

Sorting Raw Milk Cost-effectively in a Dairy Process with GeneDisc® Technology

Overview

Raw milk harbors a wide variety of microorganism flora, which potentially includes pathogenic bacteria (e.g. *Listeria*, pathogenic *Escherichia coli* and *Salmonella*), which can cause foodborne illnesses.

The contamination of raw milk by pathogenic bacteria can occur:

- By direct contact at the dairy farm or during transportation
- During the milking process, if the udder of the animal is infected

As a result, the use of raw milk being mandatory for certain cheeses with protected designation; dairy companies are facing a real challenge.

The European Rapid Alert System for Food and Feed (RASFF)¹ listed 12 alerts related to ShigaToxic *E. coli* (STEC) in dairy products in 2014 making STEC the second most common alert for this type of product after *Listeria monocytogenes* (29 alerts) and before *Salmonella* spp. (8 alerts).

In addition, global reports of recent outbreaks associated to STEC have increased focus on this pathogen.

Therefore, detection of STEC is an increasing concern for dairy industries as a recall can:

- Generate high economic losses to the dairy company due to scrapping product and recall costs
- Heavily impact brand image
- Reduce significantly customer confidence in raw milk products

The Challenge

A dairy company, producing raw and non-raw milk cheeses from both cow and ewe milk wanted to implement a quality control point for STEC on incoming raw milk. This control would allow them to select dairy farms and to sort incoming raw material for proper use:

- Raw milk cheese process for non-contaminated milk
- Pasteurized or micro-filtered² milk cheese for potentially contaminated milk.

This initial sorting would prevent a late detection of contamination on final products that would lead to batch scrapping or recall.

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





Testing methods must be rapid as storage time of incoming raw milk before being processed is limited.

Detection method for STEC should also provide the lowest rate of false positives possible because:

- Production volume of raw milk cheese is higher than pasteurized or micro-filtered milk cheese
- Added-value for raw milk cheese is higher than treated milk cheese as these products can be commercialized under a protected designation

The Solution

GeneDisc System

To address the customer's need for a rapid method to sort raw milk in regards with STEC contamination, Pall offers a polymerase chain reaction (PCR) based method using the GeneDisc system (Figure 1). This system offers sensitive and specific detection of food pathogen 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

Following reference methods

The two reference methods for STEC detection – ISO/TS 13136³ and MLG5B⁴ – follow a two-step screening:

- First, virulence factors (genes encoding for shiga toxin – stx – and intimin – eae –) are screened for
- Then, major serogroups are detected. 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.

GeneDisc comprehensive STEC product range includes ready-to-use reagents enabling these workflows to be strictly followed.

Increasing level of discrimination

As raw milk 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 5 or 7 method. With this cutting-edge approach, all targets – serogroups and virulence factors – are analyzed within one single PCR run. This solution, which relies on an accurate screening based on the association of virulence factors to serogroups, provides a lower rate of presumptive positive.

Testing Protocol

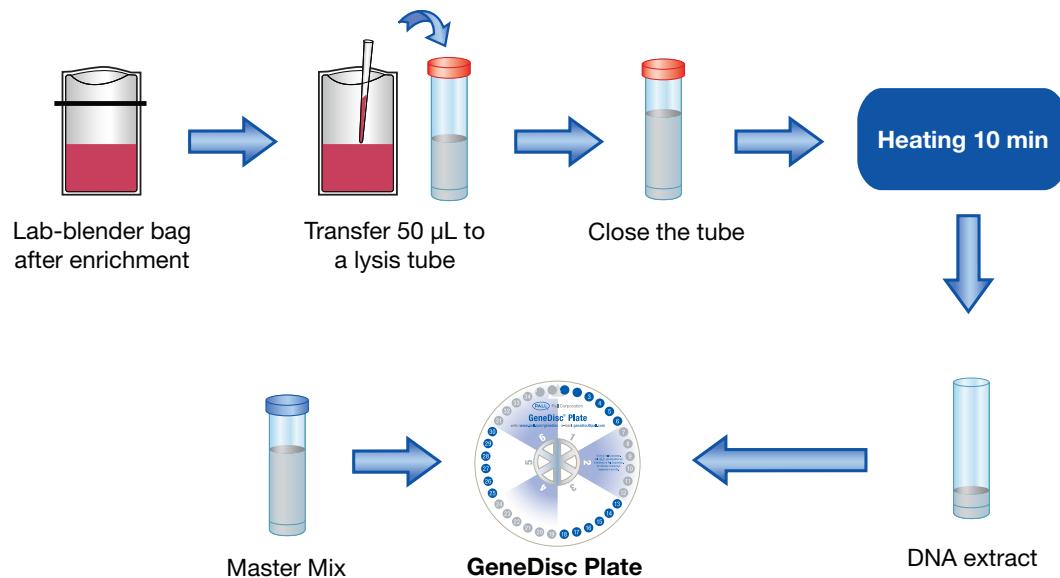
Operating procedure is identical for both GeneDisc strategies (strategy based on reference method and STEC Top 5 or 7 strategy):

- An initial enrichment of the 25 mL raw milk sample is performed in as short as 16 h
- Then, the bacterial DNA is extracted. An example of a protocol applicable to raw milk is described in Figure 2.
- Finally, DNA extracts are analyzed in less than one hour with the GeneDisc system.

A serogroup screening is to be performed for the reference method when virulence genes screening provides positive results.

A second screening after Immunomagnetic Separation (IMS) is performed for the GeneDisc STEC Top 5 or 7 method. This enables to reduce further the rate of presumptive positives.

Figure 2: Workflow of the GeneDisc STEC Top 5 or 7 Method





The overall time to results for raw milk samples (down to 18 hours) is compatible with incoming raw milk storage practices in place at the dairy company (less than 48 hours at 4 °C).

Preliminary Study

To assess the economic viability of this incoming raw milk sorting, a preliminary study was run to determine rate of presumptive positive for two GeneDisc methods: method based on ISO/TS 13136 and STEC Top 5 method.

This study was performed on 4064 raw milk samples, from both ewe (733 samples) and cow (3331 samples), from both dairy farm and collecting truck scales. Results for positive samples and positive results are summarized in table 1. One positive sample can include multiple positive results (i.e. more than one serogroup).

The GeneDisc STEC Top 5 method had a reduction in the number of samples requiring confirmation testing because of a more specific association of virulence genes to O-groups. This GeneDisc method generated only 142 confirmations (3 %) compared to 1050 (26 %) for the ISO/TS 13136 screening.

Table 1: Number of presumptive positive samples (results) per GeneDisc method

Method	Number of Samples Analyzed	Ewe	Cow	Total
		733	3331	4064
ISO/TS 13136 Standard with GeneDisc Plates STEC & EHEC 5 ID	stx+/eae+	234	452	686
	Serogroup (ISO TS 13136)	201 (396)	379 (654)	580 (1050)
GeneDisc STEC Top 5 method	1st PCR screening	112 (135)	248 (264)	360 (399)
	2nd PCR screening after IMS	23 (25)	94 (117)	117 (142)
	Isolated pathogenic STEC strains	6	13	19

Implementation of the GeneDisc Technology

This study demonstrated an 80 % reduction of presumptive positive samples using the GeneDisc STEC Top 5 method for screening compared to the ISO/TS 13136 method. As a result, a larger portion of incoming raw milk was available for higher added-value raw milk cheese process.

The GeneDisc STEC Top 5 method also leads to a lower number of confirmation procedures required to be run (approximately a reduction by factor 9). This reduction improves cost-efficiency of testing compared to the ISO/TS 13136 screening by decreasing confirmation hands-on time and media costs associated to confirmations.

GeneDisc STEC Top 5 method was economically suitable and an efficient brand protection tool for raw milk sorting use and was implemented by the customer as a routine test.

The Benefits

With the GeneDisc solution, dairy companies can now achieve a rapid routine raw milk sorting and selection of dairy farms based on an accurate microbiological assessment. Using Pall's technology, they can realize the following benefits for milk sorting:

- Higher volume of incoming milk dedicated to value-added raw milk products with the low presumptive positive rate of GeneDisc STEC Top 5 method.
- Smooth incoming milk storage flow as results are obtained in as little as 18 hours.
- As local practices by food agencies for STEC alert may vary from one country to another, GeneDisc comprehensive product range for pathogenic *E. coli* detection can fit any strategy for product release.
- Easy implementation of similar control points for *Listeria* and *Salmonella* using the GeneDisc system.
- Easy integration into all dairy company types due to proven ease of use. Minimally trained operators can achieve reliable and robust results.

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 **Rapid Alert System for Food and Feed (RASFF):** ec.europa.eu/food/food/rapidalert/index_en.htm
- ² To learn more about Pall OEM microfiltration solutions and other milk filtration products from Pall, please contact your local Pall sales representative.
- 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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