Preservative Testing – Choice of Challenge Isolates

By Dr. Philip Geis

"What you see is that the most outstanding feature of life's history is a constant domination by bacteria."~Stephen Jay Gould

An effective preservative capability is a central element of drug, cosmetic and household and institutional product microbiological risk assessment and quality. Such a capability can mitigate both incidental low-level contamination from a controlled manufacturing system, as well as introduction of contamination through typical, expected product use; establishing appropriate product quality for the life of the product. Whereas some products do not require frank preservation by design (e.g. aseptic production/single use), formulation (e.g. high ethanol contact, extremes of pH and anhydrous) or constraining dynamics of use (e.g. near term expiration dates, refrigeration), chemical preservation and preservative qualification remain a primary element of quality for the majority of aqueous consumer products.

Preservative Effectiveness Testing

As is seen with cosmetics, a relatively small set of preservatives are used to establish and maintain microbiological quality (1). Unfortunately simple addition alone or in combination is not sufficient to assure efficacy. The critical preservation capability must be demonstrated by preservative effectiveness test (PET) protocols that, as with preservatives themselves, is product category specific. Relevant PET's are USP <51> for drugs (2), AOAC protocol developed by the Personal Care Products Council for cosmetics (3), and a variety of methods (e.g. 4,5) for household and institutional products. A central element of each tests is consistency driving to a level of preservative efficacy that accommodates realities of microbial contamination risk in manufacturing

and product use.

These tests are relatively similar in that each exposes the product to an exaggerated microbial challenge, monitors survival over a 4 week period and derives a pass/fail determination-based comparison of microbial reduction observations to established criteria. The primary functional difference between category methods is the inoculum - the microbes used.

Drug PET

As drug products are typically made under conditions of stringent hygiene and enjoy protective packaging, storage and expiration labeling, USP <51> with its small number of clinical isolates and modest kill rate expectations is more aligned to low-level potential contamination in making and use. The isolates indicated by <51> (Table 1) are primarily of clinical origin and sourced from the American Type Culture Collection. With original isolations ranging from 50 to almost 80 years ago, these isolates can be considered laboratory-adapted strains. None apparently bears resistance plasmids (6) but little additional information is available. The combination of GMP's, USP 51-driven efficacy, packaging and typical use appears to have been successful in maintaining a high standard of overall microbiological quality in for this product category.

Table 1: USP PET Challenge Microorganisms

Isolate	ATCC® No.	Date Submitted	Source	Comments
Staphylococcus aureus	6538™*	1930/1940	Clinical FDA	• BSL 2 • PEG's are inhibitory • Some potential for resistance to high pH • Min. Aw for growth 0.86 • Agar grown cells more sensitive than broth grown (cationic biocides)
Pseudomonas aeruginosa	9027™*	1943	Clinical	• BSL 2 • Similar disinfectant resistance to hospital isolates • Agar grown cells more sensitive than broth grown (cationic) • Broth grown cells give more reproducability in preservative testing

Escherichia coli	8739™*	1949	Clinical	• BSL 1 • Centrifuged/washed cells more sensitive to PHMB • Agar grown cells more sensitive than broth grown (cationic) • Broth grown cells more reproducible in preservative tests
Candida albicans	10231™*	1950	Clinical	• BSL 2 • > UV resistance than USP bacteria • Mycelial formation at acid pH
Aspergillus brasiliensis	16404™*	1965	Botanical (blueberry)	• BSL 1 • Potential for biocorrosion

Cosmetic PET

Although some have used the USP method for qualification of cosmetic preservative systems (7), most cosmetic manufacturers use protocols based on the compendial method developed and qualified by the Microbiology Committee of the Personal Care Products Council (3). This protocol uses the USP 51 isolates and additional microbial isolates representative of species reported as manufacturing or in-use contaminants. A recent casual poll of major manufacturers found most using modified PCPC methodology with in-house manufacturing and consumer return isolates such as those described by Brannan et al. (8). This is not a trivial consideration as clinical isolates may be quite different from those recovered from the general environment (9). Unfortunately, commercial availability of authentic isolates is currently very limited, so most of these originated from clinical origins as with the USP isolates (Table 2). A rationale behind such additions would be that these microbes may persist at low levels in manufacturing systems so specific practical efficacy is assured. Further, some wish to establish of efficacy in context of isolates capable of developing tolerance or even resistance to preservatives. Though such capabilities may not be immediately expressed in subsequent laboratory culture, the genetic capability is presumably sustained.

Overall, cosmetic microbiology appears to have established the greatest control over microbial contamination. Reviews of FDA recall records for microbial contamination (10,11) have consistently found that annual recalls involving cosmetics were substantially fewer than those for foods or drugs.

Table 2: PCPC Additional PET Challenge Microorganisms

Isolate	ATCC® No.	Date Submitted	Source	Comments
Enterobacter gergoviae	33028™*	1948	Clinical (urine) CDC	• BSL 2 • Type species • France - Institute Pasteur
Burkholderia cepacia	25416™*	1969	Botanical (onion)	• BSL 2 • Similar disinfectant resistance to hospital isolates • Cepobactin production (nonclinical isolate) • Lipase production
Acinetobacter baumannii	19606™*	1966	Clinical (urine)	• BSL2 • Type species • Poor survival on dry environmental surfaces • Biofilm forming
Klebsiella pneumoniae	10031™*	1946	Clinical FDA	• BSL 2 • Aw 0.96 is limit for growth, • Plasmolysis observed at Aw 0.93, rapid loss of viability at 0.88 • Disinfectant resistance similar to hospital isolates • Antibiotics assay EP/USP/AOAC • Poor to no capsule formation
Staphylococcus epidermidis	12228™*	1955	Clinical FDA	• BSL 1 • Plasmids • Lipase and chitinase production • Does not form biofilm • Heavy metal resistant • Antibiotics assay

	USP/EP/AOAC

PET for Everything Else

As household and institutional products are rarely produced on manufacturing systems that establish effective microbiological control, the primary microbial component of challenge testing are manufacturing isolates. Base, unpreserved formulations of most household products would “pass” a USP 51 protocol due to formula characters including high or low pH, surfactant and salt concentrations, etc. So clinical isolates are less relevant to qualification of preservative efficacy for these products as their basic formulation, packaging and intended use militate against contamination in use. Contrast this with manufacturing where continuous challenge in absence of GMP’s that allows adaptation under relatively harsh conditions of microbes some might see as extremophiles (12,13,14). In-use contamination is a concern primary for those products whose use demands aqueous dilution. Even more than cosmetics, manufacturers in these categories rely on authentic manufacturing isolates to validate preservative efficacy.

Conclusion

Successful risk assessment of any product requires consideration of manufacturing risk, preservation, package design and intended product use. Design of preservative efficacy testing, and most importantly selection of isolates against which efficacy is demonstrated, is central to establishing and maintaining product microbial quality. Compendial inocula drive to standard measure of general efficacy and many supplement such challenge with isolates more aligned to specific microbial risks relevant to formulation, manufacturing and product use. Though some attempts have been made to source such isolates (15), these remain primarily remain in-house and proprietary. Systems developed in absence of a clear understanding of microbial risk in making, use and anticipated misuse may well suffer substantial and surprising contamination.

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Biography

Dr. Geis earned a PhD in microbiology and mycology from the University of Texas. His career in microbiology began at a clinical lab in the US Army, moving to commercial media production, and in 1981 to The Proctor & Gamble Company (P&G). Through almost three decades with P&G microbiology, Phil managed preservative and disinfectant development and studies of household and skin microbial ecologies and hygienic manufacturing. He was the first recipient of P&G's namesake award – Dr. Philip Geis Microbiology Quality Award. Dr. Geis brings unique global expertise and experience in diverse regulatory, manufacturing, product quality and consumer realities for a broad range of products from OTC drugs to fabric softeners to dog food.

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