Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

Detection of Shiga Toxin *Escherichia coli* (STEC) in Large Test Portions of Raw Ground Meat and Vegetables by Immunomagnetic Separation and qPCR

Marion Bouvier[1], Mélissa Canizares[1], Lionel Meyer[2], and Delphine Thevenot-Sergentet[1]
(1) Université de Lyon, VetAgro Sup, LNR *E. coli* (incl. STEC), Marcy l'Etoile, France,
(2) Millipore SAS, Molsheim, France.

Introduction

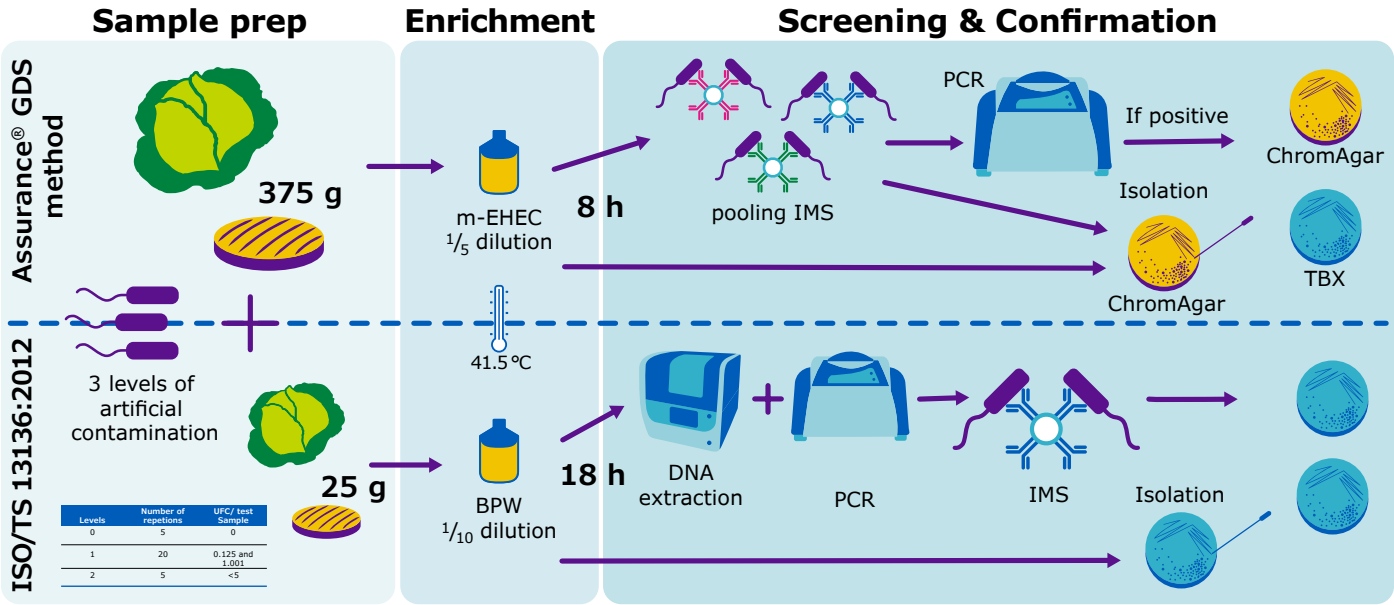
The aim of this study was to evaluate the performance of the Assurance® GDS method combining immunomagnetic separation (IMS) and qPCR for the analysis of large test portions (up to 375 g) of vegetables and meat. Performance was compared with the reference method ISO/TS-13136:2012 and the validated 25 g sample size.

Methods

A total of 120 samples of meat and vegetables were analyzed using both methods, including 20 uninoculated and 100 samples spiked with stressed cells at positive and fractional levels of different STEC strains.

The alternative method includes short, 8 h enrichment in proprietary broth ($^1/_5$ dilution) at 41.5 °C followed by screening of eae, stx1 & stx2, O157:H7 markers, and other serogroup identification. Following, cultural confirmation was performed on two selective agars by direct plating and using IMS beads.

For the reference method and sample size, enrichment was performed in BPW ($^1/_{10}$ dilution) at 41.5 ºC for 18 h.



Pathogen Screening Kit: Assurance® GDS MPX Top 7 STEC (Cat. No. 71015BC)

Results

- Results showed that 70% and 62% of the all samples spiked were detected and confirmed by the alternative method and the reference method, respectively (**Table 1**).
- The $RLOD_{50}$ ratios (= LOD_{50} alternative/LOD_{50} reference) ranged between 0.35 and 0.96 for the four food items tested.
- The performance of the methods and sample sizes were considered equivalent ($RLOD_{50}$ < 2.5 according ISO 16140-2:2020).
- 100% of PCR hits were confirmed by strain isolation on agar plates.

Matrix	Serotype	Method	Test Sample	LOD_{50}*	ROLD
Lettuce	0111:H8	LNR method adapted from TS13136	25 g	0.418	0.742
		Assurance® GDS Method	375 g	0.31	
Frozen Minced Meat	0145:H28	LNR method adapted from TS13136	25 g	.0544	.035
		Assurance® GDS Method	375 g	0.12	
Spinach	0157:H7	LNR method adapted from TS13136	25 g	0.515	0.96
		Assurance® GDS Method	375 g	0.522	
Fresh Ground Meat with 20% fat	0111:H8	LNR method adapted from TS13136	25 g	0.343	0.726
		Assurance® GDS Method	375 g	0.374	

Table 1: LOD results.

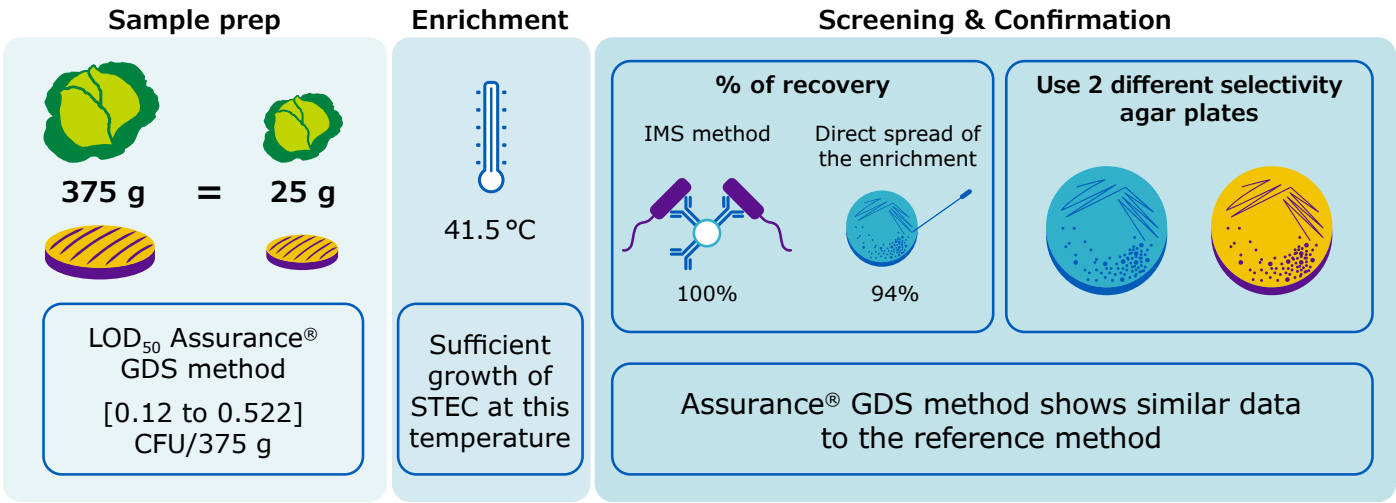
* LOD and RLOD were was calculated using the Excel file on the ISO website (http://standards.iso.org/iso/16140)

Conclusion

The alternative method using IMS + PCR technology allowed rapid STEC detection (8 h) of larger sample sizes (375 g) from beef and vegetables with equivalent performance to the reference method.

The IMS step also contributed to improve the cultural confirmation of STEC positive samples.

Key Features



Request more information

SigmaAldrich.com/assurance-gds-info

MilliporeSigma
400 Summit Drive
Burlington, MA 01803

MS_AN12249EN Ver. 2.0
49772
01/2024