Rapid Screening of Microorganisms from Ultra-High Temperature (UHT), Extended Shelf-life (ESL) and Acidic Drinks Using Hygiena's Innovate™ System

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One Health Diagnostics™

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Innovate™ | Innovate™ AUTOSAMPLER III

INTRODUCTION:

Aseptic processing is a widely used method in food and beverage processing. Traditional methods for microbial testing can take several days to weeks for results. The Innovate™ Rapid Microbial Screening System utilizing the RapiScreen™ Beverage kit can analyze adenosine triphosphate (ATP) bioluminescence for rapid microbial screening of UHT, ESL, and highly acidic drinks, providing rapid results.

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 *B. coagulans, B. subtilis, C. sporogenes, L. fermentum*, and *S. cerevisiae* in five different product matrices.

PURPOSE:

The objective of this study was to:

1. Ensure that *B. coagulans, B. subtilis, C. sporogenes, L. fermentum,* and *S. cerevisiae* are detectable using ATP methods.
2. Demonstrate the rapid detection of the five organisms spiked in five different product matrices, ranging from dairy to plant-based to fruit-flavored drinks.
3. Compare results of detection with standard agar plate methods.

REGISTERED TRADEMARKS / GLOBAL CERTIFICATIONS:

METHOD:

For each product matrix, replicate test portions were spiked at a fractional level of inoculation to achieve 5-15 positive results out of 20 tested. *B. coagulans, B. subtilis, C. sporogenes, L. fermentum*, and *S. cerevisiae* were chosen to cover a selection of different organisms. On each sampling day (Days 1, 2, 3, 5 and 7), 50 µL aliquots of enriched product were transferred to the Innovate System plate for analysis to detect the absence or presence of growth. Confirmation plates were prepared to confirm the growth of each target organism and pour plates were performed on day 15 in accordance with the ISO 4833:1:2012 method.

Table 1: Inoculation Summary of All Matrices and Organisms Tested (with each matrix, conditions, replicates, and reference methods evaluated in the matrix study).

Matrix	pH	Threshold RLU	Container Volume (mL)	Inoculation Organism (Condition)	Inoculation Level	Replicates per Method	Reference Method
ESL Plant-based drink (almond)	7.6	48	1890	*Bacillus coagulans*	Non-inoculated	5	ISO 4833-1 & BAM Chapter 3
				ATCC 7050	Fractional positive	20	
				Spores	High positive	5	
Half and half (10% fat)	6.7	5	11	*Clostridium sporogenes*	Non-inoculated	5	ISO 4833-1 & BAM Chapter 16
				ATCC 7955	Fractional positive	20	
				Spores	High positive	5	
Protein-based Drink (casein)	6.9	15	330	*Lactobacillus fermentum*	Non-inoculated	5	ISO 4833-1 & CMMEF
				ATCC 9338	Fractional positive	20	
				heat stressed	High positive	5	
Fruit-flavoured sports drink	2.9	19	500	*Saccharomyces cerevisiae*	Non-inoculated	5	ISO 4833-1 & BAM Chapter 18
				NCTC 3178	Fractional positive	20	
				heat stressed	High positive	5	
Fruit-flavoured sports drink[k]			500	*Saccharomyces cerevisiae*	Non-inoculated	5	ISO 4833-1 & BAM Chapter 18
				ATCC 9896	Fractional positive	20	
				heat stressed	High positive	5	
UHT Plant-based drink (oat)	6.8	31	1000	*Bacillus subtilis*	Non-inoculated	5	ISO 4833-1 & BAM Chapter 3
				ATCC 6633	Fractional positive	20	
				Spores	High positive	5	

[a] Matrix tested in the independent laboratory, Q-Laboratories, Cincinnati, OH.

RESULTS:

Table 2: RapiScreen™ Beverage Kit Results for the Spiked Matrixes and Respective Strains Comparing the Candidate Method to the Reference Method

Matrix	Strain	Spiked CFU per package[a]	Day	N[b]	Candidate Method			Reference Method			dPODc[f]	95% CI[g]
					x[c]	PODc[d]	95% CI	x	PODR[e]	95% CI		
ESL Plant-based drink	*Bacillus coagulans* ATCC[h] 7050	588	5	5	5	1	(0.57, 1)	5	1	(0.57, 1)	0	(-0.47, 0.47)
		1		20	10	0.5	(0.3, 0.7)	10	0.5	(0.3, 0.7)	0	(-0.13, 0.13)
		0		5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.47, 0.47)
Half and half	*Clostridium sporogenes* ATCC 7955	6300	5	5	5	1	(0.57, 1)	5	1	(0.57, 1)	0	(-0.47, 0.47)
		7		20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0	(-0.13, 0.13)
		0		5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.47, 0.47)
Protein-based Drink	*Lactobacillus fermentum* ATCC 9338	19000	5	5	5	1	(0.57, 1)	5	1	(0.57, 1)	0	(-0.47, 0.47)
		1		20	3	0.15	(0.05, 0.36)	7	0.35	(0.18, 0.57)	-0.2	(-0.41, 0.01)
		0		5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.47, 0.47)
Fruit-flavoured sports drink	*S. Cerevisiae* NCTC[i] 3178	16.2	3	5	5	1	(0.57, 1)	5	1	(0.57, 1)	0	(-0.47, 0.47)
		1.4		20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0	(-0.13, 0.13)
		0		5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.47, 0.47)
UHT Plant-based drink	*Bacillus subtilis* ATCC 6633	9.6	5	5	5	1	(0.57, 1)	5	1	(0.57, 1)	0	(-0.47, 0.47)
		0.6		20	8	0.4	(0.22, 0.61)	8	0.4	(0.22, 0.61)	0	(-0.13, 0.13)
		0		5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.47, 0.47)
Fruit-flavoured sports drink[k]	*S. Cerevisiae* ATCC 9896	2 - 10	2	5	5	1	(0.57, 1.00)	5	1	(0.57, 1.00)	0	(-0.47, 0.47)
		0.2 - 2		20	10[l]	0.5	(0.3, 0.7)	11[l]	0.55	(0.34, 0.74)	-0.05	(-0.21, 0.11)
		0		5	0	0	(0.00, 0.43)	1[k]	0.2	(0.00, 0.62)	-0.2	(-0.76, 0.36)

[a] CFU = colony forming units applied to each package.
[b] N = number of test portions.
[c] X = number of positive test portions.
[d] PODC = Candidate method presumptive positive results confirmed positive divided by the total number of trials.
[e] PODR = Reference method results divided by the total number of trials.
[f] dPODC = Difference between the candidate method and reference method POD values.
[g] 95% CI = if the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h] ATCC = American Type Culture Collection, Manassas, VA.
[i] Matrix tested in the independent laboratory, Q-Laboratories, Cincinnati, OH.
[j] NCTC = Public Health England, Salisbury, UK.
[k] Uninoculated sample became contaminated with a filamentous fungus at Day 7
[l] Sample 1 tested positive at Day 5 from contamination with a filamentous fungus - not S. cerevisiae

RESULTS:

Table 3: RapiScreen™ Beverage Kit Results Across All Tested Timepoints vs Reference Method

Matrix	RapiScreen™ Beverage Candidate Method vs Reference Method											
	Days										Reference Method	
	Day 1		Day 2		Day 3		Day 5		Day 7		Day 15	
	High +	Fractional	High +	Fractional	High +	Fractional	High +	Fractional	High +	Fractional	High +	Fractional
ESL Plant-based drink[a]	0	0	5	1	5	1	5	10	5	9	5	10
Half and half	5	0	5	0	5	0	5	7	5	7	5	7
Protein-based drink	4	0	5	1	5	1	5	3	5	7	5	7
Fruit-flavored sports drink	0	0	2	0	5	7	5	7	5	7	5	7
UHT Plant-based drink	0	0	0	0	5	3	5	8	5	7	5	8
Fruit-flavored sports drink[b]	5	0	5	10	5	10	5	11[c]	5	11[c]	5	11[c]

[a] One sample was contaminated. See results for explanation.
[b] Matrix tested in the independent laboratory, Q-Laboratories, Cincinnati, OH.
[c] Sample tested positive at Day 5 from contamination with a filamentous fungus - not S. cerevisiae.

Testing of five different matrices showed that the probability of detection for the Innovate RapiScreen Beverage kit was at 100% for high and low inoculation levels when compared to the plating method. The kit delivered detection of contaminated product packs in 7 days or less with results that are equivalent to the 15-day reference method requirement.

CONCLUSIONS AND SIGNIFICANCE:

This study demonstrated that the Innovate™ System RapiScreen™ Beverage Kit is sensitive and robust for the detection of microbial ATP across a range of dairy, plant-based, and fruit-flavored matrices, matching the reference methods. The Innovate RapiScreen method effectively detected selected microbial contaminants in five claimed matrixes: fruit-flavored sports drink, UHT plant-based drink (almond drink), half and half (10% fat), protein-based drink (casein), and ESL plant-based drink (oat drink).

Therefore, the Innovate System is a quick and easy-to-use semi-automated system applicable for rapid product testing by beverage manufacturers to confirm the quality of inventory for fast, efficient, and safe release.

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