Pathogen Test Methods – What’s New in Methodology and Detection Limits

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I’m going to take us on a trip

Hold on tight, don’t relax,
The Road Map

• Why do we want to go anyway?
• Where have we come from?
• Where are we going?
• How far are we on the journey?
• What challenges do we still face?
• What is important to us as we choose the road we take or what do we need to bring along?
• When will we get “there”? 
Why do we care about food-borne pathogens?

- People get sick, friends, family, strangers, babies and elderly, some die and we want to protect them.
- Food producers often suffer devastating economic impact, business closure, loss of jobs.
- It's not just the food manufacturer anymore who is affected and held accountable, suppliers do suffer the consequences of contaminated food that causes illness.
  - Strain-typing
  - Cereal recall
Where have we come from?

• Oh my how the methods have evolved!
  – Listeria cold enrichment culture method
  – Observing TSAYE plates for Listeria-like colonies through obtuse lighting
  – Tumbling motility and the battery of biochemical tests
  – Inoculating mice to determine pathogenicity
Where are we going?
How far are we on our journey?
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What challenges do we still face, where are the roadblocks?

• Sample preparation
  – Better ways to separate microorganisms from the food matrix
  – Continue to develop ways to concentrate microorganisms before detection
  – Continue to build the better ‘bacteria trap’ by combining different techniques together
When will we get ‘there’?

• Quantitative detection of pathogenic microorganisms for troubleshooting and problem-solving
• Simultaneous detection and/or quantification of multiple, dissimilar pathogens in minutes (its my dream) by the elimination of sample enrichment
So let’s talk about what’s new and how they work

- Phage tail fiber recombinant protein technology and ELISA
- Magnetic Particle Capture & PCR
- Isothermal DNA Amplification & Bioluminescence Detection
- Isothermal DNA Amplification & Fluorescent Detection
Phage and Sandwich ELISA – bioMerieux Industry

- The tail fiber proteins offer a unique tool to detect bacterial pathogens
- ELISA – Enzyme linked immunosorbent assay, uses antibodies and a color change to identify a substance (pathogen)
Phage and Sandwich ELISA – bioMerieux Industry

- The phage protein tail fibers are coated on the inside wall of the pipette tip.
- During the capture step, sample is drawn into the pipette tip and conjugation occurs between Salmonella receptor sites on the surface of the salmonella cells and the phage protein.
- An antibody is applied over the surface so it can bind.
- Antibody is linked to an enzyme and then a substrate is added.
- All of this causes a color change in the substrate.
Phage and Sandwich ELISA – bioMerieux Industry

• The run needs to finish before results are available
• Specificity and selectivity are sometimes challenged
• Sophisticated instruments require upkeep and preventative maintenance

• Easy to use
• Many matrices validated
• Some propriety media
Magnetic Particle Capture & PCR - BioControl

- GDS (Genetic Detection System)
- Proprietary magnetic particle capture with DNA amplification
- Technology is PCR
- Air exchange thermal cycler reduces amplification time/run
- Probes and primers detect target organism
Magnetic Particle Capture & PCR - BioControl

- Results in 21 hours for most foods, raw foods take longer
- Single enrichment
- Innovative PickPen immuno-magnetic separation device is the capture part
- No heating blocks or prep of reagents
- Proprietary Rotor-Gene Q cycler is rotary based to ensure uniform heating and cooling of all samples

- Key to faster results? The IMS step to capture and concentrate target organisms, more sample volume is analyzed therefore shorter enrichment times
Isothermal DNA Amplification & Bioluminescence Detection – 3M™

- Multiple primers to recognize distinct regions of the genome and Bst DNA polymerase to amplify genetic material, less susceptible to inhibiting substances
- Amplification occurs continuously
- Isothermal (60°C)
- By-products converted to ATP
- ATP + luciferase = LIGHT
- Bioluminescence detection less prone to noise interference from some foods and chemicals
- Faster than other systems
- Simultaneous amplification and detection allows for positives to be known before the end of the run
Isothermal DNA Amplification & Bioluminescence Detection – 3M™

- Small Footprint on lab bench
- Low maintenance, easy upkeep
- Must use proprietary enrichment media
- Multiple units can be run by one computer
- Matrix Control available
- Color coded assays to help technicians organize efficiently
- Pre-dispensed ready-to-use lysis
- Few matrices validated
  - Environmental surfaces
  - Beef hot dogs
  - FF cottage cheese
  - Bagged spinach
  - Whole cantaloupe
  - Deli turkey
Isothermal DNA Amplification & Fluorescent Detection - Neogen

- ANSR technology
- Target DNA is released during lysis after a unique enrichment
- A special molecular “beacon” is a part of the reagent mixture
- Primer targets specific regions of the target DNA
- Rapid, as short as 10 minutes
- Amplified DNA attaches to the molecular beacon
- Fluorescence occurs and the reader measures the reaction
- Again, isothermal-replicating at constant temperature
- Speedy because denaturation = time and that’s not occurring here
- Scalable therefore flexible
Detection Limit – What do we mean?

• Definition-In microbiology, the lowest number of microorganisms that can be detected.
• Laboratories, equipment manufacturers and end user customers prefer low ones.
• Everyone should insist on honest ones.
  – Hard to sometimes get, factors change them such as size and condition, matrix inhibition, assumptions are made without evidence
• limit of detection (LOD) of one viable/culturable organism per sample size
Thanks!