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Immunomagnetic separation combined with enzyme linked immunosorbent assay for quick detection of Salmonella in raw eggs

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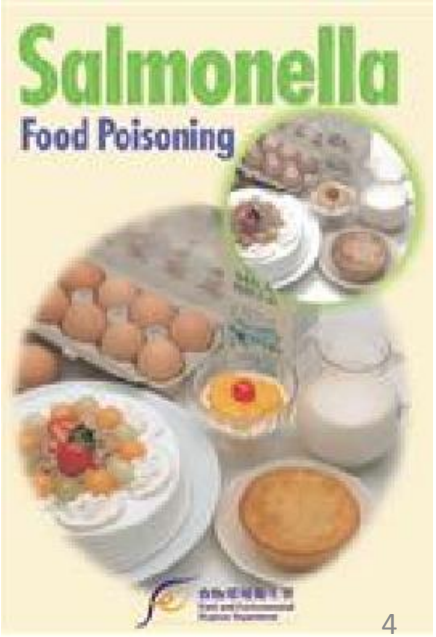
Outline

- Salmonella and challenges in detecting the bacteria in food samples
- Immunomagnetic separation
- Challenge in using immunomagnetic particles in food sample analysis
- Proposed work
- Preliminary results
- Conclusions and further work
- Acknowledgement

Salmonella and foodborne diseases

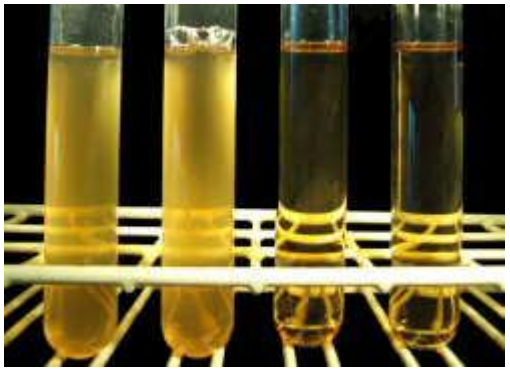
- Every year, Salmonella is estimate to cause one million illnesses in the United States, with 19,000 hospitalizations and 380 deaths (http://www.cdc.gov/salmonella/)
- Salmonella infection is usually caused by eating raw or undercooked meat, poultry, eggs or egg products
- Quick detection of Salmonella contamination is a challenge for farmers and regulatory body



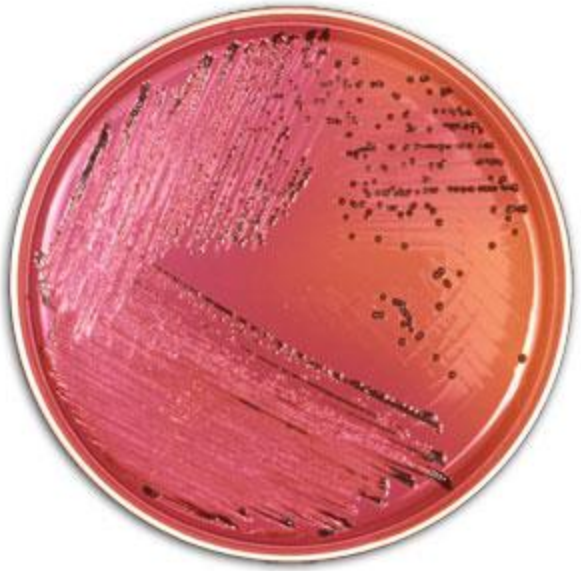


Challenges in detecting Salmonella in food safety inspection

- Sensitivity requirement
- Inhomogeneity
- Complex sample matrix

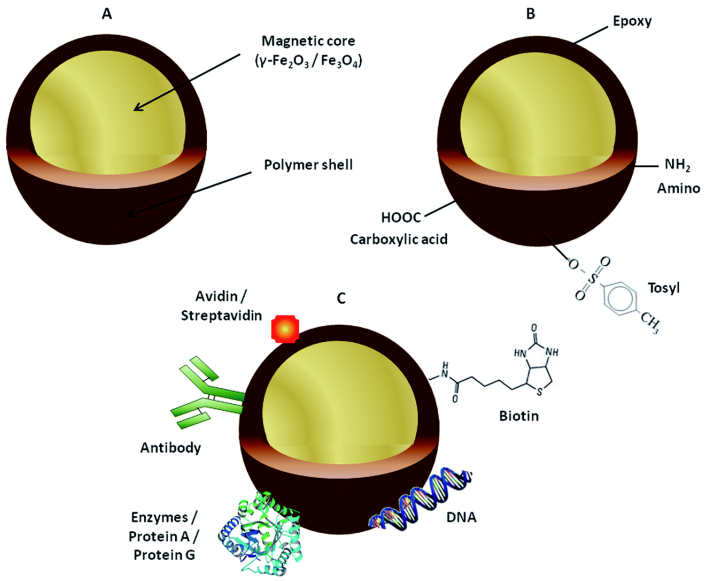


- Present standard methods based on microbiology
  - long-time incubation (>48 hours) in broth solution for increasing the number of bacteria.
  - Apply incubated solution to selective agar media in petri dish and incubation for >18-24 hours at 37 ^{o}C
  - Count colonies developed in petri dish



Immunomagnetic separation using antibody coated magnetic particles

- Paramagnetic Fe_3O_4 particles prepared with wet chemical process
- Magnetic particles are coated with a polymer (for example, silica, polystyrene, Poly(methyl methacrylate), poly(2-methacryloyloxyethyl phosphorylcholine) which provides site for bonding antibody
- Antibody coated on magnetic particles through covalent bonding with functional group on polymer's side chain
- Antibody coated magnetic particles dispersed into sample to capture antigen
- Antigen captured magnetic particles are separated from sample matrix by using a magnet





Challenges of immunomagnetic separation in food safety inspection

- Limited sample size ←→ inhomogeneous distribution of food pathogens
- Separate magnetic particles (μm diameter which is close to the size of colloidal precipitation) from food matrix is not easy even with strong magnet
- Problems in using optical technologies for direct detecting trapped antigen on magnetic particle surface after separation

Procedure for IMS separation of Salmonella bacteria from row Egg

Homogenize a raw egg with a blender with 200 mL 0.05% Triton X-100 solution

↓

Add 0.10 mL of BacTrace® Anti-*Salmonella* CSA-1 magnetic bead solution to the egg sample

↓

Incubating 45 min

↓

Place a 1" magnet sealed inside a zip bag in the sample solution

↓

The magnet stay in sample solution for 90 min

↓

Pull out magnet with the zip bag
take the magnet out of the zip bag
Rinse magnetic particles off the plastic zip bag

↓

Transfer magnetic particles into a small testing tube



Procedure for ELIZA detection

Rinse bacteria captured magnetic particles with PBS buffer solution 3 times (5 mL PBS, incubating 10 min, magnetic sample rack separation)

↓

Incubating magnetic particles with 5 mL 5% BSA solution (10 min)

↓

Rinse bacteria captured magnetic particles with PBS buffer solution 2 times

↓

Incubating magnetic particles with 40 µL of the 1 µg/mL BacTrace® Anti-*Salmonella*, CSA-1 Antibody solution and 1.0 mL PBS solution (20 min)

↓

3 time rinse magnetic particles with PBS solutioon

↓

0.60 mL of the SureBlue® TMB Microwell Peroxidase Substrate, incubating 10 min

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0.50 mL of the TMB BlueSTOP™ solution, 1.1 mL PBS

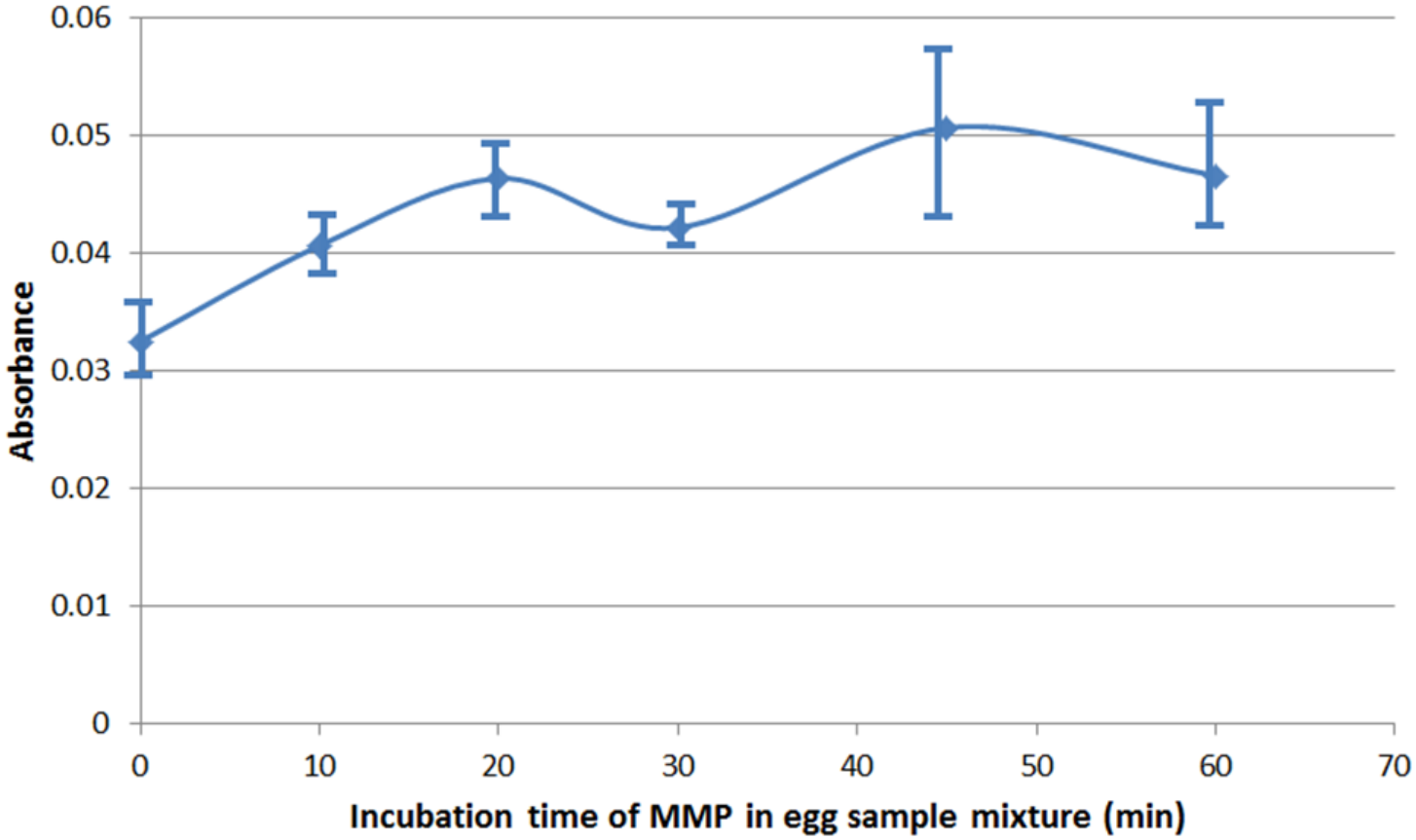
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UV/Vis measurement



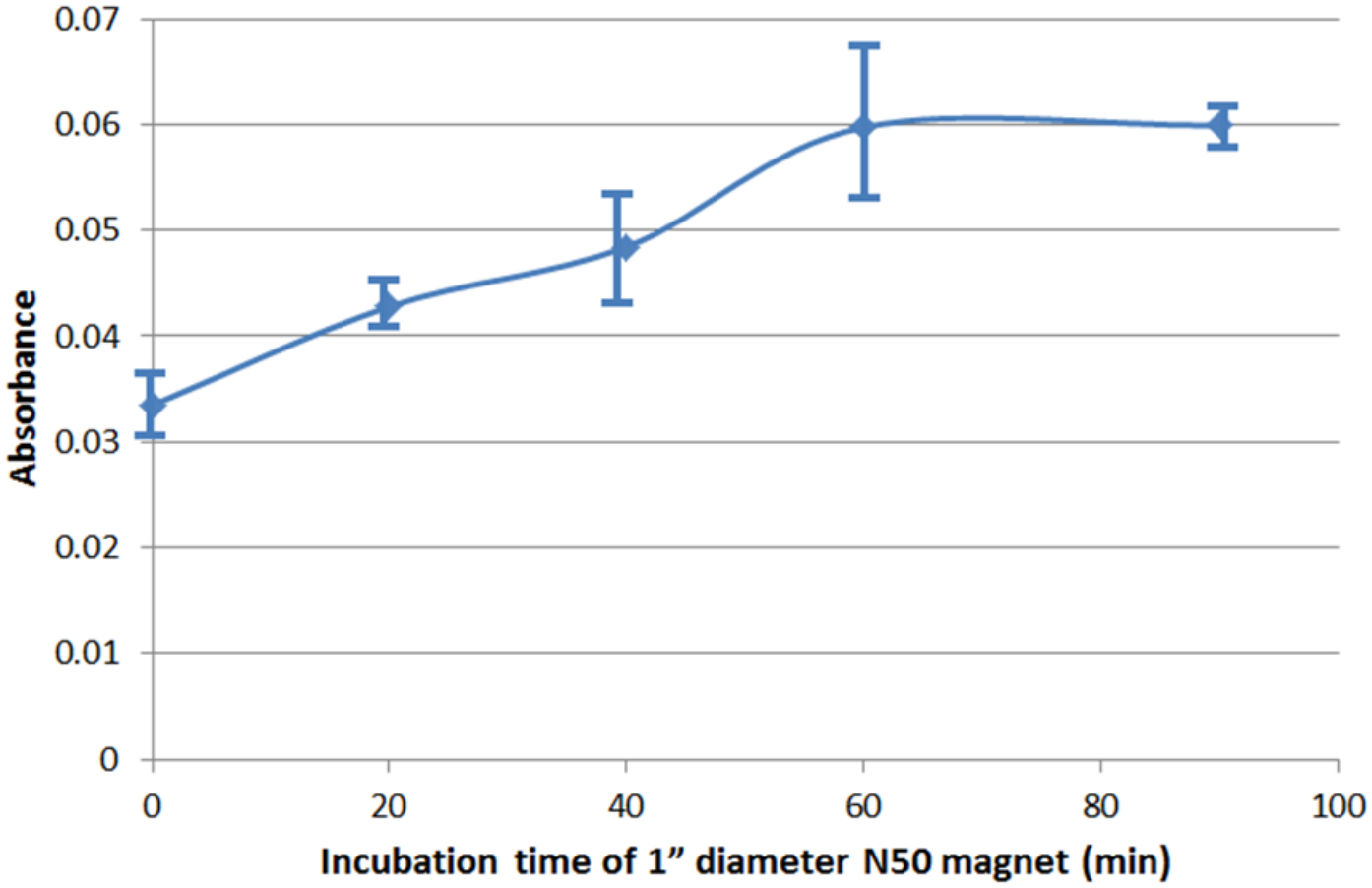


Preliminary results



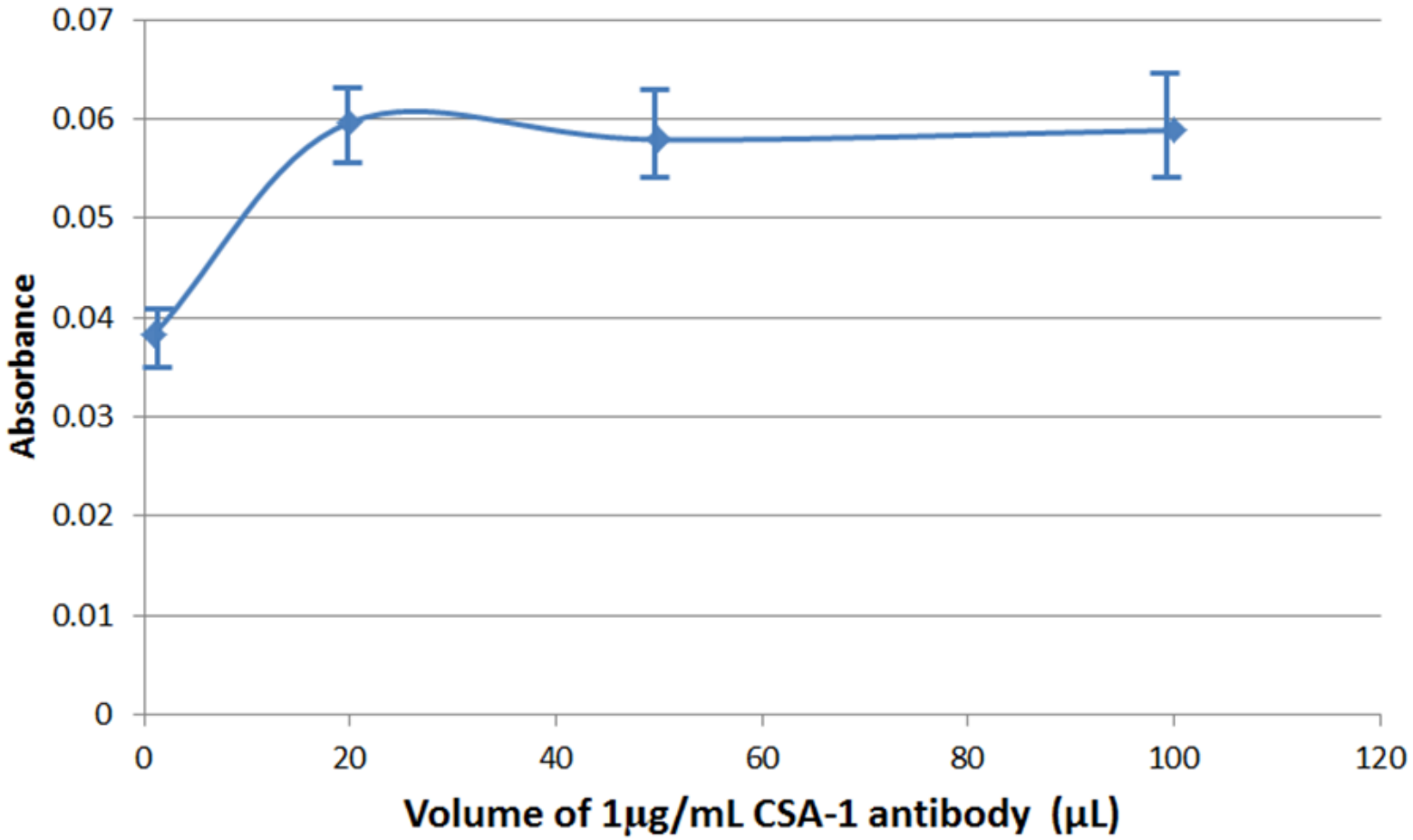
Effect of MMP/egg sample incubation time on ELISA absorbance, which indicating how much *Salmonella* added to the egg sample were trapped by MMP

Preliminary results



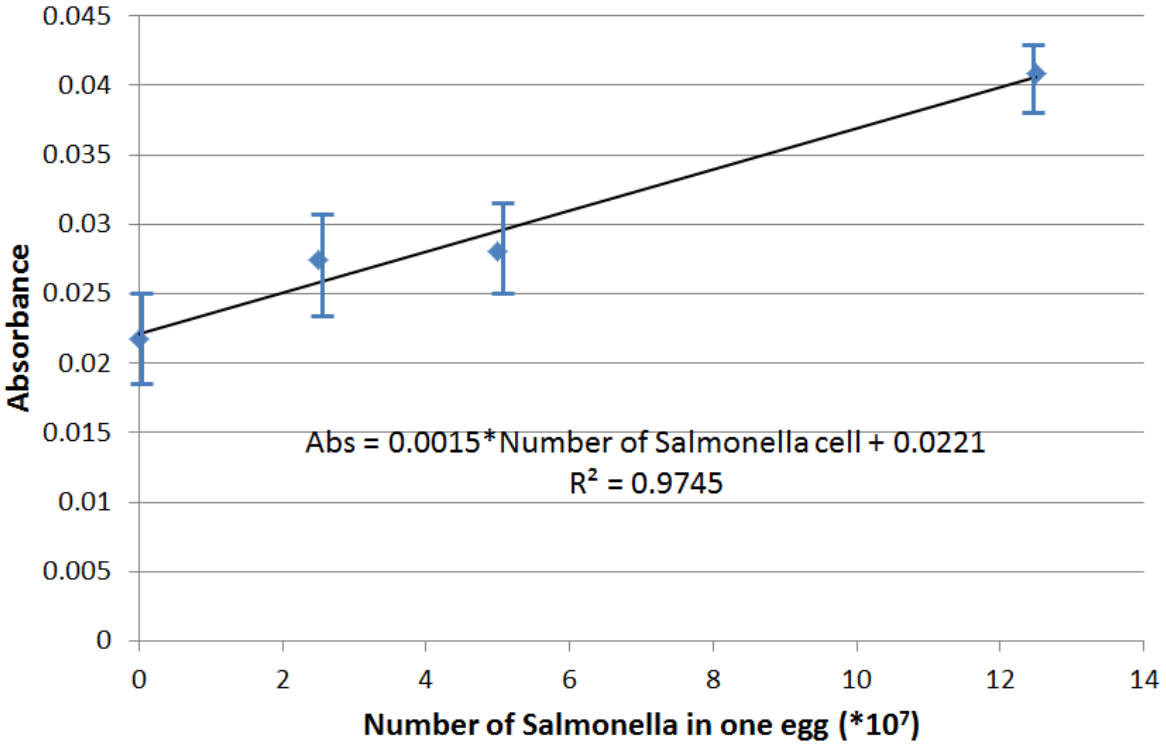
Effect of incubation time of the 1" diameter N50 magnet in egg sample mixture for pulling up *Salmonella*-trapped MMP, which is indicated by measured ELISA's optical absorbance.

Preliminary results



Effect of volume of 1 µg/mL peroxidase labeled CSA-1 antibody on the optical absorbance value of ELISA, which detects *Salmonella* trapped to the MMP.

Preliminary results



A calibration curve showing the relationship of absorbance value of the developed method with the number of *Salmonella* cells in egg samples.

Detection limit defined as the number of Salmonella cells in a raw egg which gives a signal that is three times of the standard deviation of absorbance of blank samples. The detection limit of this method is $1.4*10^7$ *Salmonella typhimurium* cells in one raw egg ($7.0*10^4$ Salmonella cells/mL in a sample mixture) without any pre-enrichment.

Conclusions

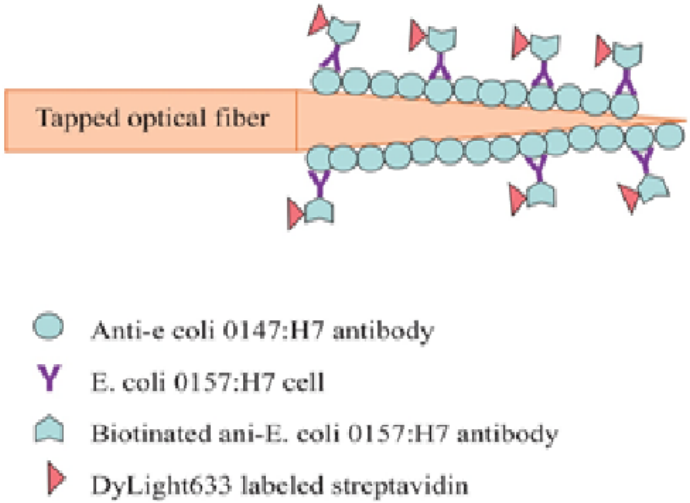
- Immunomagnetic particles have been successfully used to separate Salmonella from raw egg sample.
- Number of Salmonella bacterial in a raw egg sample can be detected within 5 hours, which is a significant improvement when compared with present standard methods
- A detection limit of $1.4*10^7$ *Salmonella typhimurium* cells in one raw egg ($7.0*10^4$ Salmonella cells/mL in a sample mixture) is achieved.

Problems

- It is difficult to directly add MMP to a raw egg to trap the bacteria and separate the MMP from the egg matrix. Raw egg sample has to be diluted to reduce its viscosity
- It is inconvenient to stir the magnetic particles added sample with a magnetic stirring bar
- It takes a long time to collect magnetic particles from sample solution.
- High blank absorbance observed when ELIZA is used for detection.

Further Work

- **Use chemical reactions to immobilize antibody on surface of optical fibers.**
- **Use AB-coated optical fibers as sampler to trap bacteria from food samples.**
- **Develop an optical fiber immunofluorescence assay method to detect trapped bacteria.**



Acknowledgement

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