CERTIFICATION

AOAC Research Institute
Performance Tested Methods℠

Certificate No.

092301

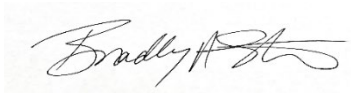
The AOAC Research Institute hereby certifies the method known as

Innovate™ RapiScreen™ Dairy Kit

manufactured by

Hygiena LLC
941 Avenida Acaso
Camarillo, CA USA

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*℠ Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*℠ certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.



Bradley A. Stawick, AOAC Research Institute Senior Director

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SUBMITTING COMPANY
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METHOD NAME
Innovate™ RapiScreen™ Dairy Kit

CATALOG NUMBERS
KIT4001 Ref. no. 82050 (Innovate RapiScreen Dairy 5000 Test Kit), Kit 4000 Ref. no. 82010 (Innovate RapiScreen Dairy 1000 Test Kit), KIT4015 Ref. no. 1311250B RapiScreen Dairy (25000) for Autosampler-Bar-Coded.

INDEPENDENT LABORATORY
Q Laboratories
1930 Radcliff Drive
Cincinnati, OH 45204

APPLICABILITY OF METHOD
Target organism – Mesophilic microorganisms.

Matrixes – ultra-high temperature (UHT) bovine milk, UHT plant-based drink (almond drink), half and half (10% fat), protein-based drink (casein), extended shelf life (ESL) plant-based drink (oat drink).

Performance claims – The study data were unable to detect a statistical difference in results between the Innovate System RapiScreen Dairy Kit and the culture-based reference methods. The method can reliably detect microbial contamination in 7 days, as compared to a 15-day reference method, and can detect contamination in some products in as few as 3 days.

REFERENCE METHODS
American Public Health Association (APHA) Standard Methods for the Examination of Dairy Products (SMEDP) Chapter 6: Microbial Count Methods, 17th edition, 2004 (2)

U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM): *Chapter 3, Aerobic Plate count*, *U.S. Food and Drug Administration*. April 2001. (3)

U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM): *Chapter 16, Clostridium perfringens*, *U.S. Food and Drug Administration*. April 2001. (4)

Compendium of Methods for the Examination of Food Products: *Chapter 19: Lactic Acid Bacteria* (no date) (5)

ISO 4833-1:2013 Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 °C by the pour plate technique (2019) ISO.

ORIGINAL CERTIFICATION DATE
September 11, 2023

CERTIFICATION RENEWAL RECORD
Renewed through December 2026.

METHOD MODIFICATION RECORD
1. **November 2023 Level 2**
2. **December 2024 Level 1**

SUMMARY OF MODIFICATION
1. **Manufacturing location change from Germany to California and a minor reformulation to Innovate RapiScreen Dary Kit components.**
2. **Editorial changes.**

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NONE

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NONE

PRINCIPLE OF THE METHOD (1)
The Innovate™ System is based on an automated benchtop luminometer capable of high throughput screening. The Innovate System works exclusively with the RapiScreen™ family. RapiScreen utilizes adenosine triphosphate (ATP) bioluminescence, where the luciferase enzyme catalyses the consumption of microbial ATP to produce light. ATP bioluminescence can detect viable microorganisms with high sensitivity, providing an objective result much faster, as compared to visible microbial growth on agar plates, resulting in a faster detection time. RapiScreen includes a sample treatment step to reduce non-microbial sources of ATP prior to performing the standard bioluminescence assay. A lysis step then releases microbial ATP for the bioluminescence reaction. The assay evaluated in this study is RapiScreen Dairy.

DISCUSSION OF THE VALIDATION STUDY (1)

The Innovate System luminometer using the RapiScreen Dairy Kit, is an easy and rapid way to detect the presence of microbial ATP in dairy and dairy alternative products. It is intended to be used to provide quicker results than standard plating methods. The Innovate System RapiScreen Dairy can reliably detect low concentrations of microbial ATP. The validation study confirmed that the Innovate System RapiScreen Dairy Kit can detect contaminated product packs in 7 days or less, depending on the product, with results that are equivalent to the 15-day reference method requirement.

Time to detection is based upon both the matrix type used and the growth rate of the organisms. The microorganism used in this study panel were stressed prior to inoculation, leading to the conclusion that healthy organisms could be detected quicker. In the inclusivity study, the Innovate System detected various types of microorganisms, including Gram-positive and Gram-negative bacteria, yeasts and moulds. The RLU signal varied between organisms due to differences in phenotype and growth rate. The method uses product-specific sterile RLU thresholds based on background ATP to determine when the organism has populated the sample. The method was able to detect all 50 inclusivity microbes at concentrations less than 5 x 10^6 cfu/mL (2.7 x 10^5 per 50 µL test aliquot) per container. Data obtained from matrix testing studies, which were closer to the designed use of the Innovate method than the inclusivity study, further supports this claim. For all tested matrixes, the Innovate System RapiScreen Dairy method produced a 100% detection rate for the high positive spike level, low fractionally positive results (5–15 positives) and negative results for the control containers. The Innovate System method was at least twice as fast, when 7-day incubation is considered, when compared to the 15-day result of the reference methods.

In order to optimise the probability of contamination detection, the Innovate method must be validated for the specific product and incubation period for the most likely microbial contaminants. The customer might also choose to validate an incubation period shorter than 7 days, as a shorter period might be sufficient to detect high and/or fractional contaminants in certain matrixes. All positive results on the Innovate System method were significantly above the threshold obtained from the matrixes' RLU baselines. Based on this study, the Innovate System using RapiScreen Dairy Kit detected microbial contaminants in the examined matrixes at least 10 days quicker than the reference method for the matrixes.

The Independent Matrix Study performed with the Innovate System RapiScreen Dairy Kit further substantiates the claim, having achieved successful detection of microbial contamination in UHT bovine milk at and after 24 h of incubation. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate method and the reference methods for all samples tested after 24 h of incubation. The RapiScreen Dairy Kit combined with the Innovate System luminometer instrument and software provides a simple and rapid method for determining the commercial sterility of manufactured products. The reagents are easy to prepare by adding a bottle of diluent to a bottle of lyophilized reagent. The software is intuitive and allows for ease of use of the Innovate System instrument. Cleaning and maintaining the Innovate System instrument is convenient as the instrument has a self-cleaning wash feature.

Further studies of robustness, instrument variation, product consistency and stability all achieved the required fractional levels of between 3 and 7 positives out of 10 and showed detection to be consistent across all conditions, independent of the examined organisms or variables introduced to the condition. Hence, the Innovate System method is sensitive and robust, with the RapiScreen Dairy Kit reagents performing consistently across their shelf life. The Instrument Variation study detected no variation in performance across three different luminometers that could affect the detection of a contaminant.

Table 2: RapiScreen Dairy Kit inclusivity results of 50 organisms. Readings were performed on the Innovate System and (+) and (-) results were given based on broth/diluent control RLU thresholds. (1)

No.	Genus	Species	Source	Origin	Candidate Method Result	cfu/sample aliquot[e]
1	*Alicyclobacillus*	*acidoterrestris*	ATCC[a] 49025	Soil	+	85,000
2	*Aspergillus*	*niger*	Wild Type[b]	Air isolate	+	2,000
3	*Bacillus*	*cereus*	ATCC 11778	Unknown	+	43,000
4	*Bacillus*	*coagulans*	NCTC[c] 3993	Soil	+	30,000
5	*Bacillus*	*licheniformis*	Wild Type	Plant-based drink	+	268,000
6	*Bacillus*	*pumilus*	Wild Type	Plant-based drink	+	56,000
7	*Bacillus*	*spizizenii*	NCTC 10400	Unknown	+	35,750
8	*Bacillus*	*subtilis*	ATCC 6051	Unknown	+	119,000
9	*Bacillus*	*thuringiensis*	Wild Type	Plant-based drink	+	69,000
10	*Byssochlamys*	*fulva*	ATCC 10099	Bottled fruit	+	22,500
11	*Candida*	*albicans*	ATCC 10231	Bronchomycosis	+	52,250
12	*Candida*	*orthopsilosis*	NCPF[d] 8798	Human	+	69,250
13	*Cellulosimicrobium*	*cellulans*	NCTC 13518	Human	+	26,800
14	*Citrobacter*	*freundii*	NCTC 9750	Unknown	+	13,600
15	*Clostridium*	*perfringens*	NCTC 8237	Water	+	223,000
16	*Clostridium*	*sporogenes*	ATCC 7955	Unknown	+	104,000
17	*Corynebacterium*	*renale*	ATCC 10848	Human	+	35,000
18	*Cronobacter*	*sakazakii*	ATCC 29544	Human	+	184,800
19	*Dekkera*	*bruxellensis*	ATCC 36234	Belgian stout	+	40,000
20	*Enterobacter*	*aerogenes*	ATCC 13048	Sputum	+	44,500
21	*Enterobacter*	*cloacae*	NCTC 10005	Spinal fluid	+	58,800
22	*Enterococcus*	*faecalis*	ATCC 19433	Piglet feces	+	35,700
23	*Escherichia*	*coli*	ATCC 8739	Feces	+	18,800
24	*Geobacillus*	*stearothermophilus*	ATCC 7953	Unknown	+	2,500
25	*Kluyveromyces*	*lactis*	ATCC 20185	Cheese	+	23,500
26	*Kluyveromyces*	*marxianus*	NCTC 3106	Creamery	+	35,750
27	*Lactobacillus*	*fermentum*	ATCC 9338	Milk	+	13,900
28	*Lactobacillus*	*fructivorans*	ATCC 8288	Unknown	+	243,000
29	*Lactobacillus*	*lactis*	ATCC 19435	Cheese	+	7,500
30	*Lactobacillus*	*paracasei*	Wild Type	Ketchup	+	9,8500
31	*Lactococcus*	*lactis*	ATCC 11454	Milk	+	9,700
32	*Leuconostoc*	*mesenteroides*	ATCC 8293	Olives	+	7,900
33	*Listeria*	*innocua*	ATCC 33090	Cow brain	+	14,300

34	*Listeria*	*monocytogenes*	ATCC 7644	Human	+	17,400
35	*Micrococcus*	*luteus*	ATCC 4698	Human	+	53,500
36	*Penicillium*	*chrysogenum*	ATCC 10106	Cheese	+	8,000
37	*Pseudomonas*	*aeruginosa*	ATCC 9027	Ear infection	+	17,500
38	*Pseudomonas*	*fluorescens*	ATCC 13525	Water	+	16,200
39	*Pseudomonas*	*putida*	ATCC 49128	Clinical isolate	+	23,000
40	*Saccharomyces*	*cerevisiae*	ATCC 9763	Distillery	+	16,500
41	*Saccharomyces*	*kudriavzevii*	ATCC 2601	Unknown	+	6,950
42	*Salmonella*	Enteritidis	ATCC 13076	Unknown	+	26,200
43	*Salmonella*	Newport	NCTC 14032	Unknown	+	22,500
44	*Salmonella*	Typhimurium	ATCC 14028	Chicken liver	+	21,000
45	*Staphylococcus*	*aureus*	ATCC 6538	Human lesion	+	12,700
46	*Talaromyces*	*pinophilus*	ATCC 36839	PVC	+	800
47	*Torulaspora*	*delbrukeii*	ATCC 10662	Unknown	+	26,500
48	*Yarrowia*	*lipolytica*	ATCC 9773	Butter	+	8,000
49	*Zygosaccharomyces*	*parabailii*	ATCC 56075	Unknown	+	52,500
50	*Zygosaccharomyces*	*rouxii*	ATCC 2623	Grape must	+	13,925

[a]American Type Culture Collection, Manassas, VA.
[b]Wild type strains isolated at Hygiena LLC Research Laboratory, Camarillo, CA.
[c]National Collection of Type Cultures, Porton Down, Salisbury, UK.
[d]National Collection of Pathogenic Fungi, Porton Down, Salisbury, UK.
[e]Sample aliquot is 50 µL.

Table 4: RapiScreen Dairy Kit results of the Spiked Matrices and Respective Strains Comparing the Candidate Method to the Reference Method. (The Innovate System read timepoint shown is described in column 4). (1)

					Candidate method			Reference method				
Matrix	Strain	Spiked cfu per pack[a]	Day	N[b]	x[c]	POD_C[d]	95% CI	x	POD_R[e]	95% CI	$dPOD_C$[f]	95% CI[g]
UHT bovine milk	*Pantoea agglomerans* ATCC[h] *27155*	11	2	5	5	1.00	(0.57, 1)	5	1.00	(0.57, 1)	0	(-0.47, 0.47)
		1.2		20	12	0.60	(0.39, 0.78)	13	0.65	(0.43, 0.82)	-0.05	(-0.21, 0.11)
		0		5	0	0.00	(0, 0.43)	0	0.00	(0, 0.43)	0	(-0.47, 0.47)
ESL plant-based drink	*Bacillus coagulans* ATCC *7050*	588	5	5	5	1.00	(0.57, 1)	5	1.00	(0.57, 1)	0	(-0.47, 0.47)
		1		20	8	0.40	(0.22, 0.61)	10	0.50	(0.3, 0.7)	-0.1	(-0.28, 0.08)
		0		5	0	0.00	(0, 0.43)	0	0.00	(0, 0.43)	0	(-0.47, 0.47)
Half and half	*Clostridium sporogenes* ATCC *7955*	6300	5	5	5	1.00	(0.57, 1)	5	1.00	(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	0.00	(0, 0.43)	0	0.00	(0, 0.43)	0	(-0.47, 0.47)
Protein-based drink	*Lactobacillus fermentum* ATCC *9338*	19000	7	5	5	1.00	(0.57, 1)	5	1.00	(0.57, 1)	0	(-0.47, 0.47)
		1		20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0	(-0.13, 0.13)
		0		5	0	0.00	(0, 0.43)	0	0.00	(0, 0.43)	0	(-0.47, 0.47)
UHT plant-based drink	*Bacillus subtilis* ATCC *6051*	9.6	5	5	5	1.00	(0.57, 1)	5	1.00	(0.57, 1)	0	(-0.47, 0.47)
		0.6		20	8	0.40	(0.22, 0.61)	8	0.40	(0.22, 0.61)	0	(-0.13, 0.13)
		0		5	0	0.00	(0, 0.43)	0	0.00	(0, 0.43)	0	(-0.47, 0.47)
UHT bovine milk[i]	*Pantoea agglomerans* CCUG 58262	2-10	2	5	5	1.00	(0.57, 1)	5	1.00	(0.57, 1)	0.00	(-0.47, 0.47)
		0.2-2		20	8	0.40	(0.22, 0.61)	8	0.35	(0.22, 0.61)	0.00	(-0.13, 0.13)
		0		5	0	0.00	(0, 0.43)	0	0.20	(0, 0.43)	0.00	(-0.47, 0.47)

[a] cfu = colony forming units applied to each package.
[b] N = number of test portions.
[c] X = number of positive test portions.
[d] POD_C = Candidate Method presumptive results confirmed positive divided by the total number of trials.
[e] POD_R = Reference Method positive results divided by the total number of trials.
[f] $dPOD_C$ = Difference between the Candidate Method results and Reference Method results 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.

DISCUSSION OF MODIFICATION APPROVED NOVEMBER 2023 (6)

The Innovate System luminometer using the RapiScreen Dairy Kit, is an easy and rapid way to detect the presence of microbial contamination in dairy and dairy alternative products. This detection method has been certified by AOAC-RI PTM program (Cert. No. 092301) for 5 claimed matrixes, UHT bovine milk, UHT plant-based drink (almond drink), half and half 10% fat, protein-based drink, ESL plant-based drink (oat drink). The Level 2 modification described here was conducted due to the shift from third-party to inhouse manufacturing, and to conform to US-specific chemical regulations. The candidate method is intended to be used to provide quicker results than standard plating methods. The Innovate System RapiScreen Dairy can reliably detect low concentrations of microbial contamination. The validation study confirmed that the Innovate System RapiScreen Dairy Kit delivers detection of contaminated product packs in 5–7 days with results that are equivalent to the 15-day reference method.

Time to detection is based upon both the matrix type used and the growth rate of the organisms. The microorganisms used in this study panel were stressed prior to inoculation, leading to the conclusion that healthy organisms would be detected quicker. In the inclusivity study, the Innovate System detected various types of microorganisms, including Gram-positive, Gram-negative bacteria, yeasts, and moulds. The RLU signal varied between organism types due to differences in phenotype and growth rate. The method uses product specific sterile RLU thresholds based on background ATP to determine whether the organism has populated the sample. The method was able to detect all 50 inclusivity microbes tested in this study.

Data obtained from matrix testing studies, which were closer to the designed use of the Innovate method than the inclusivity study, further supports this claim. For all tested matrixes, the Innovate System RapiScreen Dairy method produced a 100% detection rate for the high positive spike level, low fractionally positive results (5–15 positives) and negative results for the control containers. The Innovate System method was more than twice as fast, when 5-day incubation is considered, when compared to the 15-day result of the reference methods. At 5 days, fractional equivalence occurred in half and half. Both ESL plant-based drink and protein-based drink gave fractional results after 7days of incubation (all incubations are at 30°C).

In order to optimise the probability of contamination detection, the Innovate method must be validated for the specific product and incubation period for the most likely microbial contaminants.

Based on this study, the Innovate System using RapiScreen Dairy Kit detected microbial contaminants in the examined matrixes at least 7 days quicker than the reference method.

The RapiScreen Dairy Kit combined with the Innovate System luminometer instrument and software provides a simple and rapid method for determining the commercial sterility of manufactured products. The reagents are easy to prepare by adding a bottle of diluent to a bottle of lyophilized reagent. The software is intuitive and allows for easy use of the Innovate System instrument. Cleaning and maintaining the Innovate System instrument is convenient as the instrument has a self-cleaning wash feature.

Further studies of robustness, instrument variation, product consistency and stability all achieved the required fractional levels of between 3 and 7 positives out of 10 and showed detection to be consistent across all conditions, independent of the examined organisms or variable introduced to the condition. Hence, the Innovate System method was sensitive and robust, with the in-house manufactured RapiScreen Dairy Kit reagents performing consistently across the product shelf-life. The Instrument Variation study detected no variation in performance across three different luminometers that could affect the detection of a contaminant.

Table 2: RapiScreen Dairy Kit Inclusivity results of 50 organisms. Readings were performed on the Innovate System and positive (+) and negative (-) results were given based on broth/diluent control RLU thresholds. (6)

No.	Genus	Species	Source	Origin	Candidate Method Result	CFU/sample aliquot[e]
1	*Alicyclobacillus*	*acidoterrestris*	ATCC[a] 49025	Soil	+	4.00E+04
2	*Aspergillus*	*niger*	Wild Type[b]	Air Isolate	+	2.05E+03
3	*Bacillus*	*cereus*	ATCC 11778	Unknown	+	2.45E+03
4	*Bacillus*	*coagulans*	NCTC[c] 3993	Soil	+	2.45E+03
5	*Bacillus*	*licheniformis*	Wild Type	Plant-based drink	+	1.10E+04
6	*Bacillus*	*pumilus*	Wild Type	Plant-based drink	+	2.70E+04
7	*Bacillus*	*spizizenii*	NCTC 10400	Unknown	+	6.70E+04
8	*Bacillus*	*subtilis*	ATCC 6633	Unknown	+	1.42E+04
9	*Bacillus*	*thuringiensis*	Wild Type	Plant-based drink	+	2.80E+03
10	*Byssochlamys*	*fulva*	ATCC 10099	Bottled Fruit	+	5.00E+03
11	*Candida*	*albicans*	ATCC 10231	Bronchomycosis	+	1.25E+03
12	*Candida*	*orthopsilosis*	NCPF[d] 8798	Human	+	8.55E+03
13	*Cellulosimicrobium*	*cellulans*	NCTC 13518	Human	+	7.90E+03
14	*Citrobacter*	*freundii*	NCTC 9750	Unknown	+	7.53E+03
15	*Clostridium*	*perfringens*	NCTC 8237	Water	+	1.75E+03
16	*Clostridium*	*sporogenes*	ATCC 7955	Unknown	+	3.80E+03
17	*Corynebacterium*	*renale*	ATCC 10848	Human	+	3.00E+03
18	*Cronobacter*	*sakazakii*	ATCC 29544	Human	+	1.18E+04
19	*Dekkera*	*bruxellensis*	ATCC 36234	Belgian Stout	+	5.50E+03
20	*Enterobacter*	*aerogenes*	ATCC 13048	Sputum	+	9.90E+03
21	*Enterobacter*	*cloacae*	NCTC 10005	Spinal Fluid	+	1.42E+05
22	*Enterococcus*	*faecalis*	ATCC 19433	Piglet Faeces	+	5.17E+05
23	*Escherichia*	*coli*	ATCC 8739	Faeces	+	2.26E+04
24	*Geobacillus*	*stearothermophilus*	ATCC 7953	Unknown	+	7.50E+05
25	*Kluyveromyces*	*lactis*	ATCC 20185	Cheese	+	2.08E+03
26	*Kluyveromyces*	*marxianus*	NCTC 3106	Creamery	+	3.28E+03
27	*Lactobacillus*	*fermentum*	ATCC 9338	Milk	+	5.73E+04
28	*Lactobacillus*	*fructivorans*	ATCC 8288	Unknown	+	1.43E+04
29	*Lactobacillus*	*lactis*	ATCC 19435	Cheese	+	1.50E+03
30	*Lactobacillus*	*paracasei*	Wild Type	Ketchup	+	1.50E+03
31	*Lactococcus*	*lactis*	ATCC 11454	Milk	+	5.85E+04

32	*Leuconostoc*	*mesenteroides*	ATCC 8293	Olives	+	4.00E+05
33	*Listeria*	*innocua*	ATCC 33090	Cow Brain	+	1.14E+04
34	*Listeria*	*monocytogenes*	ATCC 7644	Human	+	2.70E+03
35	*Micrococcus*	*luteus*	ATCC 4698	Human	+	3.99E+03
36	*Penicillium*	*chrysogenum*	ATCC 10106	Cheese	+	4.50E+03
37	*Pseudomonas*	*aeruginosa*	ATCC 9027	Ear infection	+	6.50E+04
38	*Pseudomonas*	*fluorescens*	ATCC 13525	Water	+	1.15E+04
39	*Pseudomonas*	*putida*	ATCC 49128	Clinical Isolate	+	3.61E+04
40	*Saccharomyces*	*cerevisiae*	ATCC 9763	Distillery	+	3.03E+03
41	*Saccharomyces*	*kudriavzevii*	ATCC 2601	Unknown	+	1.41E+04
42	*Salmonella*	Enteritidis	ATCC 13076	Unknown	+	2.54E+04
43	*Salmonella*	Newport	NCTC 14032	Unknown	+	3.70E+03
44	*Salmonella*	Typhimurium	ATCC 14028	Chicken liver	+	4.00E+04
45	*Staphylococcus*	*aureus*	ATCC 6538	Human lesion	+	1.82E+04
46	*Talaromyces*	*pinophilus*	ATCC 36839	PVC	+	2.50E+03
47	*Torulaspora*	*delbrukeii*	ATCC 10662	Unknown	+	1.80E+04
48	*Yarrowia*	*lipolytica*	ATCC 9773	Butter	+	9.50E+03
49	*Zygosaccharomyces*	*parabailii*	ATCC 56075	Unknown	+	1.78E+04
50	*Zygosaccharomyces*	*rouxii*	ATCC 2623	Grape Must	+	1.98E+04

[a]American Type Culture Collection, Manassas, VA.
[b]Wild type strains isolated at Hygiena LLC Research Laboratory, Camarillo, CA.
[c]National Collection of Type Cultures, Porton Down, Salisbury, UK.
[d]National Collection of Pathogenic Fungi, Porton Down, Salisbury, UK.
[e]Sample aliquot is 50 µL

Table 4: RapiScreen Dairy Kit results of the Spiked Matrixes and Respective Strains comparing the Candidate Method to the Reference Method. (The Innovate System read timepoint shown is Day 5. (6)

Matrix	Strain	Spiked CFU per pack[a]	Day	N[b]	Candidate method			Reference method			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	x	POD$_R$[e]	95% CI		
ESL plant-based drink	*Bacillus coagulans* ATCCh *7050*	558	5	5	5	1	(0.57, 1)	5	1	(0.57, 1)	0	(-0.47, 0.47)
		0.4		20	14	0.7	(0.48, 0.85)	15	0.75	(0.53, 0.89)	-0.05	(-0.21, 0.11)
		0		5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.47, 0.47)
Half and half	*Clostridium sporogenes* ATCC *7955*	70	5	5	5	1	(0.57, 1)	5	1	(0.57, 1)	0	(-0.47, 0.47)
		0.1		20	6	0.3	(0.15, 0.52)	6	0.3	(0.15, 0.52)	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 (Casein)	*Lactobacillus fermentum* ATCC 9338	1410	5	5	5	1	(0.57, 1)	5	1	(0.57, 1)	0	(-0.47, 0.47)
		0.8		20	10	0.5	(0.3, 0.7)	11	0.5	(0.3, 0.7)	-0.05	(-0.29, 0.19)
		0		5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.47, 0.47)

[a] CFU = colony forming units applied to each package.
[b] N = number of test portions.
[c] X = number of positive test portions.
[d] POD$_C$ = Candidate method presumptive positive results confirmed positive divided by the total number of trials.
[e] POD$_R$ = Reference method results divided by the total number of trials.
[f] dPOD$_C$ = 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.

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