

Evaluation of a New Enrichment Broth for the Preparation of Cocoa-Containing Samples Preceding the Thermo Scientific SureTect Salmonella species PCR Assay

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Overview

Purpose: The aim of this study was to evaluate the performance of Thermo Scientific™ Oxoid™ Cocoa Sample Recovery Broth (Oxoid CSR Broth) as an enrichment medium for high cocoa chocolate and chocolate intermediates prior to testing with the Thermo Scientific™ SureTect™ Salmonella Species PCR Assay.

Methods: Eighty chocolate and chocolate intermediate samples were tested. Matrices included 85-90% cocoa chocolate, milk chocolate (27% cocoa), cocoa: powder, liquor, mass and butter. Each food matrix was artificially contaminated with *Salmonella* cells according to AOAC® and AFNOR Certification spiking protocols then combined with the appropriate enrichment broth (1:10 dilution). Post incubation, samples were processed according to the workflows in Figure 1. Oxoid CSR Broth enrichments were sub-cultured into a secondary enrichment broth before streaking onto selective agars for overall confirmation of positive or negative status.

Results: The rapid method, using Oxoid CSR Broth preceding the SureTect Salmonella Assay, showed comparable performance with a modified traditional culture method when tested with both AOAC and AFNOR Certification spiking methodologies.

Introduction

A new enrichment medium, Oxoid CSR Broth, has been developed to rapidly recover *Salmonella* spp., including injured cells, from high cocoa-containing samples without the requirement for secondary selective enrichment. Long term desiccation and heat injury during chocolate production may induce injury that can slow the rate of recovery for these organisms¹. Validation bodies such as AOAC-RI and AFNOR Certification test injured cells spiked into food to evaluate the performance of new methods in detecting food pathogens. In this study, the performance of Oxoid CSR Broth as an enrichment medium preceding the SureTect Salmonella Assay for detection of *Salmonella* spp. from chocolate and chocolate intermediates was compared to a modified traditional culture method using both AOAC and AFNOR Certification spiking methodologies.

Methods

Sample Preparation

Eighty chocolate and chocolate intermediate samples were artificially contaminated with *Salmonella* and tested with both the rapid PCR method and a modified traditional culture method (Figure 1) under three spiking scenarios (Tables 1-2). Matrices tested comprised 85-90% cocoa chocolate, milk chocolate, cocoa: powder, liquor, mass and butter. *Salmonella* isolates used for spiking were stressed according to AOAC and AFNOR Certification injury protocols: AOAC-style desiccation (14 days within matrix at room temperature) and AFNOR-style treatment with high temperature (56°C, 8 minutes). Artificial contamination generated fractional positivity post storage (AOAC) and at the point of inoculation (AFNOR certification).

Test method(s)

Samples of 25g were combined with 225ml of Oxoid CSR Broth or UHT Skimmed Milk for primary enrichment at 37°C as detailed in Figure 1. After 2 hours incubation, brilliant green dye was added to UHT Skimmed Milk enrichments. Post enrichment, Oxoid CSR Broth samples were streaked directly to Thermo Scientific™ Oxoid™ Brilliance™ Salmonella Agar and also diluted 1:5 in Buffered Peptone Water (BPW) prior to testing with the SureTect Salmonella Assay. All enrichments were sub-cultured to RVS Broth before streaking onto secondary selective plating media as part of the Modified Traditional Culture Method and for confirmation of the positive or negative status of the Oxoid CSR Broth enrichments. Typical presumptive positive colonies from the plating media were confirmed using the Thermo Scientific™ Oxoid™ Salmonella Latex Kit.

Data Analysis

A Probability of Detection (POD) analysis and the number of positive deviations between methods was calculated for samples spiked with AOAC and AFNOR Certification methodologies respectively. These statistical methods are employed by each certification body respectively during the validation of alternative methods.

Figure 1: Method Workflows

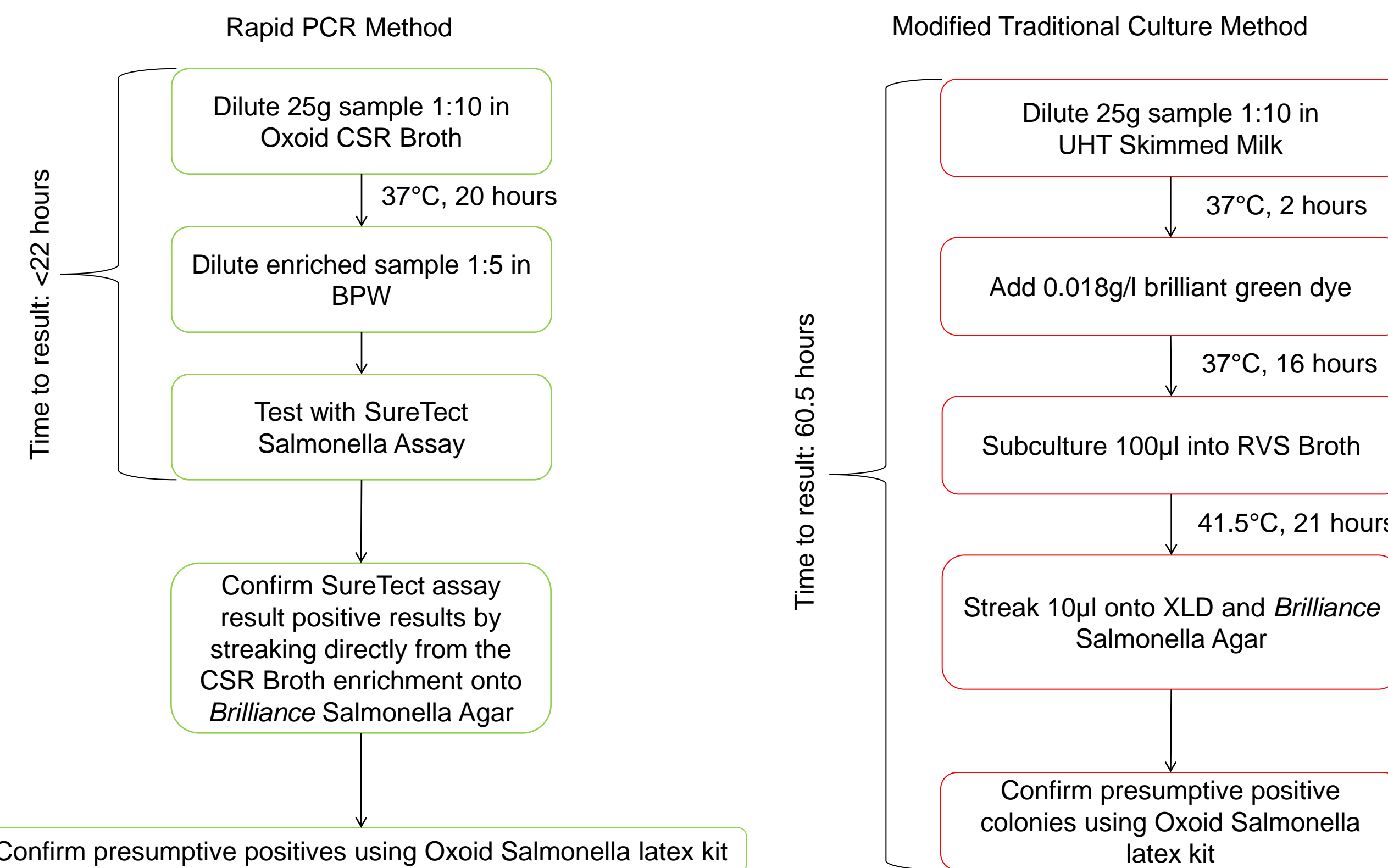


Table 1: Injury Protocols and Spike Levels (AOAC International Methodology)

Test	Matrix	Replicates per spike level	Spike organism	Inoculum Injury Method	Spike level (CFU/25g)
1	90% cocoa chocolate	20 (low)	<i>Salmonella</i> Virchow	Desiccation (14 days room temperature)	75
		3 (high)			478
		2 (unspiked)			-
2	85% cocoa chocolate	20 (low)	<i>Salmonella</i> Braenderup		1460
		5 (high)			2759
		5 (unspiked)			-

Table 2: Injury Protocols and Spike Levels (AFNOR Certification Methodology)

Test	Matrix	Replicates per spike level	Spike organism	Inoculum Injury Method	Spike level (CFU/25g)
3	90% Cocoa Chocolate	5	<i>Salmonella</i> Braenderup	Heat (56°C, 8 minutes)	1.2
	Cocoa Powder	5			1.8
	Cocoa Liquor	5			3.5
	Cocoa Mass	5			5.3
	Cocoa Butter	5			7.0

Results

Table 3: PCR vs. Traditional Culture Method (AOAC International Methodology)

Test	Matrix	Relative Spike Level	Sample Replicates	No. Positive Results			dPOD (95% CI)
				Traditional Culture Method	PCR Method	PCR Method Confirmation	
1	90% Cocoa Chocolate	Low	20	17	20	20	0.15 (-0.04; 0.36)
		High	3	3	3	3	0.00 (-0.56; 0.56)
		Unspiked	2	0	0	0	-
2	85% Cocoa Chocolate	Low	20	11	8	9	-0.10 (-0.37; 0.19)
		High	5	4	3	3	-0.20 (-0.62; 0.31)
		Unspiked	5	0	0	0	-

dPOD = $POD_{PCR\ method} - POD_{Traditional\ Method}$
Confidence limits that span the value 0 indicate no significant difference between methods

Table 4: PCR vs. Traditional Culture Method (AFNOR Certification Methodology)

Test	Matrix	Sample Replicates	No. Positive Results			Result
			Traditional Culture Method	PCR Method	PCR Method Confirmation	
3	Milk Chocolate (27% cocoa)	5	4	4	5*	1 x FN
	Cocoa Powder	5	3	4	4	1 x PD
	Cocoa Mass	5	3	5	5	2 x PD
	Cocoa Butter	5	4	5	5	1 x PD
	Cocoa Liquor	5	5	5	5	=

*: 1 additional positive result was identified through the confirmation protocol
=: equivalent result between methods, PD: Positive Deviation (i.e. rapid method +, traditional method -)

Conclusion

The proposed rapid method using Oxoid CSR Broth and the SureTect Salmonella Assay performed well compared to the modified traditional culture method for the detection of *Salmonella* spp. from high-cocoa chocolate samples and chocolate intermediates. The POD analysis for samples tested with AOAC International methodology indicated no significant difference between the methods tested. Samples tested with AFNOR Certification methodology gave four positive deviations and one false negative result, resulting in a total of three positive deviations for the proposed rapid method from the modified traditional culture method. The proposed rapid method, using Oxoid CSR Broth and the SureTect Salmonella Assay, gives rapid and reliable results while performing comparably with the modified traditional culture method.

References

- Tamminga S.K. et al, 1976. Survival of *Salmonella* Eastbourne and *Salmonella* Typhimurium in chocolate. *Journal of Hygiene*, 6 (1), 41-47.