



CERTIFICATION

AOAC Research Institute
***Performance Tested Methods*SM**

Certificate No.
082003

The AOAC Research Institute hereby certifies the method known as

iQ-Check *Enterobacteriaceae* Real-Time PCR

Corporate Location
Bio-Rad Laboratories
2000 Alfred Nobel Drive
Hercules, CA 94547 USA

Manufacturing Location
Bio-Rad Laboratories
925 Alfred Nobel Drive
Hercules, CA 94547 USA

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A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, AOAC Research Institute Senior Director

Issue Date	November 21, 2025
Expiration Date	December 31, 2026

AUTHORS ORIGINAL VALIDATION: Mike Clark and Wendy Lauer MODIFICATION JANUARY 2023: Mike Clark	SUBMITTING COMPANY Bio-Rad laboratories 2000 Alfred Nobel Dr. Hercules, CA 94547 USA
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METHOD NAME iQ-Check <i>Enterobacteriaceae</i> Real-Time PCR	CATALOG NUMBER 12003068
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INDEPENDENT LABORATORY WBA Analytical Laboratory 3609 Johnson Rd. Springdale, AR 72762 USA
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APPLICABILITY OF METHOD Analyte – <i>Enterobacteriaceae</i> (EB). Matrixes – Milk powder (10 g), powdered infant formula (10 g and 375 g), powdered infant formula with probiotics (10 g and 375 g), and stainless steel environmental swabs. Performance claims – The study data detected no statistical difference between the TECTA Combined <i>E. coli</i> and Total Coliform Test method and the reference methods.	REFERENCE METHOD ISO 21528-1:2017 Microbiology of the food chain – Horizontal method for the detection and enumeration of <i>Enterobacteriaceae</i> – Part 1: Detection of <i>Enterobacteriaceae</i> . (2)
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ORIGINAL CERTIFICATION DATE August 18, 2020	CERTIFICATION RENEWAL RECORD Renewed through December 2026.
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METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
<ol style="list-style-type: none"> 1. January 2021 Level 1 2. April 2021 Level 1 3. October 2021 Level 1 4. January 2023 Level 2 5. October 2023 Level 1 6. November 2024 Level 1 	<ol style="list-style-type: none"> 1. Editorial changes. 2. Software was updated from Version 3 to Version 4 allowing compatibility with Windows 10. 3. Editorial changes and addition of user information in French, German, Spanish, Portuguese, and Italian. 4. Addition of CFX Opus Deepwell, with CFX Manager Software, Industrial Diagnostic Edition version 3.1 using Free DNA Removal Solution and Fast APF protocols. 5. Editorial changes. 6. Editorial changes including software update to CFX Maestro Software, IDE v4.0.

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PRINCIPLE OF THE METHOD (1)

The iQ-Check *Enterobacteriaceae* real-time PCR assay is based on gene amplification and detection. The kit’s ready-to-use PCR reagents contain oligonucleotides (primers and probes) specific for EB, as well as DNA polymerase and nucleotides. Detection and data analysis are optimized for use with a Bio-Rad real-time PCR instrument, such as the CFX96 Touch Deep Well System with the CFX Manager IDE software. In addition, the iQ-Check Prep, a robotic liquid handling platform that performs DNA extraction and PCR plate set-up, can be used to perform the iQ-Check *Enterobacteriaceae* real-time PCR kit. This allows for a completely integrated automated solution for food pathogen testing. The iQ-Check Free DNA Removal Solution, provided in a separate kit, is used with the iQ-Check *Enterobacteriaceae* real-time PCR kit to optimize removal of free DNA.

The iQ-Check *Enterobacteriaceae* real-time PCR kit is provided in a ready-to-use format containing all primers, probes, and reagents (except for template DNA) required for the PCR reaction. In addition, an internal positive control (IPC) is included in the reaction mix to identify possible PCR inhibition.

DISCUSSION OF THE VALIDATION STUDY (2)

The iQ-Check *Enterobacteriaceae* real-time PCR kit successfully detected EB from 10 g sample portions of milk powder, powdered infant formula, and powdered infant formula with probiotics and stainless steel surfaces incubated with BPW. The kit also successfully detected EB, from 375 g sample portions of powdered infant formula, and powdered infant formula with probiotics incubated with BPW + PIF Supplement. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate methods and the reference methods for all samples tested. The study also demonstrated that RAPID[®] *Enterobacteriaceae* agar could be used as an alternative confirmation media after enrichment when compared to the reference method media of VRBG. In the inclusivity and exclusivity evaluations, all inclusivity organisms were correctly identified, and all exclusivity organisms were correctly excluded. The lot-to-lot consistency and stability study show no significant differences observed across the shelf life of the kits for three different lots of kits at each time point tested. Using POD analysis, the robustness study show no statistically significant differences between the 8 treatment combinations and the nominal condition for the iQ-Check *Enterobacteriaceae* kit.

The iQ-Check *Enterobacteriaceae* real-time PCR method is quick and simple to perform, providing results in less than three hours post incubation of the enrichment for up to 94 sample replicates. The use of the iQ-Check Prep instrument can provide a hands-free application that can reduce possible contamination caused by the analyst performing testing. The iQ-Check Prep instrument is able to perform DNA extraction and PCR preparation and provides added value of traceability to the lab. The CFX Manager IDE software is user friendly with the ability to track lot information and sample identification quickly and with ease. Since results are displayed in real-time, the user is able to quickly and accurately determine if results will be valid before the end of the run. The software also provides the user the option to analyze each individual Cq curves to help aid in problem solving any issues within an individual reaction.

Table 3. Inclusivity Results for the iQ-Check *Enterobacteriaceae* Assay (1)

No.	Species	Source	Origin	BPW	BPW + PIF
1	<i>Citrobacter amalonaticus</i>	ATCC ¹ 25405	Feces	+	+
2	<i>Citrobacter braakii</i>	ATCC 43162	Clinical Isolate	+	+
3	<i>Citrobacter freundii</i>	ATCC 43864	Unknown	+	+
4	<i>Citrobacter freundii</i>	ATCC 8090	Unknown	+	+
5	<i>Citrobacter koseri</i>	ATCC 27156	CDC	+	+
6	<i>Cronobacter sakazakii</i>	ATCC 29544	Infant formula	+	+
7	<i>Cronobacter sakazakii</i>	ATCC 12868	Unknown	+	+
8	<i>Cronobacter sakazakii</i>	ATCC 29004	Unknown	+	+
9	<i>Edwardsiella tarda</i>	DSMZ ² 30052	Unknown	+	+
10	<i>Enterobacter aerogenes</i>	ATCC 13048	Unknown	+	+
11	<i>Enterobacter amnigenus</i>	ATCC 33072	Soil	+	+
12	<i>Enterobacter asburiae</i>	ATCC 35953	Unknown	+	+
13	<i>Enterobacter cloacae</i>	ATCC 35030	Unknown	+	+
14	<i>Enterobacter cloacae</i>	ATCC 13047	Spinal fluid	+	+
15	<i>Enterobacter gergoviae</i>	DSMZ 9245	Unknown	+	+
16	<i>Enterobacter hormaechei</i>	ATCC 49162	Sputum	+	+
17	<i>Escherichia coli</i>	ATCC 11775	Unknown	+	+
18	<i>Escherichia coli</i> O26	ATCC BAA-1653	Unknown	+	+
19	<i>Escherichia coli</i> O157:H7	ATCC 35150	Human Feces	+	+
20	<i>Escherichia fergusonii</i>	ATCC 35469	Human feces	+	+
21	<i>Escherichia hermanii</i>	ATCC 33650	Mouse brain	+	+
22	<i>Escherichia vulneris</i>	ATCC 29943	Human wound	+	+
23	<i>Franconibacter pulveris</i>	DSMZ 19144	Unknown	+	+
24	<i>Hafnia alvei</i>	ATCC 51815	Milk	+	+
25	<i>Hafnia alvei</i>	ATCC 29926	Human	+	+
26	<i>Klebsiella oxytoca</i>	ATCC 43165	Clinical isolate	+	+
27	<i>Klebsiella pneumoniae</i>	ATCC 4352	Cow Milk	+	+
28	<i>Kluyvera cryocrescens</i>	DSMZ 4588	Unknown	+	+
29	<i>Morganella morganii</i>	ATCC 25829	Human	+	+
30	<i>Pantoea agglomerans</i>	ATCC 19552	Sewage	+	+
31	<i>Pantoea dispersa</i>	DSMZ 30073	Unknown	+	+
32	<i>Proteus mirabilis</i>	ATCC 29906	Unknown	+	+
33	<i>Proteus vulgaris</i>	DSMZ 13387	Unknown	+	+
34	<i>Proteus vulgaris</i>	ATCC 21719	Unknown	+	+
35	<i>Rahnella aquatilis</i>	ATCC 55046	Soil	+	+
36	<i>Raoultella terrigena</i>	DSMZ 2687	Unknown	+	+
37	<i>Salmonella</i> Abaetetuba	ATCC 35640	Creek water	+	+
38	<i>Salmonella</i> Hadar	ATCC 51956	Unknown	+	+
39	<i>Salmonella</i> Kahla	ATCC 17980	Unknown	+	+
40	<i>Salmonella</i> Kentucky	ATCC 9263	Unknown	+	+
41	<i>Salmonella</i> Pulorum	ATCC 13036	Egg	+	+
42	<i>Salmonella</i> Senftenberg	ATCC 43845	Unknown	+	+
43	<i>Salmonella</i> Typhimurium	ATCC 29946	Unknown	+	+
44	<i>Serratia ficaria</i>	ATCC 33106	Fig tree	+	+
45	<i>Serratia marcescens</i>	ATCC 13880	Human	+	+
46	<i>Serratia marcescens</i>	ATCC 27137	Human	+	+
47	<i>Serratia proteamaculans</i>	ATCC 35474	Unknown	+	+
48	<i>Shigella flexneri</i>	DSMZ 4782	Unknown	+	+
49	<i>Shigella sonnei</i>	DSMZ 5570	Unknown	+	+
50	<i>Yersinia aldovae</i>	ATCC 51366	Water	+	+
51	<i>Yersinia enterocolitica</i>	ATCC 9610	Human Tissue	+	+

¹American Type Culture Collection, Manassas, VA²The Leibniz Institute DSMZ, Brunswick, Germany

Table 4. Exclusivity Results for the iQ-Check *Enterobacteriaceae* Assay (1)

No.	Species	Source	Origin	Result
1	<i>Acinetobacter baumannii</i>	DSMZ ¹ 30007	Urine	-
2	<i>Acinetobacter calcoaceticus</i>	ATCC ² 23055	Unknown	-
3	<i>Aeromonas hydrophilia</i>	DSMZ 30187	Milk	-
4	<i>Aeromonas media</i>	DSMZ 4881	Fish	-
5	<i>Aeromonas sobria</i>	DSMZ 19176	Fish	-
6	<i>Alcaligenes faecalis</i>	DSMZ 30030	Unknown	-
7	<i>Bacillus megaterium</i>	ATCC 12872	Unknown	-
8	<i>Bacillus subtilis</i>	ATCC 6633	Unknown	-
9	<i>Burkholderia cepacia</i>	DSMZ 7288	Onion	-
10	<i>Burkholderia contaminans</i>	DSMZ 22706	Sheep milk	-
11	<i>Staphylococcus epidermidis</i>	ATCC 12228	Unknown	-
12	<i>Enterococcus faecalis</i>	DSMZ 20478	Unknown	-
13	<i>Lactobacillus lactis</i>	ATCC 11454	Unknown	-
14	<i>Leuconostoc mesenteroides</i>	ATCC 8293	Fermenting olives	-
15	<i>Listeria monocytogenes</i>	ATCC 19114	Animal tissue	-
16	<i>Micrococcus luteus</i>	ATCC 9341	Soil	-
17	<i>Moraxella catarrhalis</i>	DSMZ 9143	Unknown	-
18	<i>Pasteurella aerogenes</i>	DSMZ 10153	Pig	-
19	<i>Pseudomonas fluorescens</i>	DSMZ 50091	Water	-
20	<i>Pseudomonas aeruginosa</i>	ATCC 10145	Unknown	-
21	<i>Pseudomonas putida</i>	DSMZ 291	Unknown	-
22	<i>Rhodococcus equi</i>	ATCC 6939	Foal	-
23	<i>Rothia mucilaginosa</i>	DSMZ 20746	Human throat	-
24	<i>Staphylococcus aureus</i>	ATCC 6538	Human	-
25	<i>Stenotrophomonas acidaminiphila</i>	DSMZ 13117	Waste water	-
26	<i>Stenotrophomonas maltophilia</i>	DSMZ 50170	Human	-
27	<i>Streptococcus agalactiae</i>	ATCC BAA-611	Human	-
28	<i>Streptococcus faecalis</i>	ATCC 29212	Urine	-
29	<i>Flavobacterium branchiophilum</i>	DSMZ 24789	Unknown	-
30	<i>Moraxella bovis</i>	DSMZ 6328	Cow	-

¹The Leibniz Institute DSMZ, Brunswick, Germany²American Type Culture Collection, Manassas, VA

Table 6: Candidate vs. ISO 21528-1:2017 Reference Method– POD Results (1)

Matrix	Strain	Enrichment	MPN ^a Test Portion	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
					X ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Milk Powder (10 g)	<i>S. Anatum</i> USMARC 1735	BPW ^h (Paired)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.56 (0.32, 0.98)	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05	-0.32, 0.23
			1.63 (0.77, 3.44)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Milk Powder (10 g)	<i>S. Anatum</i> USMARC 1735	BPW + PIF ⁱ (Unpaired)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.56 (0.32, 0.98)	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.24, 0.33
			1.63 (0.77, 3.44)	5	3	0.60	0.23, 0.88	5	1.00	0.57, 1.00	-0.40	-0.77, 0.12
Powdered infant formula (10 g)	<i>E. coli</i> ATCC 25922	BPW (Paired)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			1.17 (0.74, 1.86)	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.27, 0.27
			5.24 (2.40, 11.48)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Powdered infant formula (10 g)	<i>E. coli</i> ATCC 25922	BPW + PIF (Unpaired)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.17 (0.74, 1.86)	20	9	0.45	0.26, 0.66	14	0.70	0.48, 0.85	-0.25	-0.50, 0.05
			5.24 (2.40, 11.48)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Powdered infant formula (375 g)	<i>E. coli</i> ATCC 25922	BPW + PIF (Unpaired)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.17 (0.74, 1.86)	20	16	0.80	0.58, 0.92	14	0.70	0.48, 0.85	0.10	-0.17, 0.35
			5.24 (2.40, 11.48)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Powdered infant formula w/ probiotics (10 g)	<i>C. sakazakii</i> ATCC 29544	BPW (Paired)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			1.65 (1.04, 2.62)	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			7.43 (3.08, 17.94)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Powdered infant formula w/ probiotics (10 g)	<i>C. sakazakii</i> ATCC 29544	BPW + PIF (Unpaired)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.65 (1.04, 2.62)	20	17	0.85	0.64, 0.95	15	0.75	0.53, 0.89	0.10	-0.15, 0.34
			7.43 (3.08, 17.94)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Powdered infant formula w/ probiotics (375 g)	<i>C. sakazakii</i> ATCC 29544	BPW + PIF (Unpaired)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.65 (1.04, 2.62)	20	19	0.95	0.76, 1.00	15	0.75	0.53, 0.89	0.20	-0.03, 0.42
			7.43 (3.08, 17.94)	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 FDA BAM, 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

^hBPW = Buffered Peptone Water

ⁱBPW + PIF = Buffered Peptone Water + PIF Supplement

Table 7: Candidate vs. ISO 21528-1:2017 Reference Method– POD Results (1)

Matrix	Strain	Enrichment	CFU ^a Test Portion	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
					χ ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Stainless Steel (4" x 4") Cellulose + D/E	<i>K. aerogenes</i> ATCC 13048 + <i>P. aeruginosa</i> ATCC 9027	BPW ^h (Paired)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			3.5 x 10 ² & 4.0 x 10 ³	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.13, 0.13
			1.7x 10 ⁴ & 1.8 x 10 ⁵	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Stainless Steel (4" x 4") Polyurethane + HiCap	<i>K. aerogenes</i> ATCC 13048 + <i>P. aeruginosa</i> ATCC 9027	BPW (Paired)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			3.5 x 10 ² & 4.0 x 10 ³	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
			1.7x 10 ⁴ & 1.8 x 10 ⁵	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aCFU = Results of the CFU/Test area were determined by plating the inoculum for each surface in triplicate

^bN = Number of test portions

^cχ = 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

^hBPW = Buffered Peptone Water

Table 8: iQ-Check *Enterobacteriaceae* Presumptive vs. Confirmed – POD Results (1)

Matrix	Strain	Enrichment	MPN ^a Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					χ ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Milk Powder (10 g)	<i>S. Anatum</i> USMARC 1735	BPW ^h	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.56 (0.32, 0.98)	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05	-0.21, 0.11
			1.63 (0.77, 3.44)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Milk Powder (10 g)	<i>S. Anatum</i> USMARC 1735	BPW + PIF ⁱ	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.56 (0.32, 0.98)	20	9	0.45	0.26, 0.66	10	0.50	0.30, 0.70	-0.05	-0.21, 0.11
			1.63 (0.77, 3.44)	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.47, 0.47
Powdered infant formula (10 g)	<i>E. coli</i> ATCC 25922	BPW	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			1.17 (0.74, 1.86)	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.13, 0.13
			5.24 (2.40, 11.48)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Powdered infant formula	<i>E. coli</i> ATCC 25922	BPW + PIF	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			1.17 (0.74, 1.86)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13

(10 g)			5.24 (2.40, 11.48)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Powdered infant formula (375 g)	<i>E. coli</i> ATCC 25922	BPW + PIF	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			1.17 (0.74, 1.86)	20	16	0.80	0.58, 0.92	16	0.80	0.58, 0.92	0.00	-0.13, 0.13
			5.24 (2.40, 11.48)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Powdered infant formula w/ probiotics (10 g)	<i>C. sakazakii</i> ATCC 29544	BPW	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			1.65 (1.04, 2.62)	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.13, 0.13
			7.43 (3.08, 17.94)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Powdered infant formula w/ probiotics (10 g)	<i>C. sakazakii</i> ATCC 29544	BPW + PIF	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			1.65 (1.04, 2.62)	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0.00	-0.13, 0.13
			7.43 (3.08, 17.94)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Powdered infant formula w/ probiotics (375 g)	<i>C. sakazakii</i> ATCC 29544	BPW + PIF	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			1.65 (1.04, 2.62)	20	20	1.00	0.84, 1.00	19	0.95	0.76, 1.00	0.05	-0.11, 0.21
			7.43 (3.08, 17.94)	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 FDA BAM, with 95% confidence interval

^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 presumptive and confirmed candidate method 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

^hBPW = Buffered Peptone Water

ⁱBPW + PIF = Buffered Peptone Water + PIF Supplement

Table 9: iQ-Check *Enterobacteriaceae* Presumptive vs. Confirmed – POD Results (1)

Matrix	Strain	Enrichment	CFU ^a Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					X ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Stainless Steel (4" x 4") Cellulose + D/E	<i>K. aerogenes</i> ATCC 13048 + <i>P. aeruginosa</i> ATCC 9027	BPW ^h	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			3.5 x 10 ² & 4.0 x 10 ³	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.13, 0.13
			1.7x 10 ⁴ & 1.8 x 10 ⁵	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Stainless Steel (4" x 4") Polyurethane + HiCap	<i>K. aerogenes</i> ATCC 13048 + <i>P. aeruginosa</i> ATCC 9027	BPW	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			3.5 x 10 ² & 4.0 x 10 ³	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
			1.7x 10 ⁴ & 1.8 x 10 ⁵	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aCFU = Results of the CFU/Test area were determined by plating the inoculum for each surface 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 presumptive and confirmed candidate method 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

^hBPW = Buffered Peptone Water

DISCUSSION OF THE MODIFICATION STUDY APPROVED JANUARY 2023 (3)

The new CFX Opus Deepwell instrument delivers the same performance as the current CFX96 Touch Deep Well instrument but with a more modern design and cloud capabilities. The improved stability of the thermal block ensures a more uniform thermal protocol. The CFX Manager Software, IDE v 3.1 brings the same performance, algorithm, and interpretation as the current CFX Manager Software, IDE v 3.0 with the only change being compatibility to both CFX96 Touch Deep Well and CFX Opus Deepwell instruments.

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