

The Keys to Successful Product Preservation

Wouldn't it be nice if someone would sit down with you and tell you everything you need to know—about life, caring for family and friends, getting along with other people, being successful in your career, being healthy—just anything and everything you need. Unfortunately, I can't tell you this. But, I can tell you a few things about Cosmetic Microbiology. This chapter discusses several articles I have written on preservation and summarizes many of the things I feel are essential to preserving products in what I call “the keys to successful product preservation.”

For me, preserving products began when I started working for a major cosmetic manufacturer in Cincinnati in 1978. I was shown how to perform the Cosmetic, Toiletry & Fragrance Association (CTFA) method for performing preservative efficacy tests¹ by the Manager of Microbiology. (Note: The CTFA name was changed to the Personal Care Products Council [PCPC] in 2008, but I will use CTFA throughout this chapter.) I compared this to the USP compendial method² and capacity tests and streak methods that had been used for preservative testing.^{3,4} All of these methods involved challenging the test samples with several microorganisms and checking for the number of surviving microorganisms at subsequent time intervals to determine how fast the preservative system inactivated the challenge microorganisms. It was possible for preservative efficacy testing to take several weeks, and the testing could take two months if rechallenge studies were performed.

Up until the early 1980s, formaldehyde and formaldehyde-donors were used as preservatives in cosmetic products. Rinse-off products (i.e., shampoos) frequently were preserved with formaldehyde and parabens, and leave-on products (i.e., creams, lotions) were preserved with formaldehyde-donors, methylparaben (MP) and propylparaben (PP). Generally,

the preservative systems in these products killed the test bacteria quickly. I performed tests initially (at time 0), at 2, 4 and 24 hours, and at 3, 5 and 7 days after adding test organisms to product sample. It was not uncommon to not recover *Pseudomonas aeruginosa* at any time point—not even initially at time 0.⁵ Most products killed 10^6 – 10^7 cfu/mL of the test bacteria in test products within a couple of days and 10^5 – 10^6 cfu/mL yeasts and molds in test products within a week.

Life in the Micro Lab was good because the bugs were killed relatively quickly, and they didn't "reappear" at later time points so I didn't need to continue trying to recover the test organisms at 2, 3 and 4 weeks, once I had determined that they had been killed. Elimination of this useless (non value-added) testing enabled me to perform full preservative efficacy tests (with *S. aureus*, *P. aeruginosa*, *Burkholderia cepacia*, *Escherichia coli*, *Bacillus* spp., *Candida albicans*, and *Aspergillus niger*) on 25–50 products each month. I didn't know how lucky I was—the powerful preservatives killed the test organisms so quickly that tests could be completed and results reported in less than two weeks, so there were virtually no micro delays in product development. Of course, this was a competitive advantage because our formulators could get results on their formulas in two weeks and not have to wait up to two months like formulators working for some of our competitors.

2.1. Overview of Preservative Efficacy Testing

Preservative efficacy testing, or challenge testing, is performed on aqueous cosmetics and drug products to determine the minimum effective concentrations of preservatives required for adequate control of microbial contamination. Products are satisfactorily preserved if they meet appropriate acceptance criteria and if they are packaged properly.⁵

Several methods of preservative efficacy testing are used including official methods such as the USP, the European Pharmacopeia (EP), and the Japanese Pharmacopeia (JP); trade association methods such as the CTFA method; and rapid methods such as the linear regression method, the accelerated preservative test, the presumptive challenge test, and the rapid screening method.^{1–8}

2.2. Linear Regression Method for Rapid Determination of Cosmetic Preservative Efficacy

With so many choices available, it seems obvious to ask which method is the best? Which is the most suitable for you or your company? The techni-

cally correct answer, of course, is the one that is the most reliable; however, people may want to introduce other factors such as speed (how fast is it?), difficulty (is it hard to do?), whether it is a compendial or trade association method used throughout the industry (which would suggest it would have tacit regulatory approval), acceptance criteria (are the criteria appropriate and in agreement with company guidelines?), cost (how much does it cost for one test?), and probably other factors.

Let's take a look at the methods to see if we can determine which one is the best. All of the methods have similarities, including the test organisms used, recovery systems, and the method of performing APCs. Differences in the methods include growth temperatures, procedures for preparing inocula, times at which APCs are determined, use of rechallenge testing, and acceptance criteria. These differences may cause variations in test results and affect whether a product passes or fails the challenge test. In addition, there has been a trend to implement automation and miniaturization of test methods in recent years. Because these things may help save time, improve reliability, and reduce costs, automation and miniaturization should also be considered.

In microbiological testing, my advice has always been to select the most conservative testing criteria because the risks are too high (e.g., destruction of batches, product recalls, bad PR, adverse consumer reactions, etc.) if we are wrong. Thus, I have always advocated stringent acceptance criteria—the ones that require the test organisms to be killed fastest during challenge testing, and the ones that require finished aqueous products to meet release criteria of < 10 cfu/mL by plating or “none found” on enrichment. This has not always been popular because some microbiologists may have to concede that they may have been wrong in recommending less stringent acceptance criteria for their companies, and test methods/procedures may need to be changed. It is the right thing to do if: 1) house organisms are recovered occasionally (or frequently) during product release testing (and this could be demonstrated by a metric such as the *retest frequency*, which is the percentage of positive samples that need to be retested), 2) you occasionally need to destroy batches of product due to microbial contamination, or 3) you have had a product recall (i.e., stock recovery or withdrawal) due to microbial contamination. The only thing I can say is... you know what to do.

Reliability is the most important consideration in selecting a method—if a test isn't reliable, then using it is a waste of time. Reliability depends on precision, sensitivity and accuracy. The precision of microbiological test results generally depends on the skill and care used by the microbiologist

(i.e., can they get the same result if they perform the test three times on different days?); whereas the sensitivity and accuracy reflect the ability of the method to recover all viable microorganisms present in the test samples. This is where validation of test methods is valuable (if done properly!). The reason I mention it this way is that validation of recovery systems (diluent and plating media or enrichment broths) typically uses recovery of low levels (1–10 cfu/mL) of selected cultures that often have been standardized to provide known levels of these microorganisms. That's fine most of the time, but what about injured microorganisms? If you have been having problems with bacterial “rebound” or the Phoenix phenomenon (see **Section 5.6**), then you may need to consider validation with injured microorganisms. Again, this is more conservative/rigorous than conventional validation, and it's not what everyone else is doing, but it may be what you need to get to the root cause of microbiological contamination problems.

Of all the methods named above, only the linear regression method uses statistical controls to ensure that the testing is “in control.” Here's how testing was done when the method was published in 1979.

Test Microorganisms: The test microorganisms used were *S. aureus* (FDA 209 strain), *P. aeruginosa* (PRD 10 strain), *Bacillus* spp. (isolated from a contaminated cosmetic product), *A. niger* and *Aspergillus flavus*. The bacteria were grown on Standard Methods Agar (SMA, Baltimore Biological Laboratories, Baltimore, MD) slants for 24 hours at 37°C, and the molds were grown on Potato Dextrose Agar (PDA, Difco Laboratories, Detroit, MI) slants for 7 days at 25°C. The inocula were prepared by transferring growth from a single slant to 9 mL sterile saline and mixing using a Vortex Genie Mixer (Scientific Products, Columbus, OH). This was repeated for each test microorganism because the method used pure culture inocula, not pooled suspensions of test microorganisms. The percentage of sporulation of the *Bacillus* spp. culture was typically 30–50% at 24 hours.

Test Procedure: A 0.1 mL amount of the saline suspension of test microorganism was added to a 50 mL portion of the test sample in a 100 mL screw capped jar. This was repeated for each test microorganism. Immediately thereafter, the jars were shaken vigorously 100 times, and 11 mL were pipetted into 99 mL of Letheen Broth (Difco Laboratories) with 0.01% (vol/vol) Triton X-100 (Sigma Chemical Co., St. Louis, MO) in milk dilution bottles. This diluent was designated LBT. The bottles were shaken vigorously 50 times, and 1:100 dilutions were made by transferring 1 mL to 99 mL of LBT and shaking 25 times. Then, 0.1 and 1.0 mL amounts of appropriate dilutions were pipetted into duplicate Petri dishes, and pour plates were prepared by adding Tryptic Soy Agar (Difco laboratories) with