Rapid Detection of Microorganisms in Soy Milk 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 this study was to evaluate the Innovate System for detection of low levels of microorganisms in soy 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:

- *Escherichia coli*
- *Staphylococcus aureus*
- *Saccharomyces cerevisiae*

Products tested:

- Standard soy 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 duplicate.

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, and 72 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 the soy milk products had an average of 6.54.

Background and Baseline Assessments
For the soy milk products tested, RLU baselines were low and consistent, allowing RLU cut-off threshold values to be set for the soy milk product. Value is shown in Table 1.

Inoculated Results
For the 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)
Soy Milk	121	*Escherichia coli*	13 CFU / pack	43,567
		Staphylococcus aureus	14 CFU / pack	49,871
		Saccharomyces cerevisiae	12 CFU / pack	599

Conclusions

Summary

The baseline studies showed successful depletion of background ATP when present, resulting in stable RLU values for the soy products tested. Stable baseline RLU values allow for the establishment of a positive/negative threshold value. This was set at 121 RLU for the soy milk product tested. RLU values above these thresholds indicate positive results. The soy milk product tested had a pH within optimal range for reagent activity.

Recommendations

The Innovate System can detect multiple microorganisms in soy milk 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.