Pall Corporation

*Better Lives. Better Planet.*SM

Pall GeneDisc® Technologies

From regulation to diagnostics

Simplified compliance to the new European Regulation for sprouted seeds

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Rotterdam, the Netherlands

From Regulation to Diagnostocs

Content:

- Intro Pall GeneDisc Technologies
- EU-Regulation: today and tomorrow
- GeneDisc Solution for STEC screening

History

- French Biotech Company, GeneSystems, founded in 2000.
- Product Offering: Molecular diagnostic solution provider in the field of food analysis, environment and forensic science.
- Proprietary technology in molecular biology (PCR instrument, PCR reagents, DNA extraction and purification).

ISO Organisation internationale de normalisation

AFNOR Validation

- Acquired by Pall Corporation in September 2008. Product development and placement across multiple Pall divisions: Medical and Food & Beverage.

A European regulation for sprouts !

And a new guidance for all foods?

European Food Safety Regulation – Context

Food Business Operators (FBO) are concerned, among others, by the following EC regulations:

- EC regulation 852/2004 which sets FBO general rules on hygiene of foodstuffs (focus on HACCP)

- EC regulation 2073/2005 which sets harmonized criteria for certain micro-organisms and how to perform tests (*e.g. Salmonella* criteria is listed for sprouted seeds)

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:338:0001:0026:EN:PDF

European Food Safety Regulation – Reasons for evolution

Several factors have occurred recently and raised concerns about Shiga-Toxic *Escherichia coli* (STEC) in sprouts:

- *E. coli* (STEC) outbreak in EU (May 2011; 3916 Human cases and 47 deaths)

- EFSA Scientific Opinion on the risk posed by STEC in seeds and sprouted seeds

Conclusion: Need to extend microbiological criteria to STEC for the sprouted seeds production chain

European Food Safety Regulation – Evolution

Following these events, Commission Regulation 209/2013 Amending EC regulation 2073/2005 has been published

12.3.2013 EN Official Journal of the European Union L 68/19

COMMISSION REGULATION (EU) No 209/2013

of 11 March 2013

amending Regulation (EC) No 2073/2005 as regards microbiological criteria for sprouts and the sampling rules for poultry carcases and fresh poultry meat

(Text with EEA relevance)

http://eur-lex.europa.eu/JOHtml.do?uri=OJ:L:2013:068:SOM:EN:HTML

What is Commission Regulation 209/2013?

This Commission Regulation establishes:

- A new microbiological testing requirement for sprouts which concerns STEC (serogroup O157, O26, O103, O111, O145 and O104:H4)
- A method for this testing (ISO TS 13136)
- An acceptance criteria (Absence in 25g)
- Sampling rules:

→Preliminary testing on a representative sample of all batches of seed

→Testing at the stage where probability of finding STEC and *Salmonella* is the highest

→ Alternative testing of spent irrigation water is allowed

→ Frequency must be at least once a month

FBO can only place sprouts on the market if the results comply with regulation acceptance criteria for *Salmonella* and STEC

Sprouts testing regulation after amendment

Food Category	Micro-organisms	Sampling plan	Limits	Analytical reference method	Stage where the criterion applies
1.18 Sprouted seeds (ready-to-eat)	*Salmonella*	n=5; c=0	Absence in 25g	EN/ISO 6579	Products placed on the market during their shelf-life
1.29 Sprouts	Shiga toxin producing *E.coli* (STEC) O157, O26, O111, O103, O145 and O104:H4	All new batches n= 5; c= 0	Absence in 25g	EN/ISO 13136	Products placed on the market during their shelf life

And a new guidance for all foods?

EUROPEAN COMMISSION
HEALTH AND CONSUMERS DIRECTORATE-GENERAL

DRAFT

GUIDANCE DOCUMENT ON THE APPLICATION OF ARTICLE 14 OF REGULATION (EC) N°178/2002 AS REGARDS FOOD WHERE SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC) HAS BEEN DETECTED

- Almost ready for publication after acceptation by the EC

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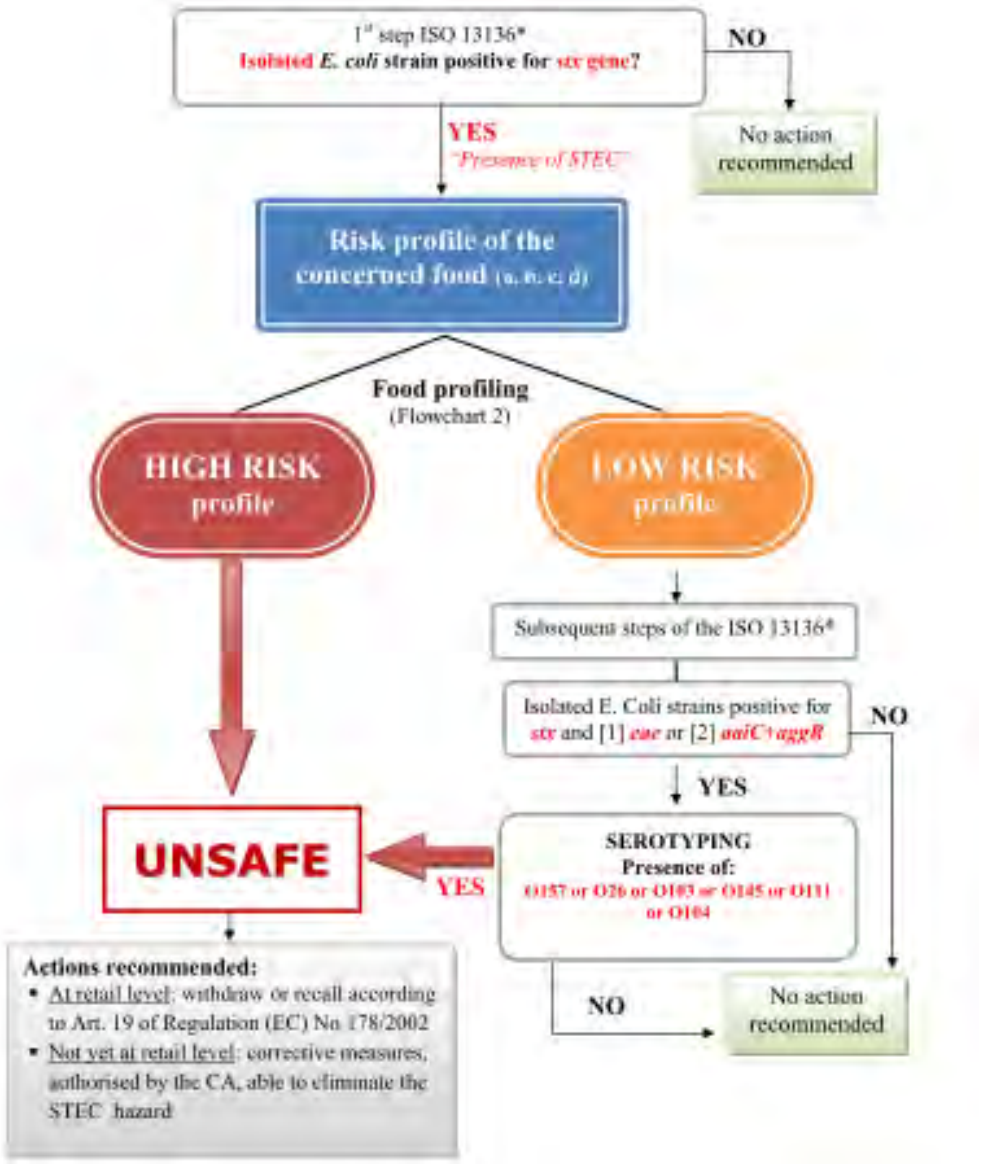
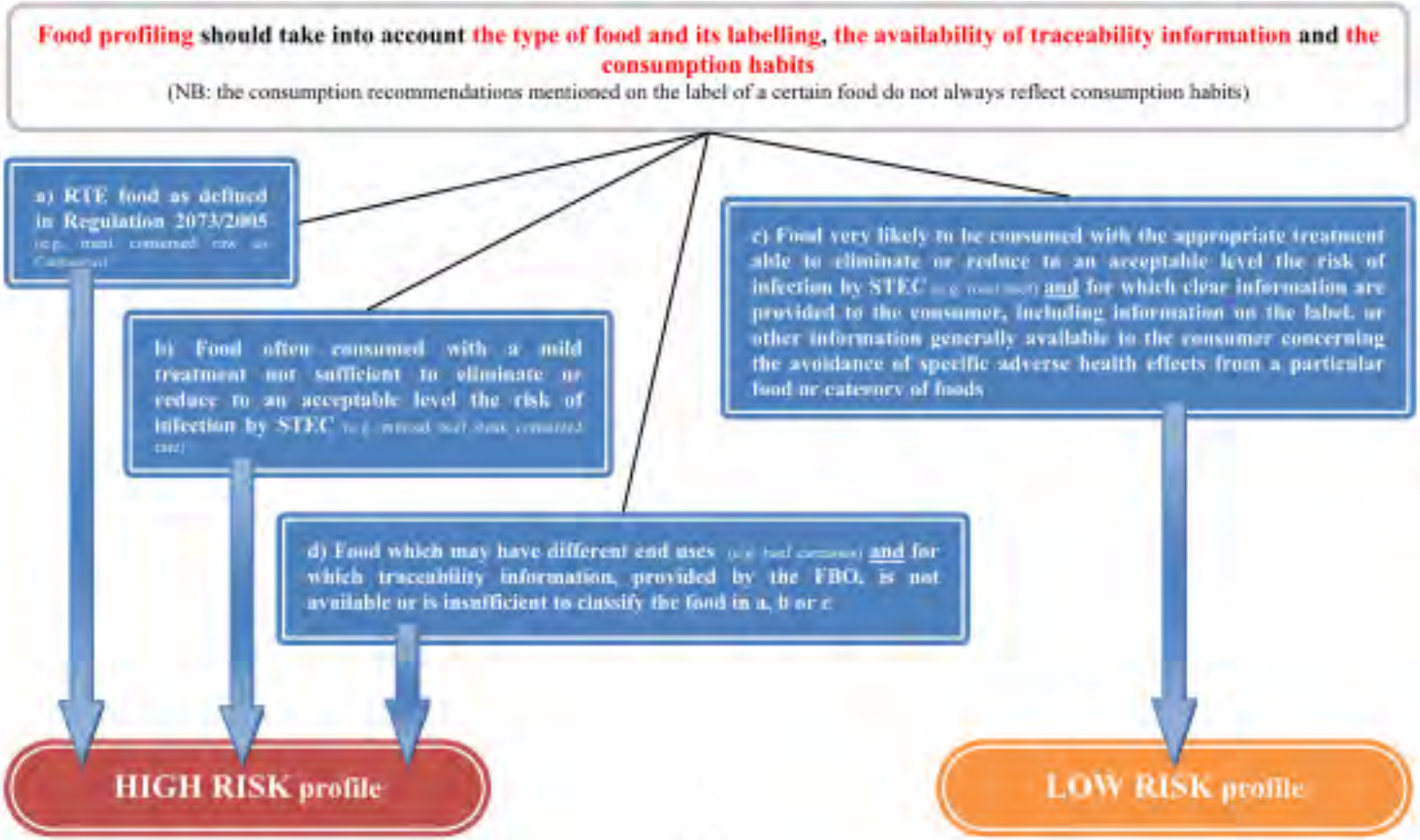
  - In the Netherlands implemented per 22 may 2014

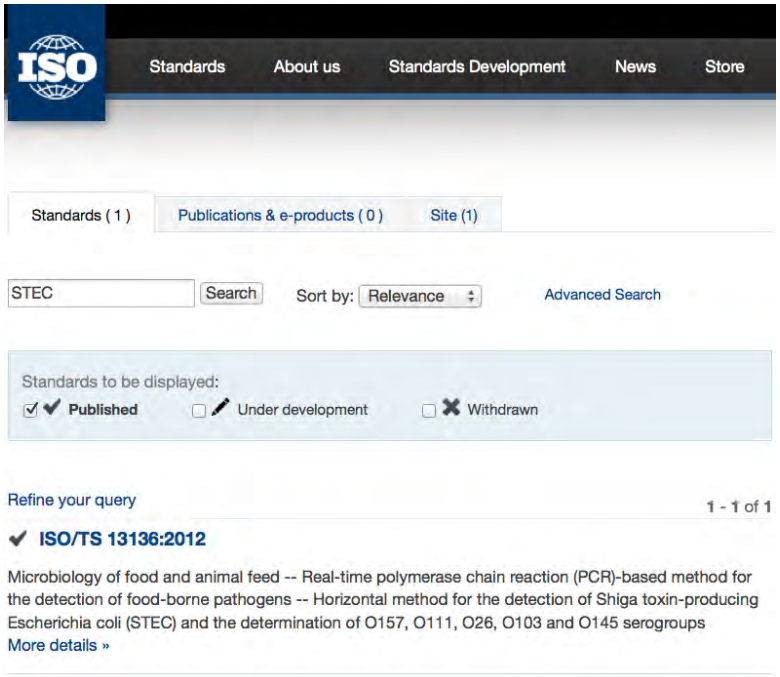
A new guidance for all foods?



A new guidance for all foods?



What is ISO TS 13136?



This technical specification published in November 2012 is relative to STEC testing and is based on Real Time PCR principle.

It describes :

- Enrichment protocol,

- DNA extraction,

- Screening for virulence factors (*stx* and *eae*),

- Identification of serogroups (O157, O111, O26, O103 and O145),

- Isolation.

O104:H4 is not included in the ISO TS 13136, but testing should be performed according to this 5 steps method for this serotype.

Flow-diagrams of ISO TS 13136 operating workflow

Flow-diagram of the screening procedure

Test portion x g or x ml

↓

9 x ml mTSB+N/A or BPW

↓

Enrichment 18 h to 24 h
(37+/-1)°C

↓

Test portion (1 ml) of the culture, DNA purification and *stx* and *eae* genes detection

↙ ↓ ↘

Positive result (*stx* and *eae*): test for serogroup associated genes

Positive result to *stx* only : result reporting

Negative result to *stx*: result reporting

↙ ↘

Positive result to *stx*, *eae* and serogroup-specific sequences: isolation

Positive result to *stx*, *eae* but negative to serogroup-specific sequences : result reporting

Flow diagram of the isolation procedure

Serogroup-specific enrichment

↓

Enrichment broth streaked on to suitable media

Incubation 18 h – 24 h at (37+/-1)°C

↓

Pick up to 50 colonies with *E. coli* morphology. Point-inolculateon nutrient agar (single colonies) and water (5 pools by 10 colonies each). Perform *stx* and *eae* detection on isolated colonies or pools.

↓

If the colony is positive for the presence of genes identified at the screening step, go to the next step. If a pool is positive, incubate nutrient agar. Test individual colonies composing the positive pool as above.

↓

Identify positive colonies as *E. coli* and check the serogroup (PCR or agglutination)

↓

Further characterization (optional): send strain to reference laboratory

↓

Result reporting

GeneDisc Solution

GeneDisc Overview

- Pall's GeneDisc system is a robust platform which allows accurate detection of microorganisms using the Real-Time Polymerase Chain Reaction (RT PCR) method
- The principle of Real-Time Polymerase Chain Reaction is the amplification of a specific DNA sequence

GeneDisc Cycler

The GeneDisc Cycler is an high throughput real time PCR instrument for the detection of microorganisms.

GeneDisc Cycler features:

- High Throughput - test up to 96 samples each hour
- Flexible and Modular - up to eight individual Real-Time PCR units can be used at once
- Rapid - Run time less than 1 hour
- Easily fits in lab - Small footprint
- Fast decision making - Real time measurement with at a glance results
- Seamless data transfer - LIMS compatible

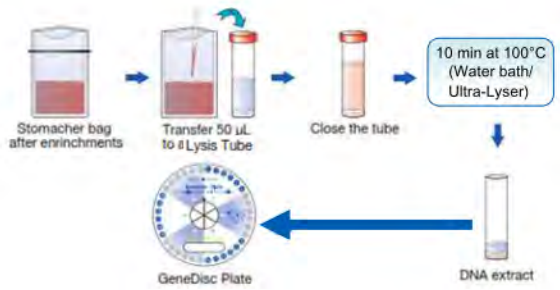
GeneDisc Plate

GeneDisc Plate features:

- Single/simultaneous detection of different organisms
- Samples to test results in hours
- Easy-to-use system, minimal operator intervention required
- Barcode control of consumables and assay parameters
- Key reagents pre-loaded in a unique, sealed plate (no operator handling of primers…)
- Unique design allows automatic filling of the plate
- High specificity, reliability and reproducibility of run

GeneDisc overview

Enrichment and DNA Extraction
From sample to DNA



20 hours
From sample
to result

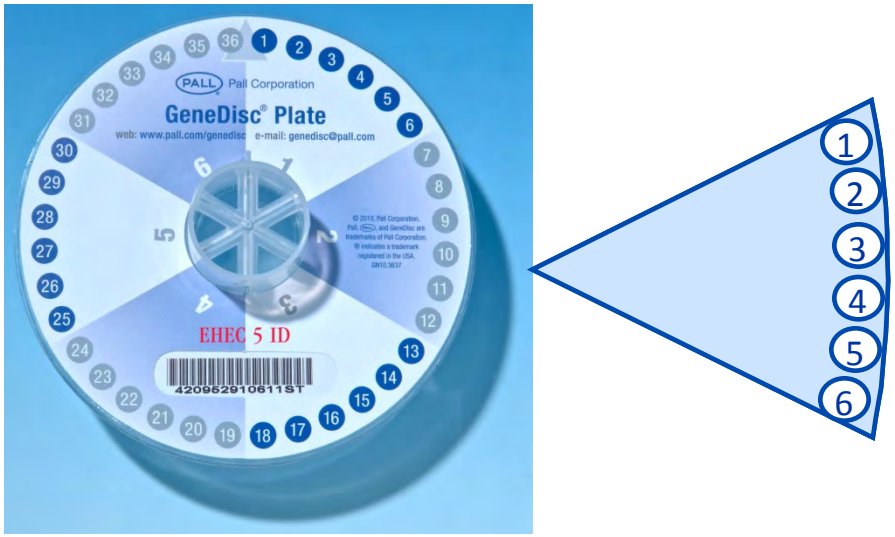
GeneDisc Cycler
From DNA to result

GeneDisc Plate
Ready to use PCR plate

GeneDisc Method for STEC

- GeneDisc method follows ISO TS 13136 requirements in term of target gene detection and workflow :

1. Screening for virulence factors + O-groups
   - Use *E.coli* TOP7 GeneDisc Plate



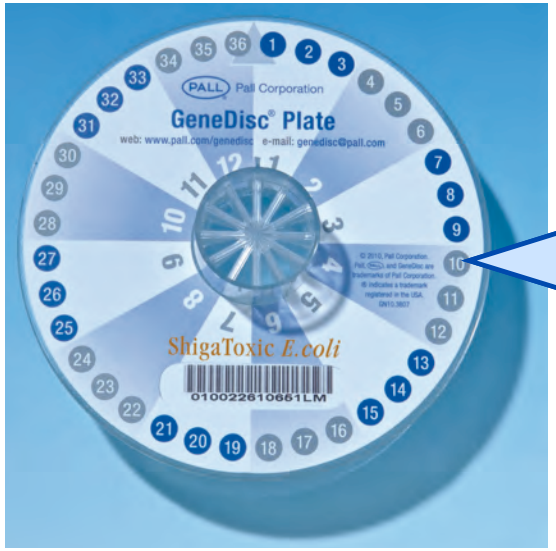
GTOP7EC106006		
Well	FAM Detection	ROX Detection
1	O145	Inhibition control
2	Vir. O45-O103-O121	stx1-2
3	Vir. O26	Vir. O145-O157
4	Vir. O111	O111
5	O157	O26
6	-	O45-O103-O121

GeneDisc Method for STEC

- GeneDisc method follows ISO TS 13136 requirements in term of target gene detection and workflow :

1. Screening for virulence factors (* eu guidance)

➢ Use ShigaToxic *E.coli* GeneDisc Plate



Well Number	FAM Fluorescence Dye Detection	ROX Fluorescence Dye Detection
1	PCR Negative control	PCR inhibition control
2	*stx2*	*stx1*
3	*E. coli* O157	*eae*

GeneDisc Method for STEC

2. Identification of Top 7 (O26, O103, O111, O145) if screening for virulence factors is positive

- Use EHEC 5 ID GeneDisc Plate

Well Number	FAM Fluorescence Dye Detection	ROX Fluorescence Dye Detection
1	PCR Negative control	PCR Inhibition control
2	*E. coli* O145	
3	Flagellar H7	*E. coli* O111
4	Flagellar H7	*E. coli* O111
5	*E. coli* O26	*E. coli* O103
6	*E. coli* O26	*E. coli* O103

3. Screening for O104:H4 when *stx2* is positive
   - Use *E. coli* O104:H4 GeneDisc Plate

ISO TS 13136 and GeneDisc Solution

Flow-diagram of the screening procedure

Test portion x g or x ml

9 x ml mTSB+N/A or BPW

Enrichment 18 h to 24 h (37+/-1)°C

Test portion (1 ml) of the culture, DNA purification and *stx* and *eae* genes detection

Positive result (*stx* and *eae*): test for serogroup associated genes

Positive result to *stx* only : result reporting

Negative result to *stx*: result reporting

Positive result to *stx*, *eae* and serogroup-specific sequences: isolation

Positive result to *stx*, *eae* but negative to serogroup-specific sequences : result reporting

Shiga Toxic *E.coli* GeneDisc Plate

EHEC 5 ID or TOP 7 GeneDisc Plate

Flow diagram of the isolation procedure

Serogroup-specific enrichment

Enrichment broth streaked on to suitable media

Incubation 18 h – 24 h at (37+/-1)°C

Pick up to 50 colonies with *E. coli* morphology. Point-inolculateon nutrient agar (single colonies) and water (5 pools by 10 colonies each). Perform *stx* and *eae* detection on isolated colonies or pools.

If the colony is positive for the presence of genes identified at the screening step, go to the next step. If a pool is positive, incubate nutrient agar. Test individual colonies composing the positive pool as above.

Identify positive colonies as *E. coli* and check the serogroup (PCR or agglutination)

Further characterization (optional): send strain to reference laboratory

Result reporting

PALL Pall Corporation

A solution to your needs

GeneDisc STEC solution

- Meet EN 2073 – All required testings performed at once :
  - Detection of all pathogenic STEC Top 7 and O104:H4
  - Simultaneous detection of STEC and *Salmonella* spp. available
- The method has been tested on seeds, sprouts and water.

GeneDisc STEC solution

- This solution enables an easy and accurate testing for STEC
- This solution is compliant to ISO/TS 13136 requirements
- This solution minimal handling of samples allows to easily perform STEC testing.
- GeneDisc method for STEC screening and serogroup identification has been AOAC approved

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 021103

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 021106

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 021105

A solution to your needs

Your needs	Our solution
✓Brand protection/Compliance to Regulation	✓STEC testing compliant to ISO TS 13136 specifications ✓*Salmonella* testing validated alternative method to ISO 6579
✓Minimal handling	✓Simultaneous STEC/*Salmonella* detection (one enrichment, one sample preparation, one run)
✓Earlier batch release	✓Rapid – Result obtained in as fast as 20h
✓Adaptive to your throughput and sampling plan	✓Modular system
✓Smooth implementation	✓Ease of use

Further information

Several external accredited Laboratories are already equipped with the the GeneDisc Solution for STEC screening.

For further information, please contact us at:

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Please, visit us at:

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Dear Customer,

Recently the ISO/TS 13136:2011 has been published:

ISO/TS 13136:2011 (E)

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

Microbiologie des aliments — Méthode basée sur la réaction de polymérisation en chaîne (PCR) en temps réel pour la détection des micro-organismes pathogènes dans les aliments — Méthode horizontale pour la détection des Escherichia coli producteurs de Shigatoxines (STEC) appartenant aux sérogroupes O157, O111, O26, O103 et O145

Pall GeneDisc Technologies SA certify that the primers and probes used in GeneDisc products listed in attachment A, are as proposed in the ISO TS 13136 for the stx1, stx2, eae, O157, O111, O103, O145, O26.

For the target genes that are patented, Pall GeneDisc Technologies SA has obtained a licence agreement from ANSES.

Therefore, GeneDisc PCR Solution is fully compliant with the primer and probes sets described in the ISO/TS 13136.

Further our GeneDisc Solutions for STEC screening has been previously approved by AOAC-PTM against the first draft of the ISO/TS 13136.

We kindly suggest you add this information to your file and hope to work closely with you on the detection of STEC in food products.

With kind regards,

Pall GeneDisc Technologies SA

Mrs Sirine Assaf
Product Manager GeneDisc

Mrs Nathalie Kerriguy
QA manager GeneDisc

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