Fit-for-purpose Verification Using Hygiena's ATP-based Innovate™ System for Sterility Testing of Chocolate-flavored Milk Drink

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Innovate™ | Innovate™ AUTOSAMPLER III

INTRODUCTION:

Commercial sterility testing is critical for validating the shelf-life stability of Ultra-High Temperature (UHT) processed, aseptically packaged products. However, detecting microbial contamination in these products can be challenging due to their low initial bioburden and potential matrix-related inhibition. Rapid detection methods can provide valuable information to support timely product release decisions. This study evaluated the performance of the Hygiena® Innovate™ Rapid Microbial Screening System, based on RapiScreen™ ATP bioluminescence technology, as a rapid alternative to conventional culture-based methods for detecting microbial contamination in a chocolate-flavored milk drink. The study assessed the limit of detection of the Innovate System for selected risk organisms, including *Bacillus cereus*, *Bacillus licheniformis*, *Enterococcus faecalis*, and *Escherichia coli*. The rapid ATP method was evaluated in parallel with traditional culture following a short enrichment incubation. Using the Innovate System, results can be obtained within 30 minutes, with approximately 5 minutes of hands-on time, offering the potential to significantly reduce the time required for microbial screening and to support faster product release decisions.

Samples of chocolate-flavored milk were inoculated with serially diluted bacterial cultures, which were then used to inoculate product packs in triplicate to achieve final concentrations ranging from 10^3 to 10^6 CFU/mL.

Samples were then tested 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 this chocolate-flavored milk.

Compared with the current in-house rapid method, the Innovate System offers greater sensitivity for testing this specific product type, with an option for scale-up using the Autosampler.

Importantly, the adoption of rapid, reliable sterility testing methods has broader implications for quality assurance and production efficiency in 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 with the option for automation, 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 the performance and fitness-for-purpose of a rapid ATP-based screening method for the detection of risk organisms such as *Bacillus cereus*, *Bacillus licheniformis*, *Enterococcus faecalis*, and *Escherichia coli* in chocolate-flavored milk samples.

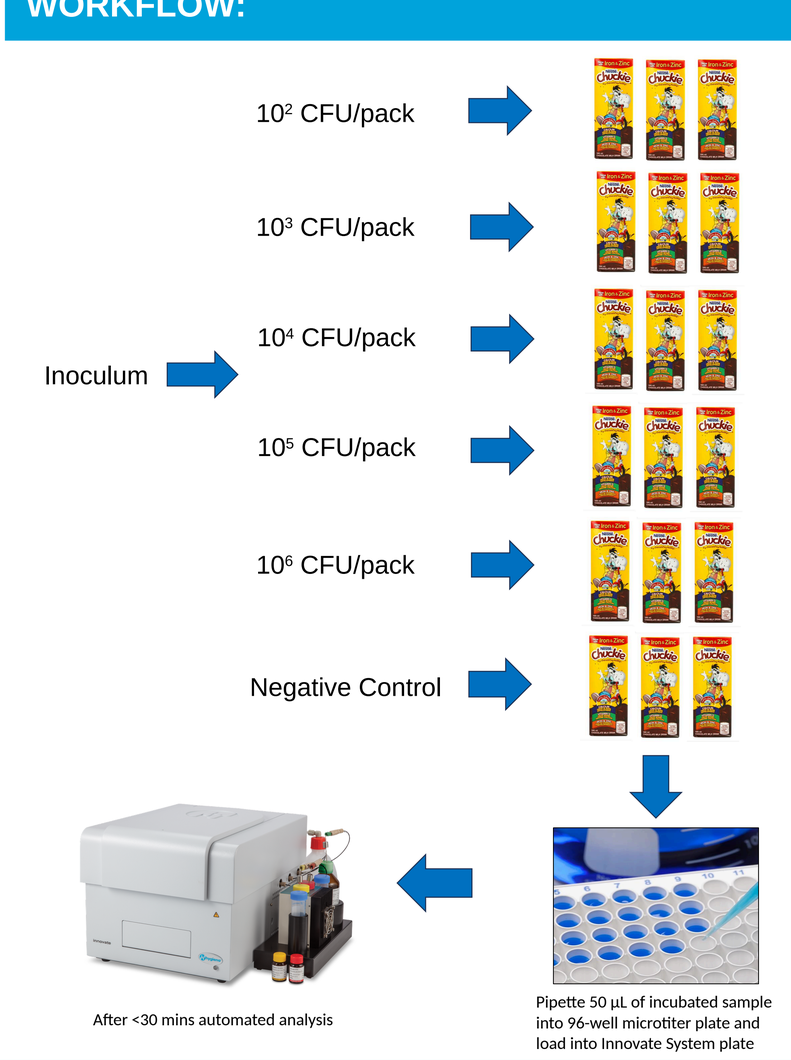
METHOD:

Initial baseline RLU levels were established in sterile Chuckie, a chocolate-flavored milk (11 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, 60 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.

Overnight-incubated microbial cultures of *Bacillus cereus*, *Bacillus licheniformis*, *Enterococcus faecalis* and *Escherichia coli* were serially diluted and used to inoculate product packs in triplicate to achieve final concentrations ranging from 10^2 to 10^6 CFU/mL.

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

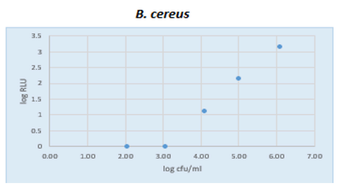
WORKFLOW:



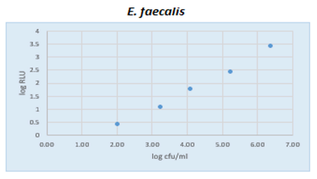
RESULTS:

Tables 1-4. Results of the Chuckie Spike Study. Positives Obtained from the Innovate System reads and the reference plating method are shown side by side.

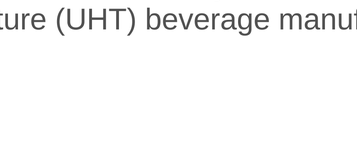
No.	Volume pipetted	Sample	*Strain*	Dilution level	RLU	CFU/mL	Direct streaking
1	50 µL	BC 0	***Bacillus Cereus***	Blank	4	0.00E+00	Sterile
2	50 µL	BC 0	***Bacillus Cereus***	Blank	3	0.00E+00	Sterile
3	50 µL	BC 0	***Bacillus Cereus***	Blank	3	0.00E+00	Sterile
4	50 µL	BC 1	***Bacillus Cereus***	0	1389	1.11E+06	Spoiled
5	50 µL	BC 1	***Bacillus Cereus***	0	1404	1.28E+06	Spoiled
6	50 µL	BC 1	***Bacillus Cereus***	0	1621	1.25E+06	Spoiled
7	50 µL	BC 2	***Bacillus Cereus***	-1	137	9.90E+04	Spoiled
8	50 µL	BC 2	***Bacillus Cereus***	-1	121	9.80E+04	Spoiled
9	50 µL	BC 2	***Bacillus Cereus***	-1	166	1.01E+05	Spoiled
10	50 µL	BC 3	***Bacillus Cereus***	-2	15	1.28E+04	Spoiled
11	50 µL	BC 3	***Bacillus Cereus***	-2	8	9.90E+03	Spoiled
12	50 µL	BC 3	***Bacillus Cereus***	-2	16	1.28E+04	Spoiled
13	50 µL	BC 4	***Bacillus Cereus***	-3	1	8.30E+02	Spoiled
14	50 µL	BC 4	***Bacillus Cereus***	-3	1	1.25E+03	Spoiled
15	50 µL	BC 4	***Bacillus Cereus***	-3	1	1.16E+03	Spoiled
16	50 µL	BC 5	***Bacillus Cereus***	-4	1	9.80E+01	Sterile
17	50 µL	BC 5	***Bacillus Cereus***	-4	1	1.04E+02	Sterile
18	50 µL	BC 5	***Bacillus Cereus***	-4	1	1.16E+02	Sterile



No.	Volume pipetted	Sample	*Strain*	Dilution level	RLU	CFU/mL	Direct streaking
1	50 µL	Ef 0	***E. faecalis***	Blank	3	0.00E+00	
2	50 µL	Ef 0	***E. faecalis***	Blank	2	0.00E+00	
3	50 µL	Ef 0	***E. faecalis***	Blank	4	0.00E+00	
4	50 µL	Ef 1	***E. faecalis***	0	2834	2.35E+06	Spoiled
5	50 µL	Ef 1	***E. faecalis***	0	2771	2.46E+06	Spoiled
6	50 µL	Ef 1	***E. faecalis***	0	2325	2.26E+06	Spoiled
7	50 µL	Ef 2	***E. faecalis***	-1	286	1.57E+05	Spoiled
8	50 µL	Ef 2	***E. faecalis***	-1	274	1.57E+05	Spoiled
9	50 µL	Ef 2	***E. faecalis***	-1	303	1.90E+05	Spoiled
10	50 µL	Ef 3	***E. faecalis***	-2	64	1.07E+04	Spoiled
11	50 µL	Ef 3	***E. faecalis***	-2	65	1.22E+04	Spoiled
12	50 µL	Ef 3	***E. faecalis***	-2	56	1.24E+04	Spoiled
13	50 µL	Ef 4	***E. faecalis***	-3	12	1.60E+03	Spoiled
14	50 µL	Ef 4	***E. faecalis***	-3	14	1.80E+03	Spoiled
15	50 µL	Ef 4	***E. faecalis***	-3	12	1.80E+03	Spoiled
16	50 µL	Ef 5	***E. faecalis***	-4	4	1.00E+02	Sterile
17	50 µL	Ef 5	***E. faecalis***	-4	1	1.00E+02	Sterile
18	50 µL	Ef 5	***E. faecalis***	-4	3	1.00E+02	Sterile



No.	Volume pipetted	Sample	*Strain*	Dilution level	RLU	CFU/mL	Direct streaking
1	50 µL	Ec 0	***B. licheniformis***	Blank	3	0.00E+00	Sterile
2	50 µL	Ec 0	***B. licheniformis***	Blank	4	0.00E+00	Sterile
3	50 µL	Ec 0	***B. licheniformis***	Blank	5	0.00E+00	Sterile
4	50 µL	Ec 1	***B. licheniformis***	0	1303	1.25E+06	Spoiled
5	50 µL	Ec 1	***B. licheniformis***	0	1403	1.12E+06	Spoiled
6	50 µL	Ec 1	***B. licheniformis***	0	1305	1.37E+06	Spoiled
7	50 µL	Ec 2	***B. licheniformis***	-1	13	9.80E+03	Spoiled
8	50 µL	Ec 2	***B. licheniformis***	-1	137	1.06E+05	Spoiled
9	50 µL	Ec 2	***B. licheniformis***	-1	133	1.35E+05	Spoiled
10	50 µL	Ec 3	***B. licheniformis***	-2	9	1.36E+04	Spoiled
11	50 µL	Ec 3	***B. licheniformis***	-2	1	8.50E+03	Spoiled
12	50 µL	Ec 3	***B. licheniformis***	-2	7	1.25E+04	Spoiled
13	50 µL	Ec 4	***B. licheniformis***	-3	1	1.20E+03	Spoiled
14	50 µL	Ec 4	***B. licheniformis***	-3	1	1.35E+03	Spoiled
15	50 µL	Ec 4	***B. licheniformis***	-3	1	9.80E+02	Spoiled
16	50 µL	Ec 5	***B. licheniformis***	-4	1	1.02E+02	Sterile
17	50 µL	Ec 5	***B. licheniformis***	-4	1	8.70E+01	Sterile
18	50 µL	Ec 5	***B. licheniformis***	-4	1	6.50E+01	Sterile



No.	Volume pipetted	Sample	*Strain*	Dilution level	RLU	CFU/mL	Direct streaking
1	50 µL	Ec 0	***E. coli***	Blank	2	-	Sterile
2	50 µL	Ec 0	***E. coli***	Blank	3	-	Sterile
3	50 µL	Ec 0	***E. coli***	Blank	3	-	Sterile
4	50 µL	Ec 1	***E. coli***	0	1081	1.64E+06	Spoiled
5	50 µL	Ec 1	***E. coli***	0	888	1.58E+06	Spoiled
6	50 µL	Ec 1	***E. coli***	0	1112	1.48E+06	Spoiled
7	50 µL	Ec 2	***E. coli***	-1	124	1.01E+05	Spoiled
8	50 µL	Ec 2	***E. coli***	-1	148	8.86E+04	Spoiled
9	50 µL	Ec 2	***E. coli***	-1	170	1.12E+05	Spoiled
10	50 µL	Ec 3	***E. coli***	-2	50	1.29E+04	Spoiled
11	50 µL	Ec 3	***E. coli***	-2	45	1.41E+04	Spoiled
12	50 µL	Ec 3	***E. coli***	-2	52	1.10E+04	Spoiled
13	50 µL	Ec 4	***E. coli***	-3	12	1.20E+03	Spoiled
14	50 µL	Ec 4	***E. coli***	-3	12	1.50E+03	Spoiled
15	50 µL	Ec 4	***E. coli***	-3	11	1.40E+03	Spoiled
16	50 µL	Ec 5	***E. coli***	-4	4	1.00E+02	Sterile
17	50 µL	Ec 5	***E. coli***	-4	6	1.00E+02	Sterile
18	50 µL	Ec 5	***E. coli***	-4	3	1.00E+02	Sterile

REFERENCES:

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

SIGNIFICANCE:

The Hygiena Innovate System, in combination with the RapiScreen Dairy kit, demonstrated robust performance and fitness-for-purpose for the rapid screening of spoilage organisms in chocolate-flavored milk beverages, achieving reliable detection at concentrations ≥10^3 CFU/mL for all four organisms tested. Baseline and background studies confirmed effective removal of background ATP and a stable baseline relative light unit (RLU) signal, enabling the establishment of reliable positive/negative RLU thresholds. By leveraging microbial ATP detection, this high-throughput approach significantly reduces time-to-result compared with conventional plating methods while maintaining analytical reliability. These findings support the potential of the Innovate System for early detection of microbial spoilage and accelerated product release in ultra-high-temperature (UHT) beverage manufacturing.

REGISTERED TRADEMARKS / GLOBAL CERTIFICATIONS:

RapiScreen™ Dairy Kit

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