

Keeping Up with Changing PCR Methods

John F. Griffith, Ph.D.

Southern California Coastal Water Research Project

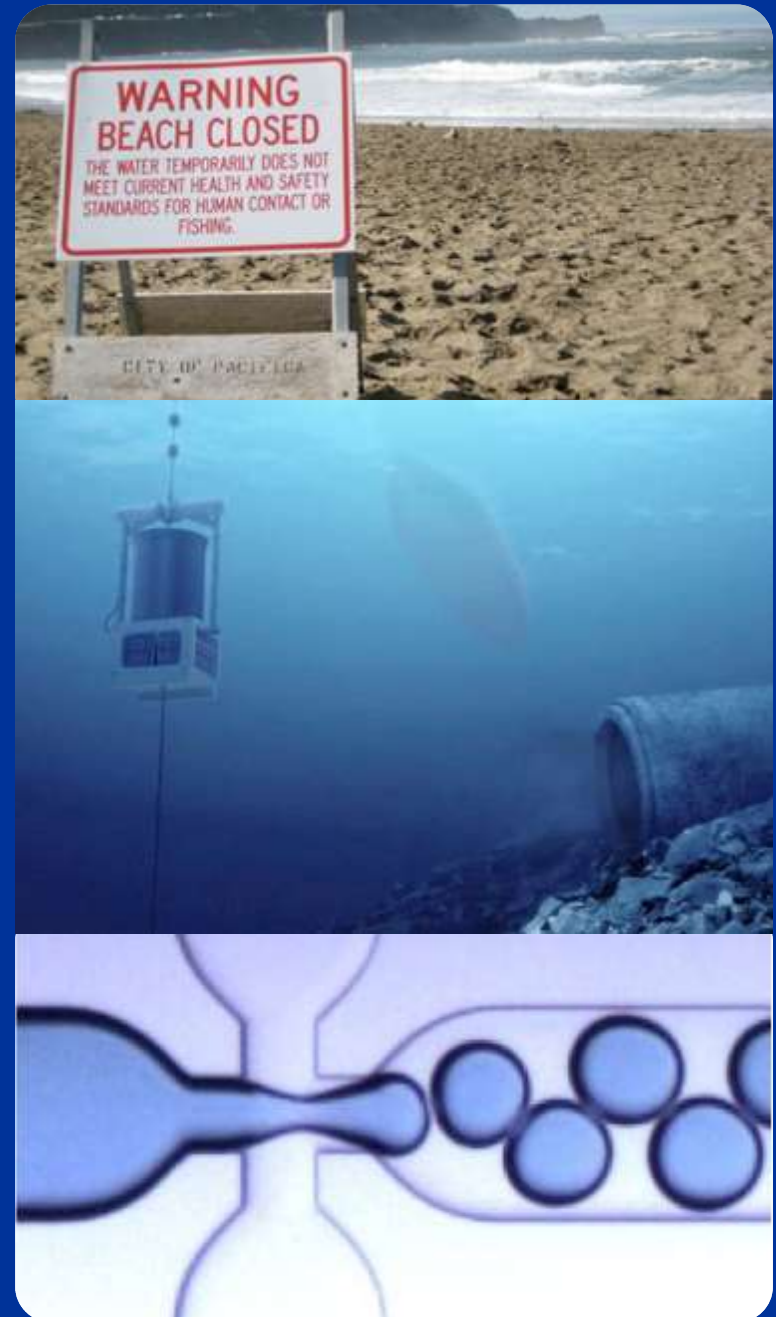


PROBLEM STATEMENT

- **Growth based methods for monitoring recreational water too slow to protect swimmers**
 - 18 to 72 hour incubation
 - Delay getting results leads to 70% error rate at California beaches
- **EPA-approved QPCR method is faster**
 - Very sensitive to environmental interferences that inhibit PCR chemistry
 - Highly variable, reliant on standard curve for quantification
 - Travel time to lab means results not out until late afternoon
- **Digital PCR more precise, less sensitive to inhibition**
 - Travel time still an impediment to timely notification of health risk
- **Need to begin processing water samples in the field to produce a meaningful answer**
 - Publicly disseminated before swimmers enter contaminated water

OUTLINE

- **Current state of qPCR for water quality monitoring**
- **Proof of concept study for automating water quality monitoring**
- **Introduction to digital PCR**
- **Development of an automated dPCR instrument**



QPCR HAS COME A LONG WAY

- 15 years of development
- Validation in multiple epidemiology studies
- Two EPA-approved methods for *Enterococcus*
 - Methods 1611 and 1609
 - *E. coli* method in use in Great Lakes

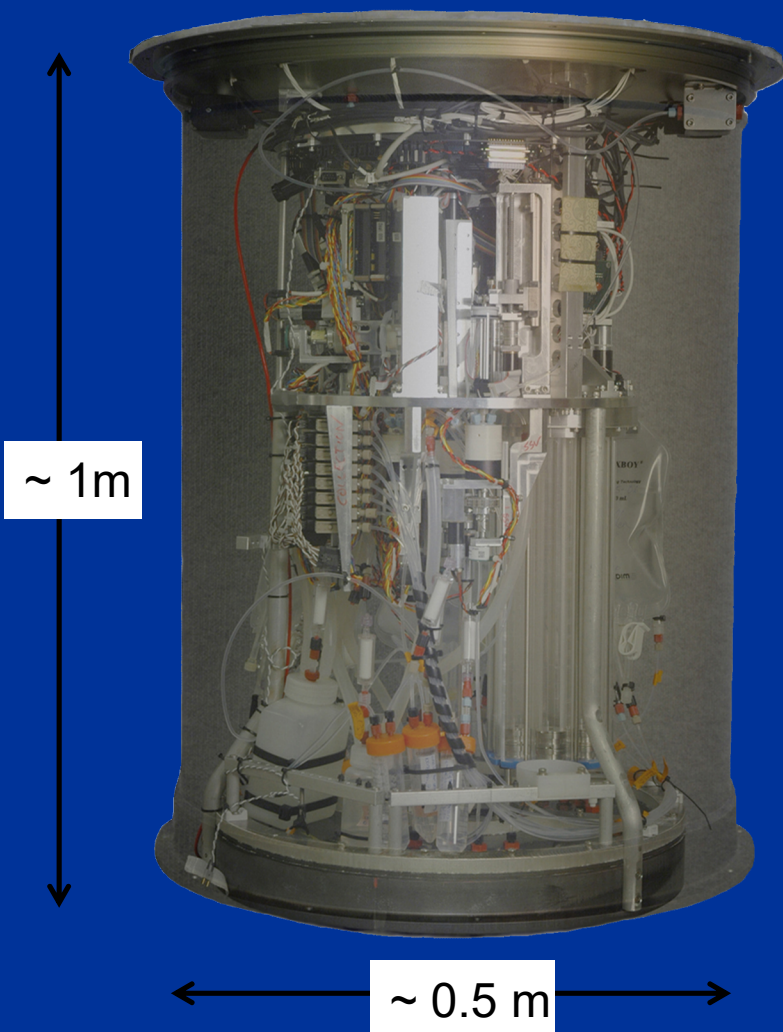
ARE WE DONE?

- **We have EPA-approved qPCR methods**
- **Have trained 14 labs in California on Method 1609**
 - Using method routinely
- **Sample collection delays timely analysis**
 - Requires additional personnel for meaningful answer

WHERE DO WE GO FROM HERE?

- **Automation can help provide answers early in the day**
- **New digital PCR technology is more accurate and less susceptible to inhibition**

2ND GENERATION ENVIRONMENTAL SAMPLE PROCESSOR (ESP)



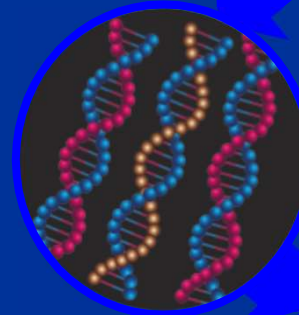
Collection



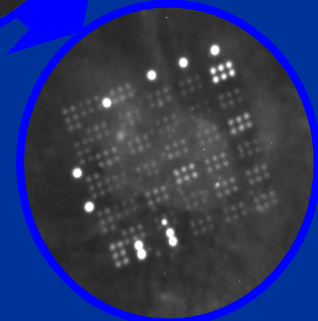
Concentration



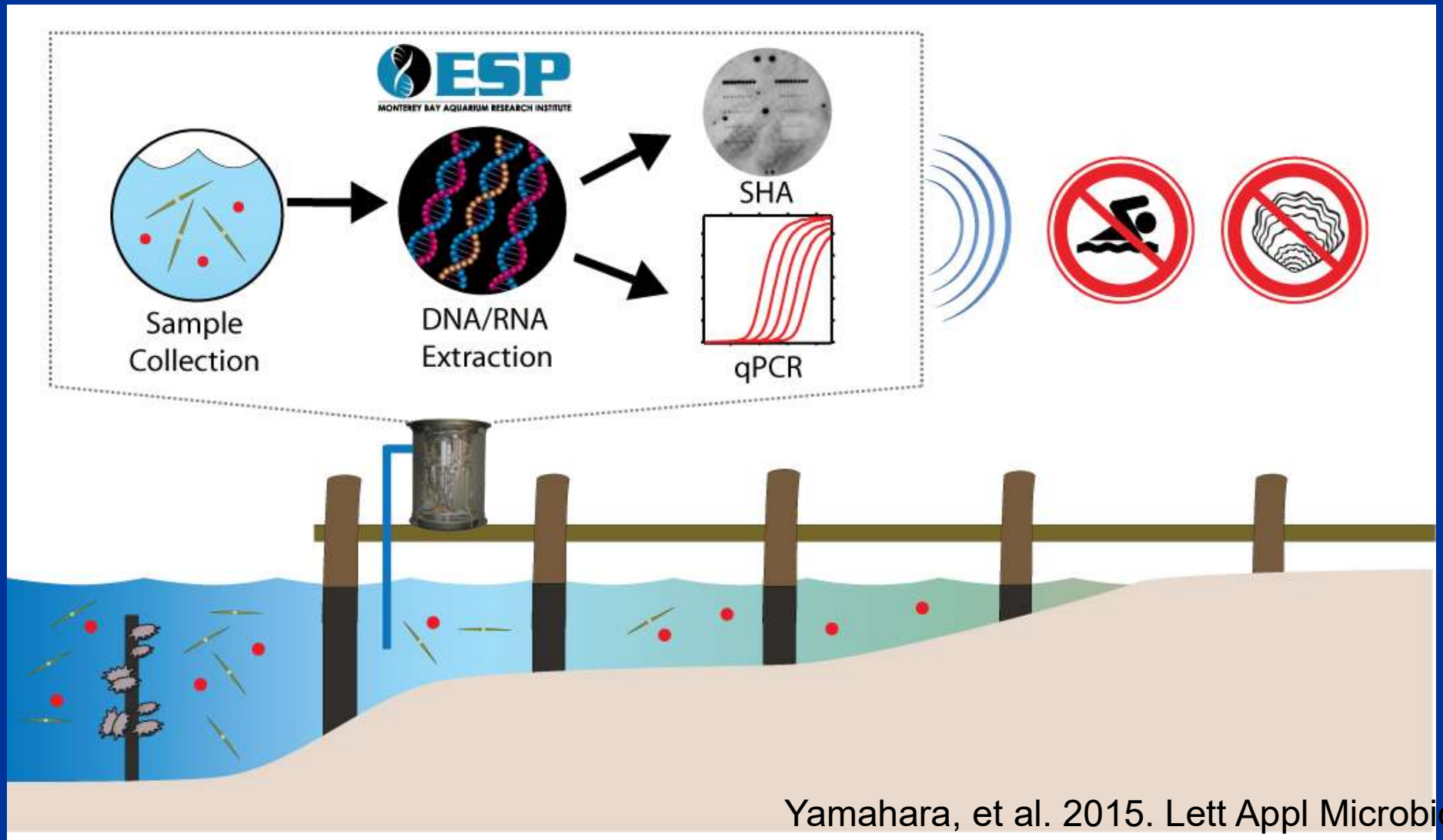
Extraction



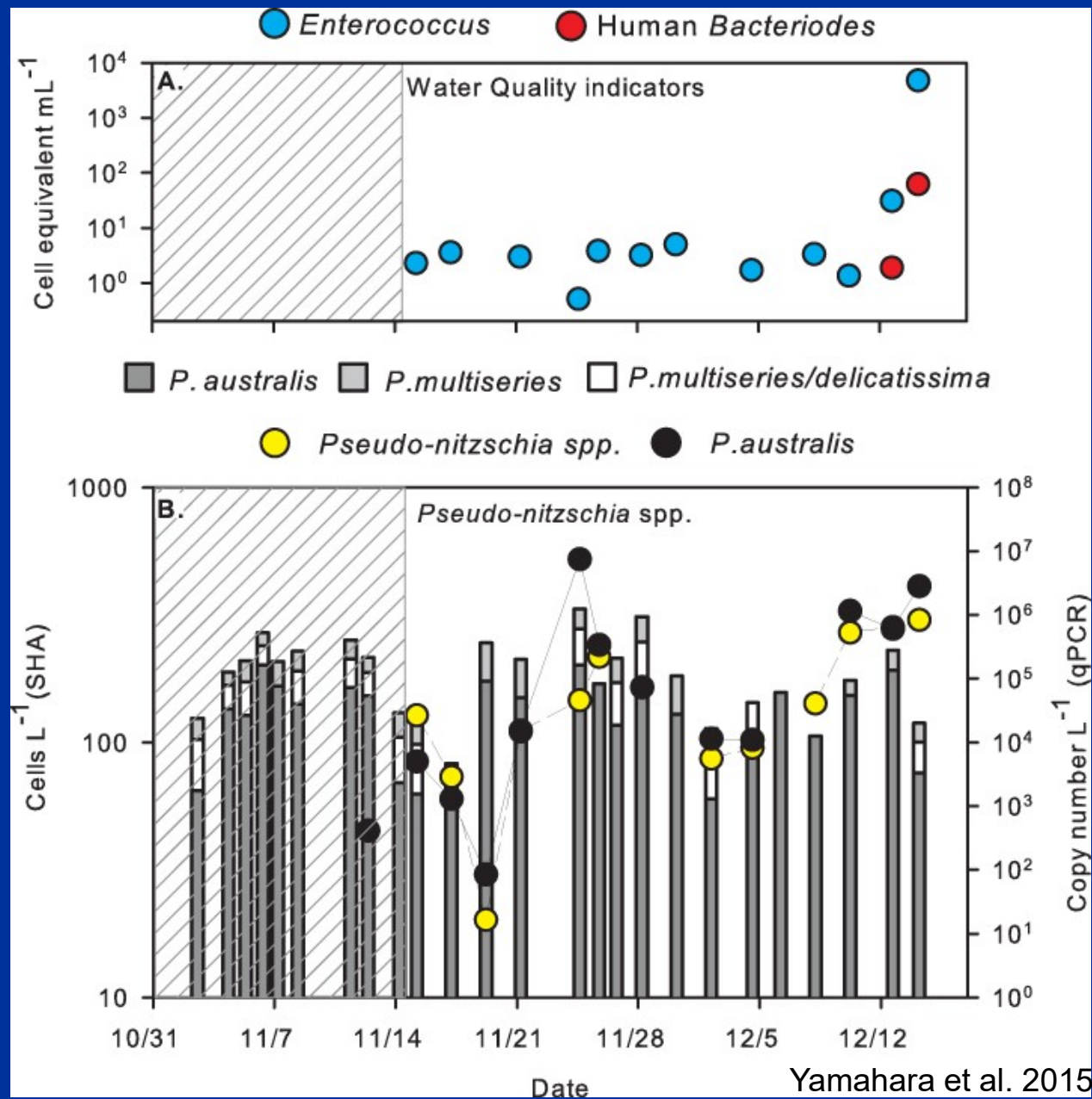
Detection



PROOF OF CONCEPT



SANTA CRUZ WHARF, CA, DEPLOYMENT



CRITIQUE OF 2ND GENERATION ESP

Pros-

- **Quantification of BOTH fecal indicators and harmful algae from the same sample**
 - Sample to results in 4 hours

Cons-

- **Extremely complicated**
 - 1980's technology
 - Requires highly skilled technicians to maintain and operate
 - qPCR very susceptible to environmental interferences
- **Not portable**
 - Similar in size to a 55 gallon drum
 - Weighs about 300 lbs.

QPCR VS. DIGITAL PCR

LIMITATIONS OF QPCR

- **Low precision when target concentration low**
- **Susceptible to inhibition**
 - May result in underestimation and false negatives
- **Difficult to implement cost-saving strategies**
 - Often difficult to measure multiple targets simultaneously in one reaction

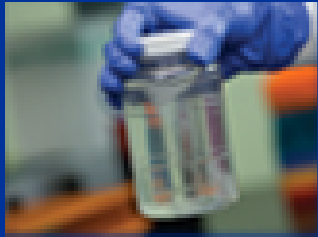
→ Digital PCR has the potential to overcome these limitations!

CHARACTERISTICS OF DIGITAL PCR

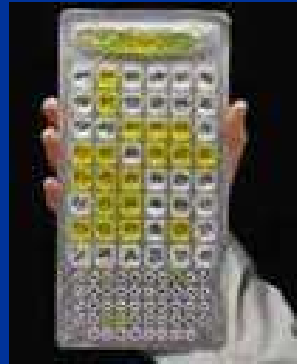
- **Direct quantification of target by counting positive droplets**
 - No standard curve needed
- **Can provide precision estimate even with one reaction**
- **More resistant to inhibition**
- **Simultaneous measurement of multiple targets in one reaction**

HOW DROPLET DIGITAL PCR WORKS

- “MPN” PCR



100ml water



96 wells



Count number of positive wells

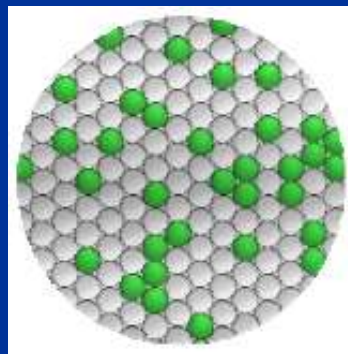
MPN Table



Direct Quantification



20ul PCR
(tube/well)



$\leq 20,000$ droplets

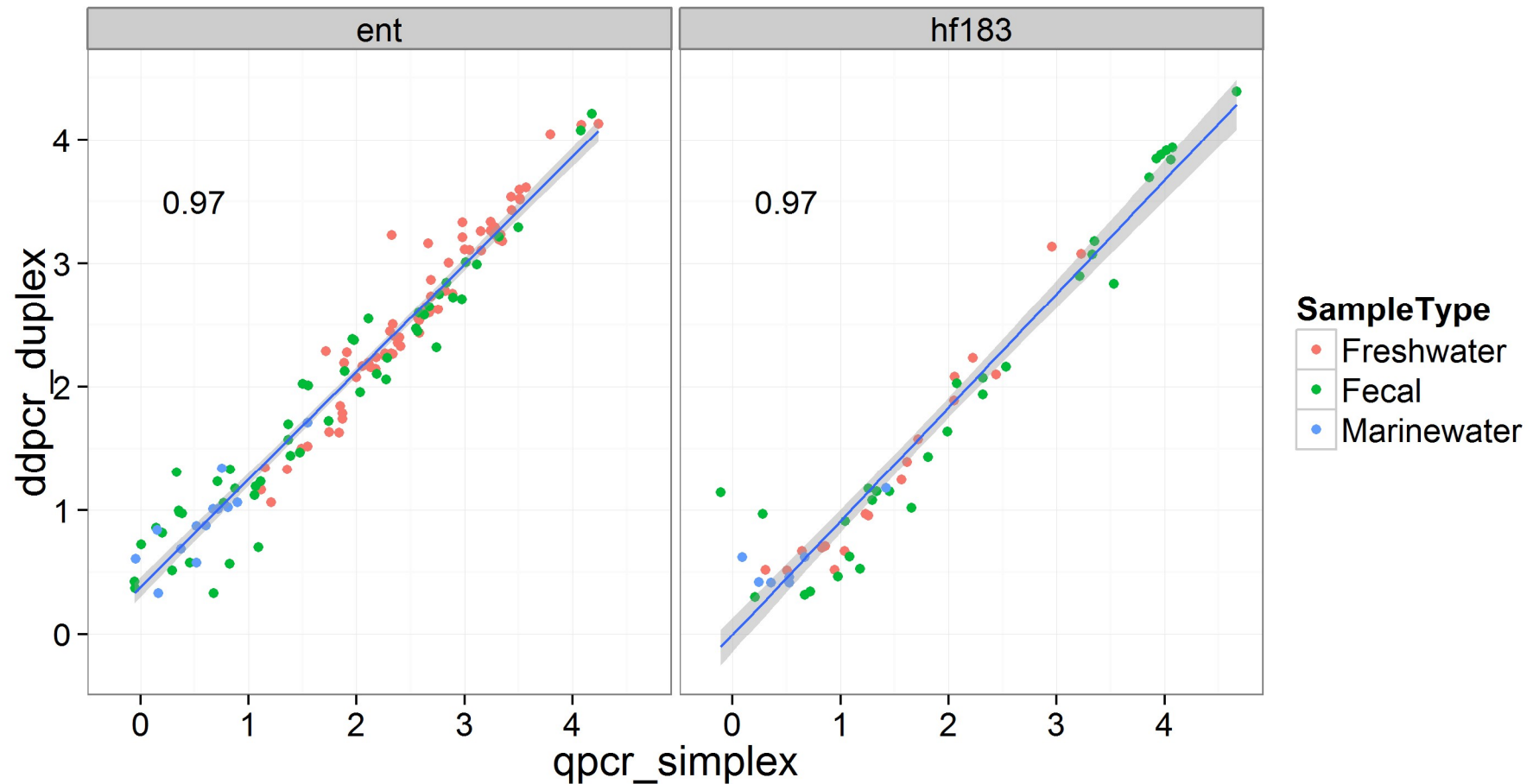


Poisson Statistics

Count positive and
negative droplets



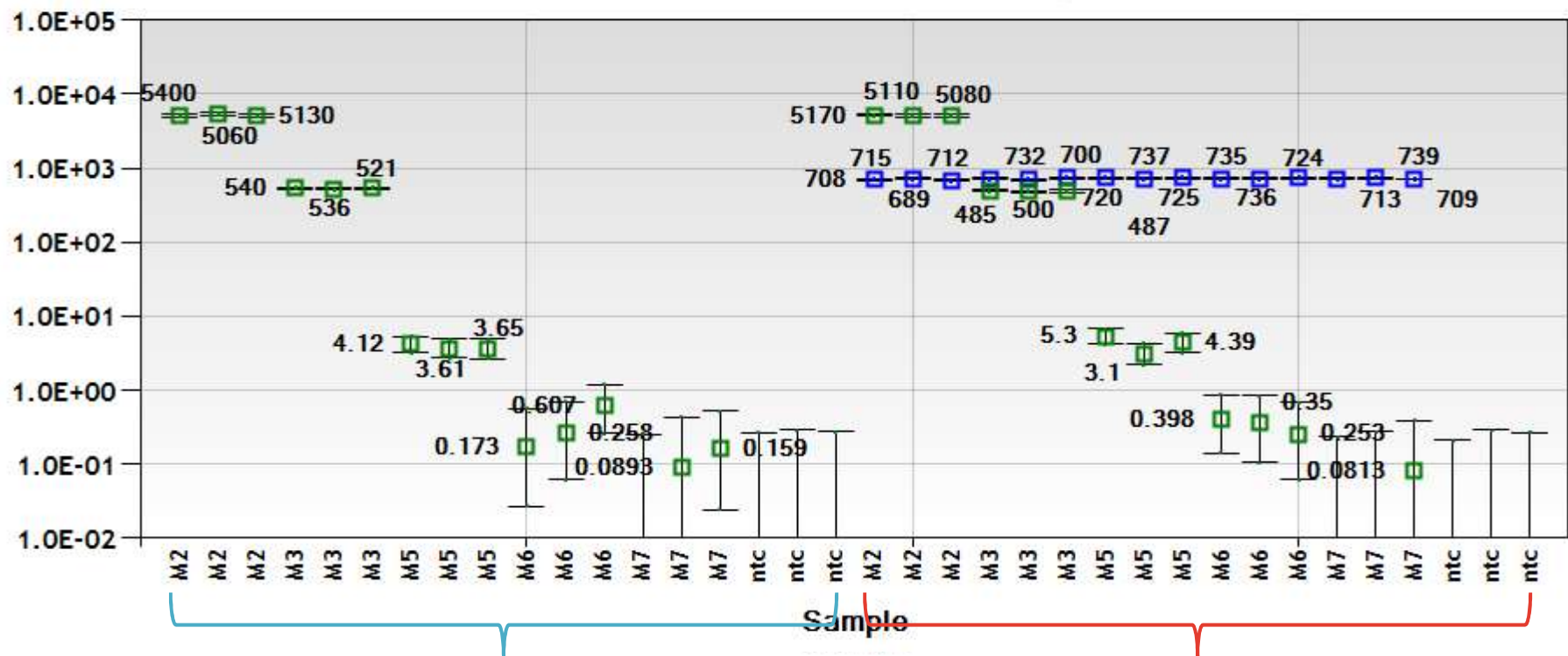
HIGHLY CORRELATED WITH EPA APPROVED QPCR METHOD FOR ENTEROCOCCUS



MEASURING *ENTEROCOCCUS* AND HF183 SIMULTANEOUSLY

- Quantification of *Enterococcus* (blue squares) and HF183 (green squares) are not affected by each other

HF183 or Enterococcus (copy per ul)



simplex

duplex

INHIBITION

- **More robust**
 - qPCR signal disappears with increased inhibitor concentration
 - ddPCR signal remains nearly constant

Humic acid concentration (ng/ul)	qPCR (HF183 copy/rxn)	ddPCR (HF183 copy/rxn)
0	1810	1810
1	1165	1680
2.5	184	1700
5	0	1870

WISH LIST FOR AUTOMATED DPCR INSTRUMENT

- **Fast**
 - Results telemetered to decision makers in < 4 hours of sampling event
- **Flexible**
 - Able to detect multiple targets (indicators, pathogens, source markers)
- **Easy to operate**
 - Able to be operated by a field technician or lifeguard
- **Portable**
 - Vehicle mounted or hand carried to sampling sites
- **Reliable**
 - Not susceptible to environmental interferences
 - Robust

NEW INSTRUMENT DESIGN CRITERIA

Sample Collection/
Processing



Analyte Detection



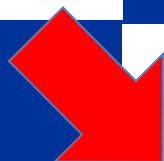
- **Detecting and Tracking sources of contamination requires mobility**
 - Engineering design for a hand-carry instrument
 - **Modular design** – separate sample collection and detection

SAMPLE COLLECTION/PROCESSING

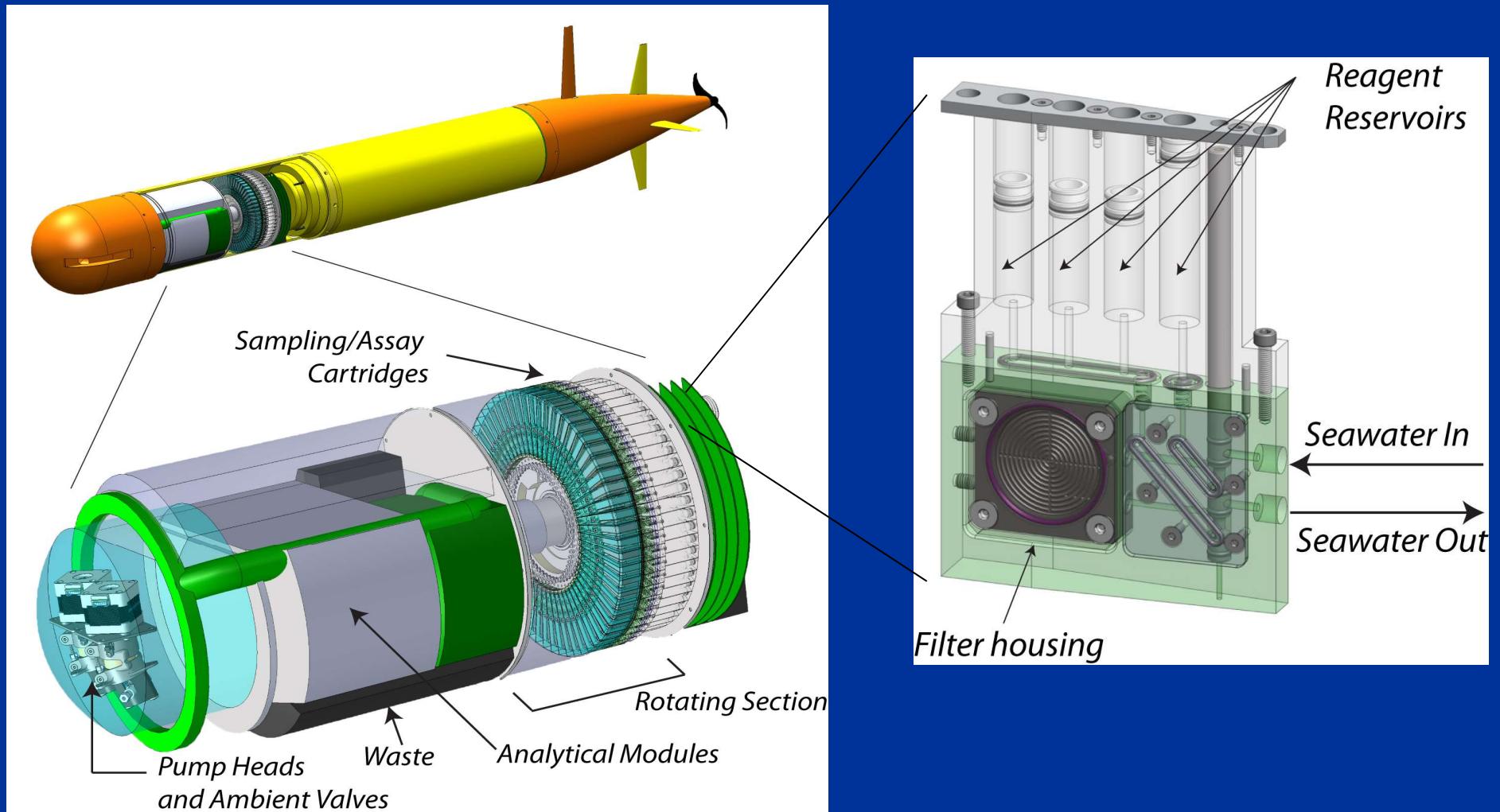
Sample Collection/
Processing



Analyte Detection



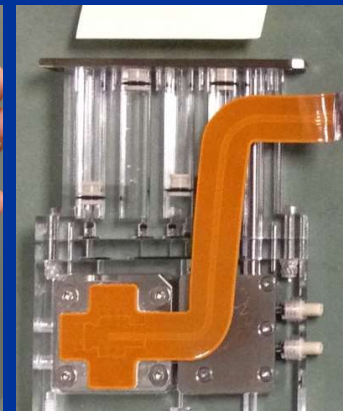
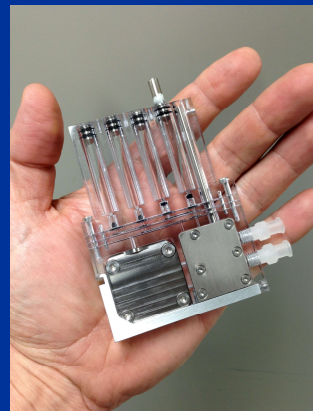
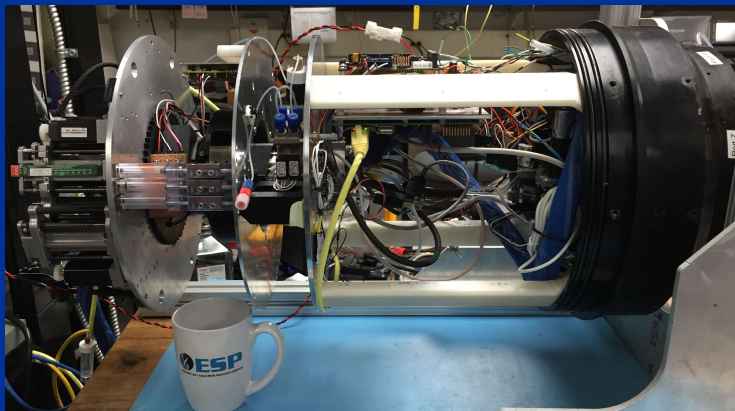
3RD GENERATION ESP SOLUTION



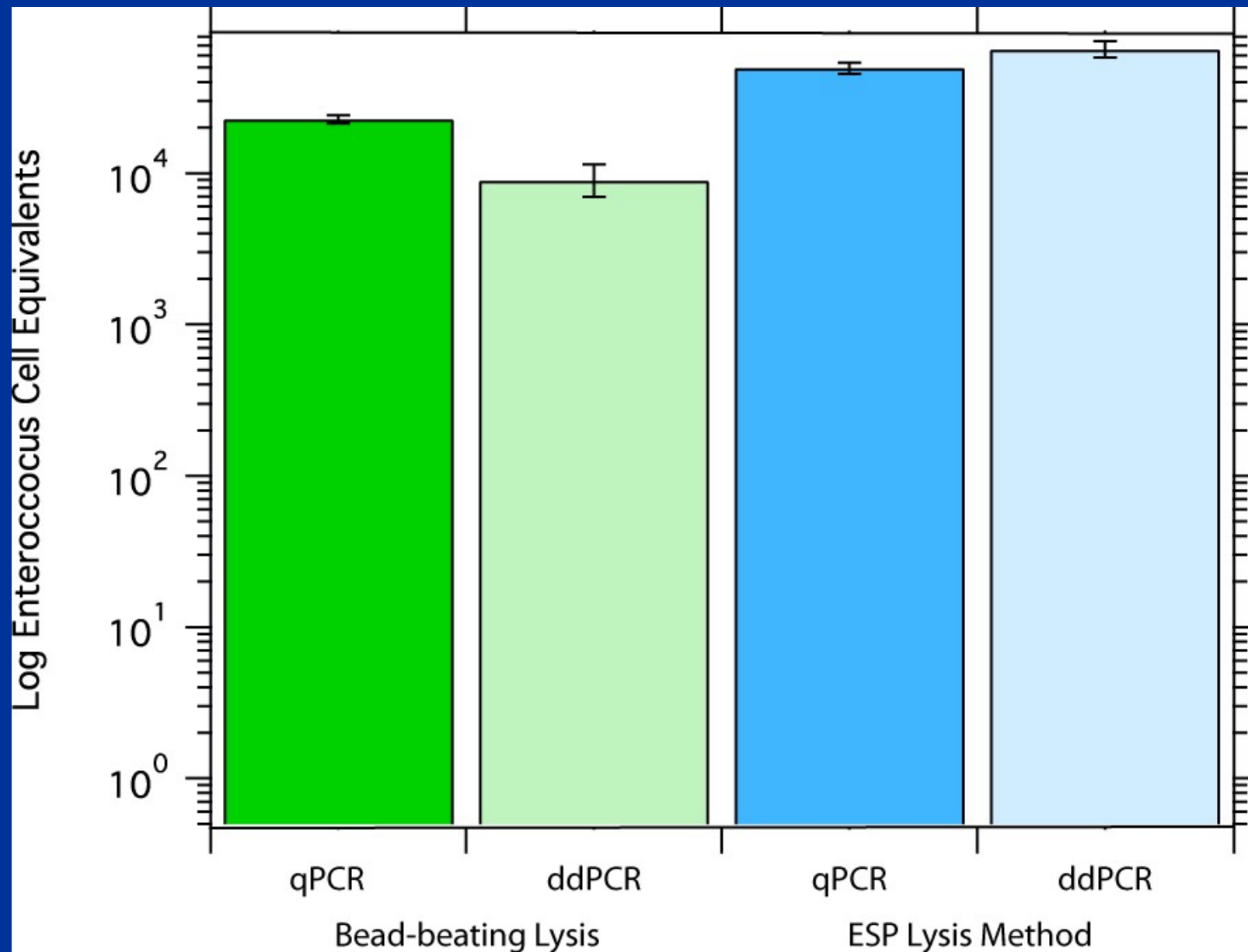
- **Same engineering concepts, different form factor**

PROTOTYPE 3RD GENERATION ESP

- **3rd Generation (3G) ESP technology**
 - Sample Collection and Processing
 - Preservation and In-situ Lysis
 - Digital PCR (ddPCR)



ESP DNA EXTRACTION COMPARISON



ANALYTE DETECTION

Sample Collection
/Processing

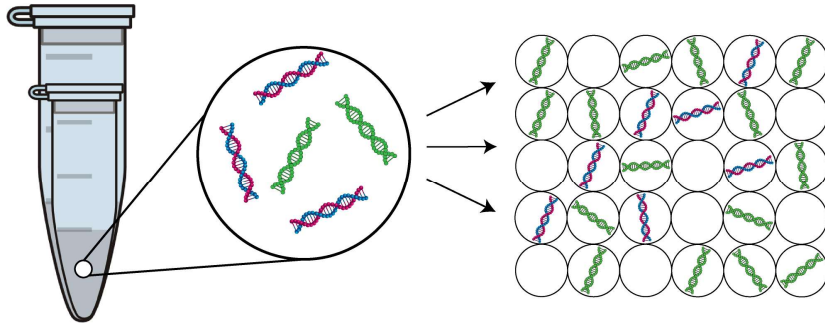


Analyte Detection

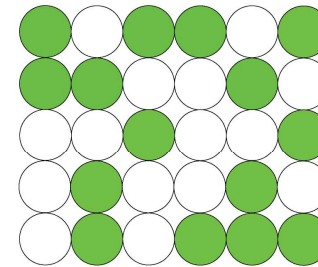


ASU DROPLET DIGITAL PCR MODULE

Partition a normal PCR reaction with many DNA templates into many individual PCR reactions

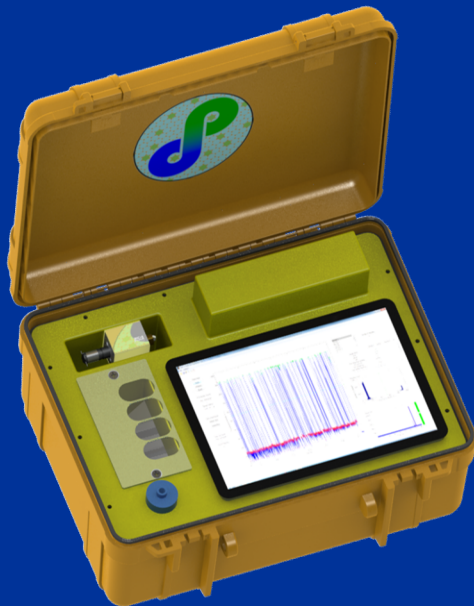


Thermal
Cycle



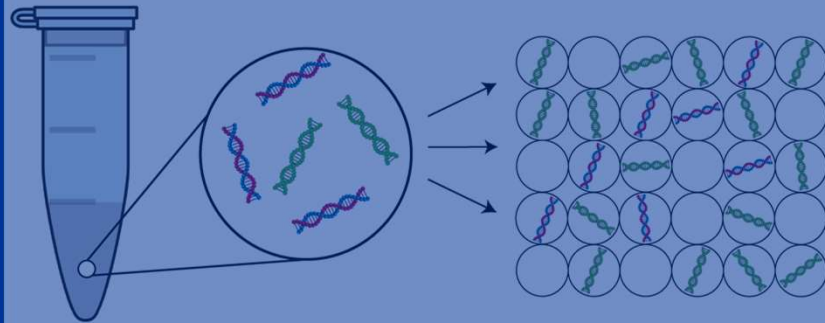
1	0	1	1	0	1
1	1	0	0	1	0
0	0	1	0	0	1
0	1	0	0	1	0
0	1	0	1	1	1

● Positive Reactions ○ Negative Reactions

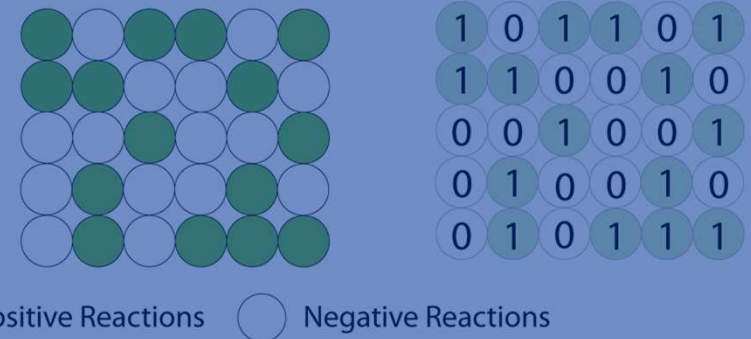


DROPLET DIGITAL PCR MODULE

Partition a normal PCR reaction with many DNA templates into many individual PCR reactions



Digital readout of positive and negative reactions provide an absolute quantification

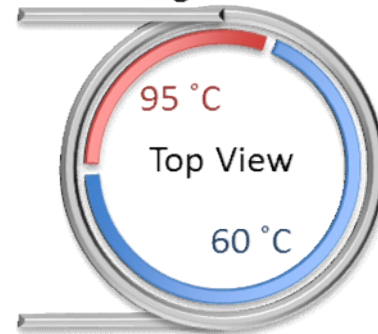


Partitioning to 1-nL Reactions



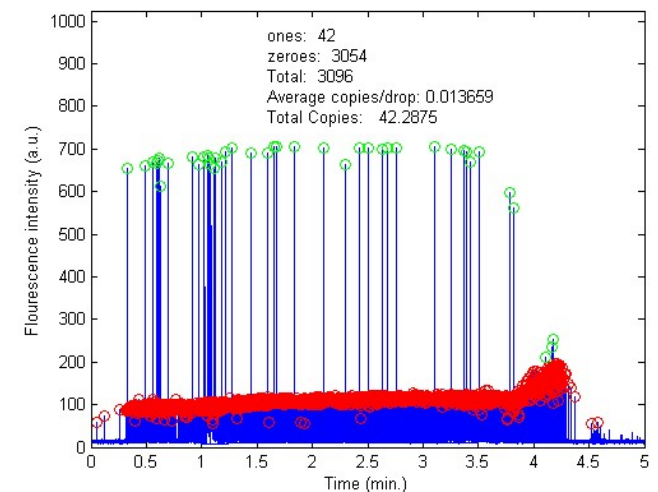
Racetrack Thermocycler

Inlet tubing

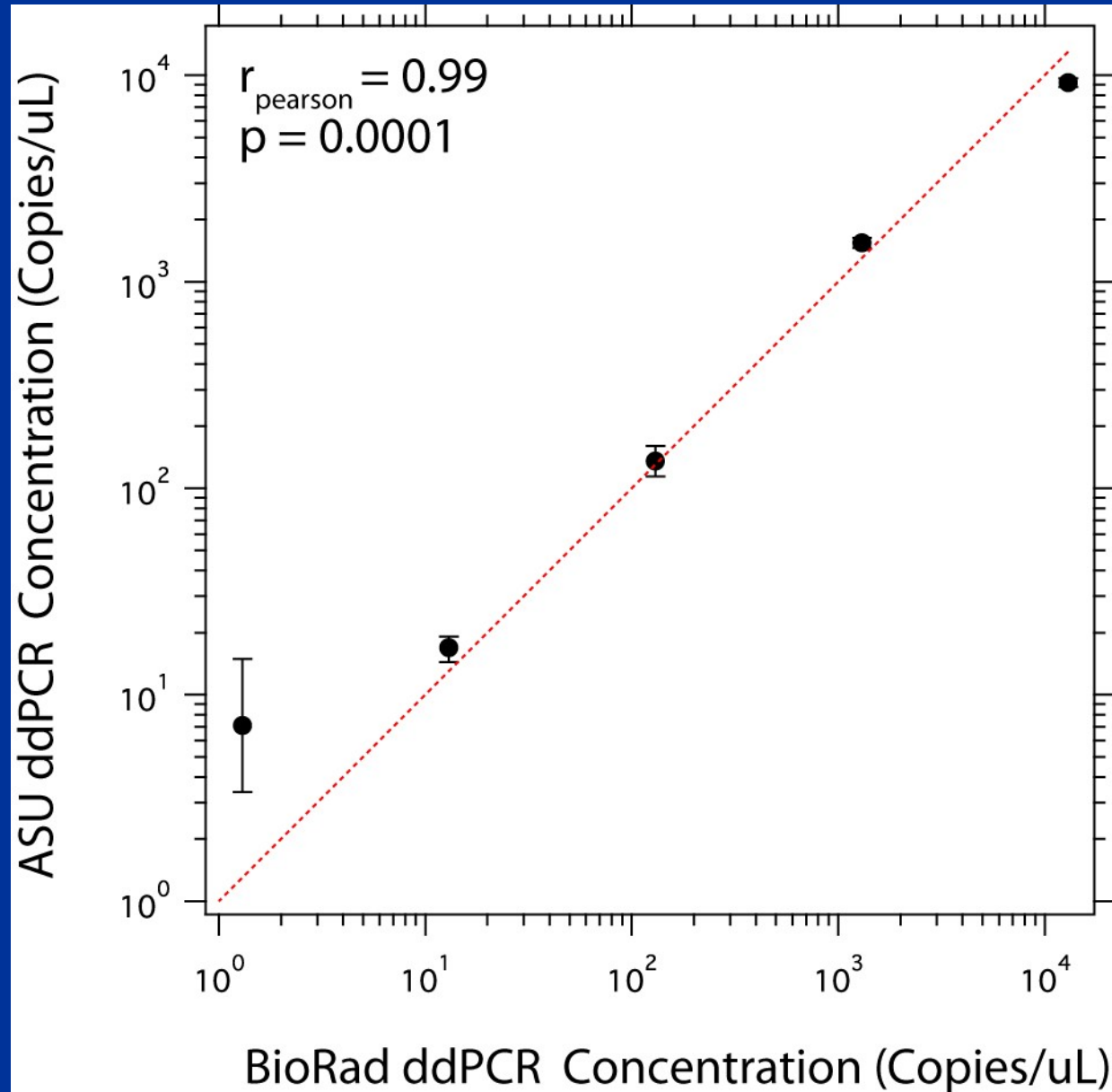


Outlet tubing

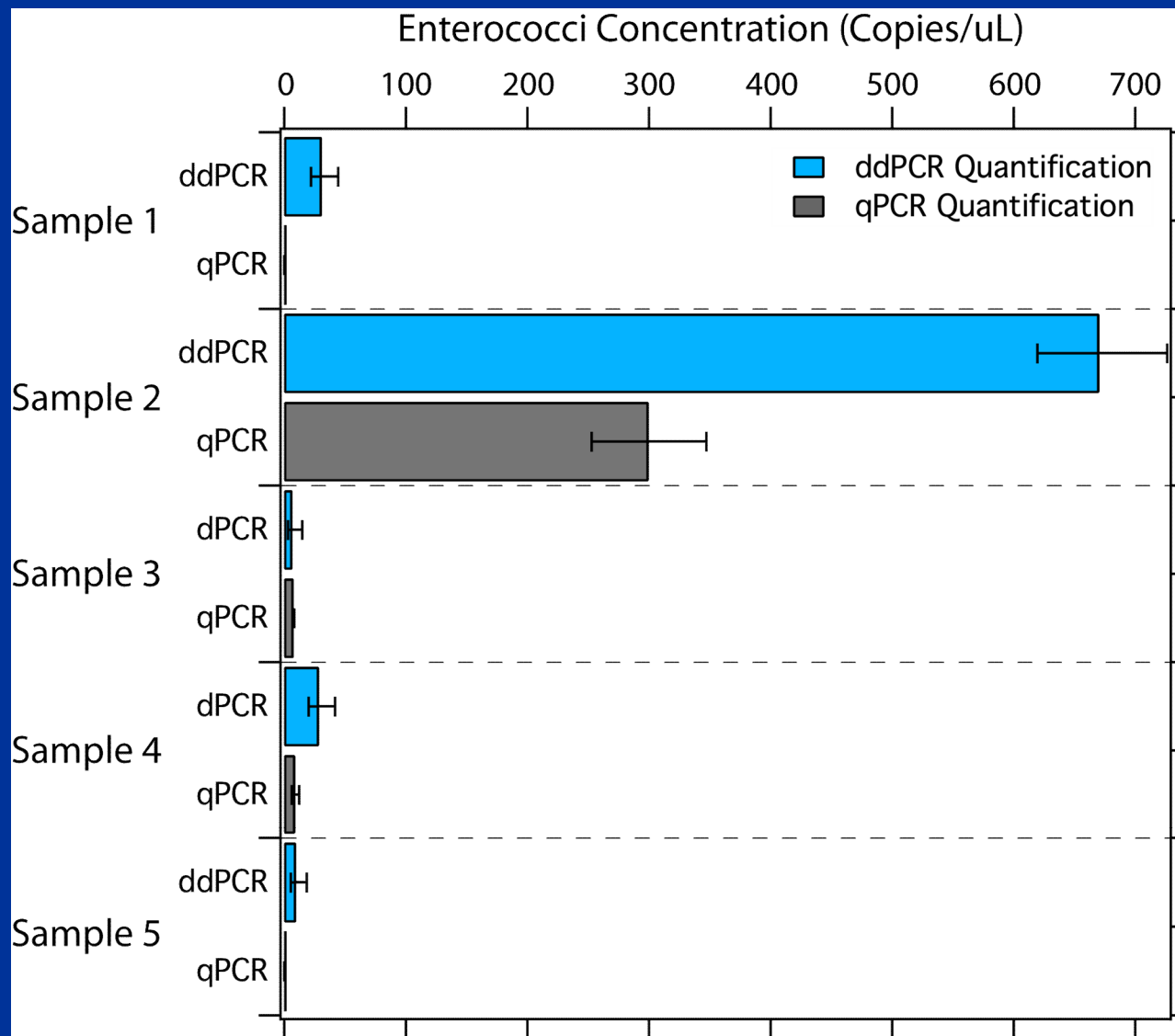
Digital Positive & Negative Droplets



DDPCR QUANTIFICATION OF ENTEROCOCCUS



QUANTIFICATION OF ENVIRONMENTAL SAMPLES USING ESP METHODS



CONCLUSIONS AND NEXT STEPS

- The *challenge of portable biological sensors* for water quality monitoring is *sample acquisition and processing for downstream analyses*
- **Modular microfluidic design makes this technology extremely adaptable to new applications**
 - Shape form extremely compact and malleable
 - Currently exploring drinking and reclaimed water applications
- **Field sampling trials will commence in late 2016 or early 2017**

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