Application Note: Analyzing the performance of the Thermo Scientific Oxoid PrecisBlue Supplement (Liquid Format) for *Salmonella* and *Cronobacter* Food Safety workflows

Introduction

The Thermo Scientific™ Oxoid™ PrecisBlue Supplement (SR0259A) is a selective novobiocin supplement included in several food safety workflows: The Thermo Scientific™ SureTect™ Salmonella species PCR Assay Method, the Thermo Scientific™ SureTect™ Cronobacter species PCR Assay Method, the Thermo Scientific™ Oxoid™ Salmonella Precis™ Method and the Thermo Scientific™ Oxoid™ Cronobacter Precis™ Method. The PrecisBlue supplement contains an inert blue dye which visually aids the end user when addition to enrichment media has been completed to prevent supplementation errors.

The supplement is used in combination with Buffered Peptone Water (BPW) only enrichments for certain matrices, as outlined in the instructions for use (IFU) for each workflow. The supplement is certified according to ISO 16140-2 by both AFNOR and MicroVal for all workflows, with performance having been assessed against the standard Thermo Scientific™ Oxoid™ Novobiocin Selective Supplement (liquid format) without dye (SR0249A) and found to be equivalent.

Three studies were conducted, comparing the performance of the new PrecisBlue supplement to the standard novobiocin without dye.

Sensitivity Study

An ISO 16140-2 style sensitivity study analyzed a total of 87 samples split across five categories (25 g meat products including poultry, 25 g dairy products, 25 g vegetables & seafood, 375 g powdered infant formula (PIF) with probiotics and 150 g animal feed) for the *Salmonella* workflows. A total of 15 samples from one category (375 g PIF and Cereals with and without probiotics including ingredients (e.g. caseinates) were analyzed for the *Cronobacter* workflows. All categories followed an unpaired study design.

Relative Limit of Detection and Probability of Detection Study

The Relative Limit of Detection (RLOD) and Probability of Detection (POD) harmonized study assessed two categories (150 g Animal feed and 25 g Ground Beef) for the *Salmonella* workflows, and one category (375 g PIF and Cereals with and without probiotics including ingredients (e.g. caseinates) for the *Cronobacter* workflows. Three contamination levels were examined per category; five replicates of uncontaminated matrix, 20 replicates of low-level contaminated matrix to yield fractionally positive results and five replicates at a high contamination level.

Inclusivity/Exclusivity

A total of 33 *Salmonella* and 23 *Cronobacter* isolates were tested for inclusivity and a total of 21 exclusivity isolates were tested across all workflows. The study design adhered to the requirements of ISO 16140-2.

Results

Performance of the PrecisBlue supplement was comparable to that of the novobiocin without dye, with the sensitivity study demonstrating comparable performance outlined in Tables 1-3. The RLOD study results for PrecisBlue were within the acceptability limit set out in the ISO16140-2 guidelines as outlined in Tables 4-6. The POD analysis of the data showed no statistically significant differences in performance between the PrecisBlue supplement for any categories across all methods when compared to the current novobiocin without dye, demonstrating comparable performance. The inclusivity/exclusivity study successfully detected and excluded all strains tested for all workflows across both the PrecisBlue and novobiocin without dye supplements as outlined in Table 7. Some strains for both the *Salmonella* and *Cronobacter* workflows required the addition of milk powder or PIF to grow, but this is not a performance concern as this was the case for both PrecisBlue and novobiocin without dye.

Table 1: Sensitivity summary results for SureTect Salmonella species PCR Assay - PrecisBlue vs novobiocin without dye for all categories

	Novobiocin without dye positive	Novobiocin without dye Negative	Sensitivity Novobiocin without dye	Sensitivity PrecisBlue
PrecisBlue Positive	29 (PA)	13 (PD)	74%	84%
PrecisBlue Negative	8 (ND)	37 (NA)		

*PA = Positive Agreement, NA = Negative Agreement, PD = Positive Deviation, ND = Negative Deviation

Table 2: Sensitivity summary results for Salmonella Precis - PrecisBlue vs novobiocin without dye for all categories

	Novobiocin without dye positive	Novobiocin without dye Negative	Sensitivity Novobiocin without dye	Sensitivity PrecisBlue
PrecisBlue Positive	25 (PA)	14 (PD)	70%	81%
PrecisBlue Negative	9 (ND)	39 (NA)		

Table 3: Sensitivity summary results for SureTect Cronobacter species PCR Assay and Cronobacter Precis[1] - PrecisBlue vs novobiocin without dye for all categories

	Novobiocin without dye positive	Novobiocin without dye Negative	Sensitivity Novobiocin without dye	Sensitivity PrecisBlue
PrecisBlue Positive	7 (PA)	2 (PD)	80%	90%
PrecisBlue Negative	1 (ND)	5 (NA)		

1. Results were identical for SureTect Cronobacter species PCR Assay and Cronobacter Precis

Table 4. RLOD and POD summary results for SureTect Salmonella species PCR Assay - PrecisBlue vs novobiocin without dye

Matrix	Timepoint	Strain	CFU level/ Test Portion[a]	N[b]	Precis Blue			Novobiocin without dye			dPOD$_C$[g]	95% CI[h]	RLOD
					X[c]	POD$_C$[d]	LOD$_{50}$[e]	X	POD$_R$[f]	LOD$_{50}$			
25 g Ground Beef	20 h	*Salmonella* Typhimurium AOOC060	0.0	5	0	0.00	0.6	0	0.00	0.9	0.00	-0.43, 0.43	0.738
			0.8	20	12	0.60		11	0.55		0.05	-0.24, 0.33	
			4.2	5	5	1.00		4	0.80		0.20	-0.28, 0.62	
50 g Dry Kibbles for Dog	20 h	*Salmonella* Derby Ad1878	0.0	5	0	0.00	0.5	0	0.00	0.6	0.00	-0.43, 0.43	0.868
			0.6	20	11	0.55		10	0.50		0.05	-0.24, 0.33	
			3.6	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. PODC = Alternative method presumptive positive outcomes confirmed positive divided by the total number of trials.
e. LOD50 = Level of detection at which 50% of samples are expected to give a positive result.
f. PODR = Reference method confirmed positive outcomes divided by the total number of trials.
g. dPODC= 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.

Table 5. RLOD and POD summary results for Salmonella Precis - PrecisBlue vs novobiocin without dye

Matrix	Timepoint	Strain	CFU level/ Test Portion	N	Precis Blue			Novobiocin without dye			dPOD$_C$	95% CI	RLOD
					X	POD$_C$	LOD$_{50}$	X	POD$_R$	LOD$_{50}$			
25 g Ground Beef	20 h	*Salmonella* Typhimurium AOOC060	0.0	5	0	0.00	0.7	0	0.00	0.9	0.00	-0.43, 0.43	0.821
			0.8	20	11	0.60		11	0.55		0.00	-0.28, 0.28	
			4.2	5	5	1.00		4	0.80		0.20	-0.28, 0.62	
50 g Dry Kibbles for Dog	20 h	*Salmonella* Derby Ad1878	0.0	5	0	0.00	0.5	0	0.00	0.6	0.00	-0.43, 0.43	0.868
			0.6	20	11	0.55		10	0.50		0.05	-0.24, 0.33	
			3.6	5	5	1.00		5	1.00		0.00	-0.43, 0.43	

Table 6. RLOD and POD summary results for SureTect Cronobacter species PCR Assay and Cronobacter Precis - PrecisBlue vs novobiocin without dye

Matrix	Timepoint	Strain	CFU level/ Test Portion	N	Precis Blue			Novobiocin without dye			dPOD$_C$	95% CI	RLOD
					X	POD$_C$	LOD$_{50}$	X	POD$_R$	LOD$_{50}$			
375 g PIF with probiotics	18 h	*Cronobacter sakazakii* Ad1446	0.0	5	0	0.00	0.2	0	0.00	0.3	0.00	-0.43, 0.43	0.868
			1.0	20	18	0.90		16	0.80		0.10	-0.13, 0.33	
			3.0	5	5	1.00		5	1.00		0.00	-0.43, 0.43	

Table 6. Inclusivity/exclusivity results for all methods

Method	Total strains tested	Without powdered milk addition		With powdered milk addition		Total	
		PrecisBlue positive results	Novobiocin without dye positive results	PrecisBlue positive results	Novobiocin without dye positive results	PrecisBlue positive results	Novobiocin without dye positive results
Inclusivity							
SureTect Salmonella	33	29	29	4	4	33	33
Salmonella Precis	33	26	26	7	9	33	33
SureTect Cronobacter	23	14	15	9	8	23	23
Cronobacter Precis	23	14	15	9	8	23	23
Exclusivity							
SureTect Salmonella	15	15	15	N/A			
Salmonella Precis	15	15	15				
SureTect Cronobacter	13	13	13				
Cronobacter Precis	13	13	13				

Conclusion

The data presented here demonstrates that the Thermo Scientific Oxoid PrecisBlue Supplement is a suitable alternative selective supplement for addition to BPW for selected matrices in Salmonella and Cronobacter food safety workflows. The liquid format of the supplement supports user-friendly addition to the enrichment media, with the inert blue dye providing visible tracking for when supplementation has occurred, preventing errors.

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