The Out-of-Pack Challenge and Screening Testing of 7 Acidic Condiments Using a Panel of Spoilage Bacteria and Yeast on the Innovate™ System

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INTRODUCTION:

The Innovate™ Rapid Microbial Screening System is based on the detection of extractable microbial ATP from organisms growing within sterile or aseptic products. ATP bioluminescence technology can detect microorganisms with great sensitivity, thereby providing a much more rapid microbial detection system than waiting for visible colonies to grow on agar plates.

Condiment testing in-pack is a slow process due to the low pH and high sugar and salt concentrations of the product matrix. This study demonstrates an "Out-of-Pack" method for the detection of spiked organisms using the Innovate System. The RapiScreen™ Beverage Kit is designed to have a strong buffering capacity to neutralize high-acid products such as condiments and is also validated on a broad range of matrices such as fruit juices, teas, energy drinks, etc.

The "Out-of-Pack" method provides an enrichment step for low-level contamination to get enriched in the nutrition-rich media followed by an easy detection using the Innovate System.

PURPOSE:

1. To demonstrate an "Out-of-Pack" enrichment method to test the growth and detection of spoilage bacteria and yeast by enrichment of organisms using three selective medias: TSB, MRS and PDB.
2. To validate the Innovate System to detect a panel of organisms in seven acidic condiments over a period of 7 days incubation following the "Out-of-Pack" enrichment step.

REGISTERED TRADEMARKS:

METHOD:

In the "Out-of-Pack" method, mayonnaise, hot sauce #1 and #2, BBQ sauce, English mustard, Dijon mustard and Thousand Island dressing were diluted into media (TSB, MRS and PDB) at a 1:10 dilution by taking 10 g of product and adding 90 mL of the respective media in a sterile container. Each of the products was tested in triplicate.

The organism panel was spiked at two levels—high spike (<1000 CFU) and low spike (<100 CFU) and incubated as follows: *Bacillus cereus* and *E. coli* with product diluted in TSB and incubated at 35 °C, *Lactobacillus fermentum* and *Lactobacillus brevis* tested with product diluted with MRS at 37 °C and *Saccharomyces cerevisiae* and *Zygosaccharomyces parabailli* tested with product diluted with PDB and incubated at 30 °C. Following incubation, the spiked products were tested on the Innovate System and plated on standard agar plates at 24 h, 36 h and 72 h, respectively.

The probability of detection was confirmed by comparing the positives on the Innovate System with the corresponding growth of the spiked bacteria on standard agar plates.

Table 1: List of Condiments Tested

List of Condiments Tested
Mayonnaise
Hot Sauce (Cholula and Frank's)
BBQ Sauce
English Mustard
Dijon Mustard
Thousand Island Dressing

Table 2: List of Organism Panel and Pre-enrichment Media

Organism Panel	ATCC	Pre-incubation Temperature	Pre-enrichment Media
Escherichia coli	8739	35 °C	TSB
Bacillus cereus	11778	35 °C	TSB
Lactobacillus brevis	14869	37 °C	MRS
Lactobacillus fermentum	9338	37 °C	MRS
Saccharomyces cerevisiae	9763	30 °C	PDB
Zygosaccharomyces parabailii	N/A	30 °C	PDB

Table 3: Out-of-Pack Innovate Testing Protocol

Incubate product sample (1:10 diluted with an enrichment media) for 24 – 72 h
↓
Aliquot the enriched sample in microplate and place in the Innovate System
↓
Reagents are auto-dispensed in sequence in the Innovate System
↓
Output is measured with results in <30 minutes

RESULTS:

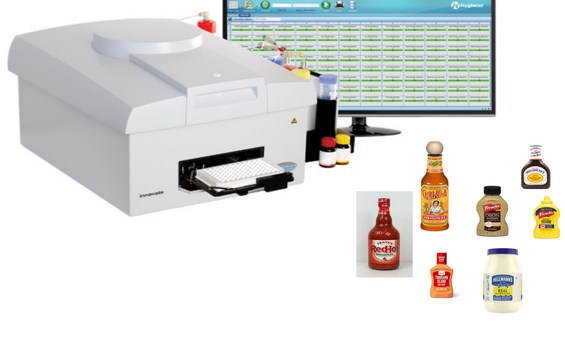
- Mayonnaise: All organisms were detected at 24 hours at the high spike levels. At low spike levels, all the organisms were detected at 24 hours, except *B. cereus* and *Z. parabailii*, which were detected at 48 hours.
- Hot Sauce #1*: B. cereus* and *E. coli* were unable to survive and be detected. The results were confirmed when the product was streaked on TSB agar plates and showed no detection on standard agar plates as well. *L. brevis,* at low and high spike levels, was detected at 48 hours, whereas *L. fermentum*, *S. cerevisiae* and *Z. parabailii* at both low and high levels were detected within 24 hours.
- Hot Sauce #2: *B. cereus* and *E. coli* were unable to survive and be detected. The rest of the organism panel was detected within 24 hours at the high spike level. At the low spike level, *L. brevis, S. cerevisiae* and *Z. parabailii* were detected in 48 hours, whereas *L. fermentum* was detected in 24 hours.
- BBQ Sauce: All organisms were detected in 24 hours at the high spike level. At the low spike level, *B. cereus* and *Z. parabailii* were detected in 48 hours, whereas the rest of the organism panel was detected in 24 hours.
- English Mustard: *B. cereus*, *E. coli* and *Zygosaccharomyces* were not detected in this product at either high or low levels, whereas *L. fermentum and L. brevis* were detected at 24 hours and *S. cerevisiae* was detected at 48 hours.
- Dijon Mustard: *B. cereus* was detected in 48 hours at a high spike level, whereas it took 72 hours at the low spike level for detection. *L. fermentum* and *L. brevis* were detected at 24 hours, whereas *Saccharomyces* and *Zygosaccharomyces* were not detected, which was confirmed by no detection on standard plates either.
- Thousand Island Dressing: At the high spike level, *L. brevis*, *L. fermentum*, *S. cerevisiae* and *Z. parabailii* were detected within 24 hours. At a low spike level, *L. brevis* was detected within 48 hours and *Zygosaccharomyces* was detected by 72 hrs.

RESULTS:

Table 4: Time to Detection of the Organism Panel Using the Innovate System

	Time to Detection*						
Product Names	**Spike level**	***B. cereus***	***E. coli***	***L. brevis***	***L. fermentum***	***S. cerevisiae***	***Z. parabailii***
Mayonnaise	High	24 hours	24 hours	24 hours	24 hours	24 hours	24 hours
	Low	48 hours	24 hours	24 hours	24 hours	24 hours	48 hours
Hot Sauce #1	High	Not Detected	Not Detected	48 hours	24 hours	24 hours	24 hours
	Low	Not Detected	Not Detected	48 hours	24 hours	24 hours	48 hours
Hot Sauce #2	High	Not Detected	Not Detected	24 hours	24 hours	24 hours	24 hours
	Low	Not Detected	Not Detected	48 hours	24 hours	48 hours	48 hours
BBQ Sauce	High	24 hours	24 hours	24 hours	24 hours	24 hours	24 hours
	Low	48 hours	24 hours	24 hours	24 hours	24 hours	48 hours
English Mustard	High	Not Detected	Not Detected	24 hours	24 hours	48 hours	Not Detected
	Low	Not Detected	Not Detected	48 hours	24 hours	Not Detected	Not Detected
Dijon Mustard	High	48 hours	24 hours	24 hours	24 hours	Not Detected	Not Detected
	Low	72 hours	48 hours	24 hours	24 hours	Not Detected	Not Detected
Thousand Island Dressing	High	N/A	N/A	24 hours	24 hours	24 hours	24 hours
	Low	N/A	N/A	48 hours	24 hours	24 hours	72 hours

* Time to detection is the time to detect the organism after the pre-incubation of 24 hours



CONCLUSIONS AND SIGNIFICANCE:

The baseline studies using the RapiScreen Beverage Kit for the Out-of-Pack method showed successful depletion of background ATP when present, resulting in stable RLU values for all 7 condiments tested. Stable baseline RLU values allowed for the establishment of positive/negative threshold values. The spiking results showed that the organisms grew in the products, producing very high RLUs above the threshold at both low spike levels (<100 CFU) and high spike levels (<1000 CFU).

The Out-of-Pack protocol is a good method for complex matrices that are highly viscous and have a low pH, whereas a normal in-pack spiking method is unable to promote the growth of bacteria. The nutrient-rich media is able to promote the of growth of low-level contaminants (bacteria, yeast and mold) in the product, making it accessible by the Innovate System to detect it with ease.

The rapid detection by an Out-of-Pack dilution in selective media followed by ATP measurements on the Innovate System will detect spoilage organisms more effectively in condiment products.