



Application Bulletin

Reducing Operating Cost for Pathogenic *E. coli* Testing in Raw Beef Meat with GeneDisc® Technology

Overview

South America is a leading region for cattle meat production with approximately 15 million tons produced annually. This large production supports high exports to various countries, South American exports for boneless cattle meat representing over 30% of the global quantity exported.¹

To sustain these exportations, producers must comply with country specific regulations, requirements and border controls. A common control point used in a growing number of countries is screening for pathogenic *Escherichia coli*. Variability of practices for this pathogen control from one country to another, combined with final customer requirements, poses a challenge for meat producers.

In 2015, all the thirteen border alerts or notifications listed by the European Rapid Alert System for Food and Feed (RASFF)² about frozen or chilled beef meat from South America were related to pathogenic *E. coli*. This highlights how critical control is to the industry.

Pall GeneDisc technology for pathogenic *E. coli* offers a flexible and cost effective 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.

The Challenge

Two South American beef meat producers export a large part of their production to the European Union, USA, Canada and Russia.

To answer border controls, customer requirements and to anticipate future local rules and regulations about pathogen testing, they both have initiated work on quality control testing for pathogenic *E. coli*.

The first one externalized testing to a service laboratory using a Polymerase Chain Reaction (PCR) system for detection of Shiga Toxic *E. coli* (STEC) virulence genes encoding for shigatoxins (*stx*) and intimin (*eae*). The drawbacks this customer experienced are extended time to results, high rate of false positive and high cost per test.

Figure 1: The GeneDisc System: GeneDisc Cycler (left) and GeneDisc Plates (right)



The second company chose to perform immunoassay tests for *E. coli* O157. As no information on virulence genes can be provided by this type of test, they additionally perform tests for virulence genes on an open PCR system. They have observed a high rate of false positive that were not confirmed by culture.

The Solution

To address the customers' need for a rapid, flexible and cost-effective method to test raw beef meat for STEC contamination, Pall offers a PCR based method using the GeneDisc system (Figure 1). This system offers sensitive and specific detection of food pathogens in a matter of hours by detecting specific DNA sequences. This extremely accurate tool can be easily implemented in an internal quality control laboratory. The equipment automation ensures reliability and ease of use for microbiological testing.

Two Testing Strategies

GeneDisc STEC product range includes ready-to-use reagents enabling two testing strategies to be followed.

Following reference methods

The reference methods for STEC detection — ISO/TS 13136³ and MLG5B⁴— are based on a two-step screening:

- First, virulence factors (*stx* and *eae*) are screened for
- Then if the virulence factors are positive, major serogroups are tested for. In Europe, STEC Top 5 serogroups include *E. coli* O157, O26, O103, O111 and O145. In the USA, serogroups O45 and O121 are added to this list making the STEC Top 7.

These workflows can be followed with dedicated GeneDisc Plates (e.g. GeneDisc Plate STEC and GeneDisc Plate EHEC 5 ID).

In addition, simultaneous detection of O157 serogroup and *Salmonella* spp. with the detection of these virulence genes is available.

Figure 2: The GeneDisc Plate STEC Top 7 associates screening of specific virulence genes to serogroups



Increasing level of discrimination

As raw beef meat may contain a pool of multiple *E. coli*, each harboring a different virulence gene (e.g. pool of 3 strains: *E. coli* 1 with *stx*, *E. coli* 2 with *eae* and *E. coli* 3 with O26), but none containing a combination of these factors, false presumptive positive can be obtained with the workflow described above.

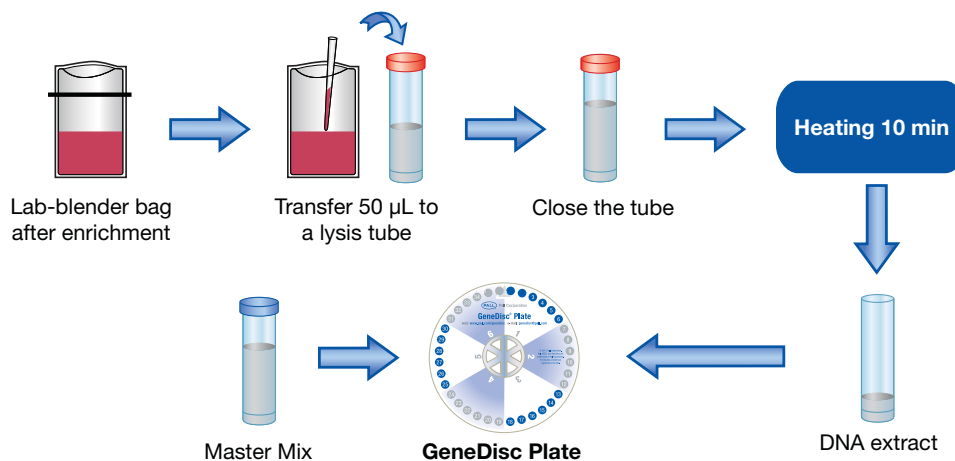
To overcome this limitation, Pall also offers a unique enhanced workflow with the GeneDisc STEC Top 7 method (Figure 2). With this cutting-edge approach, all targets — serogroups and virulence factors — are analyzed within one single PCR run. This accurate solution screens based on the association of virulence factors to serogroups and provides a lower rate of presumptive positive.

Testing Protocol

The operating procedure is identical for both GeneDisc strategies (strategy based on reference method and STEC Top 7 strategy):

- An initial enrichment of the 25 g or 375 g raw beef meat sample is performed in as short as 8 h.
- Then, the bacterial DNA is extracted. An example of a protocol applicable to raw beef meat is described in Figure 3.
- Finally, DNA extracts are analyzed in less than one hour with the GeneDisc system.

Figure 3: Workflow of the GeneDisc STEC and STEC Top 7 methods

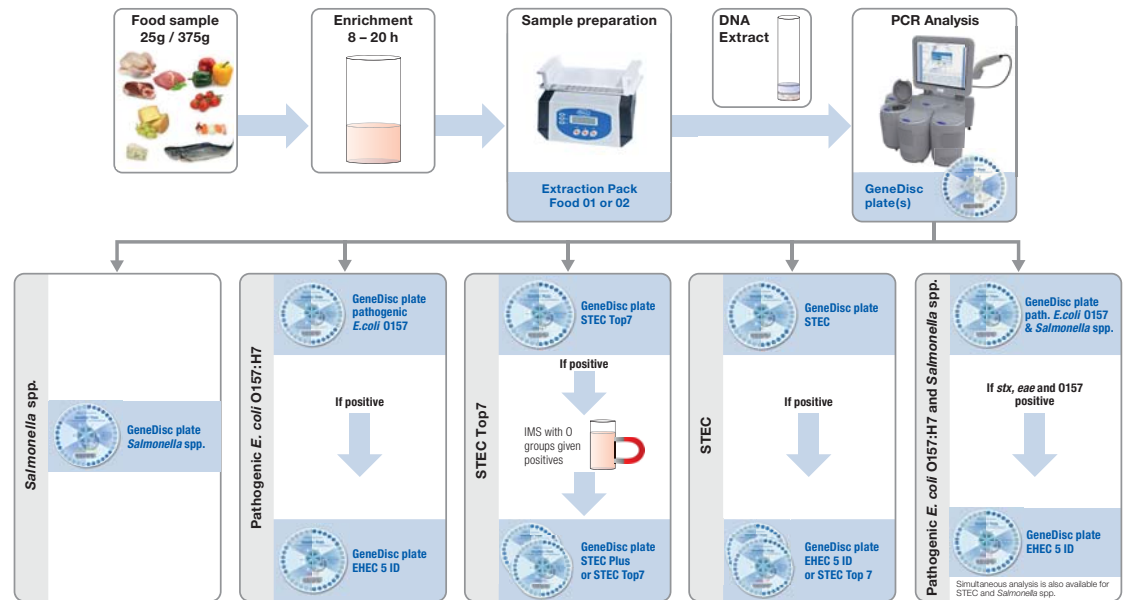


A serogroup screening is to be performed for the reference method when virulence genes screening provides positive results (Figure 4 – STEC).

A second screening after Immuno-Magnetic Separation (IMS) is performed for the GeneDisc STEC Top 7 method (Figure 4 – STEC Top 7). This enables further reduction of the rate of presumptive positives.

Simultaneous testing of STEC and *Salmonella* spp. does not require separate enrichment nor DNA extraction as it is directly performed on the same DNA extract obtained after one common enrichment (Figure 4).

Figure 4: Flexible testing options for *Salmonella* and pathogenic *E. coli* answer the various control practices faced by meat producers



The Benefits

Both producers experienced high improvements after implementing GeneDisc STEC solutions with their raw beef meat testing on raw materials, in-process samples and end products. They have reduced the cost per test by up to 70%. Cost reduction was achieved thanks to a combination of multiple factors:

- In-house testing is now possible with an easy-to-use and easy to integrate PCR platform for routine STEC testing. Only basic operator training is required to operate the system. By eliminating sample shipment time, they have reduced time to results.
- STEC Top 7 solution reduces presumptive positive rate and the cost of associated culture confirmations to perform.
- Simultaneous testing of STEC, *E. coli* O157 and *Salmonella* spp. rationalizes testing workflows and decreases hand-on cost and culture media cost.

In addition, they have reached a higher profitability by:

- Reducing risk of border or customer rejection by releasing product based on informative test results,
- Efficiently sorting their raw material and in-process products for further processing such as cooking meat if required.

Following integration, the GeneDisc system flexibility allowed the customers to expand their STEC testing to other samples such as vegetables and to move their *Listeria* testing on sausages to the GeneDisc platform.



About Pall Corporation

Pall Corporation is a global filtration, separation and purification leader providing solutions to meet the critical fluid management needs of customers across the broad spectrum of life sciences and industry. We work with our customers to advance health, safety and environmentally responsible technologies.

Pall Food and Beverage provides products and services to ensure product quality and maintain process reliability in beverage and food production. Our solutions also assist in consumer protection, waste minimization and reduction of operating costs.

References and Footnotes

- ¹ **Food and Agriculture Organization of the United Nations – Statistics Division (FAOSTAT) – 2013 Data:**
<http://faostat3.fao.org>
- ² **Rapid Alert System for Food and Feed (RASFF) :** ec.europa.eu/food/food/rapidalert/index_en.htm
- ³ **ISO/TS 13136:** Microbiology of food and animal feed — Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens — Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups
- ⁴ **U.S. Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook, MLG 5B:** Detection and Isolation of non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) from Meat Products and Carcass and Environmental Sponges



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