Rapid Detection of Microorganisms in UHT Milk Using the Innovate System

Introduction

Traditional methods for microbiological testing can take 7 – 15 days for results and requires 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 UHT processed 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)
- 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:

- *Bacillus cereus, B. spizizenii, B. subtilis*
- *Citrobacter freundii*
- *Enterobacter cloacae*
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Salmonella* Typhimurium

Products tested:

- Standard UHT milk
- Whole UHT milk
- Skimmed UHT milk
- UHT chocolate milk

Sample Preparation and Enrichment

Sample Background/Baseline Testing

Product ATP baselines were determined by incubating the product at 30°C for 24, 48, and 72 hours. The sample was shaken thoroughly to mix, and 20 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 with between 2-30 CFU (target of ~10 CFU) and analyzed after incubation for 24, 48, 72 and 120 hours at 30°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 37°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 microorganisms.

Results

pH Assessment

pH readings for all UHT milk products fell consistently between 6.6 and 7.0.

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.

Food Type	Product RLU Threshold	Organism	Spike count	24 hr RLU (Avg)
UHT Milk	39	*Enterobacter cloacae*	9 CFU / pack	2,039
		Citrobacter freundii	6 CFU / pack	2,189
Skimmed UHT Milk	36	*Bacillus subtilis*	8 CFU / pack	250
Whole UHT Milk	21	*Bacillus subtilis*	8 CFU / pack	1,664
UHT Choc Milk	15	*Bacillus cereus*	5 CFU / pack	629
		Bacillus spizizenii	7 CFU / pack	5,098
UHT Choc Milk	42	*Salmonella* Typhimurium	11 CFU / pack	19,653
		Enterobacter cloacae	9 CFU / pack	8,872
		Citrobacter freundii	9 CFU / pack	160
		Escherichia coli	8 CFU / pack	11,626
		Pseudomonas aeruginosa	15 CFU / pack	131

Conclusions

Summary

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. These were set at between 15 and 42 RLU for all UHT milk products tested. RLU values above these thresholds indicate positive results. All UHT milk products tested had a pH within optimal range for reagent activity.

Recommendations

The Innovate System can detect multiple microorganisms in UHT 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.