STUDY REPORT

Thermo Scientific SureTect Listeria species PCR Assay and Thermo Scientific SureTect Listeria monocytogenes PCR Assay method extension for use with the Applied Biosystems QuantStudio 5 Real-Time PCR Instrument AOAC-RI PTM Validation: Method Comparison Study

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Summary

Thermo Scientific™ SureTect™ Listeria monocytogenes PCR Assay and Thermo Scientific™ SureTect™ Listeria species PCR Assay (candidate methods) have been validated in accordance with the AOAC Research Institute *Performance Tested Methods*℠ (PTM) Program for the detection of Listeria from a variety of food and environmental surfaces. The candidate methods have been validated for use with the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument to perform PCR and Thermo Scientific RapidFinder™ Analysis Software v1.0 or greater for data analysis. In addition, stainless steel surface sponge (100 mL enrichment) and swab (10 mL enrichment) matrices have been added. This report details the method comparison study between the candidate methods and the International Organization for Standardization (ISO) 11290-1:2017 for a representative range of matrices.

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Methodology

The performance of the candidate method was assessed as an unpaired study in comparison to the ISO 11290-1:2017 reference method. Method developer studies were conducted by Thermo Fisher Scientific on sliced deli turkey, bagged lettuce, pasteurized 2% fat milk, stainless steel surface swabs (1"x1") and sponges (4"x4").

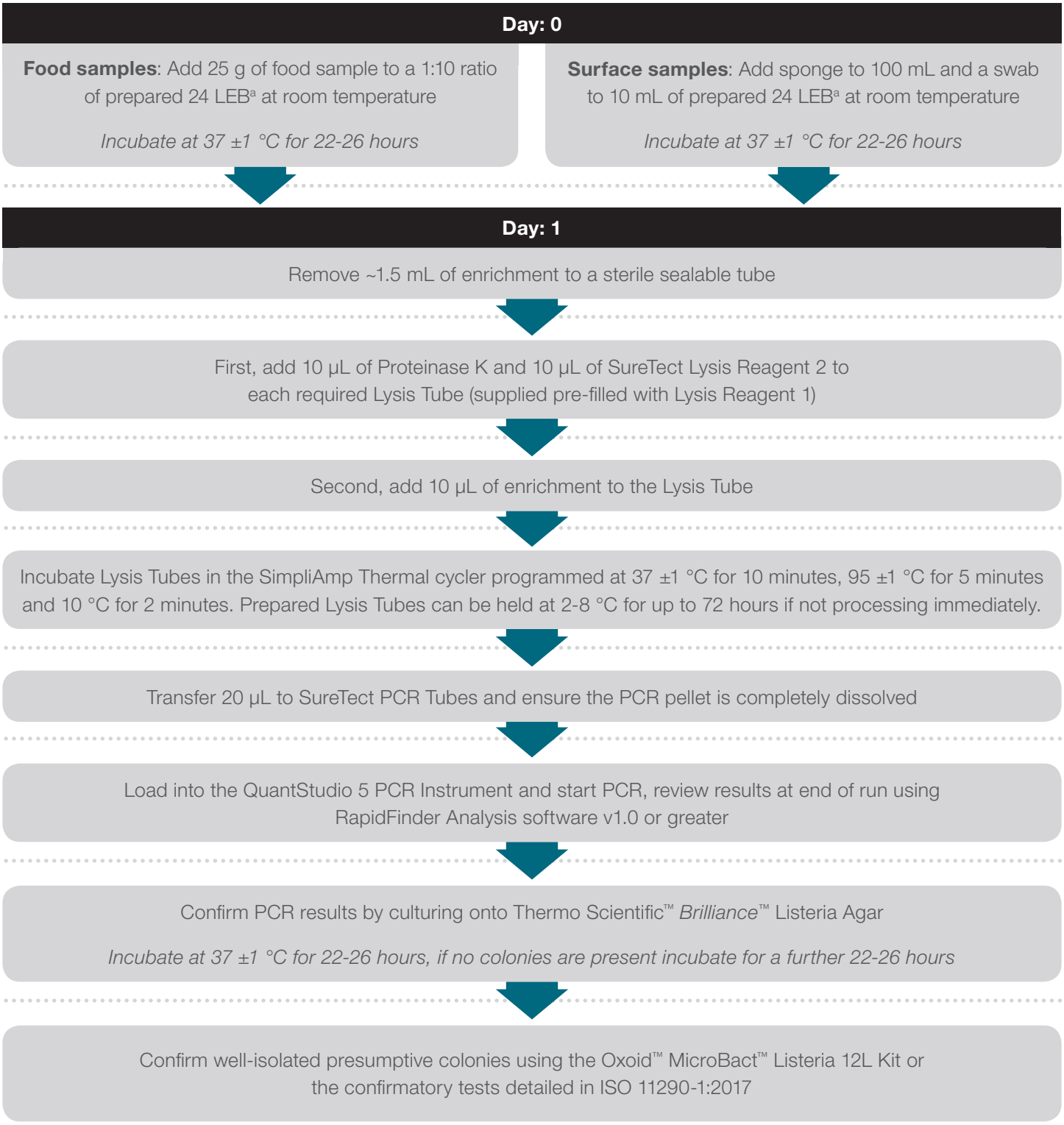
Sample Preparation

For food samples, a 25 g sample was added to a homogenizer bag with a 1:10 ratio of 24 Listeria Enrichment Broth (LEB) (supplemented with 24 LEB Selective Supplement (10 mL per liter) and 24 LEB Buffer Supplement (10 mL per 25 g of sample)) at room temperature. The sample was homogenized thoroughly and incubated at 37±1 °C for 22–26 hours.

For surface samples, sterile sampling sponges/swabs were premoistened in a suitable diluent. For sampling of areas that have been cleaned or treated with disinfectants, pre-moisten the sponge/swab with a neutralising broth, such as Dey-Engley Broth, prior to sampling. The surface was sampled by rubbing the sponge/swab in horizontal and vertical directions across the entire sampling area 4"x4" area for sponges and 1"x1" area for swabs). The sponge samples were added to 100 mL and the swab samples were added to 10 mL of 24 LEB (prepared as described for food samples) and incubated at 37±1 °C for 22-26 hours.

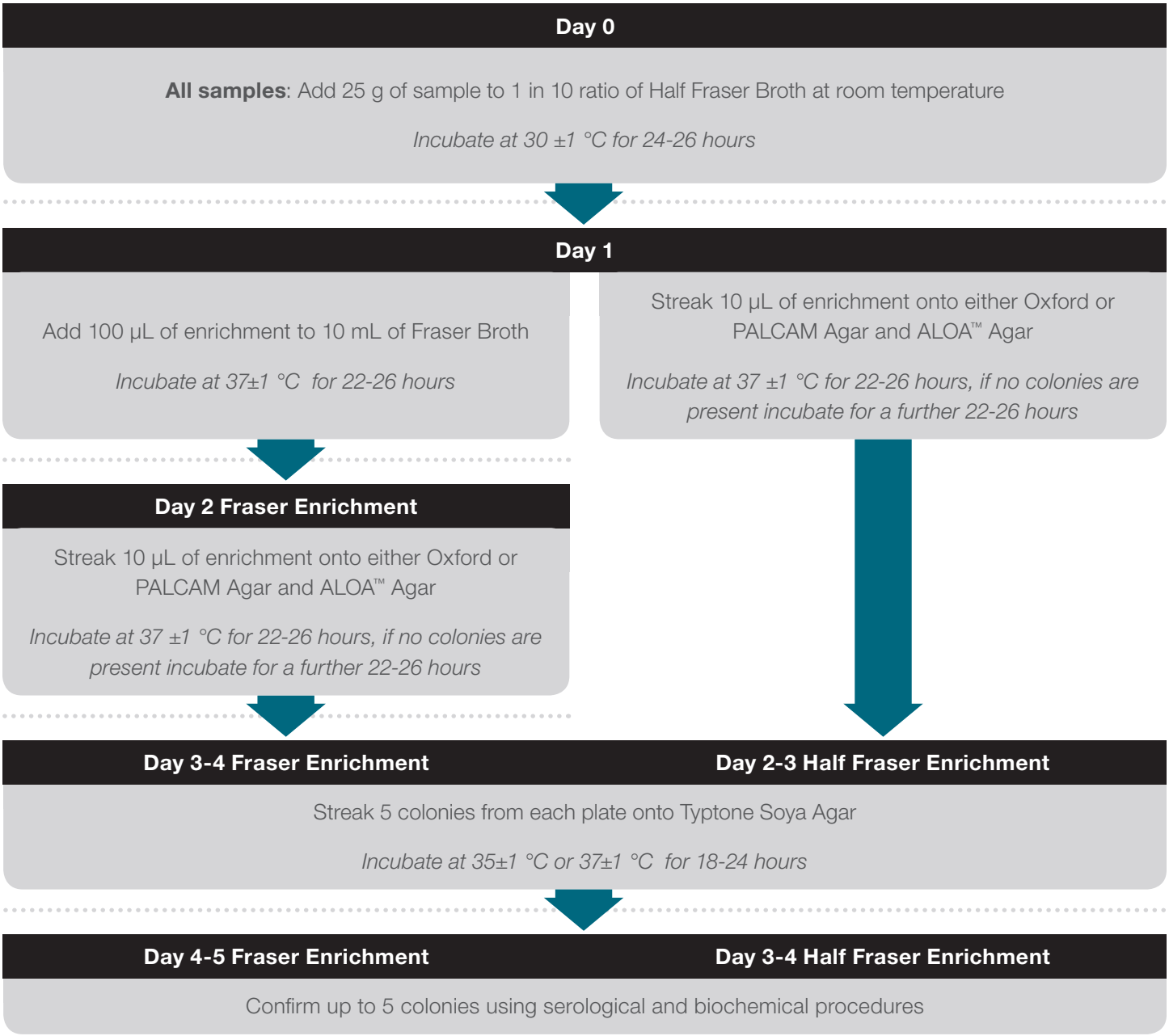
Approximately 1.5 mL of food and surface enrichment was dispensed into a new tube ready to process the sample to the next stage. Enrichment samples were stored at 2–8 °C for a maximum of 72 hours.

Figure 1. Thermo Scientific SureTect Listeria species and SureTect Listeria monocytogenes PCR Assay Protocol for the Detection of *Listeria* species from Food and Environmental Surface Samples



[a] 24 LEB was supplemented with 24 LEB Selective Supplement (10 mL per liter) and 24 LEB Buffer Supplement (10 mL per 25 g of sample).

Figure 2. ISO 11290-1:2017 Reference Method for the Detection of *Listeria* species from Food and Surface Samples



Results

The results of the candidate methods confirmed (via the candidate confirmation method) in comparison to the ISO 11290-1:2017 reference method is detailed in appendix 1.

The results from the bagged lettuce, 2% pasteurized milk, stainless steel swabs and sponges showed no statistically significant differences by POD analysis between the candidate methods (including presumptive results, and confirmed results via candidate and reference methods) and the reference method, or between the candidate presumptive result and the candidate method confirmed (via the candidate method and the reference method).

The sliced deli turkey samples were found to be naturally contaminated with a *L.* spp. strain; during the testing of the SureTect Listeria monocytogenes PCR Assay, the candidate method confirmed via the ISO 11290-1:2017 reference method, showed poor performance compared to the candidate presumptive PCR result and the candidate method confirmed result via the candidate method. During the reference method confirmation of the candidate method, the natural *L.* spp. contaminant overgrew the *L. monocytogenes* spike organism in the Fraser Broth. This overgrowth of *L.* spp. resulted in very few visible *L. monocytogenes* colonies on the OCLA or Oxford Agar plates and therefore only two confirmed positives were observed for the low spike samples. This resulted in statistically significant differences by POD analysis in favour of the candidate method (both candidate presumptive result and candidate confirmed result via the candidate method). The results from the SureTect Listeria monocytogenes PCR Assay showed no statistically significant differences between the SureTect Listeria monocytogenes PCR Assay and the reference method for the sliced deli turkey.

The results from the SureTect Listeria species PCR Assay testing of sliced deli turkey showed that the SureTect Listeria species PCR Assay candidate method (confirmed via candidate method and reference method) had superior performance to the reference method. The 24 LEB (part of the candidate method) showed an improved recovery of heat-stressed cells in comparison to the Half Fraser Broth (part of the reference method) and this resulted in a statistically significant difference by POD analysis in favor of the SureTect Listeria species PCR Assay candidate method.

Conclusion

The data presented in this report show that the SureTect Listeria monocytogenes PCR Assay and the SureTect Listeria species PCR Assay are suitable for the detection of *Listeria monocytogenes* and *Listeria* species respectively, from a variety of food and environmental surface samples when using the QuantStudio 5 Real-Time PCR Instrument and associated RapidFinder Analysis Software. POD analysis conducted during the validation study demonstrated very few statistically significant differences. The statistically significant differences observed were in favor of the candidate methods. Inclusivity and exclusivity testing demonstrated that both candidate methods successfully detected all target *Listeria* isolates and correctly excluded all non-target isolates. The AOAC-RI PTM validation certificate (License number: 061302 and 071304) is available from either www.thermofisher.com or the AOAC Research Institute at www.aoac.org.

Appendix 1

Table 1. SureTect Listeria monocytogenes PCR Assay Results: Candidate Method Confirmed (via the Candidate Method) vs Reference Method POD Summary

Matrix[a]	Inoculating strain(s)	MPN[b]/ test portion	N[c]	SureTect candidate method confirmed via the candidate method result			Reference method result			$dPOD_{CC}$[g]	95% CI[h]
				x[d]	POD_{CC}[e]	95% CI	x	POD_R[f]	95% CI		
Sliced deli turkey	TCC 1227 *L. monocytogenes*	N/A[j]	5	0	0.00	0.00, 0.43	1	0.20	0.00, 0.62	-0.20	-0.62, 0.28
		0.30	20	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	-0.05	-0.33, 0.24
		0.50	5	3	0.60	0.23, 0.88	1	0.20	0.00, 0.62	0.40	-0.16, 0.75
Bagged lettuce	TCC 1220 *L. monocytogenes*	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.20	20	10	0.50	0.30, 0.70	14	0.70	0.48, 0.85	-0.20	-0.45, 0.10
		1.10	5	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	0.20	-0.31, 0.62
2% pasteurized milk	TCC 0840 *L. monocytogenes*	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.20	20	7	0.35	0.18, 0.57	3	0.35	0.18, 0.57	0.00	-0.28, 0.28
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless steel sponge	TCC 0813 *L. monocytogenes*/ 10X *E. faecalis*	N/A[j]	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	14	0.70	0.48, 0.85	15	0.70	0.48, 0.85	0.00	-0.27, 0.24
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless steel swab	TCC 1205 *L. monocytogenes* 1/2b	N/A[j]	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

[a]Matrix = For the stainless steel surface matrices the data is shown combined for PikoReal, 7500 Fast and QuantStudio 5 PCR instruments
[b]MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval
[c]N = Number of test portions
[d]x = Number of positive test portions
[e]POD_{CC} = Candidate method confirmed via the candidate method positive outcomes divided by the total number of trials
[f]POD_R = Reference method divided by the total number of trials
[g]$dPOD_{CC}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values
[h]95% CI = If the confidence interval (CI) of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[j]N/A = Not applicable

Table 2. SureTect Listeria species PCR Assay Results: Candidate Method Confirmed (via the Candidate Method) vs Reference Method POD Summary

Matrix[a]	Inoculating strain(s)	MPN[b]/ test portion	N[c]	SureTect candidate method confirmed via the candidate method result			Reference method result			$dPOD_{CC}$[g]	95% CI[h]
				x[d]	POD_{CC}[e]	95% CI	x	POD_R[f]	95% CI		
Sliced deli turkey	TCC 1180 *L. innocua*	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.50	20	16	0.80	0.58, 0.92	6	0.30	0.15, 0.52	0.50	0.19, 0.70
		2.50	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.62
Bagged lettuce	TCC 1220 *L. monocytogenes*	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.20	20	10	0.50	0.30, 0.70	14	0.70	0.48, 0.85	-0.20	-0.45, 0.10
		1.10	5	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	0.20	-0.31, 0.62
2% pasteurized milk	TCC 0840 *L. monocytogenes*	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.20	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless steel sponge	TCC 0813 *L. monocytogenes*/ 10X *E. faecalis*	N/A[j]	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.27, 0.27
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless steel swab	TCC 1205 *L. monocytogenes* 1/2b	N/A[j]	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

[a]Matrix = For the stainless steel surface matrices the data is shown combined for PikoReal, 7500 Fast and QuantStudio 5 PCR instruments
[b]MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval
[c]N = Number of test portions
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[e]POD_{CC} = Candidate method confirmed via the candidate method positive outcomes divided by the total number of trials
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[g]$dPOD_{CC}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values
[h]95% CI = If the confidence interval (CI) of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[j]N/A = Not applicable

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