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-

gredients and excipients, used for the preparation of drugs, except for water and gases.

1.3 Bioburden: Number and type of viable microorganisms existing in nonsterile pharmaceutical products.

1.4 Action levels: Established bioburden levels that require immediate follow-up and corrective action if they are exceeded.

1.5 Alert levels: Established bioburden levels that give early warning of a potential drift from normal bioburden level, but which are not necessary grounds for definitive corrective action, though they may require follow-up investigation.

1.6 Quality management system: The procedures, operation methods and organizational structure of a manufacturer (including responsibilities, authorities and relationships between these) needed to implement quality management.

2. Scope

In general, the tests for total viable aerobic count and for the detection of specified microorganisms are not applied to antibiotic or bacteriostatic drugs. However, the tests should be done for microorganisms that are not affected by the drug. The test for total viable aerobic count is not applied to drugs containing viable microorganisms as an active ingredient.

3. Sampling plan and frequency of testing

3.1 Sampling methods

Microbial contaminants are usually not uniformly distributed throughout the batches of non-sterile pharmaceutical products or raw materials. A biased sampling plan, therefore, cannot be used to estimate the real bioburden in the product. A sampling plan which can properly reflect the status of the product batch should be established on the basis of the bioburden data obtained by retrospective validation and/or concurrent validation. In general, a mixture of samples randomly taken from at least different three portions, almost the same amount for each portion, is used for the tests of the product. When the sampling is difficult in a contamination-controlled environment, special care is required during sampling to avoid introducing microbial contamination into the product or affecting the nature of the product bioburden. If it is confirmed that the product bioburden is stable for a certain period, as in the case of nonaqueous or dried products, it is not necessary to do the tests for total viable aerobic counts and for the detection of specified microorganisms, immediately after the sampling.

3.2 Testing frequency

The frequency of the tests should be established on the basis of a variety of factors unless otherwise specified. These factors include:

a) Dosage forms of non-sterile pharmaceutical products (dosage directions);
b) Manufacturing processes;
c) Manufacturing frequency;
d) Characteristics of raw materials (natural raw material, synthetic compound, etc.);
e) Batch sizes;
f) Variations in bioburden estimates (changes in batches, seasonal variations, etc.);
g) Changes affecting the product bioburden (changes in manufacturing process, supplier of raw materials, lot number of raw materials, etc.);
h) Others.

In general, the tests may be performed at a high frequency during the initial production of a drug to get information on the microbiological attributes of the product or raw materials used for the production. However, this frequency may be reduced as bioburden data are accumulated through retrospective validation and/or concurrent validation. For example, the tests may be performed at a frequency based on time (e.g., weekly, monthly or seasonally), or on alternate batches.

4. Microbial control program

When the "Microbial Limit Test" is applied to a nonsterile pharmaceutical product, the methods for the recovery, cultivation and estimation of the bioburden from the product must be validated and a "Microbial control program" covering the items listed below must be prepared.

a) Subject pharmaceutical name (product name);
b) Frequency of sampling and testing;
c) Sampling methods (including responsible person, quantity, environment, etc. for sampling);
d) Transfer methods of the samples to the testing area (including storage condition until the tests);
e) Treatment of the samples (recovery methods of microbial contaminants);
f) Enumeration of viable microorganisms (including testing quantity, culture media, growth-supporting test of the media, culturing methods, etc.);
g) Detection of specified microorganisms (including testing quantity, culture media, growth-supporting test of the media, culturing methods, etc.);
h) Estimation of the number of and characterization of microbial contaminants;
i) Establishment of "Microbial contamination limits" (including alert level and action level);
j) Actions to be taken when the levels exceed "Microbial contamination limits";
k) Persons responsible for the testing and evaluation, etc.;
l) Other necessary items

5. Microbial contamination limits for nonsterile pharmaceutical products

By establishing "Microbial contamination limits", it is possible to evaluate at the initial processing stage of the product whether the microbiological quality of the raw materials is adequate or not. Furthermore, it is then possible to implement appropriate corrective action as needed to maintain or improve the microbiological quality of the product. The target limits of microbial levels for raw materials (systhetic compounds and minerals) are shown in Table 1.

In general, synthetic compounds have low bioburden levels due to the high temperatures, organic solvents, etc., used in their manufacturing processes. Raw materials originated from plants and animals in general have higher bioburdens than synthetic compounds.

The microbial quality of the city water or purified water used in the processing of active ingredients or nonsterile pharmaceuticals may have a direct effect on the quality of the finished dosage form. This means it is necessary to keep the level of microbial contaminants in the water as low as possible.

The microbial contamination limits for nonsterile finished dosage forms are shown in Table 2. These microbial limits

are based primarily on the type of dosage form, water activity, and so on. For oral liquids and pharmaceutical products having a high water activity, in general, low microbial contamination limits are given. In this guideline, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Candida albicans* are shown as specified microorganisms, but it is also necessary to test certain pharmaceutical products for other microorganisms (for example, certain species of *Clostridia, Pseudomonas, Burkholderia, Aspergillus*, and *Enterobacter species*) that may have the potential to present a microbial risk to patients. The selection of the specified microorganisms was based on following criteria; indicator for poor hygienic practices, pathogenic potential for route of administration, and survival profile of the microorganism and recoverability in the product. Due to the inherent precision limitations of the enumeration methods, a value exceeding the target limit by not more than 2 times.

6. Microbial contamination limits for herbal drugs

Target limits of microbial contamination for herbal drugs and herbal drug containing preparations are shown in Table 3. Category 1 indicates herbal drugs and their preparations to which boiling water is added before use, and category 2 indicates other herbal drugs and their preparations. In this guideline, enterobacteria and other gram-negative bacteria, *Escherichia coli, Salmonella*, and *Staphylococcus aureus* are mentioned as specidied microorganisms, but other microorganisms such as certain species of *Bacillus cereus, Clostridia, Pseudomonas, Burkholderia, Asperigillus* and *Enterobacter species* are also necessary to be tested depending on the origin of the herbal drug raw materials or the preparation method of the preparations.

Table 1. Microbial enumeration limits for raw materials

Microorganisms	Target limit (cfu/g or cfu/mL)
Total aerobic microbial count (TAMC)	≦1000
Total combined yeasts/molds count (TYMC)	≦100

Table 2. Microbial enumeration limits for nonsterile finished dosage forms

Route of administration	TAMC (cfu/g or cfu/mL)	TYMC (cfu/g or cfu/mL)	Examples of objectionable microorganisms
Inhalation (liquid)	≦20	≦20	*Staphylococcus aureus* *Pseudomonas aeruginosa*
Inhalation (powder)	≦100	≦50	*Staphylococcus aureus* *Pseudomonas aeruginosa*
Nasal	≦100	≦50	*Staphylococcus aureus* *Pseudomonas aeruginosa*
Varginal	≦100	≦50	*Escherichia coli* *Staphylococcus aureus* *Candida albicans*
Otic or Topical (including transdermal patches)	≦100*	≦50*	*Pseudomonas aeruginosa* *Staphylococcus aureus*
Rectal	≦1000	≦100	*Not specified*
Oral (solid)	≦1000	≦100	*Escherichia coli*
Oral (liquid)	≦100	≦50	*Escherichia coli*

* For transdermal patches, the limits are expressed as cfu per transdermal patch.

Table 3. Microbial enumeration limits for herbal drugs and their preparations

Microorganisms	Category 1 (cfu/g or cfu/mL)	Category 2 (cfu/g or cfu/mL)
Aerobic bacteria	10^7	10^5
Molds and yeasts	10^4	10^3
Enterobacteria and other gram-negative bacteria	*	10^3
Escherichia coli	10^2	not detected
Salmonella	not detected	not detected
Staphylococcus aureus	*	*

* The limits are not specified.

8. Microbiological Evaluation of Processing Areas for Sterile Pharmaceutical Products

This chapter describes the methods for the control and evaluation of microbial contamination in areas used for the processing of sterile pharmaceutical products. Such processing areas are classified into critical areas and clean areas according to the required levels of air-cleanliness. A critical area is a defined space in which the airborne particulate and microorganism levels are controlled to meet grade A. The cleanliness requirements for such a space extend to the surfaces of the facilities and equipment which form or are located within the space, as well as to the supplied raw materials, chemicals, water, etc. Environmental conditions, such as temperature, humidity, and air pressure, are also controlled in this space when required. A clean area is a controlled space such that the levels of contaminants (particulates and microorganisms) in air, gases and liquids are maintained within specified limits, which are less stringent than those of grade A. When sterile pharmaceutical products are manufac-