Surface Imprinted Polydopamine Based Magnetic Separation and Quantum Dots Based Fluorescent Detection of Foodborne Pathogenic Bacteria

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Introduction

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Food Safety

- An estimated 600 million – almost **1 in 10 people** in the world – fall ill after eating contaminated food and 420,000 die every year, resulting in the loss of 33 million healthy life years (DALYs) (WHO, 2017).
- The Department of Agriculture (USDA) estimated that foodborne illnesses cost **$15.6 billion** each year (USDA, 2017).
- It is estimated that *Salmonella* in food has caused about 1 million illnesses, 19,000 hospitalizations, and 380 deaths in the United States every year. (CDC, 2018)
- It is highly important to develop a rapid and cost-effective method for detection of foodborne pathogenic bacteria to ensure food safety.

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Current Detection Methods

- Culture plating
- Polymerase chain reaction (PCR)
- Enzyme-linked immune-sorbent assay (ELISA)
- Strips (Lateral flow immunoassay)
- Biosensors
  - Biosensing materials (enzyme, antibodies, nucleic acid probes ...)
  - Transducing methods (optical, electrochemical, piezoelectric ...)

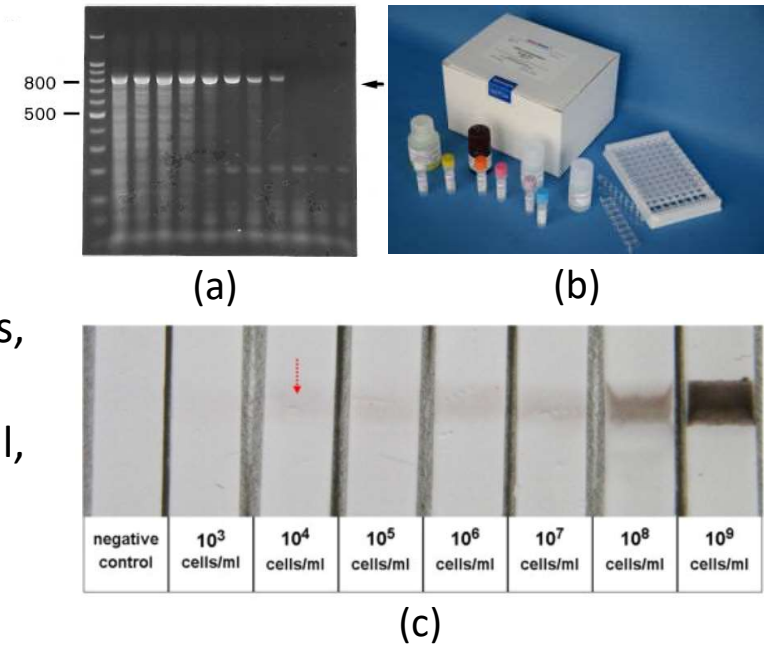

Figure 1. Examples of detection methods for *Salmonella* (a) PCR[1] (b) ELISA (c) Strips[2]

[1] Journal of medical microbiology 52.9 (2003): 773-776
[2] Food chemistry 141.3 (2013): 2526-2532 (permission pending)

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Magnetic Separation

Using magnetic nanoparticles

Advantages

- Fast and efficient separation (>90%[1])
- Little denaturation of the sample [2]
- Specific recognition of the target
- Cost efficient

Problems

- Requirement for high-gradient magnetic field
- Aggregation of magnetic particles

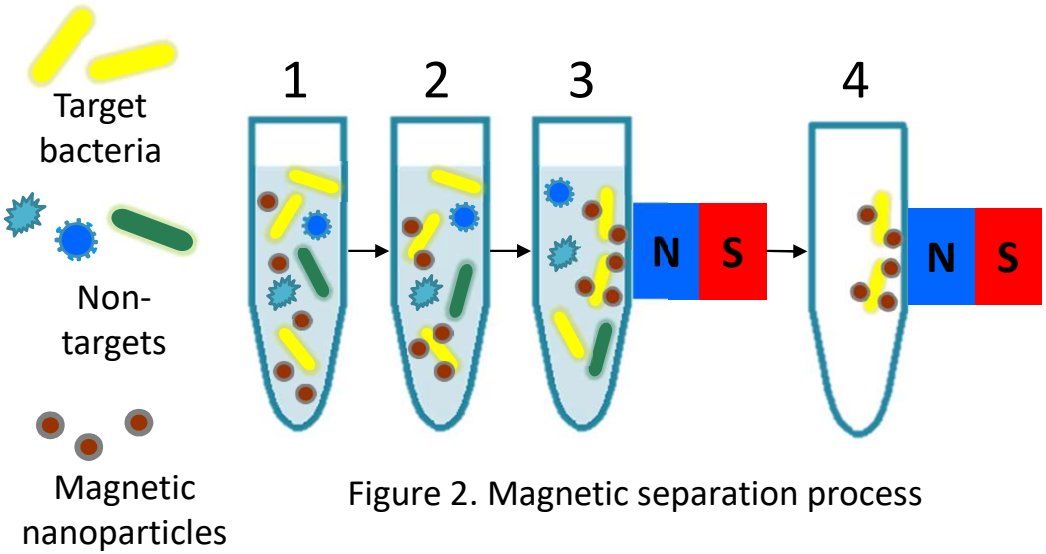

Figure 2. Magnetic separation process

[1] J. Food Prot., 2005, 68(9), 1804–1811
[2]Lab Chip 7, 1644–1659

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Fluorescent Detection

Using quantum dots

Advantages

- High detection sensitivity
- Fast response time
- Multiple assays with multi-wavelength emissions

Problems

- Difficulties in washing
- Relatively high cost (for quantum dots)

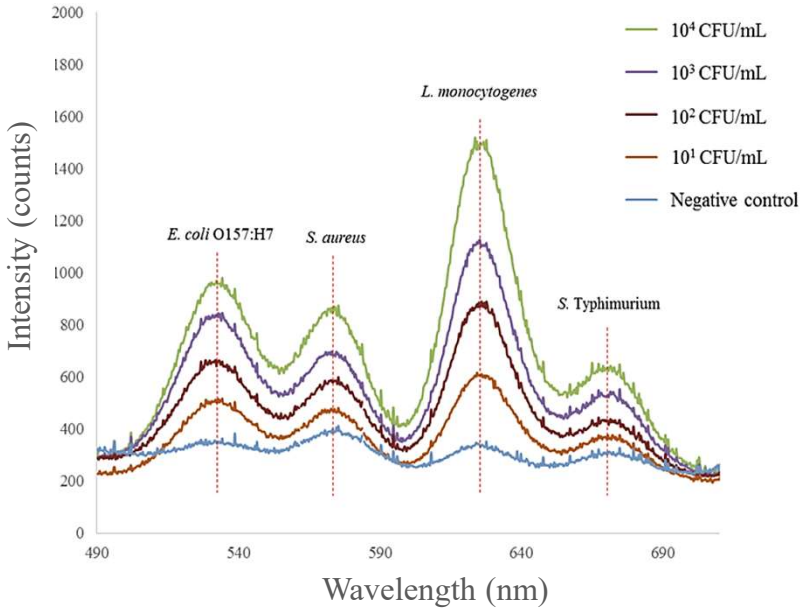

Figure 3. Fluorescence spectra of multiplex foodborne pathogenic bacteria[1]

[1] " Transactions of the ASABE 58.3 (2015): 891-906

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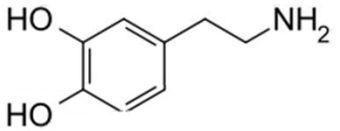
Polydopamine



The technique of using polydopamine as a kind of recognition element has been developed especially for small molecules. The advantages includes:

- Stable in extreme environments
  - ✓acids or bases
  - ✓organic solvents
  - ✓high temperatures
  - ✓high pressures
- Easy to make
- Cost-effective

Table 1. Estimated cost

item	use antibody		use PDA	
	MNP-SA	Ab	MNP	PDA
price	$149/5mg	$375/5mg	$129/50mg	$337/100g
unit price ($/mg)	29.8	75	2.58	3.37
amount/sample (ug)	20	5	20	20
price/sample ($)	0.596	0.375	0.0516	0.0674
total price/sample ($)	**0.971**		**0.119**	

Imprinted Polydopamine Film

Virus detection using imprinted polymer film on electrodes (Lu et al., 2012)

Virus **detection and concentration** using imprinted polymer film on nanobeads (Yang et al., 2017)

Bacteria **detection** using imprinted polymer film on electrodes (Chen et al., 2017)

Bacteria **detection and concentration** using imprinted polymer film on nanoparticles hasn't been reported.

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Objective

- The objective of this research is to develop an innovative method for detection of foodborne pathogenic bacteria using quantum dots as fluorescent reporter, magnetic nanoparticles and imprinted polydopamine as separation method.

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Materials and Methods

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Major Materials and Instruments

Table 2. List of major materials

material	size	feature	company
magnetic nanoparticles	100 nm	magnetic	Ocean NanoTech
quantum dots	5 nm	λ_{em} = 615 nm	Ocean NanoTech
biotin labeled antibodies	150 k D	polyclonal	Meridian Life Science
dopamine	153 D	polymerize in pH 8.0	Sigma-Aldrich

Table 3. List of major instruments

instrument	model	feature	company
magnetic separator	MS0206	≈1.0 T	Aibit Biotech Instrument
spectrometer	USB2000	200-850 nm	Ocean Optics
light source	self-assembled	λ_{ex} = 380 nm	-

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Bacteria and Plating Method

***Salmonella* Typhimurium (ATCC 14028)**

***Escherichia coli* O157:H7 (ATCC 43888)**

Escherichia coli K12 (ATCC 29425)

***Listeria monocytogenes* (ATCC 43251)**

Listeria innocua (ATCC 33090)

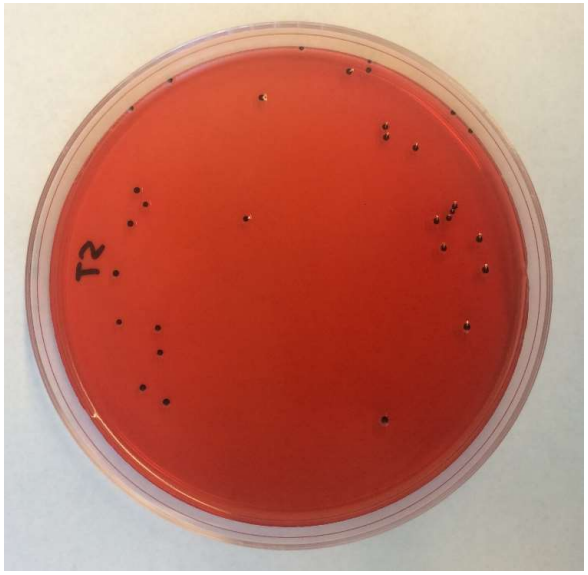

Figure 4. *Salmonella* Typhyimurium on a XLT4 plate

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Magnetic Separation

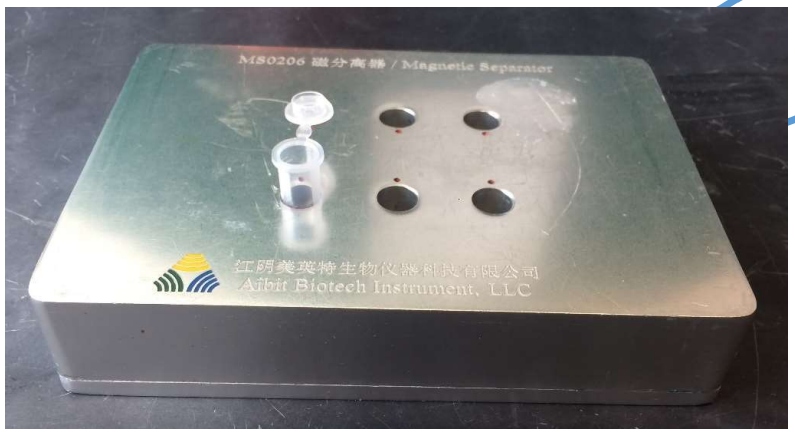

Figure 5. Magnetic separator

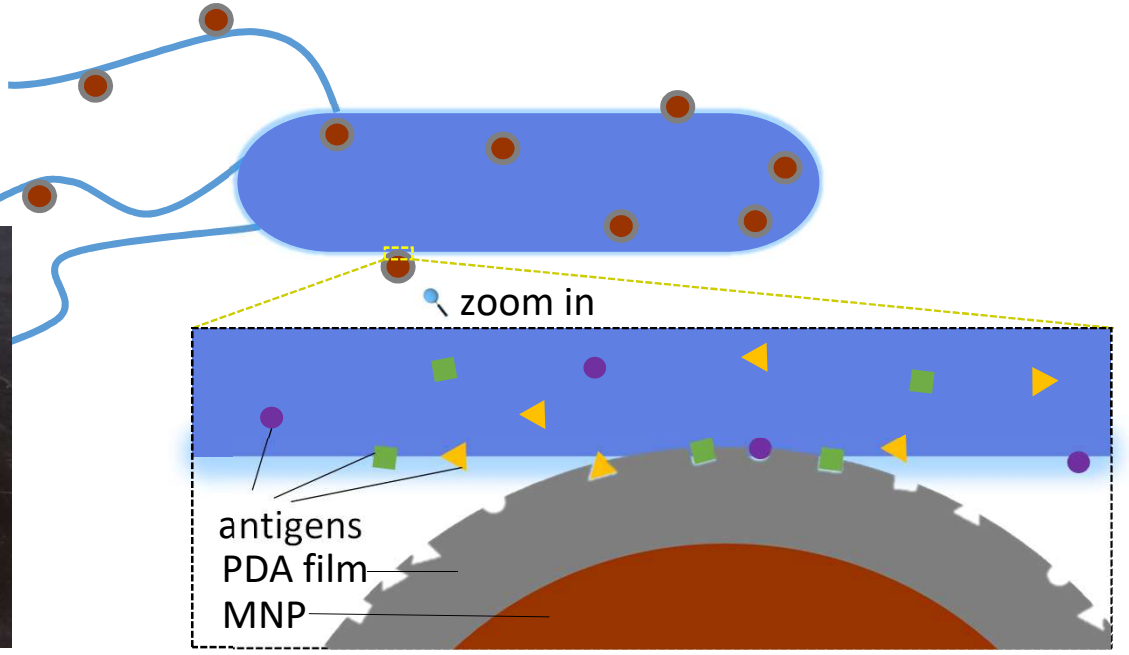

Figure 6. Schematic diagram of imprinted film

Fluorescent Detection

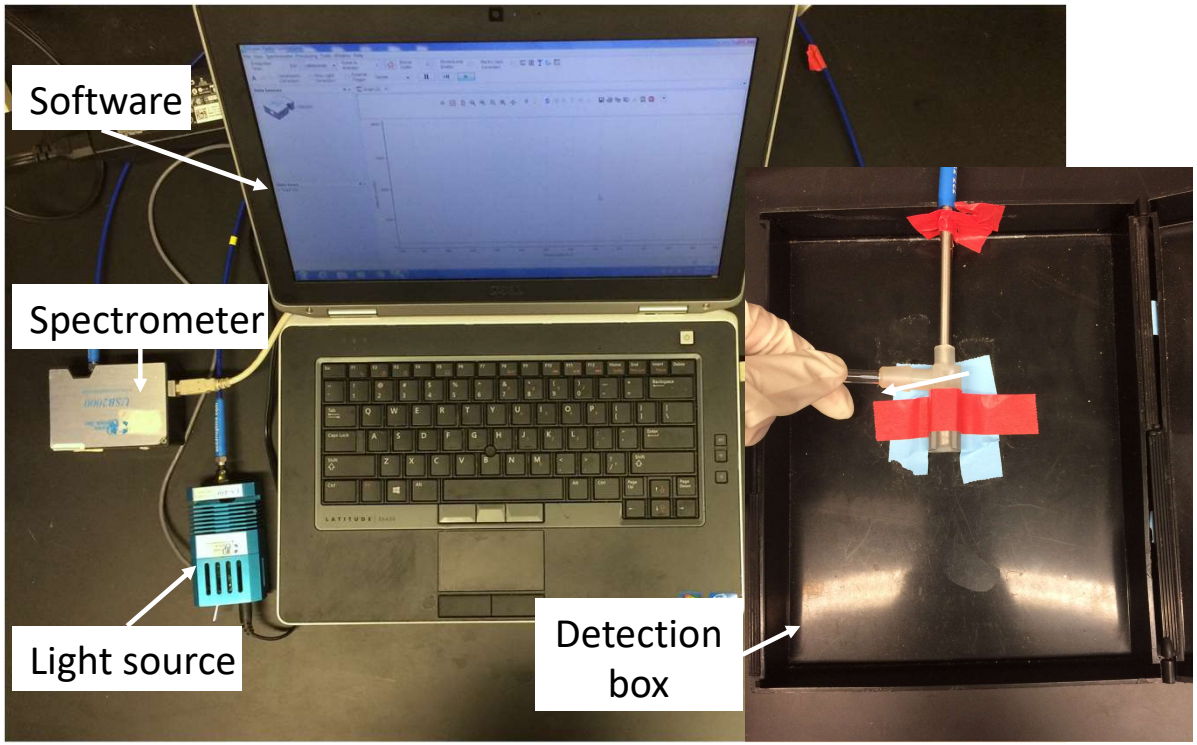

Figure 7. Schematic diagram of the fluorescent detection module

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Procedure

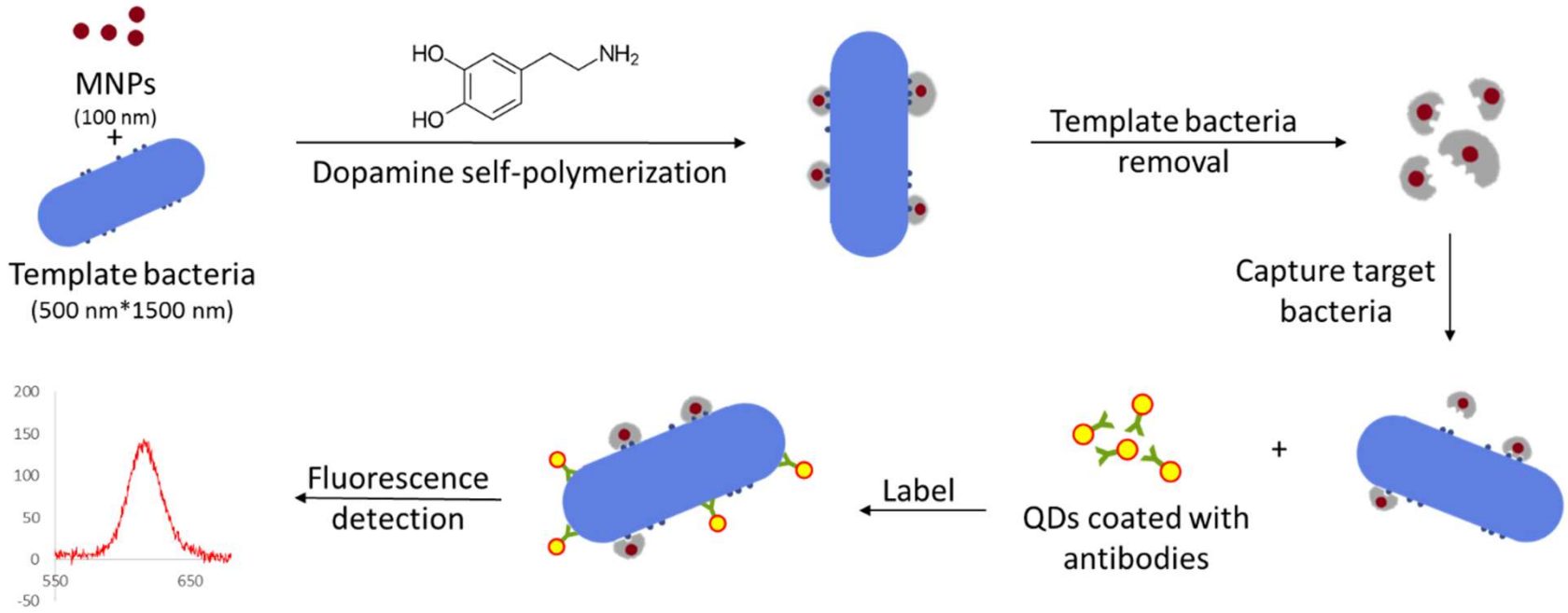

Figure 8. Process of bacteria capture and detection

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Results and Discussion

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Fluorescent Detection

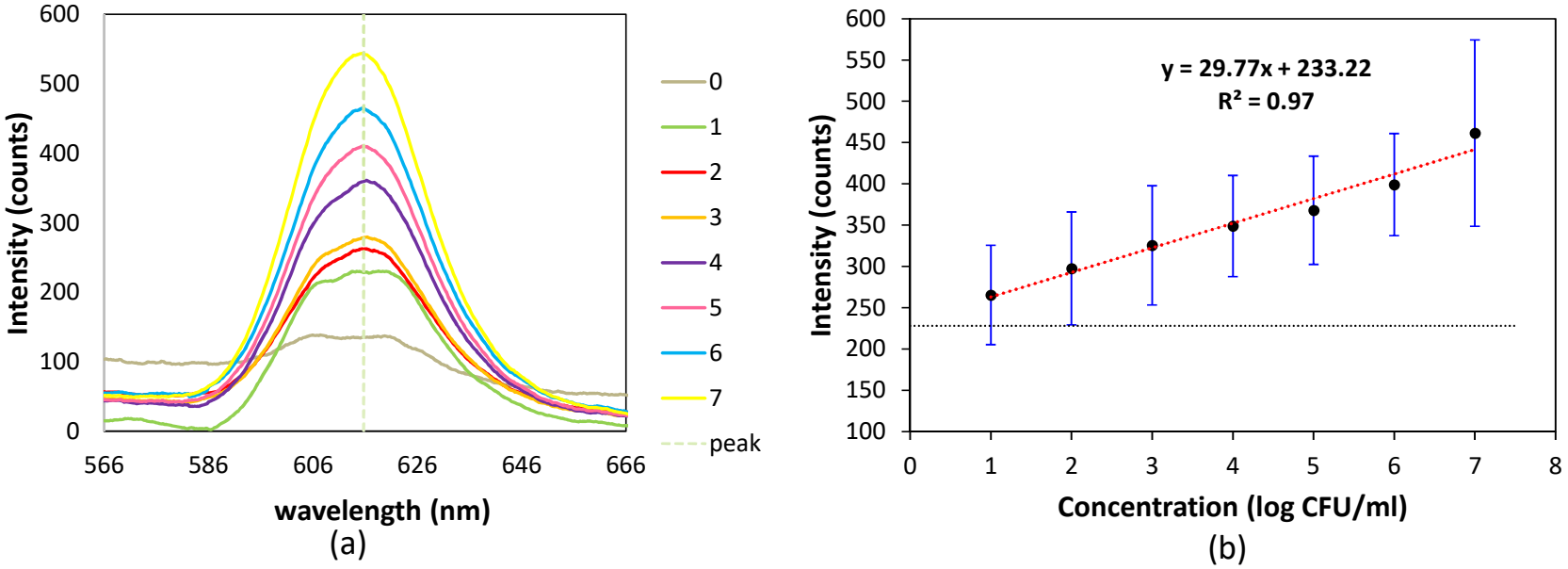

Figure 9. Fluorescence signals of *Salmonella* at different concentrations (a) typical spectrum and (b) linear regression

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Fluorescent Detection

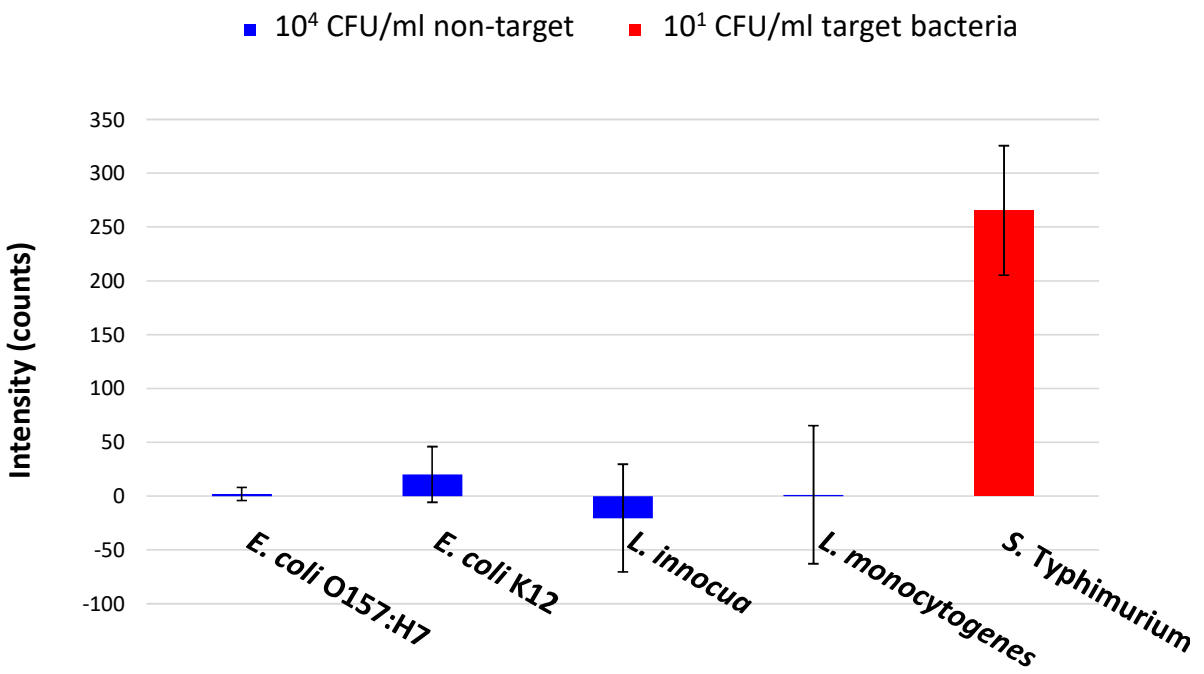

Figure 10. Specificity test using 10^4 CFU/ml non-target bacteria and 10^1 CFU/ml target bacteria

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Conclusions

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Conclusions

- A fluorescent biosensor was successfully developed for rapid detection (t < 2h) of *Salmonella* Typhimurium using surface imprinted polydopamine (PDA) on magnetic nanoparticles (MNPs) to capture target bacteria and quantum dots (QDs) as a label for detection.
- Imprinting PDA film on the nanoparticle surface to capture and concentrate bacteria was reported. Although its affinity and specificity was not as good as antibodies and aptamers, PDA is much cheaper and more stable for in-field application.
- The PDA@MNPs developed were able to capture bacteria for the biosensor to reach the detection limit as low as 10^2 CFU/ml.

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On-Going Research

- The ongoing research focuses on:
  - improvement of sensitivity – signal amplification
  - improvement of affinity – smaller structures
  - simultaneous detection – change of template

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Thank you!

Questions?

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