



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

081801

The AOAC Research Institute hereby certifies the method known as:

GENE-UP[®] Cronobacter species Assay

manufactured by

bioMérieux

595 Anglum Road

Hazelwood, MO 63042

USA

This method has been evaluated in the AOAC[®] Performance Tested MethodsSM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Performance TestedSM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above-mentioned method for a period of one calendar year from the date of this certificate (February 11, 2022 – December 31, 2022). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

February 14, 2022

Date

AUTHORS ORIGINAL VALIDATION: Ron Johnson, John Mills, Vikrant Dutta, Nikki Taylor MODIFICATION NOVEMBER 2020: Ron Johnson, Vikrant Dutta, John Mills, Deborah Briese	SUBMITTING COMPANY bioMérieux 595 Anglum Road Hazelwood, MO 63042
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METHOD NAME GENE-UP® <i>Cronobacter</i> species Assay	CATALOG NUMBERS 421920
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INDEPENDENT LABORATORY Q.Laboratories, Inc. 1400 Harrison Avenue Cincinnati, OH 45214	AOAC EXPERTS AND PEER REVIEWERS Yi Chen ¹ , Michael Brodsky ² , Wayne Ziemer ³ ¹ FDA CFSAN, College Park, MD, USA ² Brodsky Consultants, Thornhill, Ontario, Canada ³ Consultant, Loganville, GA, USA Modification November 2020 and August 2021 reviewed internally by AOAC Research Institute.
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APPLICABILITY OF METHOD Target organisms – <i>Cronobacter</i> species (<i>C. sakazakii</i> , <i>C. dubliensis</i> , <i>C. muytjensii</i> , <i>C. malonaticus</i> , <i>C. turicensis</i> , <i>C. universalis</i>)	REFERENCE METHOD International Organization for Standardization: ISO 22964:2017 <i>Microbiology of the food chain – Horizontal method for the detection of Cronobacter spp.</i> Updated: 2017. (Accessed May 2018) (2)
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Matrixes – milk-based powdered infant formula with probiotics (25 g, 375 g), milk-based powdered infant formula without probiotics (25 g, 375 g), soy-based powdered infant formula (25 g, 375 g), non-fat dry milk (100 g), soy ingredients (100 g), stainless steel (4 x 4 in sponge) and plastic (1 x1 in swab)

Performance claims - The GENE-UP® *Cronobacter* species assay is equivalent to the ISO 22964:2017 (1) for milk-based powdered infant formula with probiotics (25 g and 375 g), milk-based powdered infant formula without probiotics (25 g and 375 g), soy-based powdered infant formula (25 g and 375 g), non-fat dry milk (100 g), soy ingredients (100 g), stainless steel and plastic environmental surface samples.

ORIGINAL CERTIFICATION DATE August 02, 2018	CERTIFICATION RENEWAL RECORD Renewed annually through December 2022.
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METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
1. November 2020 Level 2	1. Addition of an environmental DNA removal step using BACTVIAB PMAxx reagent.
2. August 2021 Level 1	2. Changes include PCR tube color and new septum cap to simplify workflow.
3. February 2022 Level 1	3. Replace red dye with blue dye in GENE-UP Lysis kit.

Under this AOAC® <i>Performance Tested</i> SM License Number, 081801 this method is distributed by: NONE	Under this AOAC® <i>Performance Tested</i> SM License Number, 081801 this method is distributed as: NONE
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PRINCIPLE OF THE METHOD (1)

The GENE-UP *Cronobacter* kit is to be used with the GENE-UP Thermocycler. The GENE-UP *Cronobacter* kit contains all the necessary components for PCR, including sample-specific primers and probes and an internal amplification control. The GENE-UP Thermocycler detects fluorescence at several wavelengths (channels) to allow multi-target detection in the same reaction vessel. The fluorescent signal of the target is recorded in channel 640, while the fluorescent signal for an internal amplification control is recorded in channel 705. The software automatically interprets the results for both fluorescence channels and determines the sample result based on the outcome of the control.

Both the assay for the target and the internal amplification control utilize dual Fluorescence Resonance Energy Transfer (FRET) hybridization probes. These probes consist of two different short oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of the reaction cycle. The first probe for the sample assay is labeled at the 3' end with fluorescein; the second probe is labeled at the 5' end with LC Red 640. FRET occurs only after the two probes come in close proximity from hybridizing to the template DNA.

The resulting fluorescent signal from the FRET interaction, which forms a real-time amplification curve, is how the amplified target is detected by the GENE-UP Thermocycler. After the PCR cycling program finishes, the PCR product(s) are melted to determine the presence of the target DNA. The software uses both the real-time amplification curve and the melt peak to make a positive or negative call.

DISCUSSION OF THE VALIDATION STUDY (1)

The GENE-UP *Cronobacter* method successfully detected *Cronobacter* from milk-based powdered infant formula with and without probiotics, soy-based powdered infant formula, nonfat dried milk, soy ingredients, stainless steel and plastic. 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 matrixes.

The GENE-UP *Cronobacter* method is quick and simple to perform, providing results in less than 1.5 h post incubation of the selective enrichment for 30 sample replicates. With the new individual lyophilized pellets, it allows the user to conduct PCR without an additional step of adding the master mix, reducing the amount of hands-on time during PCR which eliminates the chance of contamination. The GENE-UP software is user friendly with the ability to track lot information and sample identification quickly and with ease. The GENE-UP software and instrument also offer the ability to utilize an open platform and set up unique runs before a run is completed. The small footprint is also desirable when working in laboratories with a limited amount of space, or a small PCR amplification room.

Table 5. GENE-UP *Cronobacter* species Inclusivity Study Results (1)

Source	Species	Origin	BPW + novobiocin enrichment
LMG ^a 262502	<i>C. Condimenti</i>	Unknown	Positive
LMG 23823	<i>C. Dublinensis ssp dublinensis</i>	Milk powder	Positive
GRE ^b :1561052	<i>C. Dublinensis ssp dublinensis</i>	Unknown	Positive
LMG 23825	<i>C. Dublinensis ssp lactaridi</i>	Milk powder	Positive
GRE:1658019	<i>C. Dublinensis ssp lactaridi</i>	Milk powder	Positive
LMG 23824	<i>C. Dublinensis ssp lausannensis</i>	Water	Positive
NCTC ^c 9844	<i>C. Dublinensis ssp lausannensis</i>	Unknown	Positive
GRE:1561049	<i>C. Dublinensis ssp lausannensis</i>	Unknown	Positive
GRE:1559055	<i>C. Dublinensis ssp lausannensis</i>	Infant food	Positive
GRE:1653013	<i>C. Malonaticus</i>	Human	Positive
GRE:1559032	<i>C. Malonaticus</i>	Milk powder	Positive
LMG 23826	<i>C. Malonaticus</i>	Abscess	Positive
GRE:1653014	<i>C. Malonaticus</i>	Unknown	Positive
GRE:1561045	<i>C. Malonaticus</i>	Unknown	Positive
GRE:1561044	<i>C. Malonaticus</i>	Unknown	Positive
API ^d :0702084	<i>C. Malonaticus</i>	Food	Positive
ATCC ^e 51329	<i>C. Muytjensii</i>	Unknown	Positive
GRE:1559116	<i>C. Muytjensii</i>	Plant gum	Positive
GRE:1561038	<i>C. Muytjensii</i>	Unknown	Positive
GRE:1561039	<i>C. Muytjensii</i>	Unknown	Positive
API:0707080	<i>C. Muytjensii</i>	Unknown	Positive
API:0707081	<i>C. Muytjensii</i>	Unknown	Positive
NCTC 8155	<i>C. Sakazakii</i>	Milk powder	Positive
GRE:1654034	<i>C. Sakazakii</i>	Sugar	Positive
GRE:1653017	<i>C. Sakazakii</i>	Peanut	Positive
GRE:1653015	<i>C. Sakazakii</i>	Unknown	Positive
GRE:1653016	<i>C. Sakazakii</i>	Milk Powder	Positive
ATCC 29544	<i>C. Sakazakii</i>	Child throat	Positive
ATCC 12868	<i>C. Sakazakii</i>	Unknown	Positive
ATCC 29004	<i>C. Sakazakii</i>	Unknown	Positive
CUETM ^f 79-105	<i>C. Sakazakii</i>	Unknown	Positive
NCTC 9238	<i>C. Sakazakii</i>	Abdominal pus	Positive
GRE:1058001	<i>C. Sakazakii</i>	Unknown	Positive
GRE:1058002	<i>C. Sakazakii</i>	Unknown	Positive
GRE:1058003	<i>C. Sakazakii</i>	Unknown	Positive
GRE:1256007	<i>C. Sakazakii</i>	Unknown	Positive
GRE:1256008	<i>C. Sakazakii</i>	Unknown	Positive
GRE:1561037	<i>C. Sakazakii</i>	Unknown	Positive
LMG 23827	<i>C. Turicensis</i>	Blood culture	Positive
GRE:1559056	<i>C. Turicensis</i>	Infant food	Positive
GRE:1561041	<i>C. Turicensis</i>	Unknown	Positive
GRE:1561040	<i>C. Turicensis</i>	Unknown	Positive
GRE:1561042	<i>C. Turicensis</i>	Unknown	Positive
API:0106057	<i>C. Turicensis</i>	Unknown	Positive
GRE:1653018	<i>C. Universalis</i>	Infant food	Positive
GRE:1561046	<i>C. Universalis</i>	Unknown	Positive
GRE:1561047	<i>C. Universalis</i>	Unknown	Positive
GRE:1561048	<i>C. Universalis</i>	Unknown	Positive
GRE:1561002	<i>C. Universalis</i>	Unknown	Positive
API:1504034	<i>C. Universalis</i>	Unknown	Positive

^aLMG: Collection of the Laboratorium voor Microbiologie en Microbiële Genetica, Rijksuniversiteit, Ledeganckstraat 35, B-9000, Gent, Belgium.^bGRE : bioMérieux Grenoble stock culture collection, Grenoble, France.^cNCTC: National Collection of Type Cultures, Porton Down, Salisbury, UK.^dAPI: bioMérieux LaBalme Stock culture collection, LaBalme, France.^eATCC: American Type Culture Collection, Manassas, VA.^fCUETM: Collection of l'Unité d'Écotoxicologie Microbienne, Institut National de la Recherche Agronomique (INRA), 369 rue Jules Guesde, B.P. 39, 59651 Villeneuve d'Ascq cedex, France.

Table 6. GENE-UP *Cronobacter* species Exclusivity Study Results (1)

Source	Strain	BMX Stock Culture Collection Number	CRO assay result
ATCC ^a 51113	<i>Citrobacter braakii</i>	GRE:1161034	Negative
ATCC 51112	<i>Citrobacter fameri</i>	GRE:1559145	Negative
ATCC 13316	<i>Citrobacter freundii</i>	GRE:0062057	Negative
ATCC 27028	<i>Citrobacter koseri</i>	GRE:1156045	Negative
ATCC 29751	<i>Enterobacter aerogenes</i>	GRE:1053039	Negative
ATCC 35953	<i>Enterobacter asburiae</i>	GRE:1558006	Negative
NCTC 13464	<i>Enterobacter cloacae</i>	GRE:1461014	Negative
ATCC BAA-2082	<i>Enterobacter hormaechei</i>	GRE:1558014	Negative
Unknown	<i>Enterobacter kobei</i>	GRE:1753005	Negative
Unknown	<i>Escherichia coli</i>	GRE:1661008	Negative
ATCC 36650	<i>Escherichia hermannii</i>	GRE:1156039	Negative
Unknown	<i>Franconibacter helveticus</i>	GRE:1653010	Negative
Unknown	<i>Franconibacter pulveris</i>	GRE:1653007	Negative
ATCC 13337	<i>Hafnia alvei</i>	GRE:0356011	Negative
ATCC 27736	<i>Klebsiella pneumoniae</i>	GRE:1053063	Negative
ATCC 51702	<i>Kluyvera georgiana</i>	GRE:1356006	Negative
ATCC 393	<i>Lactobacillus casei</i>	GRE:1661003	Negative
ATCC 23216	<i>Leclercia adecarboxylata</i>	GRE:0356006	Negative
ATCC 25830	<i>Morganella morganii</i>	GRE:1260016	Negative
ATCC 29906	<i>Proteus mirabilis</i>	GRE:0062055	Negative
ATCC 9250	<i>Providencia rettgeri</i>	GRE:1156030	Negative
NCTC ^b 13437	<i>Pseudomonas aeruginosa</i>	GRE:1554028	Negative
ATCC 19585	<i>Salmonella typhimurium</i>	GRE:1053096	Negative
Unknown	<i>Serratia ficaria</i>	GRE:1556086	Negative
ATCC 13880	<i>Serratia marcescens</i>	GRE:0662008	Negative
ATCC 12022	<i>Shigella flexneri</i>	GRE:1053103	Negative
LMG ^c 28204	<i>Siccibacter colletis</i>	GRE:1655001	Negative
CIP ^d 109627	<i>Siccibacter turicensis</i>	GRE:1653011	Negative
ATCC 14990	<i>Staphylococcus epidermidis</i>	GRE:0062018	Negative
Unknown	<i>Yersinia intermedia</i>	GRE:1054013	Negative

^aATCC: American Type Culture Collection, Manassas, VA.

^bNCTC: National Collection of Type Cultures, Porton Down, Salisbury, UK.

^cLMG: Collection of the Laboratorium voor Microbiologie en Microbiële Genetica, Rijksuniversiteit, Ledeganckstraat 35, B-9000, Gent, Belgium.

^dCIP: The Collection of Institut Pasteur, Paris France.

Table 9. Method Developer Laboratory - GENE-UP *Cronobacter* species Assay, Candidate vs. Reference – POD Results (1)

Matrix/Test Portion	Strain	Time Point	MPN ^a / Test Portion (CI)	N ^b	Candidate			Reference			dPOD _c _f	95% CI ^g			
					x ^c	POD _d	95% CI	x ^c	POD _e	95% CI					
Milk-based powdered infant formula with probiotics (25g)	<i>Cronobacter sakazakii</i> ATCC 29544	18h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			1.48 (0.94, 2.49)	2 0	13	0.65	0.43	0.82	14	0.70	0.48	0.85	-0.05	-0.32	0.23
			>11 (4.10, >11)	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Milk-based powdered infant formula without probiotics (25g)	<i>Cronobacter dublinensis</i> spp <i>dublinensis</i> BMX 112161	18h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			0.67 (0.46, 0.93)	2 0	14	0.70	0.48	0.85	13	0.65	0.43	0.82	0.05	-0.23	0.32
			5.24 (2.39, 11.48)	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Soy-based powdered infant formula (25g)	<i>Cronobacter sakazakii</i> ATCC BAA-894	18h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			0.26 (0.10, 0.47)	2 0	7	0.35	0.18	0.57	4	0.20	0.08	0.42	0.15	-0.12	0.40
			4.06 (1.93, 6.00)	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Milk-based powdered infant formula	<i>Cronobacter sakazakii</i> ATCC 29544	20h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			1.48 (0.94, 2.49)	2 0	17	0.85	0.64	0.95	14	0.70	0.48	0.85	0.15	-0.11	0.39

with probiotics (375g)			>11 (4.10, >11)	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Milk-based powdered infant formula without probiotics (375g)	<i>Cronobacter dublinensis</i> spp <i>dublinensis</i> BMX 112161	20h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			0.67 (0.46, 0.93)	2 0	16	0.80	0.58	0.92	15	0.75	0.53	0.89	0.05	-0.21	0.30
			5.24 (2.39, 11.48)	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Soy-based powdered infant formula (375g)	<i>Cronobacter sakazakii</i> ATCC BAA-894	20h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			0.26 (0.10, 0.47)	2 0	9	0.45	0.26	0.66	4	0.20	0.08	0.42	0.25	-0.04	0.49
			4.06 (1.93, 6.00)	5	4	0.80	0.38	1.00	5	1.00	0.57	1.00	-0.20	-0.62	0.28
Non-fat dry milk (100g)	<i>Cronobacter dublinensis</i> spp <i>lausannensis</i> BMX 112177	20h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			1.17 (0.41, 1.00)	2 0	20	1.00	0.84	1.00	14	0.70	0.48	0.85	0.30	0.08	0.52
			>11 (4.10, >11)	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43

Table 9 (continued). Method Developer Laboratory - GENE-UP *Cronobacter* species Assay, Candidate vs. Reference – POD Results (1)

Matrix/Test Portion	Strain	Time Point	MPN ^a / Test Portion	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g			
					x ^c	POD _c ^d	95% CI	x ^c	POD _R ^e	95% CI					
Soy Ingredients (100g)	<i>Cronobacter malonaticus</i> BMX 112172		0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			0.45 (0.24, 0.70)	2 0	1 5	0.75	0.53	0.88	16	0.80	0.58	0.91	-0.05	-0.30	0.21
			4.06 (1.93, 8.56)	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Stainless Steel 4" X 4"	<i>Cronobacter malonaticus</i> BMX 112170 10x <i>Salmonella Typhimurium</i> ATCC 13311	16h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			2 & 600	2 0	14	0.70	0.48	0.85	14	0.70	0.48	0.85	0.00	-0.27	0.27
			20 & 600	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Plastic 1" X 1"	<i>Cronobacter sakazakii</i> BMX 112147	16h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			10	2 0	14	0.70	0.48	0.85	14	0.70	0.48	0.85	0.00	-0.27	0.27
			70	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval.

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials.

^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials.

^fdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^h *Cronobacter malonaticus* CFU /Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate.

ⁱ *Salmonella Typhimurium* CFU /Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate.

Table 10. Method Developer Laboratory - GENE-UP Cronobacter species Assay, Presumptive vs. Confirmed – POD Results (1)

Matrix/Test Portion	Strain	Time Point	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g			
					χ ^c	POD _{CP} ^d	95% CI	χ ^c	POD _{CC} ^e	95% CI					
Milk-based powdered infant formula with probiotics (25g)	<i>Cronobacter sakazakii</i> ATCC 29544	18h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			1.48 (0.94, 2.49)	20	13	0.65	0.43	0.82	13	0.65	0.43	0.82	0.00	-0.28	0.28
			>11 (4.10, >11)	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Milk-based powdered infant formula without probiotics (25g)	<i>Cronobacter dublinensis</i> spp <i>dublinensis</i> BMX 112161	18h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			0.67 (0.46, 0.93)	20	14	0.70	0.48	0.85	14	0.70	0.48	0.85	0.00	-0.27	0.27
			5.24 (2.39, 11.48)	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Soy-based powdered infant formula (25g)	<i>Cronobacter sakazakii</i> ATCC BAA-894	18h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			0.26 (0.10, 0.47)	20	7	0.35	0.18	0.57	7	0.35	0.18	0.57	0.00	-0.28	0.28
			4.06 (1.93, 6.00)	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Milk-based powdered infant formula with probiotics (375g)	<i>Cronobacter sakazakii</i> ATCC 29544	20h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			1.48 (0.94, 2.49)	20	17	0.85	0.64	0.95	17	0.85	0.64	0.95	0.00	-0.23	0.23
			>11 (4.10, >11)	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Milk-based powdered infant formula without probiotics (375g)	<i>Cronobacter dublinensis</i> spp <i>dublinensis</i> BMX 112161	20h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			0.67 (0.46, 0.93)	20	16	0.80	0.58	0.92	16	0.80	0.58	0.92	0.00	-0.25	0.25
			5.24 (2.39, 11.48)	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Soy-based powdered infant formula (375g)	<i>Cronobacter sakazakii</i> ATCC BAA-894	20h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			0.26 (0.10, 0.47)	20	9	0.45	0.26	0.66	9	0.45	0.26	0.66	0.00	-0.28	0.28
			4.06 (1.93, 6.00)	5	4	0.80	0.38	1.00	4	0.80	0.38	1.00	0.00	-0.47	0.47
Non-fat dry milk (100g)	<i>Cronobacter dublinensis</i> spp <i>lausannensis</i> BMX 112177	20h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			1.17 (0.41, 1.00)	20	20	1.00	0.84	1.00	20	1.00	0.84	1.00	0.00	-0.16	0.16
			>11 (4.10, >11)	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43

Table 10 (continued). Method Developer Laboratory - GENE-UP Cronobacter species Assay, Presumptive vs. Confirmed – POD Results (1)

Matrix/Test Portion	Strain	Time Point	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g			
					χ ^c	POD _{CP} ^d	95% CI	χ ^c	POD _{CC} ^e	95% CI					
Soy Ingredients (100g)	<i>Cronobacter malonaticus</i> BMX 112172		0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			0.45 (0.24, 0.70)	20	15	0.75	0.53	0.89	15	0.75	0.53	0.89	0.00	-0.27	0.27
			4.06 (1.93, 8.56)	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Stainless Steel 4" X 4"	<i>Cronobacter malonaticus</i> BMX 112170 <i>Salmonella</i> Typhimurium ATCC 13311	16h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			2 & 600	20	14	0.70	0.48	0.85	14	0.70	0.48	0.85	0.00	-0.27	0.27
			20 & 600	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Plastic 1" X 1"	<i>Cronobacter sakazakii</i> BMX 112147	16h	0	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			10	20	14	0.70	0.48	0.85	14	0.70	0.48	0.85	0.00	-0.27	0.27
			70	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval.

^bN = Number of test portions.

^cχ = Number of positive test portions.

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^gCFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate.

Table 11: Independent Laboratory - GENE-UP *Cronobacter* species Assay, Candidate vs. Reference – POD Results (1)

Matrix	Strain	Time Point	MPN ^a / Test Portion	N ^c	Candidate			Reference			dPOD _c ^g	95% CI ^h
					x ^d	POD _c ^e	95% CI	X	POD _R ^f	95% CI		
Powdered Infant Formula with Probiotics	<i>Cronobacter sakazakii</i> ATCC 29544	20-28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.03, 1.02)	20	8	0.40	0.22, 0.61	10	0.50	0.30, 0.70	-0.10	-0.37, 0.19
			2.62 (1.20, 5.74)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Time Point	CFU ^b / Test Portion	N ^c	Candidate			Reference			dPOD _c ^g	95% CI ^h
					x ^d	POD _c ^e	95% CI	X	POD _R ^f	95% CI		
Stainless Steel, sampled with sponge	<i>Cronobacter malonaticus</i> BMX 112170 & <i>Salmonella</i> Typhimurium ATCC 13311	16-24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			71 & 920	20	7	0.35	0.18, 0.57	9	0.45	0.26, 0.66	-0.10	-0.37, 0.19
			240 & 4900	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval.

^bCFU = Results of the CFU/Test area were determined by plating the inoculum for each stainless steel.

^cN = Number of test portions.

^dx = Number of positive test portions.

^ePOD_c = Candidate method confirmed positive outcomes divided by the total number of trials.

^fPOD_R = Reference method confirmed positive outcomes divided by the total number of trials.

^gdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values.

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 12: Independent Laboratory - GENE-UP® *Cronobacter* Kit, Presumptive vs. Confirmed – POD Results (1)

Matrix	Strain	Time Point	MPN ^a / Test Portion	N ^c	Presumptive			Confirmed			dPOD _{CP} ^g	95% CI ^h
					x ^d	POD _{CP} ^e	95% CI	X	POD _{CC} ^f	95% CI		
Powdered Infant Formula with Probiotics	<i>Cronobacter sakazakii</i> ATCC 29544	20-28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.61 (0.03, 1.02)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
			2.62 (1.20, 5.74)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Matrix	Strain	Time Point	CFU ^b / Test Portion	N ^c	Presumptive			Confirmed			dPOD _{CP} ^g	95% CI ^h
x ^d	POD _{CP} ^e	95% CI	X	POD _{CC} ^f	95% CI							
Stainless steel, sampled with sponge	<i>Cronobacter malonaticus</i> BMX 112170 & <i>Salmonella</i> Typhimurium ATCC 13311	16-24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			71 & 920	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
			240 & 4900	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval.

^bCFU = Results of the CFU/Test area were determined by plating the inoculum for each stainless steel.

^cN = Number of test portions.

^dx = Number of positive test portions.

^ePOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.

^fPOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.

^gdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

DISCUSSION OF THE MODIFICATION APPROVED NOVEMBER 2020 (11)

During the modification study, no discordant results were observed for the GENE-UP method during the evaluation of the BACTVIAB PMAxx solution. No statistically significant differences were observed between the candidate method and reference method.

The GENE-UP assay is quick and simple to perform, providing results in less than 1.5 h post incubation of the selective enrichment. The addition of the BACTVIAB PMAxx Reagent now allows end users to eliminate environmental (extracellular) DNA prior to analysis, reducing the chance that a discordant result could be obtained.

Table 5. GENE-UP Results – Presumptive vs. Confirmed (11)

GENE-UP Method	Matrix/Test Portion	Inoculum	MPN ^a / Test Portion or CFU/Test Area	N ^b	x ^c	Presumptive		Confirmed		dPOD _{CP} ^f	95% CI ^g	
						POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
GENE-UP <i>Cronobacter</i>	Powdered Infant Formula with probiotics (375 g)	<i>Cronobacter muytjensii</i> ATCC 51329	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.60 (0.33, 1.01)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
			6.49 (4.10, 11.2)	5	0	1.0	0.56, 1.0	0	1.0	0.56, 1.0	0.00	-0.47, 0.47

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval. For stainless steel surfaces, the level of contamination was determined by plating the inoculum in triplicate

^bN = Number of test portions; ^cx = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive and confirmed POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

^hResults obtained at 10 h and 26 h were equivalent.

ⁱResults for sample aliquots tested with and without BACTVIAB PMAxx reagent were equivalent

Table 6. GENE-UP Results – Confirmed vs. Reference (11)

GENE-UP Method	Matrix/Test Portion	Inoculum	MPN ^a / Test Portion or CFU/Test Area	N ^b	x ^c	Candidate			Reference		dPOD _c _f	95% CI ^g
						POD _c ^d	95% CI	x	POD _R ^e	95% CI		
GENE-UP <i>Cronobacter</i>	Powdered Infant Formula with probiotics (375 g)	<i>Cronobacter muytjensii</i> ATCC 51329	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.60 (0.33, 1.01)	20	11	0.55	0.34, 0.74	10	0.50	0.30, 0.70	0.05	-0.24, 0.33
			6.49 (4.10, 11.2)	5	0	1.0	0.56, 1.0	0	1.0	0.56, 1.0	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval. For stainless steel surfaces, the colony forming unit per test area was determined by plating the inoculum in triplicate

^bN = Number of test portions; ^cx = Number of positive test portions

^dPOD_c = Candidate method true positive outcomes (presumptive and confirmed) divided by the total number of trials

^ePOD_R = Reference method positive outcomes divided by the total number of trials

^fdPOD_c = Difference between the candidate method true positives and reference method POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

^hResults obtained at 10 h and 26 h were equivalent.

ⁱResults for sample aliquots tested with and without BACTVIAB PMAxx reagent were equivalent

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