Rapid Detection of *Cronobacter* species in Non-Dairy, Plant-Based Products Using the ATP Detection Innovate™ System

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INTRODUCTION:

Cronobacter sakazakii and *Cronobacter muytjensii* are motile, gram-negative, non-spore-forming, rod-shaped coliform bacteria within the family *Enterobacteriaceae*, genus *Cronobacter*. *Cronobacter spp.* have been implicated in outbreaks of neonatal illness (premature infants), in isolated cases of severely immuno-compromised individuals and in the elderly. *Cronobacter* can survive in dry foods, like powdered infant formula, powdered milk, herbal teas and starches, even throughout the desiccation process.

Recently, *Cronobacter sakazakii* has been associated with a large voluntary recall from a nutritional and beverage products company. Preliminary root cause analysis showed that the products did not meet commercial sterility specifications.

The Innovate™ Rapid Microbial Screening System is designed for the rapid detection of microorganisms in a wide range of products. To detect very low levels of contaminants in these types of products, an enrichment step is required to ensure that there is sufficient ATP present for detection. 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.

This study demonstrates the detection of *Cronobacter sakazakii* and *Cronobacter muytjensii* in 6 different plant-based dairy alternatives.

PURPOSE:

The objective of this study was to :

1. Ensure that *Cronobacter sakazakii* and *Cronobacter muytjensii* are detectable using ATP methods in non-dairy, plant-based products.
2. Six different flavors of plant-based products were spiked at high and low levels with *Cronobacter sakazakii* and *Cronobacter muytjensii* and detection was compared with standard agar plate methods.

METHOD:

Bacteria were grown and diluted into 6 products: ESL Milk – Chocolate, Low-fat and Original; UHT Milk – Chocolate and Original; and High-fat milk (Table 1), where each milk had differing ingredients and fat content and represented a range of flavors and colors. The study was done in duplicate. Bacteria (Table 2) were added at 2 levels: a high CFU (approximately 10,000 CFU per pack or 10 CFU per 1 mL) and a low CFU (<100 per pack or <1 CFU per 10 mL). Each pack was incubated at 32 °C and aliquots were removed and examined for ATP for 3 consecutive days using the RapiScreen™ Dairy Kit, and samples were confirmed by plating on TSA plates.

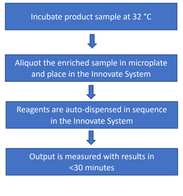
Table 1: List of Products Tested

List of Plant –Based Milks Tested
Chocolate Oat Milk -ESL
Low-Fat Oat Milk -ESL
Original Oat Milk - ESL
Barista Oat Milk - UHT
Chocolate Oat Milk - UHT
Original Oat Milk- UHT

Table 2: *Cronobacter spp.* and Pre-incubation Temperatures

Organism Panel	ATCC	Pre-incubation Temperature
Cronobacter muytjensii	51329	32 °C
Cronobacter sakazakii	29544	32 °C

Table 3: Innovate System Testing protocol



RESULTS:

As shown in Table 4, both *C. muytjensii* and *C. sakazakii* were detected using the Innovate System after 24-hour incubations in all products spiked with the organisms at low and high spike levels. As for the traditional plating method, positive results were obtained after 48 hours for all samples tested.

Each milk type produced rapid growth in the packs at 24 hours. The comparison of growth rate using RLUs shows the following: ESL and UHT Original had mean RLUs of 99,880 at high spike and 74,660 for low spike and 114,928 and 128,786 RLUs at 24 hours, respectively. At low spike, ESL and UHT Chocolate had 134 RLUs and 174 RLUs at 24 hours, rising to 90,537 RLUs and 29,407 RLUs, respectively, at 48 hours - the chocolate milk appears to slow down the growth and subsequent ATP titer. For ESL Low-fat and UHT High-fat milks, the low spike was 7,804 RLUs and 33,316 RLUs, respectively. Both species tested were easily detected in all 6 products, producing 100s of RLU above the baseline, showing detection within 24 hours.

CONCLUSIONS AND SIGNIFICANCE:

As summarized in the results section, both *C. muytjensii* and *C. sakazakii* were detected using the Innovate System after 24 hours incubation in all products spiked with the organisms. As for the traditional plating method, positive results were obtained after 48 hours for all samples tested.

Results for growth on day 3 and beyond were not completed due to the excessive growth of both organisms in all the products. By day 2, all the product containers were bloated. Due to this, the containers were disposed of after day 2 to prevent the risk of the products bursting inside the incubator. Similarly, only the low spikes for the ESL Chocolate, ESL Low-Fat, UHT Barista, and UHT Chocolate Milk types were tested due to the fast growth of both organisms in all the Oat milk products.

Aseptic processing of oat milk products may help reduce the risk of microbial contamination of the products. This is clearly shown in the low baseline values for ATP detection in uninoculated samples. In addition, *Cronobacter* contamination at low levels (<100 CFU per container) could be detected at 24 hours using the Innovate System and RapiScreen Dairy Kit. This was 24 hours faster than detection using standard plating methods.

Based on these results we can conclude that *Cronobacter* spp. can be rapidly detected in plant-based milk using the rapid Innovate System ATP method.

RESULTS :

Table 4: Time to Detection

	C. muytjensii Detection				*C. sakazakii* Detection			
	INNOVATE SYSTEM		PLATING		INNOVATE SYSTEM		PLATING	
PRODUCT	High CFU	Low CFU	High CFU	Low CFU	High CFU	Low CFU	High CFU	Low CFU
ESL Original	24 hours	24 hours	48 hours	48 hours	24 hours	24 hours	48 hours	48 hours
UHT Original	24 hours	24 hours	48 hours	48 hours	24 hours	24 hours	48 hours	48 hours
ESL Chocolate	N/A	24 hours	N/A	48 hours	N/A	24 hours	N/A	48 hours
ESL Low Fat	N/A	24 hours	N/A	48 hours	N/A	24 hours	N/A	48 hours
UHT Barista	N/A	24 hours	N/A	48 hours	N/A	24 hours	N/A	48 hours
UHT Chocolate	N/A	24 hours	N/A	48 hours	N/A	24 hours	N/A	48 hours