Optical biosensors for food pathogen detection: fiber optic sensor

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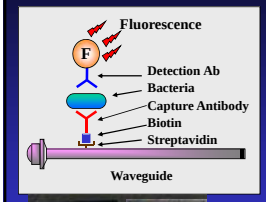
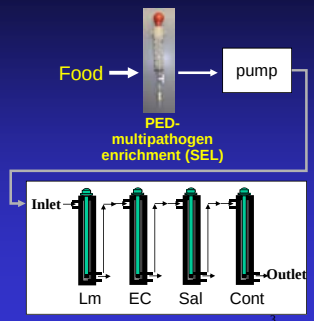
Objectives

- Develop and optimize fiber optic sensor for each food pathogen: *Listeria monocytogenes*, *E. coli* O157:H7, *Salmonella* Enteritidis
- Develop multi-pathogen fiber optic sensor for detection of three target pathogens simultaneously

Development of a Multi-Pathogen Fiber Optic Biosensor





Summary: Individual fiber optic sensor for each pathogen

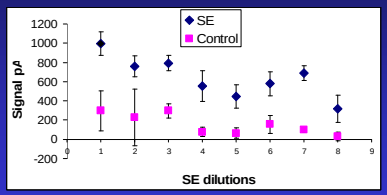
Pathogen	Detection limit	Detection time	Publications
Listeria monocytogenes	10^3 cfu/ml 10^4-10^5 cfu/ml hotdog	23 h	•Geng et al. 2004. AEM. 70: 6138 •Nanduri et al 2006. Sensors, 6:808
	bologna		•Kim et al. 2007. Fd. Sci. Biotechnol.16:337
E. coli O157: H7 Stx	10^3 cfu/ml ground beef 0.5 ug/ml	4-6 h	•Geng et al. 2006. Sensors, 6:796 •Tu et al. 2006. SPIE
Salmonella Enteritidis	10^4 cfu/ml Chicken Egg	4-6 h	•Morgan et al. 2006. Key Eng. Materials •Valadez et al (unpublished)

Antibodies for use with fiber optic biosensor

Pathogen	Capture antibody	Detection antibody
Listeria monocytogenes	Anti-Listeria PAb Anti-P66 PAb	MAb C11E9
E. coli O157: H7	KPL anti-E. coli O157:H7	KPL anti-E. coli O157:H7
Salmonella Enteritidis	KPL anti-Salmonella (CSA-1)	MAb-2F11

Fiber optic sensor for *Salmonella*

Analysis of sensitivity of the sensor with different concentrations of *S.* Enteritidis (PT4) cells suspended in PBS



1=10^9 cfu/ml
8=10^2 cfu/ml

Detection limit = 1x10^3 cfu/ml

Valadez et al (unpublished)

Specificity of the fiber-optic sensor

Bacterial culture	AVG (pA)	Control (pA)	P < 0.05	Results
S. Enteritidis	1230.486 ± 59.734	405.933 ± 277.610	-	Positive
S. Typhimurium	1152.806 ± 285.519	477.600 ± 000.000	0.5588	Positive
E. coli O157:H7	376.116 ± 2.003	188.833 ± 000.000	< 0.0001	Negative
S. aureus ATCC	1008.200 ± 319.933	319.933 ± 000.000	0.0047	Negative
E. faecalis	792.915 ± 42.640	84.099 ± 000.000	0.0141	Negative
L. gasseri	946.355 ± 144.537	270.600 ± 000.000	0.0069	Negative
L. rhamnosus	707.383 ± 115.280	424.100 ± 240.416	< 0.0001	Negative
L. monocytogenes	420.383 ± 95.860	270.600 ± 000.000	< 0.0001	Negative

Bacterial cells were used at 10^8 cfu/ml

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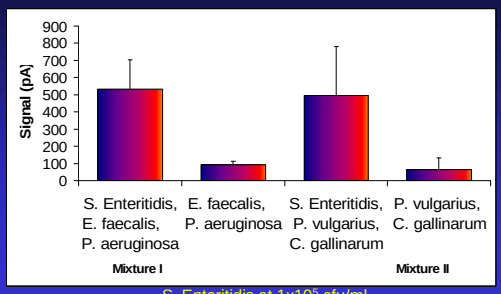
Specificity of the *Salmonella* fiber optic sensor in presence of mixed bacterial cultures



S. Enteritidis at $1x10^5$ cfu/ml
Others at $1x10^6$ cfu/ml each

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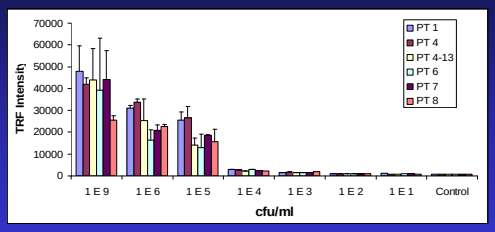
Sensitivity of *S.* Enteritidis PTs by immunomagnetic bead-time resolved immunofluorescence (IMB-TRF) assay



Detection limit = $1x10^5$ cfu/ml

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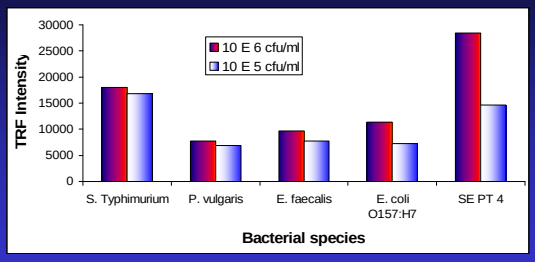
Selectivity tests of IMB-TRF



Values are an average of two wells each tested with two different concentrations of cells

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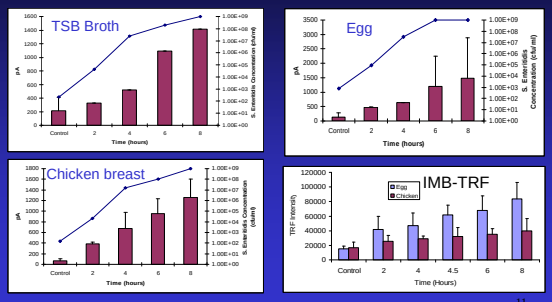
Detection of *S.* Enteritidis grown in food samples and tested every 2 h intervals using fiber optic biosensor.



Bars: Fiber optic signals; Lines: Growth curves

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Multi-Pathogen Assay Using a Fiber optic Waveguide Sensor System

Tasks

- Optimize immobilization conditions
- Screen different combination of capture and detection antibodies for optimum signal for *Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella* Enteritidis
- Run simultaneous assay for each pathogen
- Test with food samples in presence of natural competitive microorganisms
- Produce a multi-pathogen cartridge with waveguides pre-coated with the capture antibodies

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Antibodies to be used for multi-pathogen fiber optic sensor

Pathogen	Capture antibody	Detection antibody
Listeria monocytogenes	Anti-Listeria PAb Anti-P66 PAb	MAb C11E9 PAb404 PAbC639 PAb407
E. coli O157: H7	KPL anti-E. coli O157:H7	PAb Ec 8378 pET32a
Salmonella Enteritidis	KPL anti-Salmonella (CSA-1)	MAb-2F11

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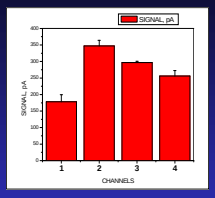
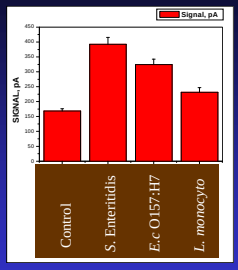
Multi-pathogen detection: Initial trial

E. coli O157:H7



Capture Ab: KPL (anti *E.coli*
Detection Ab: pET32a (Cy5)
Channels: 1: Control; 2-4: Exp



	Listeria	*Salmonella*	*E. coli*
Capture	PAb-P66	Anti-Sal (CSA-1)	Anti-E.coli (KPL)
Detection	MAb C11E9	MAb 2F11	pET32a

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Conclusions

- Sensitivity of fiber optic sensor for *Salmonella* Enteritidis is 1x10^3 cfu/ml with pure culture
- Fiber optic assay is highly selective
- *Salmonella* can be detected from spiked (10^2 cfu/g) chicken and egg samples only after 4 h of enrichment
- Specific antibodies are available for multi-pathogen fiber optic assay and initial trial show promising result in simultaneous detection of all three pathogens if present in a sample

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Next steps for multi-pathogen testing using fiber optic sensor

- Optimize assay for simultaneous detection of 3 pathogens suspended in buffer/growth media
- Determine assay sensitivity/selectivity
- Validate the assay with spiked food samples following enrichment in SEL
- Application of PED for sample preparation

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Thank you

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