Food Safety

Novel Study Design to Compare Manual and Automated PCR-setup Workflows

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Introduction

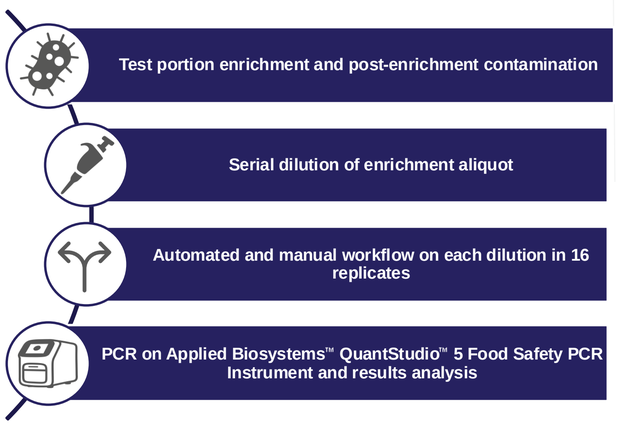
ISO 16140-2:2016[1] and AOAC Appendix J[2] certified methods often require modifications to adapt to the changing demands of the food safety industry. Certification bodies provide some guidance on modifications. However, each case is unique, and the method developer is typically best suited to develop the study design for the method modification evaluation. One popular method modification involves automating labor-intensive pipetting steps.

Thermo Fisher Scientific has recently been granted ISO 16140-2:2016 and AOAC PTM and OMA approval for its newly introduced Thermo Scientific™ SureTect™ Automation Platform. The purpose of this poster is to present the study design used to validate and certify this modification. The Thermo Scientific™ SureTect™ Salmonella species PCR Assay method has been used as a case study.

Method

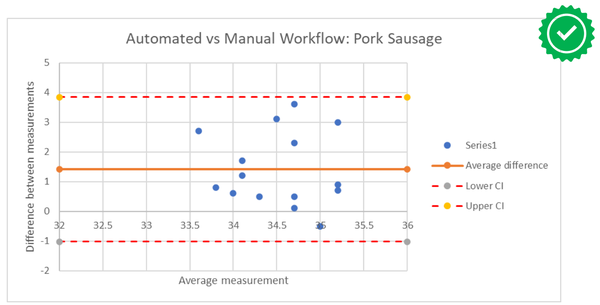
A paired study design (Figure 1) was developed to compare the automated SureTect lysis and PCR-setup workflow to the validated manual SureTect lysis and PCR-setup workflow. The study consisted of testing four challenging food matrices. Each matrix was enriched and artificially contaminated post-enrichment at a level above the limit of the detection (LOD), approximately 10^5-10^6 cfu/test portion. To achieve 'fractional positive' inoculation levels, an aliquot of the contaminated enrichment was then diluted using the non-contaminated enrichment broth to achieve levels above the limit-of-detection (LOD), at LOD, and below LOD. Each dilution was tested through PCR in 16 replicates for each workflow. For the above limit of detection (LOD) dilutions, the results were analyzed by comparing the average C_t difference between the automated and manual workflows. For the LOD and below LOD dilutions, the number of positive replicates were compared using an appropriate statistical method. The statistical method used was the AOAC Probability of Detection (POD) evaluation.

Figure 1. Study Design Overview

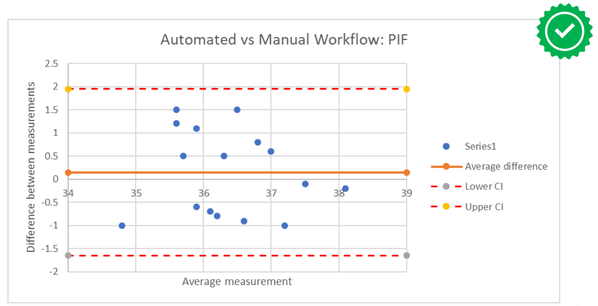


Results

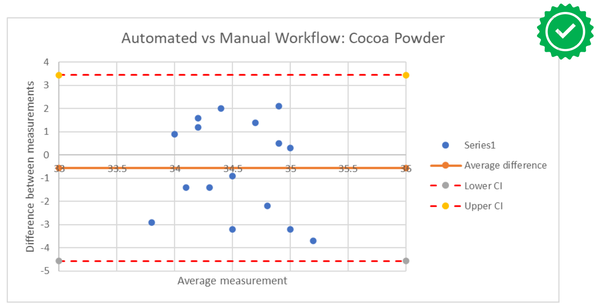
Figure 2. Bland-Altman Plots, Automated vs. Manual Workflows (A-D)



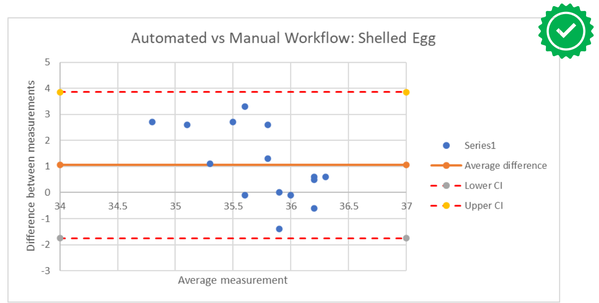
A. C_t comparison between automated and manual workflows for pork sausage.



B. C_t comparison between automated and manual workflows for powdered infant formula (PIF).



C. C_t comparison between automated and manual workflows for cocoa powder.



D. C_t comparison between automated and manual workflows for shelled eggs.

Table 2. AOAC POD Results, Automated vs. Manual Workflows

Food matrix (25 g)	Contamination Level (cfu/mL)	dPOD[a]	95% LCI[b]	95% UCI[c]	Acceptance
Raw pork sausage	Uncontaminated	0	-0.16	0.16	Pass
	Low (10^3-10^4)	-0.19	-0.43	0.06	
	High (10^5)	0	-0.16	0.16	
Cocoa powder	Uncontaminated	0	-0.16	0.16	
	Low (10^3-10^4)	-0.13	-0.35	0.10	
	High (10^5)	0	-0.16	0.16	
Powdered infant formula	Uncontaminated	0	-0.16	0.16	
	Low (10^3-10^4)	0.06	-0.14	0.26	
	High (10^5)	0	-0.16	0.16	
Shelled egg	Uncontaminated	0	-0.16	0.16	
	Low (10^3-10^4)	-0.13	-0.35	0.10	
	High (10^5)	0	-0.16	0.16	

Table 2. POD analysis comparing the automated method to the validated manual method. Lower and upper confidence intervals must contain zero to meet acceptance criteria
[a]Difference in probability of detection between automation and manual workflow. [b]Lower confidence interval. [c]Upper confidence interval.

The C_t values from the dilutions above LOD were plotted in Bland-Altman plots to demonstrate the equivalence of the procedures. All data points were within the confidence intervals (Figure 2, A-D). For the AOAC POD analysis, the difference in POD (dPOD) was within the acceptability limits (lower and upper CI contained zero) for the four matrices tested, demonstrating that the workflows have a statistically comparable limit of detection (Table 2).

Conclusions

The study design demonstrated that the automated and manual workflows had a statistically comparable performance and can be considered equivalent.

The study design provided a robust and accurate assessment for the performance of the SureTect Automation Platform (Figure 3) and could be employed in future studies where similar method changes are made.

Figure 3. Thermo Scientific™ SureTect™ Automation Platform

References

1. ISO 16140-2:2016: Microbiology of the food chain — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method
2. *Official Methods of Analysis* (2019), 21st Ed., Appendix J, AOAC INTERNATIONAL, Rockville, MD, app_j.indd (aoac.org) (accessed March 2024)

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