# A high throughput method for the detection of STEC Top7 in meat samples

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## Objective

*E. coli* O157:H7 and the top six (O26, O45, O103, O111, O121, and O145) non-O157 Shiga toxin-producing *Escherichia coli* (STEC) have emerged as important public health treats. Pall GeneDisc® technologies has always been a pioneer in the development of rapid, easy to use and accurate tests for the simultaneous detection of pathogenic STEC. The GeneDisc® method has recently received the first AOAC approval for its STEC Top6 and O157 method in 375 g ground beef and beef trim in compliance with the MLG5B method.

Here, we propose a complete work-flow combined with a one step multiplex PCR based method using GeneDisc® technology to screen STEC O157 and non-O157 in food industry. The two major advantages of this new approach are to drastically reduce the rate of presumptive positive results and the time to result. The combination of the Top7 targets to specific virulent genes, allows a higher level of discrimination, and an enhanced focus on the highest risk STEC strains.

## Experimental treatments

### 1/ Workflow

The workflow of the GeneDisc® STEC Top7 method is described in the Figure 1.
Briefly, 375g raw beef samples are enriched for 10 hours in Buffer Peptone water. DNA is extracted by 10 minutes heating using GeneDisc® Extraction Pack for Food samples. DNA extracts are then analyzed on a STEC Top 7 GeneDisc® plate and results are automatically displayed by the GeneDisc® Cycler.

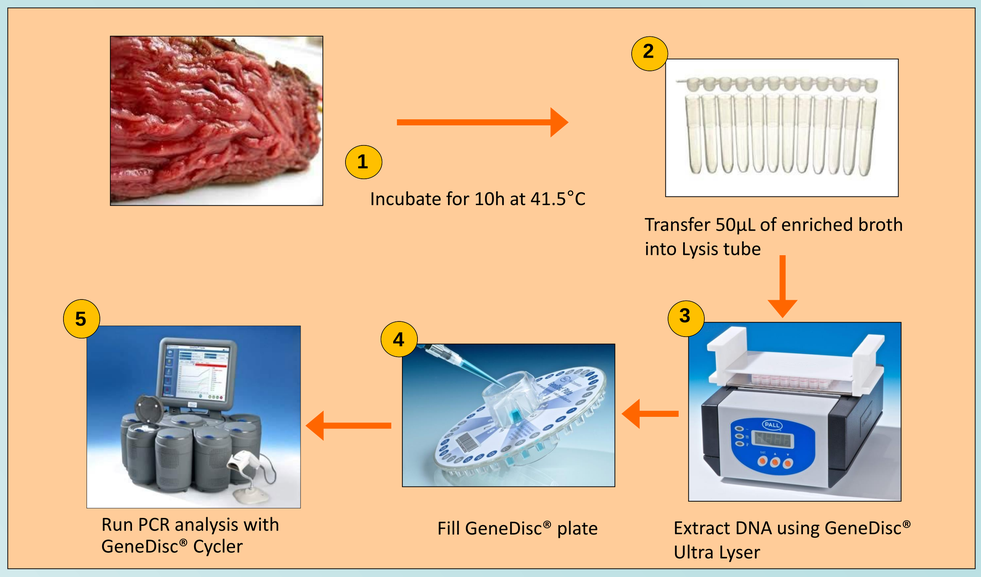


**Figure 1:** Workflow of the GeneDisc® STEC Top7 method.

### 2/ Colony testing

The STEC Top7 GeneDisc® could also be used for a rapid colony confirmation according to the protocol described in Figure 2.

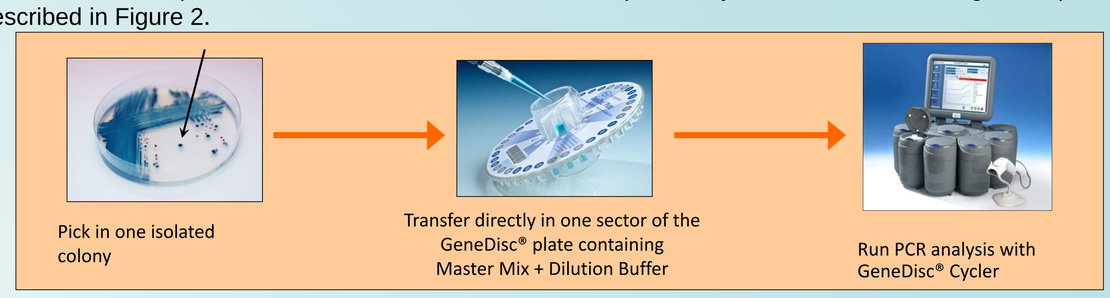


**Figure 2:** Workflow of colony confirmation.

### 3/ DNA Preparation

3.1 Specificity study

The specificity of each PCR assay has been tested by analysis of DNA extracts from 26 *E. coli* reference strains and 96 *E. coli* colonies isolated from food samples, according to the protocol described in the Figure 2. For the reference strains, DNA was extracted and purified on silica column from o/n pure cultures. DNA extracts were then calibrated by spectrophotometer measures (NanoDrop™, Thermo scientific). PCR analyses were performed with 25 and 25,000 GU/PCR well in order to test inclusivity and exclusivity, respectively.

3.2 Sensitivity study

The sensitivity of each PCR assay was evaluated with DNA extracts from enriched ground beef samples (375 g), artificially contaminated by calibrated and purified DNA. The spiking was done with DNA extracts from each pathogenic STEC Top7 strain at 10 and 25 GU/PCR well. For each DNA extract and each DNA quantity, 12 independent DNA solutions were realized and they were analyzed with 3 GeneDisc® Cyclers, *i.e.* 36 analyses/PCR assay.

3.3 Artificially contaminated samples

Validation study of the entire method was performed with ground beef and beef trim samples artificially contaminated by O26, O103 or O157 at the LOD, *i.e.* 25 GU/PCR well (3E+04 CFU/mL). The contamination was done after enrichment, in 1 mL enriched sample, and then the DNA was prepared according to the procedure described in the Figure 1.

## Validation of performances

Performances of these PCR assays have been validated in terms of specificity and sensitivity. Validation study of the entire method has been performed with artificially contaminated samples.

### 1/ Specificity

The PCR results with DNA extracts (reported in Table 1) from the reference strains and the food isolates showed that all PCR targets were successfully detected and no false positive results were obtained.

| Strain Origin | Strain type | Top 7 | | | | | | | Non Top7 |
|---|---|---|---|---|---|---|---|---|---|
| | | O26 | O45 | O103 | O111 | O121 | O145 | O157 | |
| Reference strains | Pathogenic STEC | 3 | 2 | 2 | 2 | 2 | 2 | 4 | 8 |
| | EPEC (*stx*-) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Food samples | Pathogenic STEC | 40 | 0 | 12 | 3 | 0 | 1 | 0 | 0 |
| | EPEC (*stx*-) | 39 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Results | | 100 % compliant | | | | | | | |

**Table 1:** Specificity evaluation of the STEC Top7 GeneDisc® over 118 *E. coli* strains.

### 2/ Sensitivity

The results of sensitivity study, in term of presence percentage, are summarized in the Table 2.
The limit of detection (LOD), corresponding to the lowest DNA quantity which could be detected at 90% confidence has been established at 25 GU/PCR well for each PCR assay. That is theoretically equivalent to 3E+04 CFU/ml after enrichment of 375 g ground beef samples.

| GU / well | Presence % (n = 36) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | O serogroups | | | | | | | *stx* | | |
| | 26 | 45 | 103 | 111 | 121 | 145 | 157 | 1 | 2 | 1-2 |
| 10 | 88 | 99 | 64 | 96 | 100 | nd | 100 | 73 | 98 | 100 |
| 25 | 99 | 100 | 99 | 100 | 99 | 93 | 98 | 100 | 100 | 100 |

**Table 2:** Sensitivity evaluation of the STEC Top7 GeneDisc® at 10 and 25 GU/PCR well, in ground beef samples

### 3/ Artificially contaminated samples validation

The results reported in Table 3 showed 100% presence for all targeted genes whatever the matrix was (ground beef or beef trim). For a contamination level of 25 GU/PCR well, *i.e.*, 3E+04 CFU/mL of enriched raw beef meat, the Ct values were comprised between 29.3 (O103) and 30.7 (O157), and the amplitudes are comprised between 2,626 (O157) and 5,572 RFU (O103). There was no significant difference between both matrices.

| Matrices / Spiking | Targets | n | Mean Ct | SD Ct | Mean Ampli (Relative Fluorescence Unit) | Presence % |
|---|---|---|---|---|---|---|
| Ground beef 25 GU/PCR well | Inhibition control | 18 | 30.5 | 0.4 | 2103 | 100 % |
| | O157 | 6 | 30.7 | 0.6 | 2626 | |
| | O26 | 6 | 30.3 | 0.4 | 5478 | |
| | O103 | 6 | 29.4 | 0.5 | 4879 | |
| | *stx*1-2 | 6 | 30.2 | 0.4 | 3823 | |
| Beef trim 25 GU/PCR well | Inhibition control | 18 | 30.4 | 0.3 | 1871 | 100% |
| | O157 | 6 | 30.5 | 0.5 | 3173 | |
| | O26 | 6 | 30.3 | 0.6 | 5142 | |
| | O103 | 6 | 29.3 | 0.5 | 5572 | |
| | *stx*1-2 | 6 | 30.0 | 0.3 | 5223 | |

**Table 3:** Sensitivity evaluation of the entire STEC Top7 GeneDisc® method at 25 GU/PCR well, in raw beef samples.

## Evaluation of the entire method on naturally contaminated samples

Evaluation of the entire method was also realized on 400 ground beef and 150 beef trim processed in 4 facilities across the US. The new GeneDisc® method was compared to the USDA-FSIS MLG 5B.01 method. 375 g of each sample were diluted in either 1.5 L of mTSB or 975 mL of mTSB with novobiocin (mTSBn). Samples were incubated at 42°C. The incubation time depended on the enrichment broth: 12 h for the mTSB, 15 h for the mTSBn. Presumptive positive samples were confirmed according to reference methods (USDA-FSIS MLG 5B.01 or 5.05). The results are reported in the Table 4.

Among 400 ground beef samples, the STEC Top7 GeneDisc® method gave only 1 presumptive positive sample from the mTSBn enrichment. This presumptive result was confirmed by culture method. The USDA-FSIS MLG 5B.01 method detected 6 potential positive samples. None was confirmed by the culture method*.

Among 150 beef trim samples, the GeneDisc® method detected 1 potential positive sample enriched either in mTSB or in mSTBn which were confirmed by the culture method. The USDA-FSIS MLG 5B0.1 method detected 1 other potential positive sample enriched in mTSBn which was not confirmed.

The STEC Top7 GeneDisc® approach and the reference method gave 3 and 7 presumptive positive samples, respectively. The prevalence of the GeneDisc® and the FSIS MLG5B.01 methods were 0.36 % and 1.27%, respectively.

| Sample type | Enrichment broth | GeneDisc® STEC Top7 method | | USDA-FSIS MLG 5B.01 | |
|---|---|---|---|---|---|
| | | PCR positive samples | Confirmation | PCR positive samples | Confirmation |
| Ground Beef | mTSB (12 h) | 0/400 | NA | NA | NA |
| | mTSBn (15 h) | 1/400 | 1/1 (O157) | 6/400 | 1/400* |
| Beef Trim | mTSB (12 h) | 1/150 | 1/1 (O26) | NA | NA |
| | mTSBn (15 h) | 1/150 | 1/1 (O26) | 1/150 | 0/1 |
| Prevalence | | 0.36 % | 0.36 % | 1.27 % | 0.18% |

**Table 4:** Evaluation of the entire STEC Top7 GeneDisc® method with 550 naturally contaminated raw beef meat samples.

* USDA-FSIS MLG 5B.01 does not include a screen for *E. coli* O157. However, one *E.coli* O157 has been confirmed.

## Significance

This work demonstrates that this new method for STEC Top 7 detection is specific, sensitive and reliable. Evaluation of the entire method on naturally contaminated samples also demonstrated method accuracy.

Moreover, the high throughput sample prep allows to process 96 aliquoted samples in less than 2 h. Hence, it can be used for routine screening of beef meat samples, on standard sample size used by industrials, for STEC Top7 serogroups with a time to result inferior to 12 hours.

In conclusion, the high level of discrimination of the GeneDisc® method combined with its high throughput capability provide Food industrials with a robust, complete and cost effective tool for the monitoring of STEC risk.