

Rapid Alternative Isolation and Confirmation Methods for EHEC and Salmonella after Assurance® GDS PCR Analysis

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Introduction

Reference culture methods are labor-intensive and time-consuming. Rapid alternative confirmation methods are advantageous for testing sites after presumptive positive results from PCR testing, herein Assurance® GDS. These methods were validated for Assurance® GDS for *E. coli* O157:H7 Tq assay (by MicroVal) and Assurance® GDS for *Salmonella* Tq assay (by MicroVal and AFNOR).

Purpose

The purpose of this study was to **demonstrate rapid isolation and confirmation of *E. coli* O157:H7 and *Salmonella*** by inoculated food studies and inclusivity and exclusivity studies on chromogenic agars.

Methods

Sensitivity studies enrichments were inoculated at low level, most at ≤ 5 CFU/sample. Assurance® GDS methods occur through a simple 3-Step Workflow process; see **Figure 1**. After PCR analysis, presumptive positive samples can be confirmed.

Inclusivity and exclusivity strains were plated on chromogenic agars to verify specificity. For both organisms, target colonies were confirmed from plates by latex agglutination.

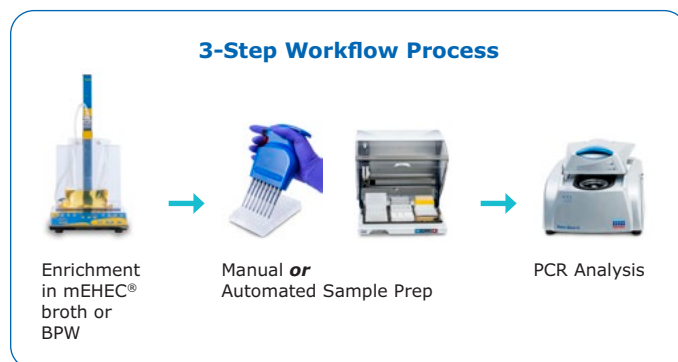


Figure 1. Assurance® GDS Method Workflow

Results: Sensitivity Study

E. coli O157:H7 (EHEC) Enriched in mEHEC®

An additional 70 inoculated representative food items (alternative enrichment only, no PCR analysis) were evaluated after 10 and 14 hours of incubation by a direct streak from the primary enrichment onto the 3 different agars, see **Figure 2**. All test portions analyzed by the alternative method were also confirmed following ISO 16654:2001 reference method at 24 hours. Reference and alternative method results were in agreement; see **Table 1**.

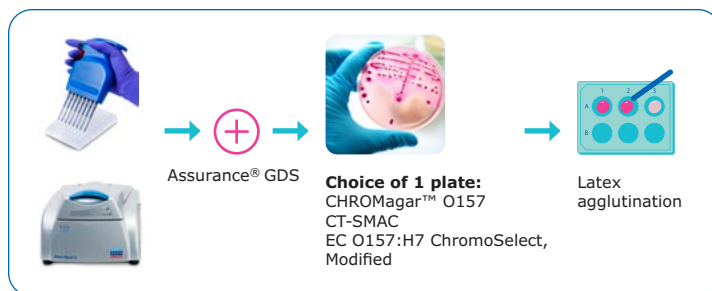


Figure 2. Assurance® GDS EHEC Alternative Confirmation Workflow

Table 1. EHEC Sample Results Between the Reference and Alternative Confirmation Methods, mEHEC®

Category	Type	PA	NA	PD	ND	PPND	PPNA
Raw beef meats (25 g)	Fresh beef meats	5	0	0	0	0	0
	Frozen then thawed beef	5	0	0	0	0	0
	Meats with seasonings	5	0	0	0	0	0
	Total	15	0	0	0	0	0
Raw beef meats (375 g)	Fresh raw beef	5	0	0	0	0	0
	Frozen then thawed beef	5	0	0	0	0	0
	Total	10	0	0	0	0	0
Dairy products (25 g)	Raw milk products	5	0	0	0	0	0
	Pasteurized products	5	0	0	0	0	0
	Raw milk cheeses	5	0	0	0	0	0
	Total	15	0	0	0	0	0
Fruits and vegetables (25 g)	Fresh and frozen produces	5	0	0	0	0	0
	Fresh and frozen sprouts or baby leafy greens	5	0	0	0	0	0
	Fresh raw, frozen fruit	5	0	0	0	0	0
	Total	15	0	0	0	0	0
Environmental samples (25 g or surface)	Surfaces	5	0	0	0	0	0
	Process water	5	0	0	0	0	0
	Dusts	5	0	0	0	0	0
	Total	15	0	0	0	0	0
All Categories		70	0	0	0	0	0

PA = Positive Agreement
NA = Negative Agreement

PD = Positive Deviation
ND = Negative Deviation

PPND = Presumptive Positive Negative Deviation
PPNA = Presumptive Positive Negative Agreement

Salmonella Enriched in mEHEC®

An additional 79 inoculated food items (alternative enrichment only, no PCR analysis) were evaluated after 12 and/or 18 hours of incubation by a direct streak from the primary enrichment onto the 3 different agars, see **Figure 3**. All test portions analyzed by the alternative method were also confirmed following ISO 6579:2017 reference method at 18 hours. Reference and alternative method results were in agreement; see **Table 2**.

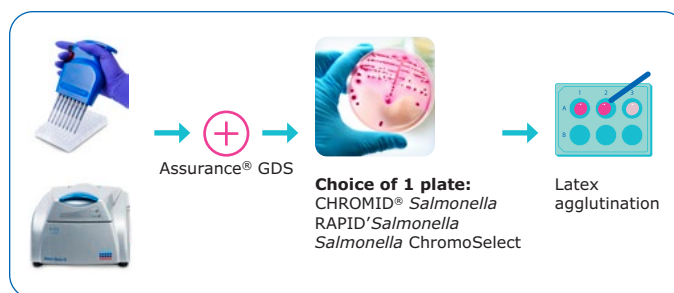


Figure 3. Assurance® GDS *Salmonella* Alternative Confirmation Workflow

Table 2. Salmonella Sample Results Between the Reference and Alternative Confirmation Methods, mEHEC®

Category	Type	PA	NA	PD	ND	PPND	PPNA
Ready-to-eat, ready-to-reheat poultry products	Cooked meat products	9	15	0	0	0	0
	Fermented or dried meat products	9	14	0	0	0	0
	Canned poultry products	10	10	0	0	0	0
	Total	28	39	0	0	0	0
Multi-component foods	Composite processed foods (cooked)	8	13	0	0	0	0
	Ready-to-reheat food; refrigerated, frozen and dry	8	12	0	0	0	0
	Composite foods with substantial raw ingredients	14	6	0	0	0	0
	Total	30	31	0	0	0	0
Raw poultry	Fresh poultry meat (un-processed)	12	8	0	0	0	0
	Ready-to-cook poultry products (processed)	9	11	0	0	0	0
	Total	21	19	0	0	0	0
All Categories		79	89	0	0	0	0

PA = Positive Agreement
NA = Negative Agreement

PD = Positive Deviation
ND = Negative Deviation

PPND = Presumptive Positive Negative Deviation
PPNA = Presumptive Positive Negative Agreement

Salmonella Enriched in BPW

An additional 115 inoculated food items (alternative enrichment only, no PCR analysis) were evaluated after 22 hours of incubation by spread plating IMS beads onto the 3 different agars, see **Figure 4**. All test portions analyzed by the alternative method were also confirmed following ISO 6579:2017 reference method 24 hours. Reference and alternative method results were in agreement; see **Table 3**.

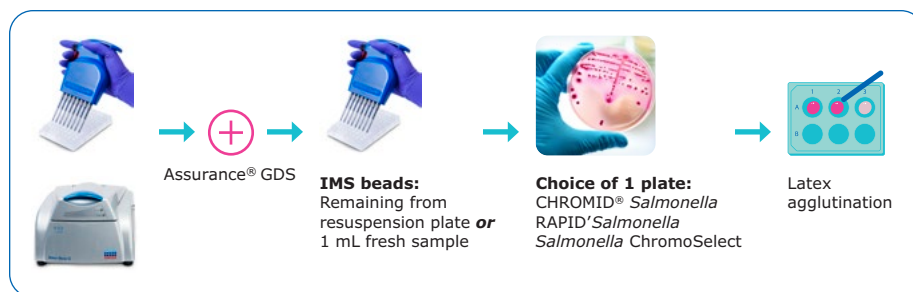


Figure 4. Assurance® GDS *Salmonella* Alternative Confirmation Workflow

Table 3. Salmonella Sample Results Between the Reference and Alternative Confirmation Methods, BPW

Interpretation	Selective Agar	PA	NA	PD	ND	Total confirmed positive samples (PA+PD)	% samples confirmed
IMS 1 mL vs. Reference Method	CHROMID® <i>Salmonella</i>	79	58	5	16	84	78
	RAPID® <i>Salmonella</i>	78	61	2	17	80	74
	<i>Salmonella</i> ChromoSelect	81	45	18	14	99	92
	All three plates	89	44	19	6	108	100
Resuspension IMS vs. Reference Method	CHROMID® <i>Salmonella</i>	76	60	3	19	79	77
	RAPID® <i>Salmonella</i>	77	60	3	18	80	78
	<i>Salmonella</i> ChromoSelect	82	49	14	13	96	94
	All three plates	86	44	16	9	102	100

PA = Positive Agreement
NA = Negative Agreement

PD = Positive Deviation
ND = Negative Deviation

Results: Specificity Study

E. coli O157:H7 (EHEC) Enriched in mEHEC®

	CHROMagar™ O157	CT-SMAC	EC O157:H7 ChromoSelect
Inclusivity	100/100	100/100	100/100
Exclusivity	0/100	0/100	0/100

A total of 100 strains were tested for inclusivity. All 100 of these strains showed the expected positive result. Zero (0) strains showed a negative result.

A total of 100 strains were tested for exclusivity. All 100 of these strains showed the expected negative result. Zero (0) strains showed a positive result.

Salmonella Enriched in mEHEC® and BPW

	CHROMID® Salmonella	RAPID® Salmonella	Salmonella ChromoSelect
Inclusivity	151/151	151/151	151/151
Exclusivity	0/100	0/100	0/100

A total of 151 strains were tested for inclusivity. All 151 of these strains showed the expected positive result. Zero (0) strains showed a negative result.

A total of 100 strains were tested for exclusivity. All 100 of these strains showed the expected negative result. Zero (0) strains showed a positive result.

Summary

For both EHEC and *Salmonella*, all contaminated samples were successfully confirmed using the alternative methods. In addition, all inclusivity strains were accurately detected, and all exclusivity strains were accurately excluded.

Significance

Fast isolation and confirmation of target pathogenic organisms is critical for the rapid release of food products. In these studies, the rapid alternative microbiological confirmation methods for *E. coli* O157:H7 and *Salmonella* utilizing chromogenic agars were selective and specific.



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