



Progress on Development of Field-Based Rapid Methods for Foodborne Pathogens

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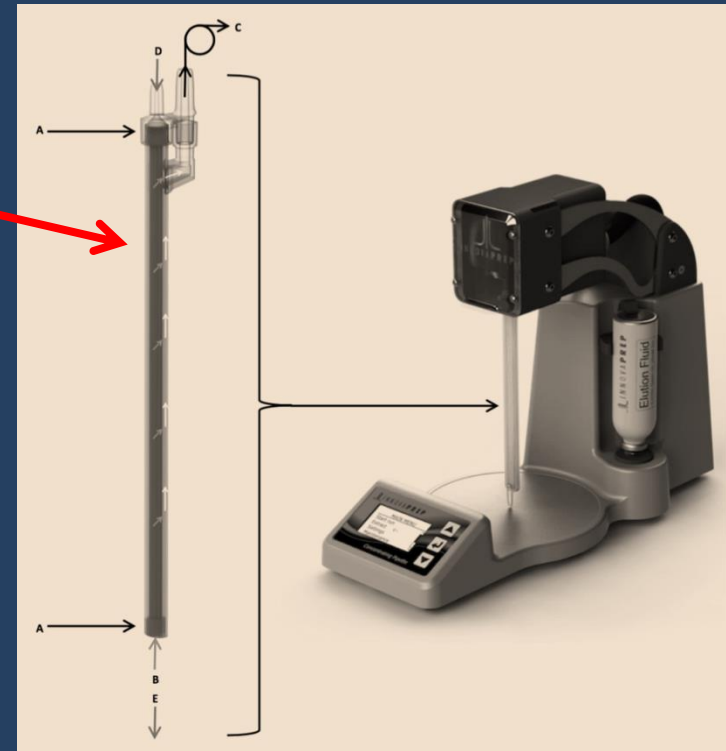
United States Department of Agriculture
Agricultural Research Service
Eastern Regional Research Service
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Plan Objective 1 (of 3)



Rapid and Quantitative Sample Preparation, No Culture Enrichment...

- InnovaPrep Concentration Pipette
- New Class of Antibody-Associated Magnetic Particles (CRADA with Abraxis, Inc)
- Ferrofluid-based targeted concentration of foodborne pathogens (Tentative agreement with Ancera, Inc, Branford, CT)
- Novel capture of nucleic acid targets; High sample volume; Live/Dead (Tentative agreement & SBIR funding with VisuGen Global, LLC, Aurora, CO)

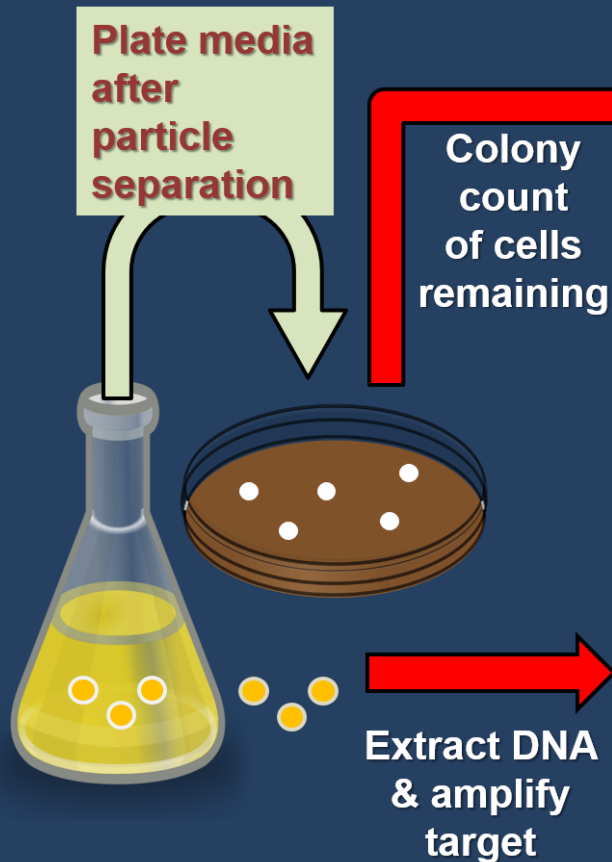




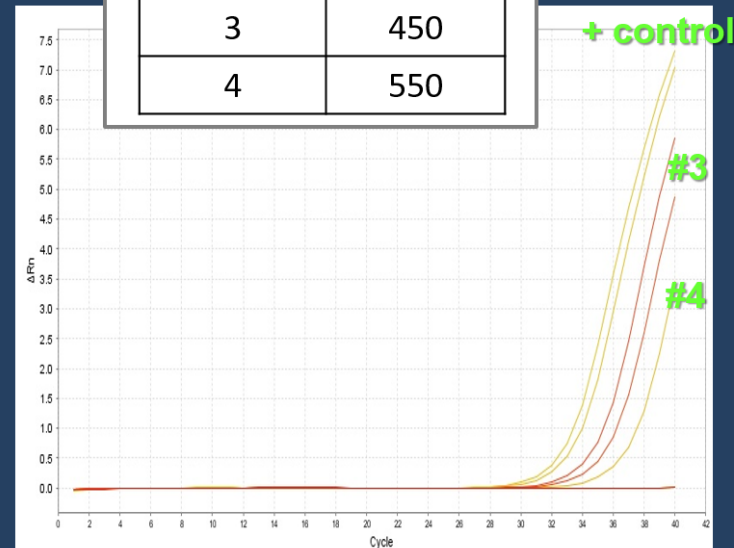
InnovaPrep Performance

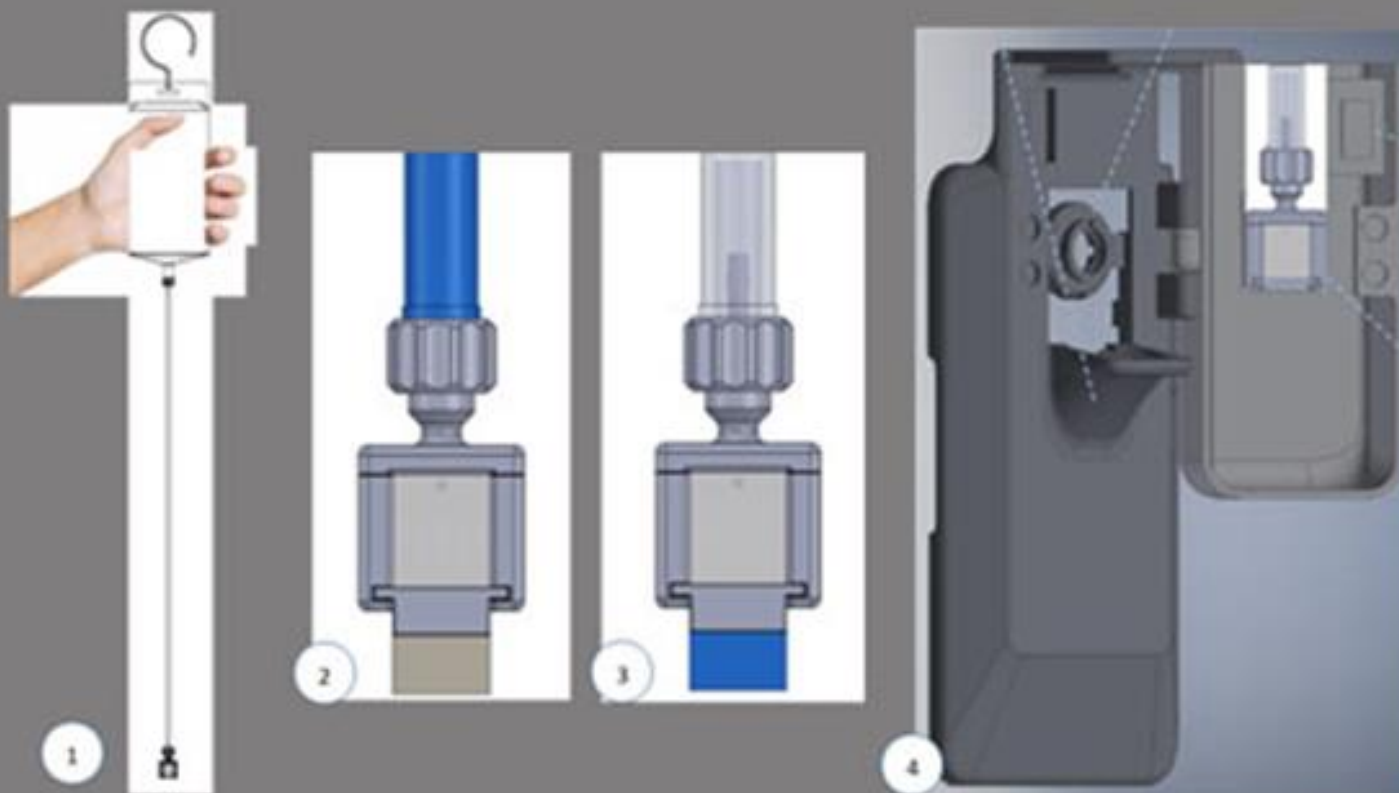
Native Bacterial Load on Frozen Vegetables ¹				Recovery			
CFU g ⁻¹ @ 30°C		CFU g ⁻¹ @ 37°C		30°C	37°C		
Control	InnovaPrep	Control	InnovaPrep				
3858	3239	2744	2434	0.840	0.887	Sampling # 1 [ca. 28.6 g]	
1671	1071	1086	914	0.641	0.842	Sampling # 2 [ca. 26.6 g]	
1500	1143	357	314	0.762	0.880	Sampling # 3 [ca. 33.7 g]	
1490	1264	775	632	0.849	0.816	Sampling # 4 [ca. 57.8 g]	
3900	2350	1282	679	0.603	0.529	Sampling # 5 [ca. 66.4 g]	
¹ "Signature Kitchens", Green Peas and Diced Carrots				\bar{x}	0.739	0.791	
					±	±	P = 0.767
				$s_{\bar{x}}$	0.0504	0.0667	

Cell Capture by Antibody-coated Magnetic Particles



Particles	Average CFU/mL
T ₀ culture	630
No cells - control	0
1	0
2	30
3	450
4	550





- 1) Add water that flows through capture chip (5 min)
- 2) Connect microparticle vial and wicking pad
- 3) Wait for wicking pad to turn blue (15 min)
- 4) Insert chip into reader to record results

Plan Objective 2 (of 3)



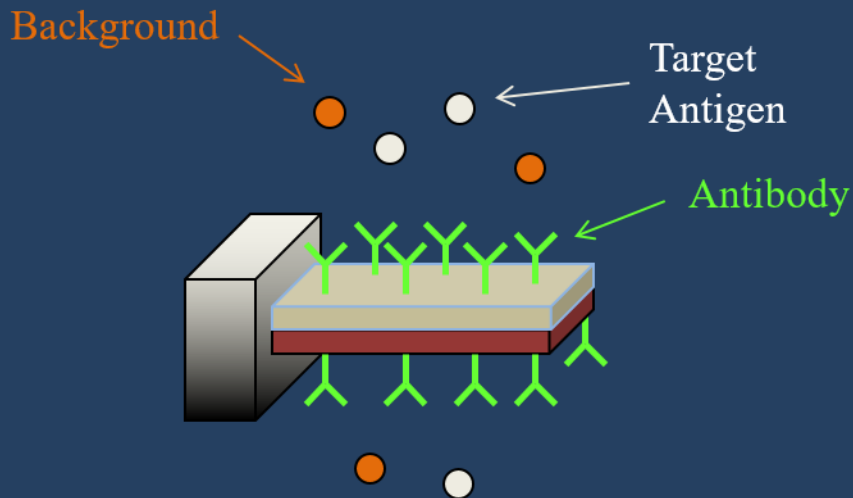
Develop rapid, quantitative detection methods...

- Piezoelectric membrane sensing (PEMS; MTRA with TBT Group Inc, Bellmawr, NJ)
- AlphaLISA (CRADA with Abraxis Inc, Warminster, PA)
- Flow-through immunoelectrochemistry

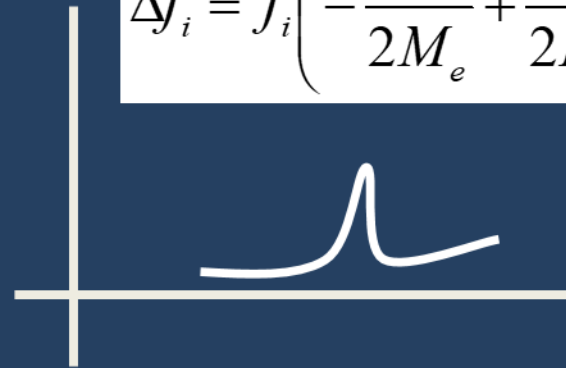
PEMS Biosensor Overview



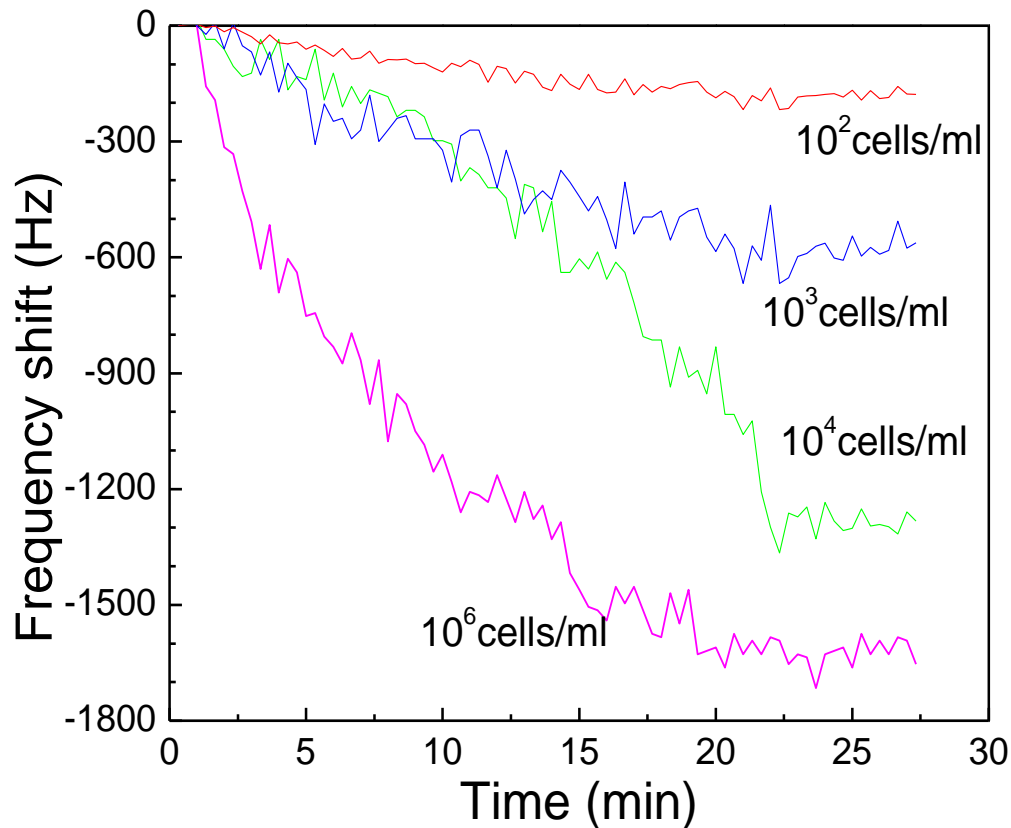
- Antibodies are immobilized on the biosensors to provide selectivity.
- Resonance frequency (f_i) of the biosensors shift (Δf_i) upon antigen attachment due to a change in mass (Δm) and spring constant (Δk) of the cantilever .
- The quantity of the attached antigens can be calculated using calibration curves



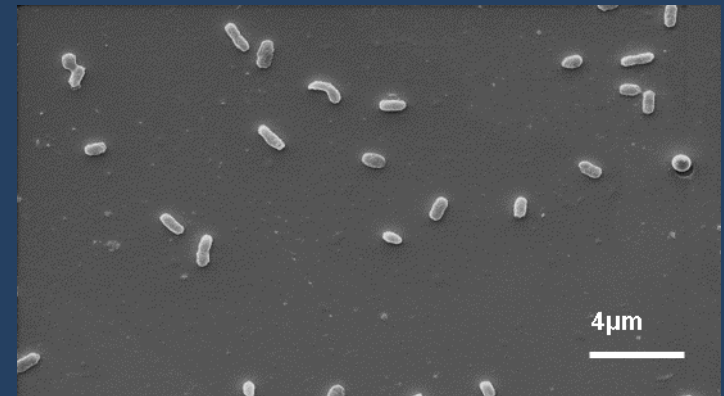
$$\Delta f_i = f_i \left(-\frac{\Delta m}{2M_e} + \frac{\Delta k}{2K} \right)$$



E. coli O157:H7 Detection



10^6 cells/ml

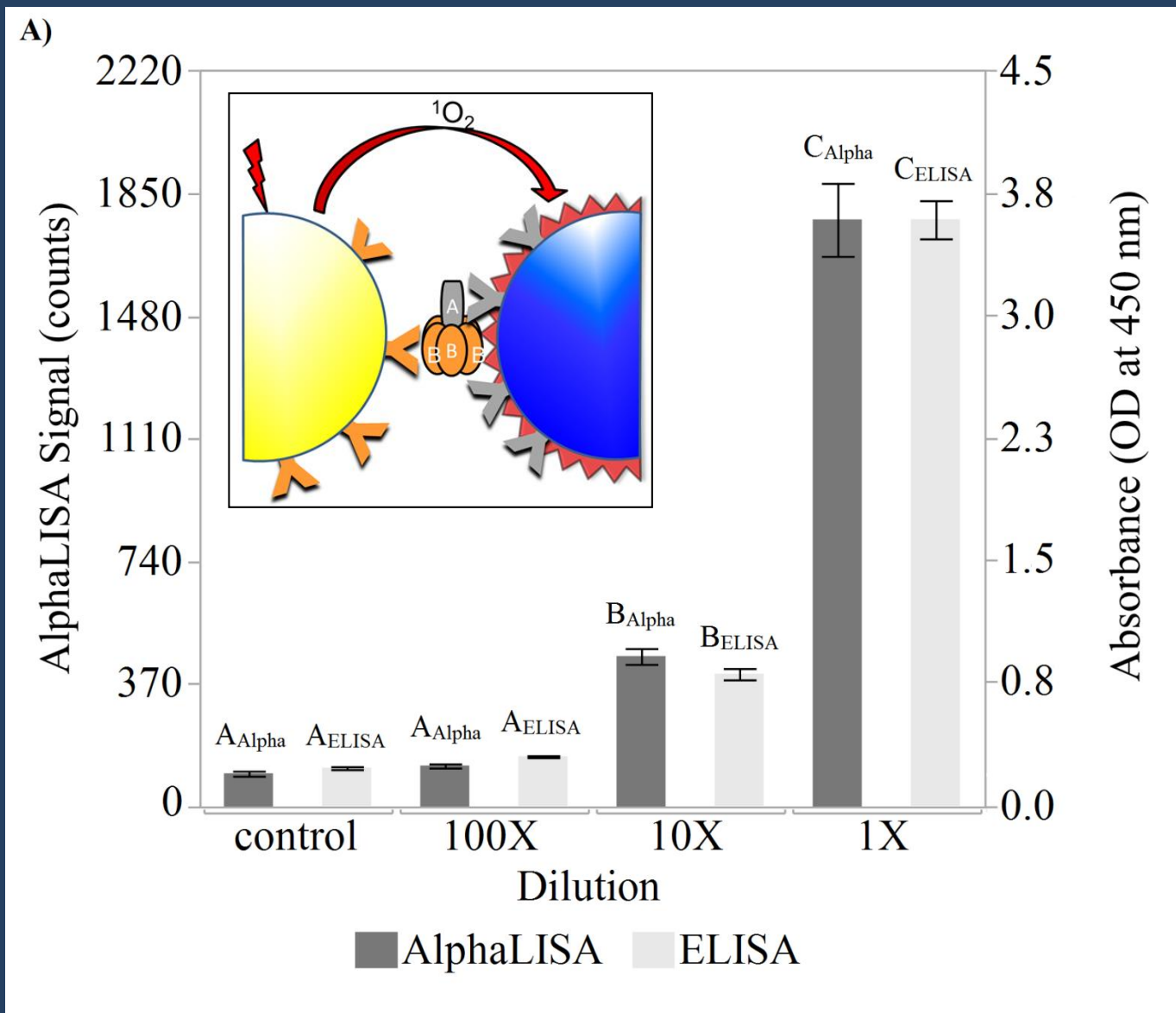


10^4 cells/ml

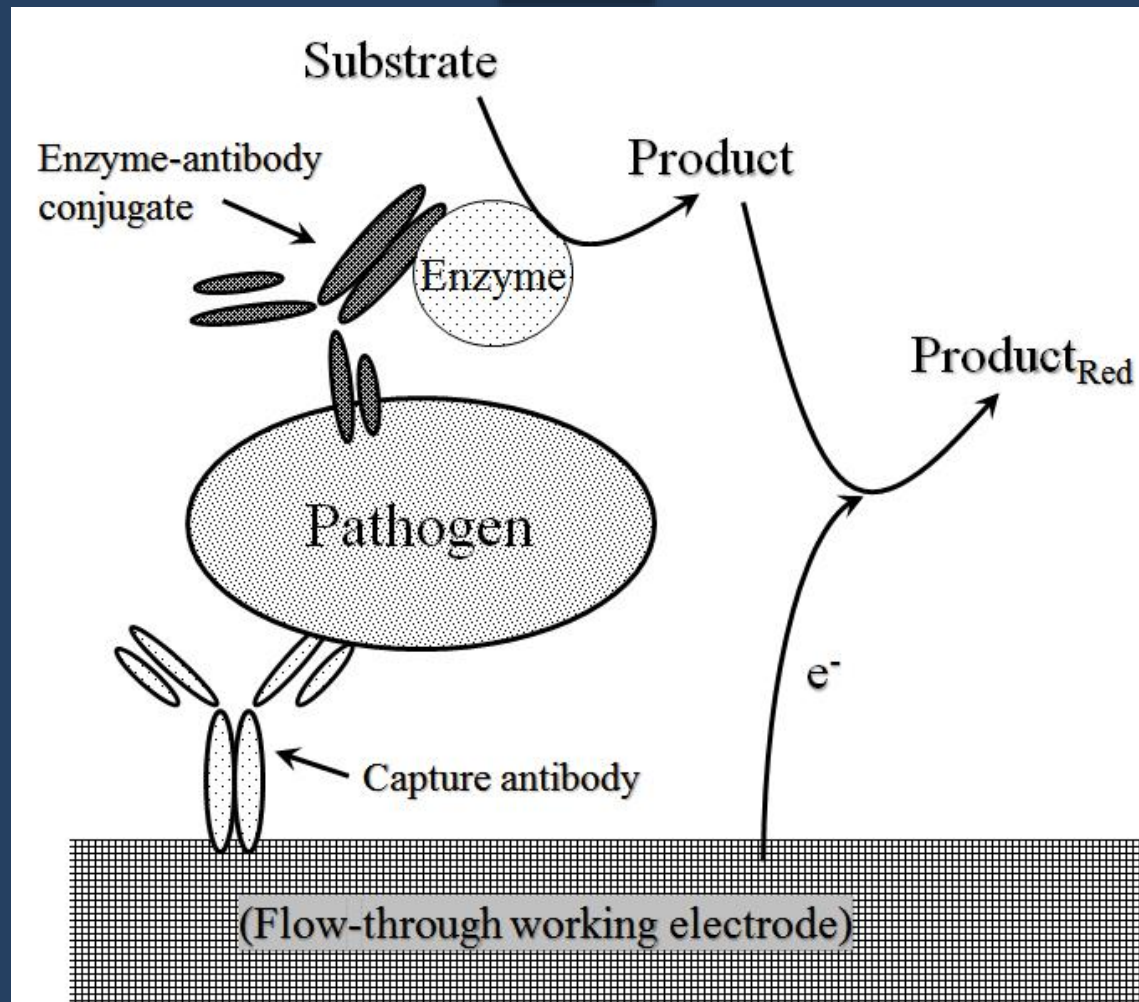




AlphaLISA vs. ELISA Detection of Stx 2 in STEC-Inoculated Romaine Lettuce

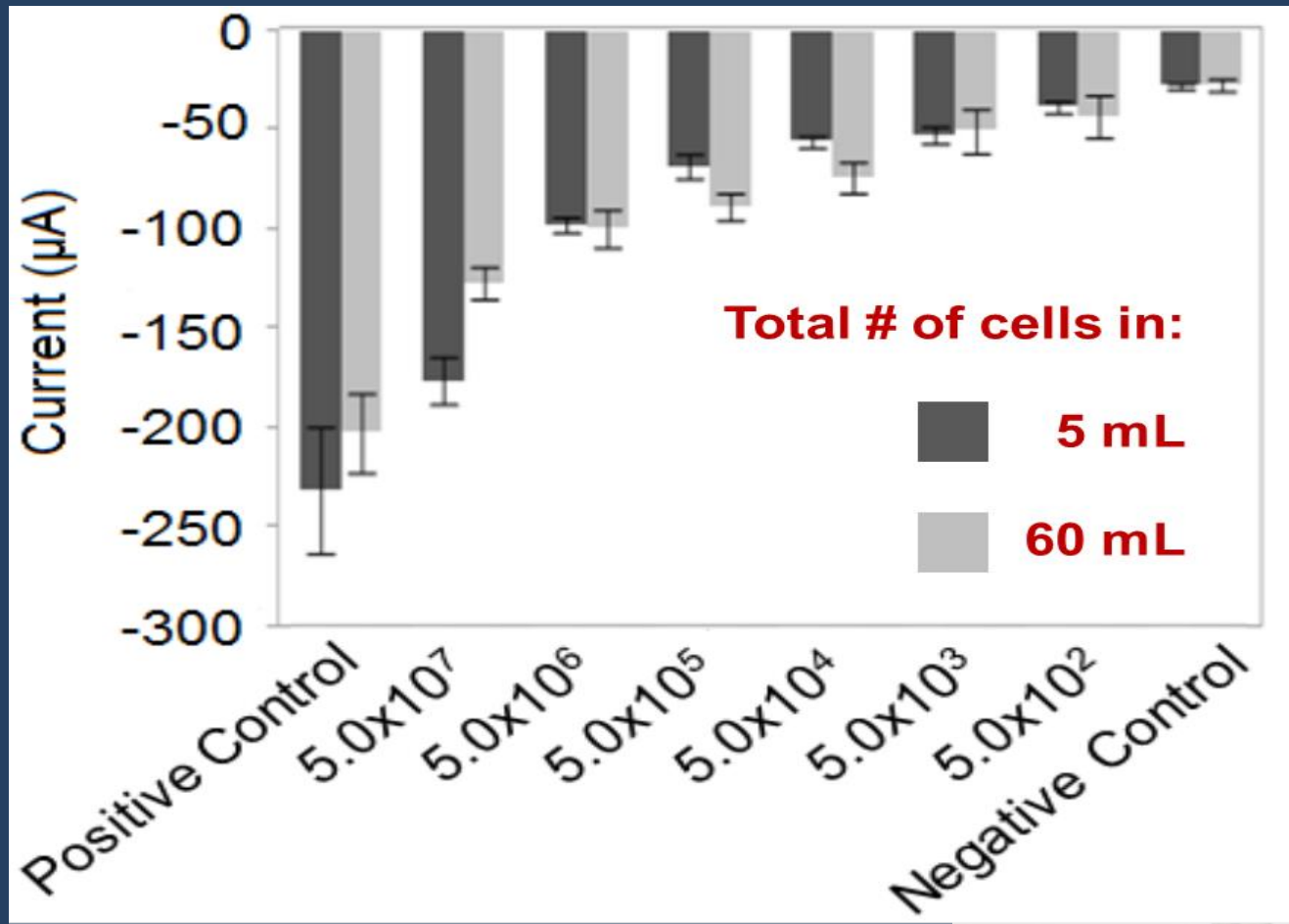


Flow-through Enzyme-Linked Immunochemical Detection





Flow-through Immunochemical Detection of *Salmonella*



Plan Objective 3 (of 3)



Develop and validate rapid identification methods...

- Agreement with BioRad (ddPCR)- comparing STEC detection versus IQ-Check and MLG
- MinION-field portable?
- Whole genome sequencing (*Campylobacter spp.*)



Sequencing of *Campylobacter spp.* Isolates (35 total) from Beef Liver and Chicken

Whole genome sequencing-- PacBio [~12 isolates] and
Illumina MiSeq

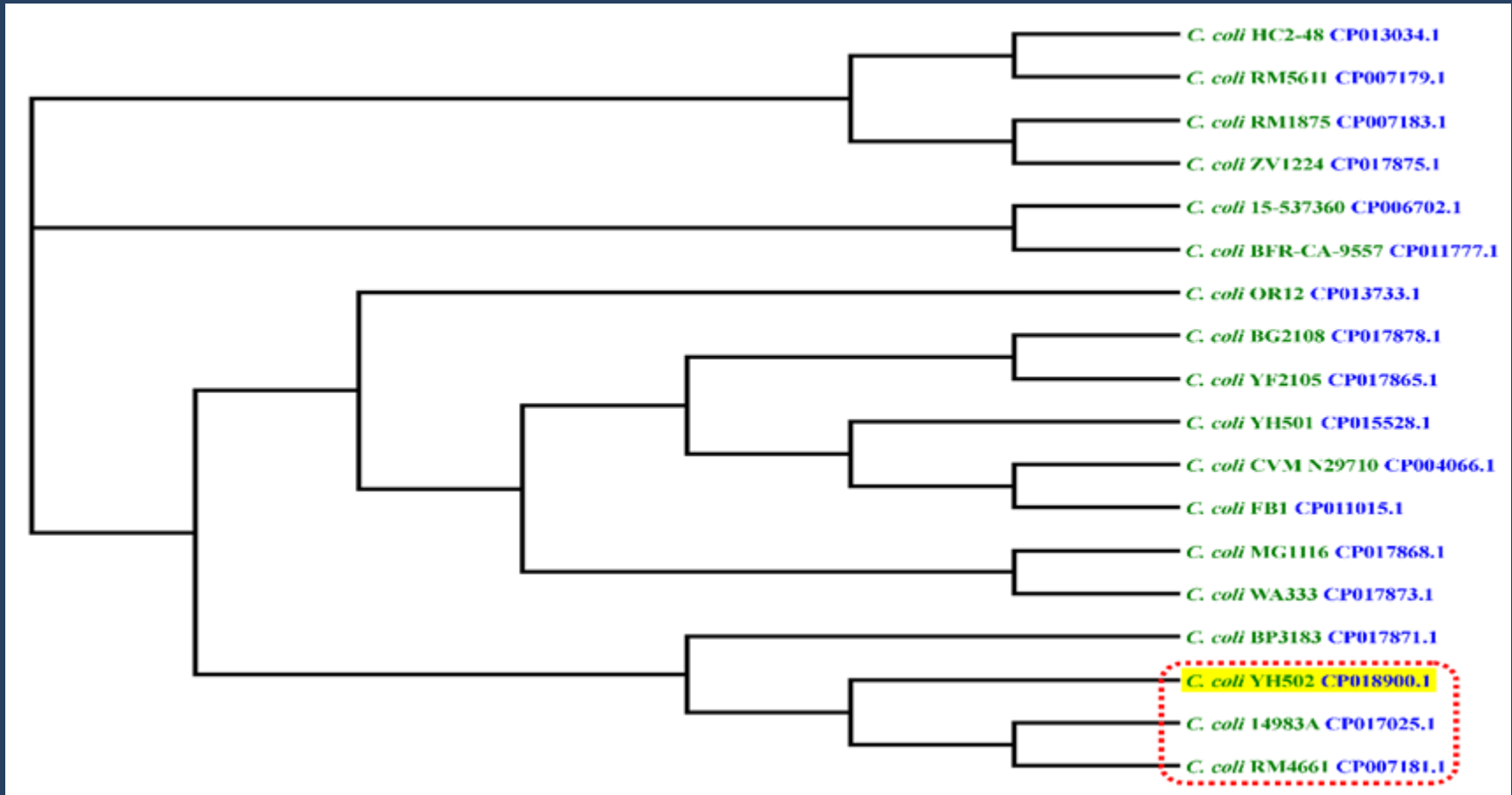
- ~12 isolates– Assembled complete genome
- 8 isolates– Type VI Secretion System
- ~14 isolates– Assessed for resistance against a panel of 13 antibiotics; Observed significant AMR for most isolates however not for gentamicin

e.g., *Campylobacter coli* YH502...

- A chromosome of 1,718,974 bp (**CP018900.1**)
- A mega-plasmid (pCOS502) of 125,964 bp (**CP018901.1**)
- GC content: 31.2%
- Contains 1931 coding sequences and 53 non-coding RNAs
- Multiple virulence genes (67) including a plasmid-borne type VI secretion system
- Antimicrobial resistance genes (beta-lactams, fluoroquinolones, and aminoglycoside)
- CRISPR sequences (14 repeats) and associated proteins (Cas1, Cas2, and Csn)



Phylogenetic Tree of *C. coli* Strains Based on Whole Genome Single Nucleotide Polymorphism Analysis



- The closest neighbor of the YH502 strain was *C. coli* strain 14983A, which was isolated from a turkey farm housefly in North Carolina.
- Multilocus sequence typing analysis of the complete genomes showed that *C. coli* YH502 and 14983A belonged to the same clonal complex (ST-828).



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