Relationship Between the Innovate System and pH for the Detection of *Pseudomonas aeruginosa*

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. In the case of pH, results are unreliable as many organisms generate little change in product acidity initially, if at all, and certain food products can mask the detection of contamination by pH due to their buffering capacity.

The objective of this study was to evaluate the Innovate System for detection of low levels of *Pseudomonas aeruginosa* in a variety of dairy, plant-based dairy alternatives and other non-dairy beverages using the Innovate System RapiScreen™ Dairy Kit for ATP detection and comparing results to pH methods (and verifying results by plate inoculation and 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 included:

- Sterile inoculating loops, pipettes, and tips
- L-shaped spreaders
- Incubator (32°C)
- Innovate RapiScreen Dairy Kit (RSD)
- Innovate System
- Standard growth plates, TSA
- *Pseudomonas aeruginosa* culture
- pH meter and electrodes
- Syringes, 1 mL Insulin Syringe U-100
- Syringes, 3 mL Luer-Lok Tips
- Precision Glide Needles, 16 gauge 1 ½"
- Precision Glide Needles, 18 gauge 1 ½"

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

A *P. aeruginosa* culture was 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.

Testing 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 100 and greater than 1,000 CFU, and analyzed after incubation for 1, 2, 3, 4, 5, 6, 7 and 10 days at 32°C ± 2°C. After each incubation period, all samples were tested on the Innovate System using the RapiScreen Dairy Kit. In addition, pH values were measured on all incubated packs as well as control packs, using a standard benchtop pH meter.

Results

Analysis

A total of 87 products from various suppliers were analyzed to determine the RLU output at each day's incubation compared to pH measurements on the same days. Products were incubated at 30-32°C to allow for maximum growth.

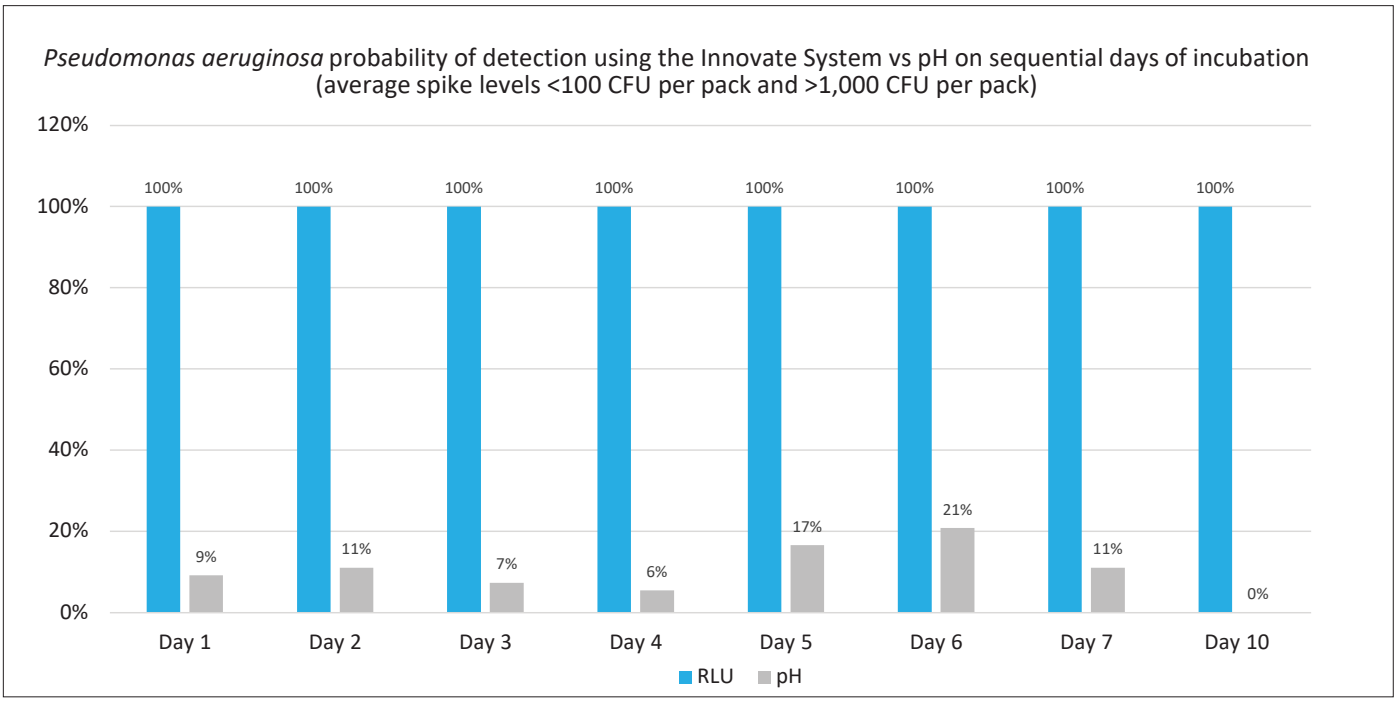
The criteria for a positive result on the Innovate System is any RLU value above the measured threshold for the specific product; thresholds were calculated as 3x the baseline RLU values. The criteria for a positive change in pH is defined as any shift in pH by 0.2 pH units from the starting pH of the incubated sterile product.

Probability of Detection (PoD)

RLU and pH values were determined for each day of incubation of each product type. Positive results were confirmed by plating and the probability of detection was calculated based on the criteria above.

Results were plotted as a percentage of positive cultures detected by either increased RLU values or pH shifts. A summary of the results is shown in Figure 1. The Innovate System was able to detect 100% of contaminated samples at each time point while pH results showed only up to 21% detection at Day 6 of incubation.

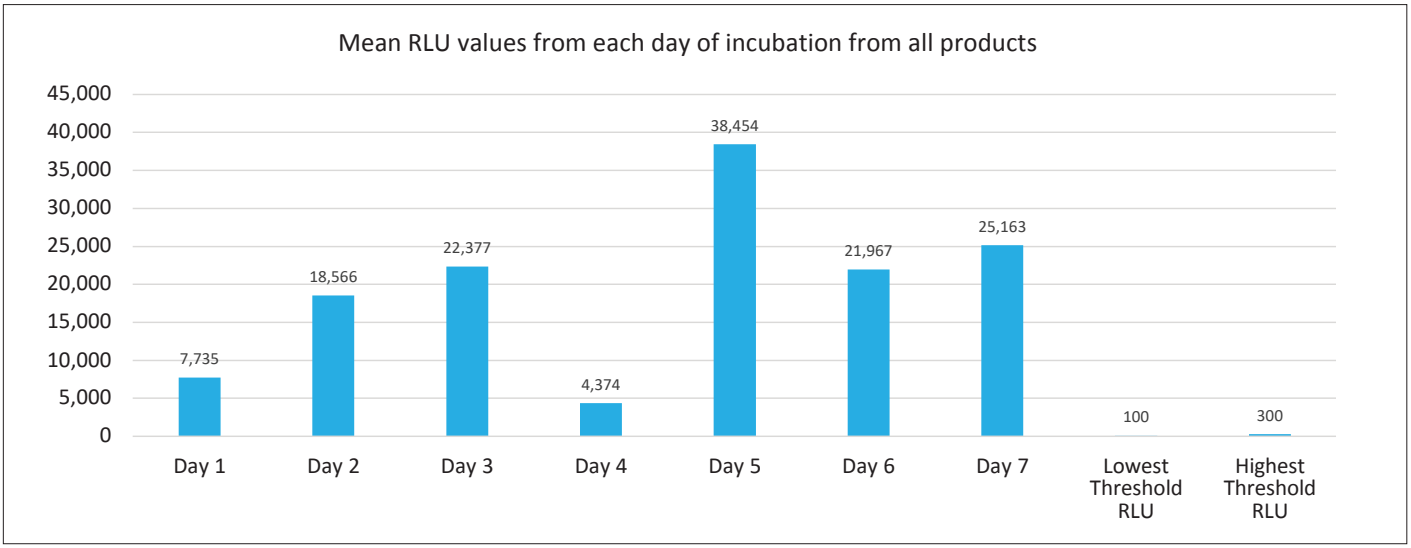
Figure 1. Calculated PoD% from all products contaminated with *P. aeruginosa*, using the Innovate System & pH measurements



RLU Values

When examining the RLU values obtained from the Innovate System, ATP levels above threshold values were seen at all time points. *P. aeruginosa* grew fairly rapidly in this cohort of products (compared to other organisms and confirmed by plating). In all products, the RapiScreen Dairy Kit was able to detect organism growth after only 24 hours of incubation. In addition, detection of growth, as determined by RLU values, remained positive extended incubation times. The mean of all RLU values for each day of incubation is shown in Figure 2.

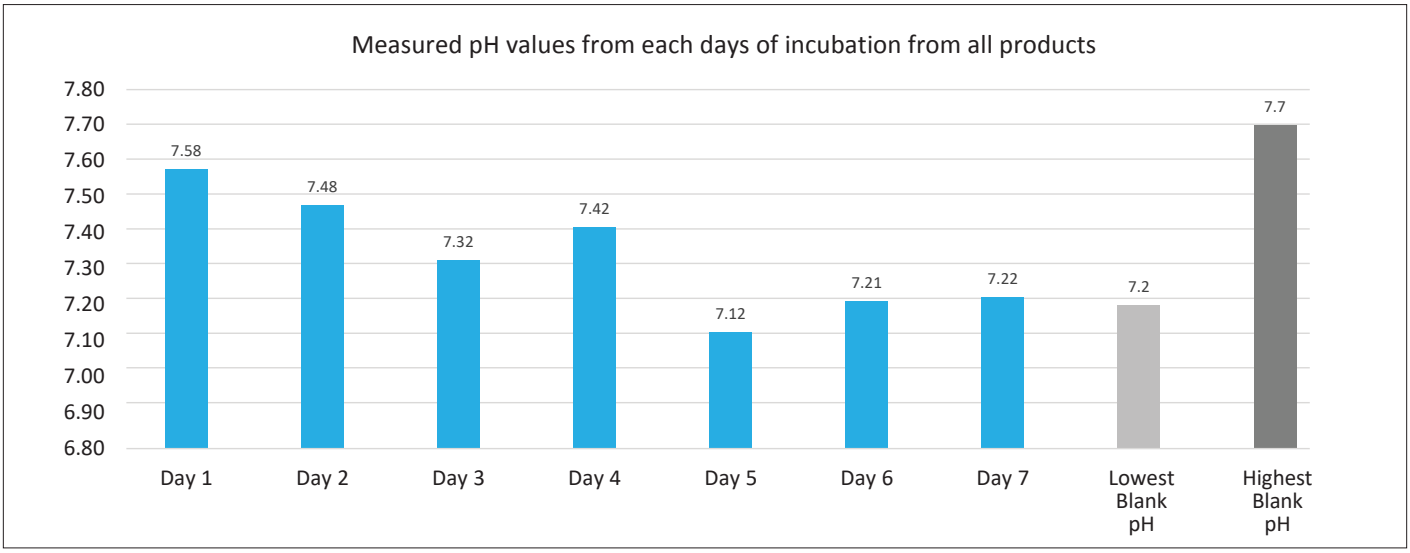
Figure 2. Measured mean RLU from all products contaminated with *P. aeruginosa* using the Innovate System



pH Values

For the products tested, the baseline pH values fell between 7.2 and 7.7. Over 7 days of incubation, the pH slowly dropped to a low mean pH of 7.12 (on day 5). However, this pH shift was not significant enough to indicate the presence of growth. This indicates that *Pseudomonas* species will not shift the pH significantly enough in a timeframe that allows for the productive use of pH measurement as an indicator of contamination. Results are show in Figure 3.

Figure 3. Measure mean pH from all products contaminated with *P. aeruginosa* using a benchtop pH meter



Conclusions

RLU vs pH Values

When examining sterile products for contamination with *P. aeruginosa*, only the Innovate System produced positive outcomes. Data confirmed detection of growth in liquid dairy and plant-based dairy alternatives in 24-48 hours using the Innovate System and was inconclusive when using pH measurements.

The level of contamination of <100 CFU per pack (mean pack size of 1,000 mL) or >1,000 CFU made no difference to either measurement system. Since the growth of *P. aeruginosa* at 32°C was fairly rapid, stationary phase will be reached in 24-48 hours and detection methods should be able to measure this growth in the same time frame. This was the case with the Innovate System; it produced enough ATP in under 24 hours to be measurable above the threshold values. However, in the case of pH, it was not a definitive measurement for contamination or spoilage in the products tested, even after 7 days of incubation.

Recommendations

The Innovate System can detect multiple microorganisms in liquid dairy and plant-based dairy alternative 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 pH, the Innovate System can streamline results for any facility needing improved time to results and reduced operational costs.