Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

New Enrichment Method for the Rapid Detection of *Salmonella* in 375g Chocolate Finished Goods

Emily Jacobs and Lisa John
MilliporeSigma, Bellevue, WA, USA

Guillaume Mesnard and François Lenestour
Laboratoire Microsept, Le Lion-d'Angers, France

Introduction

Salmonella's ability to persist in chocolate is of significant concern to the food industry as the bacteria has been found to survive through the production process, and low levels of contamination can be difficult to detect due to complex sample matrices and the presence of inhibitory substances. A new enrichment method for Assurance® GDS *Salmonella* Tq is presented for the detection of *Salmonella* in 375 g chocolate finished goods. Sensitivity and Relative Limit of Detection (RLOD) studies were conducted at Laboratoire Microsept that demonstrate equivalence between Assurance® GDS *Salmonella* and ISO 6579-1 reference method.

Purpose

To evaluate the next-day detection of *Salmonella* with the Assurance® GDS *Salmonella* Tq method in 375 g chocolate raw materials and chocolate finished goods using UHT milk or Non-Fat Dry Milk Powder + Brilliant Green (0.018 g/L) as an enrichment medium compared against ISO 6579-1.

Methods

Sensitivity and RLOD Cultures of *Salmonella* were inoculated into foods and stabilized for 48-72 hours for non-powdered chocolate raw materials and chocolate finished goods and stressed where appropriate. *Salmonella* were principally inoculated at less than 5 CFU/sample for the sensitivity study. *Salmonella* were diluted in bulk uninoculated foods to achieve partial recovery for RLOD studies. Samples were enriched 1:10 in media for minimum 24 hours and analyzed by Assurance® GDS system.

3-Step Workflow Process

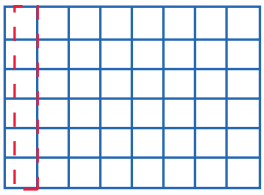
Test Portion Preparation & Enrichment

Chocolate finished goods- Aseptically weigh 375 g (mL) test portion into 3,375 mL pre-warmed (37 ± 1 °C) UHT or reconstituted Non-Fat Dried Milk. Add 0.018 g/L of Brilliant green dye. Homogenize or mix sample. Incubate at 35 °C-37 °C for 24 hours.

Reagent Prep:

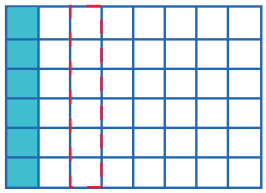
Add the appropriate volume of the specified reagent using the indicated repeater pipette tip and cover each row with an adhesive strip.

Sample Block



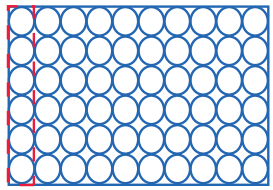
Concentration Reagent - 20 µL
(0.5 mL tip)

Sample Block



Wash Solution - 1 mL
(10 mL tip)

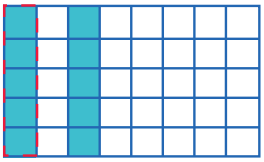
Resuspension Plate



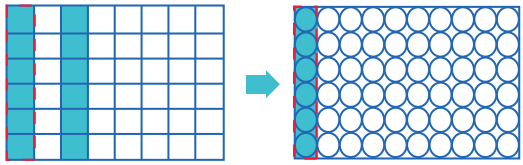
Resuspension Buffer Tq - 45 mL
(0.5 mL tip)

Reagent Prep:

Add 1 mL of enriched sample to wells
Containing concentration reagent.
Cover and vortex for 10 min.

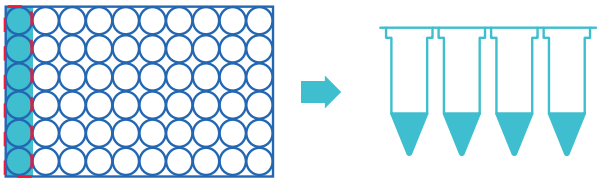


Use the PickPen® device to transfer samples through wash solution to the resuspension plate.



Amplification & Detection:

Transfer 30 µL of sample from resuspension
Plate to Amplification Tubes Tq



Place amp tubes in the Assurance® GDS Rotor-Gene and start.

See detailed Sample Processing Protocol on page 4.

Results

Sensitivity

Category	Unpaired Samples (GDS vs. ISO 6579-1)	Positive Agreement (PA)	Negative Agreement (NA)	Positive Deviation (PD)	Negative Deviation (ND)	Presumptive Positive Negative Agreement (PPNA)	Presumptive Positive Negative Deviation (PPND)	Total
Chocolate Finished Goods 375 g	Dark Chocolate Bars	6	2	0	0	0	0	8
	Milk Chocolate Bars	2	1	0	0	0	0	3
	Chocolate Bars with Nuts	4	2	3	3	0	0	12
	Chocolate Cookie Bars	6	6	0	0	0	0	12
	Chocolate Ice Cream Bars	3	2	0	1	0	0	6
	Total	27	21	5	6	1	0	60

PA: positive agreement (GDS Pres +/ GDS Confirm + ISO +)

NA: negative agreement (GDS Pres - / GDS Confirm - ISO -)

ND: negative deviation (GDS Pres - / GDS Confirm - ISO +)

PD: positive deviation (GDS Pres + / GDS Confirm + ISO -)

PPNA: positive presumptive negative agreement (GDS Pres + / GDS Confirm - ISO -)

PPND: positive presumptive negative deviation (GDS Pres + / GDS Confirm - ISO +)

Sensitivity of the Alternative Method: (PA+PD)/(PA+PD+ND) = 84.21%

Sensitivity of the Reference Method: (PA+ND)/(PA+PD+ND) = 86.84%

Relative Trueness: (PA + NA)/N = 80.00%

False Positive ratio for the Alternative Method: (FP/NA) = 0%

Acceptability Limit: (ND-PD) < 4 = 1

Relative Level of Detection (RLOD)- Unpaired

Food Type and Inoculating Organism		RLOD	RLODL	RLODU	p-value	AL
Cookie Bars with Chocolate	*Salmonella* Bongori, ATCC 43975	0.821	0.388	1.735	1.403	2.5
Cookie Bars with Caramel and Chocolate	*Salmonella* Diarizonae, ATCC 43973	1.439	0.620	3.339	0.387	
Chocolate Bars with Peanuts and Caramel	*Salmonella* Houtenae, ATCC 43974	1.000	0.467	2.140	1.000	
Chocolate Bars with Hazelnuts	*Salmonella* Salamae, ATCC 700148	2.259	0.975	5.235	0.052	
Combined		**1.236**	**0.831**	**1.838**	**0.287**	

Relative Level of Detection (RLOD) values before and after confirmation of the Alternative method results. The relative level of detection is the level of detection at P = 0.50 (LOD50) of the alternative (proprietary) method divided by the level of detection at P = 0.50 (LOD50) of the reference method. The RLOD is defined as the ratio of the alternative and reference methods:

$$RLOD = \frac{LODALT}{LODREF}$$

RLODL is the lower limit for the RLOD; RLODU is the upper limit for the RLOD

Conclusion

The Assurance® GDS for *Salmonella* Tq method detects *Salmonella* in 375g chocolate finished goods after a minimum enrichment time of 24 hours at 35 °C-37 °C when enriched 1:10 in UHT milk or Non-Fat Dry Milk powder + Brilliant Green.

A total of 60 samples were analyzed in the sensitivity study comprising 5 matrices within the chocolate finished goods category. All food types were comparable to the reference method and the RLOD values meet the Acceptability Limit which is 2.5 for an unpaired study design.

Appendix A - Directions for Use

Sample Preparation and Processing Protocol

A. Test Portion Preparation & Enrichment

a. Chocolate finished goods- Aseptically weigh 375 g (mL) test portion into 3,375 mL pre-warmed (37 ± 1°C) UHT or reconstituted Non-Fat Dried Milk. Add 0.018 g/L of Brilliant green dye. Homogenize or mix sample. Incubate at 35 °C-37 °C for 24 hours.

B. Sample Processing Protocol

Change gloves prior to handling reagents.

a. Vortex Concentration Reagent. Immediately transfer 20 µL to each of the required number of Assurance® GDS sample wells (1 well/sample) using a repeat pipette and 0.5 mL pipette tip. Cover sample wells with adhesive film strips.

b. Transfer 1.0 mL of Wash Solution to additional sample wells (1 well/sample) held in a sample well base using a repeat pipette and 10 mL pipette tip. Cover sample wells with adhesive film strips.

c. Transfer 45 µL of Resuspension Buffer Tq to the wells in the resuspension plate using a repeat pipette and 0.5 mL pipette tip. Cover resuspension plate with adhesive film strips.

d. Carefully remove adhesive film from 1 strip of sample wells. Add 1.0 mL of incubated sample to each sample well containing Concentration Reagent.

e. Avoid transferring food particles. A new pipette tip must be used for each sample. Cover each strip of sample wells with a new adhesive film strip.

f. Place sealed sample wells on the vortex mixer and vortex at approximately 900 rpm for 10 – 20 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.

g. Carefully remove and discard adhesive film strip from a strip of samples. Remove corresponding film strip from a strip of sample wells containing Wash Solution.

h. Load tips onto the PickPen® device, ensuring that the tips are firmly in place on the PickPen® tool. Extend the PickPen® magnets and insert tips into the first strip of sample wells. Stir gently for 30 s while continually moving up and down from the surface to the bottom of the well. Gently tap the PickPen® tips against the side of the sample wells to remove excess media droplets.

i. Transfer PickPen® tips to corresponding sample wells containing Wash Solution and gently swirl for 5 – 10 s (do not release particles into solution). Transfer PickPen® tips to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen® magnets and tap tips gently to release particles into the Resuspension Buffer Tq. Cover the resuspension plate with adhesive film and continue with step (C10).

j. Following incubation, transfer the particles from the BHI sample wells to the corresponding row of the prepared resuspension plate using the PickPen® tips. With tips submerged, retract the PickPen® magnets and tap tips gently to release particles into the Resuspension Buffer. Cover the resuspension plate with adhesive film and continue with step (k).

k. Repeat steps (g) through (j) for all samples using new tips for each strip of samples

Proceed to Test Procedure Section of Assurance® GDS *Salmonella* Tq DFU

MS_AN12720EN Ver. 2.0
50639
02/2024