Evaluation of the Solus One *Salmonella* method with ISO-formulated and BAM Media M192: Buffered Peptone Water (BPW) versus ISO 6579-1 Reference Method for the Detection of *Salmonella* species in Select Food and on Environmental Surfaces

Following PTM AOAC Certification of the Solus One Salmonella method (101801), a further evaluation comparing ISO-formulated and BAM Media M192: Buffered Peptone Water (BPW) to an ISO 6579-1 Reference method was carried out on two food matrixes (raw salmon fillet and shredded cheddar cheese) and a stainless steel (18 gauge: 304 food grade with a brushed finish) environmental surface. None of the selected food matrixes were naturally contaminated with target analyte.

This study evaluated ten un-paired sample test portion replicates at a low inoculation level to yield fractionally positive results (0.2–2 CFU/test portion) for the two food matrixes. Within the stainless-steel environmental surfaces, assessment comprised of ten replicate test portions at a low inoculation level to yield fractionally positive results (60 CFU/test area). A different strain of *Salmonella* species was used for the inoculation of the various food and environmental surface matrixes. In addition, the stainless-steel environmental surface was co-inoculated with a potential competitor at ten times the level of the *Salmonella* strain.

For the evaluation of the Solus One *Salmonella* method with ISO-formulated and BAM Media M192: BPW, a *Salmonella* Virchow NCTC 5472 (for raw salmon matrix), a *Salmonella* Heidelberg NCTC 5717 (for cheddar cheese matrix) and a *Salmonella* Typhimurium NCTC 5711 (for stainless steel

environmental surface matrix) inoculums were cultured by transferring a single colony from trypticase soy agar into BHI broth for 24 ± 2 h at 35 ± 2°C to obtain stationary phase cell densities. The *Salmonella* starter cultures were subsequently diluted in BHI broth, to levels that would yield fractional positives or consistently positive results at the time of sampling.

Where co-inoculation was required, a *Citrobacter farmeri* SOL 0081 strain was cultured in BHI broth for 22 ± 2 h at 35 ± 1°C and diluted to ten times the level of the *Salmonella* strain (800 CFU/test area).

Prior to inoculation of cheddar cheese matrix, the broth culture inoculum was heat stressed for 10 ± 1 min at 50 ± 1°C in a water bath. The heat stressed *Salmonella* starter culture was subsequently diluted in BHI broth, to levels that would yield fractional positives or consistently positive results at the time of sampling. Following inoculation, a bulk lot of the matrix was homogenized by hand and held for 48–72 h at 2–8°C prior to analysis, thereby allowing time for the target organism to equilibrate within the sample.

For stainless steel environmental surfaces, 4” x 4” areas were co-inoculated with 0.25 mL diluted *Salmonella* culture and ten-fold excess competitor organism, evenly distributed over the entire test area and sampled using sampling sponges (World Bioproducts EZ Reach Sponge Sampler with Hi Cap neutralizing buffer). Surfaces were dried for 16–24 h at room temperature (20–25°C) prior to sampling. Inoculation levels for the environmental surfaces were determined by plating out inoculum aliquots onto TSA in triplicate.

Each surface was sampled with respective devices using horizontal and vertical sweeping motions. Sampled sponges were placed in a sterile sampling bag containing sufficient neutralizer to cover the sponge; and swabs were placed into a sterile sampling tube. Samples were stored at 4 ± 2°C for 2 h ± 15 min prior to enrichment.

Supplemented BPW Medium was pre-warmed to 37 ± 1°C for at least 2 h prior to addition of 100 mL pre-warmed supplemented BPW Medium to sponges. Samples were subsequently incubated for 16–20 h at 41.5 ± 1°C. All stainless-steel environmental surface samples were randomized, blind

coded and then analyzed by the Solus One *Salmonella* automated preparation method.

In addition, 225 mL supplemented BPW Medium was added to 25 g food test portions. All food test samples were subsequently incubated for 20–22 h at 41.5 ± 1°C. All food test samples were randomized, blind coded and then analyzed by the Solus One *Salmonella* automated preparation method.

All enriched test samples analyzed by the Solus One *Salmonella* method, regardless of presumptive result, were culturally confirmed using an alternative confirmation procedure by directly streaking the primary enrichments to XLD agar. No differences were observed between Solus One *Salmonella* presumptive and confirmed results for all matrixes analyzed.

Comparison of the candidate Solus One *Salmonella* with ISO-formulated and BAM Media M192: BPW and ISO 6579-1 reference method for all matrixes indicated fractional levels were achieved at the low inoculation level test portions using an automated preparation method.

The POD analysis between the candidate Solus One *Salmonella* with ISO-formulated and BAM Media M192: BPW and ISO 6579-1 reference method for all matrixes indicated that there was no significant difference at the 5% level between the numbers of positive results by the candidate Solus One *Salmonella* with ISO-formulated and BAM Media M192: BPW and ISO 6579-1 reference method using an automated preparation method. A summary of POD analyses is presented in Table 1.

Table 1: Solus One *Salmonella* Method with BPW (ISO) and BAM Media M192: BPW vs. ISO 6579-1 Reference Method – POD comparison

Evaluation Method	N[a]	x[b]	POD_A[c]	95% CI		Evaluation Method	N	x	POD_B[d]	95% CI		$dPOD_{AB}$[e]	95% CI[f]	
Matrix: Cheddar cheese						*Target analyte: Salmonella* Heidelberg NCTC 5717								
Solus One *Salmonella* – BPW (ISO)	10	7	0.7	0.40,	0.89	Solus One *Salmonella* – BAM Media M192: BPW	10	9	0.9	0.60,	1.00	-0.2	-0.52,	0.16
ISO 6579-1 Reference Method	10	10	1.0	0.72,	1.00	Solus One *Salmonella* – BAM Media M192: BPW	10	9	0.9	0.60,	1.00	0.1	-0.20,	0.40
ISO 6579-1 Reference Method	10	10	1.0	0.72,	1.00	Solus One Salmonella – BPW (ISO)	10	7	0.7	0.40,	0.89	0.3	-0.04,	0.60
Matrix: Raw Salmon						*Target analyte: Salmonella* Virchow NCTC 5472								
Solus One *Salmonella* – BPW (ISO)	10	5	0.5	0.24,	0.76	Solus One *Salmonella* – BAM Media M192: BPW	10	5	0.5	0.24,	0.76	0.0	-0.37,	0.37
ISO 6579-1 Reference Method	10	7	0.7	0.40,	0.89	Solus One *Salmonella* – BAM Media M192: BPW	10	5	0.5	0.24,	0.76	0.2	-0.20,	0.53
ISO 6579-1 Reference Method	10	7	0.7	0.40,	0.89	Solus One Salmonella – BPW (ISO)	10	5	0.5	0.24,	0.76	0.2	-0.20,	0.53
Matrix: Stainless Steel Environmental surface						*Target analyte: Salmonella* Typhimurium NCTC 5711						*Non-target analyte: Citrobacter farmeri SOL 0081 Origin: Frozen Vegetables*		
Solus One *Salmonella* – BPW (ISO)	10	9	0.9	0.60,	1.00	Solus One *Salmonella* – BAM Media M192: BPW	10	9	0.9	0.60,	1.00	0.0	-0.32,	0.32
ISO 6579-1 Reference Method	10	10	1.0	0.72,	1.00	Solus One *Salmonella* – BAM Media M192: BPW	10	9	0.9	0.60,	1.00	0.1	-0.20,	0.40
ISO 6579-1 Reference Method	10	10	1.0	0.72,	1.00	Solus One Salmonella – BPW (ISO)	10	9	0.9	0.60,	1.00	0.1	-0.20,	0.40

[a]N = Number of test portions.

[b]x = Number of positive test portions. .

[c]POD_A = Positive outcomes divided by the total number of trials first member of pair.

[d]POD_B = Positive outcomes divided by the total number of trials second member of pair.

[e]$dPOD_{AB}$ = Difference in POD between the paired comparison.

[f]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.