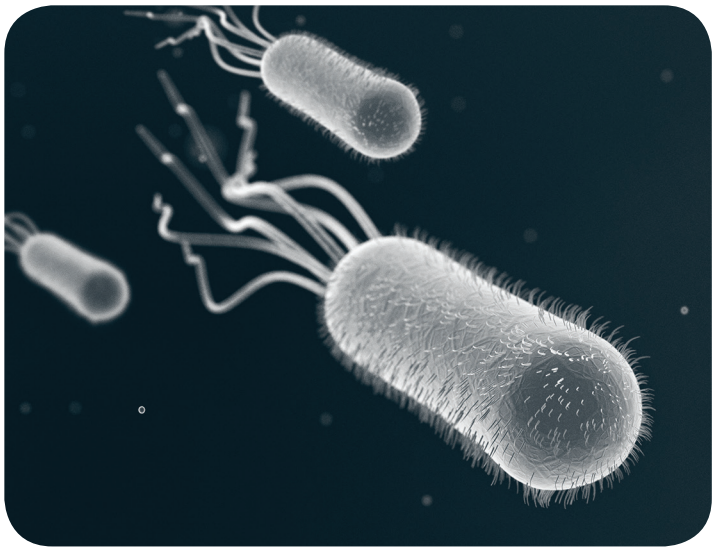
FOOD & BEVERAGE

Technical Bulletin

GeneDisc® STEC Solutions for STEC Monitoring in Raw Beef Meat Samples

Overview



Escherichia coli (E. coli) bacteria are commonly found in the gut of humans and warm-blooded animals. Most strains of *E. coli* are harmless. However, Shiga toxin-producing *E. coli* (STEC) can cause severe foodborne disease. STEC contamination occurs during the slaughter and processing of raw beef products. It is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products.

For many years the red meat industry has been interested in STEC in relation to protecting public health and to meet the requirements of countries receiving exported beef.

The Pall GeneDisc methods for STEC offer a very fast approach for testing and answering the various control practices faced by meat producers. In addition, the system's ease of use facilitates smooth implementation of the testing on-site.

History of the GeneDisc Solution for STEC Detection

Detection of STEC contamination in a sample is driven by the ISO/TS 13136 or the MLG 5C standards, describing a real-time PCR-based approach for the detection of the major serogroups: O26, O45, O103, O111, O121, O145 and O157 [1,2].

These stepwise methods consist of an enrichment step followed by DNA extraction and real-time PCR analysis for the presence of stx and eae genes, the two virulent genes necessary to detect the presence of STEC.

In a second stage, *stx*- and *eae*-positive samples are subjected to a real-time PCR-based screening targeting the O group markers.

Finally, STEC isolations should be attempted from presumptive positive samples and the presence of STEC-associated genetic markers in the *E. coli* isolates should then be confirmed by PCR. Indeed, in a complex sample which may comprise a mixture of strains, the presence of virulent genes is not always indicative of the presence of a pathogenic STEC; those genes could belong to two different *E. coli* strains.

In the ISO 13136:2012 Technical Specification and the MLG 5C standard, the primers and probes used to amplify the *eae* gene were designed in the conserved region, while a considerable heterogeneity has been identified among the DNA sequences of the *eae* gene, especially in their 3'-end region. That has led to the classification of at least 18 *eae* subtypes [3]. Among these subtypes, *eae*-γ1, *eae*-β1, *eae*-ε, and *eae*-θ are specifically associated with the seven major STEC serotypes, as described below.

Table 1: *eae* subtypes and serotypes

aea Subtype	Associated STEC Serotype
γ1	O145:H28 – O157:H7
β1	O26:H11
ε	O45:H2, O103:H2, O121:H19
θ	O111:H8

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) developed and patented specific PCR assays enable specific detection of the four *eae* variants required for detecting the STEC Top7[4]. In partnership with Pall GeneDisc Technology, those assays were successfully transferred to the GeneDisc Plates STEC Top7 and STEC Plus, in combination with the *stx*1-2 genes and the Top7 O-groups.

This approach has been demonstrated to be more discriminating than detecting "universal" *eae* gene to identify the suspect raw beef samples that should be subjected to an isolation procedure for confirmation [5]. Among 1,739 samples, 115 were presumptive positive according to the MLG 5C reference method while only 51 were classified as positive with the combination of the *eae* variants.

Figure 1 describes the GeneDisc STEC method, including different GeneDisc Plates which enables either the ISO/TS 13136 and MLG 5C approaches (GeneDisc Plate STEC or STEC/*Salmonella* spp. & GeneDisc Plate EHEC 5 ID) or the alternative GeneDisc STEC method with the GeneDisc Plate STEC Top7.

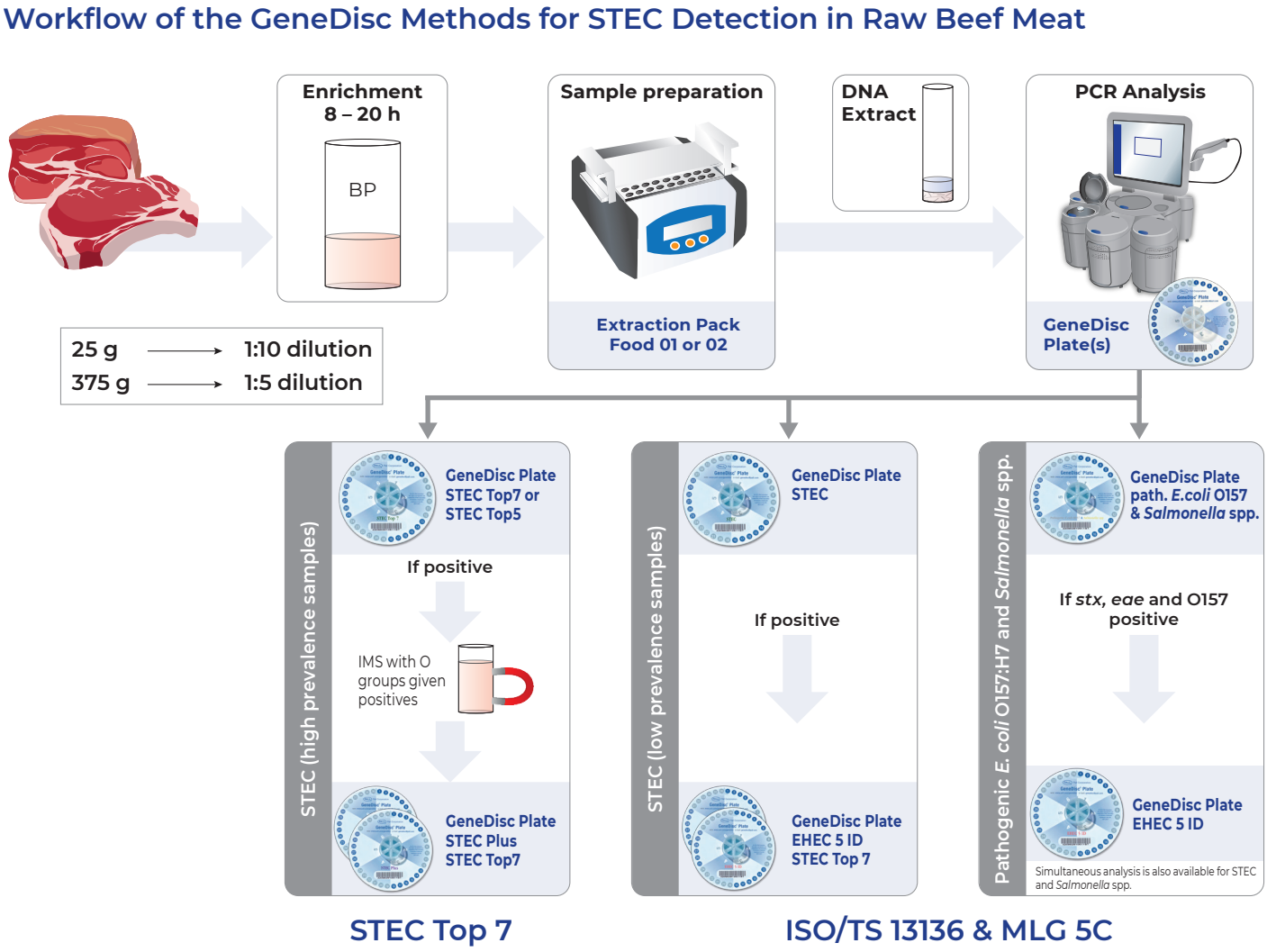


Figure 1: Workflow of the GeneDisc STEC methods.

Customer's Benefits

Two studies aiming to compare the GeneDisc method compliant with the ISO 13136 / MLG 5C and the alternative GeneDisc STEC Top7 method were carried out with 1,476 raw ground beef samples and 500 raw beef trim samples.

1) 1,476 ground beef samples (25 g) – Enrichment for 16 h at 37 °C

The results reported in Figure 2 show the high discrimination of the GeneDisc STEC Top7 screening, as the number of presumptive positive samples decreases from 71 (4.8 %) to 22 (1.4 %), compared with the methodology described in the ISO/MLG standards. Thus, the GeneDisc STEC Top7 approach enables the reduction of the number of presumptive positive samples by 69 %..

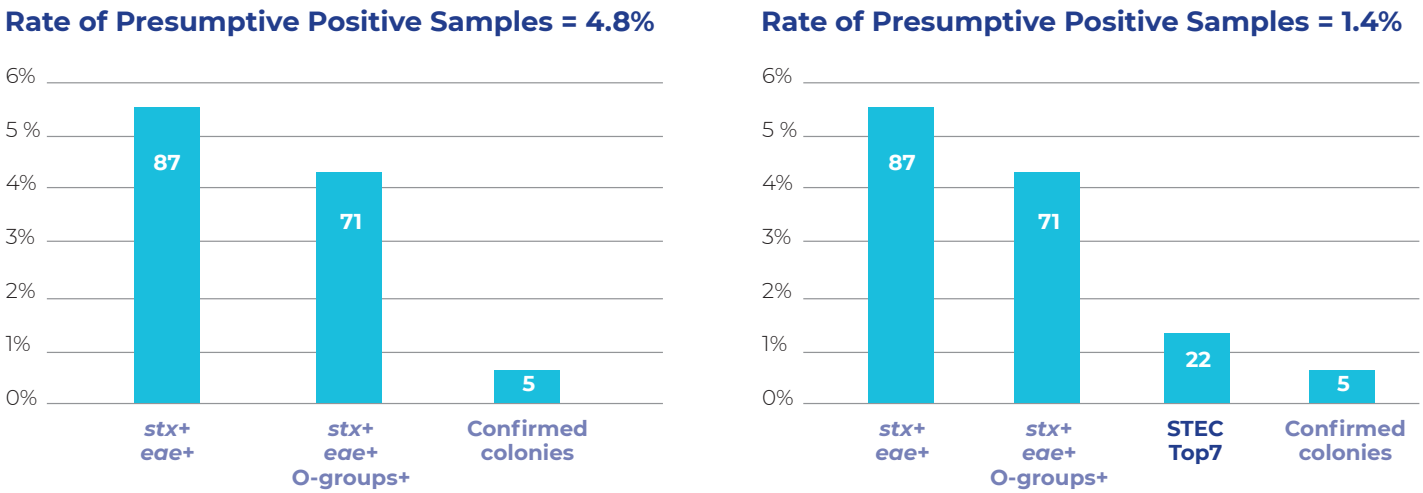


Figure 2: Rate of presumptive positive samples with (i) the GeneDisc method compliant with the ISO 13136 and/or MLG 5C standards (left) or (ii) the GeneDisc STEC Top7 method (right).

2) 500 beef trim samples (375 g) – Enrichment for 10 h at 41.5 °C

As shown in Figure 3, analysis of 500 raw beef trim samples led to 63 (12.6 %) presumptive positive samples, according to the ISO/MLG reference methods, while only 32 (6.4 %) presumptive positive samples were detected with the GeneDisc STEC Top7 method, corresponding to a reduction by 50 % of the number of samples to be confirmed by plating and PCR on characteristic colonies.

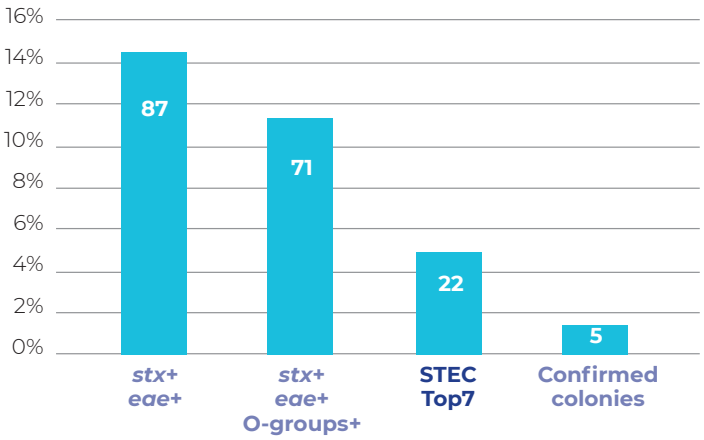


Figure 2: Rate of presumptive positive samples with the GeneDisc STEC Top7 method.

Conclusions

The GeneDisc STEC Top7 method is an accurate screening tool, based on the association of virulence factors to serogroups, which provides a lower rate of presumptive positives than any other available method.

This method enhances the workflow by significantly reducing the number of presumptive positive samples using a cutting-edge approach.

The GeneDisc Plate STEC Top7 combining all targets – serogroups and virulence factors – in a single ready-to-use device is easy to use, flexible and compatible with a high throughput routine analysis.

Bibliographic References

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