Development and Evaluation of Visual Biosensors for Rapid Detection of *Salmonella* spp. and *Listeria monocytogenes*

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Introduction

- Ongoing foodborne outbreaks have necessitated the development of modern detection methods
- Most methods (cultural, immunological, molecular) are laboratory based, and not suited for field based testing
- Need to develop field based tests, that can rapidly determine the presence of pathogens in foods
- The perfect test: sensitive, specific, rapid, robust, easy to use (integrated), cost effective

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Objective

- To develop a suite of optical biosensors for rapid detection of the foodborne pathogens *Salmonella* spp., and *Listeria monocytogenes*
- Optical biosensors: no need for instruments to read the test result (field deployable)

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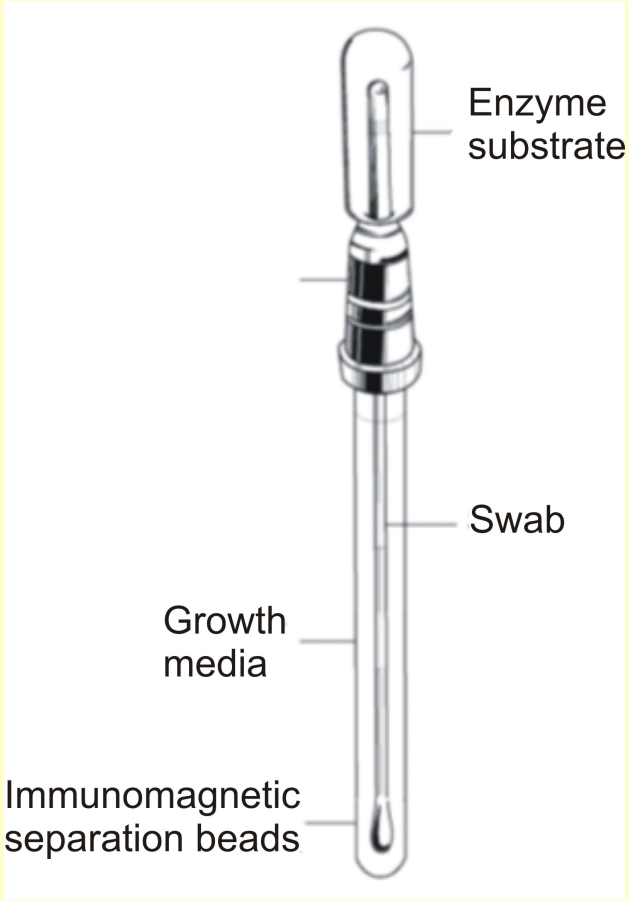
Optical Biosensor

- The Phast Swab
- Biochemical test
- Vertically integrated
- Contains a sampling device, bacterial growth media, immunomagnetic separation beads, and an enzyme substrate in a single device



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Salmonella spp.

- Biosensor based on detection of a *Salmonella* specific esterase (apeE)
- Outer membrane protein encoded by the *apeE* gene (Collin-Osdoby and Miller 1994)
- Enzyme substrate:5-Bromo-6-Chloro-3-Indolyl-Caprylate (Magenta-Caprylate)
- Cleavage of the substrate results in the formation of a magenta precipitate

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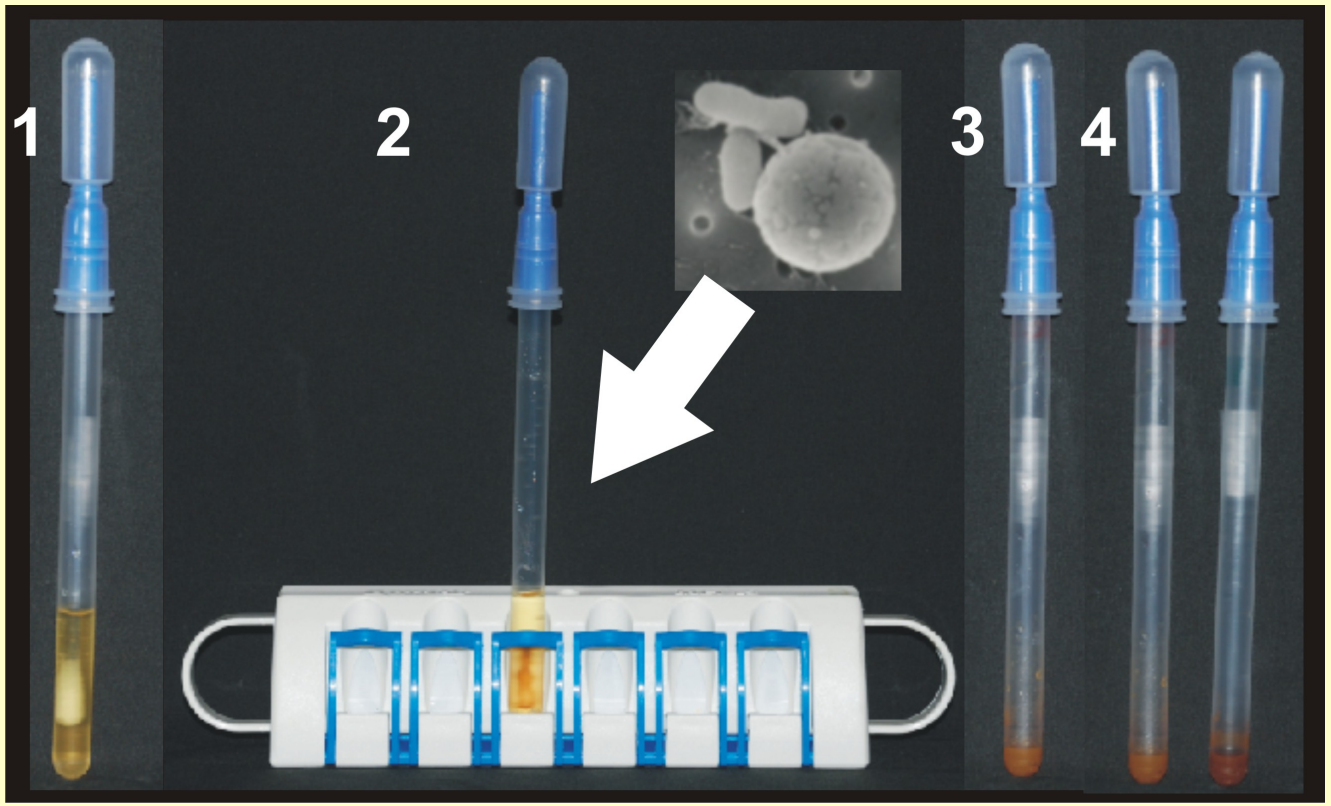
Salmonella spp.

- 20 hour test
- 13 hours of enrichment, IMS (10 minutes)
- 7 hour color development



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Salmonella spp.

- Detection of *Salmonella* in artificially spiked poultry (thigh)
- Overnight culture of *S*. Enteritidis
- 10 fold serial dilutions
- Individual 11 X 11 cm^2 thigh portions (with skin on) inoculated with 1ml of each dilution
- Poultry allowed to dry for 20 minutes
- Each thigh portion swabbed with an individual Phast Swab
- Assay conducted as described

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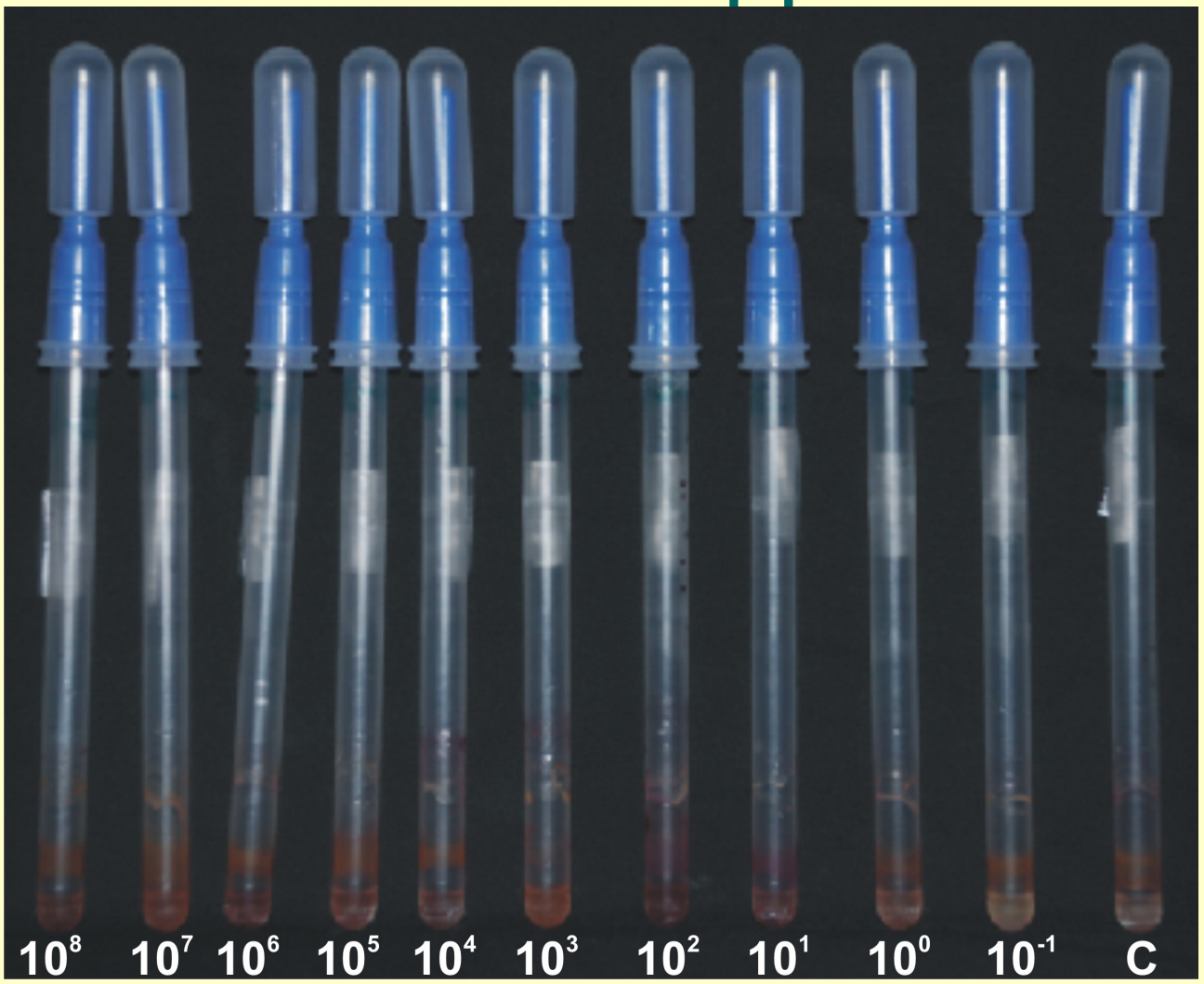
Salmonella spp.



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Salmonella spp.

- *Salmonella* Phast Swab detected 10^0 CFU/121 cm^2 of poultry (thigh) within 20 hours
- Detection limit (sensitivity): 10^7 CFU/ml
- Specificity: *Salmonella* Typhimurium, *Salmonella* Enteritidis: positive
- *E. coli* O157:H7, *E. coli* K12, *L. monocytogenes*: negative
- Color formation was not even (possibly due to fat content of the poultry)
- Alternative sampling methods

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Listeria monocytogenes

- Biosensor based on detection of phosphatidylinositol-specific phospholipase C (PI-PLC)
- Plays a critical role in escape of this human pathogen from host cell vacuoles
- Isolates lacking PI-PLC much less virulent (Camilli *et al*. 1991)
- Enzyme substrate:5-bromo-4-chloro-3-indoxyl-myo-inositol-1-phosphate (X-inp)
- Cleavage of the substrate results in the formation of a soluble blue compound

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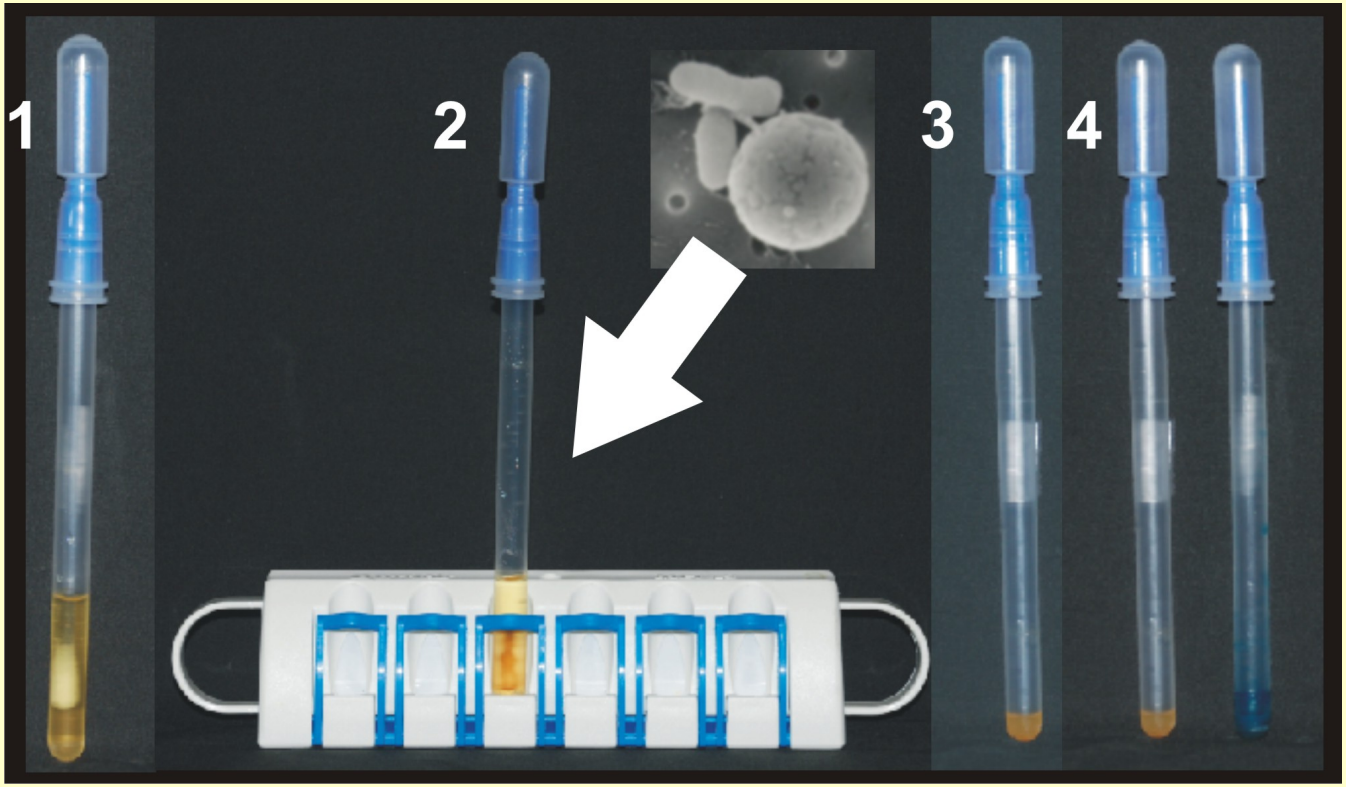
Listeria monocytogenes

- 15 hour test
- Enrichment for 10 hours, IMS (10 minutes)
- Addition of lysis buffer (PI-PLC is intracellular), substrate
- Color development for 5 hours



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Listeria monocytogenes

- Detection of *L. monocytogenes* in artificially spiked RTE turkey meat
- Overnight culture of *L. monocytogenes*
- 10 fold serial dilutions
- Individual deli slices (100 mm diameter) inoculated with 1 ml of each dilution
- Meat allowed to dry for 20 minutes
- Each deli slice swabbed with an individual Phast Swab
- Assay conducted as described

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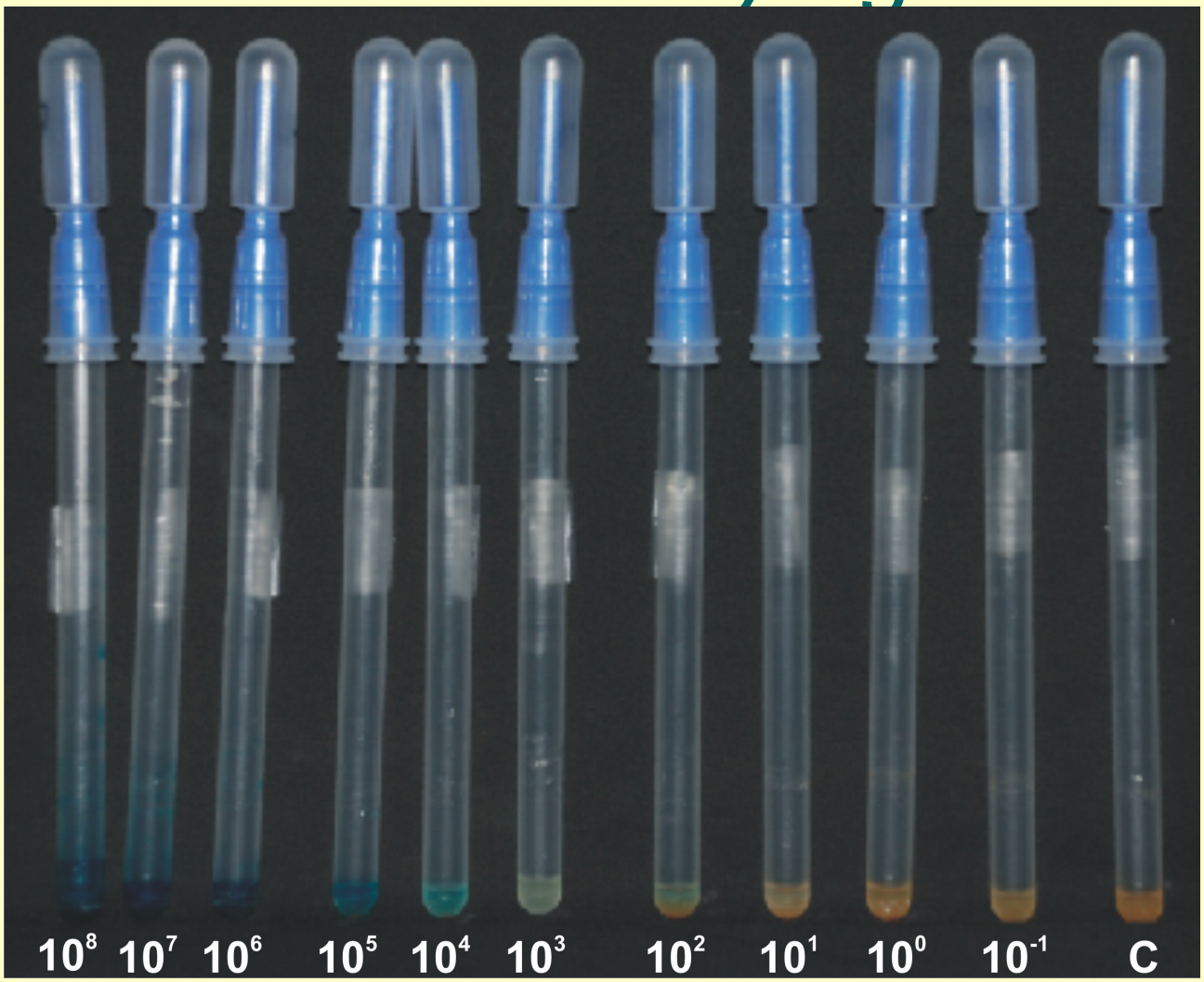
Listeria monocytogenes



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Listeria monocytogenes

- *L. monocytogenes* Phast Swab detected 10^2 CFU per RTE deli slice of turkey (100 mm diameter) within 15 hours
- Detection limit (sensitivity) 10^6 CFU/ml
- Specificity: 55 of 57 *L. monocytogenes* isolates postive, *E. coli* O157:H7, *E. coli* C, *Salmonella* spp. negative
- Future work: investigate the use of the Phast Swab to test for environmental sources of *L. monocytogenes* (within RTE meat processing plants)
- *L. monocyotgenes* is ubiquitous and is considered an indicator of the cleanliness of these plants

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Summary

- The Phast Swab is an easy to use test that can be completed in the field and requires minimal instrumentation
- Future work: make the test more sensitive by converting it to a fluorescent version

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Acknowledgements

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