Rapid Detection of Microorganisms in All-Purpose Cream Using Hygiena's ATP-based Innovate™ Rapid Microbial Screening System

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INTRODUCTION:

Commercial sterility testing is essential for confirming the shelf-life stability of Ultra-High Temperature (UHT)–processed, aseptically packaged all-purpose cream. Detecting microbial contamination in these products is challenging due to low initial bioburden, matrix-related inhibition, and delayed microbial recovery caused by emulsifiers and stabilizers. This study assesses the performance of the Hygiena® Innovate™ Rapid Microbial Screening System, which uses RapiScreen™ ATP bioluminescence technology to enable faster microbial detection compared to conventional methods.

The Hygiena Innovate System is a high-throughput microbial screening platform developed for diverse food and beverage applications. Its ATP bioluminescence-based detection, combined with product-specific enrichment, is designed to overcome inhibitory effects in complex food matrices and can deliver faster time-to-result than traditional plating techniques.

This work focuses on optimizing the detection of *Bacillus cereus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* in Crèma all-purpose cream. Both in-pack and out-of-pack incubation strategies were evaluated. While standard protocols rely on in-pack incubation, out-of-pack enrichment in nutrient-rich media was explored as a way to enhance microbial recovery and reduce overall incubation time in challenging matrices.

Samples of cream were inoculated at low levels (1, 3, and 9 CFU/pack) and incubated in-pack at 30 °C for up to 48 hours. After 24 hours, aliquots were transferred to Brain Heart Infusion (BHI) and Buffered Peptone Water (BPW) for additional incubation. Microbial growth was monitored at multiple time points using the Innovate System, with parallel agar plating as a reference method. Results, expressed in Relative Light Units (RLUs), clearly distinguished contaminated from uncontaminated samples, demonstrating the system's suitability for both liquid and high-fat, viscous dairy products.

Compared with the current in-house rapid method—which requires extended incubation followed by filtration, centrifugation, washing, and resuspension—the Innovate System offers a simplified workflow and reduced turnaround time, supporting more efficient sterility testing in dairy processing environments.

Importantly, the adoption of rapid and reliable sterility testing methods has broader implications for quality assurance and production efficiency within the dairy industry. Faster detection of contamination enables earlier decision-making, reduces product hold times, and minimizes the risk of distributing compromised products. By improving sensitivity in difficult matrices while streamlining laboratory workflows, the Innovate System has the potential to enhance both operational efficiency and food safety outcomes in modern manufacturing settings.

PURPOSE:

This study was designed to evaluate and compare a modified, shorter enrichment method for rapid ATP-based screening that produces qualitative results comparable to the traditional plating method after 42 hours of incubation for the detection of risk organisms such as *Bacillus cereus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* in all-purpose cream samples.

REFERENCES:

1. Hygiena® (2022) Innovate™ Rapid Microbial Screening System. Accessed 2026. Available from: https://www.Hygiena.com/food-safety-solutions/microbial-screening/innovate/

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RapiScreen™ Dairy Kit

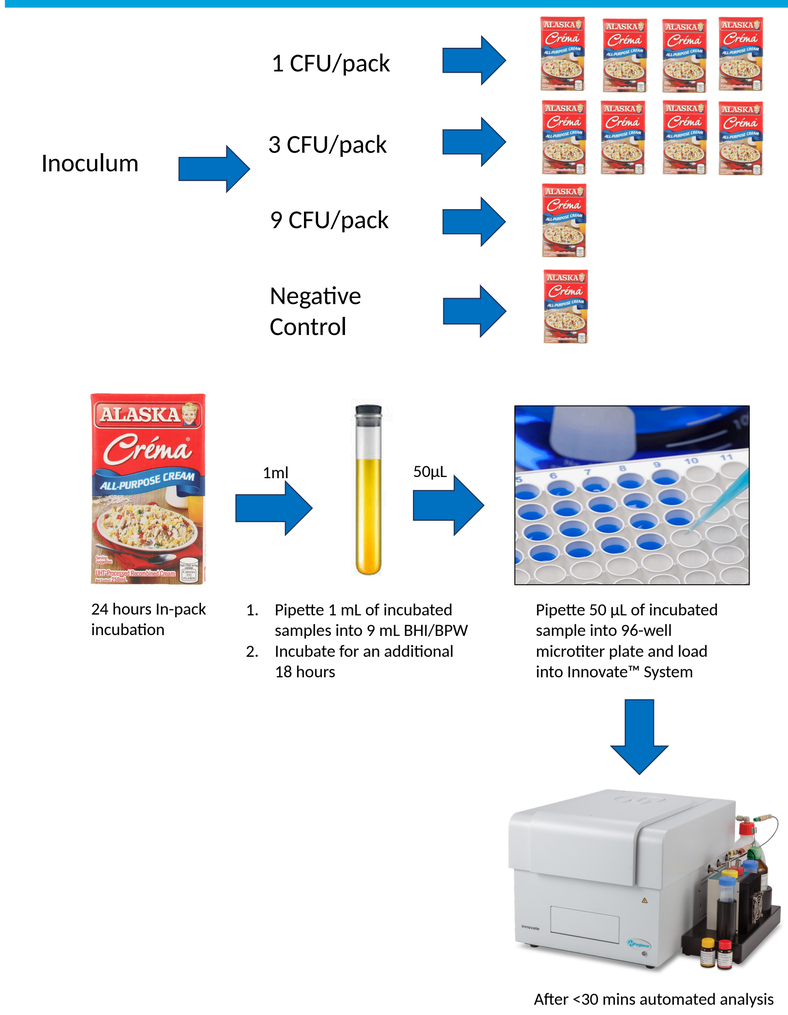
METHOD:

Initial baseline RLU levels were established in sterile Crèma, an all-purpose cream sample (14 RLU), in order to distinguish contaminated samples from non-contaminated samples. An RLU pass/fail level was established from these baselines and used to analyze spiked products. To establish the baseline, 30 non-inoculated samples from this product type were incubated at 30 °C for 48 hours as a negative control. After 48 hours of incubation, all samples were measured using the Innovate System. The threshold for each product was set according to the baseline multiplied by three. All samples with an RLU value above the threshold were considered contaminated.

Microbial cultures of *Bacillus cereus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa were* enriched in their respective enrichment broths. Defined inoculum concentrations of 1, 3 and 9 CFU/sample were spiked directly into sterile Crèma product packs. Spiked and non-inoculated products were incubated up to 48 hours at 30 °C. In a parallel experiment, additional spiked product packs were incubated in-pack for 24 hours, after which 1 mL aliquots were transferred into 9 mL of BHI and 9 mL of BPW, and the aliquots were further incubated for 18 hours.

After product incubation, aliquots were taken for parallel testing using the Innovate System and the plate-counting method. Innovate System measurements were conducted utilizing the RapiScreen (RS) Dairy Kit. The standard RS dairy protocol was used for the Innovate System measurement.

WORKFLOW:



RESULTS:

Table 1. Results of the Crèma Spike Study. (Positives obtained from the Innovate™ System reads and the reference plating method are shown side by side after 48 hours and 72 hours incubation.)

No.	Sample	Strain	Spiking Level (CFU/package)	Incubation Temperature [°C]	Number of sample Replicates	48 hrs		72 hrs	
						RLU Threshold (43 RLU)	Plating (Positive/Negative)	RLU Threshold (43 RLU)	Plating (Positive/Negative)
1	Crèma	*Bacillus cereus*	0	30	1	6	Negative	4	Negative
2			1	30	1	2043	Positive	2572	Positive
3				30	2	1	Negative	4553	Positive
4				30	3	8	Negative	10613	Positive
5				30	4	8	Negative	15661	Positive
6			3	30	1	7	Negative	16286	Positive
7				30	2	10	Negative	7807	Positive
8				30	3	6	Negative	8374	Positive
9				30	4	76	Negative	1612	Positive
10			9	30	1	15684	Positive	3127	Positive
11		*Bacillus subtilis*	0	30	1	10	Negative	5	Negative
12			1	30	1	8	Negative	1993	Positive
13				30	2	9	Negative	5596	Positive
14				30	3	10	Negative	4848	Positive
15				30	4	11	Negative	839	Positive
16			3	30	1	9	Negative	1912	Positive
17				30	2	20	Positive	5	Negative
18				30	3	9	Negative	5	Negative
19				30	4	8	Negative	1123	Positive
20			9	30	1	8	Negative	1042	Positive
21		*Pseudomonas aeruginosa*	0	30	1	8	Negative	1	Negative
22			1	30	1	37174	Positive	13217	Positive
23				30	2	24520	Positive	14152	Positive
24				30	3	23437	Positive	10590	Positive
25				30	4	28425	Positive	11660	Positive
26			3	30	1	36369	Positive	15180	Positive
27				30	2	28828	Positive	19228	Positive
28				30	3	31210	Positive	15065	Positive
29				30	4	37496	Positive	16027	Positive
30			9	30	1	32980	Positive	18120	Positive

Table 2. Results of the Crèma Spike Study. (Positives obtained from the Innovate™ System reads and the reference plating method are shown side by side after 24 + 18 hours incubation.)

No.	Sample	Strain	Spiking Level (CFU/package)	Incubation Temperature [°C]	Number of sample Replicates	42 hrs (24 hrs pre-incubate+18 hrs in BHI)		42 hrs (24 hrs pre-incubate+18 hrs in BPW)	
						RLU Threshold (43 RLU)	Plate (Positive/Negative)	RLU Threshold (43 RLU)	Plate (Positive/Negative)
						BHI		BPW	
1	Crèma (pre- incubate in package 24 hrs. then take 1 mL to enrich in media (1:10))	*Bacillus cereus*	0	30	1	13	Negative	1	Negative
2			1	30	1	113040	Positive	14658	Positive
3				30	2	117214	Positive	11898	Positive
4				30	3	102827	Positive	17010	Positive
5				30	4	100258	Positive	19389	Positive
6			3	30	1	95271	Positive	22783	Positive
7				30	2	62218	Positive	18349	Positive
8				30	3	105354	Positive	29148	Positive
9				30	4	91978	Positive	28974	Positive
10			9	30	1	96439	Positive	27184	Positive

SIGNIFICANCE:

The optimized enrichment protocol uses a hybrid in-pack and out-of-pack incubation strategy to enhance the recovery of risk organisms such as *Bacillus cereus* spiked into all-purpose cream samples, enabling reliable detection within 42 hours and demonstrating a rapid, high-throughput solution for commercial sterility testing of viscous, high-fat all-purpose cream samples. This optimized protocol achieved a detection sensitivity of 1 CFU/pack within a 42-hour incubation period at 30 °C. By reducing the turnaround time by six hours compared to the existing method, the Innovate System provides full sensitivity even in complex, high-fat matrices such as all-purpose cream. These results confirm that rapid ATP screening is a reliable and efficient tool for identifying key spoilage organisms, significantly enhancing commercial sterility testing for the dairy industry.