Innovate™ Rapid Microbial Screening System with the RapiScreen™ Beverage Kit

Claim Support Report

Product numbers: MCH4000 and KIT4010

PERFORMANCE TESTED
AOAC
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LICENSE NUMBER 052301

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1. Introduction

Overview of ATP Bioluminescence

All actively respiring organisms contain adenosine triphosphate (ATP), which is the universal energy currency in biological systems. In the presence of ATP, oxygen and magnesium, the luciferase enzyme catalyzes the conversion of luciferin to oxyluciferin (Figure 1). During this biochemical reaction, ATP is hydrolyzed to adenosine monophosphate (AMP), and stored energy is released as visible light. The luciferase reaction is very efficient—for each molecule of ATP that is converted to AMP, a photon of light is emitted.

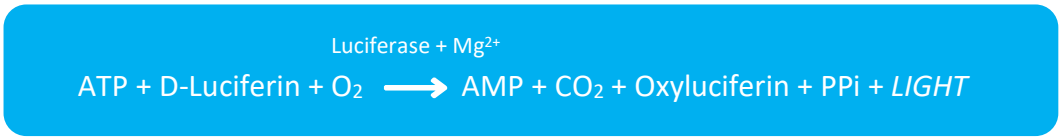

Figure 1. The Luciferase-Luciferin Reaction Produces Light.

How to Use ATP Bioluminescence for Food Safety

By measuring the light produced during the metabolism of luciferin and ATP, luciferase reactions can be used to quickly and sensitively detect the presence or absence of viable microorganisms in food and beverage samples. This method provides a more rapid detection system than waiting for visible colonies to grow on agar plates.

Hygiena's Innovate™ System RapiScreen™ Beverage Kit is designed for the rapid detection of microorganisms in a wide range of low-pH beverage products. An enrichment step is required to ensure that ATP is detectable, especially for samples with initially low levels of contaminants. Typically, the product is incubated in its own packaging for 24 – 48 hours of enrichment. Pre-established baselines obtained from uncontaminated products are used to determine positive results.

Because many beverage samples contain high levels of non-microbial ATP that must be removed before microbial ATP can be detected, the RapiScreen Beverage Kit also includes a proprietary reagent (LuminASE™) that efficiently depletes free and somatic ATP. A 10-minute treatment reduces sample background to low levels in all sample types.

Purpose of Study

This document details the validation of Hygiena's Innovate Rapid Microbial Screening System: luminometer with the RapiScreen Beverage Kit. The study primarily focused on low-pH acidic beverage products like juices and sports drinks and was designed to demonstrate the following:

- Production of stable baseline values
- Detection of microbial contamination in a diverse range of low-pH beverage products
- Detection of low-level initial contamination (1 – 10 cells or spores per package)

2. Materials

2.1 Materials

- Incubators capable of 30 ± 2 °C and 37 ± 2 °C
- Sterile plastic Petri dishes
- Sterile inoculating loops
- Sterile pipettes and tips
- 150 mL sterile containers
- 10 mL disposable syringes
- 1 mL disposable syringes
- Sterile 10 mL vials
- PP vials (supplied with reagent kit)
- 96-well microtiter plates (supplied with reagent kit)

2.2 Reagents and Media

All reagents and media were used within recommended expiration dates.

All kit reagents are manufactured by Hygiena® according to ISO 9001–approved quality standards (Neuss, Germany):

Reagents and Media
RapiScreen Beverage Kit (Product No. KIT4010) PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 052301 *
ATP Positive Control Kit (Product No. KIT4014)
Orange Serum Agar (OSA)
Sabouraud Dextrose Agar (SDA)
Tryptone Soya Agar (TSA)
de Man, Rogosa and Sharpe Broth (MRS)
Potato Dextrose Broth (PDB)

* Please see AOAC certificate for performance claims.

2.3 Microorganisms

The following microorganisms were used in the inoculation studies:

Microorganism	Reference Number
Bacteria	
Alicyclobacillus acidoterrestris	ATCC 49025
Bacillus cereus	NCTC 10320
Bacillus coagulans	ATCC 7050
Clostridium sporogenes	ATCC 7955
Enterococcus faecalis	ATCC 19433
Escherichia coli	NCTC 12923
Geobacillus stearothermophilus	ATCC 12980
Lactobacillus brevis	ATCC 14869
Lactobacillus casei	ATCC 393

Microorganism	Reference Number
Bacteria	
Lactobacillus fructivorans	ATCC 8288
Lactobacillus lactis	ATCC 12315
Pseudomonas aeruginosa	ATCC 10145
Pseudomonas putida	ATCC 49128
Staphylococcus aureus	NCTC 25923
Streptococcus salivarius subsp. *thermophilus*	ATCC 19258
Yeast	
Kluyveromyces marxianus	DSM 5418
Candida albicans	ATCC 10231
Candida orthopsilosis	NCPF 8804
Dekkera bruxellensis	ATCC 36234
Saccharomyces cerevisiae	ATCC 9763, NCPF 3178 and NCPF 3191
Zygosaccharomyces bailli	ATCC 58445
Zygosaccharomyces rouxii	NCTC 3234
Additional Fungi	
Aspergillus brasiliensis	ATCC 16404
Byssochlamys fulva	NCTC 7156
Talaromyces pinophilus	ATCC 11797

2.4 Products

The following products were used:

Product Samples	Pack Volume (mL)	pH
Apple Juice A, 100% Juice	200	3.69
Apple Juice B, 100% Juice	1,000	3.44
Mixed Berry Juice, 100% Juice	200	3.75
Red Berry Drink, 10% Juice	250	3.09
Orange Drink A, 85% Juice	200	4.08
Orange Drink B, 10% Juice	180	3.38
Prune Juice, 100% Juice	1,000	3.56
Sports Drink	500	3.25
Almond Drink	800	7.03
Oat Drink	800	6.16
Classic Chai High Concentrate	250	5.54
Classic Chai Latte Drink	250	5.58
Cold Brew Coffee & Creamer	250	6.38

Tomato Ketchup	500	5.25 (MRS), 4.67 (PDB), 4.72 (TSB)

2.5 Instrumentation

All assays were run on Hygiena's Innovate System luminometers (Serial numbers: 7316, 7030; Software version: Innovate.im™ 5.09). All appropriate positive control procedures were performed before sample testing.

3. Methods

3.1 Sample Preparation – Sample Effects

Product packs were incubated at 30 °C for 48 hours and shaken thoroughly to mix. Then, 20 mL of product was removed from the pack and placed in a sterile container for sample effects testing (pH, background and baseline assessments).

3.2 pH Assessments

Products assessed for pH were measured in duplicate to ensure accuracy of measurements. The pH meter was calibrated with pH standards before use. The average of the two values is provided in the table above for each product.

3.3 Background ATP Determination and Depletion for Baseline Measurements

Background ATP is the total non-microbial ATP concentration in any sample, while baseline ATP is the residual ATP remaining after LuminASE treatment. Very high levels of background, non-microbial ATP that sometimes occur in products can mask detection of microbial contamination. The RapiScreen Beverage Kit includes an ATPase (LuminASE) that removes non-microbial ATP, creating a stable, low-baseline RLU value before testing for microbial ATP.

Product ATP baselines as well as background values were determined by testing a sterile product after a 48-hour incubation period at the required incubation temperature. Samples were thoroughly shaken before sampling and assaying. Thirty-two (32) replicate assays from each product, with and without addition of LuminASE, were tested to measure background ATP levels and subsequent depletion of ATP by LuminASE.

3.4 Inoculum Preparation

Initial Culturing

All organisms except the *Bacillus cereus* species were plated from the Hygiena culture collection by spreading on appropriate agar medium. Following 24 – 72 hours of incubation, an overnight culture was prepared from an isolated colony. This culture was then diluted on the day of inoculation to achieve the target spiking inoculum level. *Bacillus cereus* spores were rehydrated in PBS and diluted appropriately to proper target concentrations for use.

Acid Adaptation

The following organisms were acid adapted before use in the spiking study: *D. bruxellensis*, *T. pinophilus*, *L. fructivorans*, *S. cerevisiae*, *A. acidoteresstris*, *C. orthopsilosis* and *B. fulva*. For each prepared culture, 100 µL was pipetted into a mix of 10.5 mL of apple juice A and 4.5 mL of optimal growth broth. The culture was then incubated at the organism's optimal growth temperature for 24 – 120 hours. Once turbidity was seen, 10-fold serial dilutions of the culture were prepared down to the 10^{-7} level using Maximum Recovery Diluent (MRD). The prepared dilutions were then plated on the optimal growth media and incubated at the optimal growth temperature to enumerate the cultures to ensure an accurate CFU level for the spiking study.

Spiking Study, Group 1

Organism	Reference	Products	Testing Period	Confirmation
E. coli	NCTC 12923	Apple Juice B 100% Prune Juice 100%	1, 2, 3, 4 days at 30 °C	TSA (37 °C) PDA (30 °C)
S. aureus	NCTC 25923			
B. cereus	NCTC 10320			
K. marxianus	DSM 5418			
S. cerevisiae	NCPF 3191			
Z. rouxii	NCTC 3234			

Spiking Study, Group 2

Organism	Reference	Products	Testing Period	Confirmation
A. acidoteresstris	ATCC 49025	Apple Juice A 100% Mixed Berry Juice 100% Red Berry Drink 10% Orange Drink A 85% Orange Drink B 10% Sports Drink	1, 2, 3, 4, 5, 6, 7 days at 25 °C	TSA (30 °C) PDA (30 °C) MRS (37 °C)
L. fructivorans	ATCC 8288			
C. orthopsilosis	NCPF 8804			
D. bruxellensis	ATCC 36234			
S. cerevisiae	NCPF 3178			
B. fulva	DSM 1806			
T. pinophilus	ATCC 11797			

Spiking Study, Group 3

Organism	Reference	Products	Testing Period	Confirmation
B. fulva	DSM 1806	Reinforced Almond Drink Reinforced Oat Drink	1, 2, 3, 4, 5, 6, 7 days at 25 °C * 1, 2, 3, 4, 5, 6, 7 days at 55 °C for recovery of thermophilic organisms	TSA (30 °C) PDA (30 °C) MRS (37 °C)
T. pinophilus	ATCC 11797			
B. coagulans	ATCC 7050			
E. faecalis	ATCC 19433			
P. aeruginosa	ATCC 10145			
S. thermophilus	ATCC 19258			
*G. stearothermophilus**	ATCC 12980			

Spiking Study, Group 4

Organism	Reference	Products	Testing Period	Confirmation
S. aureus	ATCC 6538	Classic Chai High Concentrate Classic Chai Latte Drink Cold Brew Coffee & Creamer	1, 2, 3, 4, 5, 6, 7 days at 30 °C	TSA (30 °C) PDA (30 °C)
P. aeruginosa	ATCC 9027			
B. cereus	ATCC 11778			
P. putida	ATCC 49128			
C. sporogenes	ATCC 7955			
A. brasiliensis	ATCC 16404			
S. cerevisiae	ATCC 9763			

Spiking Study, Group 5

Organism	Reference	Products	Testing Period	Confirmation
C. albicans	ATCC 10231	Tomato Ketchup	1, 2, 3 days at 30 °C	PDA (30 °C) MRS (30 °C)
S. cerevisiae	ATCC 9763			
Z. bailli	ATCC 58445			
L. brevis	ATCC 14869			
L. casei	ATCC 393			
L. lactis	ATCC 12315			

3.5 Sample Inoculation and Incubation

Two different methods were evaluated for the examination of recovery from the products: in-pack sterility tests and out-of-pack challenge testing.

In-pack products were aseptically inoculated at a nominal 10 CFU per pack. The product samples were then sealed with adhesive glue and swirled to mix. All product samples were incubated statically at temperatures and durations indicated in the tables. All sampled packages were assayed in duplicate on both the Innovate System and agar plates.

For out-of-pack challenge testing (the tomato ketchup study), lactic acid bacteria were spiked into product that was removed from packs and diluted into selective media. Removing product from the packs allows for better recovery of very fastidious organisms that normally would take several months to spoil the product. The selective media can be tuned for the selective growth of the organism under challenge, e.g., MRS for lactic acid bacteria.

3.6 Hygiena Protocol

Product samples were tested using the standard beverage assay protocol:

- Pipette 50 µL of each product into a sample well
- Place microtiter plate into luminometer

Injection of reagents and measurements are automatically completed by the instrument, and results are expressed in relative light units (RLUs).

3.7 Confirmation Plates

At each assay timepoint, a 100 µL sample was removed from each pack and streaked onto duplicate TSA, SDA, MRS, OSA or PDA plates as appropriate, and incubated at indicated duration and temperature.

When an organism was plated on a selective agar, an additional set of duplicate TSA plates were inoculated. This additional check allows for background flora to be enumerated. For organism growth confirmed on TSA plates, a duplicate SDA plating was also run. The presence of typical, pure colony growth of all organisms on plates confirmed the RapiScreen positive ATP bioluminescence results and confirmed that the spiked organism was indeed the organism detected.

4. Results

4.1 Background ATP Determination and Baselines

Background and Baseline Values at the 48-Hour Timepoint

Product	ATP Background (RLUs)	ATP Baseline (RLUs)	Caution Threshold (RLUs)	Positive Threshold (RLUs)
Apple Juice A, 100% Juice	1,533	2	4	6
Apple Juice B, 100% Juice	4,919	5	10	15
Mixed Berry Juice, 100% Juice	6,015	4	8	12
Red Berry Drink, 10% Juice	4,618	2	4	6
Orange Drink A, 85% Juice	878,304	32	64	96
Orange Drink B, 10% Juice	249,740	49	98	147
Prune Juice, 100% Juice	573	8	16	24
Sports Drink	3	3	6	9
Almond Drink	492	2	4	6
Oat Drink	4,045	5	10	15
Classic Chai High Concentrate	34,711	2	4	7
Classic Chai Latte Drink	23,156	2	4	6
Cold Brew Coffee & Creamer	556	10	20	30
Tomato Ketchup (1:4 PDB)	186,805	13	26	38
Tomato Ketchup (1:4 MRS)	153,860	66	132	198

4.2 Inoculation Study Results

For all results tables, RLU results are from the Innovate System. Please note, the agar plating time is at least 24 hours after plating, hence positive indication is plating time + 24 hours.

Legend for All Tables

+ = Growth on agar plates
– = No growth on agar plates

List of Results Tables

Product **Page**

Apple Juice A

200 mL, pH 3.69

Contaminant	CFU	48 Hours		72 Hours		96 Hours		120 Hours		168 Hours		Time to Detection (h)	
		RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	Innovate	Agar
D. bruxellensis	13	3	–	6	–	77	+	1,873	+	59,368	+	96	120
T. pinophilus	37	27	–	25	–	116	+	2,701	+	2,697	+	96	120
L. fructivorans	12	3	–	7	–	5	–	3	–	3	–	No growth	No growth
S. cerevisiae	8	21,629	+	Burst	Burst	Burst	Burst	Burst	Burst	Burst	Burst	48	72
C. orthopsilosis	2	38	+	15,192	+	36,365	+	61,742	+	60,048	+	48	72
B. fulva	10	3	–	3	–	3	+	16,838	+	77,836	+	120	144

Apple Juice B

1,000 mL; pH 3.44

Contaminant	CFU	24 Hours		48 Hours		72 Hours		96 Hours		Time to Detection (h)	
		RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	Innovate	Agar
E. coli	8	3	–	5	–	1	–	4	–	No growth	No growth
S. aureus	4	8	–	2	–	11	–	4	–	No growth	No growth
B. cereus	2	2	–	3	–	2	–	5	–	No growth	No growth
S. cerevisiae	2	5	+	161	+	13,778	+	29,474	+	48	48
Z. rouxii	115	15	–	8,121	+	268,596	+	Burst	+	24	72
K. marxianus	255	62	+	6,810	+	102,358	+	Burst	+	24	48
A. brasiliensis	10	8	–	37	+	1,271	+	1,804	+	48	72

Mixed Berry Juice

100% Juice, 200 mL, pH 3.75

Contaminant	CFU	48 Hours		72 Hours		96 Hours		120 Hours		168 Hours		Time to Detection (h)	
		RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	Innovate	Agar
D. bruxellensis	13	2	–	3	–	54	+	1,134	+	21,542	+	96	120
T. pinophilus	37	5	–	3	–	75	+	469	+	967	+	96	120
L. fructivorans	12	2	–	3	–	2	–	2	–	2	–	No growth	No growth
S. cerevisiae	8	9,120	+	Burst	Burst	Burst	Burst	Burst	Burst	Burst	Burst	48	72
C. orthopsilosis	2	49	+	18,937	+	30,207	+	15,484	+	42,747	+	48	72
B. fulva	10	1	–	3	–	3	–	5,198	+	22,770	+	120	144

Red Berry Drink

10% Juice, 250 mL, pH: 3.09

Contaminant	CFU	48 Hours		72 Hours		96 Hours		120 Hours		168 Hours		Time to Detection (h)	
		RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	Innovate	Agar
D. bruxellensis	13	14	–	4	–	56	+	1,093	+	31,994	+	96	120
T. pinophilus	37	6	+	63	+	154	+	1,343	+	5,102	+	72	96
L. fructivorans	49	4	–	2	–	2	–	2	–	2	–	No growth	No growth
S. cerevisiae	8	50	+	9,135	+	62,171	+	106,795	+	Burst	+	48	72
C. orthopsilosis	2	160	+	45,831	+	31,895	+	26,007	+	10,568	+	48	72
B. fulva	10	3	–	4	–	8	+	3,255	+	2,828	+	120	144

Orange Drink A

85% Juice, 200 mL, pH 4.08

Contaminant	CFU	48 Hours		72 Hours		96 Hours		120 Hours		168 Hours		Time to Detection (h)	
		RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	Innovate	Agar
D. bruxellensis	13	2,374	–	15	–	62	+	572	+	31,038	+	48	120
T. pinophilus	37	149	–	79	–	15	–	136	+	28	–	48	144
L. fructivorans	12	23	–	94	–	28	–	23	+	342	+	72	144
S. cerevisiae	<300	46,291	+	477,536	+	329,617	+	175,953	+	Burst	+	48	72
C. orthopsilosis	2	2,026	+	1,493	+	10,074	+	15,390	+	56,338	+	48	72
B. fulva	10	53	–	84	–	216	–	147	–	2,478	+	96	192

Orange Drink B

10% Juice, 180 mL, pH 3.38

Contaminant	CFU	48 Hours		72 Hours		96 Hours		120 Hours		168 Hours		Time to Detection (h)	
		RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	Innovate	Agar
D. bruxellensis	13	159	–	21	–	177	+	3,452	+	56,938	+	48	120
T. pinophilus	37	5	+	130	+	643	+	560	+	785	+	72	72
L. fructivorans	12	8	–	49	–	15	–	46	–	28	–	No growth	No growth
S. cerevisiae	8	1,361	+	79,661	+	48,141	+	233,740	+	Burst	+	48	72
C. orthopsilosis	2	33	+	1,680	+	27,244	+	72,456	+	91,809	+	48	72
B. fulva	10	436	–	5	–	64	+	34	+	150	+	48	120

Prune Juice

1,000 mL; pH 3.56

Contaminant	CFU	24 Hours		48 Hours		72 Hours		96 Hours		Time to Detection (h)	
		RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	Innovate	Agar
E. coli	8	4	–	7	–	3	–	1	–	No growth	No growth
S. aureus	4	3	–	5	–	5	–	1	–	No growth	No growth
B. cereus	11	8	–	6	–	3	–	1	–	No growth	No growth
S. cerevisiae	2	5	–	4	–	3	–	2	–	No growth	No growth
Z. rouxii	115	5	–	2	–	33	+	1,585	+	72	96
K. marxianus	255	5	–	3	+	25	+	424	+	72	72
A. brasiliensis	10	7	–	3	–	4	–	6	–	No growth	No growth

Sports Drink

500 mL, pH 3.25

Contaminant	CFU	48 Hours		72 Hours		96 Hours		120 Hours		168 Hours		Time to Detection (h)	
		RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	Innovate	Agar
A. acidoteresstris	16	1	–	1	–	3	–	1	–	1	–	No growth	No growth
T. pinophilus	40	1	–	14	+	24	+	261	+	758	+	72	96
B. fulva	10	1	–	4	–	2	–	3	–	59,099	+	168	192

Almond Drink

800 mL, pH 7.03

Contaminant	CFU	24 Hours		48 Hours		72 Hours		96 Hours		Time to Detection (h)	
		RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	Innovate	Agar
B. coagulans	20	4	–	4	+	34	+	73,039	+	72	72
G. stearothermophilus	15	47,033	+	472	+	175	+	908	+	24	48
E. faecalis	7	27	+	28,881	+	65,727	+	96,509	+	24	48
P. aeruginosa	7	27	+	28,881	+	65,727	+	96,509	+		
S. thermophilus	7	27	+	28,881	+	65,727	+	96,509	+		
B. fulva	13	3	–	3	–	225	+	11,372	+	72	96
T. pinophilus	13	3	–	3	–	225	+	11,372	+		

Oat Drink

800 mL, pH 6.16

Contaminant	CFU	24 Hours		48 Hours		72 Hours		96 Hours		Time to Detection (h)	
		RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	Innovate	Agar
B. coagulans	20	4	–	14	+	16,529	+	104,010	+	48	72
G. stearothermophilus	15	8,068	+	18	+	8	+	8	+	24	48
E. faecalis	7	16	+	120,984	+	92,171	+	91,780	+	24	48
P. aeruginosa	7	16	+	120,984	+	92,171	+	91,780	+		
S. thermophilus	7	16	+	120,984	+	92,171	+	91,780	+		
B. fulva	13	3	–	95	+	1,813	+	15,919	+	48	72
T. pinophilus	13	3	–	95	+	1,813	+	15,919	+		

Classic Chai High Concentrate

100 mL, pH 3.5

Contaminant	CFU	72 Hours		96 Hours		120 Hours		144 Hours		168 Hours		Time to Detection (h)	
		RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	Innovate	Agar
S. aureus	<100	–	–	–	–	–	–	–	–	–	–	No growth	No growth
P. aeruginosa	<100	–	–	–	–	–	–	–	–	–	–	No growth	No growth
B. cereus	<100	–	–	–	–	–	–	–	–	–	–	No growth	No growth
P. putida	<100	–	–	–	–	–	–	–	–	–	–	No growth	No growth
C. sporogenes	<100	–	–	–	–	–	–	–	–	–	–	No growth	No growth
A. brasiliensis	<100	105	+	423	+	2	–	3	–	4	–	72	96
S. cerevisiae	<100	99	+	446	+	3	NA*	4	NA*	2	NA*	72	96

* NA = not applicable (testing not done).

Classic Chai Latte Drink

100 mL, pH 3.5

Contaminant	CFU	72 Hours		96 Hours		120 Hours		144 Hours		168 Hours		Time to Detection (h)	
		RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	Innovate	Agar
S. aureus	<100	–	–	–	–	–	–	–	–	–	–	No growth	No growth
P. aeruginosa	<100	–	–	–	–	–	–	–	–	–	–	No growth	No growth
B. cereus	<100	–	–	–	–	–	–	–	–	–	–	No growth	No growth
P. putida	<100	–	–	–	–	–	–	–	–	–	–	No growth	No growth
C. sporogenes	<100	–	–	–	–	–	–	–	–	–	–	No growth	No growth
A. brasiliensis	<100	659	+	108	+	4	–	4	–	3	–	72	96
S. cerevisiae	<100	1,082	+	3,340	+	5	NA*	3	NA*	3	NA*	72	96

* NA = not applicable (testing not done).

Cold Brew Coffee with Creamer

100 mL, pH 6.3

Contaminant	CFU	24 Hours		48 Hours		72 Hours		96 Hours		120 Hours		Time to Detection (h)	
		RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	RLUs	Agar	Innovate	Agar
A. brasiliensis	278	9	–	7	–	6	–	6	–	5	–	No growth	No growth
B. cereus	128	547	+	10,737	+	17,557	+	Burst	+	Burst	+	24	48
C. sporogenes	9	10	+	557	+	285	+	Burst	+	Burst	+	24	48
P. aeruginosa	39	1,727	+	13,825	+	14,841	+	26,306	+	18,210	+	24	48
P. putida	210	9	–	7	+	5,275	+	7,645	+	4,672	+	72	72
S. cerevisiae	76	9	–	12	+	19	+	4,690	+	Burst	+	48	72
S. aureus	46	10	–	101	+	3,669	+	4,524	+	5,742	+	24	72

Tomato Ketchup

100 mL, pH 3.63; MRS pH 5.25, 1:4; PDB pH 4.67, 1:4; TSB pH 4.72

Contaminant	CFU	24 Hours		48 Hours		72 Hours		Time to Detection (h)	
		RLUs	Agar	RLUs	Agar	RLUs	Agar	Innovate	Agar
C. albicans	10	21	–	578	+	6482	+	48	72
S. cerevisiae	10	2,029	+	198,414	+	229,203	+	24	48
Z. bailli	10	460	+	71,790	+	27,219	+	24	48
L. brevis	10	78	–	158,426	+	124,790	+	24	72
L. casei	10	84	–	61,666	+	117,936	+	24	72
L. lactis	10	67	–	46	–	31	–	No growth	No growth

5. Summary

Study Overview

This comprehensive study included:

- *Food products*: Ketchup and 10 different beverage products representing a wide range of origins
- *Spiked organisms*: Diverse microbial groups that are common contaminants of beverage products but have different characteristics (15 bacteria and 11 fungi, including 8 kinds of yeast)
- *Sample numbers*: 2 – 10 sample replicates for inoculation studies and duplicate assays for each sample replicate (for both the Innovate System and appropriate agar plates)

Characteristics of Microbes Included in This Study
Gram-positive bacteria
Gram-negative bacteria
Thermophilic bacteria
Yeast
Mold
Acidophilic organisms
Spore-producing organisms

In addition, all samples were incubated statically at their appropriate temperatures for a minimum of 24 hours (for 48 hours in some cases) before testing. The target inoculation level was 10 cells with actual results ranging between 2 – 50 cells for most organisms, except for *Z. rouxii* and *K. marxianus* (115 and 255 cells, respectively).

Key Takeaways

The Innovate System detected organisms within the chosen incubation period in most instances where growth of organisms in the spiked product was confirmed on agar plates. The rare exceptions were related to molds, which can be a difficult contaminant to spike and sample when they grow in clumps that are not easy to disperse into the sample matrix.

1. This report demonstrates that the Innovate System with the RapiScreen Beverage Kit can reliably detect initial, low-level inoculums of microorganisms added to sterile product following an incubation (enrichment) period.
2. The Innovate System demonstrated an equivalent or faster time to detection when compared to traditional plate confirmation methods.
    - For bacterial and yeast contaminants, time to detection ranged between 24 and 72 hours.
    - Mold contaminants, when reliably observed, were detected between 48 and 120 hours with the Innovate System.

Additional Information and Tips

Threshold Values

Baseline studies showed that the RapiScreen Beverage reagents produced low and stable baseline values in most products. This allows establishment of positive and negative threshold values.

The Innovate System has a default positive threshold of 100 RLUs, although the majority (8 of 10) of the tested products in our studies used customized positive threshold values of <30 RLUs. For the two exceptions, Orange Drink A and Orange Drink B had positive threshold levels of 96 and 147 RLUs, respectively. This can be expected because samples with high ATP background levels (e.g., high pulp content) tend to have higher thresholds.

Tips:

- As a best practice, perform experiments to determine the threshold values for your sample types.
- Direct pipetting into the center of the well of the RapiScreen test plate before running on the Innovate System can help mitigate the effects of a high background.

Fungal Growth

Contaminating fungi that form dense masses within products (e.g., *Byssochlamys* and *Aspergillus*) provide a challenge during syringe sampling, especially when at low concentrations. This sampling difficulty is known to affect a broad range of testing methods.

In these situations, detection on the Innovate System was inconsistent and plate growth resulted in low CFU counts. For example, *Byssochlamys fulva* was detected in oat and almond products after incubation at 30 °C for 48 and 72 hours, respectively. However, after 168 hours, only one replicate from the two spiked Orange Drink A products gave an RLU result above the fail threshold, and no growth was seen on plates. When tested again after 240 hours, the Innovate System detected *B. fulva* in one of the Orange Drink A replicates, while growth was confirmed on plates from both replicates.

Tips:

- Increasing the number of sample replicates can help mitigate challenging sampling conditions of some fungi.
- When possible, physical inspection of samples can also help detect fungal growth.

Contact Information

For information, visit www.hygiena.com/contact. For technical support, visit www.hygiena.com/support.

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