Rapid Detection of Microorganisms in Human Milk Fortifier Using Hygiena® ATP-based Innovate™ Rapid Microbial Screening System

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INTRODUCTION:

Human milk fortifiers (HMF) play an important role in supporting the growth and nutritional needs of preterm and low-birth-weight infants. These products supplement essential nutrients required for early development. Because these products are used for highly vulnerable neonatal populations, maintaining commercial sterility is essential.

Traditional microbial testing often takes several days or even weeks for results, which can delay critical manufacturing and product release decisions. Rapid ATP-based microbial screening methods can offer an alternative approach for earlier detection of microbial contamination.



PURPOSE:

The objective of this study was to evaluate a rapid screening method utilizing ATP bioluminescence for the detection of spoilage organisms in Human Milk Fortifier compared to traditional culture-based methods.

METHODS:

Establishing Threshold: Five sterile sachets of human milk fortifier were incubated at 33 ˚C for 48 hours to establish the baseline signal for the matrix. Following incubation, 50 µL aliquots from each sachet were aseptically transferred into a microtiter plate (in replicates of 8) to generate 40 total data points. Relative light unit (RLU) values obtained from all wells were averaged and the mean baseline signal was multiplied by 3 to establish the product-specific detection threshold.



METHODS:

Inoculation Study: Six bacterial organisms of concern, including *Bacillus cereus* spores, *Clostridium sporogenes*, *Cronobacter sakazakii*, *E. coli*, *Salmonella enterica* (serovar Typhimurium) and *Staphylococcus aureus,* were used to inoculate 5 mL sachets of human milk fortifier. Each organism was inoculated into 3 sachets at a level between 1-10 CFU to create a total of 18 samples. Sachets were incubated at 33 ˚C and tested over a 10-day period. Sample aliquots (50 µL) were aseptically removed each day and dispensed into a microtiter plate in triplicate for microbial ATP detection using the RapiScreen™ Dairy kit. At the same time, samples were plated to confirm growth.

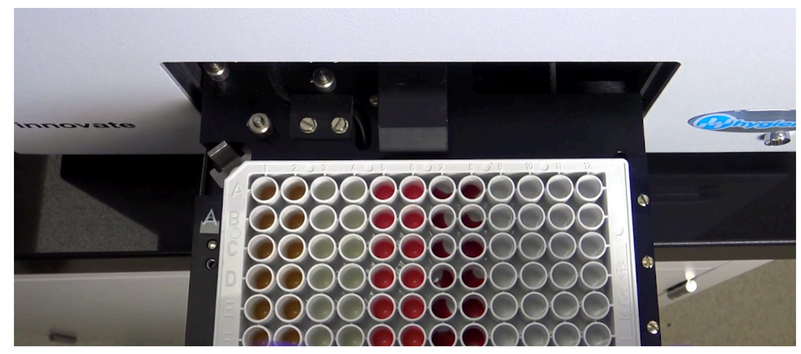


TABLE 1: Time to Detection

Product	Threshold	Organism Panel	Inoculated CFU Level per Pack	Innovate System	Avg. RLU	Plating
Human Milk Fortifier	40 RLU	*Bacillus cereus* spores	4	Day 1	20348	Day 2
		Clostridium sporogenes	4	No Detection	13	No Growth
		Cronobacter sakazakii	5	Day 1	7350	Day 2
		E. coli	5	Day 1	19133	Day 2
		Salmonella Typhimurium	10	Day 1	19141	Day 2
		Staphylococcus aureus	3	Day 1	191	Day 2

RESULTS:

HMF Detection Threshold 40 RLU

Microbial growth was detected for 5 of 6 organisms within 24 hours of incubation. The RLU signal was significantly above the threshold.

Clostridium sporogenes remained below the threshold throughout the 10-day incubation and showed no growth by culture.

Culture results were in complete agreement with ATP results for all organisms tested, but required an additional 24 hours of plate incubation for colony development.

SIGNIFICANCE:

The Innovate™ System detects low-level contamination earlier than traditional culture methods supporting faster and confident decision-making

DL Envelope

REFERENCES:

1. Innovate Rapid Microbial Screening System: https://www.hygiena.com/food-safety/commercial-sterility-testing/innovate

2. RapiScreen Dairy Kit: https://www.hygiena.com/food-safety/commercial-sterility-testing/rapiscreen-dairy-kit

REGISTERED TRADEMARKS:

Innovate™ is a trademark of Hygiena® for its line of equipment, reagents and software used to analyze samples for microbial contamination.
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