

Evaluation of a New Rapid Culture Method for Detection of *Salmonella* spp. in Cocoa-Containing Samples

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Overview

Purpose: The aim of this study was to compare the performance of a new rapid culture method using Thermo Scientific™ Oxoid™ Cocoa Sample Recovery Broth (CSR Broth) to a traditional culture method for the detection of *Salmonella* spp. from high percentage cocoa-containing samples.

Methods: Tests were carried out on 69 high cocoa-containing samples including milk chocolate, 90% cocoa chocolate, cocoa: powder, liquor, mass and butter. Each food sample (25g) was combined with enrichment broth in a 1:10 ratio and artificially contaminated with injured *Salmonella* cells. Post incubation, samples were streaked onto selective agar plates and sub-cultured into selective secondary enrichment broths. Secondary enrichment broths were streaked onto selective agars. All selective agar plates were incubated for 20+/- 2 hours at 37°C.

Results: The rapid culture method, using CSR Broth as the primary enrichment medium, generated 25 positive deviations from the ISO method.

Introduction

Studies investigating the survivability of *Salmonella* spp. in chocolate and cocoa-containing samples, using sub-lethal artificial contamination methods, show that *Salmonella* may persist through high temperature and long-term desiccation¹. Artificial contamination methods used in this study replicated these conditions.

ISO 6579:2002 details the method for the detection of *Salmonella* spp. from cocoa-containing samples using a suspension of non-fat dry milk in Buffered Peptone Water supplemented with brilliant green dye as a primary enrichment medium. This study shall use a modified traditional culture method by substituting the primary enrichment with UHT skimmed milk. A new enrichment medium, CSR Broth, has been developed as an alternative enrichment medium to be used in a proposed rapid culture method for high cocoa-containing samples that does not require secondary selective enrichment.

In this study, the performance of CSR Broth as a primary enrichment medium in a new rapid culture method for the detection of *Salmonella* spp. from cocoa-containing samples was compared against a traditional culture method.

Methods

Sample Preparation

A total of 69 cocoa-containing samples were artificially contaminated with injured *Salmonella* spp. and tested with a rapid culture method and a traditional culture method (Figure 1) under 6 different spiking scenarios (Table 1). Matrices tested comprised milk chocolate, 90% cocoa chocolate, cocoa: powder, liquor, mass and butter. Isolates used for spiking were injured according to a variety of injury protocols including high temperature (56°C, 8 mins), desiccation (≥4 hours exposure of 10µl culture to ambient air) and low temperature (-18°C, 12 days). Post injury, 25g samples were spiked with between 1.2 and 19.0 CFU.

Test method(s)

Spiked samples were combined with 225ml of CSR Broth or UHT Skimmed Milk for primary enrichment at 37°C as detailed in Figure 1. The UHT Shimmied Milk enrichments were supplemented with brilliant green dye after 2 hours incubation. Post enrichment, CSR Broth samples were streaked to one of three selective plating media (Test 1: Thermo Scientific Oxoid *Brilliance*™ Salmonella Agar, Test 2: XLD Agar, Tests 3-6: XLT-4 Agar). Due to primary enrichment samples being un-paired the enriched CSR Broths were also-sub-cultured into secondary selective broths. All secondary broths were streaked onto selective plating media post enrichment and incubated at 37°C for 20-24 hours to confirm rapid method results. Presumptive positive *Salmonella* colonies were confirmed using the Thermo Scientific Oxoid Salmonella Latex Kit from all plating media.

Data Analysis

The number of positive deviations between the rapid culture method and the traditional culture method for detection of *Salmonella* spp. in cocoa-containing products were calculated to compare performance.

Figure 1: Method Workflows

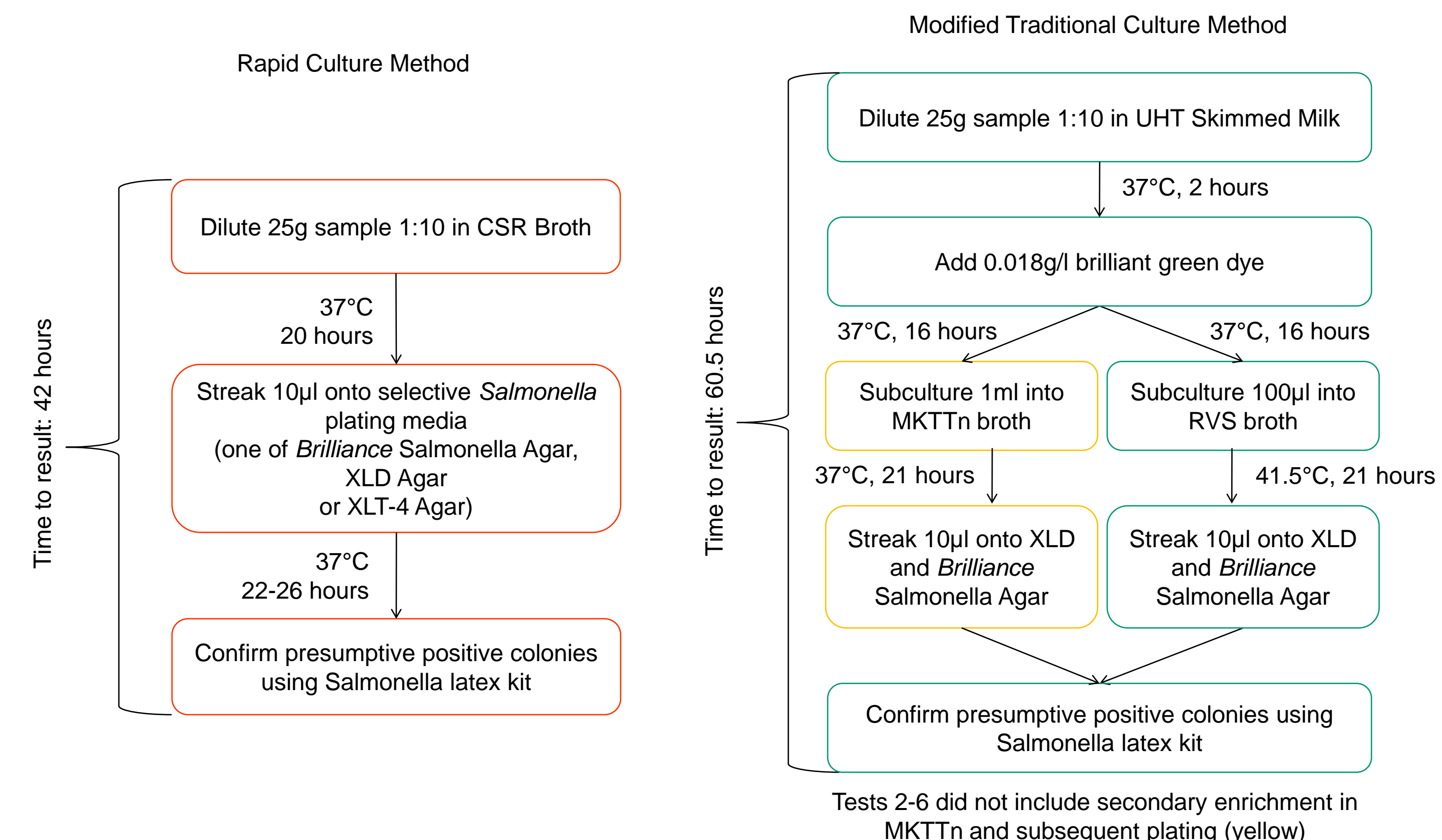


Table 1: Injury Protocols and Spike Levels

Test	Matrix	Replicates per matrix	Spike organism	Inoculum Injury Method	Injury Level [^]	Spike level (CFU/25g)	
						Rapid Culture Method	Traditional Culture Method
1	Milk Chocolate, Cocoa: powder, liquor, mass, butter	5	<i>Salmonella</i> Braenderup	Heat (56°C, 8 mins)	0.18	1.2-7.0	1.2-7.0
2	Cocoa Liquor (i)	5	<i>Salmonella</i> Tennessee	Desiccation	0.6-0.8	8.0	36.0
		5	<i>Salmonella</i> Braenderup			7.0	6.0
	Cocoa Liquor (ii)	5	<i>Salmonella</i> Tennessee			8.0	36.0
		5	<i>Salmonella</i> Braenderup			7.0	6.0
3	90% Cocoa Chocolate	5	<i>Salmonella</i> Braenderup	Desiccation	0.6-0.8	6.0	5.0
4	Cocoa Powder	5	<i>Salmonella</i> Eastbourne	Cold (-18°C, 12 days)	0.7-1.0	8.0	7.0
5	Cocoa Powder	5	<i>Salmonella</i> Tennessee	Desiccation	1.5-1.7	19.0	15.0
6	Cocoa Mass	3	<i>Salmonella</i> Tennessee	Desiccation	1.3-3.4	4.0	4.0
	Cocoa Butter	3					
	Cocoa Liquor	3					

[^]log CFU (non-selective agar count) - log CFU (selective agar count)

Results

Table 2: Results for the Rapid and Traditional Culture Methods

Test	Matrix	Sample Replicates	No. Confirmed Positive Results		Result
			Traditional Culture Method	Rapid Culture Method	
1	Milk Chocolate	5	4	4*	=
	Cocoa Powder	5	3	4	1 x PD
	Cocoa Liquor	5	3	5	2 x PD
	Cocoa Mass	5	4	5	1 x PD
2	Cocoa Butter	5	5	5	=
	Cocoa Liquor (i)	10	5	10	5 x PD
3	Cocoa Liquor (ii)	10	8	10	2 x PD
	90% Cocoa Chocolate	5	2	5	3 x PD
4	Cocoa Powder	5	3	5	2 x PD
5	Cocoa Powder	5	2	5	3 x PD
6	Cocoa Mass	3	3	3	=
	Cocoa Butter	3	0	3	3 x PD
	Cocoa Liquor	3	0	3	3 x PD

*: 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 culture method using CSR Broth performed well compared to the traditional culture method for the detection of *Salmonella* spp. from cocoa-containing samples. In Test 2, it was not possible to recover *Salmonella* Tennessee from one type of cocoa liquor (0/5 samples) using the traditional culture method whereas all spiked samples recovered the same strain from the same matrix (5/5 samples) when using CSR Broth as the *Salmonella* enrichment broth in the rapid culture method.

Across all 69 samples, the rapid culture method generated 25 positive deviations from the traditional culture method.

CSR Broth was shown to be compatible with all three primary plating media tested (*Brilliance* Salmonella Agar, XLD Agar, XLT-4 Agar), allowing for method flexibility when testing cocoa-containing samples using the rapid method.

This rapid culture method, using CSR Broth as the primary enrichment medium, gives rapid and reliable results while performing better than the traditional culture method.

References

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