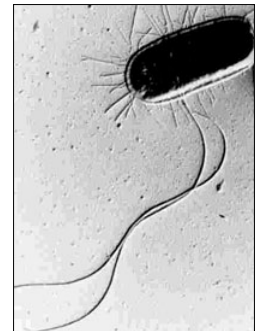


Application and Use of Immunomagnetic Separation (IMS) for Salmonella Detection

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Salmonella

- Etiologic agent of Salmonellosis in humans causing severe illness in infants, the elderly, and immunocompromised patients
- Salmonellosis symptoms: include watery diarrhea, abdominal pain, nausea, fever, headache and occasional constipation with hospitalization required in cases of severe infections.

Salmonella

- 2 species:
- *Salmonella bongori*
- *Salmonella enterica* including six subspecies:
 - enterica* (I) *
 - salamae* (II)
 - arizonae* (IIIa)
 - diarizonae* (IIIb)
 - houtenae* (IV)
 - indica* (VI)
- >2,500 serovars of *Salmonella* based on Kauffmann-White antigenic scheme for the classification (Popoff et al. 1994).

Salmonella

- Family Enterobacteriaceae
- Gram-negative, non-spore forming rod
- Facultative anaerobe that can ferment glucose
- Most strains are motile with peritrichous flagella and can reduce nitrate to nitrite
- Mesophilic with optimum growth temperature of 32 – 37°C but capable of growth from 6 – 46°C.



Scanning electron micrograph showing *S. Typhimurium* (red) invading cultured human cells: Rocky Mountain Laboratories, NIAID, NIH, USA)

Salmonella

- Ubiquitous in the environment arising from the GI tracts of animals and can be present without causing apparent illness.
- Most infections result from ingestion of contaminated foods of animal origin: beef, chicken, turkey, pork, eggs, and milk.
- Other vehicles include fresh fruits and vegetables, reptiles, water, and direct person-to-person transmission.
- Serotypes such as *S. Enteritidis* (SE), can penetrate poultry reproductive organs contaminating egg contents and has been a prominent cause of illness for several decades.

Salmonella

- Detection methods are based on physiological and biochemical markers of the organism.
- Cultural methods are based on nutrient acquisition, biochemical characteristics, and metabolic products unique to *Salmonella* spp.
- More rapid immunological and molecular screening methods of detection have been devised to detect cell surface markers and nucleic acids, respectively.

Why Continue to Use Culture Methods?

- Despite advances in rapid methods, traditional culture methods continue to be the most commonly used – “gold standard”
- High sensitivity and specificity.
- Need for an unambiguous result.
- Need to satisfy regulatory requirements.
- Need for an isolate for further analysis.



Salmonella Culture Approaches

- Multitude of options!
 - Lack of inter-laboratory consistency make *Salmonella* isolation one of the most variable procedures.
1. Enrichment in a non-selective broth (BPW)
 2. Selective Enrichment in RVS/TBG/SC/TT/MKTTn incubated at 37/42°C 18-24h.
 3. Plating onto XLD/BGS/BIS/MSRV/SS/HEK
 4. Identify suspect colonies via TSI/Urea/LIA/
Polyvalent Antisera
- 5-7 Days to result and labour intensive

Separation and Concentration

- **Separation**: removal of a target population from a complex mixture.
- **Concentration**: reduction of sample volume, while recovering the initial bacterial population of interest.
- A 100-fold \uparrow in concentration results in 2-7 hour decrease in enrichment time for zero-tolerance organisms.

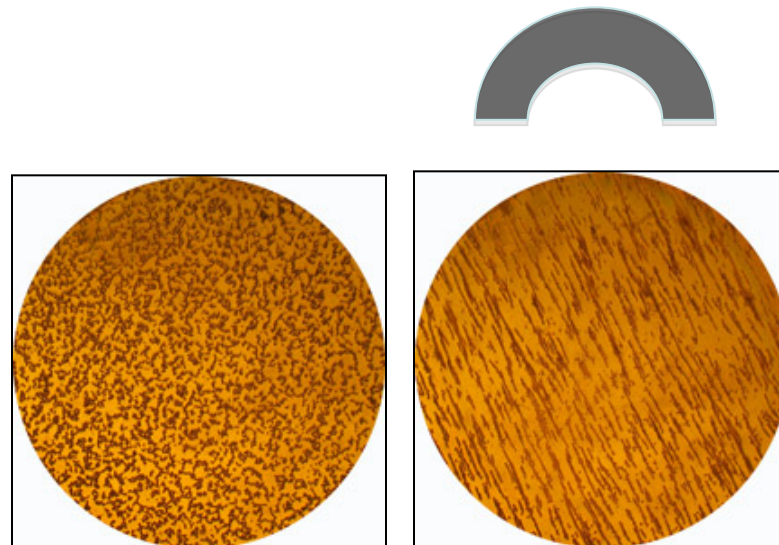
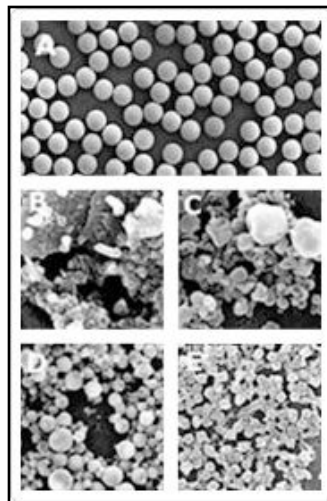
Separation and Concentration

Many Approaches:

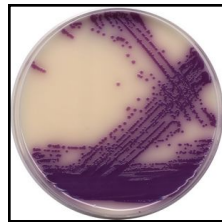
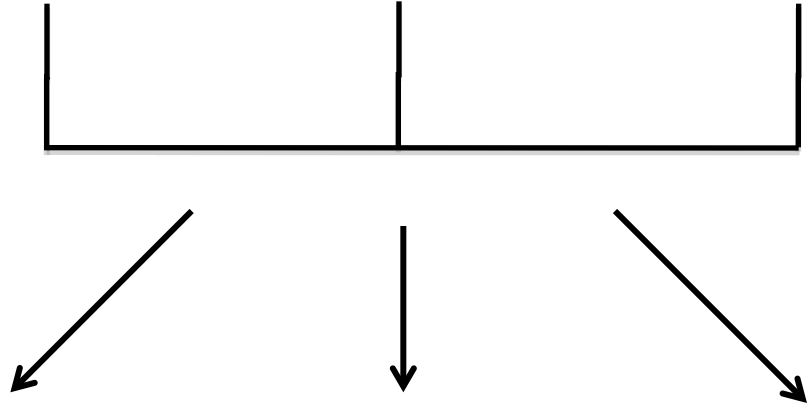
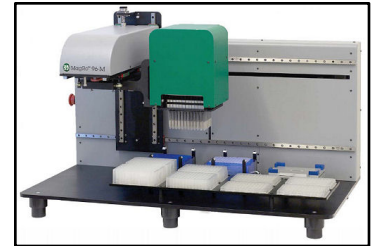
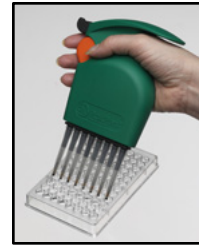
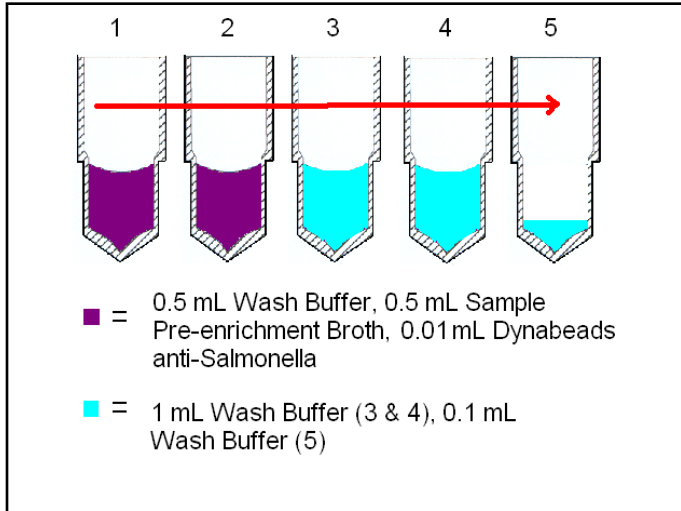
- Centrifugation (Basel et al. 1983)
- Filtration (Bobbitt et al. 1993)
- Lectin-based biosorbents (Payne et al. 1992)
- Aqueous biphasic systems (Bennett et al. 1994)
- **Most successful approach: IMS**

IMS

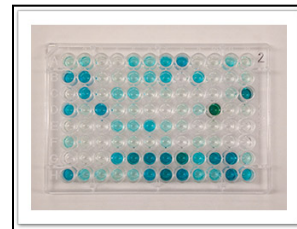
- Uniformly sized polymer particles with an iron-oxide core.
- Superparamagnetic”: magnetized only in the presence of a magnetic field.
- Bound with antibody specific for a target organism.



IMS



Culture



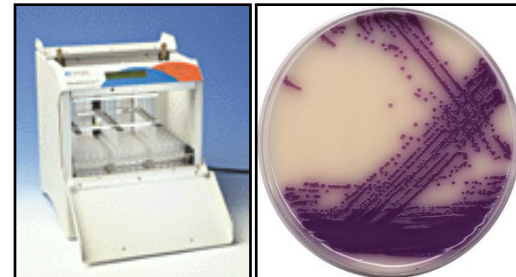
Immunoassay



PCR

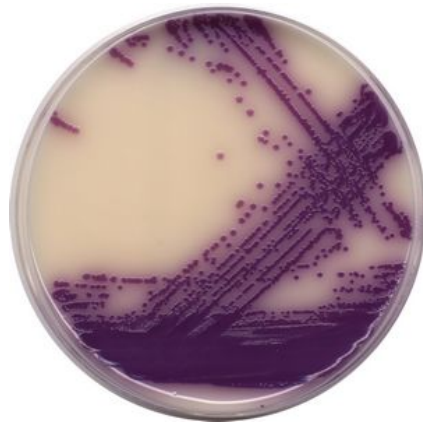
Salmonella IMS + Culture

- MFLP-84 evaluated by Lynch et. al (2004).
Journal of Microbiological Methods 58:285-288.
- Isolation of Salmonella from poultry environmental samples.
- 15.5% more sensitive than MFHPB-20
- 3-4 day turn around time vs. 4-5 for MFHPB-20
- Reduction of media by ½.

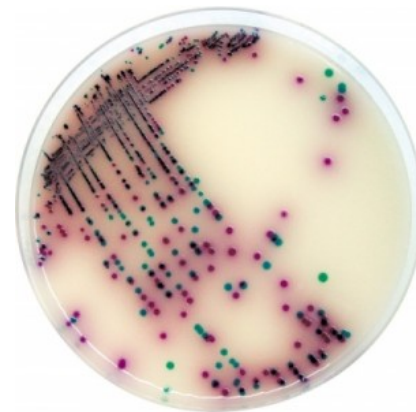


Salmonella IMS + Culture:

- Chromogenic Agars: exploit specific enzyme activity unique to the organism.
- Reduce the time and effort to screen suspect colonies and confirm identity



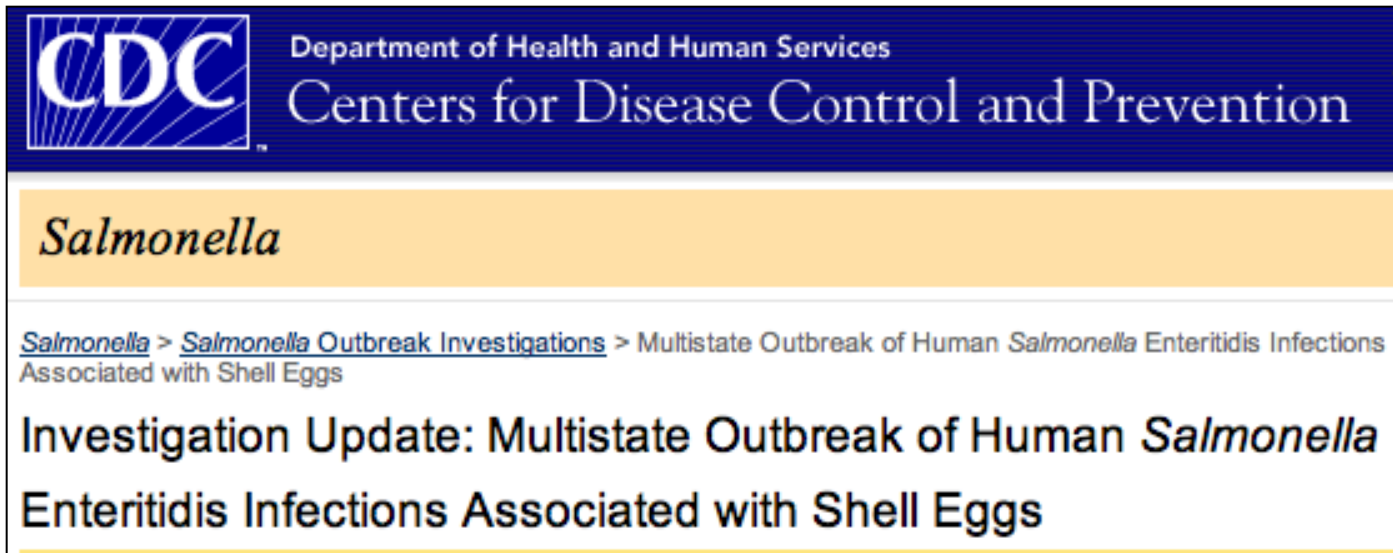
Brilliance (Oxoid)



ASAP (AES Chemunex)

- Potential to greatly impact the identification of Salmonella.

SE Outbreak



The screenshot shows the CDC logo on the left and the text 'Department of Health and Human Services' and 'Centers for Disease Control and Prevention' on the right. Below this is a yellow bar with the word 'Salmonella' in italics. Underneath is a breadcrumb trail: 'Salmonella > Salmonella Outbreak Investigations > Multistate Outbreak of Human Salmonella Enteritidis Infections Associated with Shell Eggs'. The main title of the page is 'Investigation Update: Multistate Outbreak of Human Salmonella Enteritidis Infections Associated with Shell Eggs'.

July 2010, CDC identified a nationwide sustained increase in the number of SE cases

Twenty-nine restaurant/event clusters are reported from 11 states; 15 of 29 clusters with likely egg source as Wright County Eggs (WCE); 1 cluster of 29 with likely source as Hillandale Farms.

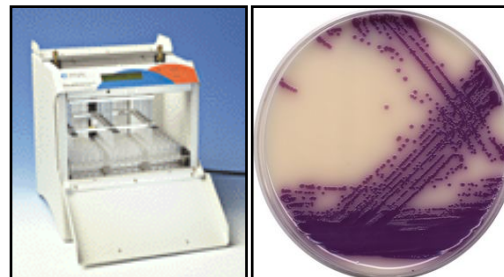


SE Outbreak

- **FDA:**
- Substantial potential for *Salmonella* to have persisted in the environment and to have contaminated eggs.
- Mandatory SE-control programs that include routine microbiological testing for producers with >50,000 laying hens.
- Immunoassays and PCR for SE now available
- **IMS for SE:** RapidChek Confirm *S. Enteritidis* (sdix).

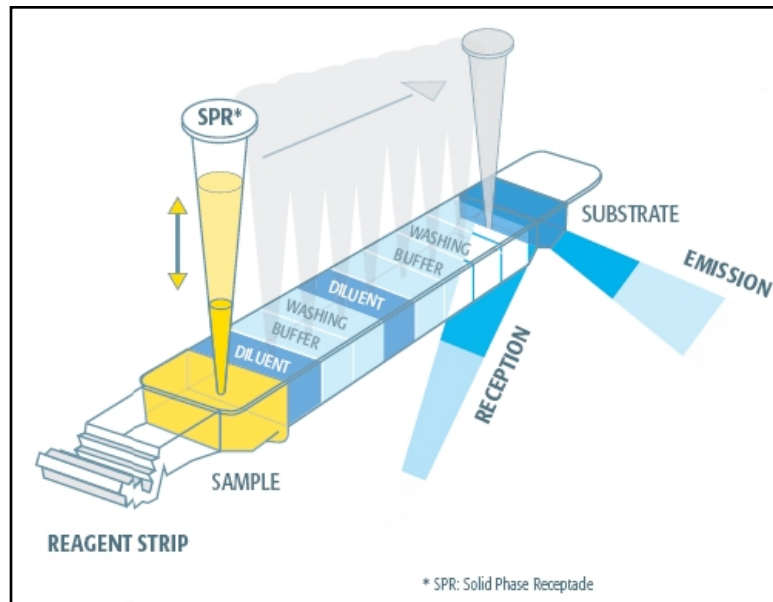
S. Enteritidis IMS + Culture

- Tested 100 swab samples (cloacae) from a +SE flock by MFHPB-20 and (SE) IMS.
- 32 Positive Salmonella spp.
- 11 Positive SE by MFHPB-20
- 19 Positive SE by (SE) IMS
- Suggests SE prevalence studies based on standard culture methods may be biased?



Salmonella ICS + ELISA

- VIDAS® - Enzyme-Linked Fluorescent Assay (ELFA) using Immuno-concentration (ICS)



miniVIDAS®

SPR* Solid Phase Receptacle (coated with Ab or other Ligand) - VIDAS®SLM

Salmonella IMS + PCR

- Salmonella Assurance GDS™ (Biocontrol)
AOAC Official Method 2009.03
AFNOR Certificate: TRA 02/12 - 01/09
AOAC Performance Tested Method 050602



Salmonella FTI + PCR

- Pathatrix (Matrix MicroScience Ltd, UK) combines IMS and a recirculation step (Flow Through Immunocapture or FTI)
- Warren et al. (2007). *J Food Prot.* 2007.70(4):1002-6.
- FTI followed by plating on XLD agar (FTI-XLD) or FTI followed by real time PCR:
- FTI-XLD was 48 h faster than standard culture
- FTI-PCR was detected *Salmonella* within 8h.



Salmonella IMS + Bacteriophages

- IMS followed by phage mediated release of adenylate kinase (AK) – assayed via luciferase/luciferin enzyme system (Bioluminescence)
Blasco et al. (1998). J Appl Microbiol, 84:661–666
Wu et al. (2001). Lett Appl Microbiol 33:311–315
- Commercialized by Alaska Food Diagnostics (UK) as fastrAK™ *Salmonella* assay.
- IMS-bacteriophage plaque formation assay
Fravrin et al. (2001). Appl Environ Microbiol 67:217–224
- IMS-Fluorescently labelled phage.
Jiang et al. (2009) Wei Sheng Wu Xue Bao. 49(3):372-7.

IMS: Factors to Consider

- The success of IMS relies upon:
 1. Specificity of antibody
 2. Bead size and surface area of the complex
 3. Recovery procedure (manual, automated)
 4. Sample matrix interference (fats)

IMS

- **Advantages**

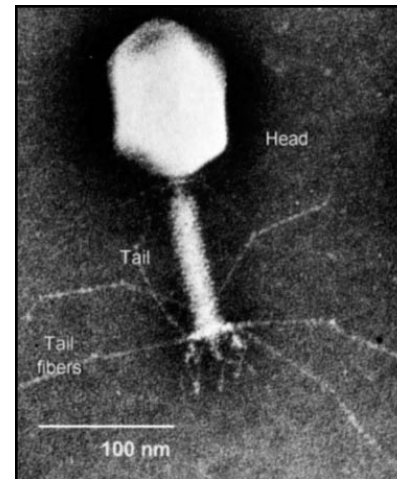
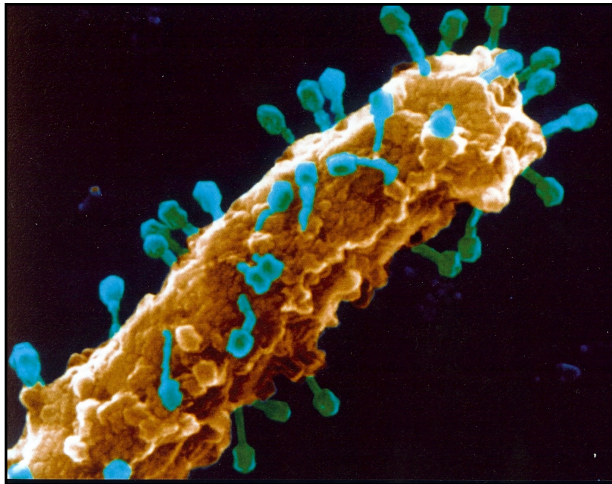
1. Effective separation of target from competitive microflora and concentration (10-100 fold).
2. Removal of food components.
3. Removal of potential inhibitors (PCR)

- **Disadvantages**

1. Non-specific binding or bacterial adherence (at $>10^6$ cfu/mL).
2. Loss of cells during wash steps.

Future Perspectives

- Harness the high specificity of bacteriophages
- Narrow to broad host range



Future Perspectives: Other Ligands

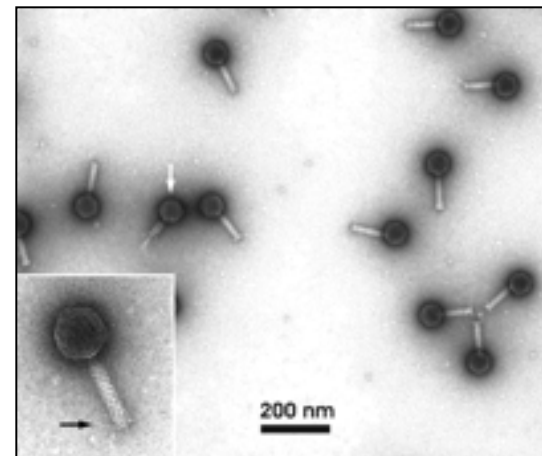
- Kretzer et al. (2007). *Appl Environ Microbiol* 73:1992–2000.
- Paramagnetic beads coated with Cell Binding Domain (CBD) from bacteriophage endolysins outperformed Ab-based magnetic beads with respect to sensitivity and % recovery.
- BioMerieux Inc. recently introduced *Salmonella* Up, an automated ELISA assay using ICS via phage recombinant protein derived from bacteriophages for the detection of *Salmonella* within 18-24 h.

Future Perspectives: Other Ligands

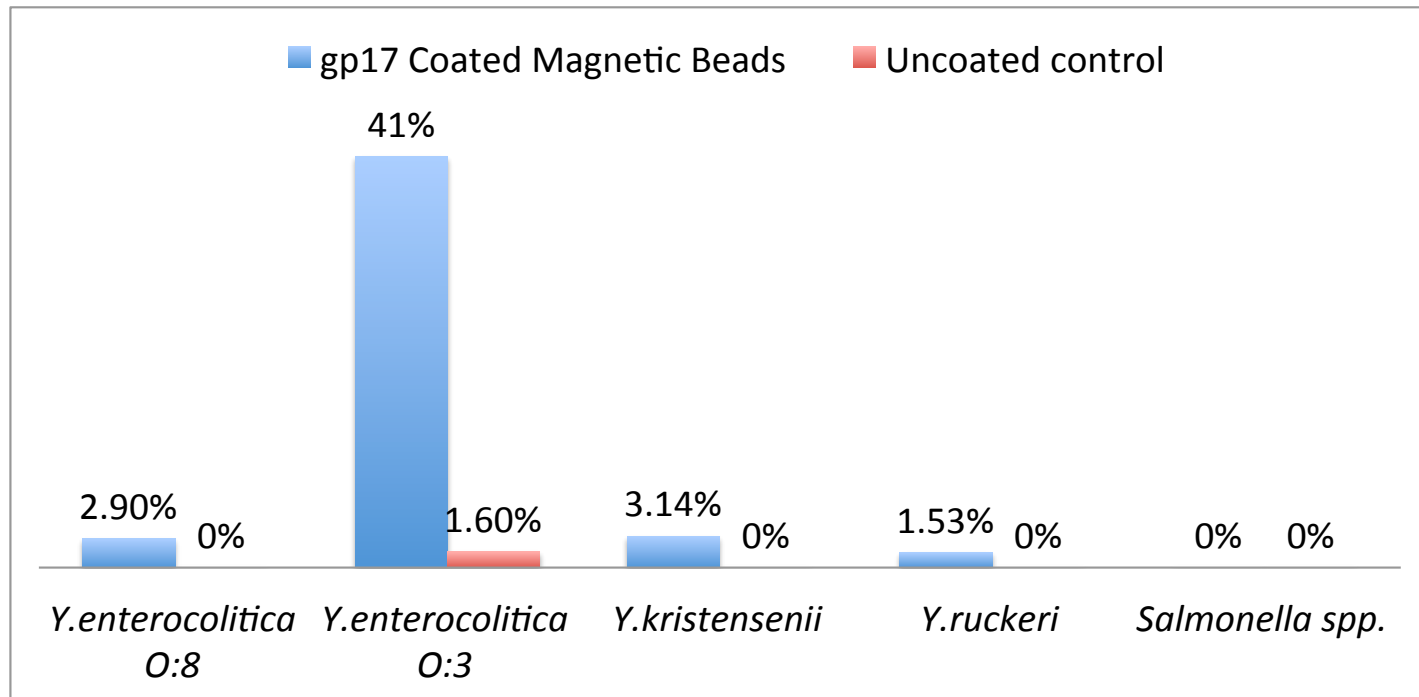
- **Example**: Isolation of *Y.enterocolitica* using Paramagnetic Beads coated with phage tail fiber protein(s) for host specificity.
- Current culture methods are inadequate and insufficiently sensitive to detect low levels in foods, water, and environmental samples.
- Direct isolation is seldom successful and time-consuming enrichment steps are utilized: cold enrichment, KOH, carbenicillin, bile salts, irgasan.

Future Perspectives: Other Ligands

- ϕ YeO3-12. Skurnik (1984)*J.Applied Bacteriol.* 56:355-363.
- Narrow host range: *Y.enterocolitica* serotype O:3, O:1, and O:2
- Tail fiber protein for host specificity (gp17), 1,938 bp. (Genbank AJ251805.1)



Future Perspectives: Other Ligands



ϕ YeO3-12 gp17 Trial: % recovery after Magnetic Separation from 1.9×10^3 CFU/mL cell suspensions

Conclusions

- Research is needed in the area of sample preparation as improvements have a direct impact on detection methods
- The need for combined and/or sequential methods is apparent, as these may continue to further improve sensitivity, specificity, and turn around times.
- The use of alternative ligands (other than Ab) for magnetic separation is promising.
- IMS-Phage based detection systems may circumvent the problem of viability presented by PCR, while promising to be more rapid than standard culture methods.

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