Preservative Efficacy Test

2020 Practitioners Survey

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Personal care products comprise a vast array of forms, intended applications, and associated consumer habits and practices (CHP). From a microbiological standpoint, the properties of personal care products occupy a unique niche:

- mild, often aqueous in composition
- intimate contact with consumer
- often subject to ongoing contamination
- extended shelf life
- little post purchase microbial control (e.g., refrigeration)

Preservation, therefore, plays a critical role in ensuring that the product, as manufactured and during intended CHP, remains safe for use and is adequately resistant to loss of product stability/function.

The role of the product development microbiologist is to design microbial robustness into the product. One of the key tools to help establish the microbial robustness is the preservative efficacy test (PET), widely practiced in the personal care industry. The basic protocol consists of inoculating product with relevant microorganisms and monitoring viability over time, as shown in Figure 1.

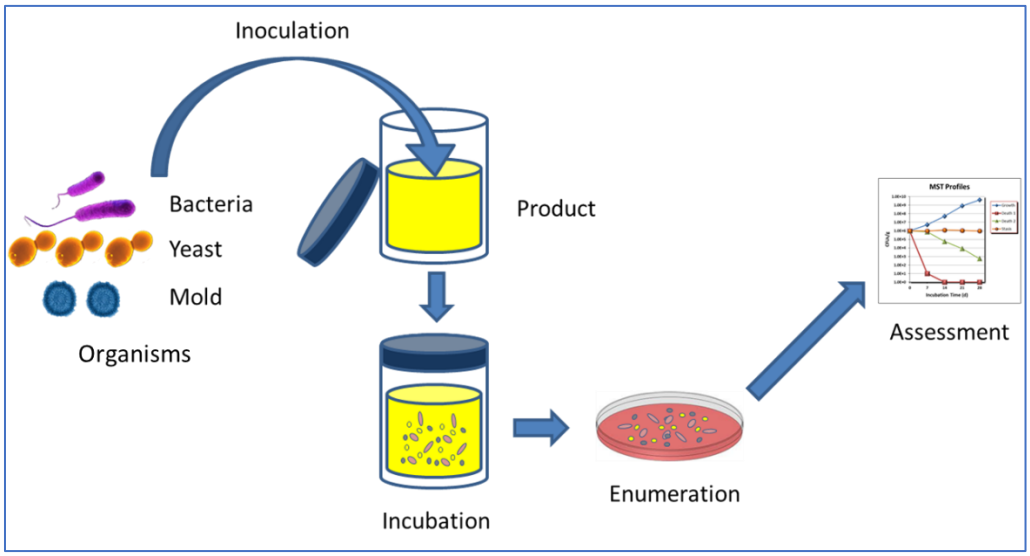

Figure 1. General schematic of preservative efficacy test (PET) protocol

However, within each step of the protocol, there is potential for a wide latitude of practice for parameters, execution and interpretation. The goal of this survey was to landscape PET protocols, practices, success criteria, assessment, and other related perspective. The survey questions were developed and vetted by the Personal Care Product Council (PCPC) Microbiology Subcommittee, and were organized into categories reflecting key parameters of PET. The survey was conducted anonymously with one collated entry per company. Most questions had between 19 and 26 company respondents, with conditional questions having 11 or more respondents.

Overall PET

The first part of the survey landscaped general practices with regard to PET testing. Companies (N=22) leverage both internal and external resources to perform PET. Just over half of companies performed PET internally only, 12% used only external testing, and 36% employed both internal and external testing resources. PET protocols are described in a number of resources, including pharmacopeias (e.g., United States Pharmacopeia (USP), European Pharmacopeia (EP)), the PCPC Microbiology Guidelines, FDA-Bacteriological Analytical Manual (BAM), etc. Companies indicated that they employed a number of PET protocols, with over 80% using protocols developed in-house, as shown in Figure 2.

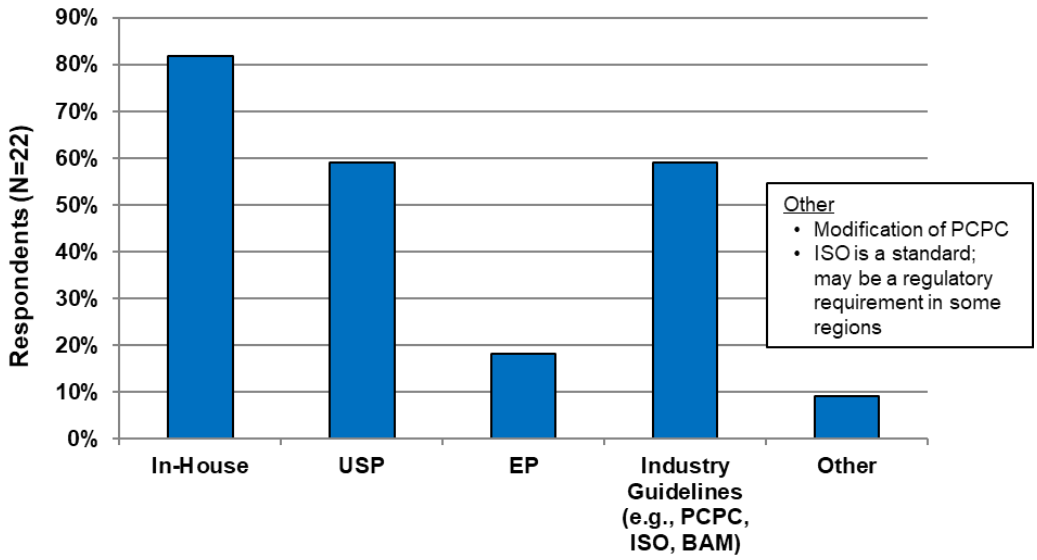

Figure 2. Protocols used for PET

Organisms

The selection of organisms to include in PET is one of the most important considerations in assessing the effectiveness of preservation. While reference protocols are somewhat consistent in identifying a core group of organisms for inclusion, the PET practitioner has wide latitude to include other organisms (both reference and environmental strains) relevant to ensuring a robust product. Companies typically have the highest degree of knowledge as to the type of organism(s) which present the highest risk throughout the product life cycle.

Over 90% of companies conducted PET using the reference strains described in the United States Pharmacopeia (*P. aeruginosa ATCC 9027; S. aureus ATCC 6538; E. coli ATCC 8739; C. albicans ATCC 10231; A. brasiliensis ATCC 16404*). Most companies (59%) also used other reference strains and 36% of companies used in-house/environmental strains. For those companies using in-house/environmental bacteria in their PET, the most frequent additions were gram-negative bacteria, with *Burkholderia*, *Enterobacter*, *Klebsiella*, and *Serratia* identified as the most common genera included (61%, 39%, 33%, and 22%, respectively). Only 14% of companies overall included an in-house/environmental fungi, with Penicillium being the only genera cited.

Inoculation

The parameters used to prepare inoculum, including microbial growth stage at harvest and whether cultures are prepared as pure or mixed cultures, are potential sources of variability in the PET. Company responses indicated a diverse array of approaches for the inoculation aspect of PET. Most companies prepared bacterial inoculum from either late exponential phase (42%) or exponential phase (26%) cultures. The remaining companies were split between using either stationary phase or mixed phase cultures for inoculum. For organisms with both spore and vegetative states (e.g., bacillus, mold), 43% of companies used spores only for inoculum, 19% used only vegetative cells, and 38% used a mix of spores and vegetative cells. Most companies (57%) introduced inoculum into the product using separate cultures. The remaining companies introduced inoculum as either separate bacterial and fungal pools (29%) or as multiple bacterial and fungal pools (14%). The final inoculum target for bacterial and fungal inoculum were closely clustered in the range of 10^5 to 10^6/g or mL, as shown in Figure 3.

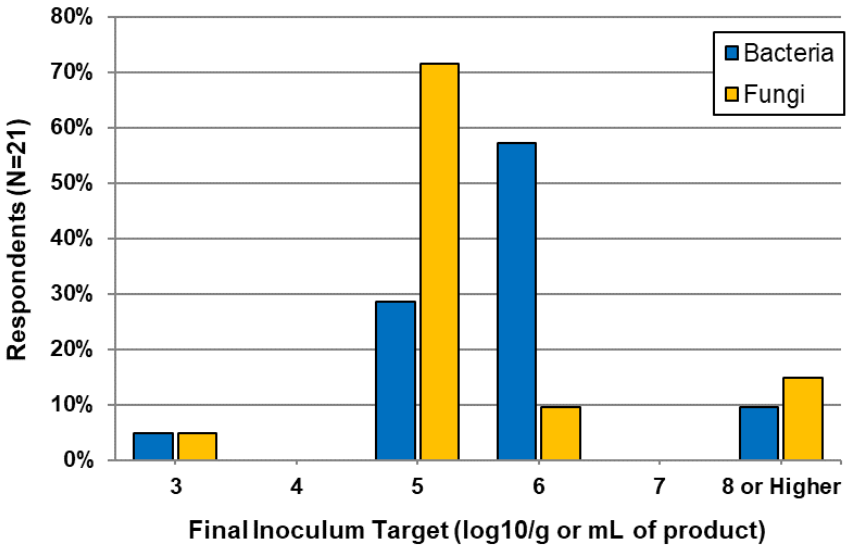

Figure 3. Bacterial and fungal inoculation targets for PET

Almost half of all companies (47%) used rechallenges as part of their PET protocols, with more than half of rechallenge practitioners using 2 or more rechallenges.

Product

Companies conducted PET during product development and throughout the scale up cycle. Companies were asked to describe their frequency of conducting PET (for both aged and unaged product) for each batch scale (i.e., lab, pilot, plant trial, production). Over 90% of companies conducted PET at lab scale. The frequency of PET conducted for pilot scale, plant trial scale and production scale was 60%, 48%, and 64%, respectively. Companies indicated that PET results were highly consistent during scale up. Significant differences in PET results during scale up occurred at a frequency of <10% for all companies, with 28% of companies indicating that they never experienced significant differences in PET results during scale-up. The majority of companies (73%) used the same PET protocol for pre-launch formulations and for confirmatory testing of products in-market. Just under 75% of companies evaluated only 1 lot of formulation during PET, while just over 20% evaluated 3 or more lots.

Most companies (63%) evaluated multiple preservative concentrations to help establish minimum efficacy levels. When asked to describe their testing strategy across product variants, 44% of companies indicated that a separate PET was conducted for each product variant. The remaining companies were evenly split (28% each) in either testing only those variants assessed having the lowest hostility, or testing product variants using a bracketed approach. For the accelerated PET, 44% of companies aged product in the final package, with the remaining companies evenly split (28% each) in either not aging product in the final package or using both approaches. When asked whether water activity (A_w) was considered as a basis for PET exemption, 58% of companies indicated that formulations having A_w of $\leq$0.60 were exempted from PET, while approximately 16% exempted formulations having A_w of $\leq$0.70 from PET. The remaining companies (26%) did not use A_w as a basis for PET exemption.

Incubation

For accelerated stability testing, most companies (72%) used an incubation temperature of 40°C, while 17% of companies used an incubation temperature of 45°C (17%). Other temperatures cited include 30°C and 50°C. Incubation periods of 1 month and 3 months were the most common time intervals for aging product prior to conducting PET. The incubation periods used in accelerated stability prior to conducting PET are shown in Figure 4.

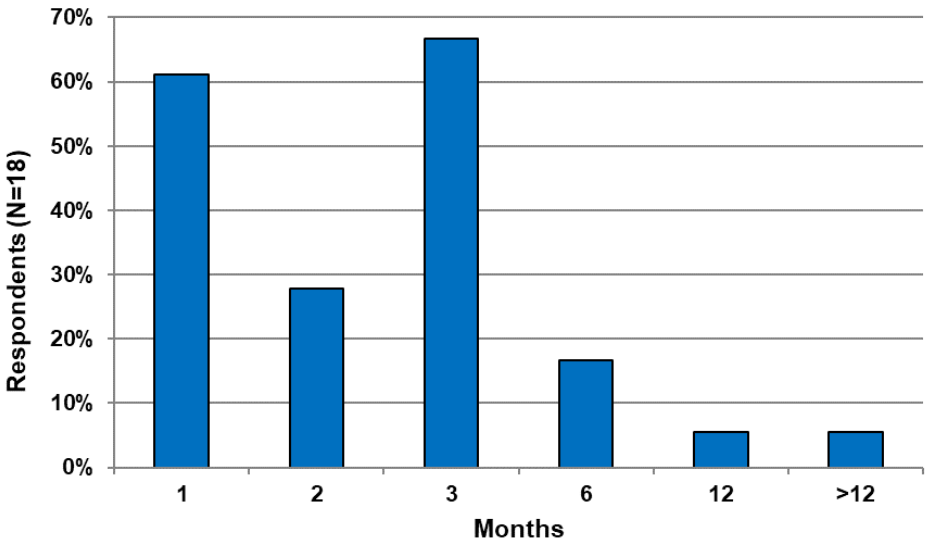

Figure 4. Incubation periods prior to conducting PET during accelerated stability

Enumeration

Companies indicated a wide variety of enumeration timepoints within the PET, ranging from time zero (at inoculation) up to 8 weeks. The most frequent enumeration timepoints cited by companies were at Time 0, 1 week, 2 weeks, 3 weeks, and 4 weeks. as shown in Figure 5.

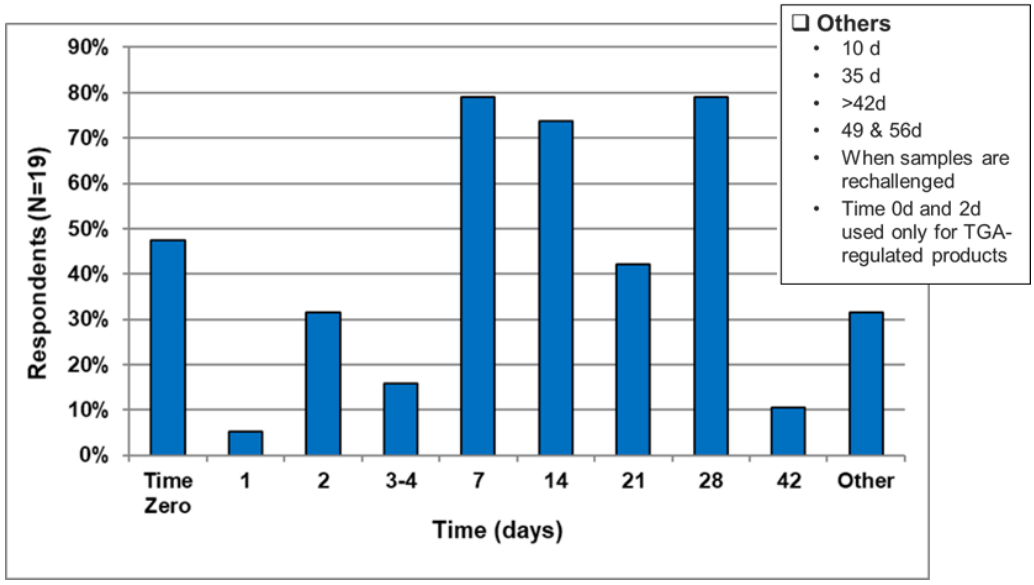

Figure 5. Timepoints for organism enumeration within a PET

Rapid Methods

With the broad array of organisms, protocol variations, and many enumeration timepoints, companies were asked whether they leveraged rapid or alternative methods

(RAM) in place of traditional agar plating techniques. Most companies (67%) did not use RAM as part of their PET protocols. Those companies using RAM focused use of these techniques mainly on lab scale formulation development. For companies currently using or strongly considering RAM for PET, there was no clear favorite technology. The 2 highest preferences – automated MPN and spiral plating – were practiced by only 2 companies each. Companies were asked to identify the biggest challenges (choosing up to 3) for implementing RAM for PET. The most cited challenge was method validation (72%), followed by regulatory acceptance and data translation vs. traditional methods at 50% each, as shown in Figure 6.

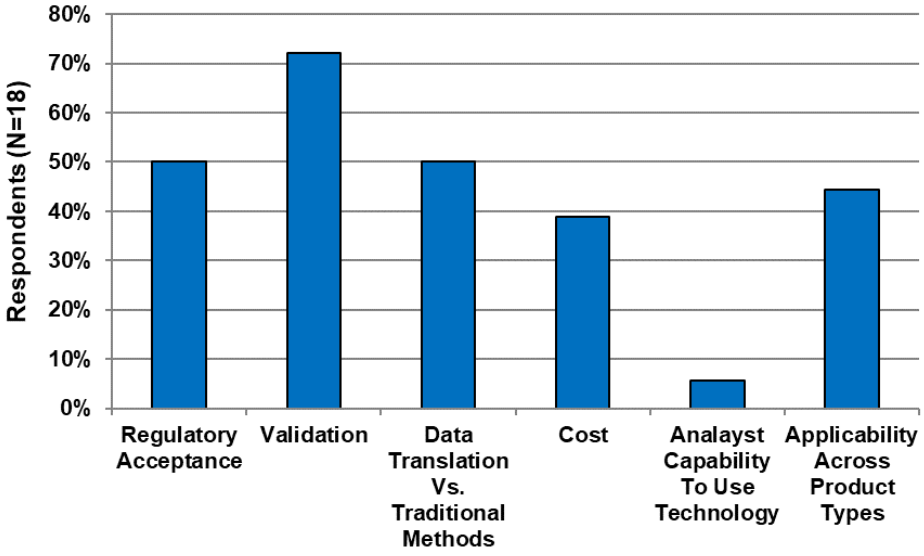

Figure 6. Biggest challenges for implementing RAM for PET

Chemical analysis of preservatives

PET can be viewed as a measure of the microbiological preservation capacity within a product. PET provides a reasonable, but somewhat inexact measure of that capacity using a biological endpoint – demonstrating stasis or loss of viability of a target microorganism. PET will often detect a catastrophic loss of preservative stability. However, a slower loss of stability may go unnoticed against the limited sensitivity of the PET. One means of gaining a more sensitive understanding of preservative stability is via direct chemical analysis of the preservative(s). A majority of companies (65%) used chemical analysis of preservatives to complement PET in evaluating preservative stability. Of those companies, 33% used chemical analysis of preservatives routinely for regulated products, 27% used it routinely for all products, and 27% used it only at key milestones. The remaining 13% of companies used chemical analysis of preservatives only during troubleshooting.

Success Criteria

Just as with a number of other parameters, many companies develop their own set of PET success criteria. Companies were asked to identify factors which influenced their PET success criteria, checking all that applied, shown in Figure 7.

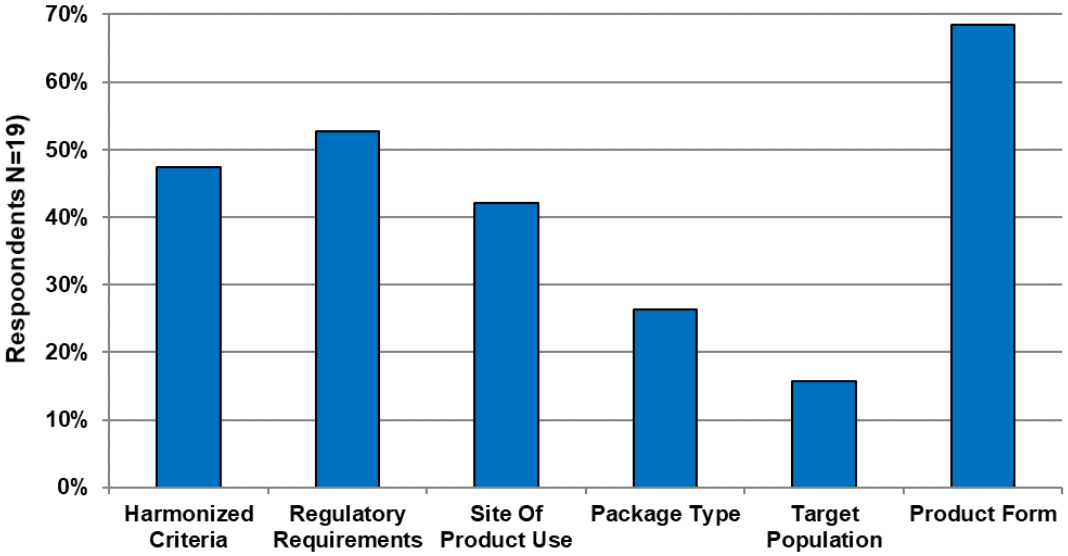

Figure 7. Factors influencing PET success criteria

Given the inherent subjectivity and customization by companies in developing PET success criteria, the survey was not able to specifically delineate exact PET success criteria for bacteria, yeast and mold for each company. Instead, companies were asked to identify their most stringent PET success criteria condition. For the purposes of the survey, this was defined as the shortest time needed to achieve the highest desired log reduction in their success criteria. While it is not possible to directly link a given log reduction to a given time, the corresponding patterns provide a directional indication for success criteria of the companies surveyed. The criteria for typical aqueous formulations are shown in Figure 8.

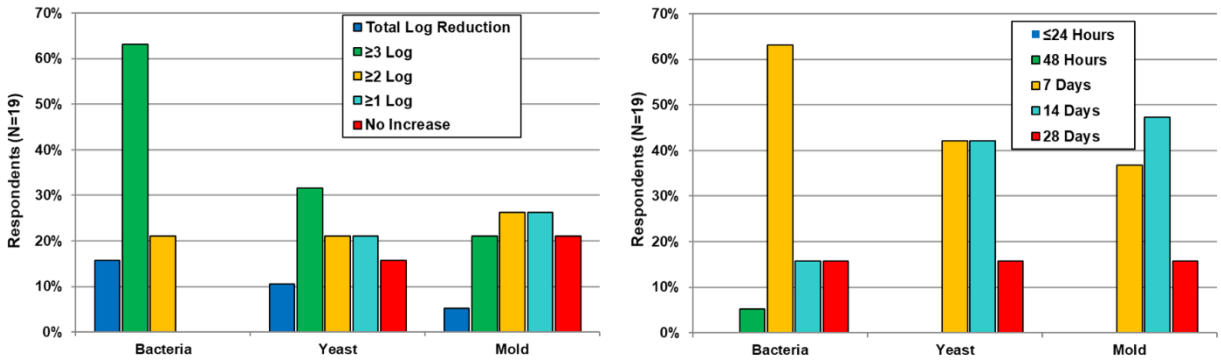

Figure 8. Most stringent PET success criteria - highest desired log reduction (on left) and shortest desired time (on right) – for typical aqueous formulations

For bacteria, the most stringent criteria was focused around a $\geq$ 3 log reduction and a 7 day time period. The criteria for log reduction for yeast was lower compared to bacteria, along with a corresponding longer pattern of timing to achieve the log reduction. In parallel, the criteria for log reduction for mold was lower compared to yeast, with a

corresponding longer pattern of timing to achieve the log reduction for mold. Companies were asked to identify their response in the event of a marginally failing PET success criteria or obtaining an unexpected PET result. Most companies (56%) indicated they would reject the formulation, 28% would use additional factors to determine adequacy of preservation, while 16% would retest the formulation.

Most companies (73%) indicated that they varied their PET protocols to address unique product types. For those companies varying their PET, a variety of product attributes formed the basis for varying PET protocols, as shown in Figure 9.

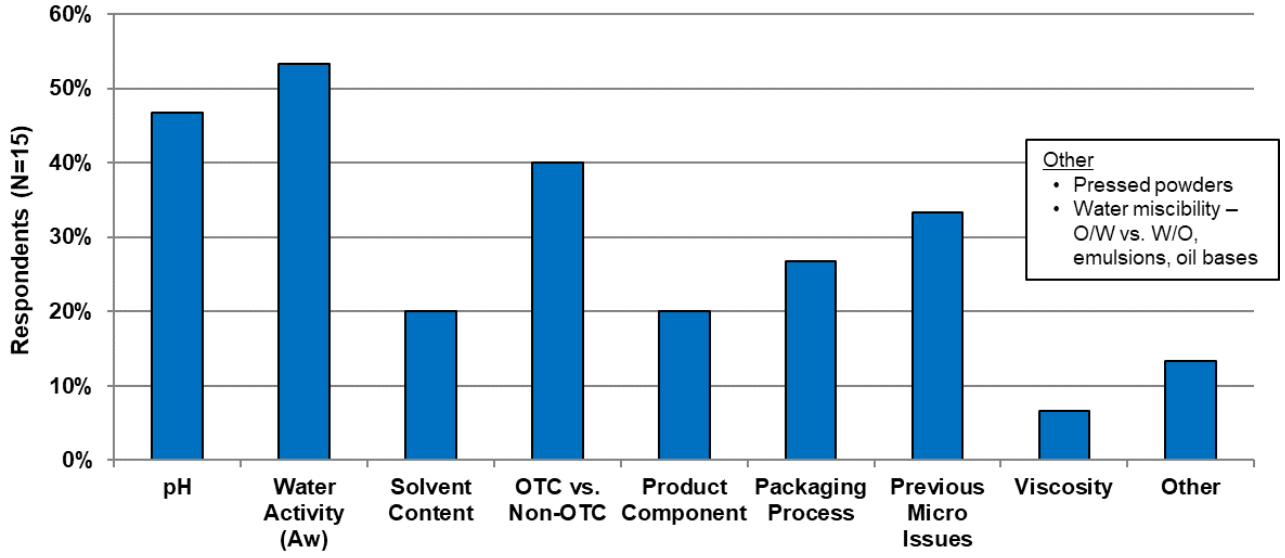

Figure 9. Product attributes used as a basis for variations in PET protocol

The companies surveyed indicated that a number of variations of PET protocols were implemented to address unique product types, as shown in Figure 10.

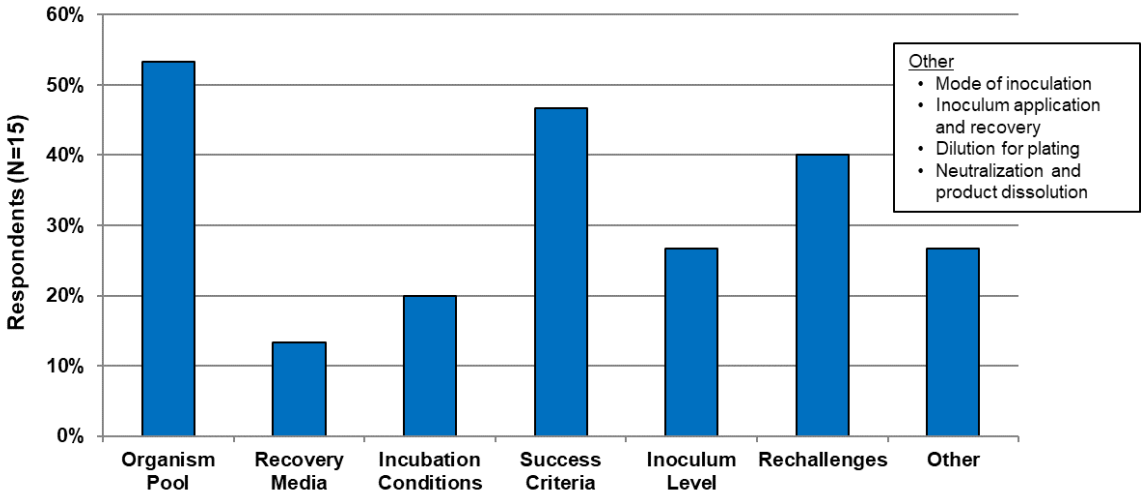

Figure 10. PET protocol variation used to address unique product types

Conclusion

The survey provided a view into the rich array of company practices within the basic framework of the PET protocol. Of note are the following:

- There is a wide latitude of practice for PET
    - Protocols are flexible to customize risk understanding.
    - Varying protocols to yield the best information for product preservation is consistent with company responsibility to ensure safety for the products that they manufacture and the risks that they manage.
- Many companies establish additional data beyond standard PET protocols in assessing preservation efficacy, including:
    - Early and non-traditional enumeration data points within a PET, including T=0 and pre 1 week timepoints
    - Common use of rechallenge to more closely approximate consumer contamination and understand preservation capacity
    - Common PET analysis of multiple preservative levels during product development to establish minimum effectiveness
    - Leveraging chemical analysis of preservatives to help evaluate preservative effectiveness and stability
- The most stringent PET success criteria for bacteria is centered on a >3 log reduction by 7d, consistent with ISO and PCPC guidance.
- Companies did not indicate wide practice in rapid or alternative methods (RAM), citing a number of technical and regulatory hurdles. Accelerating the slow adoption and acceptance of RAM presents a potential opportunity in microbiological methods innovation.