BPW and UHT Enrichment Protocols to Detect *Salmonella* in Chocolate Using the Assurance® GDS for *Salmonella* Tq

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Abstract

Introduction: *Salmonella* can persist in chocolate matrices. Due to complex matrices and the presence of inhibitory substances in chocolate, *Salmonella* can be difficult to detect. This is a significant concern to the food industry. Two new enrichment methods for Assurance® GDS for *Salmonella* Tq (GDS *Salmonella*) are presented for the detection of *Salmonella* in 375 g chocolate raw materials and finished products.

Purpose: To validate specific protocols to detect *Salmonella* in raw and finished chocolate products using the GDS *Salmonella* method.

Methods: Sensitivity and Relative Limit of Detection (RLOD) studies were conducted at external expert laboratory. Raw and finished chocolate products (375 g test portion) were inoculated by *Salmonella* strains and enriched for 24-32 h at 34-38 °C using BPW (1:10 dilution ratio) or UHT milk containing 0.018 g/L of Brilliant Green (UHT+BG) dye (1:10 ratio). Enriched samples were diluted in wash buffer (100 µL/900 µL for BPW and 300 µL/700 µL for UHT+BG) before analysis. The GDS *Salmonella* method was performed, followed by ISO method confirmations. These protocols were compared to the ISO 6579-1 reference method for detection of *Salmonella*.

Results: The sensitivity study and the RLOD values meet the Acceptability Limits (respectively 3 and 2.5) of an unpaired study design. For example, 21 samples were analyzed using UHT milk + BG protocols. 2 positive deviations and 3 negative deviations were obtained (ND-PD=-1<AL). Statistics support there is no significative difference between the GDS *Salmonella* method for both enrichment methods and the ISO reference method.

Significance: These data demonstrate allowance of two different enrichment methods for the detection of *Salmonella* in chocolate products by Assurance® GDS for *Salmonella* Tq. The GDS method detects *Salmonella* in 375 g chocolate raw materials and finished goods after a minimum enrichment time of 24 h in UHT+ BG or BPW broths.

Introduction

Salmonella strains have the ability to persist in cocoa and chocolate matrices. Due to complex sample matrices and the presence of inhibitory substances such as polyphenols, low levels of contamination can be difficult to detect.

Two new enrichment methods for Assurance® GDS *Salmonella* Tq are presented for the detection of *Salmonella* in 375 g cocoa and chocolate raw materials and chocolate finished goods. Sensitivity and Relative Limit of Detection (RLOD) studies were conducted at external expert laboratory and demonstrate equivalence between Assurance® GDS *Salmonella* Tq and the reference method ISO 6579-1.

Methodology

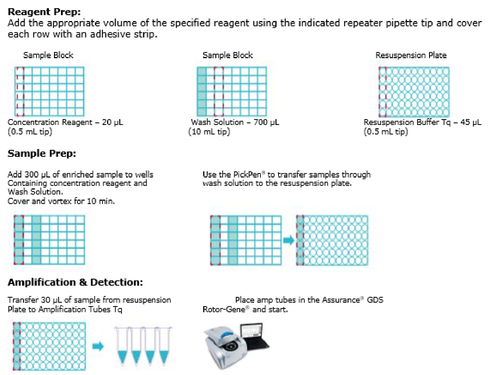
Study design:

Reference method: EN ISO 6579-1:2017 method: Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp. and its amendment A1 (March 2020)
Matrices: raw and finished chocolate products
Assays: sensitivity and RLOD for alternative methods in comparison of the ISO reference one

GDS protocols (alternative method):

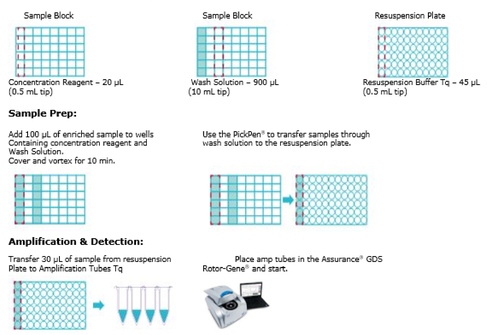
UHT milk + Brilliant Green (BG) 1/10 ratio:

1. 375 g (mL) of sample to 3375 mL (ratio 1/10) of pre-warmed UHT or reconstituted non-fat dried milk + 0.018 g/L of Brilliant green dye
2. Incubation at 34-38 °C for 24-32 h
3. Assurance® GDS workflow



BPW 1/10 ratio:

1. 375 g (mL) of sample to 3375 mL (ratio 1/10) of Buffered Peptone Water
2. Incubation at 34-38 °C for 24-32 h
3. Assurance® GDS workflow



Sensitivity results

Sensitivity studies were performed on 15 raw and 6 finished chocolate products using UHT + BG or BPW enrichment protocols followed by a dilution of enriched samples. The protocols were compared to the ISO 6579-1 protocol.

Table 1. Sensitivity on raw and finished products

Protocol	PA	NA	PD	ND	AL (ND - PD)
UHT + BG 1/10 ratio 300 µL of sample	15	1	2	3	1
BPW 1/10 ratio 100 µL of sample	16	2	1	2	1

For one category, Acceptance Limit (AL) is a maximum of 3 regarding ISO 16140-2. Each protocol tested showed an AL of 1. The sensitivity of these two protocols seems to be equivalent to the reference protocol of the ISO 6579-1.

RLOD results

All food types were comparable to the reference method for the sensitivity study. The RLOD values meet the Acceptability Limit of an unpaired study design.

Table 2. RLOD assay on chocolate powder 11% fat with UHT protocol

Inoculation	Mesured stress	Inoculation level CFU/sample	Reference method ISO 6579 and 6887 requirements: RVS XLD	RVS SALMA	MKTTn XLD	MKTTn SALMA	Confirmation API20E	Result	Number positive samples	Assurance® GDS Salmonella spp - UHT MILK+BG 1:10 at 37 °C - 24h: PCR TEST CT	XLD IMS beads	Latex test	Result	Number positive samples	ISO 16140 confirmations: RVS/MKTTn: RVS XLD	RVS SALMA	MKTTn XLD	MKTTn SALMA	Result
Salmonella Give (stress 6 minutes at 50°C)	0,5	0,0	Ø	Ø	Ø	Ø	/	-	0/2	-	Ø	/	-	0/2	Ø	Ø	- (EL)	Ø	-
			Ø	Ø	Ø	Ø	/	-		-	Ø	/	-		Ø	Ø	- (EL)	Ø	-
		0,8	+ (BH)	+ (BH)	+ (BH)	+ (BH)	+	+	7/10	+ 20,56	+ (AL)	+	+	9/10	+ (BH)	+ (BH)	+ (BH)	+ (BH)	+
			Ø	Ø	Ø	Ø	/	-		+ 18,91	+ (AL)	+	+		+ (AH)	+ (AH)	+ (AH)	+ (AH)	+
			+ (AH)	+ (AH)	+ (BH)	+ (BH)	+	+		+ 16,58	+ (AL)	+	+		+ (AH)	+ (AH)	+ (AH)	+ (AH)	+
			Ø	Ø	Ø	Ø	/	-		+ 18,19	+ (AL)	+	+		+ (BH)	+ (BH)	+ (BH)	+ (BH)	+
			+ (BH)	+ (BH)	+ (BH)	+ (BH)	+	+		+ 19,19	+ (AL)	+	+		+ (AH)	+ (AH)	+ (AH)	+ (AH)	+
			+ (BH)	+ (BH)	+ (BH)	+ (BH)	+	+		-	Ø	/	-		Ø	Ø	- (EL)	Ø	-
			+ (BH)	+ (BH)	+ (BH)	+ (BH)	+	+		+ 17,36	+ (AL)	+	+		+ (AH)	+ (AH)	+ (AH)	+ (AH)	+
			+ (BH)	+ (BH)	+ (BH)	+ (BH)	+	+		+ 15,97	+ (AL)	+	+		+ (BH)	+ (BH)	+ (BH)	+ (BH)	+
			Ø	Ø	Ø	Ø	/	-		+ 17,16	+ (AL)	+	+		+ (AH)	+ (AH)	+ (AH)	+ (AH)	+
			+ (BH)	+ (BH)	+ (BH)	+ (BH)	+	+		+ 17,56	+ (AL)	+	+		+ (AH)	+ (AH)	+ (AH)	+ (AH)	+
		2,6	+ (BH)	+ (BH)	+ (BH)	+ (BH)	+	+	2/2	+ 15,69	+ (AL)	+	+	2/2	+ (BH)	+ (BH)	+ (BH)	+ (BH)	+
			+ (BH)	+ (BH)	+ (BH)	+ (BH)	+	+		+ 16,92	+ (AL)	+	+		+ (AH)	+ (AH)	+ (AH)	+ (AH)	+

Bacterial flora
Ø: no culture
L = low
M = moderate
H = high

Distribution of flora
A = pure culture of suspect colonies
B = mixture with a majority of suspect colonies
C = mixture with a minority of suspect colonies
D = mixture with rare suspect colonies
E = absence of suspect colonies
(x): x colonies characteristic of target if x ≤ 5

Table 3. RLOD assay chocolate powder 21% fat with BPW protocol

Inoculation	Mesured stress	Inoculation level CFU/sample	Reference method ISO 6579 and 6887 requirements: RVS XLD	RVS SALMA	MKTTn XLD	MKTTn SALMA	Confirmation API20E	Result	Number positive samples	Assurance® GDS *Salmonella* spp - BPW 1:10at 37 °C - 24h: PCR TEST CT	XLD IMS beads	Latex test	Result	Number positive samples	ISO 16140 confirmations: RVS/MKTTn: RVS XLD	RVS SALMA	MKTTn XLD	MKTTn SALMA	Result
Salmonella Kentucky (stress 5 minutes at 50 °C)	0,5	0,0	+ (AL)	+ (AL)	+ (AL)	+ (AL)	/	-	0/2	-	Ø	/	-	0/2	Ø	Ø	Ø	Ø	-
			+ (AL)	+ (AL)	+ (AL)	+ (AL)	/	-		-	Ø	/	-		Ø	Ø	Ø	Ø	-
		0,6	+ (AM)	+ (AM)	+ (AM)	+ (AM)	+	+	6/10	+ 15,56	+ (AL)	+	+	9/10	+ (AM)	+ (AM)	+ (AM)	+ (AM)	+
			+ (AM)	+ (AM)	+ (AM)	+ (AM)	+	+		+ 15,00	+ (AL)	+	+		+ (AM)	+ (AM)	+ (AM)	+ (AM)	+
			+ (AM)	+ (AM)	+ (AM)	+ (AM)	+	+		+ 18,67	+ (AL)	+	+		+ (AM)	+ (AM)	+ (AM)	+ (AM)	+
			+ (AL)	+ (AL)	+ (AL)	+ (AL)	/	-		+ 15,15	+ (AL)	+	+		+ (AM)	+ (AM)	+ (AM)	+ (AM)	+
			+ (AL)	+ (AL)	+ (AL)	+ (AL)	/	-		+ 17,65	+ (AL)	+	+		+ (AM)	+ (AM)	+ (AM)	+ (AM)	+
			+ (AM)	+ (AM)	+ (AM)	+ (AM)	+	+		+ 19,91	+ (AL)	+	+		+ (AM)	+ (AM)	+ (AM)	+ (AM)	+
			+ (AL)	+ (AL)	+ (AL)	+ (AL)	/	-		+ 21,6	+ (AL)	+	+		+ (AM)	+ (AM)	+ (AM)	+ (AM)	+
			+ (AM)	+ (AM)	+ (AM)	+ (AM)	+	+		+ 16,02	+ (AL)	+	+		+ (AM)	+ (AM)	+ (AM)	+ (AM)	+
			+ (AM)	+ (AM)	+ (AM)	+ (AM)	+	+		-	Ø	/	-		Ø	Ø	Ø	Ø	-
			+ (AL)	+ (AL)	+ (AL)	+ (AL)	/	-		+ 15,13	+ (AL)	+	+		+ (AM)	+ (AM)	+ (AM)	+ (AM)	+
		2,8	+ (AM)	+ (AM)	+ (AM)	+ (AM)	+	+	2/2	+ 14,9	+ (AL)	+	+	2/2	+ (AM)	+ (AM)	+ (AM)	+ (AM)	+

The RLOD values meet the Acceptability Limit of an unpaired study design on the different food types.

Conclusions

The Assurance® GDS for *Salmonella* Tq method detects *Salmonella* strains in 375 g chocolate raw materials and chocolate finished goods after a minimum enrichment time of 24 hours at 35-37 °C when enriched 1:10 in UHT milk or Non-Fat Dry Milk powder + Brilliant Green or BPW broth followed by a dilution in Assurance® GDS wash buffer without additional subculture or modification steps.

This project shows the possibility to use our alternative method to detect *Salmonella* in difficult matrices. Two different enrichment protocols are available to help customers to reduce time-to-result and allow flexibility in regard to enrichment broth.

To complete this work, more data will be generated during the Method Comparison Study to obtain the AFNOR certification according ISO 16140-2.

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