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Evaluation of an Alternative Method for *Salmonella* Detection from 375 g Cocoa-Containing Matrices

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ABSTRACT

Introduction: The Limit of Detection is a key indicator when assessing the performance of pathogen detection methods. Two models are frequently used to assess detection capability: the AOAC International Guidelines (Appendix J, 2012) prescribe the calculation of the Probability of Detection (POD), and the ISO 16140-2:2016 standard offers calculation of the Relative Limit of Detection (RLOD).

Purpose: This study introduces POD and RLOD concepts for education of testing laboratories in method selection.

Methods: Cocoa powder was tested with the Thermo Scientific™ SureTect™ Salmonella species PCR Assay workflow (alternative method) and the ISO 6579-1:2017 reference method in both a paired and unpaired study.

The alternative method tested 375 g test portions of cocoa powder and was conducted with both ISO 6887-4:2017 enrichment media (paired) and prewarmed BPW (unpaired).
Five blank test portions, 20 inoculated at a low level to achieve fractional recovery, and five inoculated at a higher level were analyzed in both studies.

Interpretation: POD calculations report the difference of PODs (dPOD) between the alternative and reference methods (Table 1, Figures 1 and 2). A dPOD equal to or higher than 0 confirms equivalent or better performance for the alternative method. RLOD calculations report a ratio between the levels of detection of the alternative and reference methods (Table 2). RLOD values lower than the Acceptability Limits (AL) indicate comparable or improved performance of the alternative method to the reference method. The AL differ between paired and unpaired studies.

Table 1: POD calculations for the paired and unpaired study on cocoa powder

Study design	Inoculation level	Alternative Method			Reference Method			dPOD	95% LCL	95% UCL
		POD(c)	95% LCL	95% UCL	POD(r)	95% LCL	95% UCL			
Paired study	Uninoculated	0,00	0,00	0,43	0,00	0,00	0,43	0,00	-0,47	0,47
	Low	0,75	0,53	0,89	0,75	0,53	0,89	0,00	-0,13	0,13
	High	1,00	0,57	1,00	1,00	0,57	1,00	0,00	-0,47	0,47
Unpaired study	Uninoculated	0,00	0,00	0,43	0,00	0,00	0,43	0,00	-0,43	0,43
	Low	0,75	0,53	0,89	0,65	0,43	0,82	0,10	-0,18	0,36
	High	1,00	0,57	1,00	1,00	0,57	1,00	0,00	-0,43	0,43

Figure 1: POD (top) and dPOD (bottom) plots for the paired study

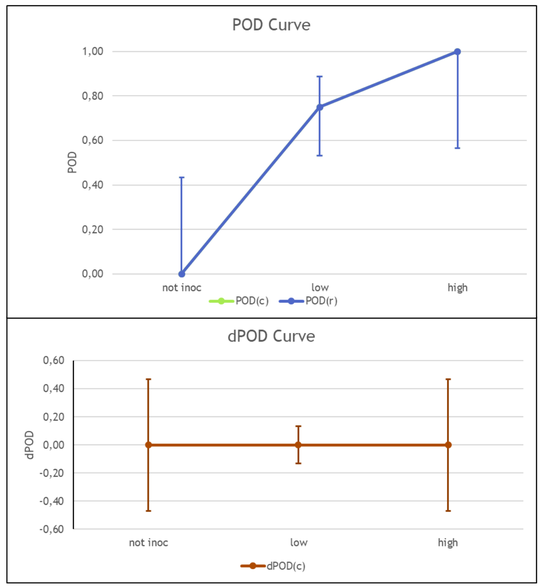

Figure 2: POD (top) and dPOD (bottom) for the unpaired study

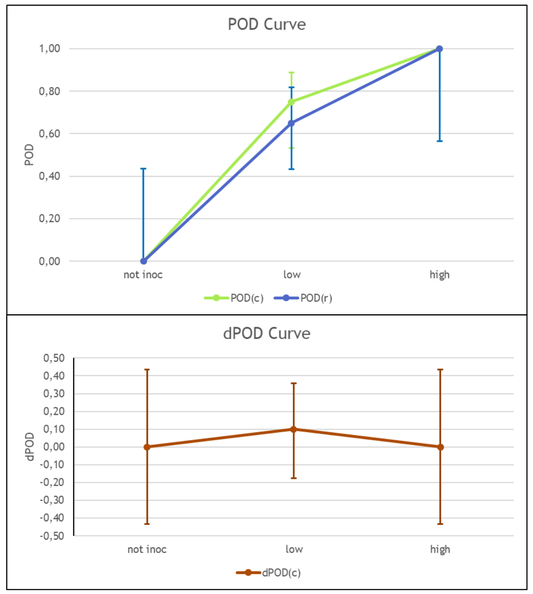

Table 2: RLOD calculations for the paired and unpaired studies

Study design	RLOD	AL	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statistic	p-value
Paired study	1.000	1.5	0.466	2.145	0.000	0.382	0.000	1.000
Unpaired study	0,757	2,5	0,338	1,695	-0,278	0,403	0,690	1,510

SIGNIFICANCE

The POD and RLOD values for the paired study showed 100% concordance between the compared methods (Tables 1 and 2); this is highlighted with visual POD and dPOD curves (Figure 1) and an RLOD value equal to 1 (Table 2). In the unpaired study, the alternative method showed a better limit of the detection compared to the reference method; the dPOD curve and RLOD value both highlight the improved performance of the alternative method (Figure 2). The RLOD values were both below the defined acceptability limits of the ISO 16140-2:2016 standard (Table 2).

The alternative method SureTect kit contains pre-filled reagent tubes for 96 samples in one box with all components stored at 4-8°C. The PCR assays were run on the Applied Biosystems™ QuantStudio 5 thermal cycler using the Thermo Scientific™ RapidFinder™ Analysis Software.

CONCLUSIONS

Performances: The SureTect Salmonella species PCR Assay alternative method had equivalent or better detection levels than the ISO 6579 reference method using the ISO 6887-7:2017 enrichment broths or pre-warmed BPW.

Time to Result: The SureTect workflow provided a next-day result for cocoa and chocolate products.

Large Test Portion: The alternative method for 375 g samples performed comparably to the reference method.

Simplicity & Flexibility: The alternative method workflow was simple and easy to conduct compared to the reference and other rapid methods; the workflow benefits from minimal handling steps, and harmonized lysis and PCR cycling parameters between screening and identification kits.