

## ABSTRACT

Over-the-counter (OTC) drug products are routinely evaluated for the absence of microbial contamination prior to release. The current testing method using agar plates requires 3 to 5 days to detect product contamination events. An alternative method was investigated in order to expedite detection of potential contamination events in OTC cough syrups. Four different brands of pediatric cold and cough syrups containing the active ingredients chlorpheniramine maleate, dextromethorphan HBr, and pseudoephedrine HCl were analyzed. Five replicate samples of each brand were prepared by adding 1 mL cough syrup to 99 mL Tryptic Soy Broth + Soy Lecithin + Tween 20. In order to simulate a contamination event, 4 of the replicates of each brand were individually inoculated with the following microorganisms: *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538) and *Candida albicans* (ATCC 10231) at <100 cfu/volume. The inocula were spread-plated on Tryptic Soy Agar (TSA) and incubated 24-48 hours at 30°C for confirmation of inoculum levels. The fifth replicate of each brand of cough syrup was not spiked and served as the negative control. Subsequently, all spiked and non-spiked samples were enriched at 30°C for 18-22 hours. After enrichment, all samples were diluted 1:1000 in phosphate buffer and analyzed on Advanced Analytical's RBD 3000 using the FASTest Total Viable Organism detection kit, specific for enumerating viable microorganisms in liquid matrices. Following enrichment, each sample was streak-plated on TSA for confirmation to the RBD 3000 result. Cough syrup samples were considered positive for microbial contamination on the RBD 3000 if the result was 5-times greater than the negative control and if the TSA streak-plate was positive for microbial growth. All spiked cough syrup samples were positive according to the RBD 3000 and correlated with the TSA streak-plate results. Additionally, the TSA plates were negative for all non-spiked samples. The RBD 3000 provided accurate detection of simulated contamination events in pediatric cold and cough syrup within 24 hours.

## MATERIALS

**Bacterial Cultures:** *Escherichia coli* #8739, *Pseudomonas aeruginosa* #9027, *Staphylococcus aureus* #6538 and *Candida albicans* #10231 (ATCC, Manassas, VA). Tryptic Soy Broth (TSB) was used for culturing and Tryptic Soy Agar (TSA) for plating (Difco, Sparks, MD). TSB + Soy Lecithin + Tween 20 (TSBST) was used as the enrichment media. **Products:** Four different brands of pediatric cold and cough syrups containing the active ingredients chlorpheniramine maleate, dextromethorphan HBr, and pseudoephedrine HCl were analyzed. **Detection:** FASTest Total Viable Organism (TVO) Kit and fully automated RBD 3000 (Advanced Analytical Technologies, Inc. Ames, IA).

## METHODS

**Enrichment:** Five replicate samples of each brand of cough syrup were prepared by suspending 1 mL of cough syrup in 99 mL of TSBST. All samples were incubated at 30°C for 30 minutes to neutralize the preservatives in the cough syrup. Four replicate samples of each brand were individually inoculated/spiked with  $\leq 100$  cfu of *E. coli*, *Ps. aeruginosa*, *S. aureus*, or *C. albicans*. All inocula were spread-plated on TSA, incubated at 30°C for 24-48 hours to determine exact inoculum levels. The fifth replicate sample of each cough syrup brand was not inoculated and served as the negative control. All samples were enriched at 30°C with shaking for 18-22 hours. **Sample Processing:** Following enrichment, samples were diluted 1:1000 in 10mM phosphate buffer and loaded into the RBD 3000 for detection of microorganisms using the TVO kit. Enriched samples were also streak-plated onto TSA and incubated at 30°C for 24-48 hours for verification of the RBD 3000 results. Enriched samples were considered positive for microbial contamination on the RBD 3000 if the results were five-times greater than the negative control and if the TSA streak-plate was positive for microbial growth.

## RESULTS

**Table 1.** Results of microorganism detection in spiked and non-spiked over-the-counter pediatric cough syrups using the RBD 3000 and the agar plate method.

Inocula	Total Spike Level per mL Cough Syrup	Enrichment Time	Detection by RBD 3000				Detection by Agar Plate			
			Cough Syrup Brand 1	Cough Syrup Brand 2	Cough Syrup Brand 3	Cough Syrup Brand 4	Cough Syrup Brand 1	Cough Syrup Brand 2	Cough Syrup Brand 3	Cough Syrup Brand 4
<i>E. coli</i>	9 cfu	18 hrs	+	+	+	+	+	+	+	+
<i>Ps. aeruginosa</i>	13 cfu	18 hrs	+	+	+	+	+	+	+	+
<i>S. aureus</i>	10 cfu	18 hrs	+	+	+	+	+	+	+	+
<i>C. albicans</i>	89 cfu	22 hrs	+	+	+	+	+	+	+	+
None	0	18 hours	-	-	-	-	-	-	-	-
None	0	22 hours	-	-	-	-	-	-	-	-

## DISCUSSION/CONCLUSIONS

- The RBD 3000 provided accurate detection of simulated contamination events in 4 different brands of pediatric cold and cough syrups within 24 hours.
- The RBD 3000 results were in agreement with the TSA plate results in all cases and provided clear, objective results within 15-minutes of sampling.
- All spiked cough syrup samples were positive according to the RBD 3000 and were positive for microbial growth on the TSA plates.
- All non-spiked, negative control samples were negative according to the RBD 3000 and had no microbial growth on the TSA plates.