Evaluating the performance of the SureTect Salmonella species PCR assay to detect *Salmonella* spp. from PPS samples when Tetrathionate (TT) Broth and a regrowth step is used as the enrichment procedure

Introduction

Salmonella causes greater than 200 million infections worldwide annually, resulting in 155,000 deaths[1]. Sensitive and reliable methods to detect *Salmonella* spp. in livestock farms is quintessential to reducing global disease burden. These methods must be suitable for application for the detection of *Salmonella* spp. in primary production samples (PPS) and offer robust confirmation techniques.

The Thermo Scientific™ SureTect™ Salmonella species PCR Assay is validated for a broad range of foods, environmental samples and pet food by ISO 16140-2:2016[2], AOAC PTM and AOAC OMA. The assay can be run on the Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR System using RapidFinder™ Analysis Software v2.0 or later or run on the Applied Biosystems™ 7500 Fast Food Safety 96-well Real-Time PCR instrument using RapidFinder™ Express Software v2.0 or later. After completing the run, interpreted results are clearly displayed and can be reported, stored, printed, or downloaded as required.

The SureTect Salmonella Species PCR Assay has also recently been certified by NF Validation for the detection of *Salmonella* spp. in PPS when using buffered peptone water (BPW) (ISO) with 20 mg/L of novobiocin, followed by a regrowth step, as the enrichment procedure.

This study proved the performance and ability of the SureTect Salmonella species PCR Assay to detect *Salmonella* spp. from PPS samples when Tetrathionate (TT) Broth and a regrowth step is used as the enrichment procedure. The enrichment can also be used with the Thermo Scientific™ RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit in the event of a positive *Salmonella* spp. screening result to simultaneously analytically confirm *Salmonella* Typhimurium and/or *Salmonella* Enteritidis.

Materials and Methods

Two studies were conducted, comparing the alternative method (figure 1) to the ISO 6579-1:2017[3] and ISO 6579-1:2017/Amd 1:2020[4] reference method (figure 2) using primary production samples (PPS) from poultry, porcine, and bovine origin.

In the first ISO 16140-2 sensitivity style study, 15 samples comprising faecal and non-faecal PPS were artificially contaminated with six different *Salmonella* spp. isolates at 3.8 – 8.4 CFU/test portion. In the second ISO 16140-2 RLOD/ AOAC POD harmonized study, poultry faeces was inoculated with *Salmonella* Infantis at three contamination levels; low (20 replicates at 0.9 CFU/test portion), high (five replicates at 3.3 CFU/test portion) and negative control (five replicates left uninoculated). All samples were allowed to equilibrate at ambient temperature (23 ± 5°C) for 24 hours.

Samples were enriched and tested on the SureTect Salmonella PCR Assay on both QuantStudio 5 and 7500 Fast PCR Instruments; all samples were culturally confirmed (figure 1). Both studies were compared to the ISO reference method (figure 2) following an unpaired study design.

Alternative Workflow

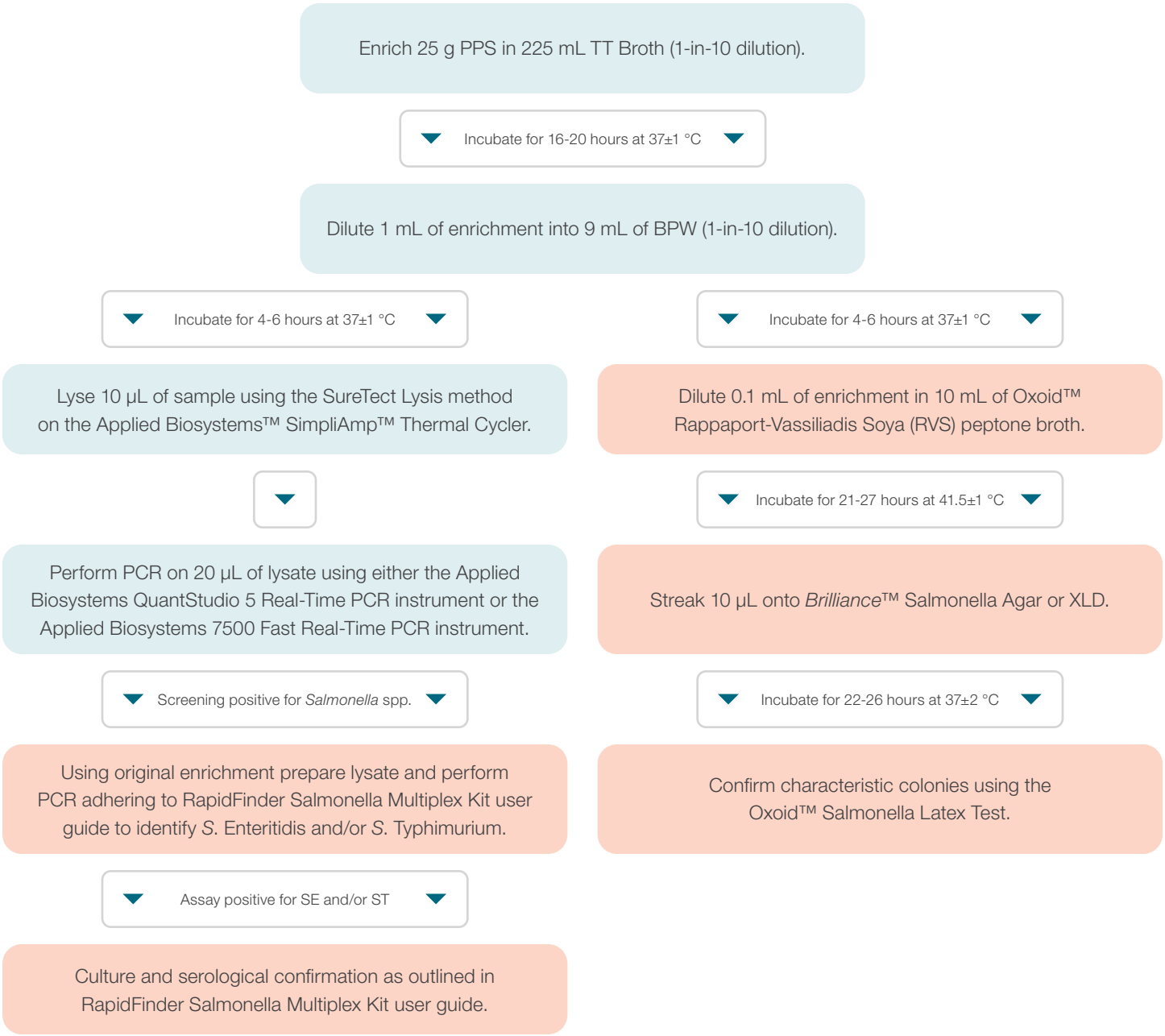

Figure 1: SureTect Salmonella Species PCR Assay workflow for PPS Samples using TT Broth. (Blue = Screening/detection steps, Red = Confirmation steps).

Reference Workflow

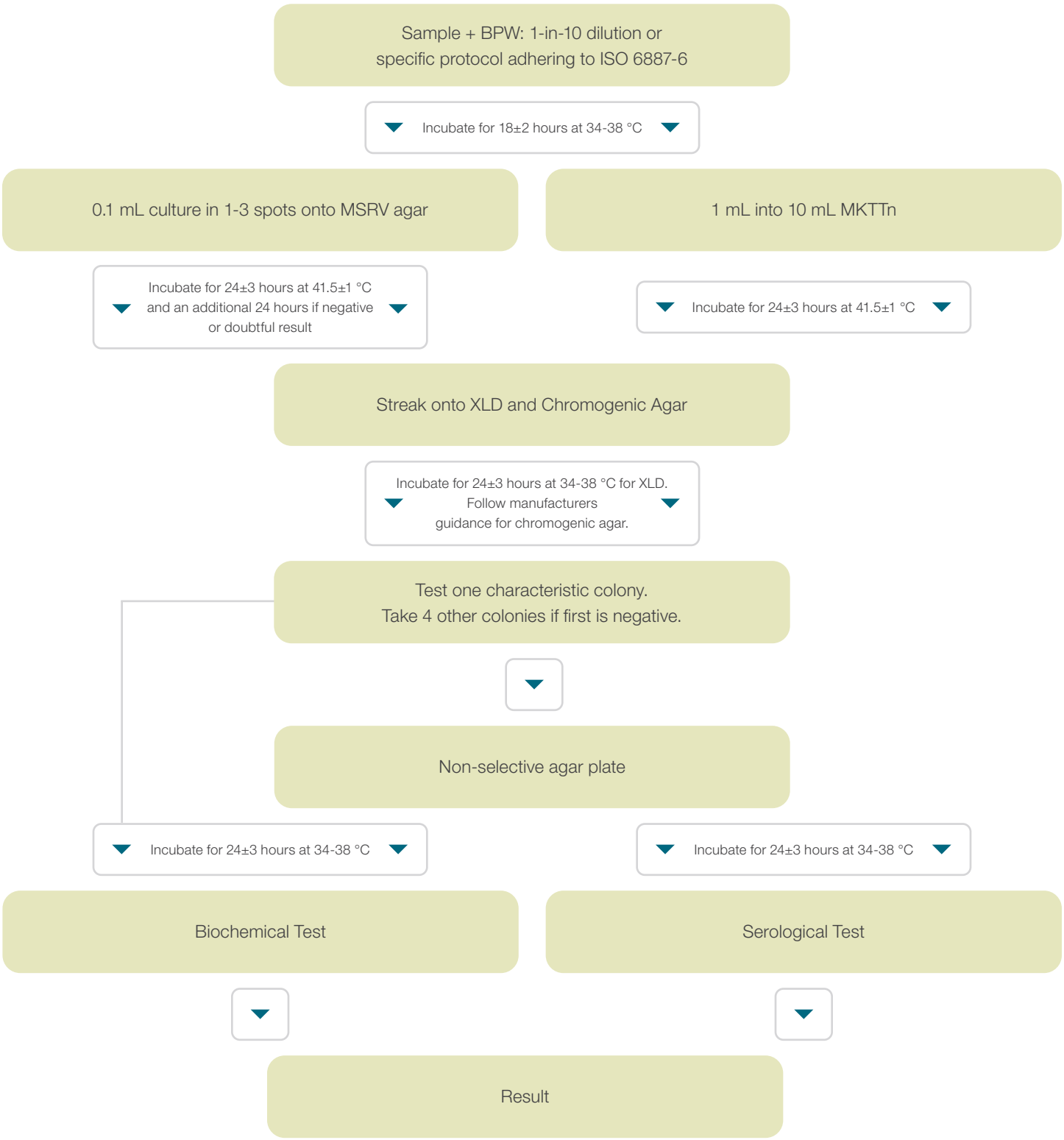

Figure 2: Workflow for ISO 6579-1:2017 & ISO 6579-1/A1:2020 for PPS Samples.

Results

Performance of the alternative method was shown to be comparable to the reference method for the sensitivity study and within the ISO16140-2 acceptability limits for the RLOD study as set out in Tables 1 and 2 respectively. For the sensitivity study one negative deviation (ND) was observed with a faecal sample on the 7500 Fast only, indicating the sample was close to the limit of detection of the SureTect Salmonella PCR Assay.

For the RLOD study, performance was skewed in favor of the alternative method; the RLOD was well below the acceptability limit of 2.5. The POD analysis showed no statistically significant differences in performance between the alternative method and the reference method at the 95% confidence interval for all contamination levels.

Table 1: Sensitivity summary results for SureTect Salmonella species PCR Assay vs ISO 6579-1:2017 reference method – Applied Biosystems 7500 Fast Real-Time PCR Instrument and Applied Biosystems QuantStudio 5 Real-Time PCR Instrument for initial testing timepoint and after 72 h storage.

	ISO 6579:1-2017 positive	ISO 6579:1-2017 negative	Sensitivity reference method	Sensitivity alternative method
SureTect Salmonella species PCR Assay Positive	15 (PA)*	0 (PD)	100%	100%
SureTect Salmonella species PCR Assay Negative	0 (ND)*	(NA)		

PA = Positive Agreement NA = Negative Agreement PD = Positive Deviation ND = Negative Deviation
*One sample on the 7500 Fast turned from a PA to a ND

Table 2. RLOD and POD summary results for SureTect Salmonella species PCR Assay vs ISO 6579-1:2017 reference method – Applied Biosystems QuantStudio 5 and Applied Biosystems 7500 Fast Real-Time PCR Instruments

Matrix	Timepoint	Strain	MPN[a]/ Test Portion	N[b]	Alternative[c]			Reference			dPOD$_C$[g]	95% CI[h]	RLOD
					X[c]	POD$_C$[d]	LOD$_{50}$[e]	X	POD$_R$[f]	LOD$_{50}$			
25 g Poultry Bootsocks	16 hr + 4 hr Regrowth	Salmonella Infantis Ad1404[5]	N/A[i]	5	0	0.00	0.45	0	0.00	0.65	0.00	-0.43, 0.43	0.661
			0.9	20	15	0.15		12	0.60		0.15	-0.13, 0.40	
			3.3	5	5	1.00		5	1.00		0.00	-0.43, 0.43	

a. MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
b. N = Number of test portions.
c. x = Number of positive test portions.
d. POD_C = Alternative method presumptive positive outcomes confirmed positive divided by the total number of trials.
e. LOD_{50} = Level of detection at which 50% of samples are expected to give a positive result.
f. POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
g. $dPOD_C$= Difference between the confirmed alternative method result and reference method confirmed result POD values.
h. 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
i. N/A = Not applicable.

Conclusions

The results show that enrichment of primary production samples in TT broth for 16 hours followed by a short regrowth step is compatible with the SureTect Salmonella species PCR Assay. The RapidFinder Salmonella Multiplex PCR Kit is suitable for use as an analytical confirmation method.

References

1. Gorski, L., Parker, C. T., Liang, A., Cooley, M. B., Jay-Russell, M. T., Gordus, A. G., Atwill, E., R., and Mandrell, R. E. (2011)." Prevalence, Distribution, and Diversity of *Salmonella enterica* in a Major Produce Region of California". Appl. Environ. Microbiology. vol. 77 no. 8 2734-2748
2. 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 (2016), ISO, https://www.iso.org/standard/54870.html
3. ISO 6579-1:2017 Microbiology of the food chain-Horizontal method for the detection, enumeration, and serotyping of *Salmonella* – part 1: Detection of *Salmonella* spp.
4. ISO 6579-1:2017/Amd 1:2020 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp. Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC
5. ADRIA Culture Collection, 20 Av Plage des Gueux Creach Gwen, 29196 Quimper, France.

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