

# Microbial Bioburden on Oral Solid Dosage Forms

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Most pharmaceutical companies test oral solid dosage forms for microbial bioburden on a routine basis. However, **routine testing of many products is not necessary** because the low water activity of these products will not promote the proliferation of microorganisms. Furthermore, various manufacturing processes for oral solid dosage forms create hostile environments for microorganisms. Pharmaceutical manufacturing processes are designed to prevent objectionable microorganisms in drug products not required to be sterile. Hence, it is expected that oral solid dosage forms will have a natural microbial load of nonobjectionable microorganisms.

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Life first appeared on Earth in the form of bacteria-like organisms. Cellular fossils, recovered from rocks that have been dated to 3.5 billion years old, have been identified as prokaryotic cells (bacteria and cyanophytes) and stromatolites (mats of sediment trapped and glued together by prokaryotic cells in shallow marine waters) (1,2). For billions of years, bacterial life has remained the most common and most successful form of life on Earth.

Some overzealous humans view microorganisms, and especially all bacteria, as alien foes that must be exterminated. However, sterility is an unnatural state on this planet, except for some areas of the human or animal body. From the ocean depths to the tallest mountains, from the coldest place in Antarctica to the hottest geyser in Yellowstone Park, microbial life is all around us. Whether we like it or not, this fact never will change.

Natural evolution has caused humans to adapt to cope with bacteria. In fact, to maintain good health we need bacteria in our intestines. The normal human has approximately  $10^{12}$  bacteria on some areas of the skin,  $10^{10}$  in the mouth, and  $10^{14}$  in the gastrointestinal tract (3). The number of bacteria in our body greatly surpasses that of our own eukaryotic cells.

## Normal flora of the human oral cavity

The presence of food residues, dead epithelial cells, secretions, and optimal growth temperature makes the mouth a favorable habitat for a wide variety of bacteria. Oral bacteria include streptococci, lactobacilli, staphylococci, and corynebacteria. The largest numbers are anaerobes, mostly from the genus *Bacteroides*.

## Normal flora of the gastrointestinal tract

The bacterial population of the human gastrointestinal tract constitutes a complex ecosystem. More than 400 bacterial species have been identified in the feces of a single person. The upper gastrointestinal tract (the stomach, duodenum, jejunum, and upper ileum) normally contains a sparse microflora; the bacterial concentration is  $<10^4$  organisms/mL of intestinal secretions (4).

The flora of the colon is qualitatively similar to that in human feces (see Table I). Bacteria in the colon reach levels of  $10^{11}$  bacteria/g of stool. Coliforms are prominent; streptococci, clostridia, and lactobacilli can be found regularly, but the predominant species are anaerobic *Bacteroides* and certain anaer-



**Table I: Bacteria found in the colons of humans.\***

Bacterium	Range of Incidence (%)
<i>Bacteroides fragilis</i>	100
<i>Bacteroides melaninogenicus</i>	100
<i>Bacteroides oralis</i>	100
<i>Lactobacillus</i> spp.	20–60
<i>Clostridium</i> spp.	1–35
<i>Bifidobacterium bifidum</i>	30–70
<i>Staphylococcus aureus</i>	30–50
<i>Streptococcus faecalis</i>	100
<i>Escherichia coli</i>	100
<i>Salmonella enteritidis</i>	3–7
<i>Klebsiella</i> spp.	40–80
<i>Enterobacter</i> spp.	40–80
<i>Proteus mirabilis</i>	5–55
<i>Pseudomonas aeruginosa</i>	3–11
<i>Anaerobic cocci</i>	common

\* Adapted from: K. Todar, "The Normal Bacterial Flora of Animals," in *Bacteriology* 303, Department of Bacteriology, University of Wisconsin–Madison (1997).

**Table II: Significance of microorganisms in nonsterile pharmaceutical products.**

Finished Product	Indicator Organism
Natural plant, animal, or mineral products	<i>Salmonella</i> spp.
Finished products that have raw materials from plant, animal, or mineral origin	<i>Salmonella</i> spp.
Oral suspensions and solutions	<i>Escherichia coli</i>
Topical products	<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i>
Urethral and rectal administration	yeast and molds

obic lactobacilli (bifidobacteria). These organisms can outnumber *Escherichia coli* by 1000:1 (4).

### Microbial bioburden specifications for oral solid dosage forms

The FDA guide concerning oral solid dosage forms (OSDFs) does not provide specifications about microbiological-quality criteria. Current good manufacturing practice (CGMP) requirements as specified in 21 CFR Part 211.113, Control of Microbiological Contamination, state that "appropriate written procedures, designed to prevent objectionable microorganisms in drug products not required to be sterile, shall be established and followed."

The two key words are *objectionable microorganisms*. But what is their definition? One of the few documents that addresses this concern appears on the Center for Drug Evaluation and Research's Web site (5). There, Paul Motise answers the question, "What does *objectionable* mean?" by saying:

The meaning of *objectionable* has several facets that need to be evaluated on a case-by-case basis by each drug manu-

facturer. The primary meaning relates to microbial contaminants that, based on microbial species, numbers of organisms, dosage form, intended use, patient population, and route of administration, would adversely affect product safety. Of course, most objectionable would be organisms that pose a threat of patient infection or mortality.

According to USP 24 (1111) "Microbiological Attributes of Nonsterile Pharmaceutical Products," the significance of microorganisms in nonsterile pharmaceutical products should be evaluated in terms of the use of the product, the nature of the product, and the potential hazard to the user. The publication also takes into account the processing of the product in relation to meeting acceptable standards for pharmaceutical purposes. USP 24 suggests testing for the presence of USP-specified indicator organisms for a finished product (see Table II).

In the 1960s and 1970s, several cases of contamination in OSDFs were documented, including outbreaks of *Salmonella* spp. Those cases resulted from gross violations of sanitary practices. I have not found any references to the isolation of microorganisms — following the enforcement of CGMPs — that could pose a major threat of patient infection or mortality from OSDFs.

In my evaluations I always look for examples and references from the food industry. Because tablets and capsules are oral dosage forms, the use of references from the food industry is scientifically sound. For example, "The Grade A Pasteurized Milk Ordinance" (1997 R-5 Revision, Publication No. 229) issued by the Public Health Service–FDA includes the following specifications for pasteurized milk:

- bacterial limits: 20,000 cfu/mL or g (total aerobic count)
- coliforms: not to exceed 10 cfu/mL or g.

By always using the pasteurized milk specification as an evaluation guideline, I am relying on the knowledge and experience of food scientists, microbiologists, epidemiologists, and FDA personnel who have been conducting microbial contamination and hazard analyses since 1924.

From the pharmaceutical industry point of view, the limits that are cited in the pasteurized milk specification are very high. They also are unacceptable to most industry microbiologists. Nevertheless, the limits have been approved by FDA's Center for Food Safety and Applied Nutrition. If we assume that the average person drinks 8 oz. (237 mL) of milk per day with a total aerobic count (TAC) of 5000 cfu/mL and a coliform count of 2 cfu/mL, then we can conclude that milk could provide to the average person a total of 1,200,000 cfu heterotrophic bacteria and 480 cfu coliforms per day. What difference would a tablet with 10 cfu heterotrophic bacteria make?

Tables III and IV provide two more good references — current FDA guidelines for microbiological quality criteria for infant formula (6) and medical foods (7). *Medical food* is food that is formulated to be consumed or administered entirely under the supervision of a physician. It is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements based on recognized scientific principles have been established by medical evaluation.



**Table III: FDA microbiological criteria for infant formula.**

Organism	Action Levels or Violative Criteria
TAC	>10,000 organisms/g
<i>Salmonella</i> spp.	presence
<i>Listeria monocitogenes</i>	presence
<i>Escherichia coli</i>	>3 organisms/g
<i>Salmonella aureus</i>	>3 organisms/g
<i>Bacillus cereus</i>	>1000 organisms/g

**Table IV: FDA microbiological criteria for medical foods.**

Organism	Action Levels or Violative Criteria
TAC	>10,000 organisms/g
<i>Salmonella</i> spp.	presence
<i>Listeria monocitogenes</i>	presence
<i>Escherichia coli</i>	>3 organisms/g
<i>Staphylococcus aureus</i>	>3 organisms/g
<i>Bacillus cereus</i>	>100 organisms/g
<i>Yersinia enterocolitica</i>	presence
Coliforms	>3 organisms/g

Medical foods also are specially formulated and processed for patients who are seriously ill.

The FDA guidelines made no mention of the presence of *Pseudomonas* spp., which therefore could be deemed tolerable if the total count is <10,000 organisms/g. From these guidelines we can infer that *P. aeruginosa* is not considered a pathogen when it is administered orally in concentrations <10,000 organisms/g.

It also is necessary to determine the acceptable TAC for OSDFs. If we follow the examples of the food industry, then we can say that  $\leq 20,000$  cfu/g/mL is acceptable. However, in the microbiology world, establishing precise numbers could be misleading. In addition, the pharmaceutical industry is very conservative. I therefore would prefer to settle for alert and action levels of 1000 cfu/g/mL and 10,000 cfu/g/mL, respectively. A TAC >20,000 cfu/g/mL would be unacceptable. However, when OSDFs are intended for known immunocompromised patients, as a safety factor both the alert and the action levels should be reduced by one or two log; that is, the alert level would be 100 cfu/g/mL and the action level would be 1000 cfu/g/mL.

Within the past 10 years USP has tried to improve the regulatory guidance concerning OSDFs. Its several symposia concerning this problem, however, have had limited success. A significant step forward was presented by Robert R. Friedel and Dr. Anthony M. Cundel in *Pharmacoepial Forum*, March–April, 1998. In the “Stimuli to Revision Process” section, Friedel and Cundel presented in a very rational and practical way the concept of water activity (*A<sub>w</sub>*) in the article “The Application of Water Activity Measurement to the Microbiological Attributes Testing of Nonsterile Over-the-Counter Drug Products.” They recommended that products with *A<sub>w</sub>* <0.60 such as direct-compression tablets, liquid-filled capsules, nonaqueous liquid products, and so forth, would be excellent candidates for the elimination of routine microbiological testing because of their low water activity (8).

In May 1998, Cundel, speaking at the USP Conference of Microbiology for the 21st Century, said:

The water activity strategy outlined in the *USP* stimuli article that I coauthored is that you routinely determine the water activity of your product. If the material has a water activity less than 0.85, vegetative organisms and USP indicators would not survive in that product. I submit that we would eliminate total aerobic microbial count and absence of USP indicator from the product testing. If the water activity is less than 0.75, which is below the limit for yeast and molds, I would suggest that no testing of that product be done. Products like liquid-filled capsules, nonaqueous liquid suspensions, syrups, tablets, and capsules are not really candidates for testing because of their low water activity.

In 1999, USP issued the draft of a new proposal for “Microbiological Attributes of Nonsterile Pharmaceutical Products” (1111). Although it was well intended, it went overboard on the requirements. This preview lists *Burkholderia* spp., *Pseudomonas* spp., *Candida* spp., and *Clostridium* spp. as objectionable microorganisms in tablets and capsules for immunocompromised individuals and individuals with cystic fibrosis and ulcers.

The proposed draft of *USP* (1111) was excessive for several reasons. First, *Burkholderia* spp. and *Pseudomonas* spp. are opportunistic pathogens of the lower respiratory tract in immunocompromised individuals and cystic fibrosis patients (9,10). The source of the organisms is the upper respiratory tract, not the gastrointestinal tract. The bacteria enter the lungs by inhalation, not by ingestion. As mentioned earlier in this article, exposure to these bacteria in water and food is common, and they are transient in the gastrointestinal tract (11–14). If the proposed draft of *USP* (1111) is applied to normal life, then something as harmless as taking a shower can be deemed dangerous because *Pseudomonas* spp. and *Aeromonas* spp. frequently are found in potable water. Similarly, will we have to prevent people from swimming in lakes, rivers, or oceans? Will we require that food and water be sterile for immunocompromised individuals and cystic fibrosis patients? Will they have to sterilize their toothbrushes after each use? What about kitchen utensils?

Second, *Candida albicans* infections are associated with immunocompromised patients, especially those on hyperalimentation, ventilators, catheters, and so forth. Because most patients are colonized by *C. albicans*, it is obvious that a patient is at a greater risk from their own microbiota than from the consumption of a contaminated tablet or capsule.

Third, *Clostridium* spp. is ubiquitous in soil, water, and of course, food. As indicated in Table I, *Clostridium* spp. colonize the gastrointestinal tract. With the exception of *C. botulinum*, which is monitored in the food-canning process, this genus is so ubiquitous that it cannot be used as a microbiological criterion. Again, with the exception of *C. difficile*, which proliferates only as a result of antibiotic treatment, no evidence exists that immunocompromised individuals have an increased risk of infection from *Clostridium* spp., and it may be impossible to ensure that they will not be exposed to it anyway. The same applies to *Burkholderia* spp., *Pseudomonas* spp., and *Candida* spp.

Fourth, none of these organisms would survive and prolifer-



**Table V: Minimum  $A_w$  for growth of representative microorganisms.\***

Organism (Bacterium, Fungus, or Yeast)	$A_w$
<i>Pseudomonas</i> spp.	0.95
<i>Escherichia coli</i>	0.95
<i>Salmonella</i> spp.	0.92
<i>Bacillus</i> spp.	0.90–0.95
<i>Micrococcus</i> spp.	0.86–0.93
<i>Staphylococcus aureus</i>	0.86
<i>Aspergillus niger</i>	0.77
<i>Aspergillus fumigatus</i>	0.82
<i>Penicillium chrysogenum</i>	0.79
<i>Saccharomyces cerevisiae</i>	0.80
<i>Xeromyces bisporus</i> (xerophilic)	0.61
<i>Zygosaccharomyces roussi</i> (osmophilic)	0.62

\* Adapted from Friedel and Cundel, "The Application of Water Activity Measurement to the Microbiological Attributes Testing of Nonsterile Over-the-Counter Drug Products," in "Stimuli to Revision Process," *Pharmaceutical Forum* (March–April, 1998).

ate in OSDFs because these products typically have  $A_w < 0.6$ . The decision tree in the "Preview of USP <1111>" has a cutoff value of  $A_w < 0.6$ . However, only a few xerophilic fungi and osmophilic yeasts can grow at  $A_w < 0.75$ , and these organisms can be isolated only in unique environments and are not normally associated with infection. Isolation of xerophilic fungi and osmophilic yeasts requires specialized microbiological media with a reduced water activity to be cultivated. Therefore, they cannot be isolated in routine or compendial microbiological media (15,16).

### The significance of water activity

The measurement of water activity has been used widely in the food industry to assess methods for preserving food. 21 CFR 110, Current Good Manufacturing Practice in Manufacturing, Packing, or Holding Human Food, defines water activity as "a measure of the free moisture in a food and ... the coefficient of the water vapor pressure of the substance divided by the vapor pressure of pure water at the same temperature."

Water activity is a critical factor for determining the shelf life of solid foods, and it is therefore logical to apply it to OSDFs. Whereas temperature, pH, and several other factors can influence the amount and speed of growth of organisms in a product, reducing the water activity levels may be the most important factor for controlling microbial growth and spoilage. Most bacteria, for example, do not grow at  $A_w < 0.91$ , and most molds cease to grow at  $A_w < 0.80$ . By measuring water activity, it is possible to predict which microorganisms will and will not be potential sources of contamination and spoilage. Water activity, not water content, determines the lower limit of available water for microbial growth (16–19). It is common wisdom within the pharmaceutical industry that OSDFs (tablets and capsules) typically have  $A_w$  of 0.3–0.6.

Water activity instruments measure the amount of free water present in a sample. A portion of the total water content in a product is bound strongly to specific sites on the chemicals that

make up the product. These sites can include the hydroxyl groups of polysaccharides, the carbonyl and amino groups of proteins, and other polar sites. Hydrogen bonds, ion–dipole bonds, and other strong chemical bonds hold water in products. Some water is bound less tightly, but it is still not available as a solvent for water-soluble food components. Drying, concentration, dehydration, and freeze-drying are widely available processes for reducing the amount of free water in both food and pharmaceutical products. Because water is present in free and bound states to varying degrees, analytical methods that attempt to measure the total amount of water in a sample do not always yield the same results. Hence, water activity truly indicates the amount of water available for microbial growth (18). A safe moisture level is specified by 21 CFR Part 110 as

a level of moisture low enough to prevent the growth of undesirable microorganisms in the finished product under the intended conditions of manufacturing, storage, and distribution. The maximum safe moisture level for food is based on its water activity ( $A_w$ ). An  $A_w$  will be considered safe for a food if adequate data are available that demonstrate that the food at or below the given  $A_w$  will not support the growth of undesirable microorganisms.

### The most-common isolated bacteria from OSDFs: pseudomonads

Pseudomonads are distributed widely in nature and thrive wherever there is water (20,21). Therefore, not surprisingly, they are isolated from products not intended to be sterile. Some are classified as opportunistic pathogens of humans. An *opportunistic pathogen* is defined as any microorganism capable of adapting and causing disease in a tissue or host other than the normal one (22). *P. aeruginosa* is the type species of the genus *Pseudomonas*. *P. aeruginosa* and like bacteria are so common that they can be isolated routinely from fruits and raw vegetables intended for human consumption (11,23). These organisms ordinarily are not recognized as pathogens when they are administered orally. In fact, *P. aeruginosa* can be isolated from healthy humans. It is not uncommon to find it in the microbiota in the upper respiratory tract, the large intestine, and on the skin.

Most of the time *Pseudomonas* spp. and like bacteria are recovered from enrichment procedures intended to isolate *E. coli*, *Salmonella* spp., and *S. aureus*. Enrichment procedures are intended to allow the proliferation of small numbers of microorganisms that may be present in a sample and that cannot be detected by routine TACs. If only one bacterium is present in the sample (usually 10 g), then it will multiply to millions of bacteria within a couple of days. Therefore, the enrichment procedure is not representative of the true microbial load of a sample. In most cases enrichment procedures have a sensitivity of  $\geq 1$  cfu/g, which is at least 10 times higher than the sensitivity of the TAC ( $\geq 10$  cfu/g).

### The hazard to consumers

OSDFs with low water activity will not promote the proliferation of pseudomonads or other microorganisms. *Pseudomonas* spp. cannot proliferate at  $A_w < 0.9$ . No microorganism can pro-





liferate at  $A_w < 0.6$ , which is the cutoff point for all microbial life.

OSDFs are seldom, if ever, the cause of infections in hospitals, where the predominant sources of infections are surgical and invasive procedures and nosocomial infections. Also, pharmaceutical products are not considered the cause of infections in the immunocompromised population and cystic fibrosis patients. The major risks to these individuals come from the environment (e.g., air, water, food, pets, and humans) and hospitals.

Infectious diseases acquired orally are dose dependent. For example, as few as 10 bacteria are required to cause bacillary dysentery (dysentery caused by *Shigella* spp.) (24). On the other hand, depending on the strain, the number of cells needed to cause salmonellosis may range from 15 to more than 100,000 salmonella cells (25,26). *P. aeruginosa* ordinarily is not recognized as a pathogen when it is administered orally, and for that reason an infectious dose does not exist.

Unfortunately, the pathogenic characteristics of *Pseudomonas* spp. are highly overstated. Iglewski reported that  $\sim 10^9$  bacteria of *P. aeruginosa* are required to initiate an infection in healthy eyes of mice, whereas in a scratched eye from  $10^4$  to  $10^5$  bacteria are required (27). Both figures are very high when they are compared with the infectious dose for *Bacillus anthracis*. Only one spore or cell from this bacterium is capable of starting an infection in healthy humans. In another experiment, *P. aeruginosa* was given orally to volunteers at a concentration of as much as  $2 \times 10^8$  bacteria. Although the bacteria were recovered from stools, no clinical illness resulted (28).

FDA's Center for Food Safety and Applied Nutrition did not include *P. aeruginosa* in the "Bad Bug Book" (*Foodborne Pathogenic Microorganisms and Natural Toxins Handbook*, 1992) (26,27). Furthermore, Doyle et al. did not include *P. aeruginosa* in their chapter about foodborne pathogenic bacteria (29). More examples exist that are similar to those that are presented in this article. Hence, according to current literature, *P. aeruginosa* is not an oral pathogen.

As an example of the pharmaceutical industry's misconceptions about bacterial contaminants, during an FDA inspection I was asked about the danger of endotoxins in oral dosage forms. I replied that it is pretty obvious that absorption of endotoxins in large quantities through the intestines is incompatible with life in mammals because most of the bacteria inside the intestines are Gram negative. It was not the first time that I have heard that question, even from colleagues. It is an empirical fact of life that microbial endotoxins are normally present in the human gut without causing harmful effects (30). Otherwise, the human race would not survive.

## The hazard to products

The bacteriological hazard to humans from a product is negligible because of the very low levels of pseudomonads ( $\leq 10$  cfu/g) usually found in OSDFs. Also, the low water activity of these products will not promote the proliferation of *Pseudomonas* spp. or other microorganisms. As time passes, any surviving bacteria in the product will die.

In addition, we must consider the manufacturing processes for OSDFs. The two most common stepwise procedures in

manufacturing processes are dry blending of raw materials and tableting-encapsulation; and fluid-bed granulation, milling, blending, and tableting-encapsulation. Contrary to some perceptions, manufacturing processes for OSDFs provide hostile environments for microorganisms because of the conditions created during the various manufacturing steps. The following paragraphs describe these stepwise procedures in more detail.

**Dry blending of raw materials and tableting-encapsulation.** In this manufacturing process, no water is added. Therefore, the water activity of the final blend results from the interaction of the raw materials. In most cases, the water activity is dictated largely by the raw material with the highest concentration (e.g., lactose or microcrystalline cellulose). The final step is either tableting in a rotary tablet press or encapsulation. Tableting could reduce the amount of viable organisms by as much as 100% (31,32). This reduction is achieved by at least three factors. The first two factors are the high pressure and heat formation typical in tablet compression. The third factor is the reduction of the amount of water in the product, which thereby diminishes the water activity of the final product. Encapsulation is not as harsh a process as tableting, but it still does not create conditions conducive to microbial proliferation.

**Fluid-bed granulation, milling, blending, and tableting-encapsulation.** Contrary to what might be expected, microorganisms are killed during fluid-bed granulation and drying processes. More than 70% of the microbial bioburden is killed during drying (33,34). Tableting further decreases the amount of microorganisms present in the product.

## The cost of reducing bacteria

In the past, most disinfectants and antibacterials were used only in hospitals. However, within recent years these substances have been added to regular detergents and soaps intended for household use. According to a popular consumer magazine, between 1997 and 1999, manufacturers introduced more than 700 everyday products labeled *antibacterial* or *disinfectant* (35). This trend induces consumers to think they should sterilize their homes to prevent diseases. No evidence exists that the addition of these substances to household products reduces the risk of contracting diseases. On the contrary, the use of antibacterial cleaners and disinfectants at home may contribute to the problem of antibiotic resistance. "Even if these products really could [destroy all bacteria]," said Stuart B. Levy, MD, director of the Center for Adaptation Genetics and Drug Resistance at the Tufts University School of Medicine, "it's best to preserve the bacteria we're biologically adapted to rather than change our microbial environment and perhaps let unfamiliar, potentially harmful bacteria emerge" (36).

Many people, including government and industry officials, think that food and oral drugs also must be sterile. However, this attitude is not in the best interest of the consumer. Sterilization of food and drugs is expensive, and the extra cost, when it is not necessary, is paid by the consumer and is a waste of money. The same applies when an OSDF product is rejected because it contains low levels of pseudomonads. The product is fit for use; it has no pathogens. The sterility syndrome, how-



ever, induces people to think that the product is a hazard to the consumer. The extra cost of manufacturing another lot and complying with additional requirements is passed to the consumer. "The time has come for global society to accept bacteria as normal, generally beneficial components of the world and not to try to eliminate them, except when they give rise to disease," Levy stated (36).

## Conclusion

Low levels of *Pseudomonas* spp. and similar microorganisms administered orally are very unlikely to present a risk to patients taking OSDFs. Neither are OSDFs considered the cause of infections in immunocompromised and cystic fibrosis patients. The major risks to these patients come from the environment and hospitals.

It should be noted that FDA and USP have no regulatory requirements or specifications about testing for the absence of pseudomonads in OSDFs. This activity should be considered valueless except when required in a specific monograph. Therefore, it is a questionable practice to isolate and identify bacteria that do not show the characteristic colonial morphology in the specified media when subcultured from the enrichment media for *Salmonella* spp., *S. aureus*, and *E. coli*.

## Recommendations

For OSDFs that have  $A_w < 0.85$ , testing for TAC and USP indicator organisms should not be performed. If  $A_w < 0.75$  exists, then no microbiological testing of that product should be done. Data to support this approach must come from development and validation activities.

Acceptable TAC for OSDFs should be established in terms of alert and action levels, which could be 1000 cfu/g/mL and 10,000 cfu/g/mL, respectively. A TAC that is  $> 20,000$  cfu/g/mL is unacceptable. For OSDFs intended to be used by known immunocompromised patients, as a safety factor the alert and action levels should be reduced by one or two log.

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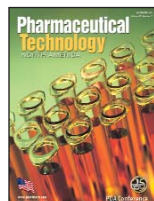
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