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⟨1111⟩ MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: ACCEPTANCE CRITERIA FOR PHARMACEUTICAL PREPARATIONS AND SUBSTANCES FOR PHARMACEUTICAL USE

The presence of certain microorganisms in nonsterile 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.

Microbial examination of nonsterile products is performed according to the methods given in the texts on *Microbial Enumeration Tests* ⟨61⟩ and *Tests for Specified Microorganisms* ⟨62⟩. Acceptance criteria for nonsterile pharmaceutical products based upon the total aerobic microbial count (TAMC) and the total combined yeasts and molds count (TYMC) are given in *Tables 1* and *2*. 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.

Table 1 includes a list of specified microorganisms 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 microorganisms depending on the nature of the starting materials and the manufacturing process.

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

Table 1. Acceptance Criteria for Microbiological Quality of Nonsterile Dosage Forms

Route of Administration	Total Aerobic Microbial Count (cfu/g or cfu/mL)	Total Combined Yeasts/Molds Count (cfu/g or cfu/mL)	Specified Microorganism(s)
Nonaqueous 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	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL)
			Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
Gingival use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL)
			Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
Cutaneous use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL)
			Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
Nasal use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL)
			Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
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 (1 g or 1 mL)

Table 2. Acceptance Criteria for Microbiological Quality of Nonsterile Substances for Pharmaceutical Use

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

In addition to the microorganisms listed in *Table 1,* the significance of other microorganisms recovered should be evaluated in terms of the following:

- 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.
- The 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 in the interpretation of microbiological data. For raw materials, the assessment takes account of the processing to which the product is subjected, the current technology of testing, and the availability of materials of the desired quality.

⟨1112⟩ APPLICATION OF WATER ACTIVITY DETERMINATION TO NONSTERILE PHARMACEUTICAL PRODUCTS

The determination of the water activity of nonsterile pharmaceutical dosage forms aids in the decisions relating to the following:

(a) optimizing product formulations to improve antimicrobial effectiveness of preservative systems,
(b) reducing the degradation of active pharmaceutical ingredients within product formulations susceptible to chemical hydrolysis,
(c) reducing the susceptibility of formulations (especially liquids, ointments, lotions, and creams) to microbial contamination, and
(d) providing a tool for the rationale for reducing the frequency of microbial limit testing and screening for objectionable microorganisms for product release and stability testing using methods contained in the general test chapter *Microbial Enumeration Tests* ⟨61⟩ and *Tests for Specified Microorganisms* ⟨62⟩.

Reduced water activity (a_w) will greatly assist in the prevention of microbial proliferation in pharmaceutical products; and the formulation, manufacturing steps, and testing of nonsterile dosage forms should reflect this parameter.

Low water activity has traditionally been used to control microbial deterioration of foodstuffs. Examples where the available moisture is reduced are dried fruit, syrups, and pickled meats and vegetables. Low water activities make these materials self-preserved. Low water activity will also prevent microbial growth within pharmaceutical drug products. Other product attributes, for example, low or high pH, absence of nutrients, presence of surfactants, and addition of antimicrobial agents, as well as low water activity, help to prevent microbial growth. However, it should be noted that more resistant microorganisms, including spore-forming *Clostridium* spp., *Bacillus* spp., *Salmonella* spp. and filamentous fungi, although they may not proliferate in a drug product with a low water activity, may persist within the product.

When formulating an aqueous oral or topical dosage form, candidate formulations should be evaluated for water activity so that the drug product may be self-preserving, if possible. For example, small changes in the concentration of sodium chloride, sucrose, alcohol, propylene glycol, or glycerin in a formulation may result in the creation of a drug product with a lower water activity that can discourage the proliferation of microorganisms in the product. This is particularly valuable with a multiple-use product that may be contaminated by the user. Packaging studies should be conducted to test product stability and to determine that the container–closure system protects the product from moisture gains that would increase the water activity during storage.

Reduced microbial limits testing may be justified through risk assessment. This reduction in testing, when justified, may entail forgoing full microbial limits testing, implementing skip-lot testing, or eliminating routine testing.

Nonaqueous liquids or dry solid dosage forms will not support spore germination or microbial growth due to their low water activity. The frequency of their microbial monitoring can be determined by a review of the historic testing database of the product and the demonstrated effectiveness of microbial contamination control of the raw materials, ingredient water, manufacturing process, formulation, and packaging system. The testing history would include microbial monitoring during product development, scale-up, process validation, and routine testing of sufficient marketed product lots (e.g., up to 20 lots) to ensure that the product has little or no potential for microbial contamination. Because the water activity requirements for different Gram-reactive bacteria, bacterial spores, yeasts, and molds are well described in the literature,[1] the appropriate microbial limit testing program for products of differing water activities can be established. For example, Gram-negative bacteria including the specific objectionable microorganisms, *Pseudomonas aeruginosa, Escherichia coli,* and *Salmonella* species will not proliferate or survive in preserved products with water activities below 0.91, while Gram-positive bacteria such as *Staphylococcus aureus* will not proliferate below 0.86, and *Aspergillus niger* will not proliferate below 0.77. Furthermore, even the most osmophilic yeast and xerophilic fungi will not proliferate below 0.60, and they cannot be isolated on compendial microbiological media.[1] The water activity requirements measured at 25° for the growth of a range of representative microorganisms are presented in *Table 1*.

[1] J. A. Troller, D. T. Bernard, and V. W. Scott. Measurement of Water Activity. In: *Compendium of Methods for the Microbiological Examination of Foods*. American Public Health Association, Washington, DC, 1984 pp.124–134.