

ABSTRACT

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 24-48 hours. Fifty-five different product samples including emulsions, excipients, personal care (PCP), over-the-counter (OTC), household, beverage and cosmetic products were tested using the new procedure to demonstrate this rapid detection method. Product suspensions were prepared by diluting samples 1:10 aseptically in phosphate buffer (PB) or GEM and mixing to obtain homogeneous suspensions. One-milliliter of the diluted product suspensions or 1mL of undiluted excipient was aseptically transferred into 19mL of GEM containing a substrate and incubated for 30 minutes at 30°C ± 2°C for preservative neutralization. In order to simulate contamination events, neutralized product samples were individually inoculated with the following microorganisms: *Escherichia coli* (ATCC 8739 or 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), *Candida albicans* (ATCC 10231), and *Aspergillus niger* spores (ATCC 16404) at 2 to 78 cfu/volume. The inocula were spread-plated on tryptic soy agar and incubated 24-48 hours at 30°C ± 2°C for confirmation of inoculum levels. Additional product samples were not inoculated to serve as negative controls. Subsequently, the spiked and non-spiked samples were enriched by incubating for 24-48 hours at 30°C ± 2°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™* Detection System for the presence or absence of microorganisms. Samples were considered positive for microbial contamination if the post-enrichment result of the spiked product sample was ≥3 times the baseline. This alternative method of screening products for microbial contamination yielded positive results for 87% of the spiked products within 24 hours, 91% within 30 hours, and 100% within 48 hours for the 55 various products tested.

MATERIALS

Bacterial Cultures: *Escherichia coli* #25922 or 8739, *Pseudomonas aeruginosa* #9027, *Staphylococcus aureus* #6538, *Candida albicans* #10231 (ATCC, Manassas, VA), and *Aspergillus niger* #16404 (Remel, Lenexa, KS). Tryptic Soy Broth (TSB) and Yeast Extract and Malt Extract (YM) broth (Difco, Sparks, MD) were used for culturing. Tryptic Soy Agar (TSA, Difco) was used for plating.

Enrichment: *Micro PRO™* Media Kit (MPMK) (Advanced Analytical, Ames, IA). **Products:** Emulsions (n=3), excipients (n=14), personal care (n=12), over-the-counter (n=14), household (n=6), beverage (n=4) and cosmetic (n=2). **Detection:** *Micro PRO™* Reagent Kit and the *Micro PRO™* Detection System (Advanced Analytical).

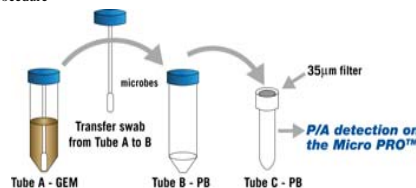
METHODS

Enrichment: Product suspensions were prepared by diluting samples 1:10 aseptically in PB or GEM and mixed to obtain homogeneous suspensions. One-milliliter of the diluted product suspensions or 1mL of undiluted excipient was aseptically transferred to Tube A of the MPMK and incubated for 30 minutes at 30°C ± 2°C for preservative neutralization. Neutralized product samples were individually inoculated with the above microorganisms at 2 to 78 cfu/volume. The inocula were spread-plated on TSA and incubated 24-48 hours at 30°C ± 2°C for confirmation of inoculum levels. Additional product samples were not inoculated to serve as negative controls. Subsequently, the spiked and non-spiked samples were enriched by incubating for 24-48 hours at 30°C ± 2°C with agitation.

METHODS CONTINUED

Sample Processing: Following enrichment, samples were processed as shown in Figure 1. The substrate/swab was transferred to Tube B of the MPMK and vortexed to elute the microorganisms from the substrate. One-hundred microliters were then added to Tube C of the MPMK through a 35-micron filter cap. All samples were analyzed on the *Micro PRO™* Detection System for the presence or absence of microorganisms.

Figure 1. Illustration of the *Micro PRO™* Media Kit Product Screening Procedure



DISCUSSION/CONCLUSIONS

- This alternative method of screening products for microbial contamination yielded positive results for 87% of the spiked products in 24 hours, 91% within 30 hours and 100% within 48 hours for the 55 products tested.
- Samples were considered positive for microbial contamination if the post-enrichment result of the spiked product sample was ≥3 times the baseline. The baseline is calculated as the average non-spiked product results plus the standard deviation of these results.
- All inoculated samples were ≥3 the baseline post-enrichment.
- The *Micro PRO™* Detection System in conjunction with the *Micro PRO™* Reagent kit provides a rapid alternative for screening products for microbial contamination.

RESULTS

Table 1. Product Screening Results showing detection of low-levels of microorganisms in various product types.

Product Type	Inoculum Range	Time to Result
Emulsions (n=3)	15 - 46	24-48 hrs
Excipients (n=14)	8 - 66	24 hrs
PCP (n=12)	2 - 78	24-30 hrs
OTC (n=14)	9 - 78	24-30 hrs
Household (n=6)	2 - 75	24-48 hrs
Beverage (n=4)	6 - 41	24 hrs
Cosmetics (n=2)	12 - 61	48 hrs