an investigation regarding the cause is required, and initial qualification media fills are to be repeated. When the result of each media fill run is less than the alert level, the initial qualification media fills meet the requirement of the MFT.

Table 1. Alert and action levels for large numbers of media filled units

Number of units*1	Number of contaminated units		
	Acceptance levels	Alert levels	Action levels
3,000	0	not applicable	≧ 1
4,750	0	1	≧ 2
6,300	0	1 - 2	≧ 3
7,760	0	1 - 3	≧ 4
9,160	0	1 - 4	≧ 5
10,520	1	2 - 5	≧ 6
11,850	1	2 - 6	≧ 7
13,150	1 - 2	3 - 7	≧ 8
14,440	1 - 2	3 - 8	≧ 9
15,710	1 - 3	4 - 9	≧10
16,970	1 - 3	4 - 10	≧11

*1 It is not necessary to relate the number of units with lot size of actual products.

Table 2. Upper 95% confidence limit of a Poisson variable for numbers of contaminated units

Observed numbers of contaminated units (k)	Upper 95% confidence limit (U)
0	2.9957
1	4.7439
2	6.2958
3	7.7537
4	9.1537
5	10.5130
6	11.8424
7	13.1481
8	14.4346
9	15.7052
10	16.9622

Using the 95% confidence limit (U) in Table 2, the contamination rate (P) of observed numbers of contaminated units (k) per filled units (n) can be calculated as $P = U/n$ (equation 1). For example:

If 5,000 units were filled and two contaminated units were observed, $n = 5{,}000$ and $U = 6.30$ ($k = 2$) are substituted in the equation 1; $P = 6.30/5{,}000 = 0.0013$. The upper 95% confidence limit for the contamination rate would be 0.13%.

Table 3. Initial performance qualification: Media fills

Production lot size	Numbers of media fill runs	Alert level and action required	Action level and action required
< 500	A minimum of 10 media fill runs using the maximum lot size of the product	One contaminated unit in any run. Investigate cause.	Two contaminated units in single run, or one each in two runs. Investigate cause and repeat initial qualification media fills.
500 - 2,999	A minimum of 3 media fill runs using the maximum lot size of the product	The same as above	The same as above
≧ 3,000	A minimum of 3 media fill runs using at least 3,000 units	When any of the media fill runs exceeds the alert level shown in Table 1, take the action set out in 2.1.1.	When any of the media fill runs exceeds the action level shown in Table 1, take the action set out in 2.1.1

Table 4. Periodic performance requalification: Media fills

Production lot size	Numbers of media fill runs	Alert level and action required	Action level and action required
< 500	A minimum of 3 media fill runs using the maximum lot size of the product		One contaminated unit in any run. Investigate cause and repeat initial qualification media fill runs.
500 - 2,999	One media fill run using the maximum lot size of the product		One contaminated unit. Investigate cause and repeat initial qualification media fill runs.
≧ 3,000	One media fill run using at least 3,000 units	When the media fill run exceeds the alert level shown in Table 1, take the action set out in 2. 1. 1.	When the media fill run exceeds the action level shown in Table 1, take the action set out in 2. 1. 1

2.1.2 Requalification

1) When the result of the media fill run is less than the alert level, the media fill run meets the requirement of the MFT.

2) When the result of the media fill run exceeds the alert level, an investigation regarding the cause is required, and one more media fill run is to be done. If the result is less than the alert level, the media fill run meets the requirement of the MFT.

3) When the result of the media fill run exceeds the action level, a prompt review of all appropriate records relating to aseptic production between the current media fill and the last successful one, and an investigation regarding the cause must be conducted simultaneously. If necessary, appropriate action to sequester stored and/or distributed products should be taken. After investigation regarding the cause, repeat three serial media fill runs. If the results are less than the alert level, the media fill runs meet the requirement of the MFT.

2.2 Parameters which affect sterility

When media fill alert and action levels are exceeded, an investigation should be conducted regarding the cause, taking into consideration the following points:

1) Microbial environmental monitoring data
2) Particulate monitoring data
3) Personnel monitoring data (microbial monitoring data on gloves, gowns, etc. at the end of work)
4) Sterilization cycles for media, commodities, equipment, etc.
5) Calibration of sterilization equipment
6) Storage conditions of sterile commodities
7) HEPA filter evaluation (airborne particulate levels, DOP test, velocity measurements, etc.)
8) Pre and post filter integrity test data (including filter housing assembly)
9) Room air flow patterns and pressures
10) Unusual events that occurred during the media fill run
11) Characterization of contaminants
12) Hygienic control and training programs
13) Gowning procedures and training programs
14) Aseptic processing technique and training programs
15) Operator's health status (especially coughing, sneezing, etc., due to respiratory diseases)
16) Other factors that affect sterility

3. Data guidance for media fills

Each media fill run should be fully documented and the following information recorded:

1) Data and time of media fill
2) Identification of filling room and filling line used
3) Container/closure type and size
4) Volume filled per container
5) Filling speed
6) Filter lot and catalogue number
7) Type of media filled
8) Number of units filled
9) Number of units not incubated and reason
10) Number of units incubated
11) Number of units positive
12) Incubation time and temperature
13) Procedures used to simulate any step of a normal production fill (e.g., mock lyophilization or substitution of vial headspace gas)
14) Microbiological monitoring data obtained during the media fill set-up and run
15) List of personnel who took part in the media fill
16) Growth promotion results of the media (in case of powder fill, an antimicrobial activity test for the powder is necessary)
17) Characterization of the microorganisms from any positive units
18) Review

4. Media fill procedures

Methods to validate aseptic processing of liquid, powder and freeze-dried products are described. Basically, it is possible to apply media fill procedures for liquid products to other dosage forms and container configurations.

4.1 Media selection and growth promotion

Soybean-casein digest medium or other suitable media are used. As growth promotion testing microorganisms, strains listed in the Sterility Test and, if necessary, one to two representative microorganisms which are frequently isolated in environmental monitoring should be used. The media inoculated with 10 to 100 viable microorganisms of each strain should show obvious growth when incubated at the predetermined temperature for 5 days.

4.2 Sterile medium preparation

The medium is sterilized according to the pre-validated method.

4.3 Incubation and inspection of media filled units

Leaking or damaged media fill evaluation units should be removed and recorded prior to incubation of media filled units. Incubate at 20 - 25°C for 1 week, and then at 30 - 35°C for 1 week (or at 30 - 35°C for 1 week, and then at 20 - 25°C for 1 week), or at 30 - 35°C for 2 weeks. Observe the media filled units for growth of microorganisms at least once between the third day and seventh day and on the last

day of the test period, twice in total. Microorganisms present in contaminated units should be characterized.

A. Liquid products

Media fill procedure

Media fill should include normal facility/equipment operations and clean-up routines. Containers, closures, parts of the filling machine, trays, etc. are washed and sterilized according to the standard operating procedures. Media fills should be conducted under processing conditions that include "worst case" conditions, e.g., correction of line stoppage, repair or replacement of filling needles/tubes, replacement of on-line filters, permitted interventions, duration and size of run, number of personnel involved, etc.

A predetermined volume of medium is filled into sterilized containers at a predetermined filling speed and the containers are sealed. The media are contacted with all product contact surfaces in the containers by an appropriate method, and then incubated at the predetermined temperature.

B. Powder products

B.1 Powder selection and antimicrobial activity test

Actual products or placebo powder are used. In general, lactose monahydrate, D-mannitol, polyethylene glycol 6,000, carboxymethyl cellulose salts or media powder, etc. are used as placebo powders. Prior to employing any of the powders, evaluate whether the powder has antimicrobial activity. Media powders are dissolved in water and other powders in liquid medium, and the solutions are inoculated with 10 to 100 viable microorganisms of each kind, shown in 4.1, for the growth promotion test. If obvious growth appears in the medium incubated at the predetermined temperature for 5 days, the powder has no antimicrobial activity and is available for the media fill test.

B.2 Sterilization of powders

Dry powders are bagged in suitable containers (e.g. double heat-sealed polyethylene bags), and are subjected to radiation sterilization.

B.3 Sterility of filling powders

The powders must pass the Sterility Test. However, if the sterilization is fully validated, sterility testing of the powders can be omitted.

B.4 Media fill procedures

Chose a suitable procedure from among the following procedures.

1) Fill sterilized liquid media into containers by suitable methods, and then fill actual products or sterilized placebo powder with the powder filling machine. If sterilized powder media are used as a placebo powder, fill sterilized water instead of sterilized liquid media.

2) Distribute liquid media into containers, and then sterilize them in an autoclave. Remove the containers to the filling area, and then fill actual products or sterilized placebo powder into the containers with the powder filling machine.

3) Fill actual products or sterilized placebo powder into containers with the powder filling machine, and then fill sterilized liquid media into the containers by appropriate methods. If sterilized powder media are used as a placebo powder, fill sterilized water instead of sterilized liquid media.

C. Lyophilized products

In the case of lyophilized products, it may be impossible to conduct a media fill run in the same way as used for actual processing of lyophilized products. The process of freezing and lyophilization of the solution may kill contaminant organisms and change the characteristics of the media too. The use of inert gas as a blanket gas may inhibit the growth of aerobic bacteria and fungi. Therefore, in general, the actual freezing and lyophilization process should be avoided and air used as the blanket gas.

Media fill procedures

Use the following method or other methods considered to be equivalent to these methods.

1) After filling of the media into containers by the filling machine, cap the containers loosely and collect them in presterilized trays.

2) After placing the trays in the lyophilizer, close the chamber door, and conduct lyophilization according to the procedures for production operation. Hold them without freezing under weak vacuum for the predetermined time.

3) After the vacuum process, break the vacuum, and seal the stoppers.

4) Contact the media with all product contact surfaces in the containers by appropriate methods, and then cultivate them at the predetermined temperature.

References

1) Good manufacturing practices for pharmaceutical products (WHO-GMP, 1992)
2) ISO 13408-1 (Aseptic processing of health care products: Generals)

7. Microbial Attributes of Nonsterile Pharmaceutical Products

The presence of microbial contaminants in nonsterile pharmaceutical products can reduce or even inactivate the therapeutic activity of the product and has the potential to affect adversely the health of patients. Manufacturers, therefore, should ensure as low as possible a contamination level for finished dosage forms, raw materials and packaging components to maintain appropriate quality, safety and efficacy of nonsterile pharmaceutical products. This chapter provides guidelines for acceptable limits of viable microorganisms (bacteria and fungi) existing in raw materials and nonsterile pharmaceutical products. Testing methods for the counting of total viable microorganisms and methods for the detection and identification of specified microorganisms (*Escherichia coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus*, etc.) are given under the "Microbial Limit Test". When these tests are carried out, a microbial control program must be established as an important part of the quality management system of the product. Personnel responsible for conducting the tests should have specialized training in microbiology and in the interpretation of the testing results.

1. Definitions

1.1 Nonsterile pharmaceutical products: Nonsterile drugs shown in monographs of the JP and nonsterile products including intermediate products and finished dosage forms.

1.2 Raw materials: All materials, including raw in-