30-Minute Screening of Microorganisms for Plant- and Milk-Based Dessert Products with the Hygiena® Innovate™ System

One Health Diagnostics™

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Innovate™ Innovate™ AUTOSAMPLER III

INTRODUCTION:

The conversion of adenosine triphosphate (ATP) from the metabolic processes of living cells to adenosine diphosphate (ADP) can be measured using light detection systems. The Innovate™ Rapid Microbial Screening System is based on this bioluminescence-based method for fast and reliable detection of microbial contaminations. After product incubation for the enrichment of microorganisms in the package, it requires only 30 minutes with five minutes of hands-on time to decide if products can be released.

In this study, six different dessert products were inoculated with low-level spiking, and measured after 24, 48 and 72 hours of product incubation at 30 °C with the Innovate System and plated in parallel onto agar. The values, measured in RLUs (Relative Light Units), show a clear difference between uncontaminated and contaminated products and demonstrate that the Innovate System is an alternative sterility control option not only for liquid products, but also for viscous dessert products.

In comparison to the plate count method, which needs an additional incubation step for at least two days, this method can detect contamination in dessert products within 48 to 72 hours, regardless of whether it is an ESL, UHT, or a dairy or plant-based dessert.

PURPOSE:

The objective of this study was to show that the 30-minute Innovate-based sterility test for milk- and plant-based desserts, produces qualitative results comparable to the plate count-based reference method after 72 hours incubation.

REGISTERED TRADEMARKS / GLOBAL CERTIFICATIONS:

RapiScreen™ Dairy Kit

METHOD:

Microbial cultures from six gram-positive and gram-negative bacteria, spore-formers and yeasts were enriched in corresponding enrichment broth. Defined inoculum concentrations from 1 to 20 CFUs/Sample were spiked directly into six different dessert product packs. Spiked and non-inoculated products were incubated up to three days at 30 °C. After product incubation, aliquots of the product were taken for parallel testing using the Innovate System and the plate counting method. Due to the viscosity of the samples, 33.5 µL of α-amylase was added to 3 g of sample and shaken at 110 rpm for 10 minutes to simplify pipetting. Tryptic soy agar, Sabouraud dextrose agar and Columbia Agar with Sheep Blood were used for plate-count testing. Innovate System measurements were conducted according to the RapiScreen (RS) Dairy Kit. The standard RS dairy protocol was used for the Innovate System measurement.

In order to distinguish contaminated samples from non-contaminated samples, a threshold was set for each product: non-inoculated products 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. Four columns (32 wells) were measured to establish the baseline. The threshold for each product was set according to the baseline multiplied by three. All samples with an RLU value higher than the threshold value were considered contaminated.

Table 1: Enrichment/ Measurement Steps

Enrichment	Inoculum Concentration	1 - 20 CFUs/Sample
	Time	24 - 72 hours
	Temperature:	30 ± 2 °C
Measurement with Innovate	Kit	Rapiscreen™ Dairy Kit
	Sample Volume	50 µL
	Protocol	Standard dairy protocol
	Time	30 min
Confirmation	Method	According to ISO 4833-2:2013 Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 2: Colony count at 30 °C by the surface plating technique
	Sample Volume	100 µL
	Time	24 - 72 hours

Table 2: Strains

	Milk-Based Products	Plant-Based Products
Bacillus cereus	-	+
Bacillus subtilis	+	-
Saccharomyces cerevisiae	+	+
Escherichia coli	+	+
Pseudomonas aeruginosa	+	+
Streptococcus thermophilus	+	+
Clostridium sporogenes	+	+

RESULTS:

Table 3-5: Results of milk-based dessert products

[pos = contaminated samples, neg= contamination free samples, pos in orange = CFU/mL < 10^4, pos in red = CFU/mL > 10^4]

Cream Chocolate — Threshold: 30 RLU

Spore/Organism	Inoculation Level (CFUs/sample)	Incubation Temperature [°C]	24 hrs: Innovate System Mean RLUs	24 hrs: ISO (pos/neg)	48 hrs: Innovate System Mean RLUs	48 hrs: ISO (pos/neg)	72 hrs: Innovate System Mean RLUs	72 hrs: ISO (pos/neg)
Bacillus subtilis	11	30 °C	4,796	pos	23,544	pos	14,641	pos
			7,300	pos	19,828	pos	10,857	pos
Saccharomyces cerevisiae	5.6	30 °C	15	pos	35,920	pos	burst	
			17	pos	38,305	pos		
Escherichia coli	15	30 °C	2,072	pos	3,864	pos	1,331	pos
			54	pos	2,720	pos	775	pos
Pseudomonas aeruginosa	7	30 °C	22	pos	10,000	pos	9,373	pos
			10	pos	10,225	pos	10,593	pos
Streptococcus thermophilus	20	30 °C	83	pos	20,604	pos	7,895	pos
			11	pos	21,957	pos	7,294	pos
Clostridium sporogenes	12	30 °C	8	neg	8	neg	10	neg
			7	neg	9	neg	7	neg

Cream Vanilla — Threshold: 42 RLU

Spore/Organism	Inoculation Level (CFUs/sample)	Incubation Temperature [°C]	24 hrs: Innovate System Mean RLUs	24 hrs: ISO (pos/neg)	48 hrs: Innovate System Mean RLUs	48 hrs: ISO (pos/neg)	72 hrs: Innovate System Mean RLUs	72 hrs: ISO (pos/neg)
Bacillus subtilis	11	30 °C	43,658	pos	80,056	pos	76,155	pos
			43,209	pos	83,225	pos	61,573	pos
Saccharomyces cerevisiae	5.6	30 °C	62	pos	435,069	pos	burst	
			56	pos	384,967	pos		
Escherichia coli	15	30 °C	10,884	pos	57,533	pos	21,306	pos
			12,452	pos	62,761	pos	24,326	pos
Pseudomonas aeruginosa	7	30 °C	557	pos	44,795	pos	39,106	pos
			399	pos	55,240	pos	46,737	pos
Streptococcus thermophilus	20	30 °C	120	pos	73,959	pos	55,454	pos
			79	pos	78,567	pos	54,786	pos
Clostridium sporogenes	12	30 °C	11	neg	13	neg	11	neg
			12	neg	12	neg	10	neg

Mousse Chocolate — Threshold: 36 RLU

Spore/Organism	Inoculation Level (CFUs/sample)	Incubation Temperature [°C]	24 hrs: Innovate System Mean RLUs	24 hrs: ISO (pos/neg)	48 hrs: Innovate System Mean RLUs	48 hrs: ISO (pos/neg)	72 hrs: Innovate System Mean RLUs	72 hrs: ISO (pos/neg)
Bacillus subtilis	11	30 °C	244	pos	17,582	pos	2,708	pos
			428	pos	13,695	pos	437	pos
Saccharomyces cerevisiae	5.6	30 °C	13	pos	12,747	pos	Burst	
			12	pos	13,751	pos		
Escherichia coli	15	30 °C	6	pos	970	pos	1,920	pos
			18	pos	944	pos	1,588	pos
Pseudomonas aeruginosa	7	30 °C	12	pos	1,214	pos	1,337	pos
			12	pos	1,042	pos	1,645	pos
Streptococcus thermophilus	20	30 °C	77	pos	3,422	pos	1,716	pos
			20	pos	4,104	pos	2,303	pos
Clostridium sporogenes	12	30 °C	5	neg	8	neg	6	neg
			8	neg	11	neg	6	neg

Table 6-8: Results of milk-based protein-rich dessert products

[pos = contaminated samples, neg= contamination free samples, pos in orange = CFU/mL < 10^4, pos in red = CFU/mL > 10^4]

Protein Pudding Hazelnut chocolate — Threshold: 33 RLU

Spore/Organism	Inoculation Level (CFUs/sample)	Incubation Temperature [°C]	24 hrs: Innovate System Mean RLUs	24 hrs: ISO (pos/neg)	48 hrs: Innovate System Mean RLUs	48 hrs: ISO (pos/neg)	72 hrs: Innovate System Mean RLUs	72 hrs: ISO (pos/neg)
Bacillus subtilis	11	30 °C	6,718	pos	18,293	pos	40,381	pos
			5,400	pos	21,950	pos	43,586	pos
Saccharomyces cerevisiae	5,6	30 °C	10	pos	173	pos	63	pos
			17	pos	196	pos	73	pos
Escherichia coli	15	30 °C	17	pos	129	pos	48	pos
			3,977	pos	66	pos	45	pos
Pseudomonas aeruginosa	7	30 °C	116	pos	2,325	pos	2,579	pos
			425	pos	1,125	pos	22,021	pos
Streptococcus thermophilus	20	30 °C	529	pos	10,244	pos	2,199	pos
			257	pos	90,745	pos	2,065	pos
Clostridium sporogenes	12	30 °C	6	neg	7	neg	7	neg
			8	neg	8	neg	8	neg

Protein Pudding Vanille — Threshold: 51 RLU

Spore/Organism	Inoculation Level (CFUs/sample)	Incubation Temperature [°C]	24 hrs: Innovate System Mean RLUs	24 hrs: ISO (pos/neg)	48 hrs: Innovate System Mean RLUs	48 hrs: ISO (pos/neg)	72 hrs: Innovate System Mean RLUs	72 hrs: ISO (pos/neg)
Bacillus subtilis	11	30 °C	18,248	pos	9,381	pos	14,302	pos
			12,053	pos	8,029	pos	12150	pos
Saccharomyces cerevisiae	5.6	30 °C	17	pos	76	pos	1,243	pos
			21	pos	24	pos	78	pos
Escherichia coli	15	30 °C	444	pos	1,268	pos	8,764	pos
			353	pos	4,680	pos	180	pos
Pseudomonas aeruginosa	7	30 °C	1,107	pos	4,404	pos	10,193	pos
			117	pos	6,184	pos	9723	pos
Streptococcus thermophilus	20	30 °C	135	pos	32,967	pos	24,797	pos
			138	pos	32,212	pos	27,432	pos
Clostridium sporogenes	12	30 °C	13	neg	13	neg	12	neg
			17	neg	6	neg	11	neg

Table 8: Results of the plant-based dessert products

pos = contaminated samples, neg= contamination free samples, pos in orange = CFU/mL < 10^4, pos in red = CFU/mL > 10^4]

Dark Chocolate (plant-based) — Threshold: 30 RLU

Spore/Organism	Inoculation Level (CFUs/sample)	Incubation Temperature [°C]	24 hrs: Innovate System Mean RLUs	24 hrs: ISO (pos/neg)	48 hrs: Innovate Mean RLUs	48 hrs: ISO (pos/neg)	72 hrs: Innovate System Mean RLUs	72 hrs: ISO (pos/neg)
Bacillus cereus	6.6	30 °C	3	pos	16,326	pos	11,006	pos
			3	neg	4	neg	4	neg
Saccharomyces cerevisiae	8	30 °C	7	pos	13	pos	7,130	pos
			11	pos	10	pos	195	pos
Escherichia coli	8.2	30 °C	439	pos	1,870	pos	1,537	pos
			9/6	pos	1,693	pos	1,794	pos
Pseudomonas aeruginosa	3.9	30 °C	12	pos	9	pos	323	pos
			11	pos	9	pos	433	pos
Streptococcus thermophilus	6.5	30 °C	8	neg	3,005	pos	4,356	pos
			9	neg	127	pos	523	pos
Clostridium sporogenes	12	30 °C	8	neg	838	pos	624	pos
			8	neg	880	pos	955	pos

After 48 hours of static incubation of the inoculated dessert product packs at 30 °C, all products were able to be analyzed correctly. The Innovate System results corresponded 100% to the plate-count measurements, which required a second plate incubation after enrichment in the product.

Clostridium sporogenes was not detected in milk-based products either with the Innovate System nor by plate growth.

For better pipetting, measurements were also taken with and without an amylase treatment. The amylase had no effect on the measurements, but it led to a comfortable handling of the samples. (Data not shown in the tables).

The 100% agreement of the results after 48 hours of incubation between ISO and the alternative Innovate System method were independent of plant-based or milk-based production, extended-shelf-life (ESL) or ultra-heat-treated (UHT) products.

SIGNIFICANCE:

ATP detection with the Innovate System RapiScreen Dairy system is a rapid, accurate and safe 30-minute testing for processed dessert products. This demonstrates that the Innovate system is suitable for commercial sterility testing of challenging dessert matrices, even chocolate cream products.

Without a time-consuming second plate incubation, the Innovate System generates comparable results to the reference method and shortens product release by more than 3 days, especially for ESL dessert products.