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

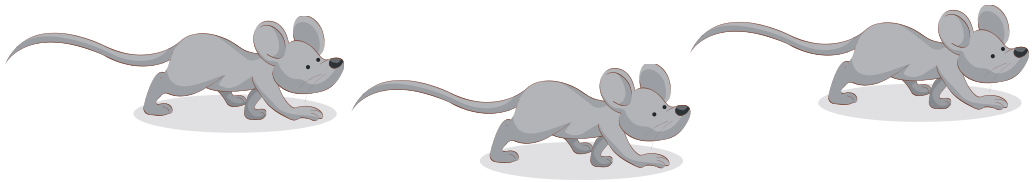
- Why do we want to go anyway?
- Where have we come from?
- Where are we going?
- How far are we on the journey*?*
- What challenges do we still face?
- What is important to us as we choose the road we take or what do we need to bring along?
- When will we get “there”?

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
- Its 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

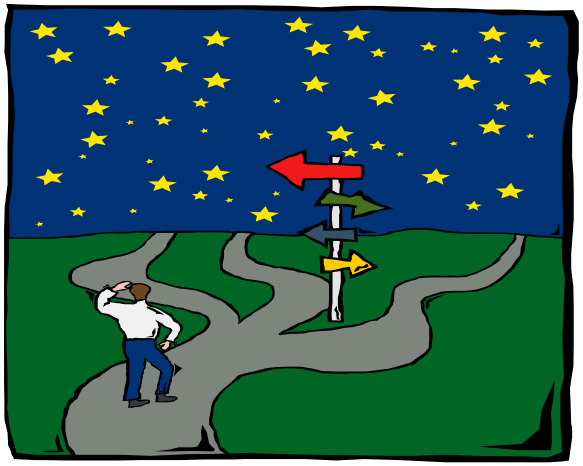
Where have we come from?

- 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?



What is important to take along?

- Approvals
- Specificity
- Sensitivity
- Robustness
- Speed
- Ease of Use
- Cost effective
- Volume of throughput

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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’?

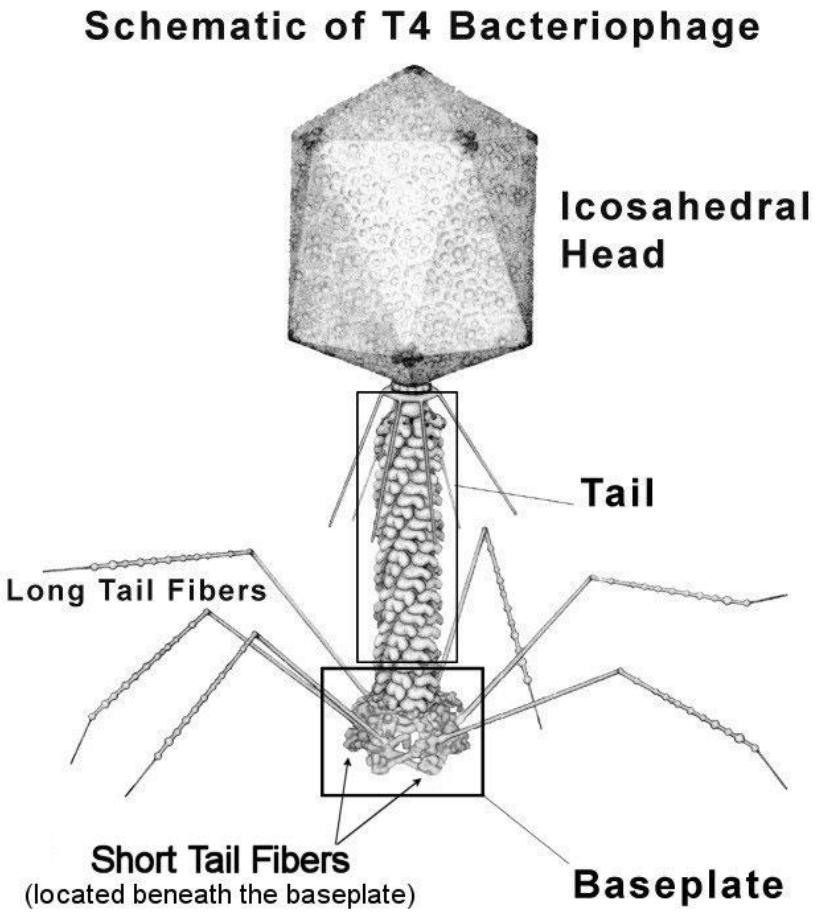
- Quantitative detection of pathogenic microorganisms for troubleshooting and problem-solving
- Simultaneous detection and/or quantification of multiple, dissimilar pathogens in minutes (its my dream) by the elimination of sample enrichment

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

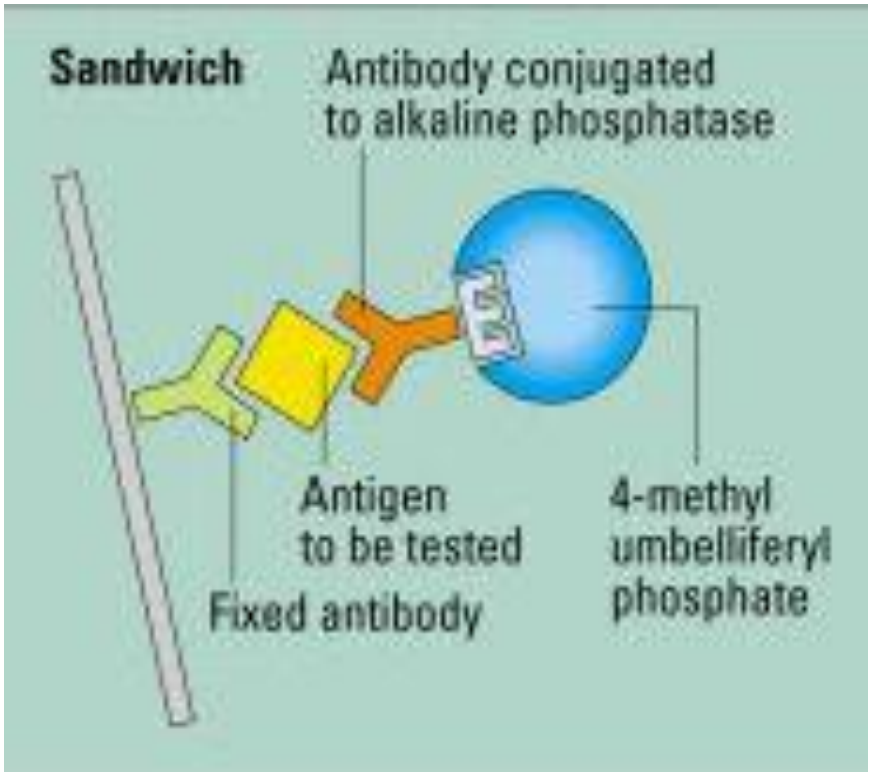
Phage and Sandwich ELISA – bioMerieux Industry

- 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

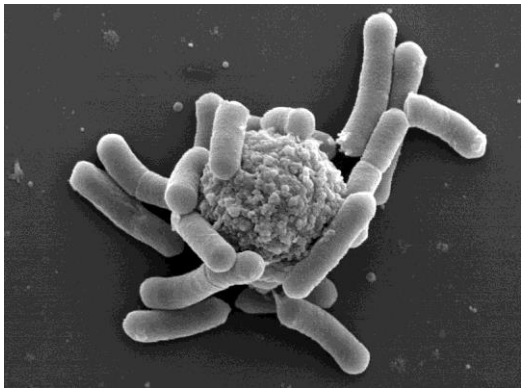


Phage and Sandwich ELISA – bioMerieux Industry

- 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

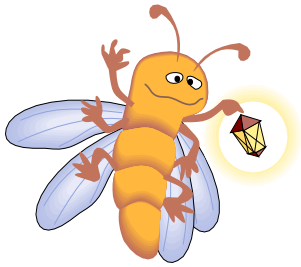


Magnetic Particle Capture & PCR - BioControl

- 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 (60C)
- 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!