



Development of Portable Detection and Quantification Technologies for Foodborne Pathogens

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Objective 1: Develop rapid and efficient techniques that separate and concentrate and/or quantify targeted pathogens from food matrices.



- Subobjective 1A. Apply rapid and high volume centrifugal flow concentration to the separation of bacteria from food matrices.
- Subobjective 1B. Partition and concentrate bacteria using immunomagnetic separation with a new class of antibody-coated paramagnetic particles.
- Subobjective 1C. Compare and contrast bacteria separation and concentration with flow-through filtration systems.
- Subobjective 1D. Develop and validate MPN-multiplex qPCR methods for rapid and quantitative detection of multiple foodborne pathogens.

Objective 2: Develop and validate field testing kits that rapidly screen for the presence and quantification of pathogens and/or indicator microorganisms in foods at the initial processing level.



- Subobjective 2A. Generate portable, label-free sensors (e.g., next generation cantilever microbalance) for rapid in-line or near-line screening of foods.
- Subobjective 2B. Generate portable antibody and/or phage-based multiplex assays including integrated comprehensive droplet digital detection (IC 3D).
- Subobjective 2C. Develop an AlphaLISA detection protocol for target pathogens.
- Subobjective 2D. Develop a flow-through immunoelectrochemical detection device for field portable detection of target pathogens

Objective 3: Develop and validate rapid methods for the identification of pathogens and/or indicator microorganisms in foods for application in either the field or testing laboratories.



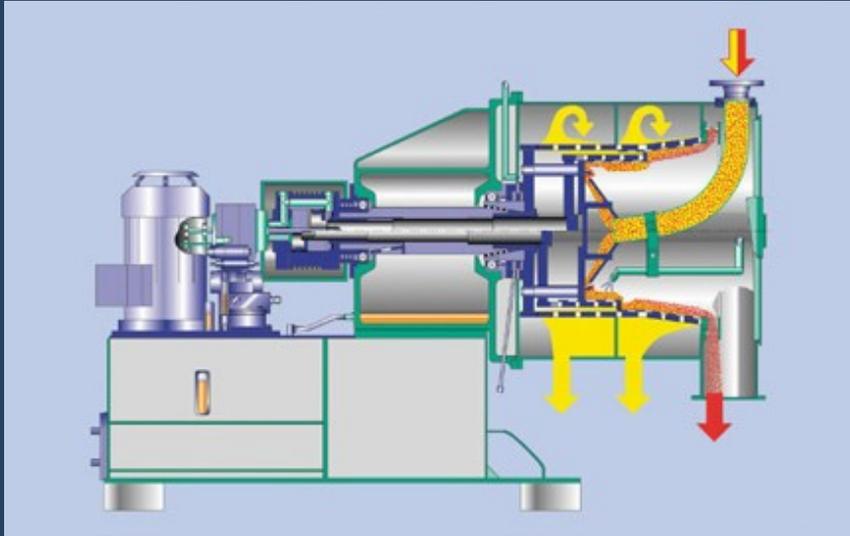
- Subobjective 3A. Generate phage and/or antibody typing arrays.
- Subobjective 3B. Generate pathogen databases and improve the accuracy of the Beam (formerly BActerial Rapid Detection using Optical scattering Technology or BARDOT) system.
- Subobjective 3C. Direct typing (colony isolates not required) of enriched samples using a targeted-sequencing method.
- Subobjective 3D. Generate genome sequence-based typing and identification schemes using next-generation sequencing technology (e.g., MiSeq, Ion Torrent PGM, and MinION), and characterize virulence and antibiotic resistance of microbial pathogens.

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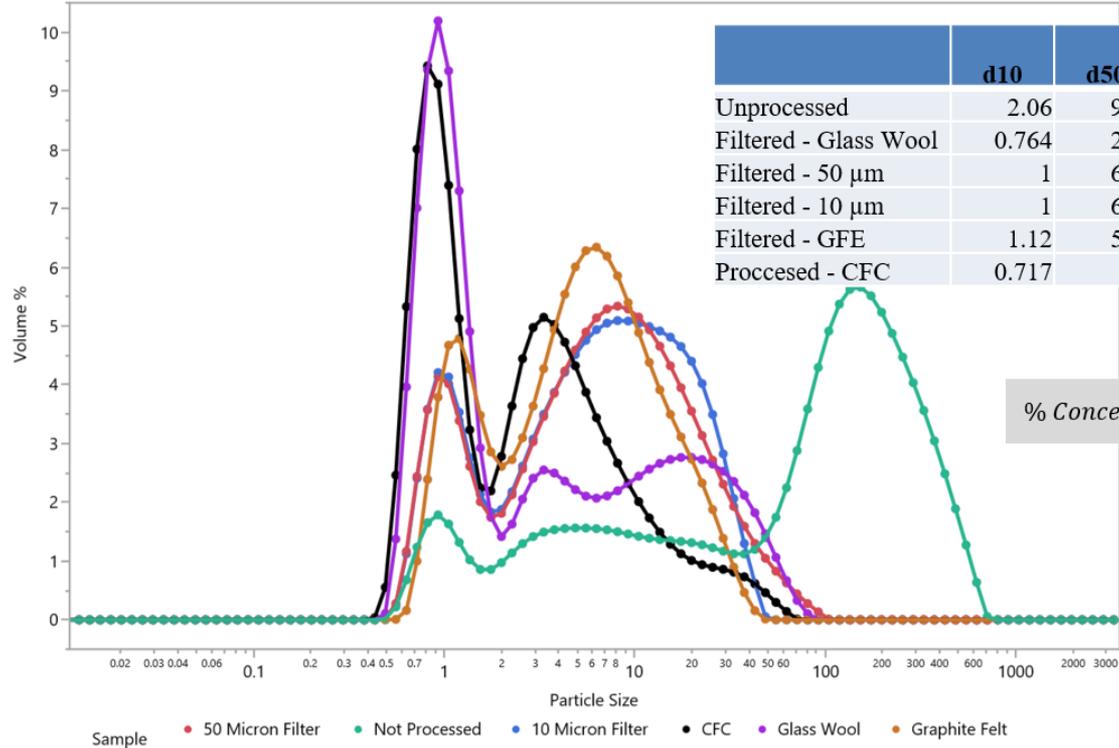
Continuous Flow Through Centrifugation (CFC)





Sample Preparation for 1L (325 g ground beef in 975 mL broth)

Particle Size Distribution - Before and After Treatments

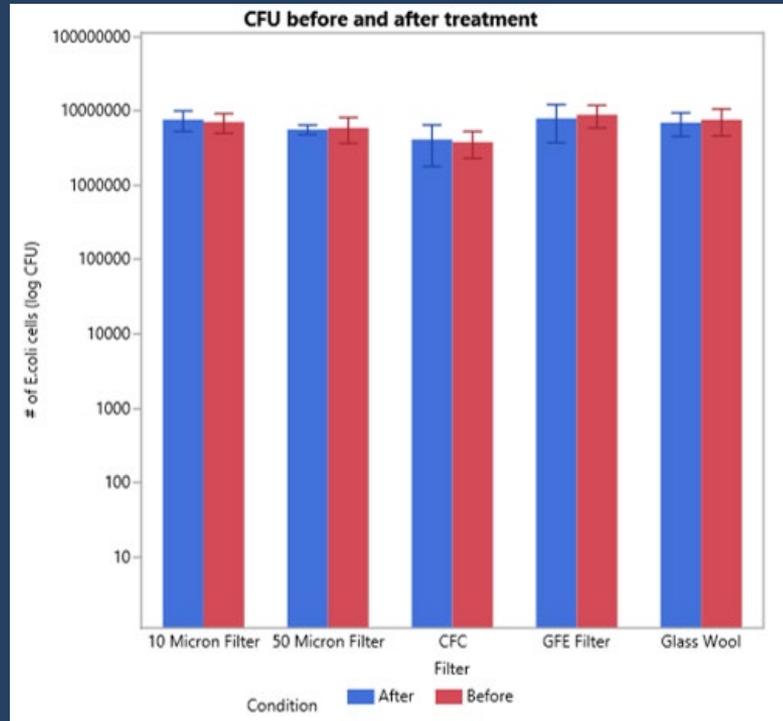


	d10	d50	d90	% Concentration Change
Unprocessed	2.06	94.5	320	0.00%
Filtered - Glass Wool	0.764	2.19	28	-86.27%
Filtered - 50 µm	1	6.54	25.5	-81.05%
Filtered - 10 µm	1	6.46	22.8	-83.01%
Filtered - GFE	1.12	5.18	17.1	-86.93%
Processed - CFC	0.717	2.3	11.5	-97.39%

$$\% \text{ Concentration Change} = \frac{(C_T - C_U)}{C_U}$$



Sample Preparation for 1L



	Average CFU	Average CFU	P value (student's t)
50	Before	5892857	0.7784
50	After	5619047	0.7784
10	Before	7083333	0.5449
10	After	7642857	0.5449
GFE	Before	8845238	0.5387
GFE	After	7916666	0.5387
Glass Wool	Before	7595238	0.5772
Glass Wool	After	6964285	0.5772
CFC	Before	3797619	0.6918
CFC	after	4119047	0.6918

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Superparamagnetic Particles



Magnetic Activated Cell
Sorting (MACS)
100 nanometers



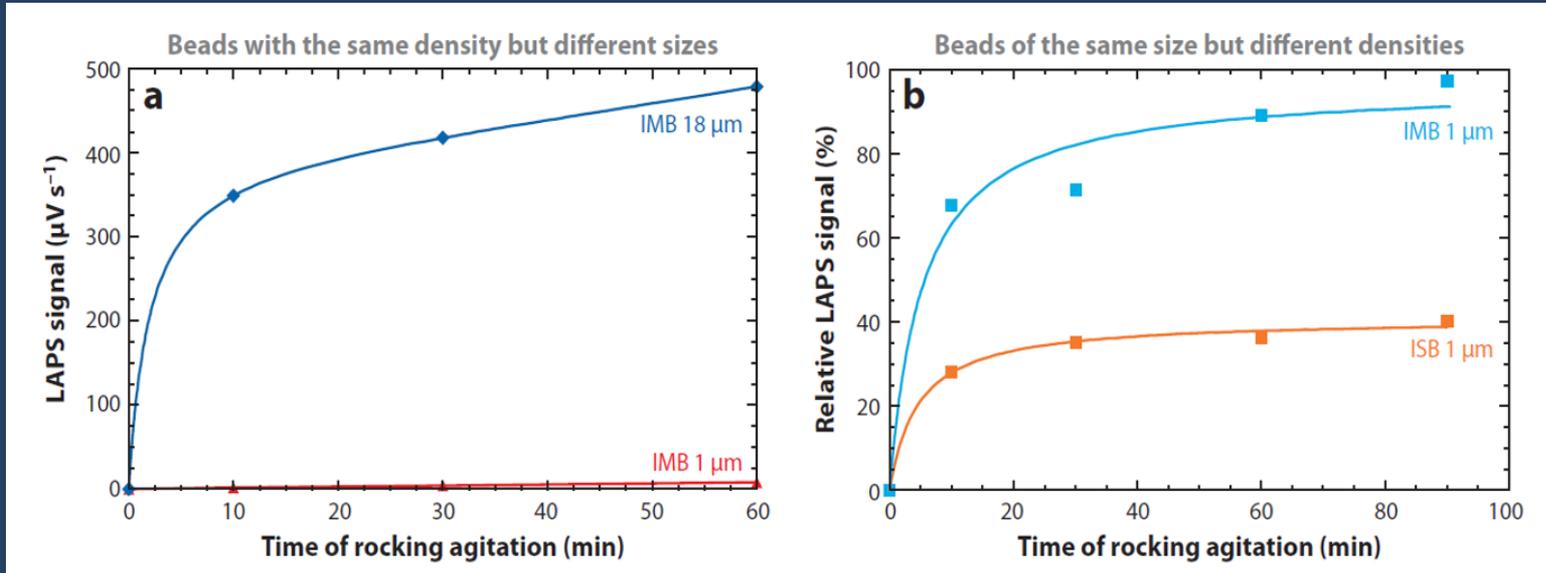
Dynabeads
1-5 micrometers

Particle Cost Becomes Prohibitive for Querying Large Volumes



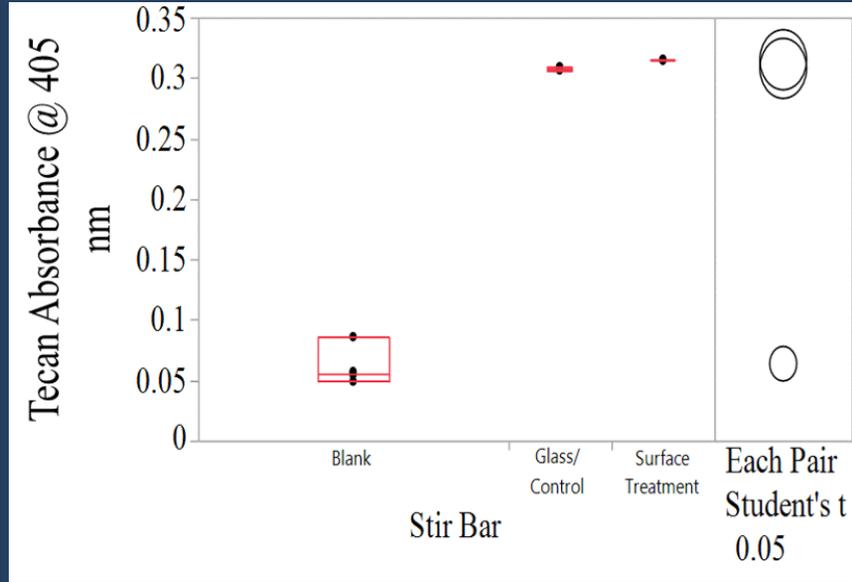
Kit	Surface Coating	Cost	Bead volume per kit	Volume used per test	Sample size	Query volume per kit
DynaBeads	Protein A	\$192	1 ml	50 μ L	100-1000 μ L	2 - 20 mL
DynaBeads	Protein G	\$192	1 ml	50 μ L	100-1000 μ L	2 - 20 mL
MagnaBind	Streptavidin	\$393	5 mL	1 mL	500-1500 μ L	2.5 -7.5 mL
Thermo	Ni-NTA	\$212	2 mL	40 μ L	400 μ L	20 mL
DynaBeads	Sheep anti-Rabbit IgG	\$419	2 mL	50 μ L	25 μ g target antigen	1000 μ g
DynaBeads	anti- <i>E. coli</i> O157	\$239	1 mL	50 μ L	100-1000 μ L	2 - 20 mL

Varying Bead Size/Density Alters Their Capture Efficiency



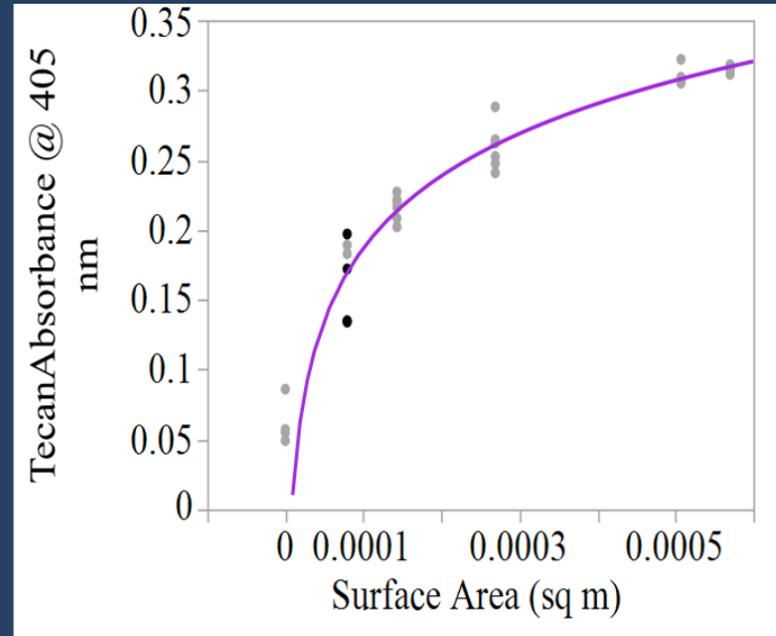
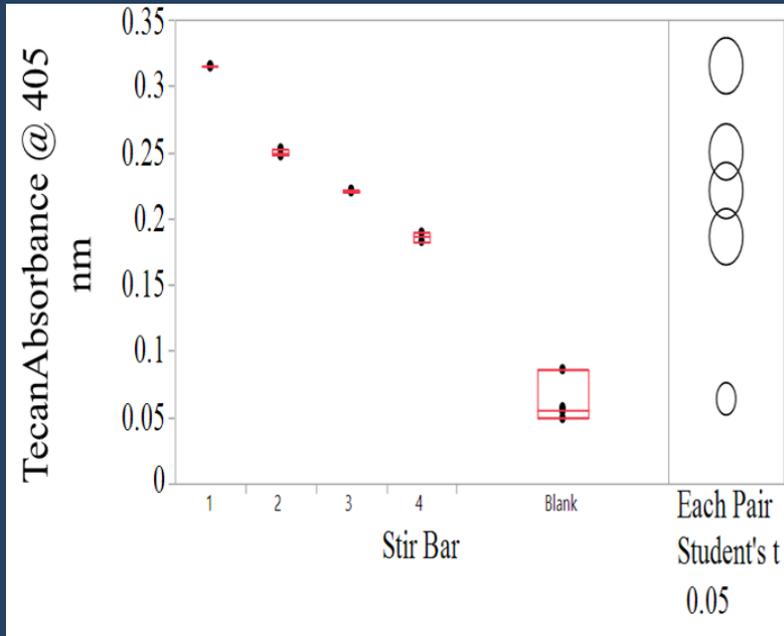
Tu et al., 2003

Novel Surface Treatment Allows Antibody Conjugation



- Anti-*E. coli* (HRP)
- Add tetramethylbenzidine
- Measure absorbance

Number of Antibodies Directly Correlated With Total Surface Area of Particles





Protocol for Ground Beef Samples



Add *E. coli* O157:H7-PC to ground beef



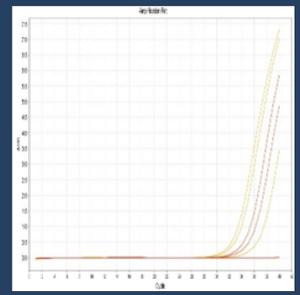
Stomach (2 min) and pour/pipet sample from bag



Add capture devices (cost ~\$0.15-0.75) and spin (10 min)



Remove capture devices, wash, and lyse sample (12 min)

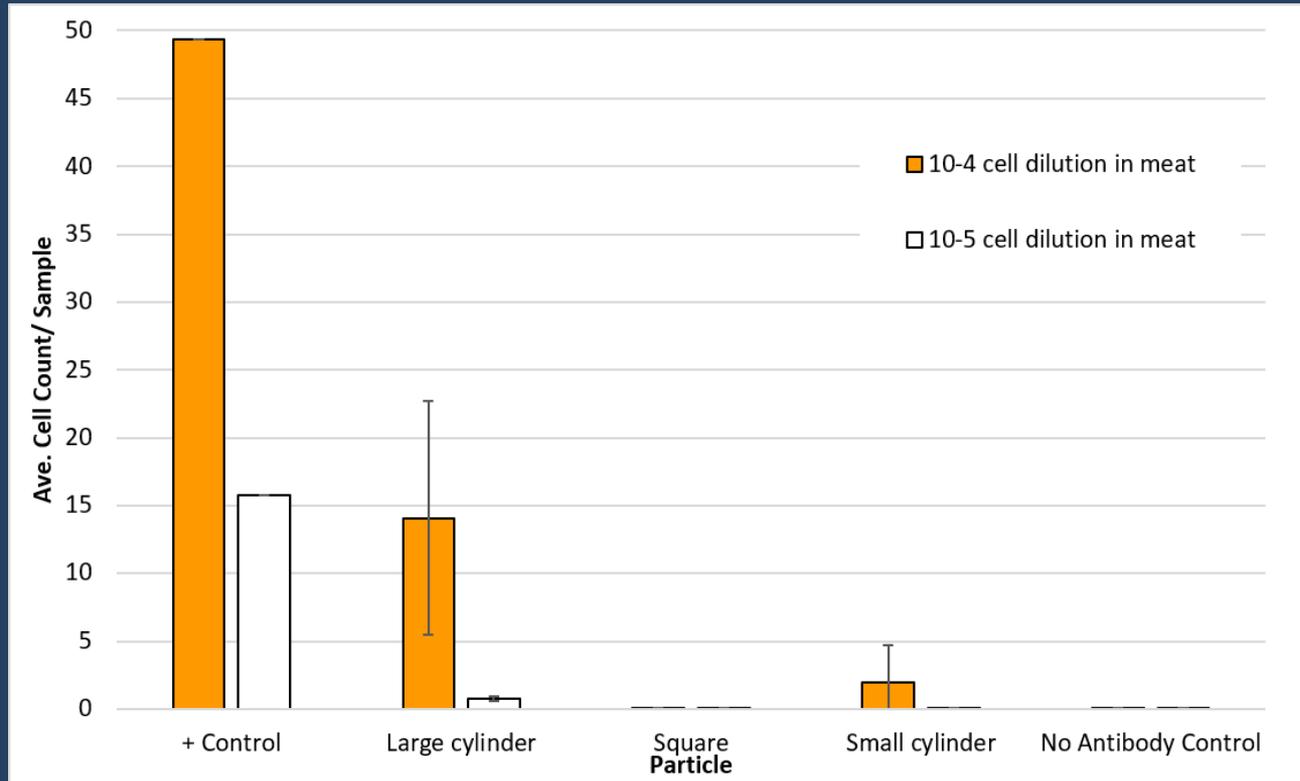


Amplify barcode via PCR (60 min)

Total time = 2 + 10 + 2 + 10 + 60 = 84 min



E. coli Cell Capture in Ground Beef Using Different Particle Sizes/Geometries



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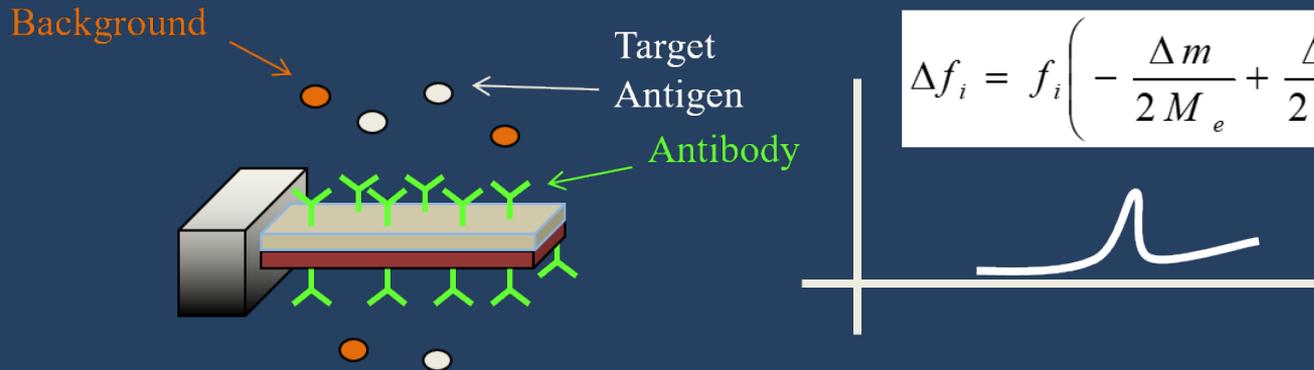
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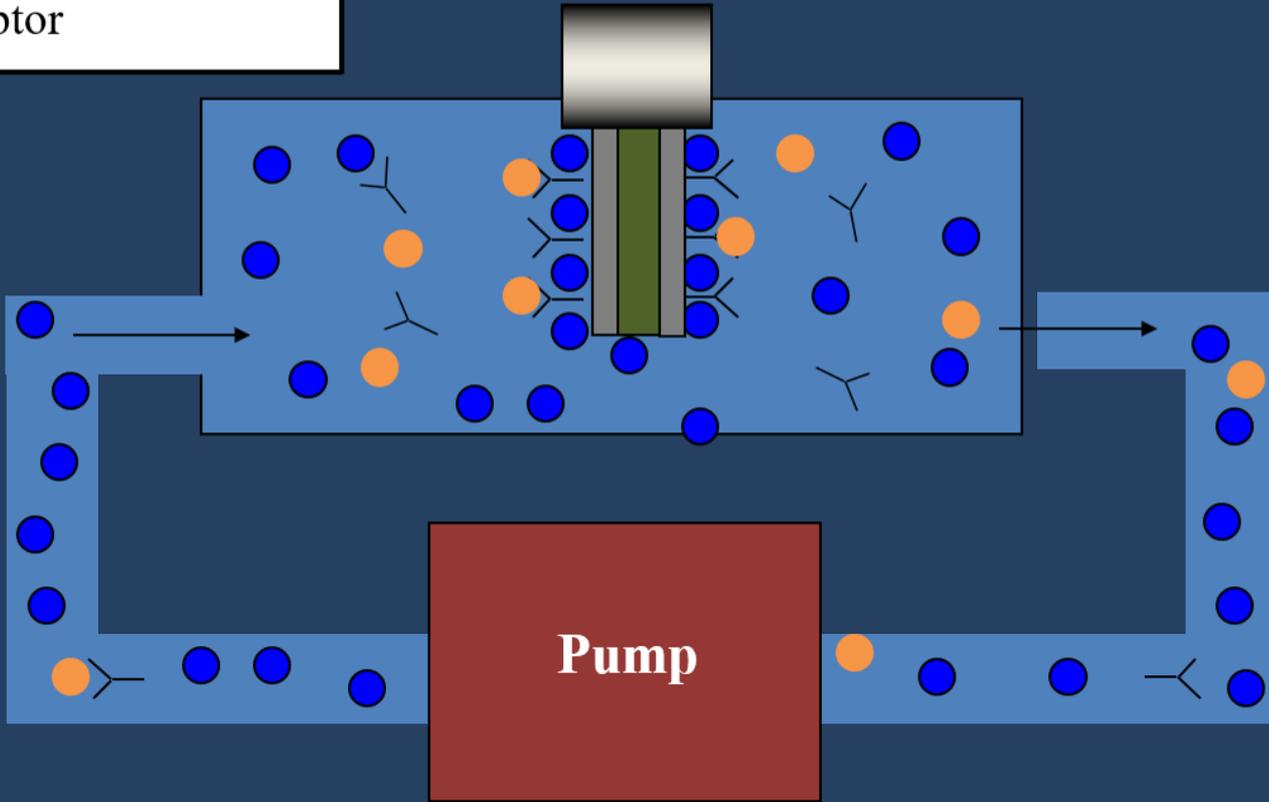
Piezoelectric Membrane Biosensor Overview

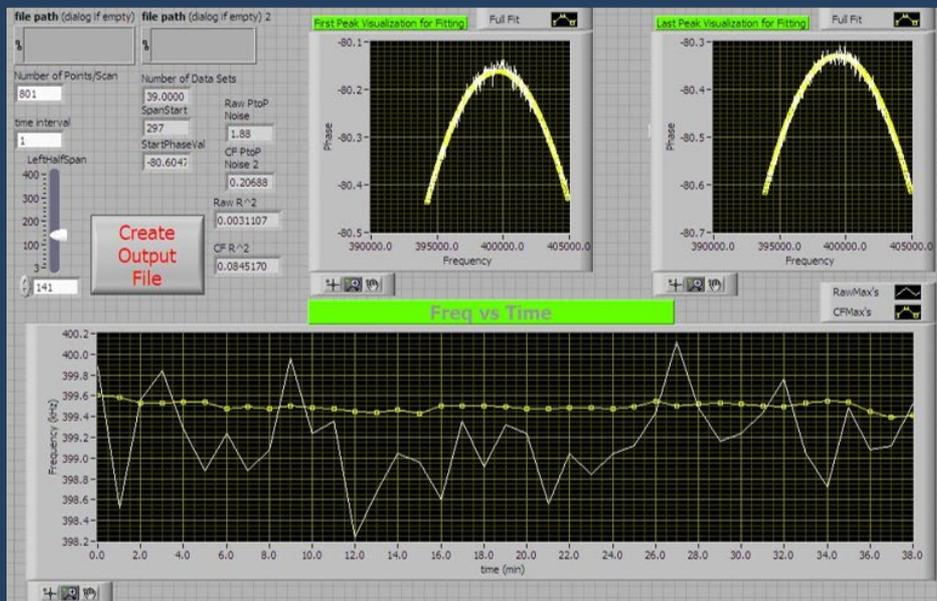
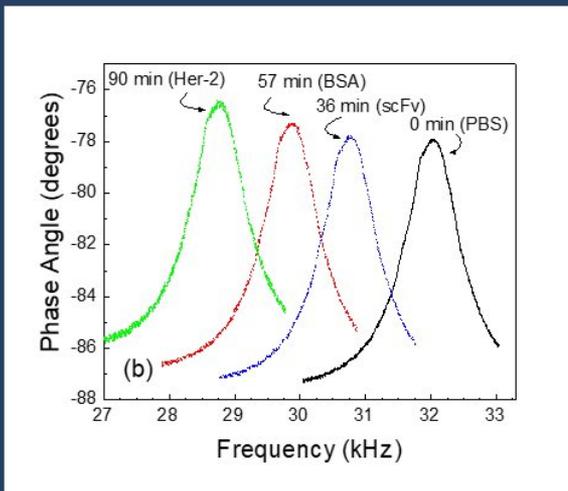
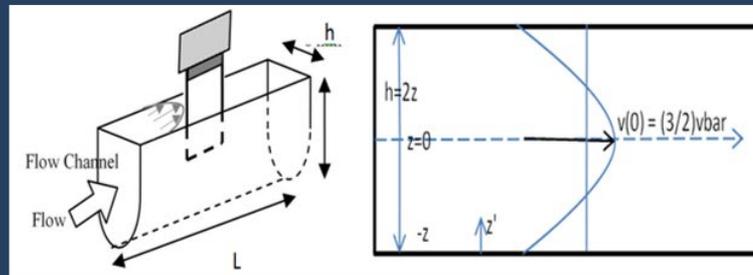
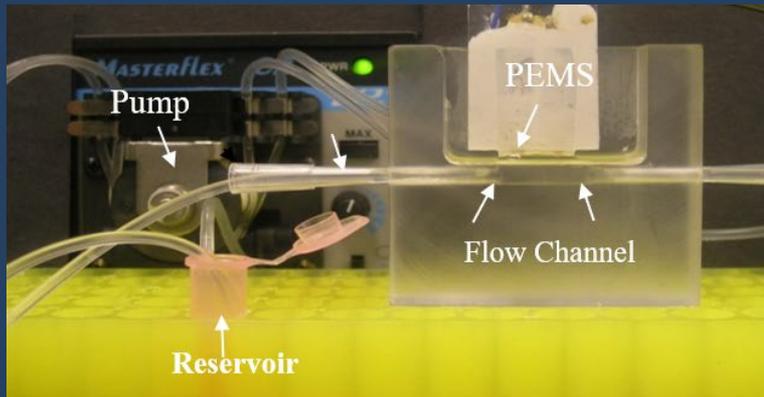
- Antibodies are immobilized on the biosensors to provide selectivity.
- Resonance frequency (f_i) of the biosensors shift (Δf_i) upon antigen attachment due to a change in mass (Δm) and spring constant (Δk) of the cantilever .
- The quantity of the attached antigens can be calculated using calibration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

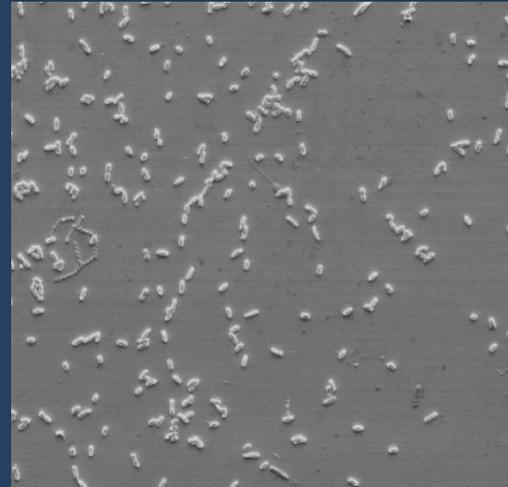
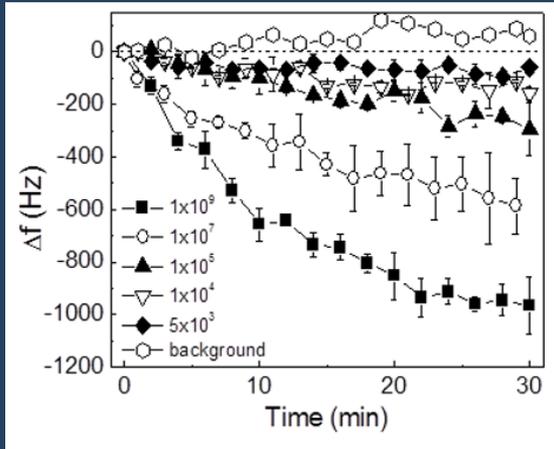
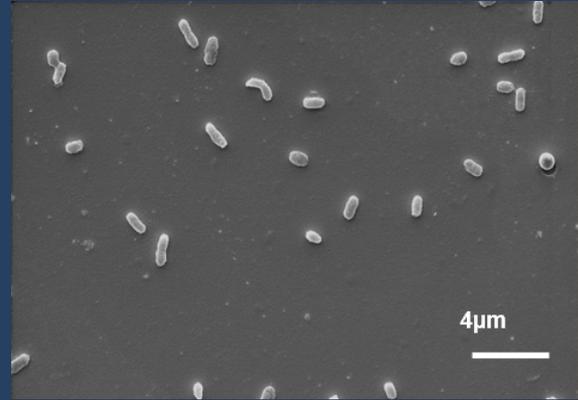
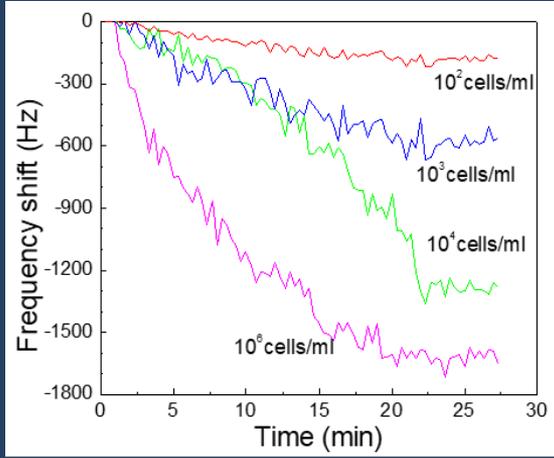




- Background/Blocking
- Target
- Y Receptor





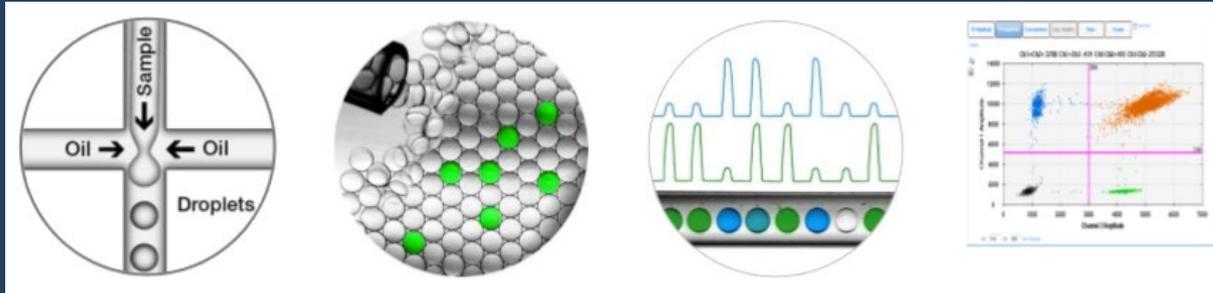


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Droplet Digital PCR (ddPCR)

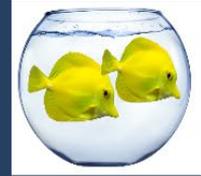


- Each droplet is an independent end-point assay
- Positive and Negative drops are counted
 - Data is absolute quantification
 - Does not require standard curve

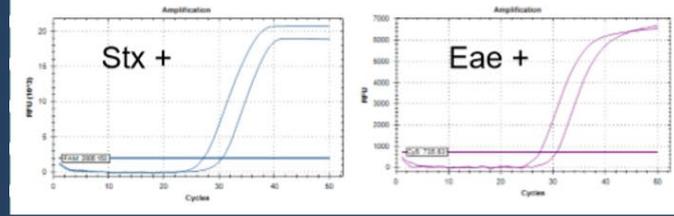
ddPCR – Power is in Partitioning



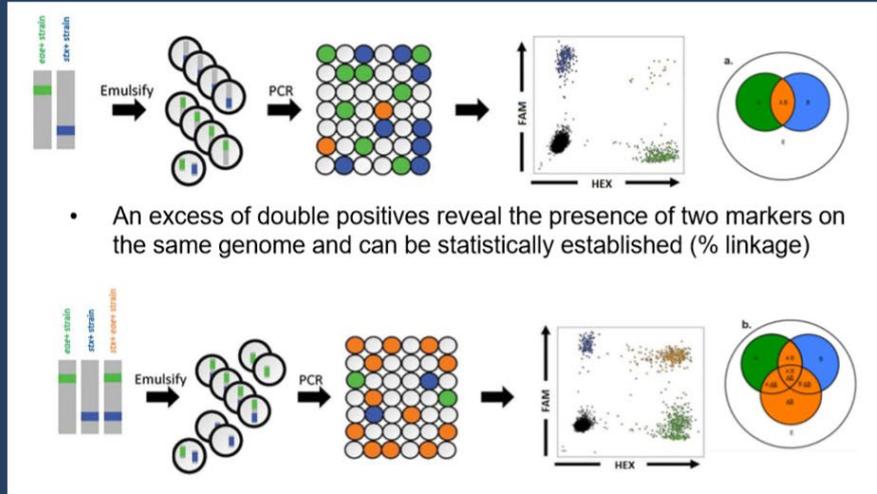
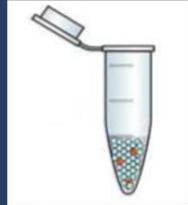
Dilute
Background



qPCR

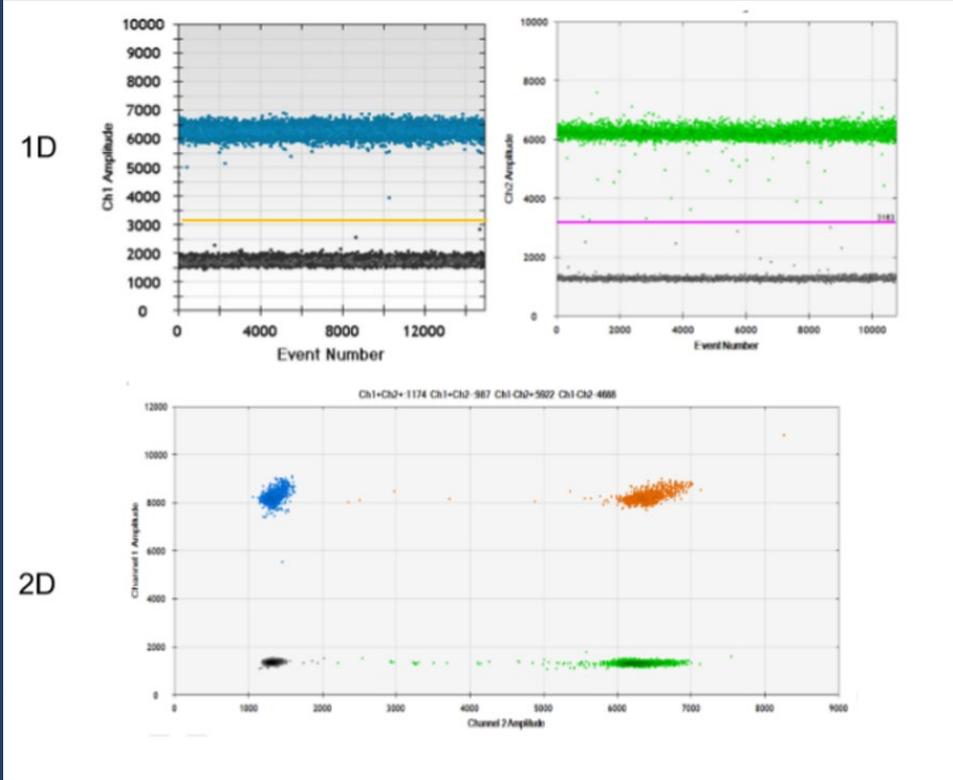


ddPCR





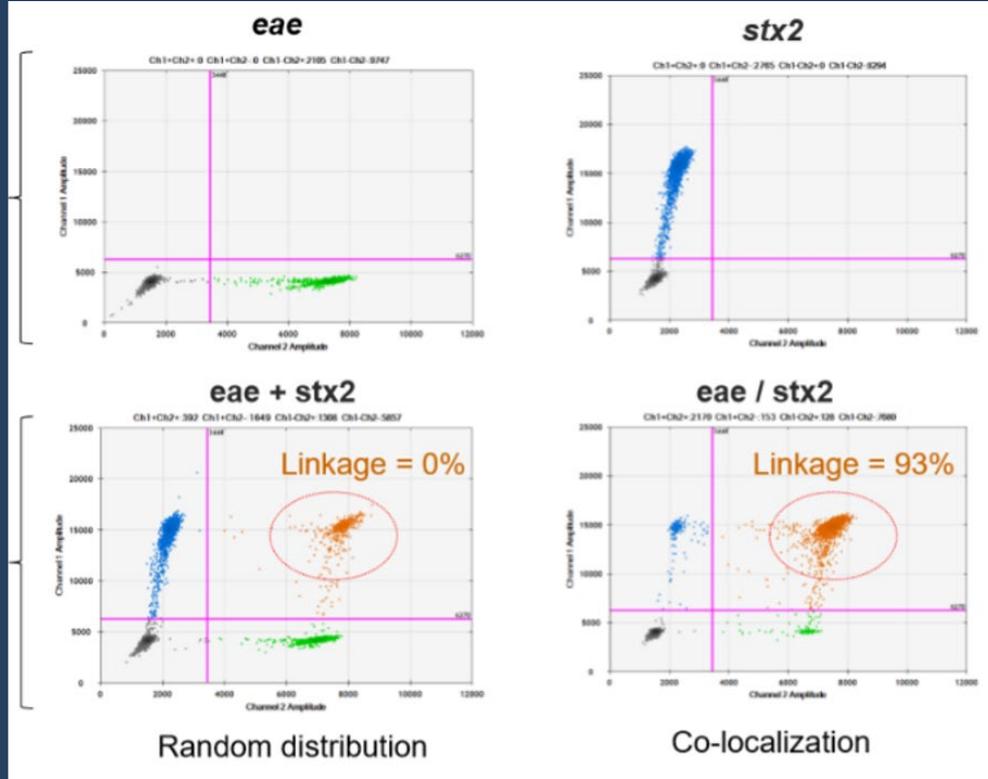
Droplet Count Data





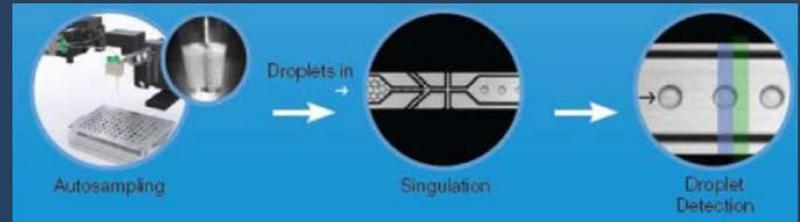
Droplet Count Data (2)

(single target strains)



(multiple target strains)

ddPCR's Simple Workflow



Droplet Analysis - Linkage



Data interpretation category

Target detection	N/A	No results
	Not enough droplet	Not enough droplet for the analysis. Please perform again the ddPCR test.
	Negative	Stx and eae targets are not detected in the sample
	Simple positive stx	Stx1 or 2 are detected in the sample
	Simple positive eae	Eae are detected in the sample
	Double positives	Both stx and eae are detected in the sample
Linkage interpretation (for double positives only)	No interpretation (low target amount)	Interpretation not possible due to low target concentration (< 10 ⁴ CFU/ml). Perform again the experiment by duplicating the number of well.
	Sample to be diluted	The target is too concentrated for the analysis. Perform again the testing by diluting 1/10 and 1/100 in BPW.
	Unlinked positives	Both stx and eae are detected but are not colocalized on the same bacteria
	Linked positives	Stx and eae showed positive linkage due to a colocalisation on the same genome.

Well	Sample	Quasasoft data							Analysis				
		stx1/2 cp/well (FAM)	FAM+ HEX+ (N(A+B))	FAM+ HEX- (NA)	FAM- HEX+ (NB)	FAM- HEX- (NE)	Accepted Droplets (Ntot)	eae cp/well (HEX)	Ratio stx/eae	% <u>AB</u>	Target detection	Linkage interpretation	Needed dilution
A01	Meat Control - No Inoculation	3.6	0	2	0	13141	13143	0			Negative	N/A	
E01	O1013:H2 + O45	20000000	11929	0	0	0	11929	2E+07			double positives	Sample to be diluted	1000.0
C02	O45:H2 05-6545	4040	1448	308	153	9204	11113	3660	1.1	84.9	double positives	Linked positives	
E02	O103:H2 5.1688 + O45 NRRL B59812	2000	131	1062	1553	11858	14604	2880	0.7	-0.5	double positives	Unlinked positives	
B01	CA22 + CA25 + CA44 (O111++) (O121eae+) (O103	20000000	7317	0	0	0	7317	2E+07			Not enough droplet	N/A	
A02	O121:H19 3056-85 + O141:426 II9	2.8	0	2	421	16896	17319	580	0.0	0.0	Simple positive eae	N/A	
C02	O177:H25 DD4	20	13	1	0	16784	16798	18	1.1	96.3	double positives	No interpretation (low target amount)	
E02	O45:H2 5.0623 DebRoy + O45:H2 1.2622 DebRoy	790	0	520	0	15211	15731	0			Simple positive stx	N/A	
F01	O121:H19 3056-85 + O174:H8 AA1	502	5	304	154	14153	14616	258	1.9	0.7	double positives	Unlinked positives	
C02	O177:H25 DD4	290	131	27	6	12755	12919	250	1.2	88.7	double positives	Linked positives	

STEC inclusivity/exclusivity assay



- Evaluated 37 unique strains of *E.coli* in PBS
 - 25 regulated O-groups; 12 nonregulated O-groups
 - 16 *stx1*, 19 *stx2*, and 9 *stx*⁻
 - 26 *eae*⁺ and 11 *eae*⁻
- ddPCR can identify *eae* and all of the *stx* subtypes, with the exception of *stx2f*

Spiked Sample Experiment Design



Expected ddPCR Result	Expected qPCR result	Strain description
Negative	Negative	Meat Sample – Not Spiked
Unlinked positives	Potential (+) with O-groups (+)	Mix of two strains simple positive for <i>stx1/2</i> or <i>eae</i> with at least one of them coming from a 7 major O-groups (O157:H7, O26, O111, O103, O121, O45, and O145)
Unlinked positives	<i>stx/eae</i> (+) with O-groups (-)	Mix of two strains simple positive for <i>stx1/2</i> or <i>eae</i> with both of them coming from other O-groups
Linked positives	Potential (+) with O-groups (+)	Strains containing <i>stx1</i> or <i>stx2</i> (different variant) and <i>eae</i> genes from the 7 major O-groups (O157:H7, O26, O111, O103, O121, O45, and O145)
Linked positives	<i>stx/eae</i> (+) with O-groups (-)	Strains containing <i>stx1</i> or <i>stx2</i> (different variants) and <i>eae</i> genes from other O-groups



Sample Enrichment

Inoculate aseptically prepared ground beef sample

Sample incubated in BPW + STEC supplement (1:3)

Screening

MLG protocol

DNA Extraction

BAX® STEC PCR (*stx*, *eae*)

BAX® STEC Panel 1/ Panel 2

Bio-Rad iQCheck® method for STEC

DNA Extraction

iQCheck® VirX PCR (*stx*, *eae*)

iQCheck® SerO PCR (O-groups)

Bio-Rad ddPCR STEC method

Sample purification

ddPCR STEC PCR (*stx*, *eae*)

Confirmation of Potential Positives (MLG 5)

IMS and microbiological confirmation on isolated colonies

Identify double positives *stx* and *eae* confirmed isolated colonies

PCR Screening Results From Beef



Sample	# Tested	BAX		IQ Check		dd PCR	
		Potential Positive	Confirmed Positive	Potential Positive	Confirmed Positive	Potential Positive	Confirmed Positive
Linked (eae, stx) Regulated O- group	15	15	15	15	15	15	15
Linked (eae, stx) Nonregulated O-group	7	0	0	1	0	7	7
Unlinked (eae, stx) Regulated O-group	11	9	0	11	0	0	0
Negative	7	0	0	0	0	0	0

ddPCR identified double positives
 ← ddPCR would reduce unlinked identified as PP

Sample	BAX									IQ Check								dd PCR						
	Screen		Panel 1			Panel 2			O157		VirX		SerO1		SerO2		SerO3		stx1/2		eae		Result	linkage
	stx (Ct)	eae (Ct)	O26 (Ct)	O111 (Ct)	O121 (Ct)	O45 (Ct)	O103 (Ct)	O145 (Ct)	S (Ct)	W (Ct)	eae (Ct)	stx (Ct)	O157 (Ct)	O111 (Ct)	O26 (Ct)	O103 (Ct)	O145 (Ct)	O45 (Ct)	O121 (Ct)	stx1/2 (cp/well)	eae (cp/well)			
O26 (stx,eae) + O45 (-,eae)	33.6	33.4	37.0	0.0	0.0	33.7	0.0	0.0	0.0	0.0	20.0	22.6	0.0	0.0	20.8	0.0	0.0	20.5	0.0	1840.0	3700.0	double positives	Linked positives	
O111 (stx, eae) + O121 (-,eae) + O103 (stx, -)	31.0	30.4	0.0	33.9	36.1	0.0	33.7	0.0	0.0	0.0	18.8	18.9	0.0	19.1	0.0	20.2	20.2	0.0	21.7	1100.0	864.0	double positives	Linked positives	
O55 (stx, eae) + O121 (-)	33.6	32.3	0.0	0.0	32.9	0.0	0.0	0.0	0.0	0.0	20.2	22.7	0.0	0.0	0.0	0.0	0.0	0.0	19.3	1960.0	2580.0	double positives	Linked positives	
O121 (-)	0.0	0.0	0.0	0.0	33.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.2	2.0	4.0	Negative	N/A	
O51:H49 * Clinical Isolate	33.3	25.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.9	24.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4300	4780	double positives	Linked positives	
Not inoculated	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Negative	N/A	

Advantages of ddPCR



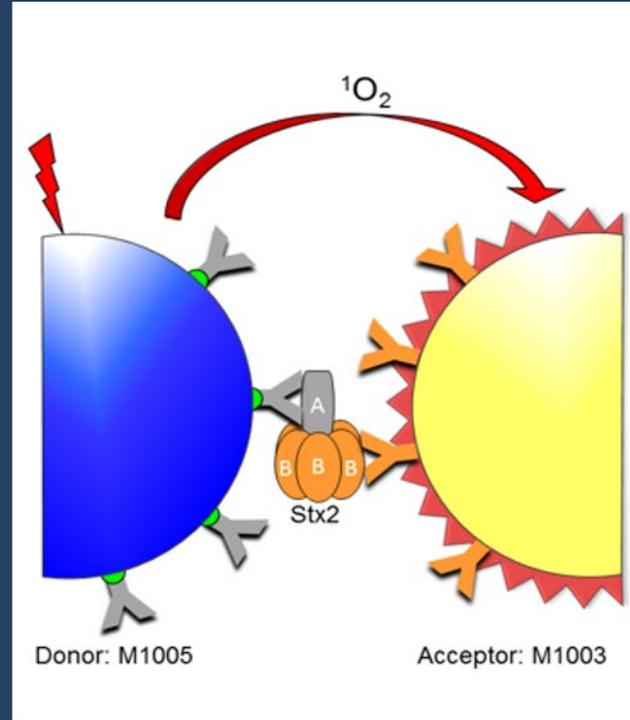
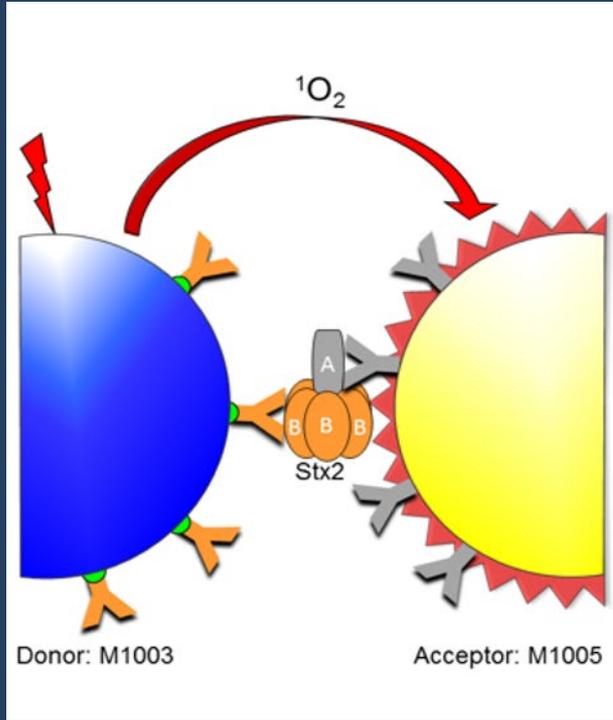
- Absolute quantitation without the need for a calibration curve
- Apparently equal accuracy yet better repeatability and a greater tolerance for inhibitors than qPCR
- Unambiguous identification of microorganism in one single amplification run (co-localization of markers)
- Detection/enumeration of living cells
- In the food domain, successfully applied to GMOs, viruses & bacteria quantification

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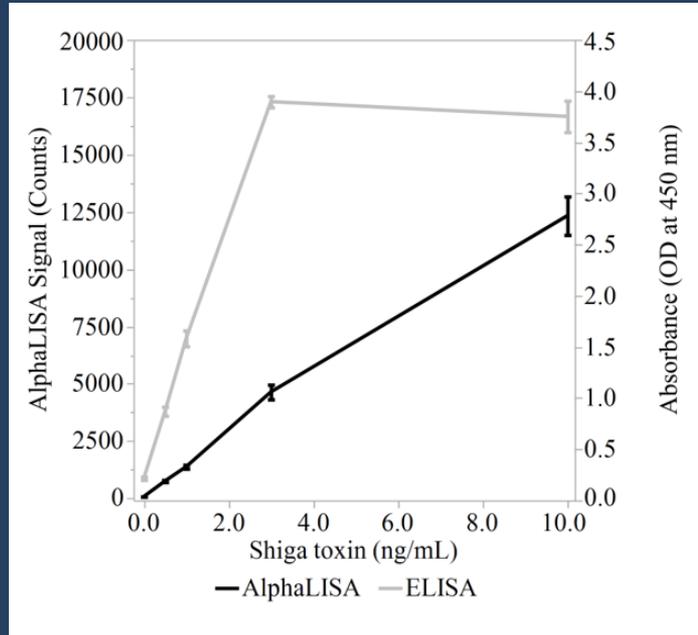


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Shiga-toxin Specific AlphaLISA



Purified Stx2 detected using the AlphaLISA and the ELISA



Comparisons of the limit of detection and signal-to-noise ratios of the AlphaLISA versus the ELISA

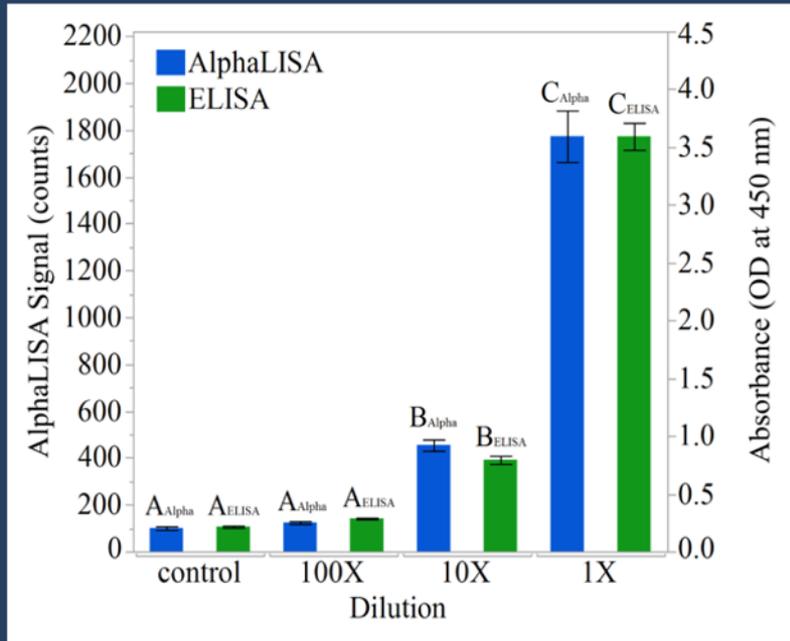


Assay	Average zero signal	3X SD	LOD (ng/ml)	Signal to Noise ratio 0.5 ng/mL	Signal to Noise ratio 1 ng/mL	Signal to Noise ratio 3 ng/mL	Signal to Noise ratio 10 ng/mL
AlphaLISA A	75.92	63.285	0.100	10	18	61	163
ELISA	0.21767	0.1665	0.143	4	7	18	17

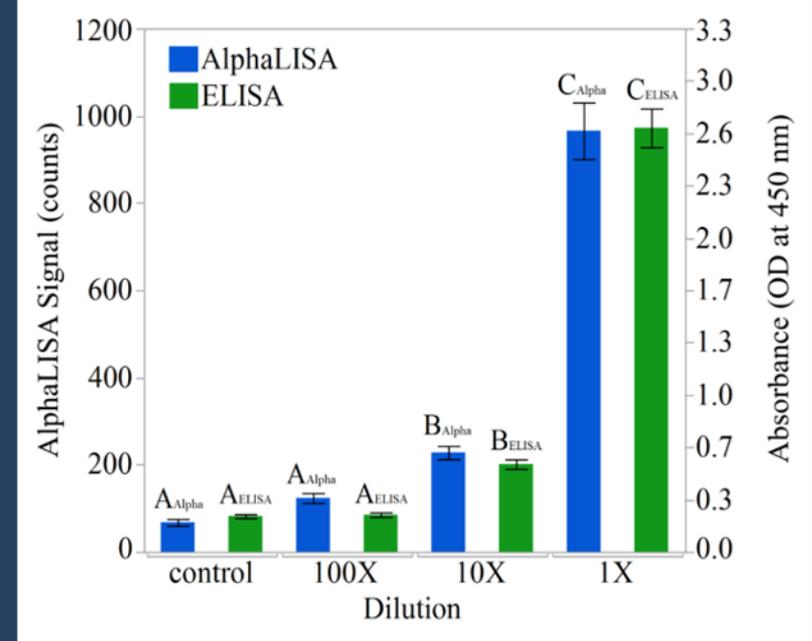


Detection of Stx2 in STEC-inoculated foods using the AlphaLISA and ELISA

A) Romaine Lettuce

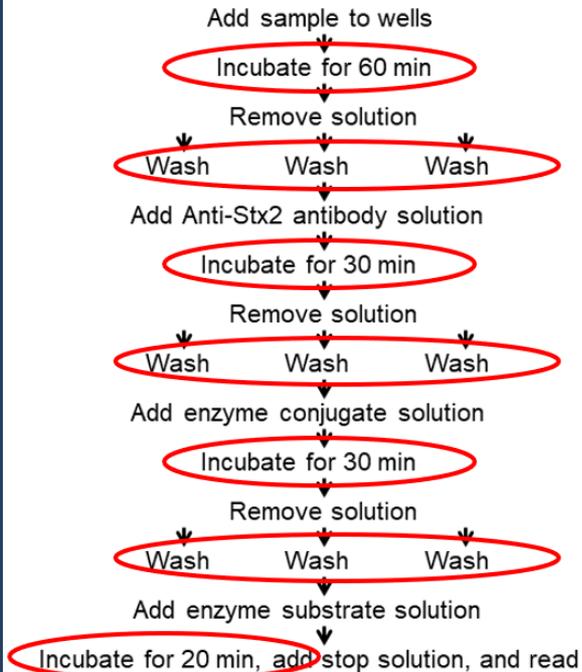


B) Ground Beef

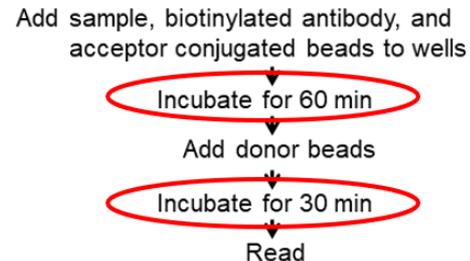


AlphaLISA Protocol is Shorter and Simpler

ELISA



AlphaLISA



Advantages of the AlphaLISA



- Faster
- Highly automatable- Less manual manipulation
- Larger dynamic range
- Recognizes intact toxin

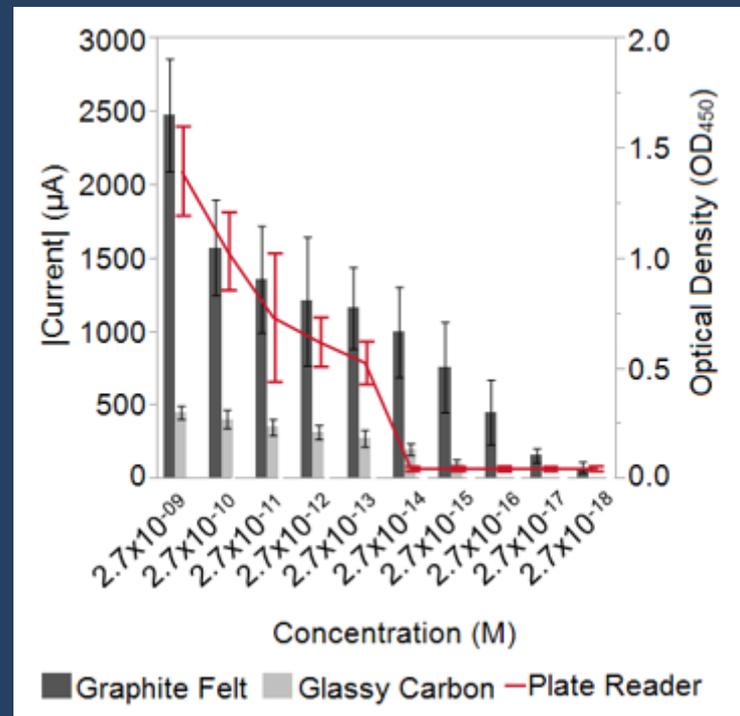
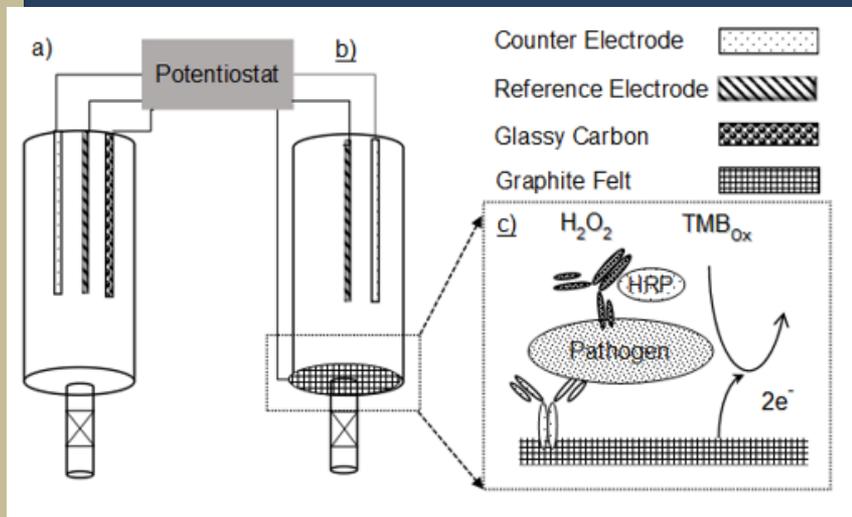
Objective 2: Develop and validate field testing kits that rapidly screen for the presence and quantification of pathogens and/or indicator microorganisms in foods at the initial processing level. (5)



- Subobjective 2A. Generate portable, label-free sensors (e.g., next generation cantilever microbalance) for rapid in-line or near-line screening of foods.
- Subobjective 2B. Generate portable antibody and/or phage-based multiplex assays including integrated comprehensive droplet digital detection (IC 3D).
- Subobjective 2C. Develop an AlphaLISA detection protocol for target pathogens.
- **Subobjective 2D. Develop a flow-through immunoelectrochemical detection device for field portable detection of target pathogens**

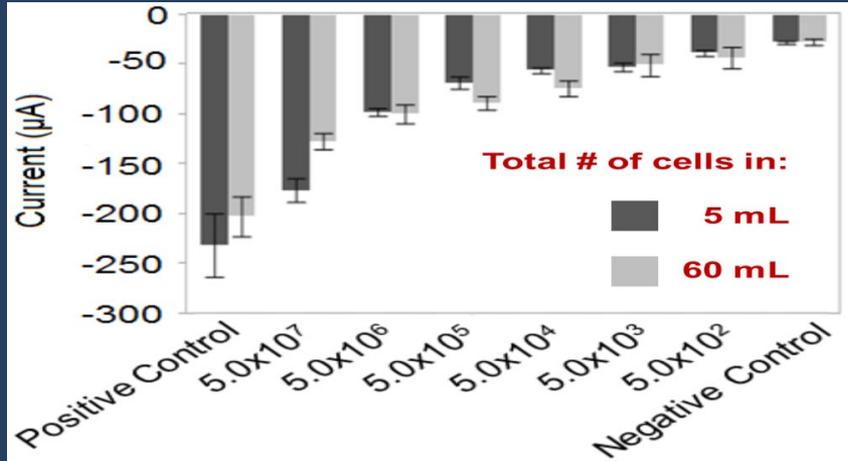


Flow Through Electrochemical Sensor

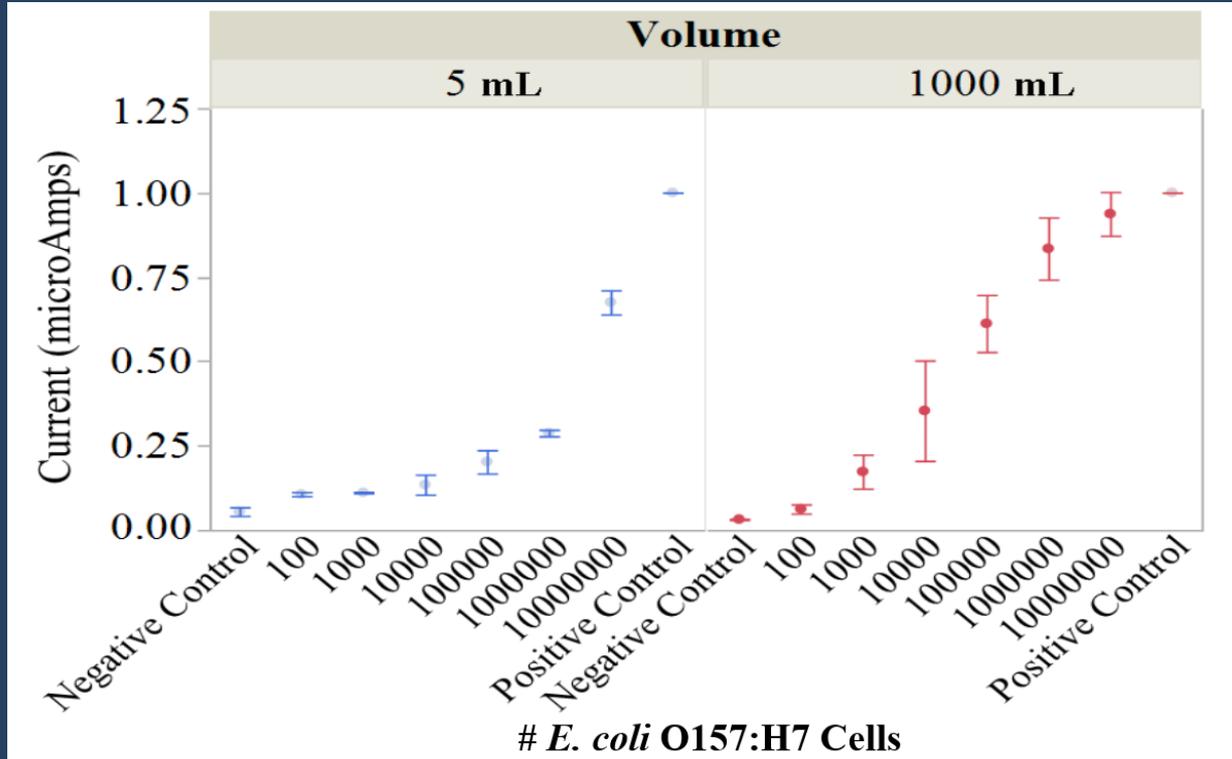




Flow-through Immunochemical Detection of *Salmonella*

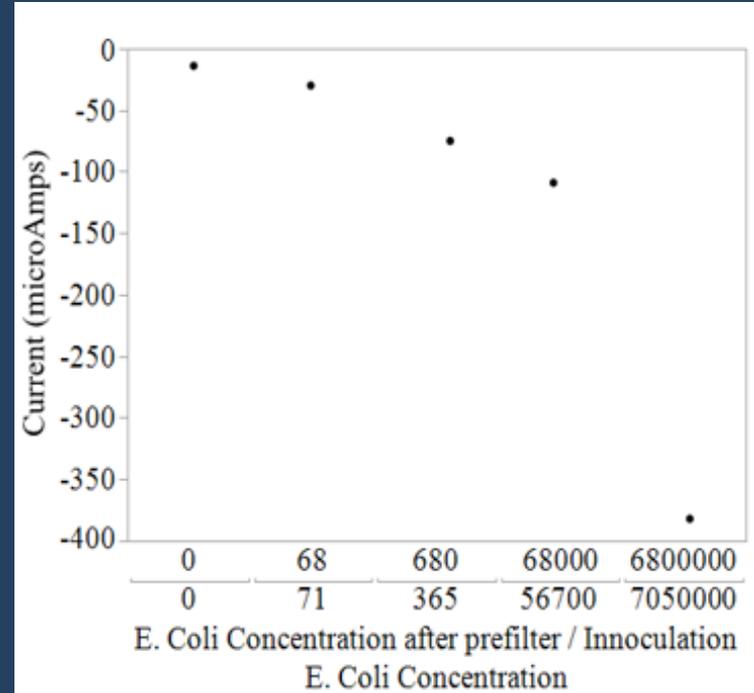
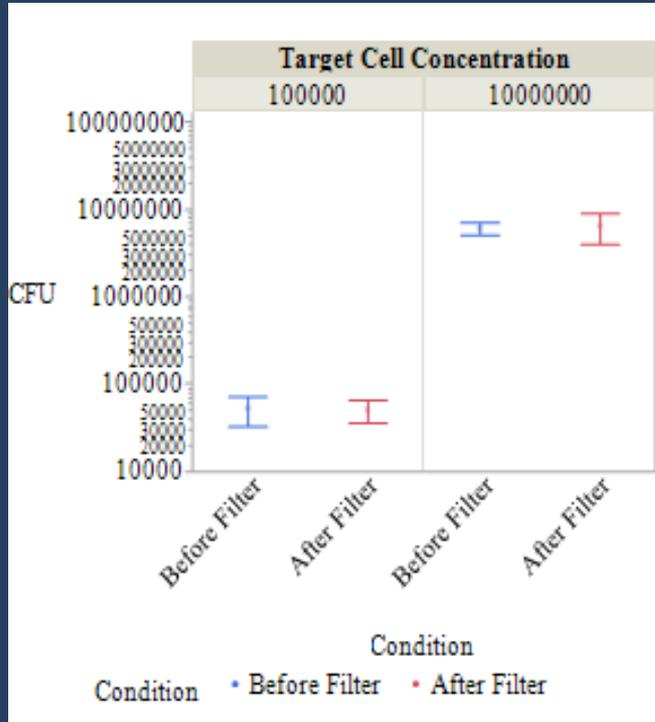


Normalized Immunochemical Response



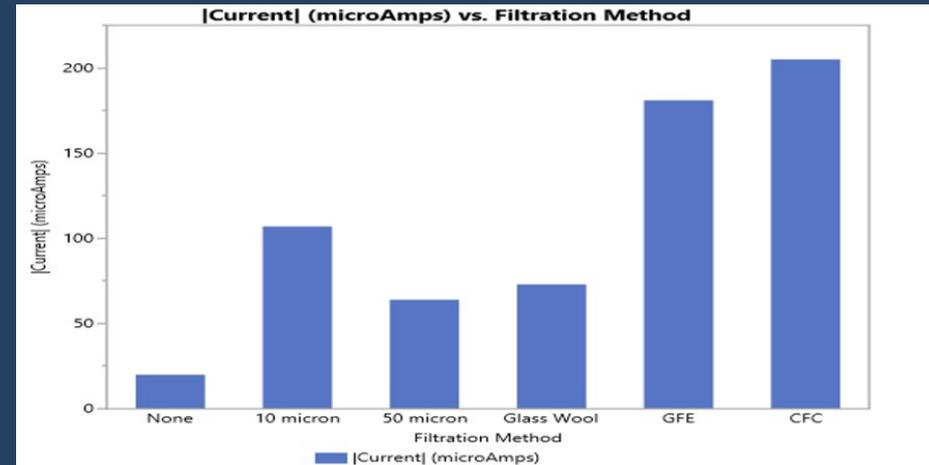


E. coli O157:H7 Detection in Food (25 g Ground Beef/75 mL of Growth Media)



Electrochemical detection of HRP/TMB in filtered ground beef (325 g/1 L)

Filtration	Flow rate (mL/min)	Clog?
<u>Glass Wool</u>	<u>6.1</u>	<u>Flow stopped</u>
<u>CFC</u>	<u>5.3</u>	<u>No</u>
<u>50 micron</u>	<u>4.1</u>	<u>Flow stopped</u>
<u>10 micron</u>	<u>6.1</u>	<u>Flow stopped</u>



Objective 3: Develop and validate rapid methods for the identification of pathogens and/or indicator microorganisms in foods for application in either the field or testing laboratories. (2)



- **Subobjective 3A. Generate phage and/or antibody typing arrays.**
- Subobjective 3B. Generate pathogen databases and improve the accuracy of the Beam (formerly BActerial Rapid Detection using Optical scattering Technology or BARDOT) system.
- Subobjective 3C. Direct typing (colony isolates not required) of enriched samples using a targeted-sequencing method.
- Subobjective 3D. Generate genome sequence-based typing and identification schemes using next-generation sequencing technology (e.g., MiSeq, Ion Torrent PGM, and MinION), and characterize virulence and antibiotic resistance of microbial pathogens.

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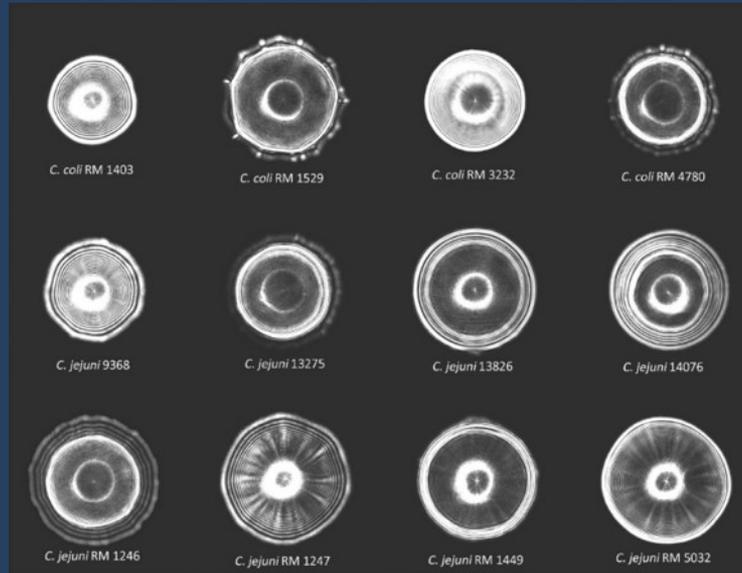
Identification of *Campylobacter* using BARDOT system

Bacteria Rapid Detection using Optical scattering Technology

- Applies a laser to a bacterial colony on an agar plate
- Capture light scattering images by a CCD chip
- Identify *Campylobacter* colonies using image processing and scatter pattern analysis software



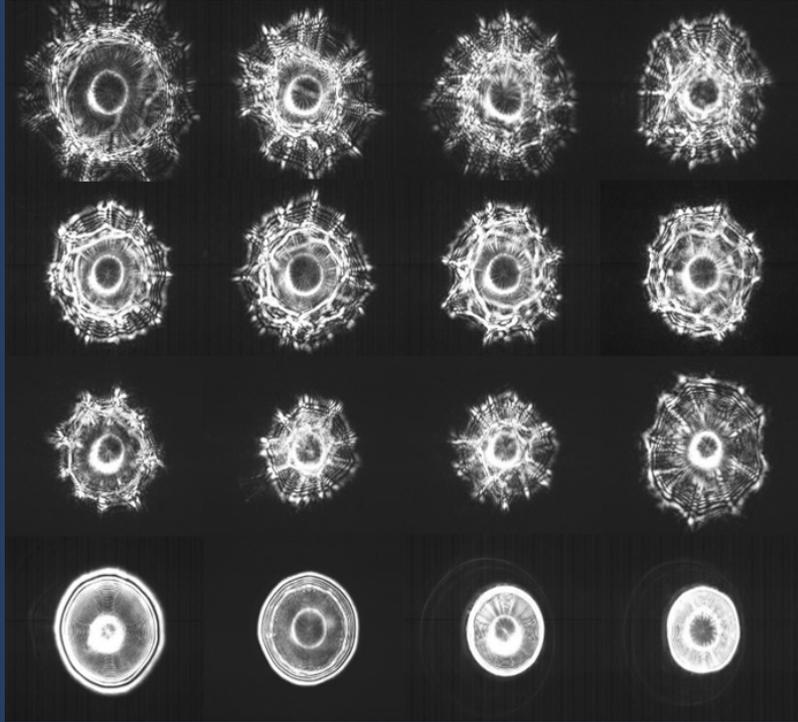
Light scattering patterns of *C. jejuni* & *C. coli* colonies from pure culture



A total of 2248 *Campylobacter* scatter images were collected from 17 strains of *C. jejuni* and 9 strains of *C. coli* grown on Brucella agar.

A *Campylobacter* library was built upon BARDOT scanning 50-100 colonies of each strain.

Light scattering patterns of major foodborne pathogens



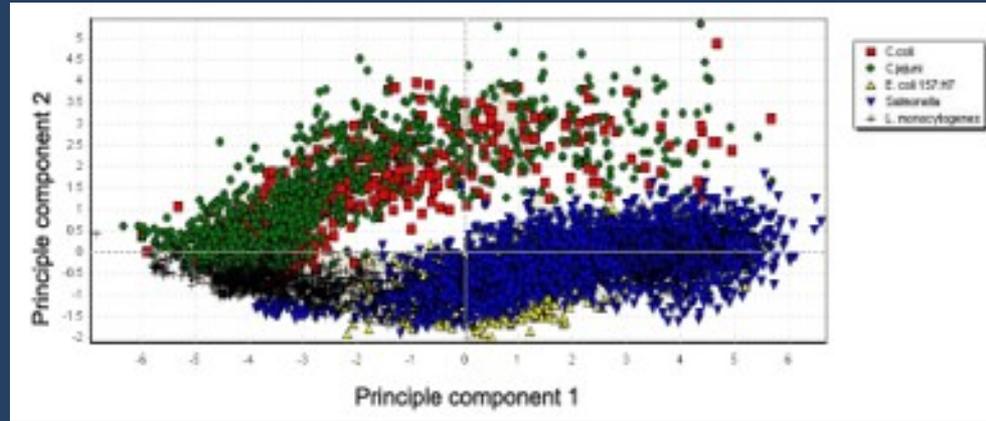
E. coli O157:H7

Salmonella spp.

L. monocytogenes

C. jejuni & *C. coli*

Principle component analysis of bacterial scatter images in *Campylobacter* library



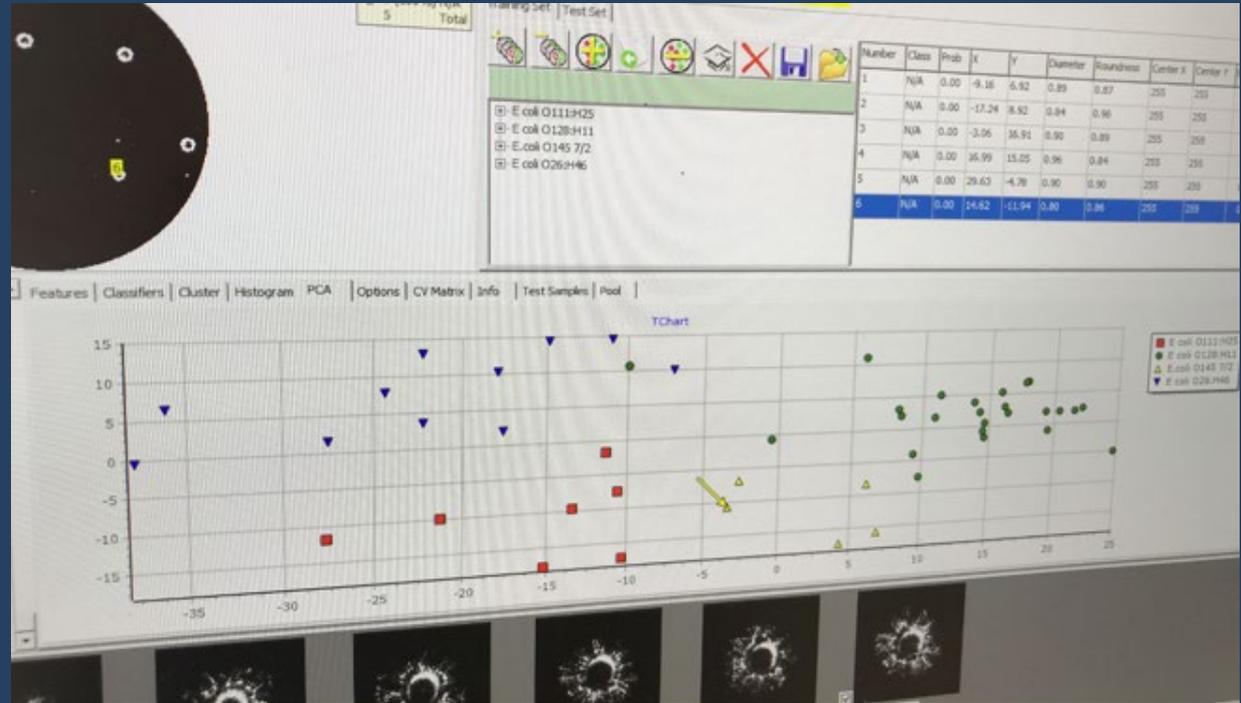
	<i>C. coli</i>	<i>C. jejuni</i>	<i>E. coli</i> O157:H7	<i>Salmonella</i> spp.	<i>L. monocytogenes</i>
<i>C. coli</i>	79.2	20.3	0.3	0.1	0.2
<i>C. jejuni</i>	15.1	84	0.1	0.4	0.5
<i>E. coli</i> O157:H7	0.1	0	90.1	9.2	0.5
<i>Salmonella</i> spp.	0	0	4	94.8	1.1
<i>L. monocytogenes</i>	0.1	0.3	2.8	6.8	90



BARDOT/BEAM Collaboration with Lincoln and Purdue Universities

PCA-*E. coli* Serotypes

- O111:H25
- O128:H11
- O145
- O26:H46





CV Matrix- *E. coli* serotypes

	O111:H25	O128:H11	O145	O26:H46
O111:H25	100	0	0	0
O128:H11	0	93.8	2.2	4
O145	0	0	100	0
O26:H46	0	5.1	0	94.9



CV Matrix- *Yersinia* spp.

	Y.enterocolitica 22h 5/2	Y.pseudotuberculosis 22h 5/2	Y.frederiksenii 22h 5/2	Y.intermedia 22h 5/2	Y.kristensenii 22h 5/2
Y.enterocolitica 22h 5/2	99.5	0.1	0.4	0	0
Y.pseudotuberculosis 22h 5/2	3.9	94.4	0	0	1.7
Y.frederiksenii 22h 5/2	6.4	0	92.1	1.5	0
Y.intermedia 22h 5/2	3.7	0	0.9	95.4	0
Y.kristensenii 22h 5/2	7.1	0	0	0	92.9

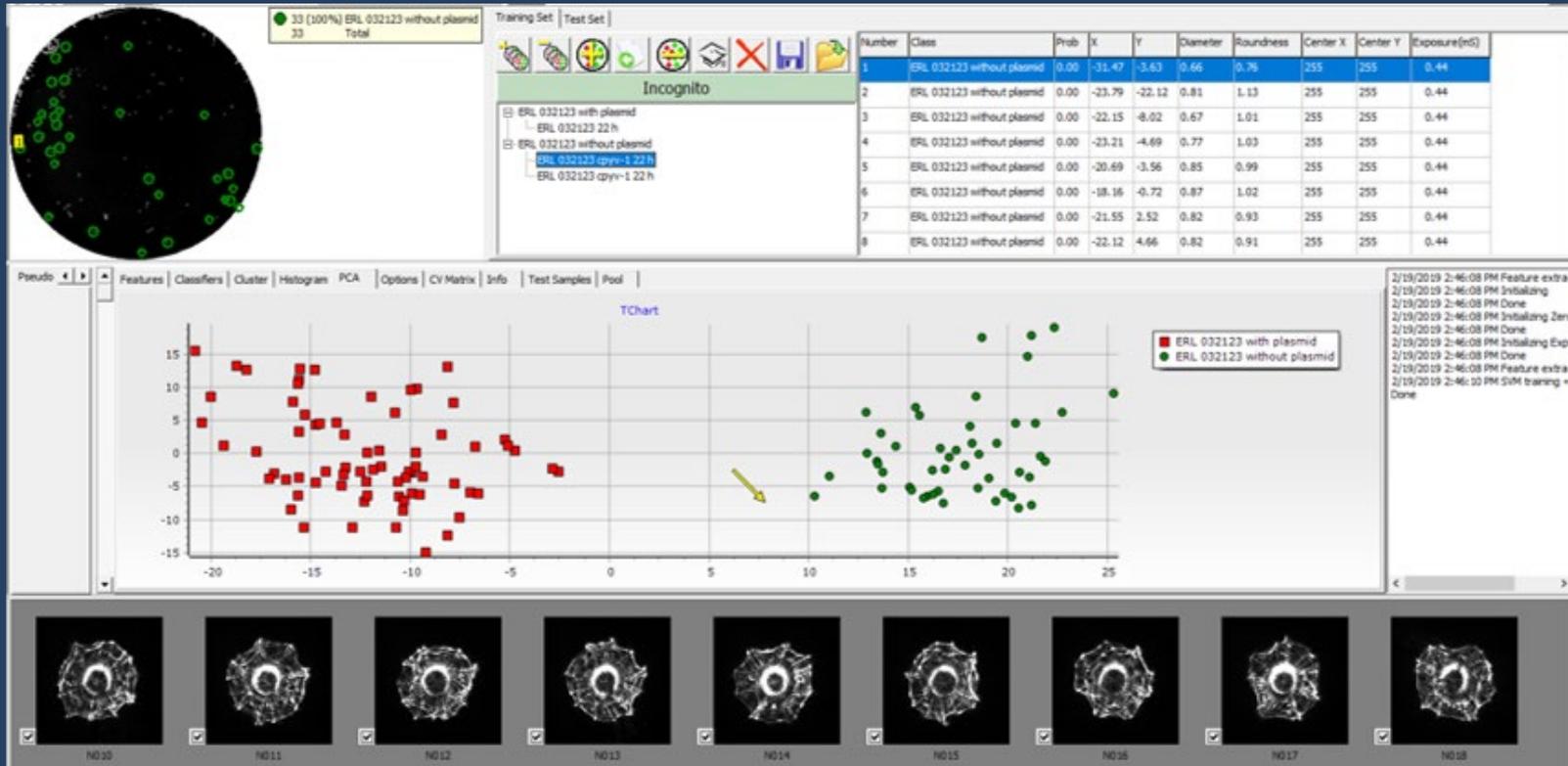


CV Matrix- *Yersinia enterocolitica* biotypes

	Y enterocolitica Biotype 1A 2X	Y enterocolitica Biotype 1B 2X	Y enterocolitica Biotype 2 2X	Y enterocolitica Biotype 3 2X	Y enterocolitica Biotype 4 2X
Y enterocolitica Biotype 1A 2X	91.7	0	8.3	0	0
Y enterocolitica Biotype 1B 2X	0.4	93	1.1	2.1	3.4
Y enterocolitica Biotype 2 2X	0	1.7	91.9	0	6.4
Y enterocolitica Biotype 3 2X	1.1	4.4	0	94.4	0
Y enterocolitica Biotype 4 2X	0	1.5	4.5	0	94

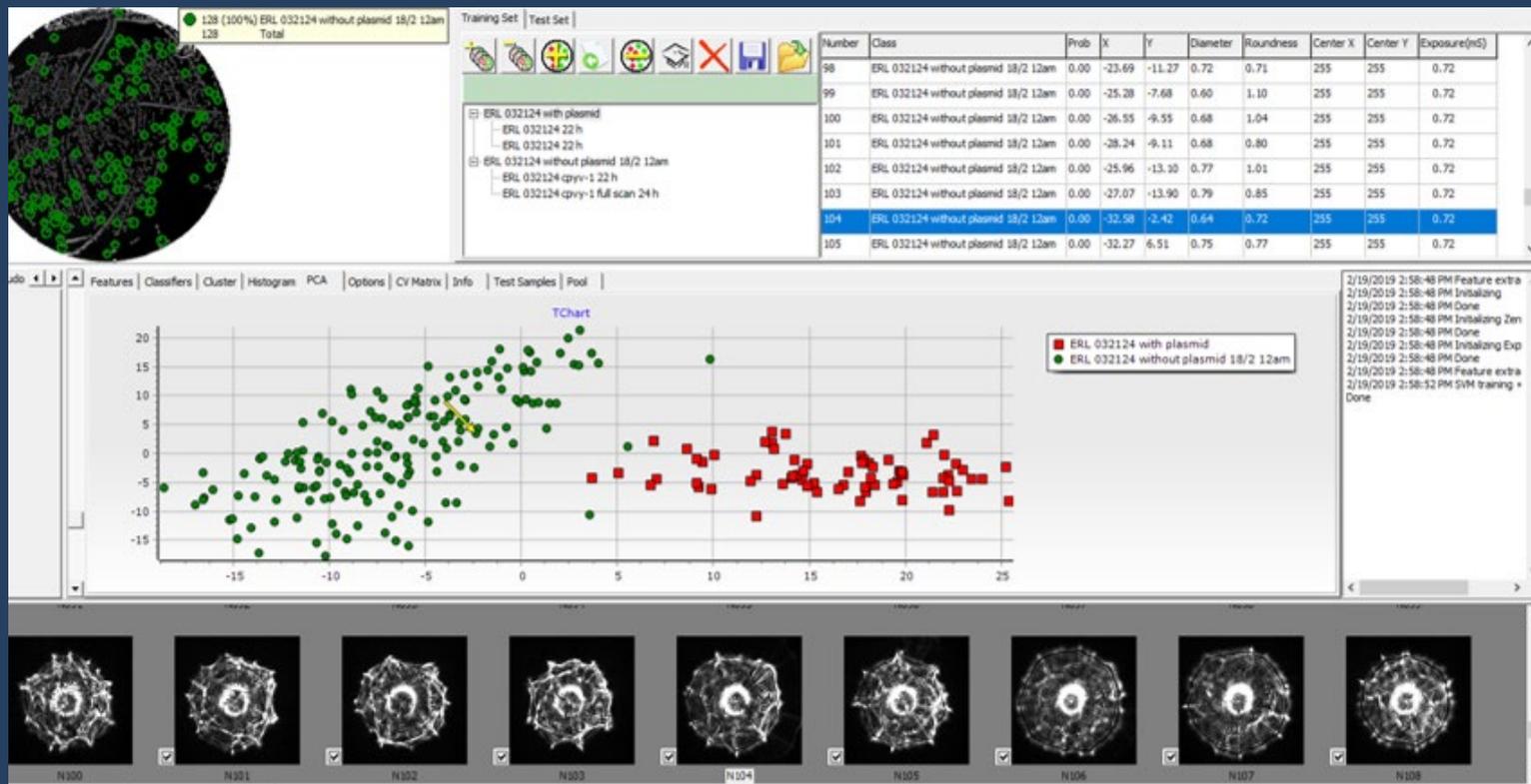


Yersinia enterocolitica ERL 032123 ± virulence plasmid





Yersinia enterocolitica ERL 032124 ± virulence plasmid



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- **Subobjective 3C. Direct typing (colony isolates not required) of enriched samples using a targeted-sequencing method.**
- Subobjective 3D. Generate genome sequence-based typing and identification schemes using next-generation sequencing technology (e.g., MiSeq, Ion Torrent PGM, and MinION), and characterize virulence and antibiotic resistance of microbial pathogens.

Research Note

Enrichment, Amplification, and Sequence-Based Typing of *Salmonella enterica* and Other Foodborne Pathogens

TOM EDLIND,¹ JEFFREY D. BREWSTER,² AND GEORGE C. PAOLI^{2*}

¹MicrobiType LLC, 5110 Campus Drive, Plymouth Meeting, Pennsylvania 19462; and ²U.S. Department of Agriculture, Agricultural Research Service, Molecular Characterization of Foodborne Pathogens Research Unit, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

MS 16-014; Received 8 January 2016/Accepted 27 July 2016/Published Online 21 December 2016

ABSTRACT

Detection of *Salmonella enterica* in foods typically involves microbiological enrichment, molecular-based assay, and subsequent isolation and identification of a pure culture. This is ideally followed by strain typing, which provides information critical to the investigation of outbreaks and the attribution of their sources. Pulsed-field gel electrophoresis is the “gold standard” for *S. enterica* strain typing, but its limitations have encouraged the search for alternative methods, including whole genome sequencing. Both methods typically require a pure culture, which adds to the cost and turnaround time. A more rapid and cost-effective method with sufficient discriminatory power would benefit food industries, regulatory agencies, and public health laboratories. To address this need, a novel enrichment, amplification, and sequence-based typing (EAST) approach was developed involving (i) overnight enrichment and total DNA preparation, (ii) amplification of polymorphic tandem repeat-containing loci with electrophoretic detection, and (iii) DNA sequencing and bioinformatic analysis to identify related strains. EAST requires 3 days or less and provides a strain resolution that exceeds serotyping and is comparable to pulsed-field gel electrophoresis. Evaluation with spiked ground turkey demonstrated its sensitivity (with a starting inoculum of ≤ 1 CFU/g) and specificity (with unique or nearly unique alleles relative to databases of $>1,000$ strains). In tests with unspiked retail chicken parts, 3 of 11 samples yielded *S. enterica*-specific PCR products. Sequence analysis of three distinct typing targets (SeMT1, SeCRISPR1, and SeCRISPR2) revealed consistent similarities to specific serotype Schwarzengrund, Montevideo, and Typhimurium strains. EAST provides a time-saving and cost-effective approach for detecting and typing foodborne *S. enterica*, and postenrichment steps can be commercially outsourced to facilitate its implementation. Initial studies with *Listeria*

Objective 3: Develop and validate rapid methods for the identification of pathogens and/or indicator microorganisms in foods for application in either the field or testing laboratories. (5)



- Subobjective 3A. Generate phage and/or antibody typing arrays.
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Published online 2017 Feb 6. doi: [10.1016/j.gdata.2017.02.005](https://doi.org/10.1016/j.gdata.2017.02.005)

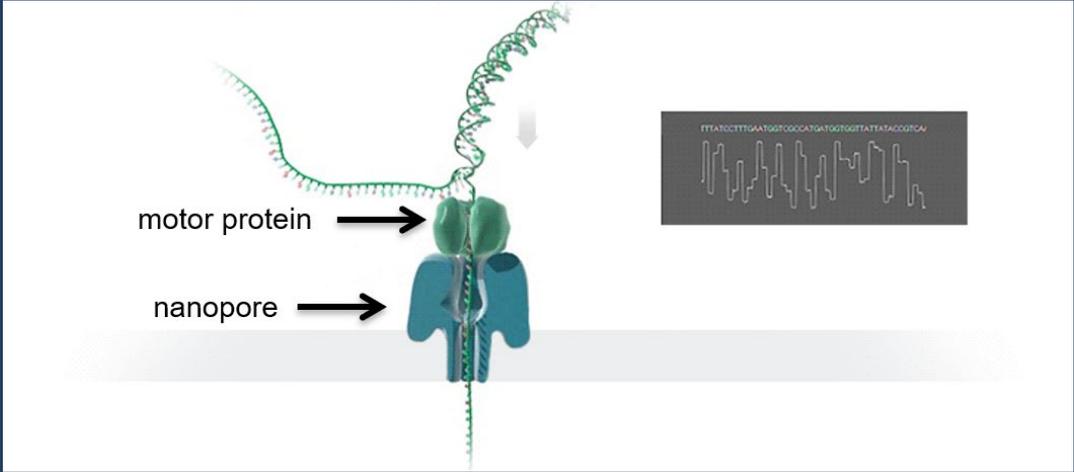
Whole genome sequencing and analysis of *Campylobacter coli* YH502 from retail chicken reveals a plasmid-borne type VI secretion system

[Sandeep Ghatak](#),^a [Yiping He](#),^{b,*} [Sue Reed](#),^b [Terence Strobaugh, Jr.](#),^b and [Peter Irwin](#)^b

- A chromosome of 1,718,974 bp
- A mega-plasmid (pCOS502) of 125,964 bp
- 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)



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Analytical and Bioanalytical Chemistry

September 2018, Volume 410, Issue 22, pp 5439–5444 | [Cite as](#)

Serogroup-level resolution of the “Super-7” Shiga toxin-producing *Escherichia coli* using nanopore single-molecule DNA sequencing

Authors

[Authors and affiliations](#)

Adam Peritz , George C. Paoli , Chin-Yi Chen, Andrew G. Gehring

Communication

First Online: 27 January 2018

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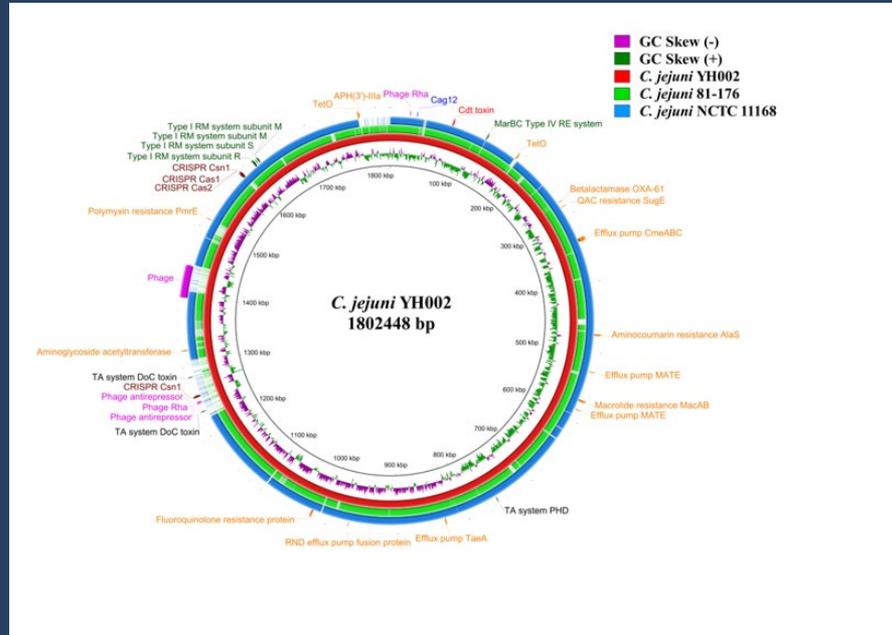
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Abstract

DNA sequencing and other DNA-based methods are now broadly used for detection and identification of bacterial foodborne pathogens. For the identification of foodborne bacterial pathogens, taxonomic assignments must be made to the species or even subspecies level. Long-read DNA sequencing provides finer taxonomic resolution than short-read sequencing. Here,

Comparative analysis of genome and methylome of a multidrug resistant *Campylobacter jejuni* strain YH002 from retail beef liver

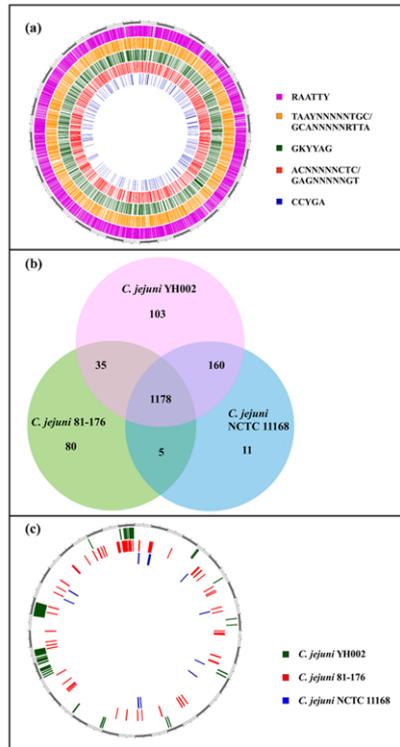




Comparative analysis of genome and methylome of a multidrug resistant *Campylobacter jejuni* strain YH002 from retail beef liver (2)

Antibiotic class	<i>C. jejuni</i> YH002		<i>C. jejuni</i> 81-176		<i>C. jejuni</i> NCTC 11168	
	MIC (µg/mL)	Gene(s)	MIC (µg/mL)	Gene(s)	MIC (µg/mL)	Gene(s)
Aminoglycoside	GEN 32.000 (R)	<i>APH(3')-IIIa, AAC-(3)</i>	GEN 0.120 (S)	<i>AAC-(3)</i>	GEN 0.120 (S)	<i>AAC-(3)</i>
Beta-lactam	AMX 32.000	<i>blaOXA-61</i>	AMX 0.190	-	AMX 1.500	<i>blaOXA-61</i>
Carbapenem	ERT 0.007	-	ERT 0.004	-	ERT 0.002	-
Ketolides	TEL 0.120 (S)	-	TEL 0.120 (S)	-	TEL 0.015 (S)	-
Lincosamide	CLI 0.060 (S)	-	CLI 0.060 (S)	-	CLI 0.030 (S)	-
Macrolide	AZI 0.030 (S)	<i>macA, macB</i>	AZI 0.015 (S)	<i>macA, macB</i>	AZI 0.015 (S)	<i>macA, macB</i>
	ERY 0.060 (S)		ERY 0.120 (S)		ERY 0.020 (S)	
Phenicols	FLO 0.120 (S)	-	FLO 0.250 (S)	-	FLO 0.060 (S)	-
Polypeptides	POL 6.000	<i>pmrE</i>	POL 8.000	-	POL 16.000	<i>pmrE</i>
Quinolone	CIP 0.030 (S)	<i>mfd</i>	CIP 0.060 (S)	<i>mfd</i>	CIP 0.030 (S)	<i>mfd</i>
	NAL 8.000 (S)		NAL 4.000 (S)		NAL 8.000 (S)	
Tetracyclines	TET 16.000 (R)	<i>tetO</i>	TET 0.120 (S)	<i>tetO</i>	TET 0.120 (S)	

Methylation analysis of *C. jejuni* YH002, 81-176, and NCTC 11168.



Comparative analysis of genome and methylome of a multidrug resistant *Campylobacter jejuni* strain YH002 from retail beef liver (3)



Summary

- Genome and methylome of a multidrug resistant *C. jejuni* strain YH002 was unraveled.
- Curious co-existence of bacteriophage and *mcrBC* type RM system was observed in the genome.
- Possible first description of PHD-DoC type TA system in *C. jejuni*.
- Specific patterns of amino acids changes altered the motility of *C. jejuni* YH002.
- A novel methylation motif (CGCGA) of type II RM system was identified.

Objective 1: Develop rapid and efficient techniques that separate and concentrate and/or quantify targeted pathogens from food matrices. (2)



- Magnetic Bar Capture Device, Armstrong, C.M., Capobianco, J.A., and Gehring, A. USDA Docket Number 68.18, patent application 62/737,212 was filed September 27, 2018.
- Bacterial cell recovery after hollow fiber microfiltration sample concentration: most probable bacterial composition in frozen vegetables. P Irwin, J. Capobianco, Y. He, L. Nguyen, M.Gehring, A. Gehring, and C. Chen. submitted to Food Control
- Accelerating Sample Preparation through Enzyme-Assisted Microfiltration of *Salmonella* in Chicken Extract. Vibbert, H.B., Ku, S., Li, X., Liu, X., Ximenes, C., Kreke, T., Ladisch, M.R., Deering, A.J., and Gehring, A.G. Biotechnol. Progress 31(6):1551-1562. 2015.

Objective 2: Develop and validate field testing kits that rapidly screen for the presence and quantification of pathogens and/or indicator microorganisms in foods at the initial processing level. (6)



- Rapid detection of *Salmonella enterica* serotype Typhimurium in large volume samples using porous electrodes in a flow-through, enzyme-amplified immunoelectrochemical sensor. Capobianco, J.A., Lee, J., Armstrong, C.M, and Gehring, A. Analytical and Bioanalytical Chemistry; Manuscript ID: ABC-02295-2017.R1, Accepted 2018. (*print delayed by ARS OTT*)
- Detection of shiga toxin 2 produced by *Escherichia coli* in foods using a novel AlphaLISA. Armstrong, C.M., Ruth, L., Capobianco, J.A., Strobaugh, T.P., Rubio, F. and Gehring, A. Armstrong, C. M., Ruth, L. E., Capobianco, J. A., Strobaugh, T. P., Rubio, F. M., & Gehring, A. G. *Toxins*, 10(11), 422, 2018.
- Flow-Through Immunoelectrochemical Sensor. Capobianco, J.A., Gehring, A., Armstrong, C.M., and Lee, J., USDA Docket Number 21.18, Patent Application under review by ARS OTT.
- Evaluation of ELISA tests specific for Shiga toxin 1 and 2 in food and water samples. Gehring, A.G., Fratamico, P.M., Lee, J., Ruth, L., He, X., He, Y., Paoli, G., Stanker, L.H., Rubio, F.M... *Food Control*. 77:145-149, 2017.
- Shiga and Shiga-like Toxins (Stx). Presumptive Analysis 8.2.14.1. Kong, Q., Patfield, S.A., Skinner, C.B., Stanker, L.H., Gehring, A.G., Fratamico, P.M., Rubio, F., Qi, W., and He, X., US Environmental Protection Agency, EPA/600/R-17/356, CAS RN: 75757-64-1 (Stx). September, 2017.
- Multilayer Devices and Methods of Manufacturing. Capobianco, J., Martorano, C., DeClement, D. PCT Application 62/452,335, 2017.
- Validation of Two New Immunoassays for Sensitive Detection of a Broad Range of Shiga Toxins. *Austin Immunology*. Kong, Q., Patfield, S.A., Skinner, C.B., Stanker, L.H., Gehring, A.G., Fratamico, P.M., Rubio, F., Qi, W., and He, X. 1(2):1007, 2016.

Objective 3: Develop and validate rapid methods for the identification of pathogens and/or indicator microorganisms in foods for application in either the field or testing laboratories. (6)



- Serogroup-level resolution of the “Super-7” Shiga toxin-producing *Escherichia coli* using nanopore single-molecule DNA sequencing. Peritz, A., Chen, C., Paoli, G., Gehring, A.G. *Analytical and Bioanalytical Chemistry*, 2018.
- Whole-Genome Sequence Data and Analysis of a *Staphylococcus aureus* Strain SJTUF J27 Isolated from Seaweed. Xie, Y., He, Y., Ghatak, S., Irwin, P., Yan, X., Strobaugh, T.P., Jr., and Gehring A. *Data in Brief* 20:894-898. 2018.
- Enrichment, Amplification, and Sequence-based Typing of *Salmonella Enterica* and other Foodborne Pathogens. Edlind, T., Brewster, J.D., Paoli, G.C. *J. Food Prot.*, 80(1):15-24, 2017.
- Advantages of Virulotyping Pathogens over Traditional Identification and Characterization Methods. Gurtler, J.B., Doyle, M.P., Kornacki, J.L., Fratamico, P.M., Gehring, A.G., Paoli, G.C. In Gurtler, J.B., Doyle, M.P., and Kornacki, J.L. (eds.) *Foodborne Pathogens: Virulence Factors and Host Susceptibility*, Springer Nature, Switzerland AG, pp 3-40. 2017.
- The use of a novel nanoLuc-based reporter phage for the detection of *Escherichia coli* O157:H7. Zhng, D., Coronel-Aguilera, C., Romero, P., Perry, L., Minocha, U., Rosenfield, C., Gehring, A.G., Paoli, G., Bhunia, A.K., Applegate, B. *Scientific Reports*, 2016.
- Rapid Identification and Classification of *Campylobacter* Spp. Using Laser Optical Scattering Technology. He, Y., Reed, S., Bhunia, A.K., Gehring, A., Nguyen, L.-H., and Irwin, P.L. *Food Microbiol.* 47:28-35, 2015.

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Thank you!

Question/Comments/Concerns