



One Health Diagnostics™

# Detection of Shiga Toxin-producing *Escherichia coli* (STEC) on MicroTally® Swabs and in 375 g Samples of Ground Beef, Beef Trim and Leafy Greens by Real-Time PCR

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BAX® System X5

BAX® System Q7

foodproof®

microproof®

## Introduction

STEC are named after their ability to produce toxin genes known as *Shiga toxin 1* (*stx1*) and *Shiga toxin 2* (*stx2*). These toxins are associated with bloody diarrhea and potentially fatal hemolytic uremic syndrome. In addition, STEC can carry other virulence factors, like the *eae* gene, which codes for the adherence protein intimin. This protein, in combination with other virulence factors, is responsible for hemorrhagic colitis.

STEC are most commonly transmitted through contaminated water or food. The main source of STEC infections are cattle-based food products like raw ground beef and beef trim. Other foods such as raw or inadequately pasteurized milk, sprouts and vegetables can also cause STEC outbreaks.

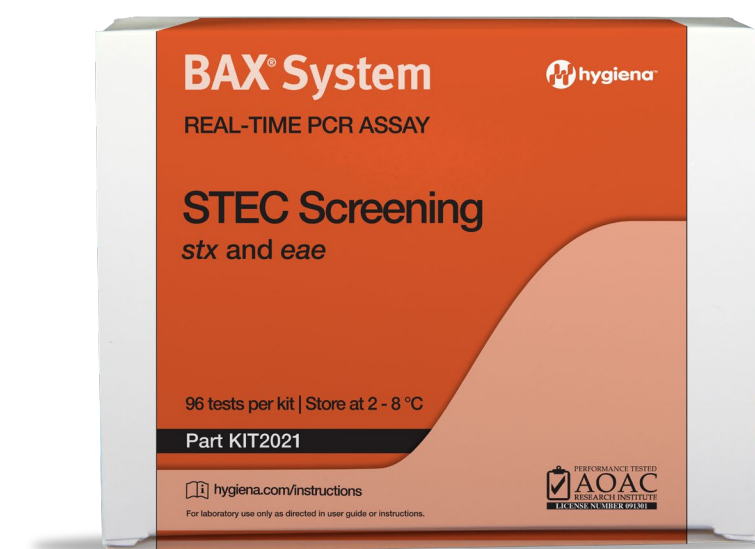
## Purpose

The aim of this study was to evaluate the compatibility of the foodproof® STEC Screening LyoKit in combination with the BAX® System MP enrichment broth, the BAX System lysis reagents and the BAX System Q7 Instrument compared to the BAX System procedure using the BAX System STEC Screening kit.

Manufactured by: Hygiena Diagnostics GmbH, Potsdam, Germany

BAX® System MP Media (2.5 kg: MED2003, 10 kg: MED2029)  
 BAX® System Lysis Reagents (Lysis Buffer: ASY2011, Protease: ASY2012)  
 BAX® System Real-Time STEC Screening (KIT2021)  
 foodproof® STEC Screening LyoKit (low profile: KIT230077)

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## Method

MicroTally® swabs, raw ground beef, raw beef trim and different types of leafy green samples were artificially contaminated with each of the seven major STEC serogroups (O157, O26, O45, O103, O111, O121, O145) at two target levels. Per sample type and strain, two samples were inoculated at a high level (10–20 CFU/test portion) and five samples at a low level (0.5–2 CFU/test portion). One sample per test matrix served as a negative control. Following inoculation, samples were refrigerated at 4 °C for 48 h to allow microorganisms to stabilize. MicroTally swabs were diluted in 250 mL or 400 mL BAX MP Media and 375 g samples of ground beef, beef trim and leafy greens in 1125 mL or 1500 mL pre-warmed BAX System MP Media and incubated at 42 °C for 7–24 h. Following enrichment, DNA extraction was performed using the BAX System lysis reagents. PCR analysis with the foodproof STEC Screening LyoKit and the BAX System STEC Screening Kit was conducted on a BAX System Q7 Instrument. In total, 400 enrichment cultures were analyzed at four different incubation times.

## Results

Both real-time PCR assays detected *stx* and *eae* genes according to the genetic characteristics of the STEC strains tested with comparable Ct values. Moreover, the *stx1* and *stx2* genes were correctly differentiated by the foodproof STEC Screening LyoKit. Exemplary results are presented in Tables 1 and 2.

## Significance

With the foodproof STEC Screening LyoKit, Hygiena offers the food industry a real-time PCR assay that detects the *eae* gene and the *stx* genes in one PCR reaction, with differentiation between the Shiga toxin genes *stx1* and *stx2*, and which is suitable for the analysis of a broad range of matrices with the workflow tested.

Table 1a. MicroTally Swabs Inoculated with *E. coli* O157:H7 and Analyzed with the BAX System STEC Screening Kit

Sample	Ct values - 7 h incubation time				Result	Ct values - 8 h incubation time				Result	Ct values - 10 h incubation time				Result	Ct values - 24 h incubation time				Result
	<i>stx</i>	<i>eae</i>	IPC			<i>stx</i>	<i>eae</i>	IPC			<i>stx</i>	<i>eae</i>	IPC			<i>stx</i>	<i>eae</i>	IPC		
1 neg	-	-	37.6		neg	-	-	37.8		neg	-	-	37.9		neg	-	-	36.9		neg
2 low	-	-	37.4		neg	-	-	37.4		neg	-	-	37.7		neg	-	-	37.8		neg
3 low	36.3	35.7	-		pos	35.1	34.5	-		pos	34.7	34.0	-		pos	30.0	29.5	-		pos
4 low	-	-	38.1		neg	-	-	36.9		neg	-	-	37.6		neg	-	-	36.8		neg
5 low	-	-	37.8		neg	-	-	37.1		neg	-	-	37.6		neg	-	-	37.5		neg
6 low	-	-	38.2		neg	-	-	37.4		neg	-	-	37.4		neg	-	-	37.0		neg
7 high	35.5	34.6	-		pos	33.6	32.9	-		pos	33.5	33.0	-		pos	30.1	29.6	-		pos
8 high	32.5	31.5	-		pos	30.6	29.8	-		pos	28.8	28.4	-		pos	26.6	26.1	-		pos

Table 1b. MicroTally Swabs Inoculated with *E. coli* O157:H7 and Analyzed with the foodproof STEC Screening LyoKit

Sample	Ct values - 7 h incubation time				Result	Ct values - 8 h incubation time				Result	Ct values - 10 h incubation time				Result	Ct values - 24 h incubation time				Result
	<i>stx1</i>	<i>stx2</i>	<i>eae</i>	IPC		<i>stx1</i>	<i>stx2</i>	<i>eae</i>	IPC		<i>stx1</i>	<i>stx2</i>	<i>eae</i>	IPC		<i>stx1</i>	<i>stx2</i>	<i>eae</i>	IPC	
1 neg	-	-	-	30.6	neg	-	-	-	30.9	neg	-	-	-	30.7	neg	-	-	-	30.9	neg
2 low	-	-	-	30.6	neg	-	-	-	30.6	neg	-	-	-	30.5	neg	-	-	-	31.0	neg
3 low	36.0	33.6	36.3	30.5	pos	34.7	33.2	34.9	30.5	pos	34.5	32.4	34.0	30.5	pos	30.0	28.0	29.3	32.4	pos
4 low	-	-	-	30.6	neg	-	-	-	30.8	neg	-	-	-	30.8	neg	-	-	-	30.8	neg
5 low	-	-	-	30.9	neg	-	-	-	30.6	neg	-	-	-	30.6	neg	-	-	-	31.0	neg
6 low	-	-	-	30.6	neg	-	-	-	30.8	neg	-	-	-	30.7	neg	-	-	-	30.9	neg
7 high	35.2	33.3	35.6	30.7	pos	33.2	31.7	32.6	31.1	pos	32.9	31.4	32.4	30.9	pos	29.9	28.1	29.1	32.2	pos
8 high	31.9	29.6	31.0	32.4	pos	30.2	28.4	29.1	32.0	pos	28.2	26.6	26.7	31.2	pos	26.1	24.4	24.9	-	pos

Table 2a. Kale Samples Inoculated with *E. coli* O157:H7 and Analyzed with the BAX System STEC Screening Kit

Sample	Ct values - 7 h incubation time				Result	Ct values - 8 h incubation time				Result	Ct values - 10 h incubation time				Result	Ct values - 24 h incubation time				Result
	<i>stx</i>	<i>eae</i>	IPC			<i>stx</i>	<i>eae</i>	IPC			<i>stx</i>	<i>eae</i>	IPC			<i>stx</i>	<i>eae</i>	IPC		
1 neg	-	-	37.4		neg	-	-	38.0		neg	-	-	37.6		neg	-	-	37.0		neg
2 low	-	-	37.6		neg	-	-	38.3		neg	-	-	37.9		neg	-	-	37.2		neg
3 low	-	-	37.6		neg	-	-	38.4		neg	41.1	33.1	-		pos	39.4	30.0	-		pos
4 low	-	-	37.4		neg	-	-	38.5		neg	-	-	38.6		neg	-	-	37.6		neg
5 low	-	-	37.1		neg	37.0	36.1	-		pos	33.8	32.6	-		pos	32.4	31.4	-		pos
6 low	-	-	37.5		neg	38.5	37.2	-		pos	32.1	31.1	-		pos	31.5	30.1	-		pos
7 high	-	-	38.0		neg	39.4	38.7	-		pos	29.4	28.8	-		pos	31.3	30.5	-		pos
8 high	41.8	41.6	36.7		pos	31.4	30.8	-		pos	29.3	28.7	-		pos	34.4	33.7	-		pos

Table 2b. Kale Samples Inoculated with *E. coli* O157:H7 and Analyzed with the foodproof STEC Screening LyoKit

Sample	Ct values - 7 h incubation time				Result	Ct values - 8 h incubation time				Result	Ct values - 10 h incubation time				Result	Ct values - 24 h incubation time				Result
	<i>stx1</i>	<i>stx2</i>	<i>eae</i>	IPC		<i>stx1</i>	<i>stx2</i>	<i>eae</i>	IPC		<i>stx1</i>	<i>stx2</i>	<i>eae</i>	IPC		<i>stx1</i>	<i>stx2</i>	<i>eae</i>	IPC	
1 neg	-	-	-	30.8	neg	-	-	-	30.6	neg	-	-	36.6	30.5	neg	-	-	-	30.9	neg
2 low	-	-	-	31.1	neg	-	-	-	31.4	neg	-	-	-	30.6	neg	-	-	-	31.2	neg
3 low	-	-	-	31.2	neg	-	-	41.0	31.5	neg	42.4	41.3	31.3	30.8	pos	42.2	-	28.4	31.2	pos
4 low	-	-	-	31.1	neg	-	-	-	31.2	neg	-	-	-	31.0	neg	-	-	-	31.3	neg
5 low	-	-	-	31.1	neg	36.4	33.5	36.7	30.7	pos	32.9	31.3	31.8	31.0	pos	32.5	31.6	33.1	31.6	pos
6 low	-	-	-	31.1	neg	37.9	36.1	37.8	31.0	pos	31.4	29.7	30.1	32.3	pos	31.4	30.2	31.7	32.7	pos
7 high	-	-	-	31.2	neg	38.1	37.2	38.5	31.0	pos	28.7	26.6	27.3	31.9	pos	31.4	30.1	32.9	32.4	pos
8 high	41.4	38.8	36.7	30.9	pos	31.0	28.3	30.1	32.9	pos	29.1	27.1	27.7	31.9	pos	33.4	32.0	34.9	31.1	pos