12. Microbial Attributes of Nonsterile Pharmaceutical Products

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

The presence of certain micro-organisms in non-sterile preparations may have the potential to reduce or even inactivate the therapeutic activity of the product and has a potential to adversely affect the health of the patient. Manufacturers have therefore to ensure a low bioburden of finished dosage forms by implementing current guidelines on Good Manufacturing Practice during the manufacture, storage and distribution of pharmaceutical preparations.◆This chapter provides guidelines for acceptable limits of viable micro-organisms (bacteria and fungi) existing in raw materials and nonsterile pharmaceutical products.◆ Microbial examination of non-sterile products is performed according to the methods given in Microbiological Examination of Non-sterile Products <4.05> on *Microbial Enumeration Tests* and *Tests for Specified Micro-organisms*. ◆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, biosafety measures and in the interpretation of the testing results. ◆

◆1. Definitions

1.1 Non-sterile pharmaceutical products: Non-sterile drugs shown in monographs of the JP and non-sterile finished dosage forms.

1.2 Raw materials: All materials, including raw ingredients and excipients, used for the preparation of drugs, except for water and gases.

1.3 Bioburden: Number and type of viable micro-organisms existing in non-sterile 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 test for total viable aerobic count is not applied to drugs containing viable micro-organisms 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 clean area, 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 non-aqueous or dried products, it is not necessary to do the tests, 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 (usage);
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, batch 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 "Microbiological Examination of Non-Sterile Products *<4.05>*" is applied to a non-sterile 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 micro-organisms (including testing quantity, culture media, growth-supporting test of the media, culturing methods, etc.);
g) Detection of specified micro-organisms (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 acceptance criteria" (including alert level and action level);
j) Actions to be taken when the levels exceed "Microbial acceptance criteria";
k) Persons responsible for the testing and evaluation, etc.;
l) Other necessary items.◆

5. Microbial acceptance criteria for non-sterile pharmaceutical products

By establishing "Microbial acceptance criteria" for non-sterile pharmaceutical products based upon the total aerobic microbial count (TAMC) and the total combined yeasts/moulds count (TYMC), ◆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 (synthetic 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 non-sterile 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.◆

Acceptance criteria for microbiological quality for non-sterile 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 acceptance criteria are given.◆

Table 1. Acceptance criteria for Microbiological Quality of Non-Sterile Substances for Pharmaceutical use

	Total Aerobic Microbial Count (CFU/g or CFU/ mL)	Total Combined Yeasts/Moulds Count (CFU/g or CFU/ mL)
Substances for pharmaceutical use	10^3	10^2

Table 2 includes a list of specified micro-organisms for which acceptance criteria are set. The list is not necessarily exhaustive and for a given preparation it may be necessary to test for other micro-organisms depending on the nature of the starting materials and the manufacturing process.

Table 2. – *Acceptance criteria for microbiological quality of non-sterile dosage forms*

Route of administration	Total Aerobic Microbial Count (CFU/g or CFU/ mL)	Total Combined Yeasts/Moulds Count (CFU/g or CFU/ mL)	Specified Micro-organism
Non-aqueous preparations for oral use	10^3	10^2	Absence of *Escherichia coli* (1 g or 1 mL)
Aqueous preparations for oral use	10^2	10^1	Absence of *Escherichia coli* (1 g or 1 mL)
Rectal use	10^3	10^2	—
Oromucosal use Gingival use Cutaneous use Nasal use Auricular use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
Vaginal use	10^2	10^1	Absence of *Pseudomonas aeruginosa* (1 g or 1 mL) Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Candida albicans* (1 g or 1 mL)
Transdermal patches (limits for one patch including adhesive layer and backing)	10^2	10^1	Absence of *Staphylococcus aureus* (1 patch) Absence of *Pseudomonas aeruginosa* (1 patch)
Inhalation use (special requirements apply to liquid preparations for nebulization)	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL) Absence of bile-tolerant gram-negative bacteria (1g or 1 mL)

If it has been shown that none of the prescribed tests will allow valid enumeration of micro-organisms at the level prescribed, a validated method with a limit of detection as close as possible to the indicated acceptance criterion is used.

In addition to the micro-organisms listed in Table 2, the significance of other micro-organisms recovered should be evaluated in terms of:

- the use of the product: hazard varies according to the route of administration (eye, nose, respiratory tract);
- the nature of the product: does the product support growth, does it have adequate antimicrobial preservation?
- the method of application;
- the intended recipient: risk may differ for neonates, infants, the debilitated
- use of immunosuppressive agents, corticosteroids;
- presence of disease, wounds, organ damage.

Where warranted, a risk-based assessment of the relevant factors is conducted by personnel with specialized training in microbiology and the interpretation of microbiological data. For raw materials, the assessment takes account of processing to which the product is subjected, the current technology of testing and the availability of materials of the

desired quality. Acceptance criteria are based on individual results or on the average of replicate counts when replicate counts are performed (e.g. direct plating methods).

When an acceptance criterion for microbiological quality is prescribed it is interpreted as follows:

— 10^1 CFU: maximum acceptable count = 20,
— 10^2 CFU: maximum acceptable count = 200,
— 10^3 CFU: maximum acceptable count = 2000, and so forth.

◆6. Acceptance criteria 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 specified micro-organisms, but other micro-organisms 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 3. Acceptance criteria for herbal drugs and their preparations

Micro-organisms	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.◆