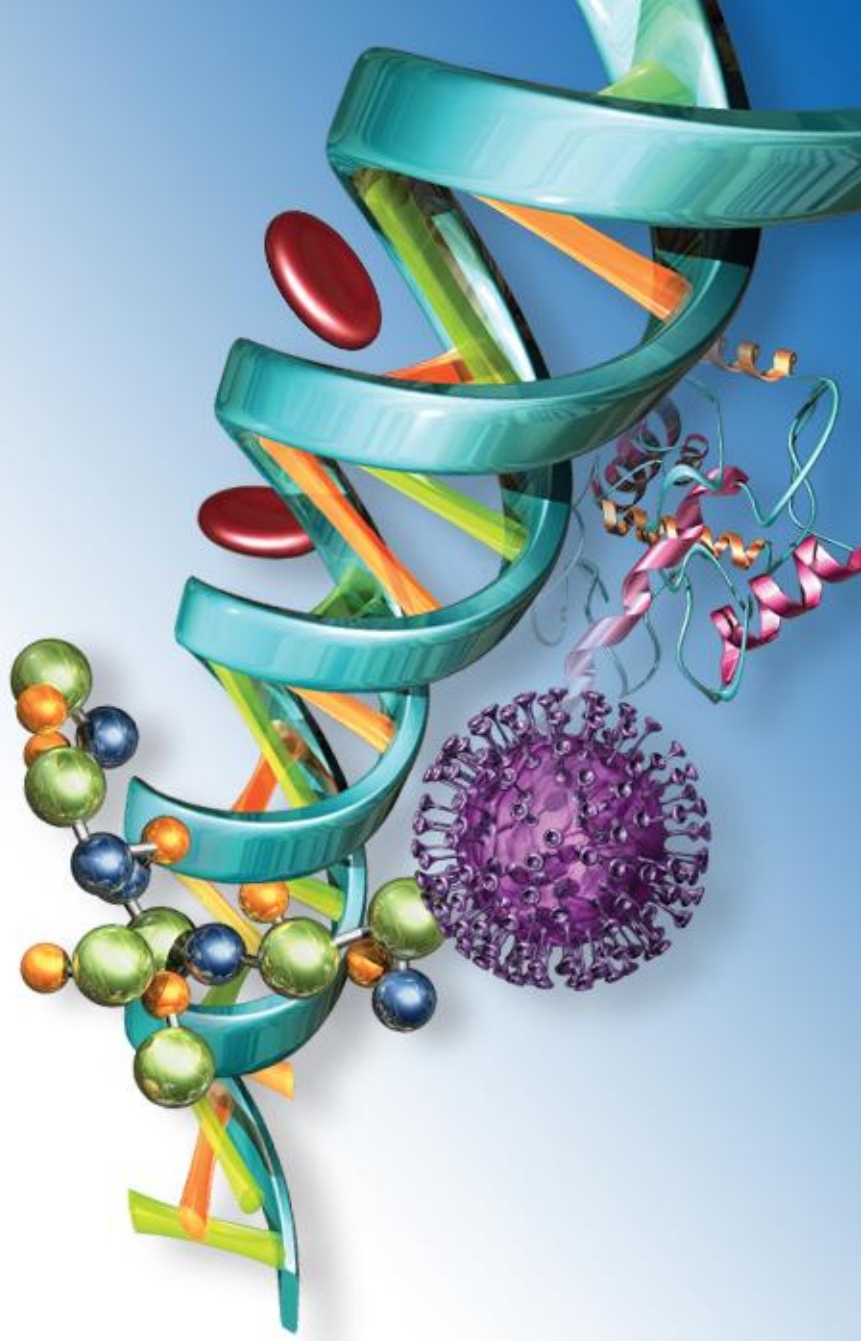
GeneDisc® Rapid Microbiology System

A revolutionary step in advanced microbial quality monitoring



ISGA
October 2011

PALL Life Sciences

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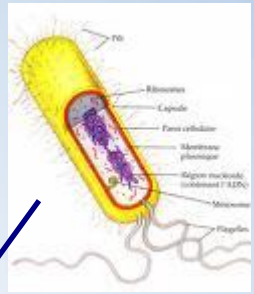
GeneDisc

A multi-parametric food pathogen testing system

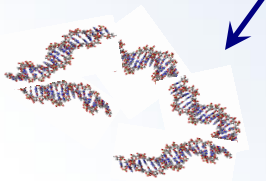
- Easy to use, no need for strong technical skills
- Flexible, modular system, scale to your test volume
- Real time PCR
- At-a-glance display of results, enables rapid decisions
- LIMS connectivity for data management
- Salmonella and E.coli test results on the same sample

Real time PCR Overview

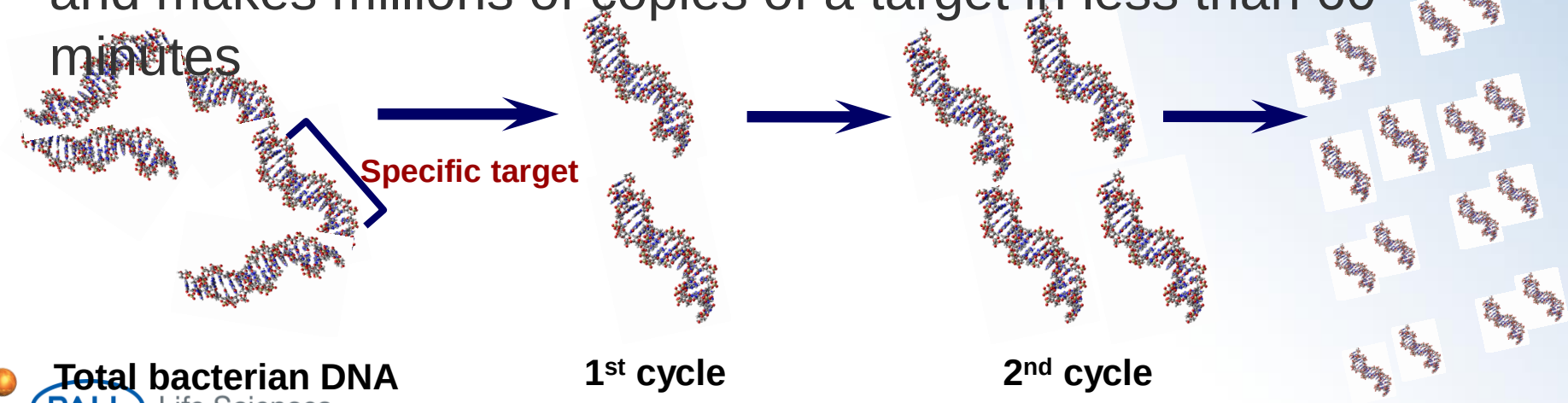
- All microorganisms contain unique DNA sequences (targets)



- After DNA Extraction,



- Polymerase Chain Reaction (PCR) copies these targets and makes millions of copies of a target in less than 60 minutes



Outline Description of the System

- There are three core components to the system:
  - The Extraction Pack and associated consumables

  Universal method for microbial DNA extraction in all food matrices
  - The GeneDisc plate and associated consumables

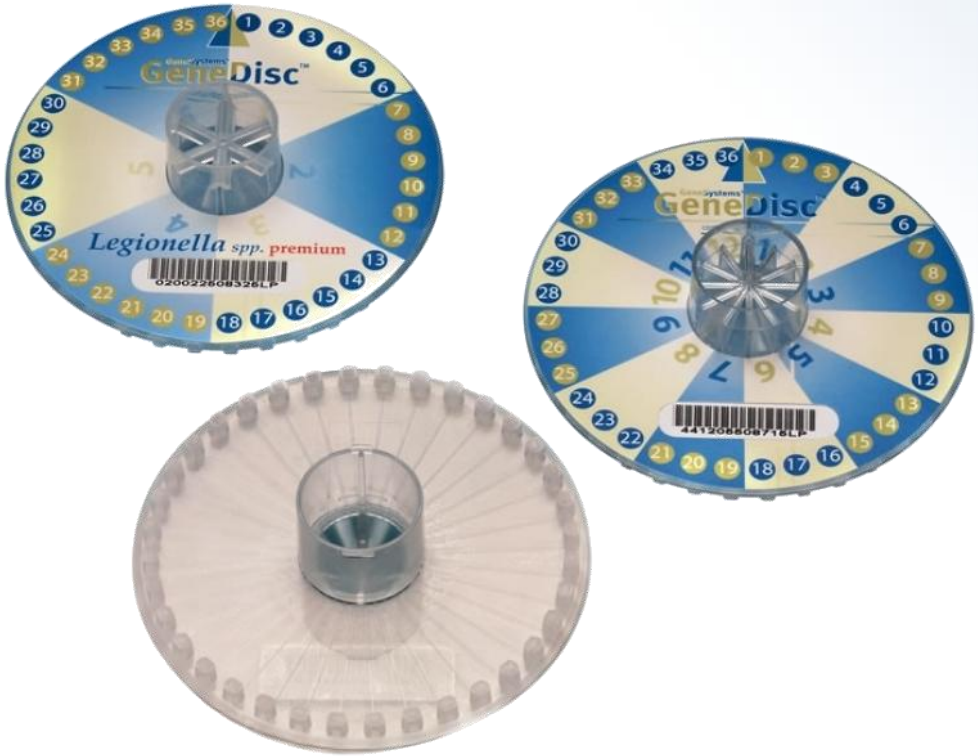
  To assay extracted microbial DNA
  - The GeneDisc Cycler

  To run assays to detect or quantify this DNA

Plate:
the qPCR assay consumable

Cycler:
the qPCR assay instrument

GeneDisc® Real time PCR technology



- Assay Consumable
  - GeneDisc® Plate
    - TaqMan technology
    - Primer/Probes preloaded
    - Inhibition control/sample
    - Negative control/sample
    - Plate for 6 or 12 samples
    - Bar code for traceability
    - 1 tube of master mix / plate
    - Minimal volume to work with 20 µL
    - Limited risk of cross contamination

GeneDisc® Real time PCR technology

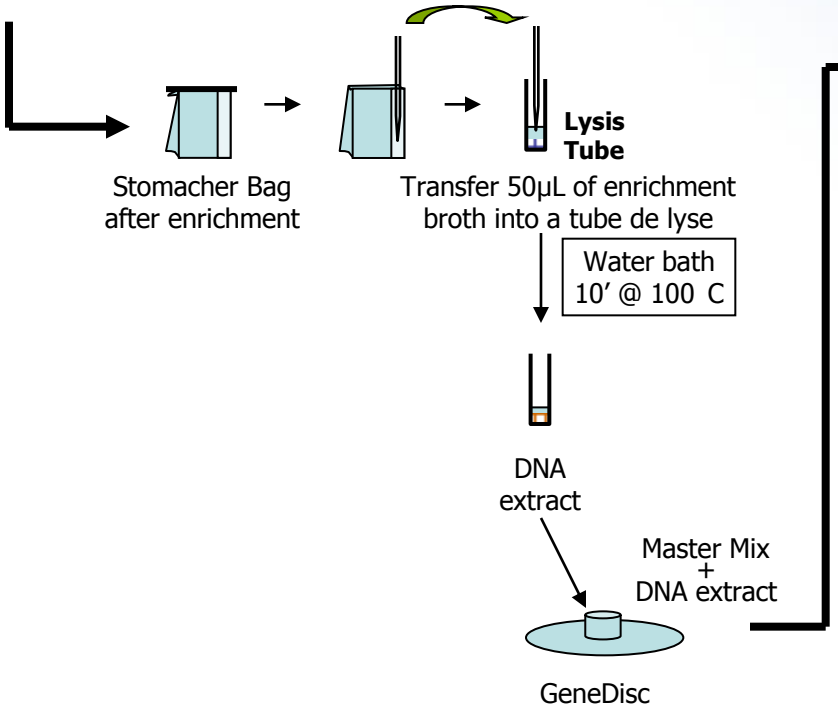
- Real-Time PCR instrument
  - GeneDisc® Cyclers

    Designed for routine analysis
    - Adaptable, from 1 to 8 units
    - 1 to 8 GeneDisc plates simultaneously, ie. 1 to 96 samples

Process flow

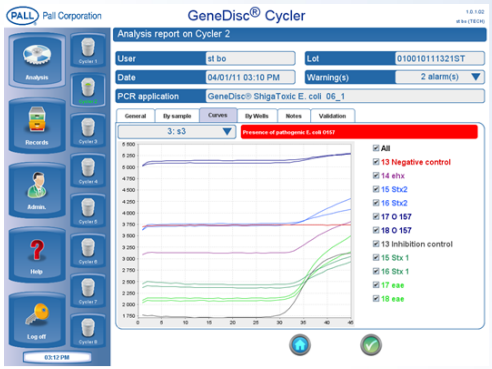
Enrichment & DNA extraction

Pall Food DNA Extraction kit



Screening by real time PCR

GeneDisc & GeneDisc Cycler



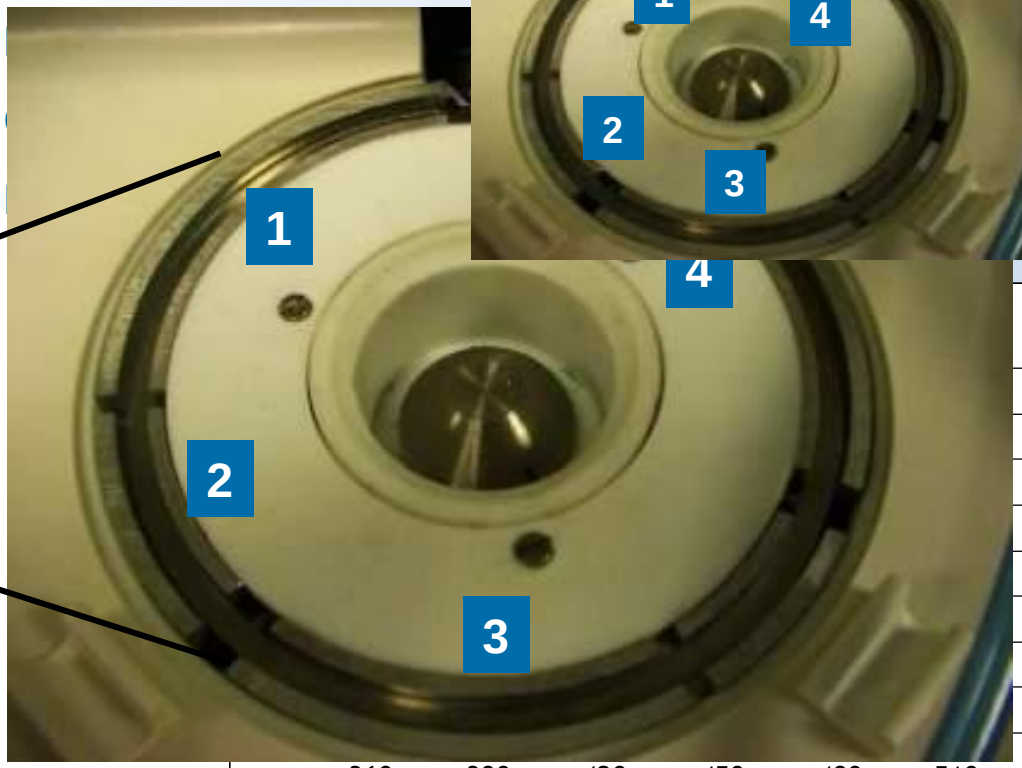
~10 hr

1 hr

GeneDisc Cycler Heating Process :

- IP
- Consistent heating well to well

1
2
3



4 heating sectors

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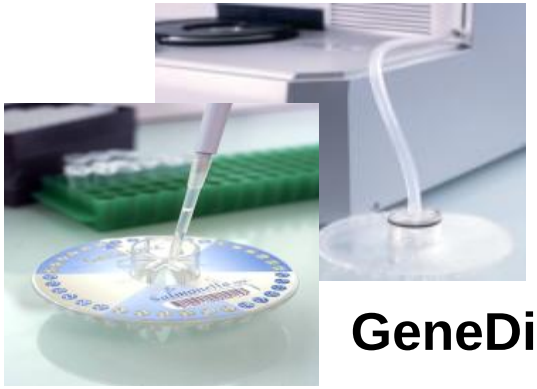
PCR Operations with GeneDisc Plate

Barcode Reading

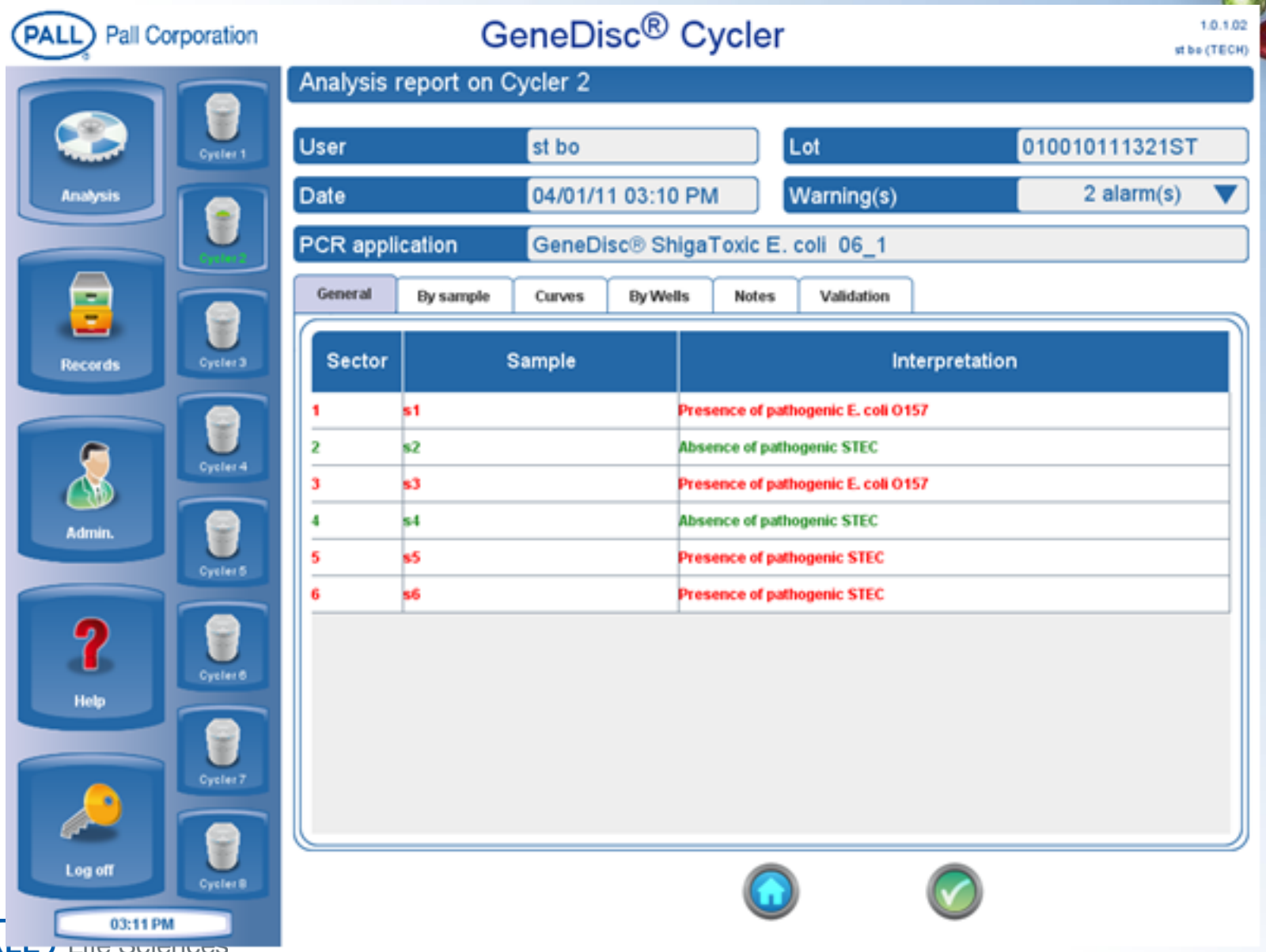


GeneDisc Filling

GeneDisc Loading – PCR run starts.

- GeneDisc bar-coded;
- Barcode selects appropriate qPCR parameters.
- 1 Master Mix tube ↔ 1 GeneDisc - Limited risk of contamination;
- GeneDisc filled (vacuum system), placed into GD Cycler, lid closed
- Assay starts automatically – no further operator intervention
- Disc completely sealed, minimising contamination risk.

PCR Results – Result Overview



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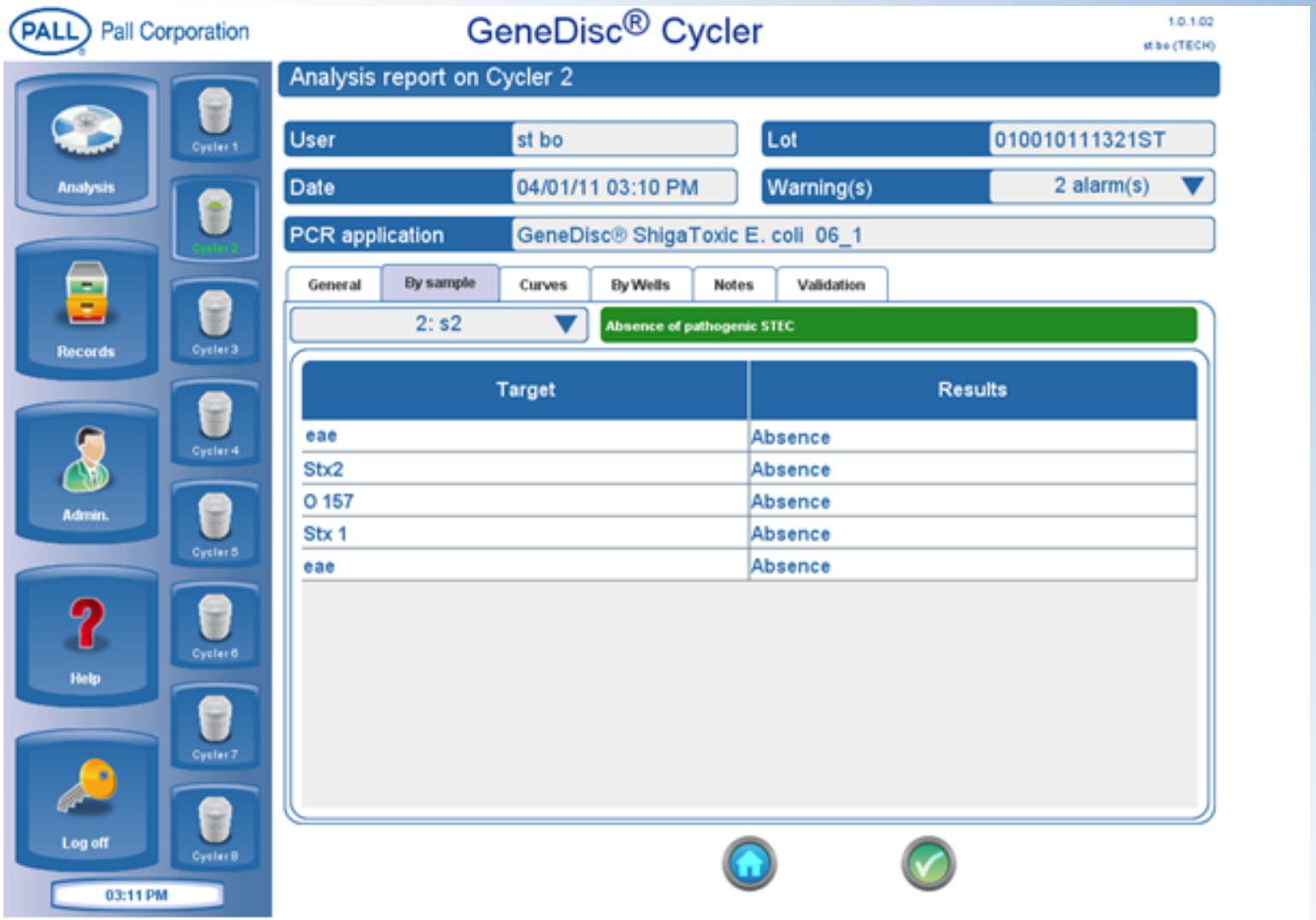

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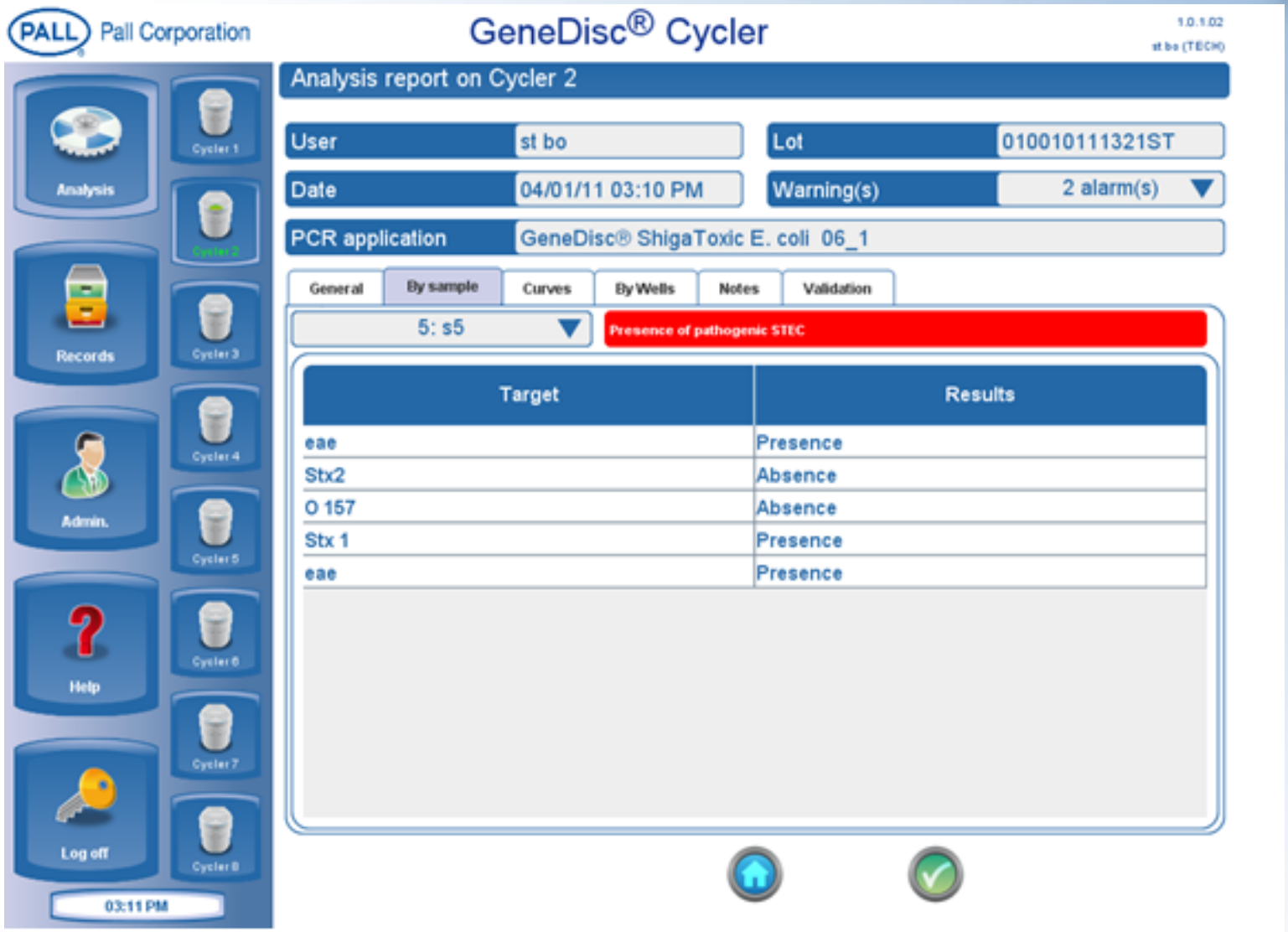
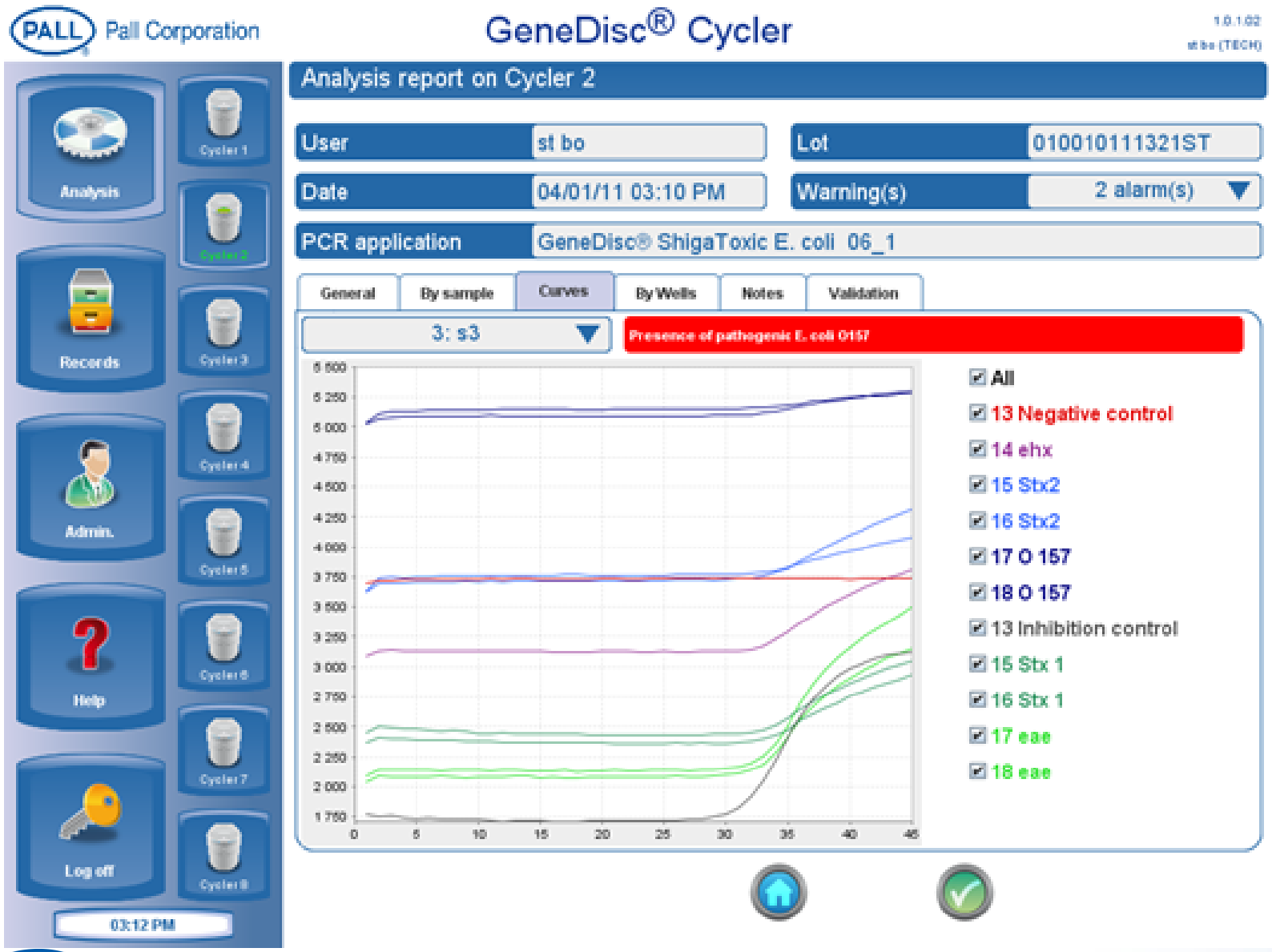


PCR Results – Result Overview



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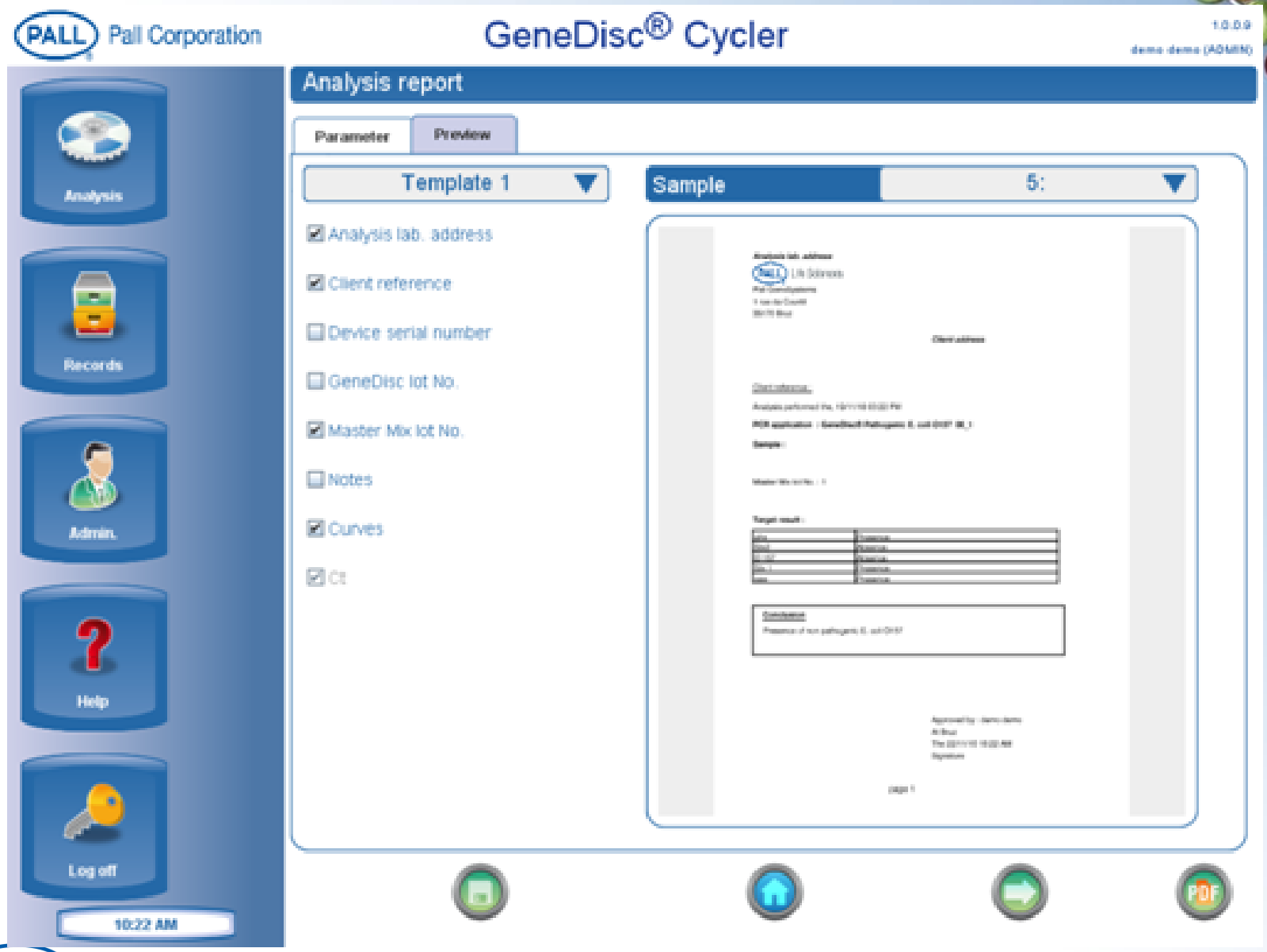
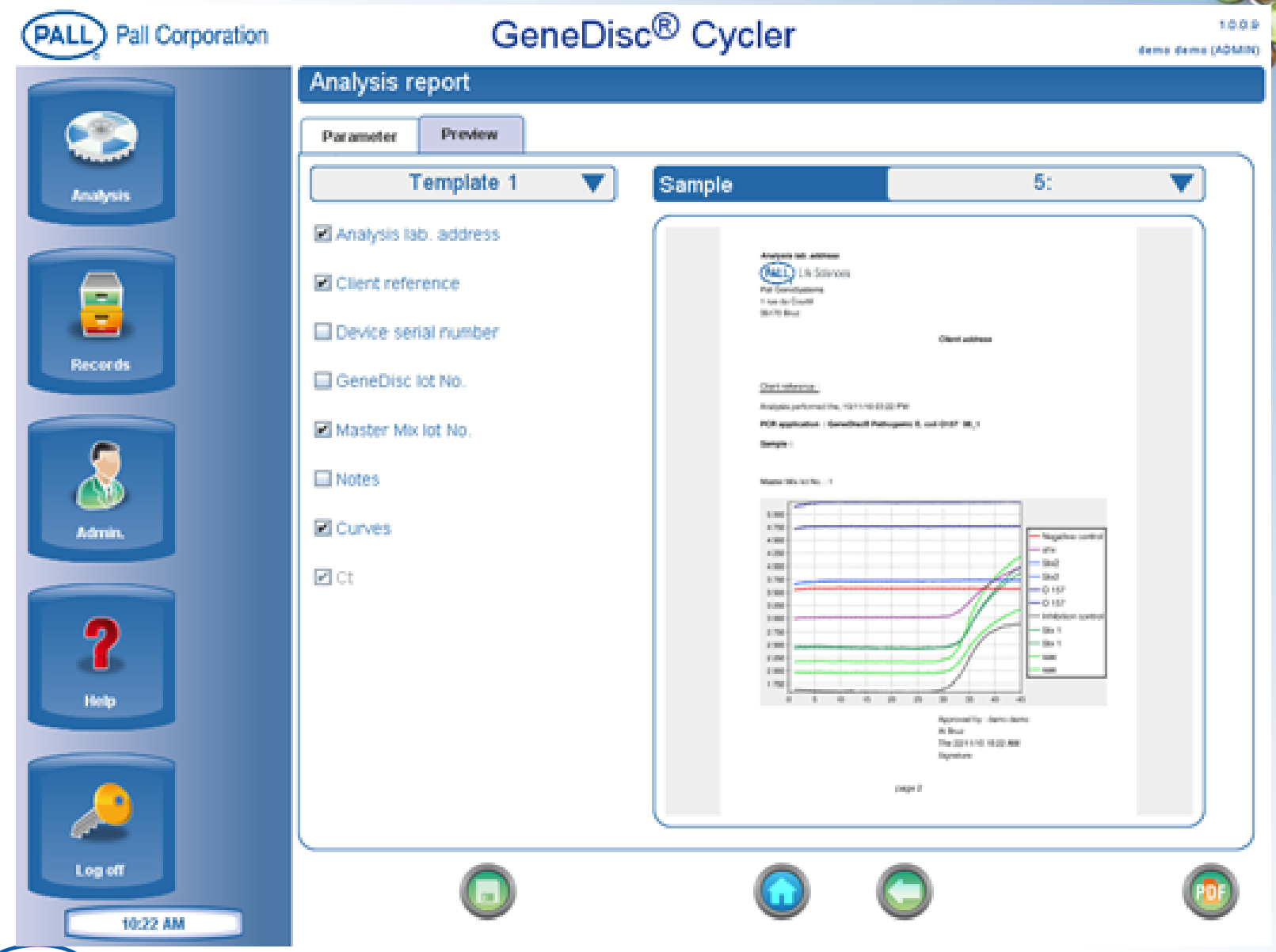
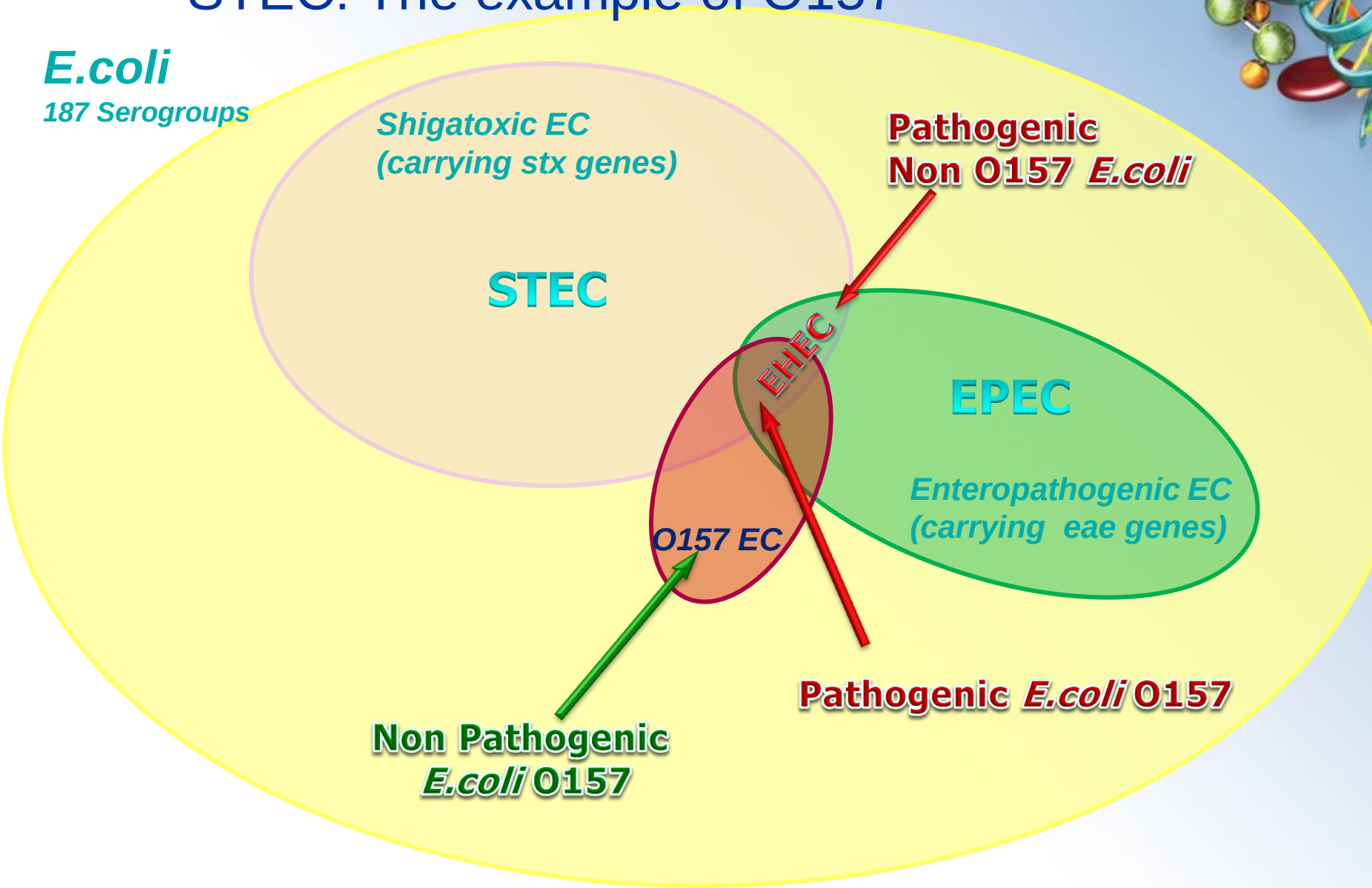
PCR Results – Result Overview



PCR Results – Result Overview

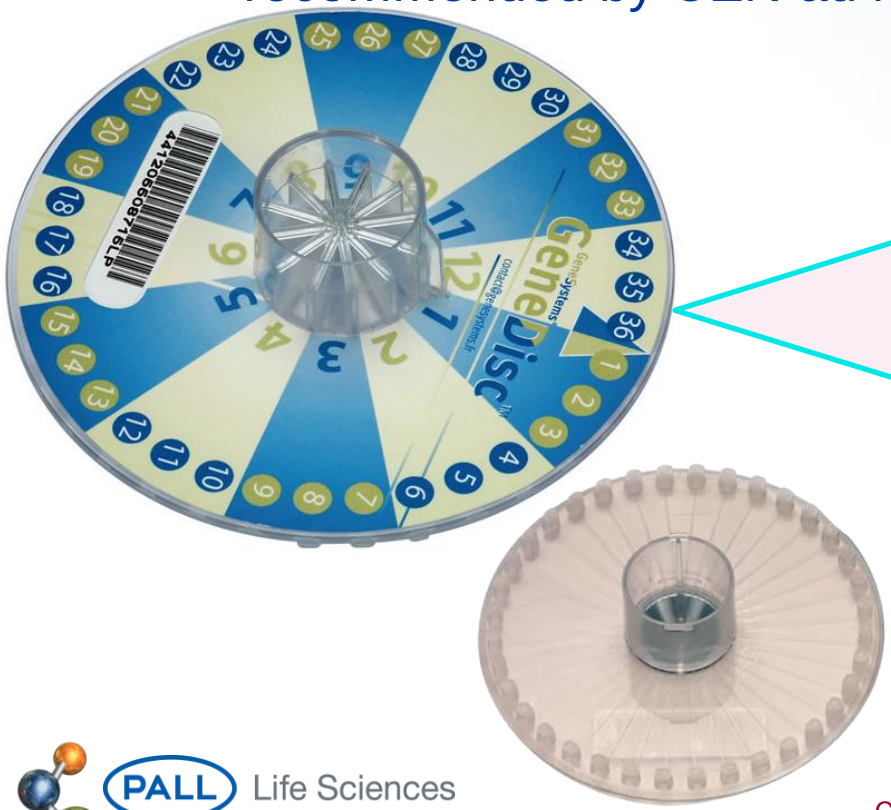


STEC: The example of O157



Design of the GeneDisc STEC & *E. coli* O157

- GeneDisc
  - Simultaneously detection of *E. coli* O157, STEC & *Salmonella* spp
  - Primers and probes design used for pathogenic genes are recommended by CEN ad hoc group for their high specificity



Well #	FAM detection	ROX detection
1	Negative CTRL	Inhibition CTRL
2	*iroB* (*Salmonella*)	*stx1/stx2**
3	$rfbE_{O157}$*	*eae**

***Primers and probes used are those proposed by the "STEC ad hoc group" of the European Committee for Standardization (CEN) for a future ISO standard**

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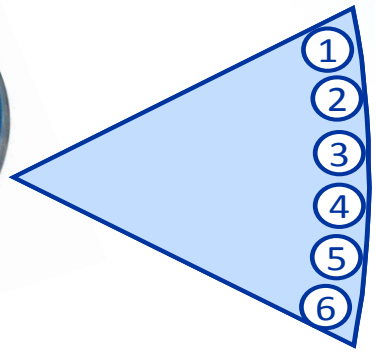
*Perelle S., et. al., Molec. Cell. Probes 18: 185-92 (2004)
*Nielsen & Andersen , J. Clin. Microbiol., 41:2884-93 (2003)

Focus on Pathogenic *E.coli* O157 and non-O157 STEC

- Simultaneous detection of the « big 6 » non-O157 STEC (O26, O103, O111, O145, O121, O45)
- The additional primers and probes (O121 & O45) are those recommended by USDA ARS (Dr Pina Fratamico)
- Added O104:H4 plate for testing related to outbreak in Germany



1	*E. coli* O145	-
2	Negative CTRL	Inhibition CTRL
3	*E. coli* O157	*E. coli* O111
4	*E. coli* O26	*E. coli* O103
5	*E. coli* O91	*E. coli* O113
6	*E. coli* O121	*E. coli* O45

Chronology of *E.Coli* O104:H4 project

1. **End of May 2011** → Outbreak started being reported to media
   - Important pressure on German and EU authorities;
   - No official analytical method available for the outbreak strain, *E. coli* O104:H4.

2. **Former study run with GD technology had some available sequences**
   - Work closely to the French Food Safety Agency (Anses) for developing the right targets;
   - Adaptation on the GD technology and production of the first batch for quick validation by Anses.

3. **By June 2011, German lab (Bfr Berlin managed by Dr Lothar Beutin) got the GD O104:H4 for food sample screening**
   - Investigations of 100's of samples;
   - Food survey identified fenugreek seeds as the most probable source of infection.

4. **By end of June 2011, Pall was used for the French outbreak investigations linked to *E. coli* O104:H4.**

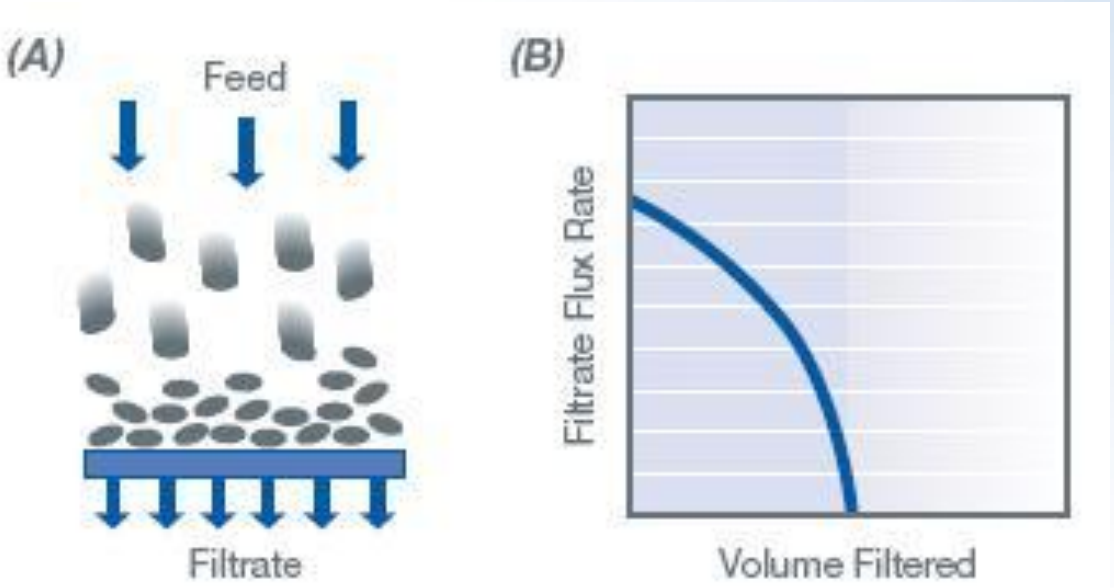
Pall GeneDisc Technologies – STEC O104:H4

One Step → detection of various virulence gene and O104:H4 genes

- *stx2*
- *wzx* O104
- *flic-H4*
- *ter* (tellurite resistance)
- *aggR* (transcriptional regulator)
- Inhibition and negative control per sample

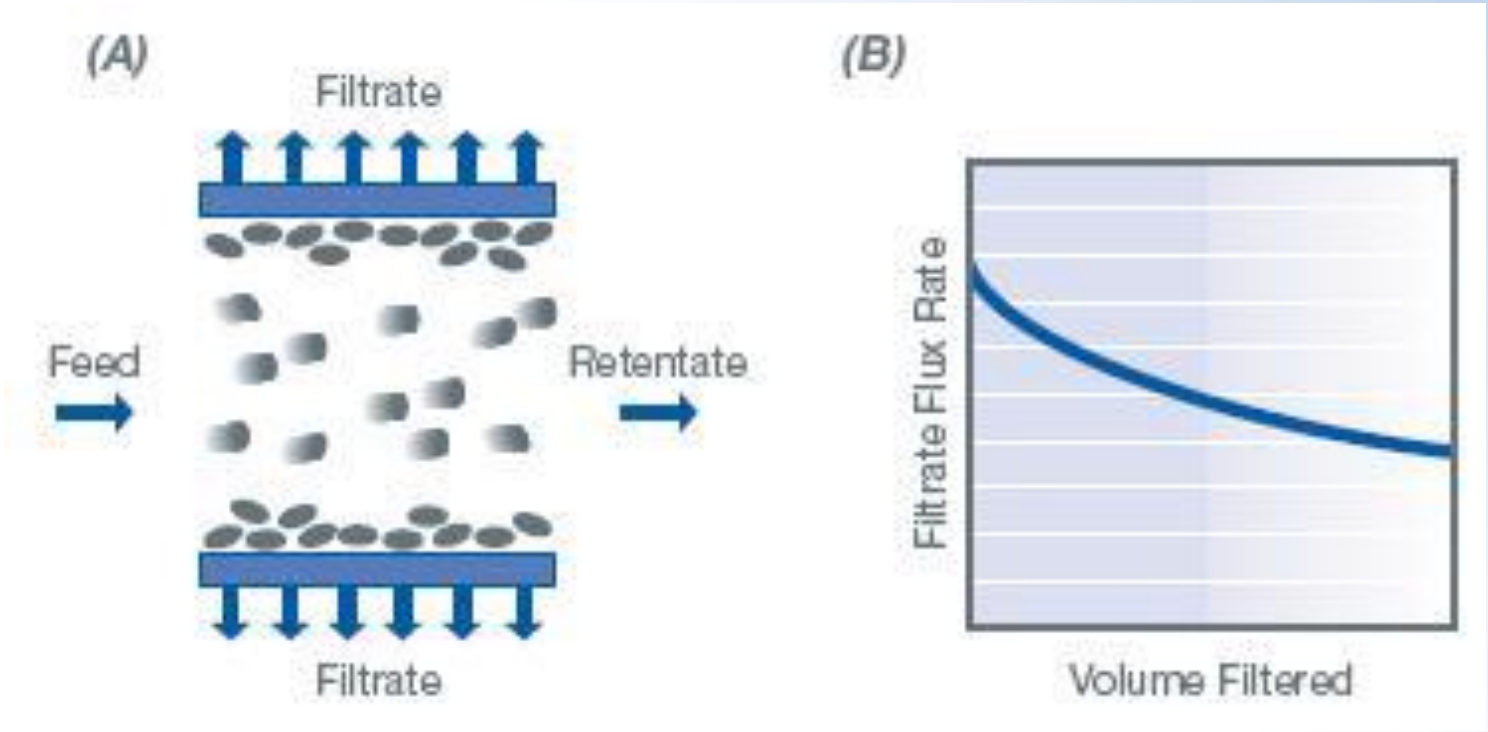
Filtering Sprout Irrigation Water

- Test more water without running more tests
- Increase sample size from 1 liter to 5 or 10 liters by using a concentration step. Direct flow – enrich filters



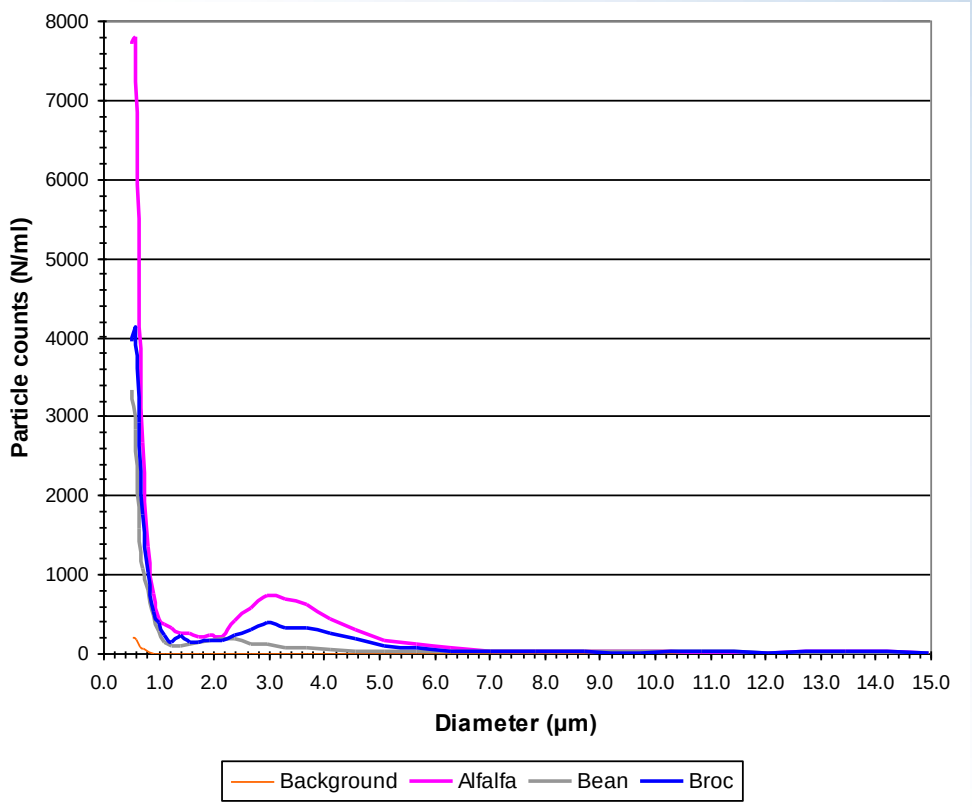
Filtering Sprout Irrigation Water

- Test more water without running more tests
- Increase sample size from 1 liter to 5 or 10 liters by using a concentration step. Tangential Flow – enrich concentrate.



Irrigation Water Challenges

Particle Size Distribution



Jonathan's Sprout Irrigation Water

Irrigation Water Challenges

Irrigation Water Concentration Factors		
Factor	Direct Flow	Tangential Flow
Time	Sizing	Sizing
Cost	Lower	Higher
Cross Contamination	Single Use	Sanitize and Reuse
Testing	Material Retained on Filters	Concentrate

Thank you

Any Questions ?

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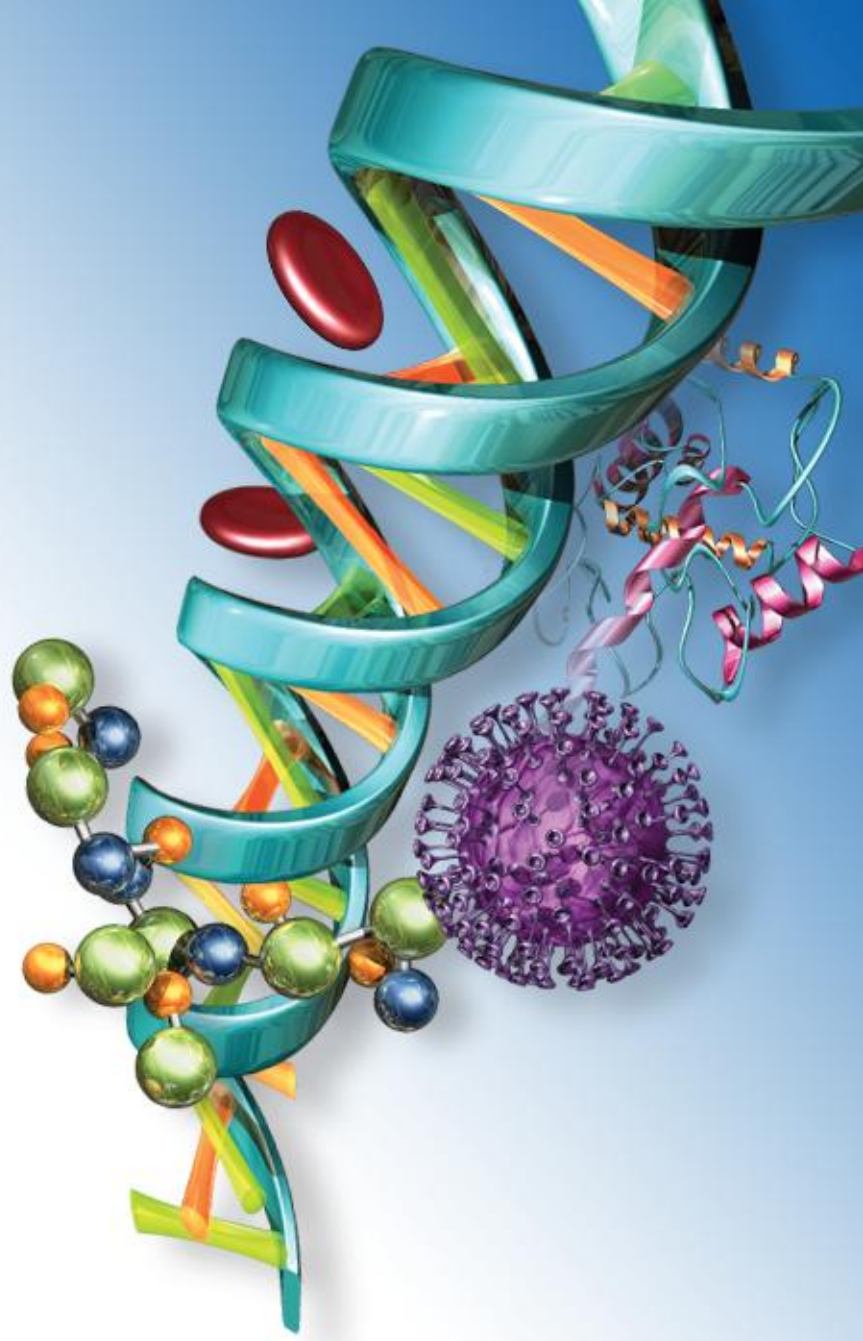
An innovative step in advanced microbial quality monitoring



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