

PATHOGENS DETECTION VIA PCR WITH AUTOMATED SAMPLE PREPARATION BASED ON IMMUNOMAGNETIC SEPARATION (IMS)

mRAMA Workshop – Barcelona 22 November 2017

MERCK

Assurance GDS – Genetic Detection System

A unique molecular method for Food Pathogen detection



Advanced PCR-based pathogen screening method for various Food pathogens

Developed to meet the daily rigors & requirements specific to food testing labs

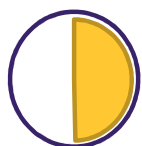
Designed to provide the most accurate and fast results – validated across a wide range of matrices

Automated sample prep now available

Assays available

Same Day Results

Salmonella – ISO 16140 validation



12 hours

Select foods and
environmental
samples



20 hours

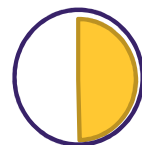
All samples

E. coli O157:H7 – ISO 16140 validation



10 hours

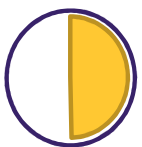
25 g samples



12 hours

375 g samples

Top STEC (ShigaToxins producing E.coli) – AOAC validation



12 hours

25g and 375 g
samples

Assays available

Next Day Results

Listeria spp. (ISO 16140 validation)



28 hours

Foods and
environmental
samples

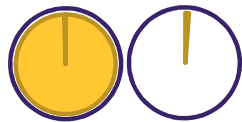
Listeria monocytogenes (ISO 16140 validation)



28 hours

Foods and
environmental
samples

Cronobacter (ISO 16140 validation on going)



26 hours

Infant formula and
ingredients



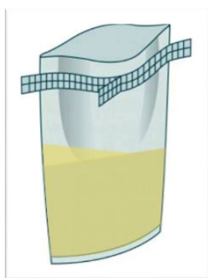
TECHNOLOGY OVERVIEW

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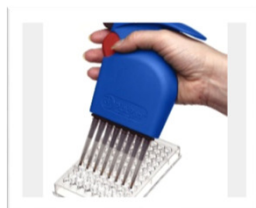
Assurance GDS

Basic Workflow

3-Step Process



Enrichment



Manual or
Automated
Sample Prep



PCR
Analysis

Enrichment step

Sample Preparation to let the target bacterium grow at a detectable level

Begins with sample enrichment:



Sample of food/env
matrix sent to lab



Add sample to
enrichment broth



Masticate sample



Incubate

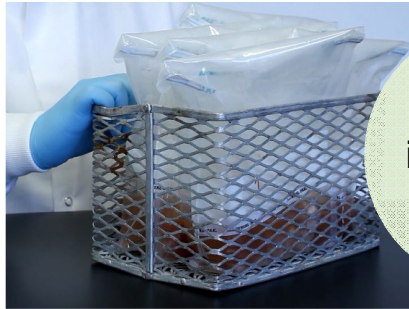
Common food test volumes are 25 g and 375 g

Common env. samples are swabs (10 mL)

Common food to enrichment ratios are 1:10 and 1:5

Sample prep for PCR

Sample Preparation to have the target microorganism be ready for PCR



From
incubated
sample

But how do you get the
sample ready for PCR?

To amp
tube



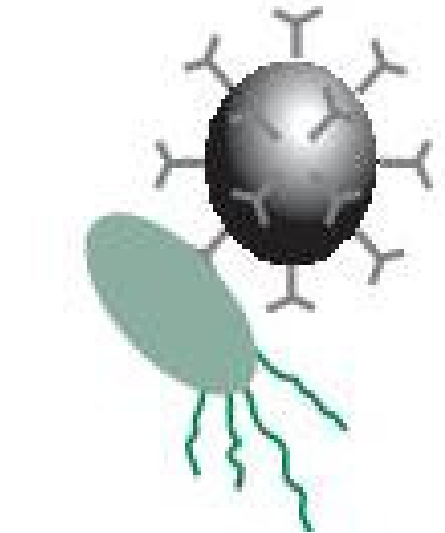
ImmunoMagnetic Separation (IMS) The Core of GDS Technology

IMS protocol - **selectively** captures and separates target pathogens

Specific antibodies for the target microorganism are coated on paramagnetic particles

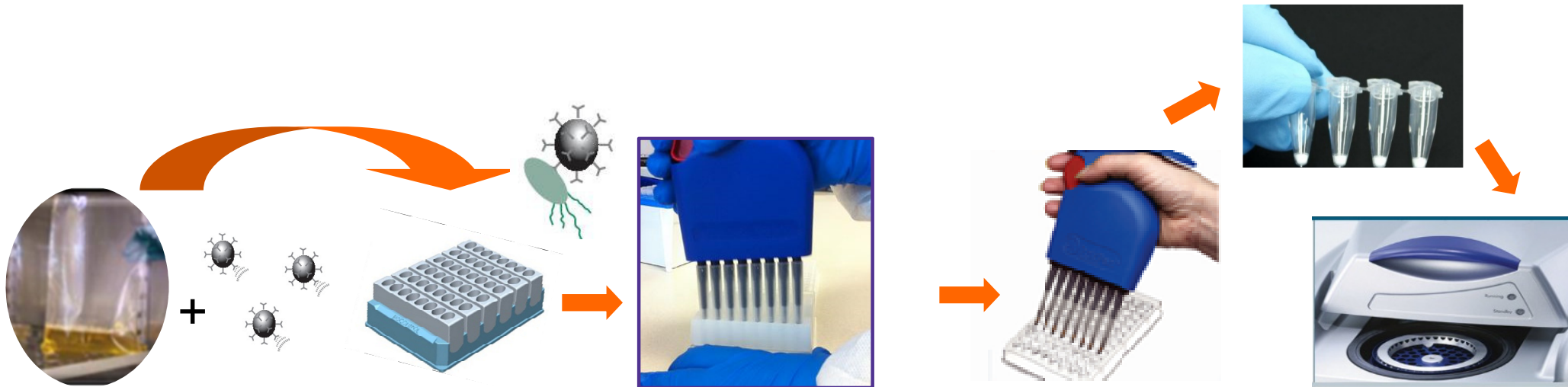
The target is captured and bound to the IMS particles based on antibody-antigen reaction

Magnets on a special device called PickPen collect particles with captured organisms



IMS

Assurance GDS employs an antibody specific intrasolution IMS protocol to capture and separate organisms



1 mL of enriched sample is added to magnetic beads coated with antibodies against the target bacteria

Pathogen is captured and bound to magnetic beads based on antibody-antigen reaction

PickPen magnets collect beads with captured organisms

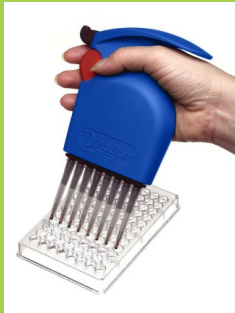
Beads are resuspended in a buffer and transferred to amplification tubes

Amplification tubes are loaded in the thermocycler

IMS

Advantages

PickPen IMS Sample Preparation

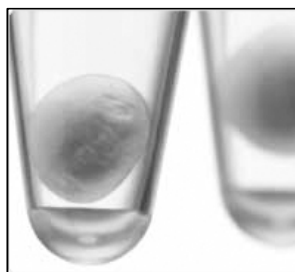


- **Improves accuracy** – captures and concentrates target pathogens (1st level of specificity), decrease carryover of background microflora
- **Prevents PCR inhibition** – physically separates pathogens from potential inhibitory compounds (sample matrix, enrichment media)

PCR Reagents system

Assurance GDS probe-based detection

- All reagents lyophilized inside amplification tubes
- No preparation needed

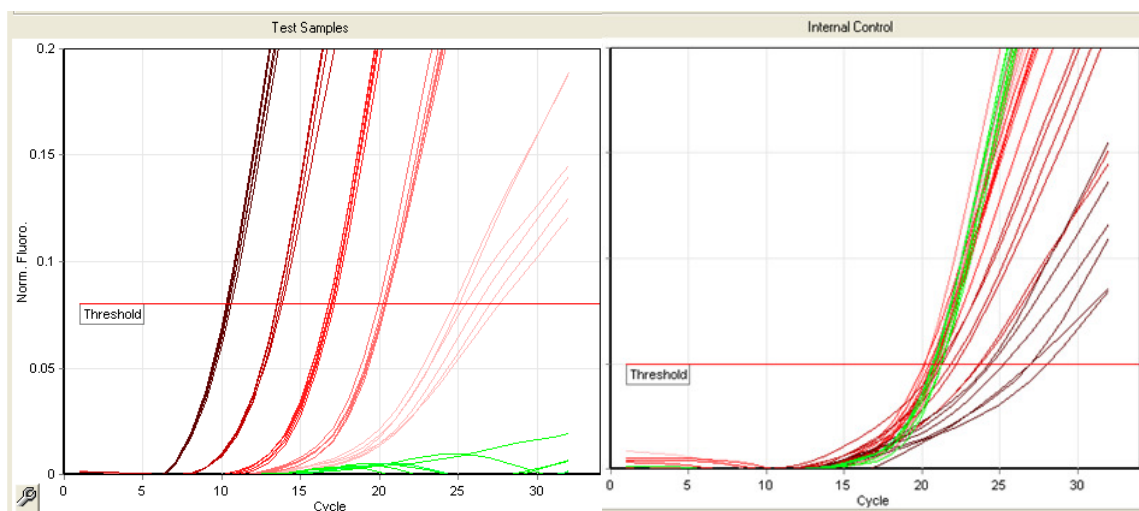


Internal control

- Primers and probes in each tube
- Signal produced on a different wavelength than target DNA to prevent identification confusion

Additional levels of specificity

- Target specific primers ensure that only target DNA is amplified
- MGB Eclipse Probe ensures that only target DNA is detected



The Probe

How the MGB Eclipse Probe Works

Probe in solution

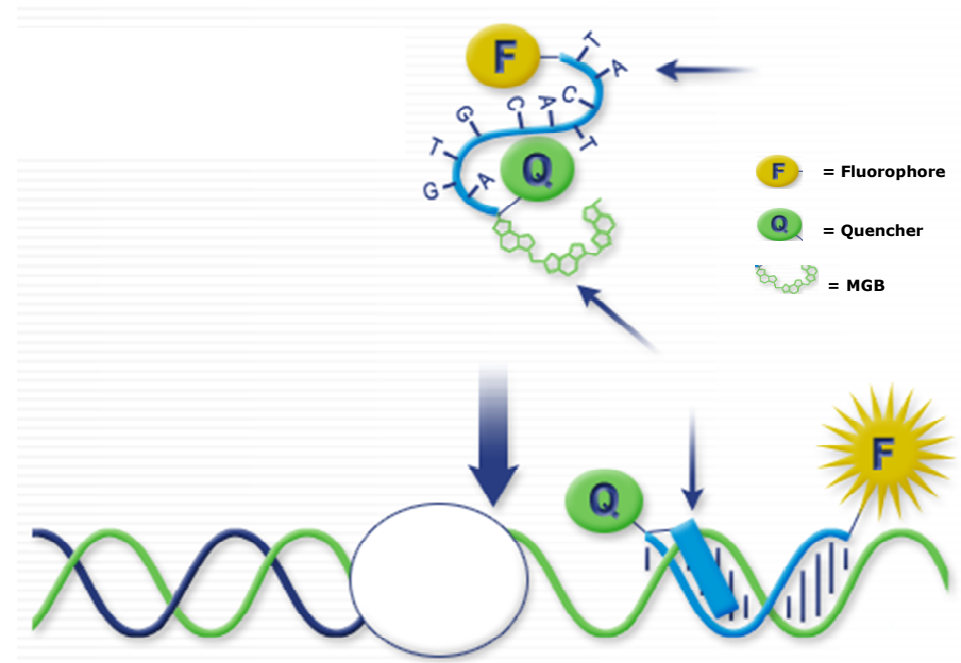
- Arranged in a random coil
- Fluorescent marker quenched

Probe binds to specific target DNA

- Minor Groove Binder (MGB) molecule attaches to DNA helix for stability

Probe bound to target

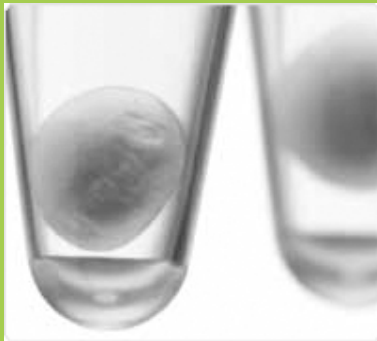
- Becomes linear
- Fluorescent marker exposed, emits signal



Assurance GDS

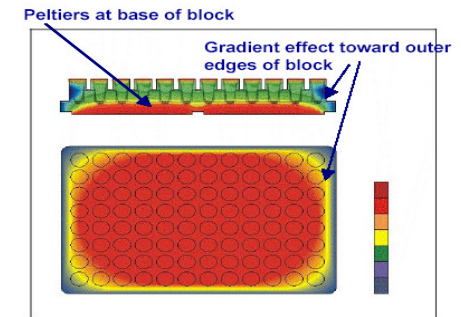
PCR Amplification & Detection Advantages

Probe-based PCR Detection



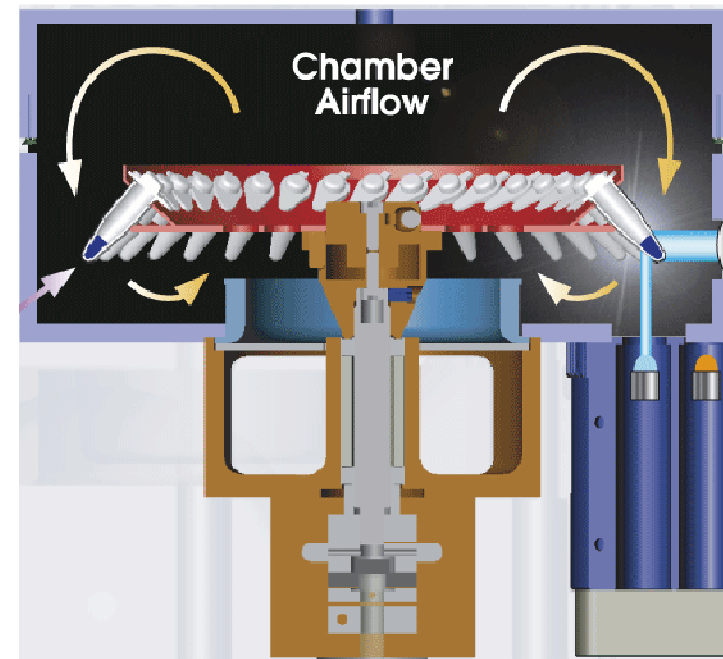
- **Superior specificity** – use of both primers and probes to add 2 additional levels of specificity
- **Reliable results** – true internal control in every reaction tube, will know amplification occurred
- **Consistency & stability** – PCR reagents in ready-to-use pellet in each reaction tube

Assurance GDS RotorGene Q Thermocycler



Rotary-based Design

- Samples arranged in a rotary format for direct heating / cooling
- Centrifugal motion with constant air exchange
- Ensures uniform temperature among all reactions
- Eliminates lengthy dwell times required by block based systems
- Rotors options: 36 or 72 samples



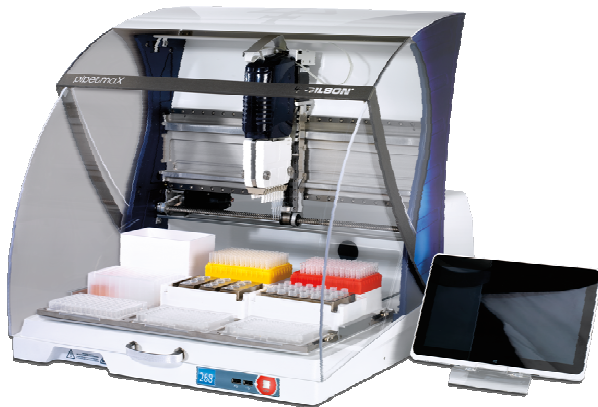


AUTOMATION OPTION

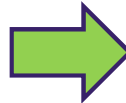
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Assurance GDS AUTOMATION With PichPen PipetMax (PPMX)

Sample Prep



PickPen PipetMax PPMX



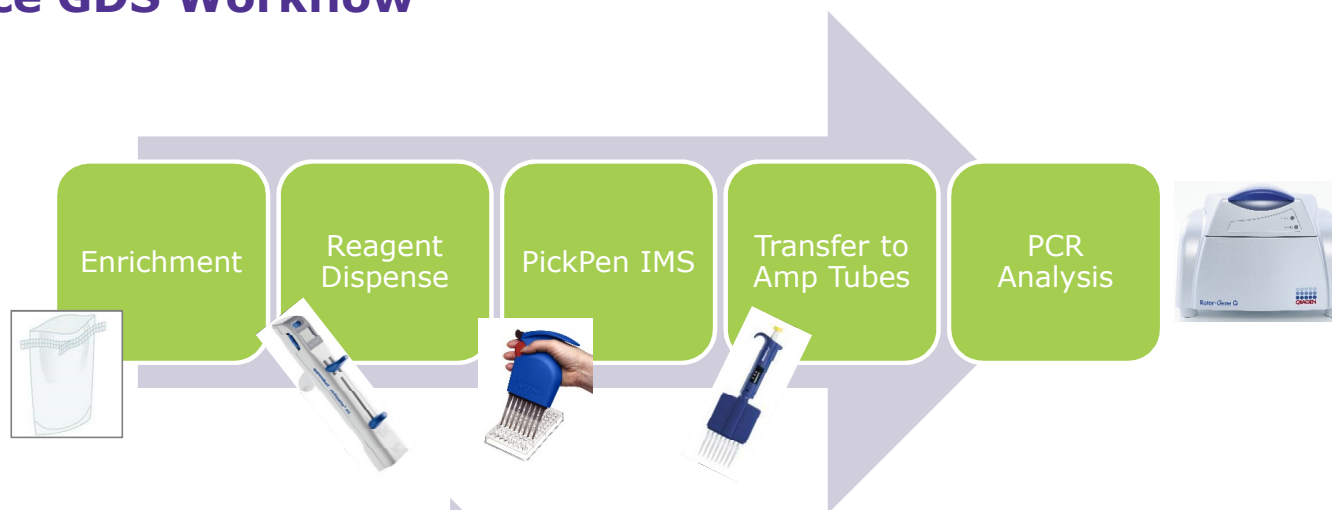
PCR Analysis



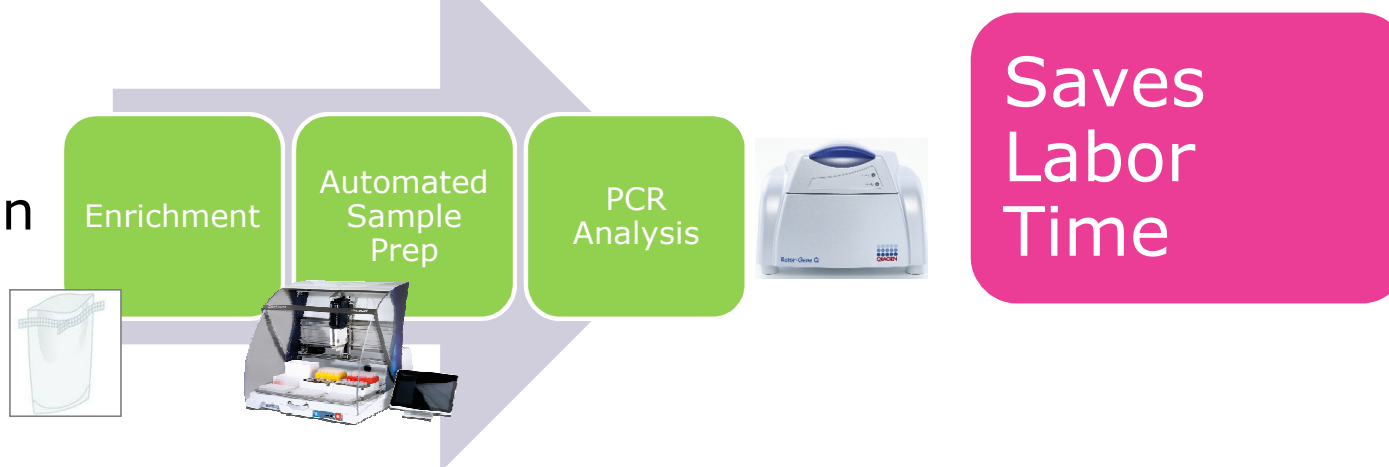
RotorGene Q Thermocycler

Assurance GDS Workflow

Manual



Automation



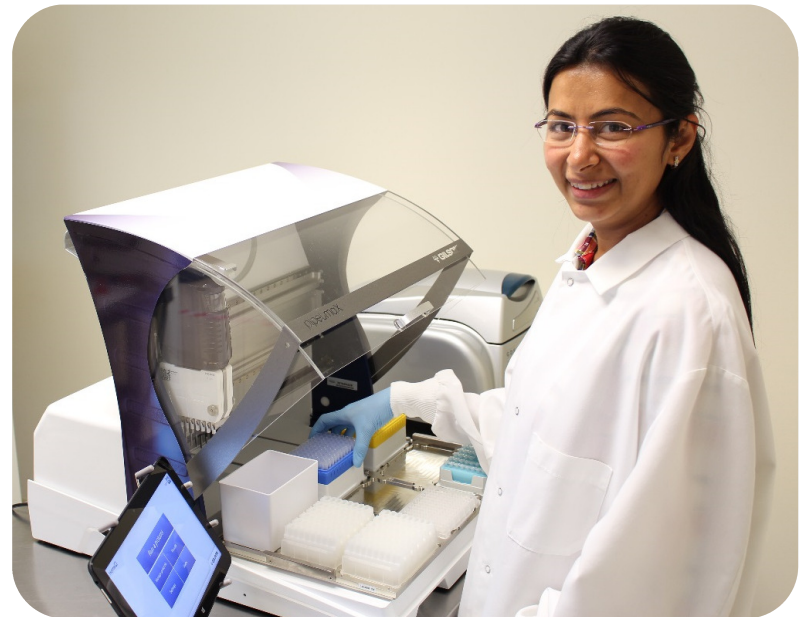
Assurance GDS

Automated Sample Prep

What does the PPMX do?

- Reagent dispensing
- Vortex mixing
- PickPen IMS
- Transfer to amplification tubes

GDS PickPen® PIPETMAX® (PPMX)



PPMX Features

Simple installation, setup & unloading

- Removable tray
- No tubes or priming

Small footprint

- W 54 cm x D 67cm x H 53 cm

Minimal maintenance required

Pre-programmed with Assurance GDS protocols

Touchscreen tablet with easy to use software

- Step-by-Step Wizard option to guide users

Bi-directional LIMS compatibility

- Barcode reader compatible



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Assurance GDS

PPMX Advantages

Increased Accuracy & Consistency

- Removes user variation
- Lowers risk of cross-contamination (aseptic travel path)
- Simplifies user training

Reduction in Labor Time

- Automates routine pipetting & PickPen IMS – freeing up technicians for other tasks
- Saves up to **50%** of labor time depending on protocol

Conclusions

- Assurance GDS is a PCR based system providing same day or next day results for Food Pathogens
- The different assays methods are validated according ISO16140
- IMS allows:
 - to work easily with difficult matrices that usually may inhibit PCR reaction
 - to concentrate the sample, enhancing sensitivity and providing reliable results
 - to work with PCR even without a DNA extraction or lysis and without the need to work in a dedicated cabinet
- It is a real time PCR method with 3 levels of specificity: 1st IMS, 2nd Primers, 3rd Probe.
- In each amp. tube there is an Internal Control that allows to trust in negative result
- Sample preparation can be automated to save labor time and increase productivity of the lab

A complete solution for Food testing lab: Fast, Reliable and Automated

And now ...



PickPen PipetMax Live !

