TECHNICAL INFORMATION

STEC Detection and Identification

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NeoSEEK™ approach to STEC detection and identification

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Introduction

Enterohaemorrhagic *Escherichia coli* (EHEC) are recognized as the primary cause of hemorrhagic colitis (HC) or bloody diarrhea, which can progress to the potentially fatal hemolytic uremic syndrome (HUS). EHEC are typified by the production of Shiga toxins (Stx). There are many serotypes of Stx-producing *E. coli* (STEC), but only those that have been clinically associated with HC are designated as EHEC. As a group, EHEC are a subset of STEC and comprised of pathogenic strains, of which *E. coli* O157:H7 is the prototypic strain.

Prior to June 2012, only *E. coli* O157:H7 was regulated as an adulterant by United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS). However, there are several well-known EHEC strains that have caused illness worldwide (e.g., O26, O45, O103, O111, O121 and O145). After June 2012, these six EHEC strains were declared adulterants by USDA-FSIS and now are required to be tested for in beef trim. Situations may arise, such as food testing associated with a non-O157 EHEC illness outbreak, where the analyst may need to pursue the identification of these probable STECs (Feng and Weagant, 2011). Given the testing guidelines for these non-O157 STEC, there are several limitations that exist, including the number of genetic markers that can be determined by real-time polymerase chain reaction, the requirement for a pure culture isolate, expensive labeled primers, time, cost of reagents, inconsistent performance of antibody-coated beads, and the lack of effective selective or differential media to aid in identification.

Purpose

To develop a technology, identified as NeoSEEK, that allows for the inclusion of more genetic markers to help build a profile of the organisms to allow for quick and reliable detection and identification of STECs without the need of obtaining an isolate.

Methods

Neogen has adapted the Sequenom platform that is currently in use for high throughput SNP genotyping at GeneSeek®, Inc., a Neogen company based in Lincoln, Neb. This platform is used to detect the presence and identity of STEC from a sample. This method relies on MALDI-TOF Mass Spectrometry-based multiplexing. Specifically, this platform has three steps: 1) PCR amplification; 2) primer extension reaction to generate allele-specific DNA products of different masses; and 3) chip-based mass spectrometry for analysis of the extension products. The test is performed by looking for the presence/absence pattern of a particular set of target genes based on genetic profile Neogen has developed through PCR for the STECs of interest. Target genes include O-group, Stx1, 2, Eae, fliC targets for the most prevalent H-types and other virulence associated genes. A total of 70 independent targets are assayed including probing of two distinct sites for several of the above mentioned targets. The number and types of targets were selected to be able to gather enough evidence to make an identification of O-group (if present) and whether the O-group(s) detected are associated with pathogenic strains, without the need for single colony isolation. This method is one of the most error-free technologies for high throughput SNP typing. The goal was to develop a method that could be applied to, minimally, an isolated organism (colony), but preferably also to be used to detect and identify STECs in a complex beef sample enrichment matrix.

The PCR primers and extension probe primers that have been developed have an extremely high specificity for their targeted regions with very little to no background observed when testing from beef enrichments. This allows for reliable identification to be made.

Two studies were carried out, one in irradiated ground beef, the other in off-the-shelf ground beef from a local store. Single and double organism spikes were performed for each beef type in modified Tryptic Soy Broth (TSB) with novobiocin and in unsupplemented TSB. For the double spikes, two organisms from the O-groups of interest were used with at least one being an STEC. The spiked samples were blind coded and the mass spec results were read to identify the spiked organism(s) as well as STEC/non-STEC differentiation.

Data

Table 1. Summary of identification of blind-coded samples from two beef trials

			% Correct identification	
Matrix	**Media**	**No. of Spikes**	**O-group**	**STEC/non-STEC**
Irradiated beef	mTSBn	Single 28	28/28	28/28
	TSB	Single 28	28/28	28/28
Irradiated beef	mTSBn	Double 24	48/48	48/48
	TSB	Double 24	48/48	48/48
Off-the-shelf	mTSBn	Single 28	28/28	28/28
	TSB	Single 28	28/28	28/28
Off-the-shelf	mTSBn	Double 24	48/48	48/48
	TSB	Double 24	48/48	48/48

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Letter of No Objection

In September of 2012, Neogen received a letter of no objection for the use of NeoSEEK for STEC as a confirmation tool to detect Shiga toxin-producing strains of *E. coli* (STECs). For the letter of no objection, a study was conducted consisting of blinded spiked (low and high levels) ground beef trim samples. The analysis was done in accordance with MLG 5B.01. Aliquots of the enrichment cultures were sent to NeoSEEK for STEC analysis.

Table 2. Summary of results from the low and high inoculated samples. Inoculations of *E. coli* O157:H7 were removed from the analysis.

Low inoculum (1-2 cfu/test portion)	Correct identification MLG 5B.01	Correct identification NeoSEEK
True positive STEC (non-O157:H7)	15	17
False positive STEC	0	0
True negative (non-STECs)	4	4
False negative	2	0
Total inoculations	21	21
High inoculum (5-10 cfu/test portion)	**Correct identification MLG 5B.01**	**Correct identification NeoSEEK**
True positive STEC (non-O157:H7)	28	31
False positive STEC	0	0
True negative (non-STECs)	4	4
False negative	3	0
Total inoculations	35	35

Table 3. Comparison of results

	True positive (NeoSEEK vs. MLG 5B.01)		True negative (NeoSEEK vs. MLG 5B.01)	
Reported positive	48	43	0	0
Reported negative	0	5	8	8
Total	48	48	8	8
Relative sensitivity	100%	88.6%		
Relative specificity			100%	100%

Conclusion

- NeoSEEK for STEC assay is accurate. An STEC/EHEC presence or absence call can be accurately made from an enrichment culture without the need to obtain an isolate or to confirm the identification of an isolate.
- In the validation study, NeoSEEK for STEC met or exceeded MLG confirmatory method.
- NeoSEEK for STEC is a rapid assay. Results are available within 24 hours of receipt of the sample compared to greater than three days with MLG confirmation.
- GeneSeek, Inc, is accredited by the American Association of Laboratory Accreditation (A2LA) to perform the NeoSEEK for STEC test. GeneSeek has held an accreditation by A2LA to ISO/IEC 17025 since 2008.
- **Only confirmatory method with a letter of no objection.**

Reference

Feng, P and Weagant, S. D. (2011). Diarrheagenic Escherichia coli. In United States Food and Drug Administration Bacteriological Analytical Manual (online), www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM070080

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