



One Health Diagnostics™

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Relationship Between Hygiena's Innovate™ System RLU Detection and pH for All Data Using *Staphylococcus aureus* as an Example

Innovate™

INTRODUCTION:

Staphylococcus aureus and similar organisms are used in routine challenge testing of UHT and ESL products. The growth of these organisms is rapid but the production of acids during growth can lag behind the production of ATP. A survey of a wide variety of products ranging from dairy beverages, plant-based dairy beverage alternatives to other non-dairy beverages were tested using the Innovate System and compared to pH measurements.

The measurement of pH is a simple and easy method to determine spoilage as most organisms will reduce sugars to simple acids pushing the pH down, but some organisms will generate acid slowly, little acid or not at all.

This means the detectability of spoilage requires a better measure such as in the poster demonstrating ATP as a quicker, more reliable method.

PURPOSE:

To demonstrate how bacterial growth is detected using the Innovate™ System vs pH changes within incubated product packs and how ATP detection can be better at detecting these organisms (*S. aureus* as an example for this study), both during challenge testing and routine testing in comparison with pH measurements.

REGISTERED TRADEMARKS:

Innovate™ is a registered trademarks of Hygiena.

METHOD:

87 products tested from the last 12 months were spiked with *Staphylococcus aureus* at 2 different levels; the higher level at 100 CFU/mL and the lower level at 0.1 CFU/mL. The incubations were all in-pack with product types broken down into the following groupings – dairy (42%), non-dairy alternatives (25%), soups (15%), sports drinks (15%) and high protein shakes (3%).

Packs were incubated at 32 °C ± 1 °C and assayed every 24 hours for extractable ATP RLUs and pH measurements. The data was analyzed to examine the RLU output at each day's incubation compared to pH measurements on the same days. The criteria for positivity for the Innovate System was any RLU above the measured threshold tested from the uninoculated product and for pH it was any shift in pH by 0.2 pH units from the starting pH of the incubated sterile product.

RESULTS:

Resultant RLU (Relative Light Units) measurements and pH measurements can be used to calculate the PoD% (probability of detection) when compared to standard plating confirmations run from each aliquot.

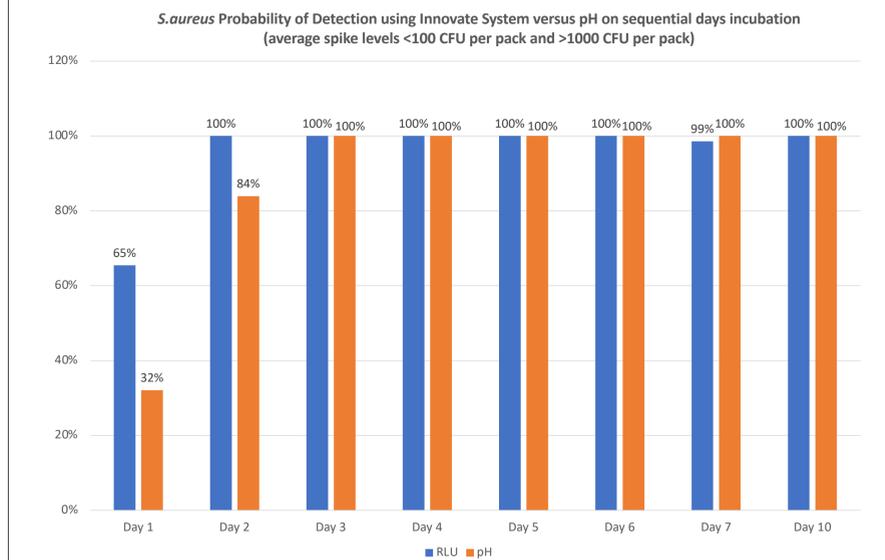
The PoD% for the ATP system and pH was as follows, at 24 hours 65% and 32%, at 48 hours 100% and 84% and for all other further incubation periods, results were 100% for both methods.

The mean RLU and pH measurements were as follows, at 24 hours 14,486 RLU and pH 7.2; at 48 hours, 37,415 RLUs and pH 6.66. The RLU level remained consistent through Day 7, with a RLU value of 49,107. The pH continued to drop on each day with the lowest pH being 5.93 on Day 7.

The results indicated that both the Innovate System and pH measurements will produce positive outcomes when measuring contamination due to *S. aureus* in the tested beverages in 24 hours using the Innovate System and in 48 hours using pH measurements.

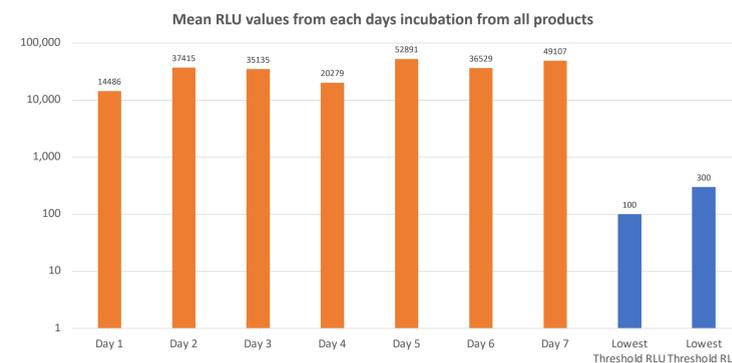
RESULTS:

Graph 1: Calculated PoD% (Probability of Detection) from All Products Contaminated with *S. aureus* Using the Innovate System and pH Measurements

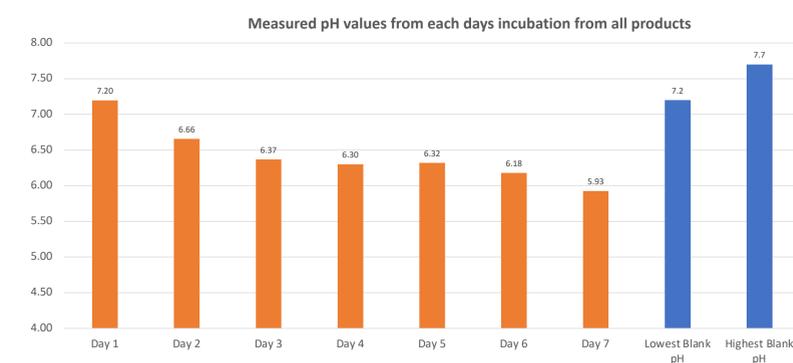


RESULTS:

Graph 2: Measured Mean RLU from All Products Contaminated with *S. aureus* Using the Innovate System



Graph 3: Measured Mean pH from All Products Contaminated with *S. aureus* Using a Benchtop pH Meter



SIGNIFICANCE:

The use of ATP measurement is a very rapid, sensitive and extremely efficient method for detecting very low-level contamination levels compared to pH when a testing regime is implemented. Even though the growth of *Staphylococcus aureus* is relatively slow, it will produce enough ATP in under 24 hours to be measurable above the threshold values.

