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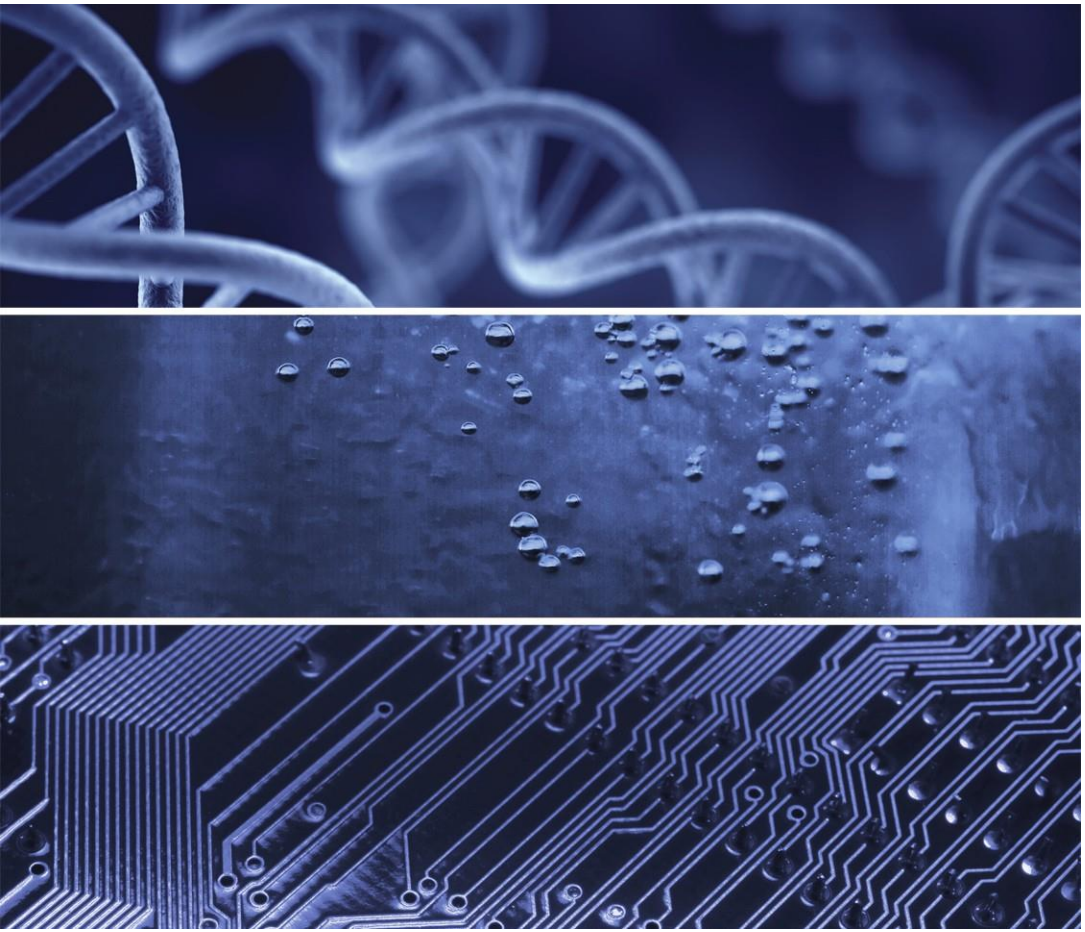

GeneDisc® Methods for the Detection of Food Pathogens

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Food Safety Today

Recall

Cost Savin

AOAC

USDA

on Reference Methods

HACCP

Outbreal

Storage Cost

Sal

mer Specification

Microbiology

O157 AFNOR

Extended Shel

STEC

Processed Food

Supplier Qualifi

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Pse

FDA

Contamination

Time To Result

Discover How GeneDisc Solutions Can Help

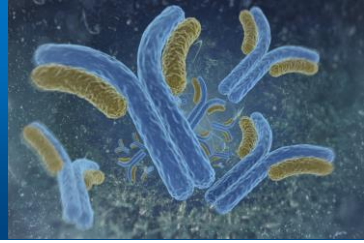
Food Pathogens Detection Methods Available

Culture Methods

- Culture method is still widely used for pathogen detection due to its low price
- BUT:
  - **Time consuming**: For ISO; approx. 4 days to a *Listeria* negative result, approx. 3 days to a *Salmonella* negative result and approx. 2 days to an *E. coli* O157 negative results
  - **Demanding workflows:** Sub-culture, confirmation of a presumptive positive results
  - **Variability:** Culture media quality, operator to identify characteristic colonies
  - **Storage:** Reagents, incubations
  - **Outdated compared to new standards (gene based)**

Chromogenic media provide faster answers but overall face same limitations

Immunoassay-based Methods



- Compared to culture methods, immunoassays based methods are faster and easier to use thanks to less demanding workflows.

- But compared to molecular methods (including GeneDisc technology):
  - Time to results can be longer (48 h) for some applications
  - Throughput limitation
  - Sensitivity limitation
  - Specificity (cross reactivity of assays)
  - Outdated compared to new MLG, ISO which are gene (PCR) based
  - Does not distinguish between pathogenic and non-pathogenic *E. coli* O157

Molecular Assay-based Methods

- Molecular detection methods (*e.g.* PCR) are:
  - Fast
  - Highly sensitive
  - Highly specific
  - Flexible in terms of throughput
  - Cornerstone for new standards

- But open systems can be:
  - Complex to use (primer/probes handling, results interpretation…)
  - Difficult to use / cross contamination sensitive (96 wells plate filling, PCR control management)
- And SYBR Green assays:
  - Detect all double-stranded DNA (including non-specific reaction products)
  - Require expertise for result interpretation (*e.g.* melting curve analysis)

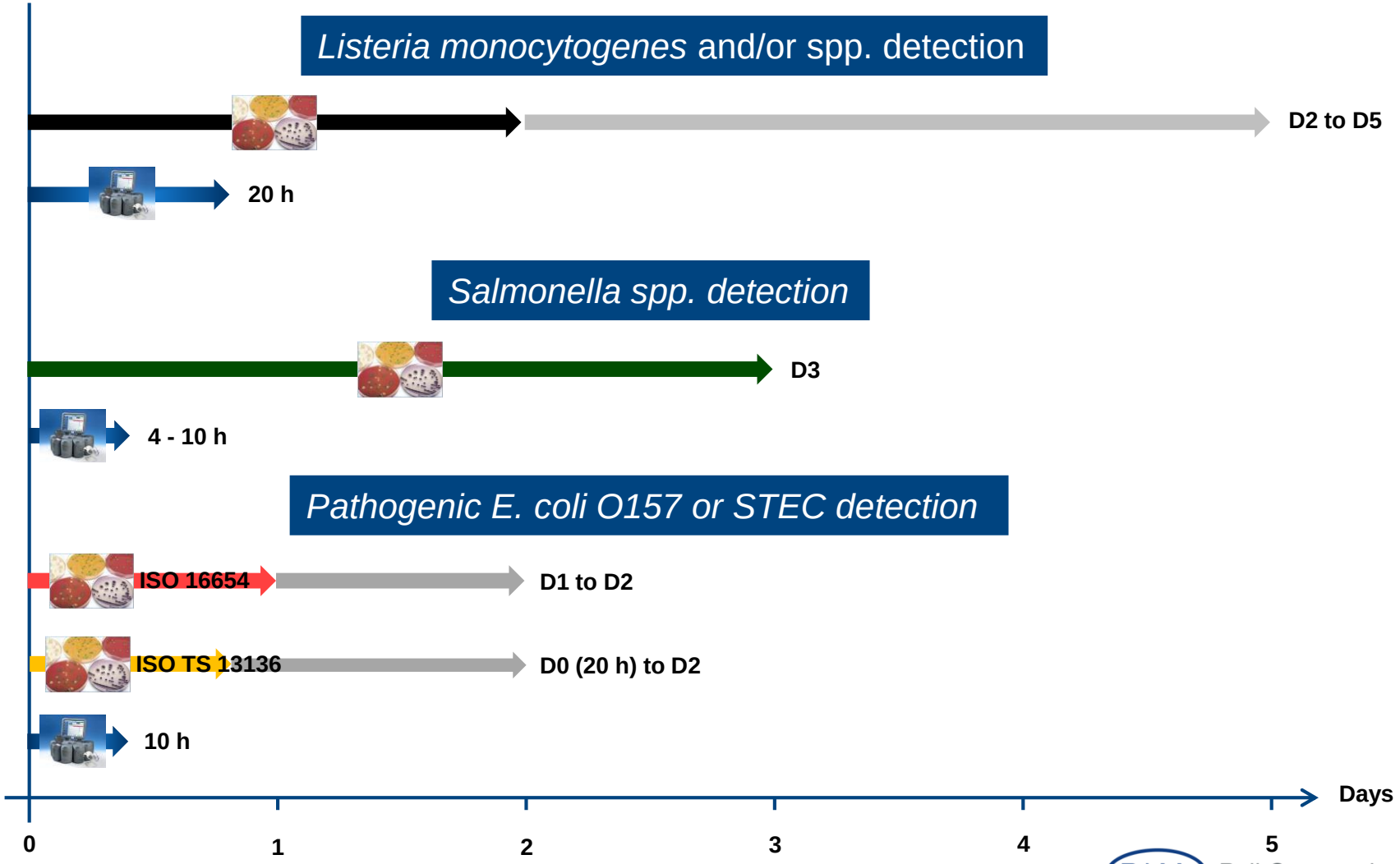
GeneDisc Methods

- GeneDisc methods combine :
  - all benefits of PCR
  - with a unique design which ensures ease of use and control of cross contamination. Minimally trained technicians can use the system.
  - assays based on Taqman Real-Time PCR ensure high specificity

The GeneDisc Solution

GeneDisc Solution : Reduce your Time to Result

Minimum Total Time to GeneDisc Result* *vs.* Total Time to Negative Results (ISO)



*Minimum total time to GeneDisc result depends on the tested matrix

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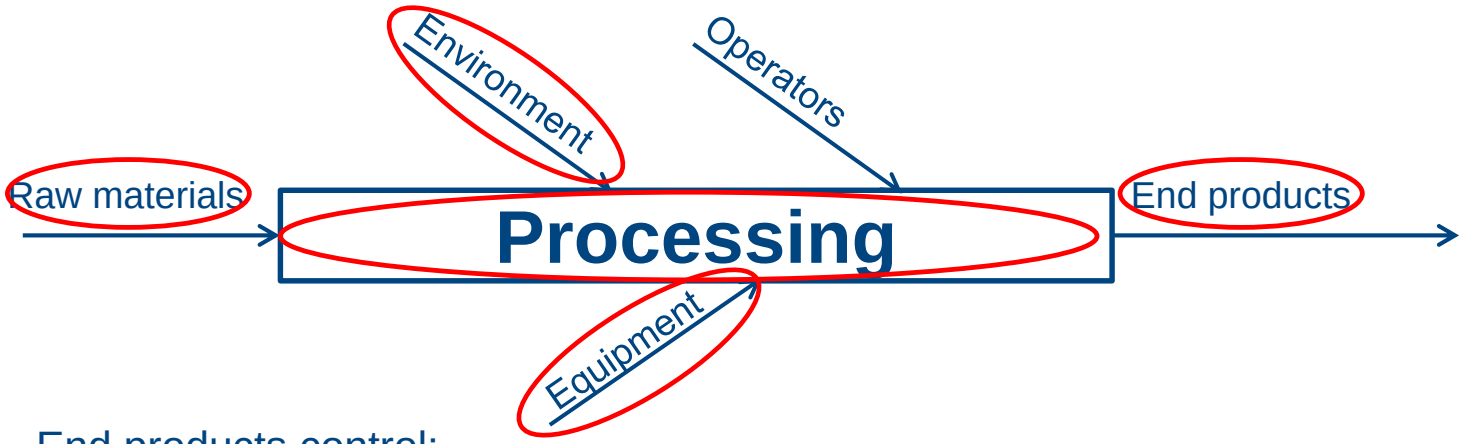
GeneDisc Solution : Control Your Process

The GeneDisc system time to result and ease of use make it a perfect fit for:



- End products control:
  - Comply with regulations
  - Answer customer specifications
  - Smoothen logistic flow with an earlier release
- In-process control:
  - Monitor corrective actions in real time
  - Apply hazard plan (*e.g.* HACCP)
  - Identify contamination early
- Raw material control:
  - Qualify suppliers
  - Direct raw material to proper use

GeneDisc Solution : The Right Tool for Food Safety Quality Controls

- **Reduce costs and increase profitability**
  - ✓Accelerated batch release
  - ✓Reduced storage costs (raw materials and end-products)
- **Secure product release**
  - ✓Earlier results make implementation of batch release test possible
  - ✓Validated performance for GeneDisc Food Safety solutions (AOAC-PTM and NF Validation)
- **Easily implement controls and real time monitoring**
  - ✓Easy to use instruments
  - ✓Ready to use reagents for a safe and easy handling
  - ✓No high technical skills required to run the GeneDisc system
- **Adapt GeneDisc solutions to your needs**
  - ✓Flexibility for testing - extraction protocols flexible throughput, multiparametric plates…
  - ✓System's modularity - scalable system to match your throughput.

The GeneDisc Method for the Detection and Identification of Foodborne Pathogens:

HOW DOES IT WORK?

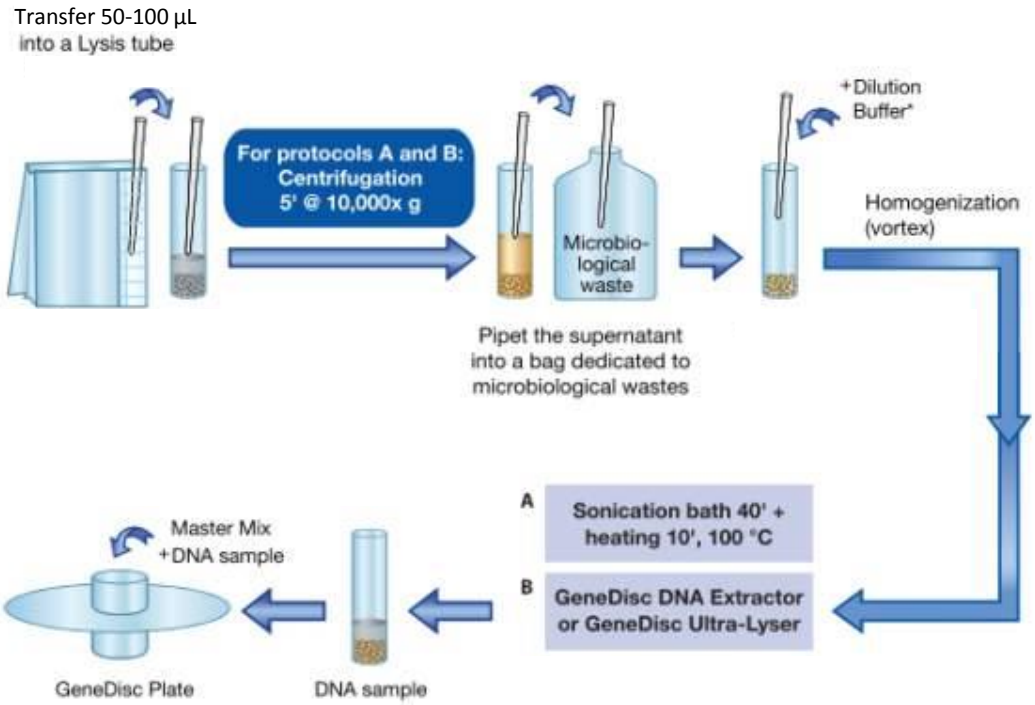
The GeneDisc System : From Sample to Result

- Real time PCR based system for the detection of bacteria in only three simple steps from sample to result:
  - **Step 1** - **Extract DNA after enrichment**
    Universal extraction protocols
    Frees DNA from microbial cells

  - **Step 2** - **Fill the GeneDisc Plate**
    Ready-to-use consumable
    Pre-loaded with all primers and probes

  - **Step 3** - **Run the Real-Time PCR test**
    Using the GeneDisc Cycler

Step 1 – Extract DNA after Enrichment GeneDisc Method for *Listeria*



Step	Minimum Time
STEP 0 : Enrichment	18 h
STEP 1 : DNA extraction	< 1 h for 48 samples
STEP 2 : GeneDisc Plate filling	15 min
STEP 3 : PCR	< 1 h
Total TTR	20 h

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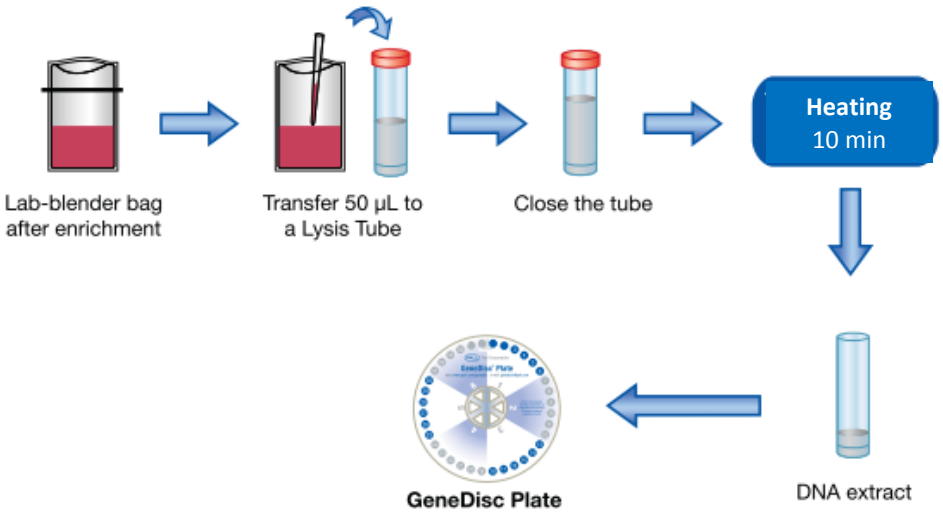
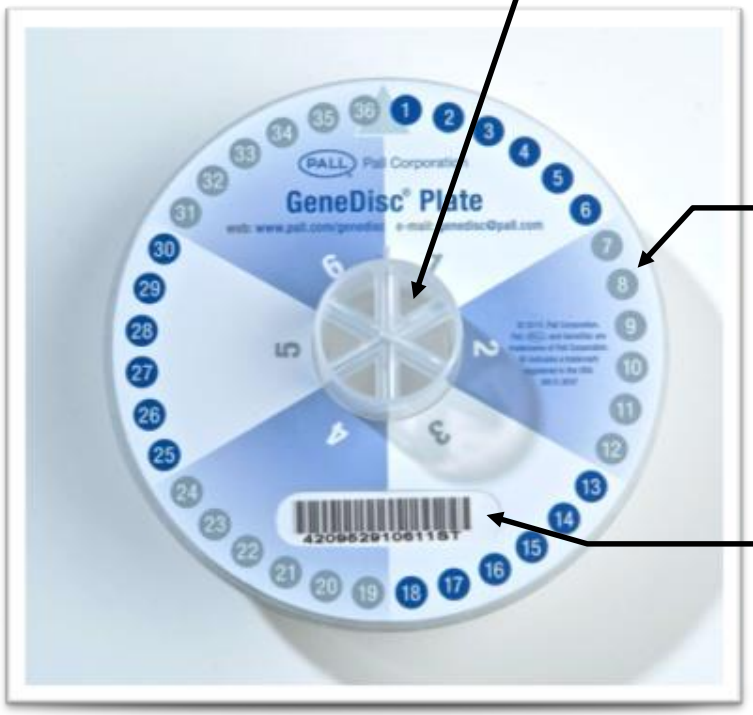
Step 1 – Extract DNA after Enrichment GeneDisc Method for *Salmonella* and Pathogenic *E. coli* (STEC and *E. coli* O157)



Step	Minimum Time
STEP 0 : Enrichment	8 h
STEP 1 : DNA extraction	< 1 h for 48 or 96 samples
STEP 2 : GeneDisc Plate filling	15 min
STEP 3 : PCR	< 1 h
Total TTR	10 h

High throughput DNA extraction solution available

Step 2 - Fill the GeneDisc Plate



The GeneDisc Plate

Sector

- One sector receives one sample DNA
- A 6 sectors GeneDisc Plate allow to test for 6 different samples
- A 12 sectors GeneDisc Plate allow to test for 12 different samples

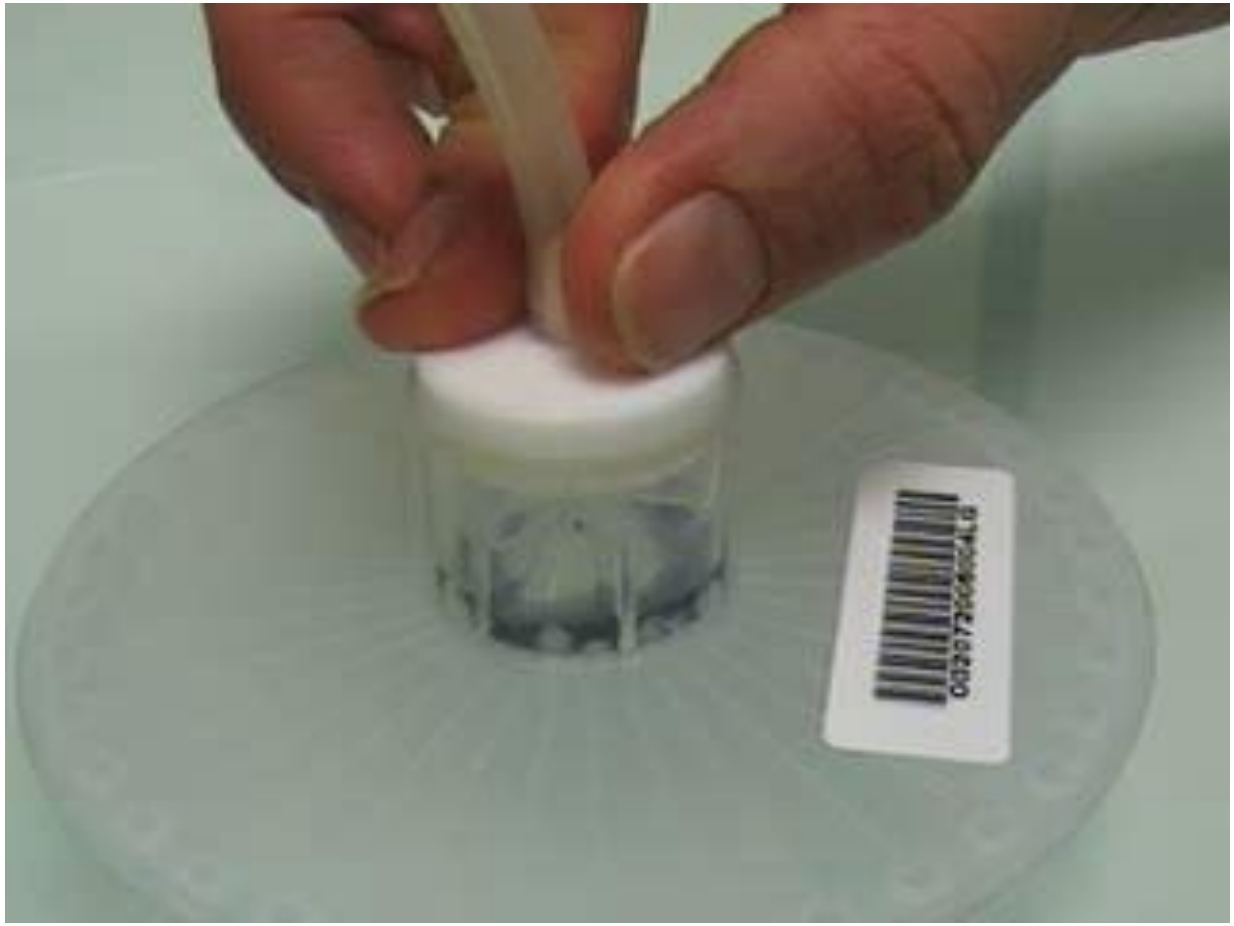
Well

- Each sector is related to 6 peripheral wells by microchannels
- Each well contains all necessary reagent (Primers and probes) for a specific PCR reaction

Barcode

- Traceability of the product
- Recognized by the GeneDisc Cycler for automatic setup of the right PCR program and results interpretation

Step 2 - Fill the GeneDisc Plate

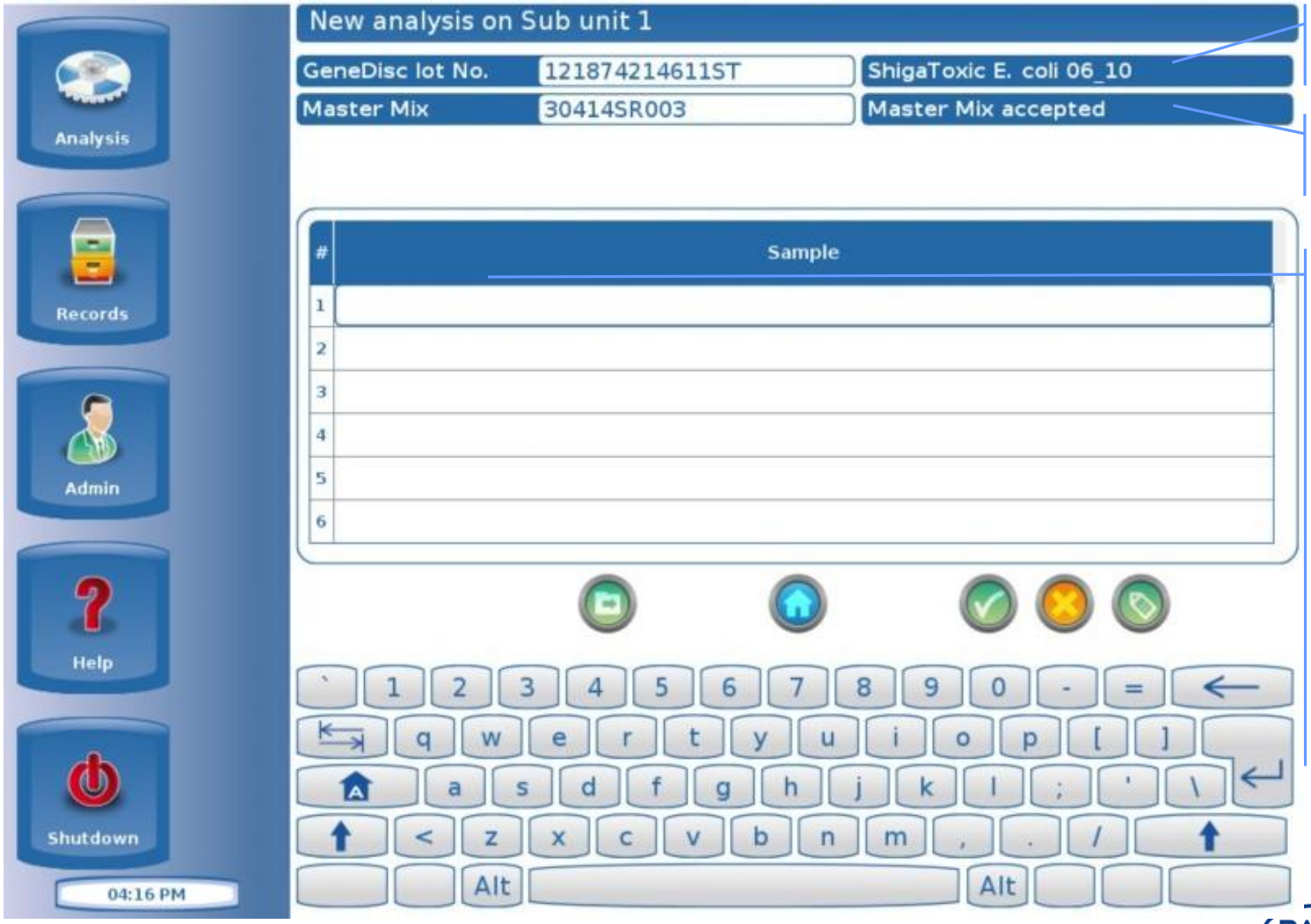


Step 3 - Run the Real-Time PCR Test

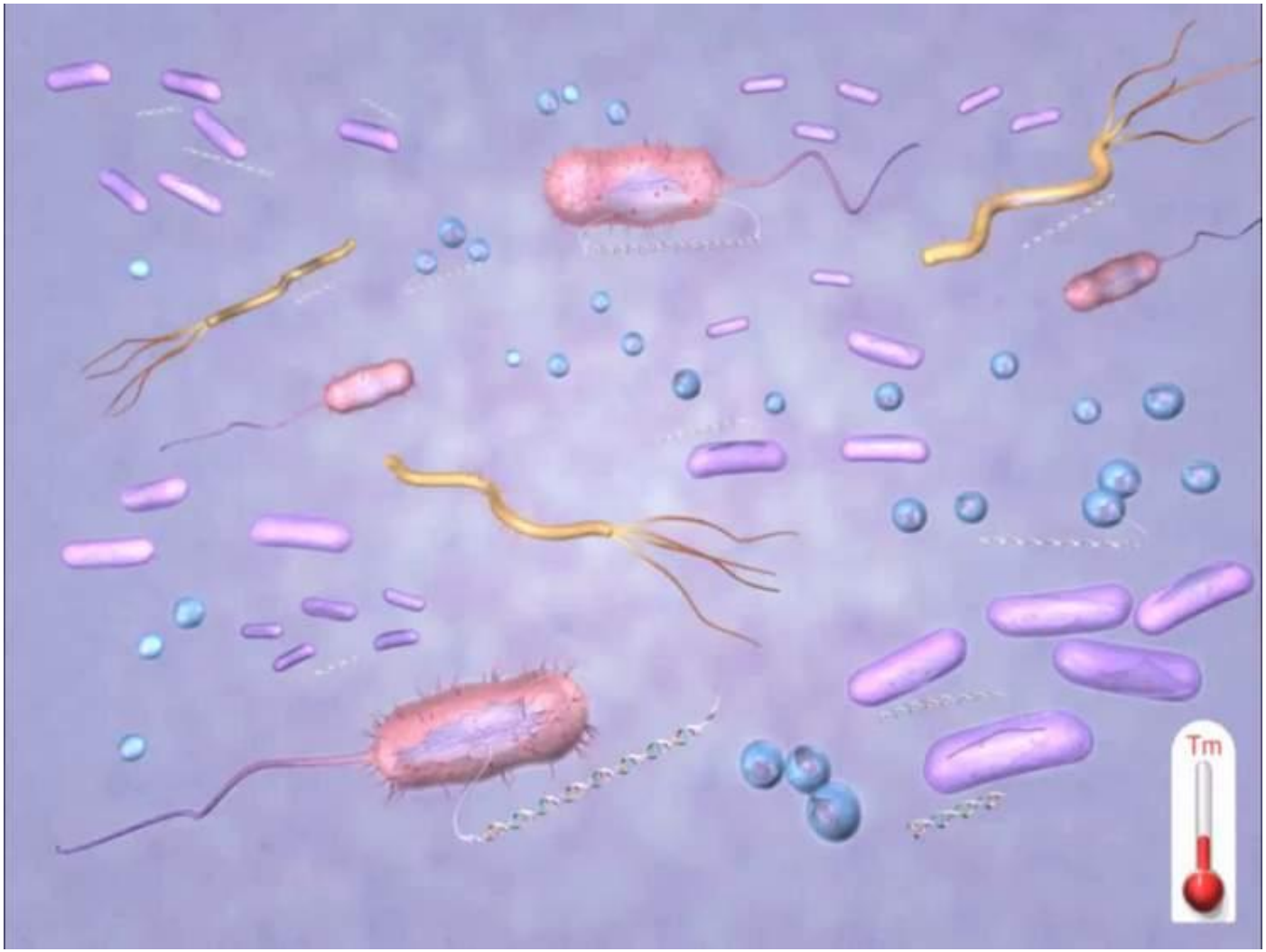
- The GeneDisc Plate is positioned in the GeneDisc Cycler
- One run is less than an hour

Step 3 - Run the Real-Time PCR Test

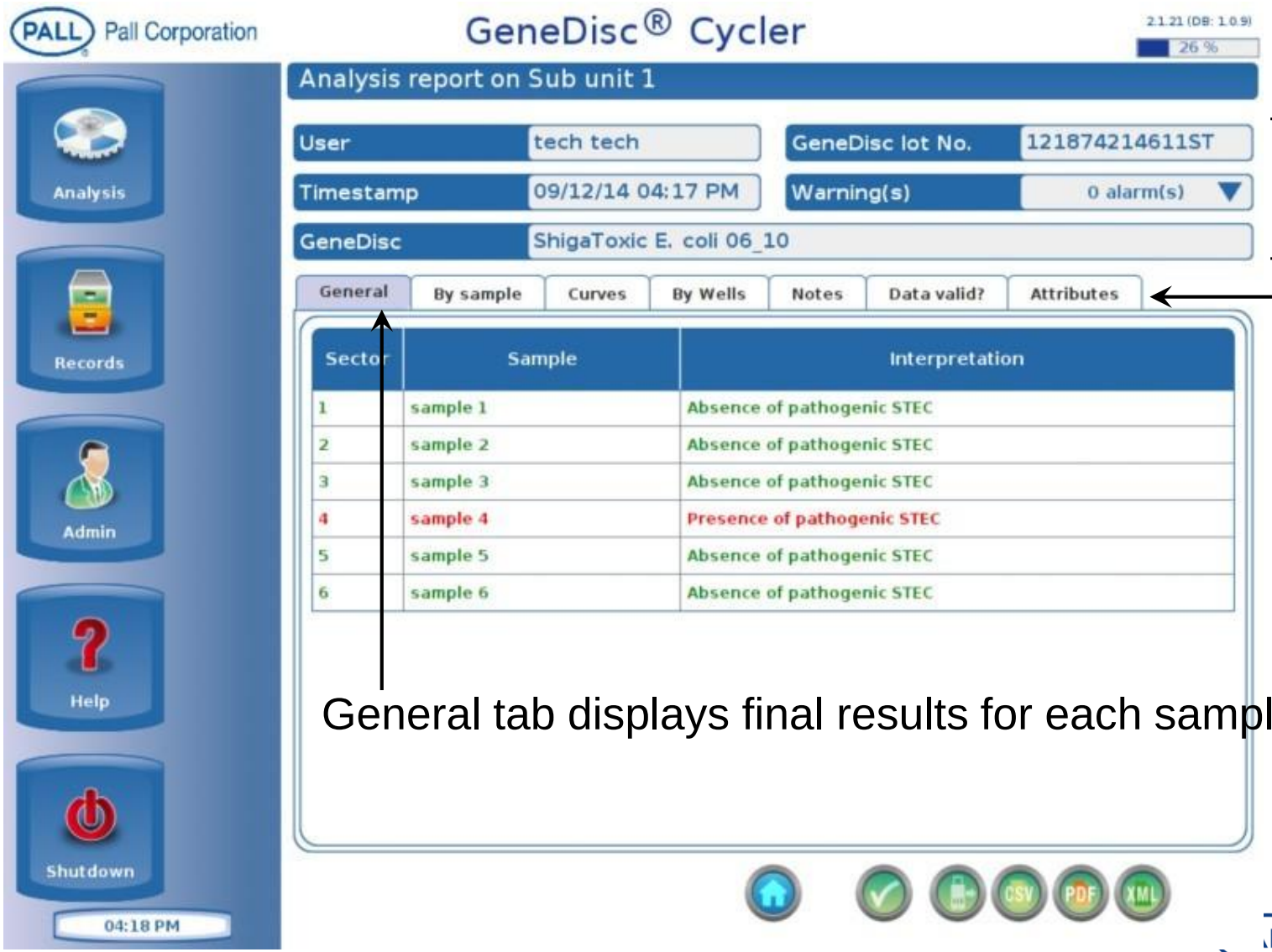
Set up Cycler with sample information



Step 3 - Run the Real-Time PCR Test



Step 3 - Run the Real-Time PCR Test

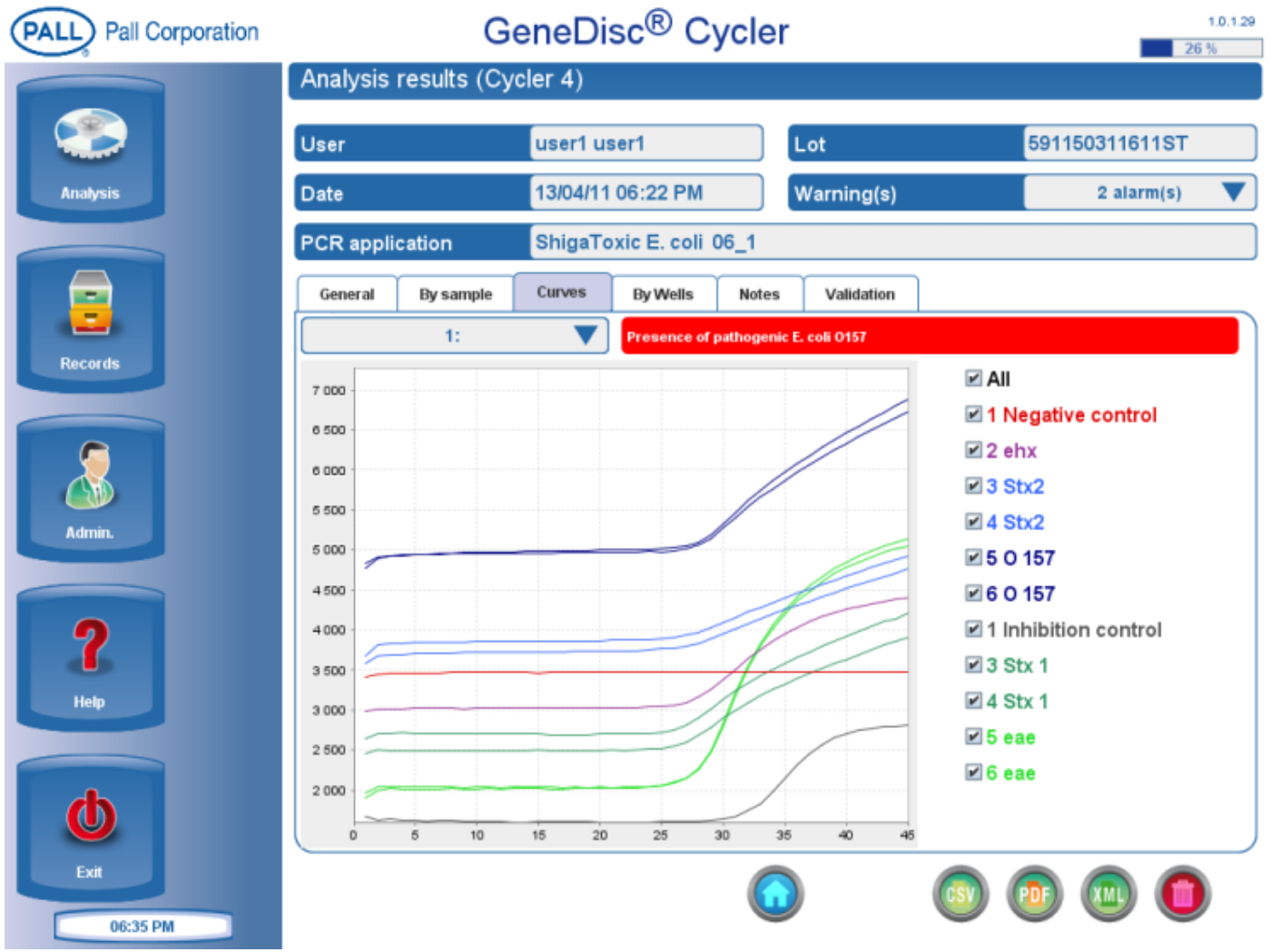


Traceability information

Different tabs providing different views of the data

General tab displays final results for each sample

GeneDisc Methods for Food Safety: From Sample To Result

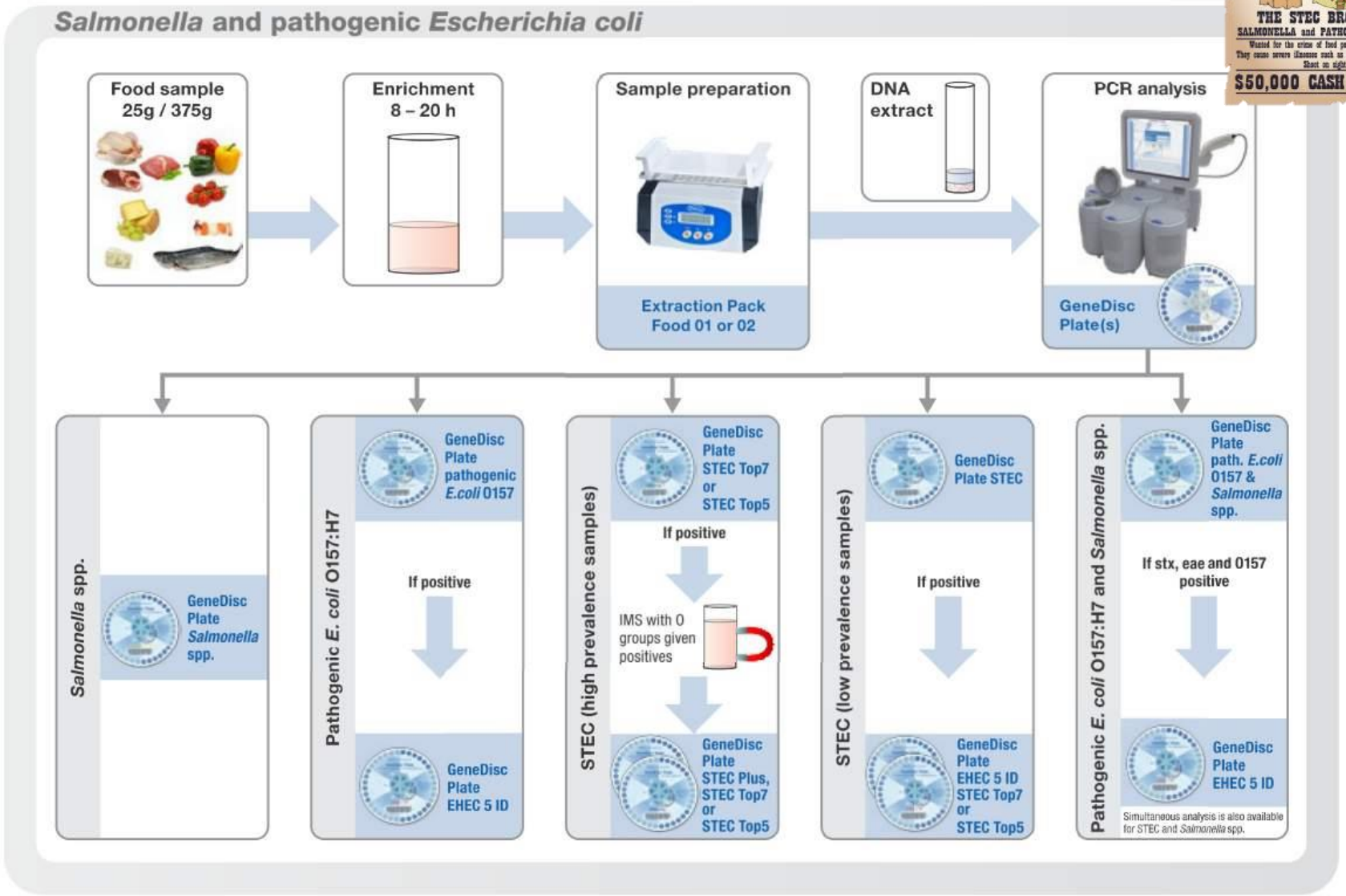


The GeneDisc Method for the Detection and Identification of Foodborne Pathogens:

TEST PERFOMANCE & VALIDATION

GeneDisc Method for Salmonella spp. and Pathogenic E. coli Detection

GeneDisc Method for *Salmonella* and Pathogenic *E. coli* Detection



GeneDisc Method for Salmonella spp. Detection

GeneDisc Method for *Salmonella* spp. Detection

Enrichment Time	Down to 8 hours
Sample Preparation Time	< 1 hour for 96 samples
PCR Cycle Time	< 1 hour
Total Turnaround Time	Down to 10 hours
Hands On Time	About 30 minutes for 96 samples (<30 s/sample)
Limit Of Detection	1 bacteria in 25 g of food sample 1 bacteria in 375 g of food sample
Specificity	Wide range of strains tested for inclusivity and exclusivity
Internal Positive Control Per Sample Analysis	Detects presence of inhibitors in each sample DNA extract

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GeneDisc Method for Pathogenic E. coli O157 Detection

GeneDisc Method for Pathogenic *E. coli* O157 Detection

Enrichment Time	Down to 8 hours
Sample Preparation Time	< 1 hour for 96 samples
PCR Cycle Time	< 1 hour
Total Turnaround Time	Down to 10 hours
Hands On Time	About 30 minutes for 96 samples (<30 s/sample)
Limit Of Detection	1 bacteria in 25 g of food sample 1 bacteria in 375 g of raw ground beef or raw beef trim
Specificity	Wide range of strains tested for inclusivity and exclusivity
Internal Positive Control Per Sample Analysis	Detects presence of inhibitors in each sample DNA extract

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GeneDisc Method for ShigaToxic E. coli (STEC) Detection

GeneDisc Method for STEC Detection

Enrichment Time	Down to 8 hours
Sample Preparation Time	< 1 hour for 96 samples
PCR Cycle Time	< 1 hour
Total Turnaround Time	Down to 10 hours
Limit Of Detection	1 bacteria in 25 g of food sample 1 bacteria in 375 g of raw beef meat
Specificity	Wide range of strains tested for inclusivity and exclusivity
Internal Positive Control Per Sample Analysis	Detects presence of inhibitors in each sample DNA extract

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GeneDisc Method for STEC Detection – Choose Your GeneDisc Solution (1/2)

Recommended for low prevalence samples

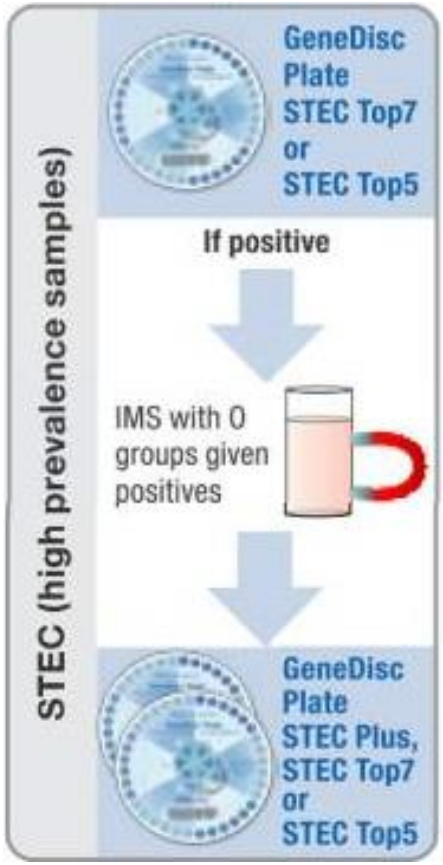
Follow reference method

In line with MLG 5B and ISO/TS 13136 – With this method, a systematic screening based on virulence factors allows to discriminate pathogenic strains from non pathogenic ones. If result is positive, an identification of the Top 7 or Top 5 serogroups is performed.

Test *Salmonella* spp. simultaneously – Analysis with *Salmonella* spp. is available and does not require any additional hands-on time nor enrichment.

GeneDisc Method for STEC Detection – Choose Your GeneDisc Solution (2/2)

Recommended for high prevalence samples



Reduce your rate of presumptive positive

Enhanced workflow – This method enables to reduce the number of presumptive positive sample using a cutting edge approach. With this method, all targets – serogroups and virulence factors – are analyzed within one GeneDisc plate.

High level of discrimination – An accurate virulence factor screening based on the association of these factors to serogroups provides a lower rate of presumptive positive than any other available method.

GeneDisc Method for Listeria Detection

GeneDisc Methods for *Listeria* Detection

Listeria

Food sample 25g Environmental samples

Enrichment 18 – 26 h

Sample preparation

Extraction Pack Food 01

DNA extract

PCR analysis

GeneDisc Plate *Listeria*

Listeria spp.

GeneDisc Plate *Listeria* spp.

Listeria monocytogenes

GeneDisc Plate *Listeria monocytogenes*

L.monocytogenes & *Listeria* spp.

GeneDisc Plate *Listeria* DUO

GeneDisc Method for *Listeria* Detection

Enrichment Time	Down to 18 hours
Sample Preparation Time	< 1 hour for 48 samples
PCR Cycle Time	< 1 hour
Total Turnaround Time	Down to 20 hours
Hands On Time	About 45 minutes for 48 samples (<1 min/sample)
Limit Of Detection	1 bacteria in 25 g of food sample and in environmental samples
Specificity	Wide range of strains tested for inclusivity and exclusivity
Internal Positive Control Per Sample Analysis	Detects presence of inhibitors in DNA extract sample

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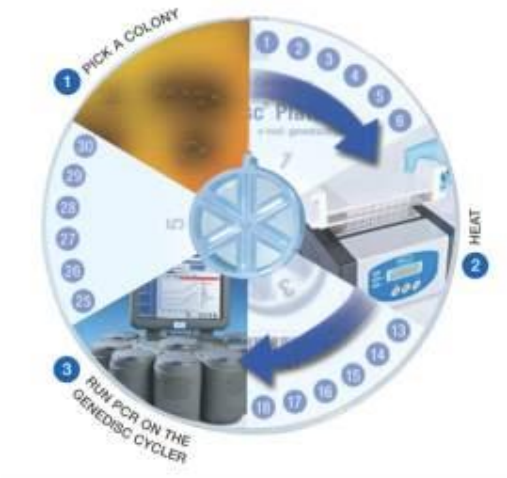
GeneDisc Method for Listeria Identification

GeneDisc Method for *Listeria* identification

GeneDisc Method for *Listeria* identification



From colony on plate, direct identification of:

- *L. monocytogenes*
- *L. grayi*
- *L. seeligeri*
- *L. innocua*
- *L. ivanovii*
- *L. welshimeri*

Sample Preparation Time	< 15 minutes
PCR Cycle Time	< 45 minutes
Total Turnaround Time	< 1 hour
Specificity	Wide range of strains tested for inclusivity and exclusivity
Internal Positive Control Per Sample Analysis	Detects presence of inhibitors in DNA extract sample
Validation	AOAC approved for colony confirmation from all major enrichment media

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The GeneDisc Method for the Detection and Identification of Foodborne Pathogens:

IN CONCLUSION

GeneDisc Methods – Validations Summary

Application	Minimum Time to Result	NF VALIDATION	AOAC-PTM
Listeria monocytogenes and/or spp. detection	20 h	✓	✓
Listeria identification	1 h		✓
Salmonella spp. detection	4 -10 h	✓	✓
Pathogenic *E. coli* O157 or STEC detection	10 h	✓ (only *E. coli* O157:H7)	✓
STEC Top 7 detection	10 h	✓ (only *E. coli* O157:H7)	✓

In Summary…

RELEASE FASTER

- Fast decision making tool for:
  - Product release
  - Test upstream in the process.

SECURE RELEASE

- Reliable, accurate third party validated performance:
  - Specificity/Sensitivity
  - Validation on real samples.

TEST EASILY

- No technical skills required to obtain high quality results:
  - Easy to use by design
  - Automatic data interpretation.

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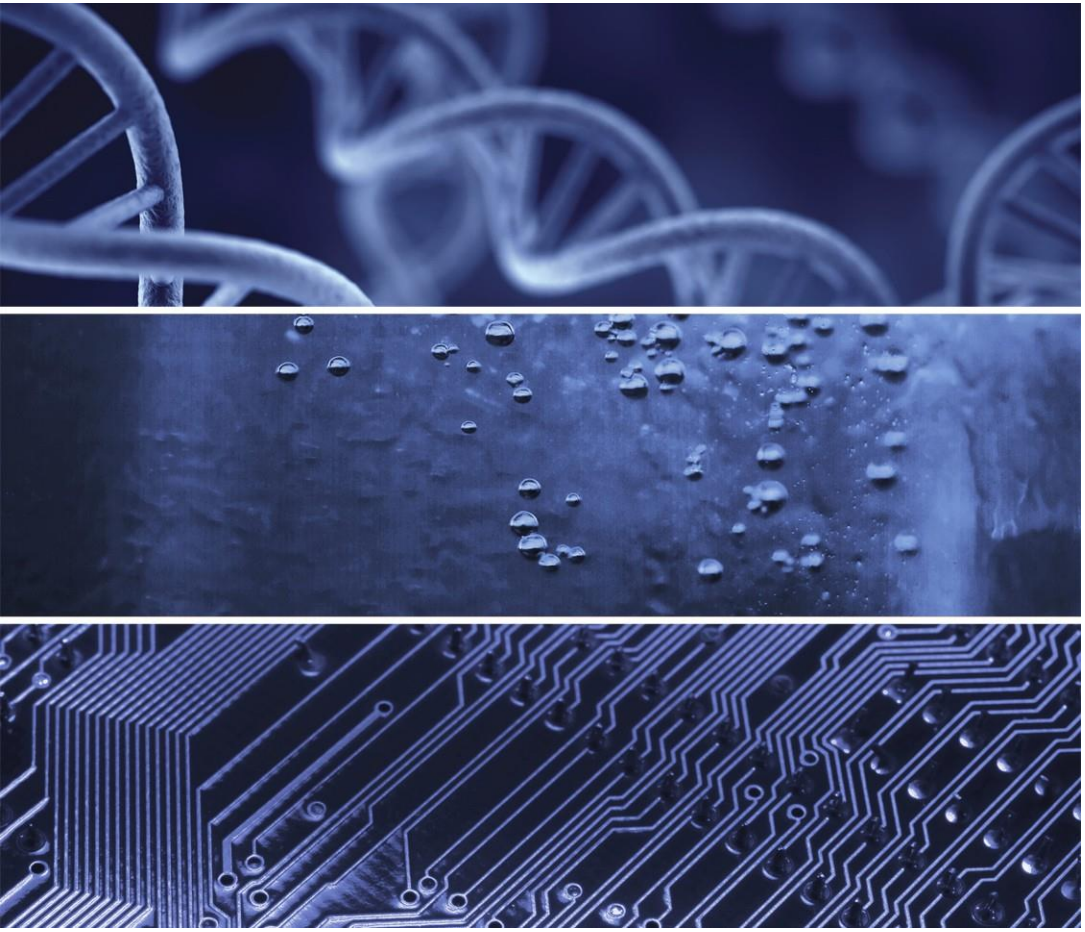

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