Rapid Detection of Microorganisms in Oat Milk Products Using the Innovate System

Introduction

Traditional methods for microbiological testing can take 7 – 15 days for results and require manual processes which are prone to technician error. In addition, results are not quantitative and require visual inspection for interpretation. To reduce time to results and streamline laboratory testing, the Hygiena™ Innovate System, can provide results in less than 30 minutes for up to 96 samples with no secondary incubation.

The objective of these studies was to evaluate the Innovate System for detection of low levels of microorganisms in three different types of oat milk using the RapiScreen™ Dairy kit for ATP detection, and comparing results to other methods, including pH and plate inoculation/growth.

Typically, a product is incubated in its own packaging to enrich the ATP from any contaminating microbial cells. Pre-established baselines obtained from uncontaminated product are used to determine positive results.

Equipment, Supplies and Reagents

Necessary materials and equipment varied depending on the organism being tested but included:

- Sterile inoculating loops, pipettes, and tips
- L-shaped spreaders
- Incubators (30°C and 37°C) and anaerobic gas generating system
- Innovate RapiScreen™ Dairy Kit (RSD)
- Innovate System
- Sabouraud Dextrose Agar
- Tryptic Soy Agar (TSA)
- Tryptic Soy Broth (TSB)
- Maximum Recovery Diluent (MRD)
- pH meter and electrodes
- Syringes, 1mL Insulin Syringe U-100
- Syringes, 3mL Luer-Lok Tips
- Precision Glide Needles, 16 gauge 1 ½”
- Precision Glide Needles, 18 gauge 1 ½”

Microorganisms tested:

- *Aspergillus brasiliensis*
- *Bacillus cereus* endospores
- *Clostridium sporogenes* vegetative
- *Geobacillus stearothermophilus*
- *Pseudomonas aeruginosa*
- *Saccharomyces cerevisiae*
- *Salmonella* Typhimurium
- *Staphylococcus aureus*

Products tested:

- UHT chocolate oat milk
- ESL oat milk
- UHT oat milk

Sample Preparation and Enrichment

Sample Background/Baseline Testing

Product ATP baselines were determined by incubating the product at 32°C for 48 hours. The sample was shaken thoroughly to mix, and 25 mL of product was removed from the sample and placed in a sterile container for pH and background/baseline testing. The background ATP level of each product was determined by running an assay using ATX buffer solution in place of reconstituted ATX reagent. The assay was then repeated using reconstituted ATX to allow for the depletion of the background ATP signal. These results are referred to as the Baseline RLU values. To calculate a product specific RLU threshold the average baseline RLU reading is multiplied by 3 to give the cutoff for a contaminated sample. For pH assessments, products were tested in triplicate to ensure accuracy of measurements.

Inoculum Preparation

All microorganisms were prepared by inoculating a single colony into 5 mL of TSB. The broth was then incubated at 37˚C for 24 hours. A ten-fold serial dilution set was then made using MRD, and plate counts were prepared on TSA plates to determine the concentration of the organisms spiked in the product. The plates were incubated at 37˚C and counted after 24 hours.

Test Methodology

The microorganisms were spiked using a syringe through the top of the product and re-sealed with adhesive glue. A non-inoculated product, spiked with sterile MRD, was incubated with each inoculated product as a negative control. The product samples were spiked at two levels, less than 10 and 100 cfu and analyzed after incubation for 24, 48, 72, 96, 120 and 168 hours at 32°C ± 1°C. After each incubation period, all samples were tested on the Innovate system using the RapiScreen™ Dairy Kit.

At each time point, 100 µL of the product sample was removed and streaked with L-shaped spreaders onto TSA plates and incubated at 32°C ± 2°C for up to 72 hours, as well as on Sabouraud Dextrose Agar and incubated at 30°C for up to 72 hours. Growth seen on these confirmation plates was checked to ensure it matched the morphology of the spiked microorganism. Gas Pak™ EZ Anaerobe Gas Generating Pouches were used to incubate confirmation plates streaked with *C. sporogenes* to enable the organism to grow effectively if present. Growth seen on confirmation plates matched the morphology of the spiked microorganisms.

Results

pH Assessment

pH readings for all UHT milk products had an average of 7.52.

Background and Baseline Assessments

For all UHT milk products tested, RLU baselines were low and consistent, allowing RLU cut-off threshold values to be set for the UHT milk products. Values are shown in Table 1.

Inoculated Results

For all organisms tested, the Innovate System was able to detect low spike levels (~10 CFU per pack) at the 24 hour time point. RLU values exceeded the product RLU thresholds (x 3). Results are shown in Table 1.

Table 1.

Product	Product Threshold (RLU)	Reagent Kit Used	Organism	Spike Count	24hr RLU (average)
UHT Choc Oat Milk	14	RSD	*Bacillus cereus (high spike)*	60CFU / 100mL	85,070
			Bacillus cereus (low spike)	0.6 CFU / 100 mL	50,544
			Salmonella Typhimurium *(high spike)*	810 CFU / 100 mL	16,911
			Salmonella Typhimurium *(low spike)*	8 CFU / 100 mL	11,031
			Pseudomonas aeruginosa (high spike)	32 CFU / 100 mL	5,207
			Pseudomonas aeruginosa (low spike)	3 CFU / 100 mL	3,247
			Staphylococcus aureus (high spike)	770 CFU / 100 mL	26,738
			Staphylococcus aureus (low spike)	8 CFU / 100 mL	138
			Clostridium sporogenes (high spike)	50 CFU / 100 mL	69
			Clostridium sporogenes (low spike)	5 CFU / 100 mL	55
			Geobacillus stearothermophilus (high spike)	570 CFU / 100 mL	868
			Geobacillus stearothermophilus (low spike)	6 CFU / 100 mL	1,144
ESL Barista Oat Milk	62	RSD	*Bacillus cereus (high spike)*	25 CFU / 100 mL	343,694
			Bacillus cereus (low spike)	0.25 CFU / 100 mL	141,394
			Pseudomonas aeruginosa (high spike)	440 CFU / 100 mL	10,940
			Pseudomonas aeruginosa (low spike)	4.4 CFU / 100 mL	1,932
			Staphylococcus aureus (high spike)	760 CFU / 100 mL	1,703
			Salmonella Typhimurium *(high spike)*	1520 CFU / 100 mL	104,107
			Salmonella Typhimurium *(low spike)*	15.2 CFU / 100 mL	39,376
UHT Oat Milk	64	RSD	*Bacillus cereus (high spike)*	260 CFU / 100 mL	333,525
			Bacillus cereus (low spike)	2.6 CFU / 100 mL	295,677
			Salmonella Typhimurium *(high spike)*	990 CFU / 100 mL	143,854
			Salmonella Typhimurium *(low spike)*	9.9 CFU / 100 mL	119,276
			Pseudomonas aeruginosa (high spike)	480 CFU / 100 mL	7,212
			Pseudomonas aeruginosa (low spike)	4.8 CFU / 100 mL	1,253
			Staphylococcus aureus (high spike)	670 CFU / 100 mL	62,895
			Staphylococcus aureus (low spike)	6.7 CFU / 100 mL	1,164
			Clostridium sporogenes (high spike)	170 CFU / 100 mL	679
			Geobacillus stearothermophilus (high spike)	197 CFU / 100 mL	9,849
			Geobacillus stearothermophilus (low spike)	1.97 CFU / 100 mL	3,387

Time to Detection Comparison

For UHT chocolate oat milk, all organisms were detected at 24 hours on the Innovate System in comparison to plating which provided results at 48 hours, independent of inoculation levels - low (~10 CFU) or high (~100 CFU). pH detection was variable, depending on the organism.

	Time to Detection in Hours (hrs)					
	Innovate System		Plating		pH	
Organism Panel	**High CFU**	**Low CFU**	**High CFU**	**Low CFU**	**High CFU**	**Low CFU**
Bacillus cereus endospores	24 hrs	24 hrs	48 hrs	48 hrs	24 hrs	24 hrs
Clostridium sporogenes vegetative	24 hrs	24 hrs	48 hrs	48 hrs	48 hrs	48 hrs
Geobacillus stearothermophilus	24 hrs	24 hrs	48 hrs	48 hrs	24 hrs	24 hrs
Pseudomanas aeruginosa	24 hrs	24 hrs	48 hrs	48 hrs	48 hrs	48 hrs
Salmonella Typhimurium	24 hrs	24 hrs	48 hrs	48 hrs	24 hrs	24 hrs
Staphylococcus aureus	24 hrs	24 hrs	48 hrs	48 hrs	24 hrs	48 hrs

For ESL oat milk, most organisms were detected at 24 hours on the Innovate System in comparison to plating which provided results at 48 - 72 hours, independent of inoculation levels - low (~10 CFU) or high (~100 CFU). pH detection was variable, depending on the organism. *Clostridium sporogenes* was not detectable with any method, suggesting either poor growth of the organism or inability to grow in the ESL milk products.

	Time to Detection in Hours (hrs)					
	Innovate System		Plating		pH	
Organism Panel	**High CFU**	**Low CFU**	**High CFU**	**Low CFU**	**High CFU**	**Low CFU**
Aspergillus brasiliensis	48 hrs	48 hrs	72 hrs	72 hrs	120 hrs	120 hrs
Bacillus cereus endospores	24 hrs	24 hrs	48 hrs	48 hrs	24 hrs	24 hrs
Clostridium sporogenes vegetative	120 hrs	No detection	No detection	No detection	No detection	No detection
Pseudomonas aeruginosa	24 hrs	24 hrs	48 hrs	48 hrs	72 hrs	72 hrs
Saccharomyces cerevisiae	24 hrs	48 hrs	48 hrs	48 hrs	48 hrs	72 hrs
Salmonella Typhimurium	24 hrs	24 hrs	48 hrs	48 hrs	24 hrs	24 hrs
Staphylococcus aureus	24 hrs	24 hrs	48 hrs	48 hrs	48 hrs	48 hrs

For UHT oat milk, all organisms were detected at 24 hours in comparison to plating which provided results at 48 hours, independent of inoculation levels - low (~10 CFU) or high (~100 CFU). pH detection was variable 24 – 48 hr, depending on the organism.

	Time to Detection in Hours (hrs)					
	Innovate System		Plating		pH	
Organism Panel	**High CFU**	**Low CFU**	**High CFU**	**Low CFU**	**High CFU**	**Low CFU**
Bacillus cereus endospores	24 hrs	24 hrs	48 hrs	48 hrs	24 hrs	24 hrs
Clostridium sporogenes vegetative	24 hrs	48 hrs	48 hrs	48 hrs	48 hrs	48 hrs
Geobacillus stearothermophilus	24 hrs	24 hrs	48 hrs	48 hrs	24 hrs	24 hrs
Pseudomanas aeruginosa	24 hrs	24 hrs	48 hrs	48 hrs	48 hrs	48 hrs
Salmonella Typhimurium	24 hrs	24 hrs	48 hrs	48 hrs	24 hrs	24 hrs
Staphylococcus aureus	24 hrs	24 hrs	48 hrs	48 hrs	24 hrs	48 hrs

Conclusions

RLU values

The baseline studies showed successful depletion of background ATP when present, resulting in stable RLU values for all three products. Stable baseline RLU values allow for the establishment of a positive/negative threshold values. The product threshold values for UHT chocolate milk was 14, UHT oat milk was 64 and ESL milk was 62, respectively. RLU values above these thresholds indicate positive results. All UHT and ESL milk products tested had a pH within optimal range for reagent activity.

Time to Detection

The Innovate System consistently detected microorganisms at 24 hours, independent of low or high inoculation levels. This is at least 24 hours quicker than conventional plating, reducing time to results. The exception to this was *Aspergillus brasiliensis,* which was only detectable at 48 hours but not detectable by plating until 72 hours and by pH at 120 hours.

Recommendations

The Innovate System can detect multiple microorganisms in oat milk products after 24 hours of incubation. RLU levels were significantly above the baseline, ensuring testing results could reflect contamination even when low levels of microorganisms are present. Paired with the time savings provided when compared to conventional culture, the Innovate System can streamline results for any facility needing improved time to results and reduced operational costs.

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