Advantages of Rapid ATP Sterility Testing Using Hygiena's Innovate™ System for Rapid Detection of *Geobacillus stearothermophilus* in Non-Dairy Milk Alternatives

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One Health Diagnostics™

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Innovate™

INTRODUCTION:

The Innovate™ System is based around the detection of extractable microbial ATP from organisms growing within sterile or aseptic products. Nutritious non-dairy milk alternatives such as fat-based milk drink will support the growth of most organisms, but the highest spoilage risk comes via gram-positive spore formers. One such organism is the thermotolerant, *Geobacillus stearothermophilus.* This organism will survive UHT treatment and produce spoilage under incorrect storage conditions. It is very important to consider the time to detection and temperature profile of this organism as this organism will grow at elevated temperatures only.

Geobacillus stearothermophilus grows very rapidly in the product, the time to result is based around the highest possibility of detecting both low and high CFU levels. Due to this factor, the incubation time to detection needs to be kept as short as possible at 24-48 hours.

The subsequent growth and senescence of this organism needs to be considered when designing a rapid ATP-based detection program. The traditional plating methods could take 7-15 days to detect confirmed contamination, whereas the Innovate System can be used to generate results in 24 -48 hrs, depending on the organism, level of contamination and matrix being evaluated.

PURPOSE:

To demonstrate the rapid growth of *Geobacillus stearothermophilus* in non-dairy milk alternatives and evaluate the following :

1) Potential of the Innovate System to detect contamination when spiked into the product throughout the different incubation days.
2) Highlight the importance of using the rapid detection using ATP-based methods based on organism such as *Geobacillus*, which is a thermotolerant spore-former.

REGISTERED TRADEMARKS:

METHOD:

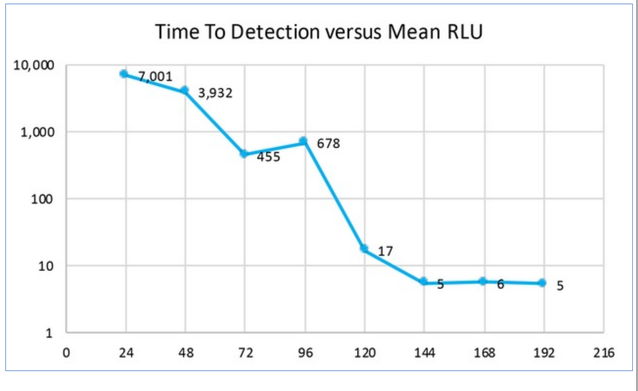
Wild-type *Geobacillus* was isolated from customer supplied non-dairy milk which was confirmed to 99% ID using the RiboPrinter™ system.
The bacteria were grown overnight and inoculated into 1 L product packs at <10 CFU per pack. Growth of this organism was followed for 7 days at 55 °C. Triplicate packs were incubated and the resultant RLUs measured. Plate confirmations were run at each incubation time alongside pH measurements. This bacteria were also inoculated at each dilution from -1 through -9 into 100 mL of milk decanted into sterile vials. These were then incubated at 55 °C and assayed for RLU values at 4, 8, 12, 24 and 48 hours.

RESULTS:

The mean RLU for in-pack incubation is greatest at 24 hours at 7,001 RLUs. This value declines daily until the baseline RLU is reached. This baseline RLU would be considered negative but since the packs have gone through the growth cycle, this will be mainly spores with no vegetative cells. The out-of-pack incubation shows that growth proceeds rapidly and significant RLUs are measurable above the baseline at 4 hours for higher dilutions (-1), (-2) and (-3) with the lower dilutions (-5), (-6) and (-7) being detected at 8 hours incubation time. Stationary phase was reached at 8 to 12 hours in this matrix.

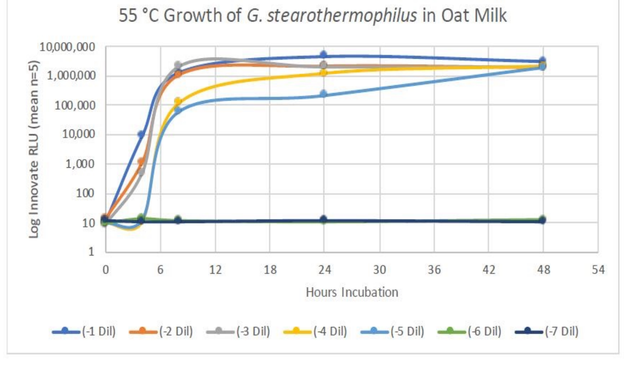
RESULTS:

Graph 1: Mean RLU from the Innovate System at Each Tested Incubation Period (24 hours)



RESULTS:

Graph 2: Time Course Growth of *G.stearothermophilus* at 55 °C Measured for ATP Content using the Innovate System Assay Early Incubation Periods of 4 hours, 8 hours, 24 hours and 48 hours



CONCLUSIONS:

Geobacillus stearothermophilus does not grow at psychrophilic or mesophilic temperatures. This organism only grows at elevated temperatures in this experiment at 55 °C and complete the growth in non-dairy milk exceptionally quickly and undergoes senescent behaviour. The sporulation produces an appreciable decline in the measured RLUs which can be seen as a declining RLU from 24 hours onwards. This decline continues as the organism fully sporulates. The Innovate System will continue to detect this organism until the thresholds set up are undermined by the reduction in ATP.

The optimum time to detection for spore-formers should be limited to 72 hours, but shorter incubation times are preferred and have a higher degree of certainty. Based on this data, Hygiena advocates an incubation of 48 hours. This will result in a higher probability of detection for a broader range of organisms and minimize the risk of false negatives from overgrowth.