

## An Alternative Method for Rapid Screening of Products for Microbial Contamination

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Testing for microbial contamination in raw materials, in-process and finished products is necessary to monitor product quality. However, traditional agar methods require several days for results, thereby requiring the manufacturer to hold the materials until results are available. An alternative method has been developed utilizing a proprietary growth enhancement media (GEM) in conjunction with a novel transferable substrate. This combination promotes rapid microbial growth and allows for the detection of bacteria, yeast and/or mold in a single assay within 30 hours. Thirty-five finished products and excipient samples from the personal care, household, and over-the-counter pharmaceutical industries were tested using the new procedure to demonstrate this rapid detection method. Product suspensions were prepared by aseptically adding 10g of product to 90mL of phosphate buffer (PB) or GEM and mixed to obtain homogeneous suspensions. One-milliliter of the diluted product suspensions or 1mL of undiluted excipients was aseptically transferred into 19mL of GEM containing a substrate and incubated for 30 minutes at  $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for preservative neutralization. In order to simulate contamination events, neutralized product or excipient samples were individually inoculated with the following microorganisms: *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), *Candida albicans* (ATCC 10231), and *Aspergillus niger* spores (ATCC 16404) at 10 to 100 cfu/volume. The inocula were spread-plated on tryptic soy agar and incubated 24-48 hours at  $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for confirmation of inoculum levels. Additional product or excipient samples were not inoculated to serve as negative controls. Subsequently, the spiked and non-spiked samples were enriched by incubating for 24-30 hours at  $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$  with agitation. Following enrichment, the substrate was transferred from the enrichment tube to a tube containing 2mL PB and vortexed for 15 seconds to elute the microorganisms from the substrate. A 1:30 dilution of the vortexed sample was prepared by filtering 0.1mL of the sample through a 35-micron filter cap directly into 2.9mL of PB. All samples were analyzed on Advanced Analytical's *Micro PRO*<sup>TM</sup> detection system for the presence or absence of microorganisms. Samples were considered positive for microbial contamination if the signal to noise ratio of the spiked product or excipient sample result was  $\geq 4$  times the negative product or excipient control. Utilizing this alternative method, in conjunction with the *Micro PRO*<sup>TM</sup> detection system, results for the presence or absence of microbial contamination were obtained within 30 hours in all 35 finished products and excipient samples tested.