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.

Table 1. Water Activities (a_W) Required to Support the Growth of Representative Microorganisms

Bacteria	Water Activity (a_W)	Molds and Yeast	Water Activity (a_W)
Pseudomonas aeruginosa	0.97	*Rhyzopus nigricans*	0.93
Bacillus cereus	0.95	*Mucor plumbeus*	0.92
Clostridium botulinum, Type A	0.95	*Rhodotorula mucilaginosa*	0.92
Escherichia coli	0.95	*Saccharomyces cerevisiae*	0.90
Clostridium perfringens	0.95	*Paecilomyces variotti*	0.84
Lactobacillus viridescens	0.95	*Penicillium chrysogenum*	0.83
Salmonella spp.	0.95	*Aspergillus fumigatus*	0.82
Enterobacter aerogenes	0.94	*Penicillium glabrum*	0.81
Bacillus subtilis	0.90	*Aspergillus flavus*	0.78
Micrococcus lysodekticus	0.93	*Aspergillus niger*	0.77
Staphylococcus aureus	0.86	*Zygosachharomyces rouxii* (osmophilic yeast)	0.62
Halobacterium halobium (halophilic bacterium)	0.75	*Xeromyces bisporus* (xerophilic fungi)	0.61

Table 2. Microbial Limit Testing Strategy for Representative Pharmaceutical and OTC Drug Products Based on Water Activity

Products	Water Activity (a_W)	Greatest Potential Contaminants	Testing Recommended
Nasal inhalant	0.99	Gram-negative bacteria	TAMC,* TCYMC, absence of *S. aureus* and *P. aeruginosa*
Hair shampoo	0.99	Gram-negative bacteria	TAMC, TCYMC, absence of *S. aureus* and *P. aeruginosa*
Antacid	0.99	Gram-negative bacteria	TAMC, TCYMC, absence of *E. coli* and *Salmonella* spp.
Topical cream	0.97	Gram-positive bacteria	TAMC, TCYMC, absence of *S. aureus* and *P. aeruginosa*
Oral liquid	0.90	Gram-positive bacteria and fungi	TAMC and TCYMC
Oral suspension	0.87	Fungi	TAMC and TCYMC
Topical ointment	0.55	None	Reduced testing
Lip balm	0.36	None	Reduced testing
Vaginal and rectal suppositories	0.30	None	Reduced testing
Compressed tablets	0.36	None	Reduced testing
Liquid-filled capsule	0.30	None	Reduced testing

* TAMC = Total aerobic microbial count; TCYMC = Total combined yeast and mold count.

NOTE—The water activities cited in *Table 2* for the different dosage forms are representative, and companies are urged to test their individual products before developing a testing strategy.

Pharmaceutical drug products with water activities well below 0.75 (e.g., direct compression tablets, powder and liquid-filled capsules, nonaqueous liquid products, ointments, and rectal suppositories) would be excellent candidates for reduced microbial limit testing for product release and stability evaluation. This is especially true when pharmaceutical products are made from ingredients of good microbial quality, when manufacturing environments do not foster microbial contamination, when there are processes that inherently reduce the microbial content, when the formulation of the drug product has antimicrobial activity, and when manufacturing sites have an established testing history of low bioburden associated with their products. *Table 2* contains suggested microbial limit testing strategies for typical pharmaceutical and over-the-counter (OTC) drug products based on water activity. Other considerations, as listed above, would be applied when setting up the microbial limits testing program for individual drug products because water activity measurements cannot solely be used to justify the elimination of microbial content testing for product release.

Similar arguments could be made for the microbial limits testing of pharmaceutical ingredients. However, this would require pharmaceutical manufacturers to have a comprehensive knowledge of the pharmaceutical ingredient manufacturer's manufacturing processes, quality programs, and testing record. This could be obtained through a supplier audit program.

Water activity, a_W, is the ratio of vapor pressure of H_2O in product (P) to vapor pressure of pure H_2O (Po) at the same temperature. It is numerically equal to 1/100 of the relative humidity (RH) generated by the product in a closed system. RH can be calculated from direct measurements of partial vapor pressure or dew point or indirect measurement by sensors whose physical or electric characteristics are altered by the RH to which they are exposed.

The relationship between a_W and equilibrium relative humidity (ERH) is represented by the following equations:

$$a_W = P/Po \text{ and } ERH(\%) = a_W \times 100$$

The a_W measurement may be conducted using the dew point/chilled mirror method.[2] A polished, chilled mirror is used as the condensing surface. The cooling system is electronically linked to a photoelectric cell into which light is reflected from the condensing mirror. An air stream, in equilibrium with the test sample, is directed at the mirror which cools until condensation occurs on the mirror. The temperature at which this condensation begins is the dew point from which the ERH is determined. Sample preparation should be considered as it may affect the water activity level of the material tested. Commercially available instruments using the dew point/chilled mirror method or other technologies need to be evaluated for suitability, validated, and calibrated when used to make water activity determinations.

[2]AOAC International Official Method 978.18. In: *Official Methods of Analysis of AOAC International,* 17th edition, AOAC International, Gaithersburg, Maryland.

These instruments are typically calibrated using saturated salt solutions at 25°, as listed in *Table 3.*

Table 3. Standard Saturated Salt Solutions Used to Calibrate Water Activity Determination Instruments

Saturated Salt Solutions	ERH (%)	a_W
Potassium sulfate (K_2SO_4)	97.3	0.973
Barium chloride ($BaCl_2$)	90.2	0.902
Sodium chloride (NaCl)	75.3	0.753
Magnesium nitrate [$Mg(NO_3)_2$]	52.9	0.529
Magnesium chloride ($MgCl_2$)	32.8	0.328

Add the following:

▲⟨1113⟩ MICROBIAL CHARACTERIZATION, IDENTIFICATION, AND STRAIN TYPING

INTRODUCTION

Microorganisms, if detected in drug substances, excipients, water for pharmaceutical use, the manufacturing environment, intermediates, and finished drug products, typically undergo characterization. This may include identification and strain typing, as appropriate. [NOTE—A *Glossary of Terms* is provided at the end of this chapter.] Routine characterization of microorganisms may include the determination of colony morphology, cellular morphology (rods, cocci, cell groupings, modes of sporulation, etc.), Gram reaction or other differential staining techniques, and certain key biochemical reactions (e.g., oxidase, catalase, and coagulase activity) that can be diagnostic. Microbial characterization to this level is sufficient for many risk-assessment purposes in nonsterile pharmaceutical manufacturing operations and in some sterile product manufacturing environments.

In some cases a more definitive identification of the microorganisms yields genus- and species-level identification. Beyond this, available methodologies can perform strain-level identification, which can be useful in an investigation to determine the source of the microorganism. Identification is especially common when organisms are recovered at atypically high rates or in numbers that exceed recommended levels for specific categories of products. Additionally, microbial identification is useful in aseptic processing and is necessary where sterility test positives have occurred and in the assessment of contamination recovered from failed aseptic process simulations, i.e., media fills.

Microbiological identification systems are based on different analytical methodologies, and limitations may be inherent to the method and/or arise from database limitations. Identification is accomplished by matching characteristics (genotypic and/or phenotypic) to an established standard (reference) organism such as a type strain. If a microorganism is not included in the database it will not be identified, so manufacturers should review the breadth of the database of the identification system they plan to use and its applicability to their needs. Users should consider which microbiological identification system(s) is (are) most applicable to their requirements. Bearing in mind both these limitations and the level of identification required (genus, species, strain), users also must select the appropriate technology to use in routine microbiological identification testing.

The need for microbial identification is specifically cited in USP general test chapter *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* ⟨62⟩. This chapter indicates a requirement for confirmatory identification tests for organisms that grow on selective or diagnostic media and demonstrate defined morphological characteristics. Also, USP general test chapter *Sterility Tests* ⟨71⟩ allows for invalidation of the test, if after identification of the microorganisms isolated from the test, the growth of this (or these) species may be unequivocally ascribed to faults with respect to the material and/or the technique used in conducting the sterility test procedure. USP general information chapter *Microbiological Evaluation of Clean Rooms and Other Controlled Environments* ⟨1116⟩ recommends that microbial isolates be identified at a rate sufficient to support the environmental monitoring program.

ISOLATION OF PURE CULTURES

The first step in identification is to isolate a pure culture for analysis. This is typically accomplished by successive streaking of the colony of interest in a quadrant pattern on appropriate general microbiological solid media with the objective of obtaining discreet colonies that usually yield pure cultures. This technique also allows phenotypic expression and growth of sufficient inoculum for succeeding identification procedures. Analysts should recognize that expression of the microbial phenotype (i.e., cell size and shape, sporulation, cellular composition, antigenicity, biochemical activity, and sensitivity to antimicrobial agents) may be affected by isolate origins, media selection, and growth conditions (see *Table 1*). Therefore, the preparatory media for identification and the number of subcultures may affect the results of phenotype identification methods.

Table 1. Phenotypic Characteristics Used in Microbial Taxonomy

Categories	Characteristics
Culture	Colony morphology, colony color, shape and size, pigment production
Morphological	Cellular morphology, cell size, cell shape, flagella type, reserve material, Gram reaction, spore and acid-fast staining, mode of sporulation
Physiological	Oxygen tolerance, pH range, temperature optimum and range, salinity tolerance
Biochemical	Carbon utilization, carbohydrate oxidation or fermentation, enzyme patterns
Inhibition	Bile salt-tolerance, antibiotic susceptibility, dye tolerance
Serological	Agglutination, fluorescent antibody
Chemo-taxonomic	Fatty acid profile, microbial toxins, whole cell composition
Ecological	Origin of the organism