Automated ATP Testing Mitigates the Limitations and Delays of pH Measurement in UHT and ESL Product Release

Objective

In the dairy industry, pH is measured to assess quality, to identify and control impurities and microbial activity, to prolong shelf life and to control for taste. Acidity in milk results from both naturally occurring citrates, phosphates, and dissolved CO_2 used in processing as well as developed acidity which is due to bacterial degradation of lactose.

Measurement of pH indicates the strength of acid (concentration of hydrogen ions) in a dairy sample by either a calorimetric or more commonly an electrometric method. Because samples are rich in protein, precipitation can be a problem. Precipitates can form clogs and make sensor cleaning difficult, increasing the likelihood of sensor fouling. The fat content also decreases aqueous electrolyte miscibility thereby increasing response time. These factors can lead to unstable pH readings and inaccurate results. Moreover, the rate of significant pH change as a function of bacterial contamination is microorganism and matrix dependent, whereas non-fermentative organisms (e.g., *Pseudomonoadaceae*), filamentous fungi, and products with a high buffering capacity (e.g., high protein, salt) may not reliably shift pH levels despite contamination.

Rapid release of UHT and ESL products is vital to efficient workflow and revenue targets for the dairy industry. With these goals in mind, the objective of this analysis is to determine the probability of microorganism detection over time utilizing traditional pH testing versus the Hygiena™ Innovate System with RapiScreen™ ATP technology.

Method

Eighty-seven product types, including dairy, plant-based dairy alternatives, and other non-dairy beverages were spiked at <100 CFU and >1,000 CFU per pack. Products were incubated at 30-32 ºC for a minimum of 24 hours to accommodate both mesophilic and opportunistic psychrophilic organisms. Thermophilic *G. stearothermophilus* was incubated at 55 ºC.

Organism	Products Tested	Data Points Generated
Aspergillus brasiliensis	87	783
Bacillus cereus	87	783
Clostridium spp.	87	783
Geobacillus stearothermophilus	52	532
Pseudomonas aeruginosa	87	783
Saccharomyces spp.	87	783
Salmonella spp.	87	783
Staphylococcus aureus	87	783

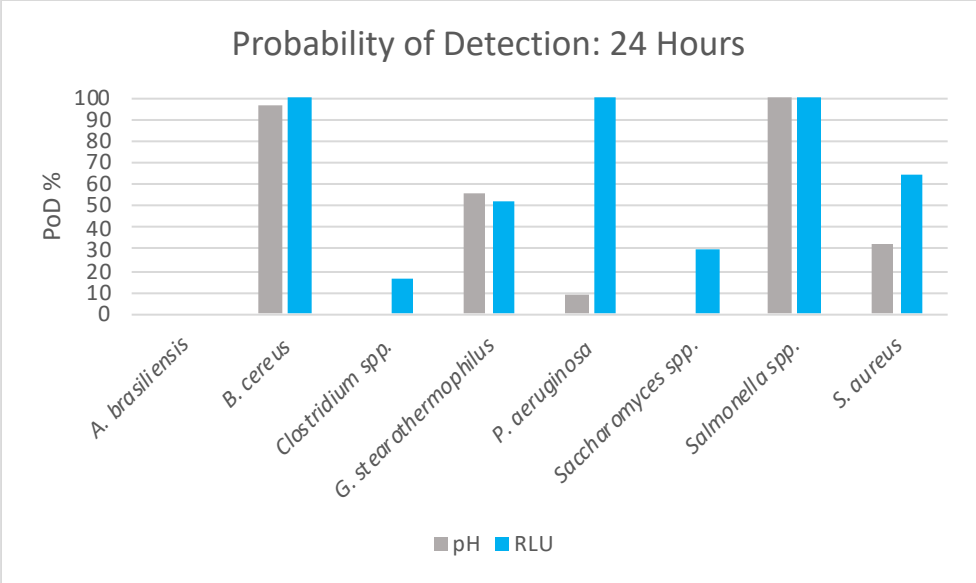
Positive criteria for the Innovate (ATP) System is 3X baseline while pH is 0.2 units from the baseline incubated sterile product.

Results

In this study, similar Probability of Detection (PoD) was seen at 24 hours utilizing both the Innovate System ATP (RLU) and pH for the following organisms:

- *B. cereus*, 97% PoD versus 100% for pH within 24 hours
- *G. stearothermophilus*, 52% PoD versus 56% for pH within 24 hours
- *Salmonella* spp., 100% PoD versus 100% for pH within 24 hours

Figure 1. Summary Data, 24 Hours



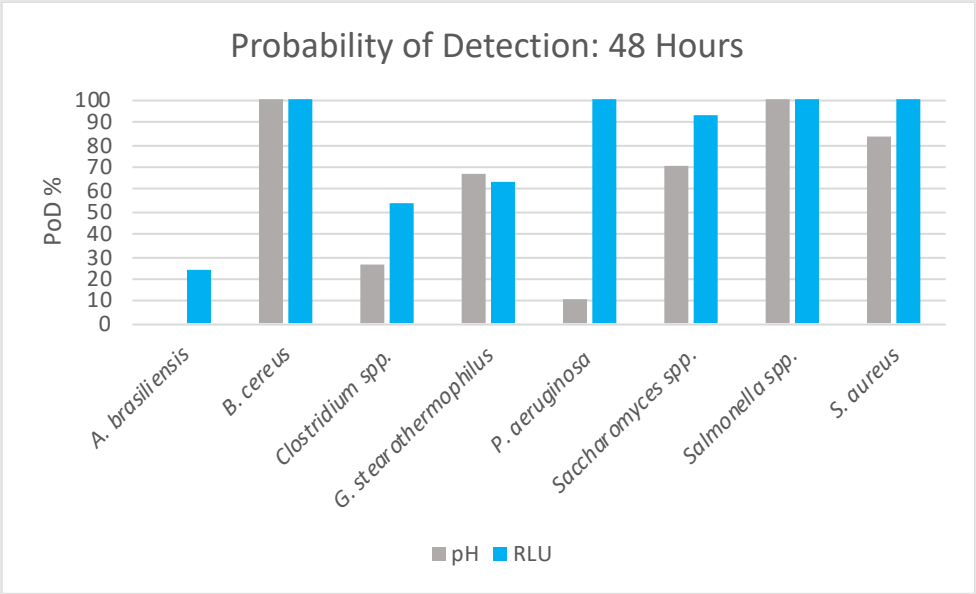
However, significantly improved PoD was seen with the Innovate System ATP (RLU) versus pH in 24 hours for the following organisms:

- *Clostridium* species, 16% PoD versus 2% for pH within 24 hours
- *Pseudomonas aeruginosa*, 100% PoD versus 9% for pH within 24 hours
- *Staphylococcus aureus*, 65% PoD versus 32% for pH within 24 hours

At 48 hours, the probability of detection was similar for the same three organisms:

- *B. cereus*, 100% PoD versus 100% for pH at 48 hours
- *G. stearothermophilis* spp., 63% PoD versus 67% for pH at 48 hours
- *Salmonella* spp., 100% PoD versus 100% for pH at 48 hours

Figure 2. Summary Data, 48 Hours



However, significantly improved PoD was seen with the Innovate System ATP (RLU) versus pH in 48 hours for the following organisms:

- *A. brasiliensis*, 24% PoD versus 0% for pH within 48 hours
- *Clostridium* spp., 54% PoD versus 26% for pH within 48 hours
- *Saccharomyces* spp., 93% PoD versus 70% for pH within 48 hours

Table 1. PoD (%) of pH and RLU Data for Each Organism Tested

Incubation Time	Day 1		Day 2		Day 3		Day 7	
Organism	**pH**	**RLU**	**pH**	**RLU**	**pH**	**RLU**	**pH**	**RLU**
Aspergillus brasiliensis	0	0	0	24	0	36	30	39
Bacillus cereus	97	100	100	100	100	100	100	97
Clostridium spp.	2	16	26	54	35	50	18	21
*Geobacillus stearothermophilus**	56	52	67	63	69	52	84	27
Pseudomonas aeruginosa	9	100	11	100	7	100	11	100
Saccharomyces spp.	0	30	70	93	89	78	50	50
Salmonella spp.	100	100	100	100	100	100	100	100
Staphylococcus aureus	32	65	84	100	100	100	100	99

*Results shown for 30-32 ºC incubation. At 55 ºC, the PoD for both pH and ATP (RLU) is 100% at 24 hours.

Discussion

Highlights of the ATP (RLU) versus pH Probabilities of Detection data for the eight organisms tested are as follows:

- Detection of the filamentous fungi, *Aspergillus brasiliensis*, is achieved at 48 hours using the Innovate System RapiScreen™ method and requires a minimum 200 spore count, whereas pH measurement cannot detect this organism until day 7.
- In the case of the obligate anaerobe, *Clostridium* spp., growth across inoculated samples is inconsistent. The Innovate System method, however, was able to detect growth with a 54% PoD in 48 hours while pH did not reach its maximum until 72 hours and only provided a 35% PoD.
- Because non-fermenting *Pseudomonas aeruginos*a does not produce a significant change in pH, this method does not offer a reliable option for detection. In contrast, the Innovate System method achieved 100% PoD within 24 hours.
- *Saccharomyces* spp. more rapidly achieved a high PoD (93% at 48 hours) using the Innovate System method compared with a delayed yet similar pH method PoD of 89% at 72 hours.
- Small, non-motile and slow growing *Staphylococcus aureus* produces detectable ATP more rapidly in the early growth phase (65% at 24 hours, 100% within 48 hours) compared with a slower pH shift (32% PoD at 24 hours), making the Innovate System a more expeditious screening option.
- The rapid growth rate of *Bacillus cereus* and *Salmonella* spp. provides a very high probability of detection (97-100% within 24 hours) using both the Innovate System method and the benchtop electrometric pH meter utilized in this study.
- Thermophilic *Geobacillis stearothermophilus*, allows for similar PoD (52-67%) for both ATP and pH in 24-48 hours. However, both methods demonstrated 100% PoD by 24 hours when incubated at 55 ºC.

Conclusions

Two organisms (*B. cereus* and *Salmonella* spp.) of the eight tested achieved similarly high probabilities of detection within 24 hours using both the Innovate System ATP method and the electrometric pH method. Thermophilic *G. stearothermophilis* also performed similarly for the two methods. The balance of the organisms tested (63%) were detected more rapidly and consistently when tested using the Innovate System, demonstrating a significant benefit toward expedited product release by using ATP as a measure of growth.

This data demonstrates that the detection of contamination using ATP detection is more reliable, rapid, and efficient than pH for the detection of a wide variety of contaminants that could potentially impact a broad range of ESL/UHT products. Hygiena's Innovate System therefore allows for more consistent risk assessment, greater quality confidence, and more rapid product release in the dairy, dairy-alternative, and beverage industries.