Fluidion® ALERT:

Comprehensive In-situ Planktonic and Aggregate-Bound E.coli Monitoring for Reliable Risk Assessment

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Presented at:

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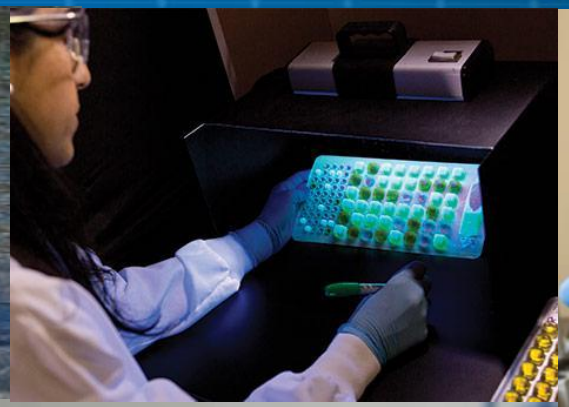
Water Intelligence

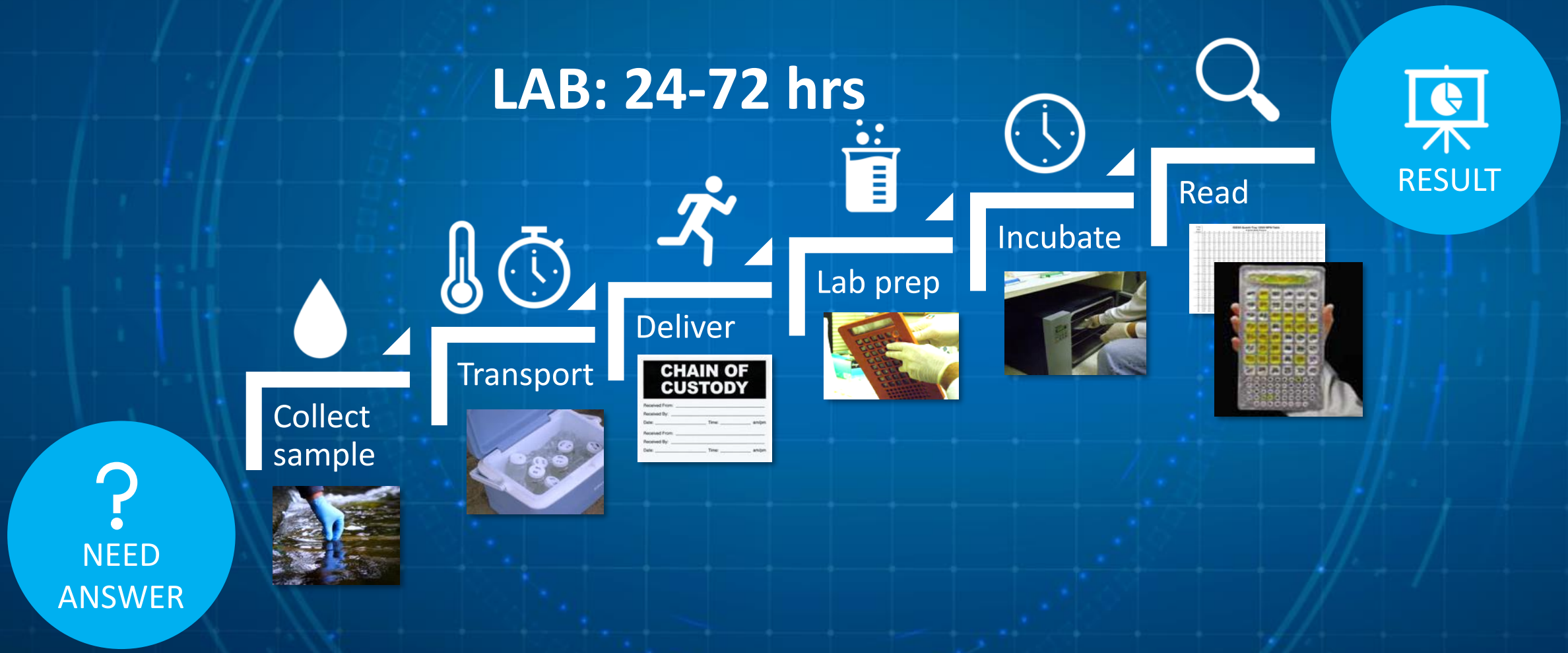
www.fluidion.com

Water sampling is personnel-intensive and costly

Fecal indicator bacteria (FIB) results today arrive too late for preventive action



Lab analysis is slow and involves complicated logistics



Lab analysis is slow and involves complicated logistics

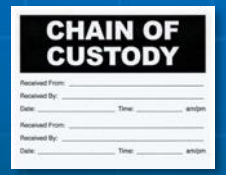
Fluidion delivers accurate results rapidly, automatically, online

LAB: 24-72 hrs

Collect sample

Transport

Deliver



Lab prep

Incubate

Read

RESULT

NEED ANSWER

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ALERT: 2-12 hrs

Fluidion® **ALERT**: Fully-automated microbiology in the field

Bring the lab to the sample, not the sample to the lab

ALERT V2

In-situ field analyzer
7 samples

- In-situ and portable measurement
- Multiple FIB targets: *E.coli*, coliforms, enterococci
- Automated sampling and remote data reporting
- Rapid results directly in the field
- Battery operated, GPS-tagged, IoT-enabled

ALERT LAB

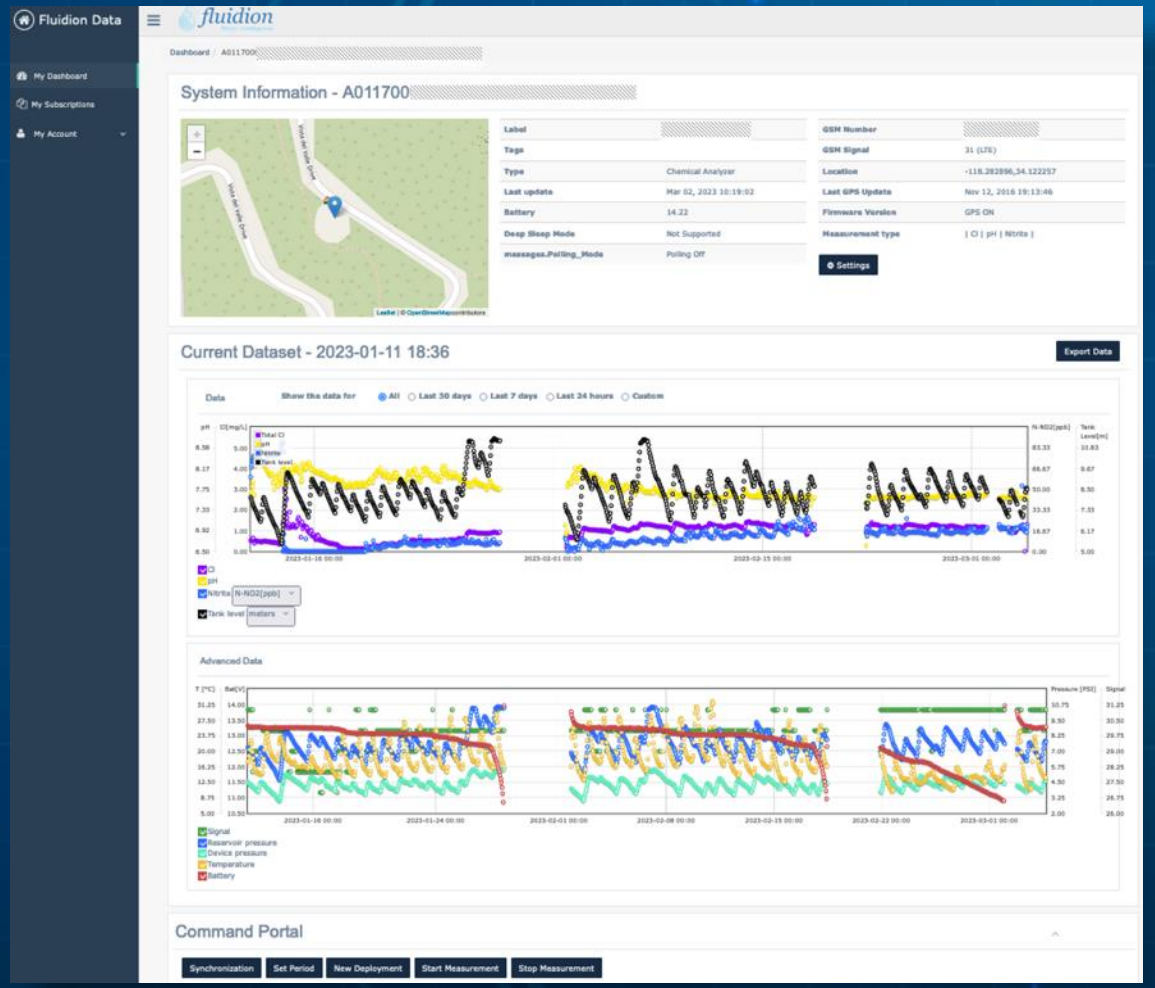
Portable field analyzer
6 samples

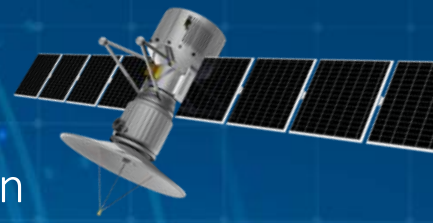
ALERT ONE

Handheld field analyzer
One sample

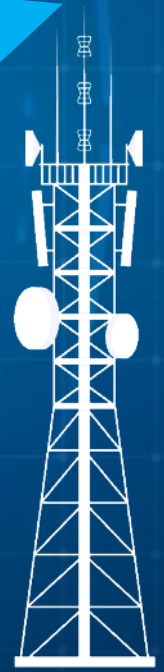
Powerful Cloud Data Analytics Interface

Next-generation IoT real-time control and remote data



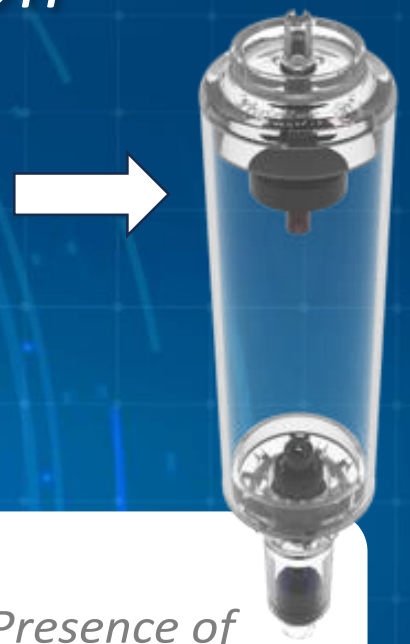


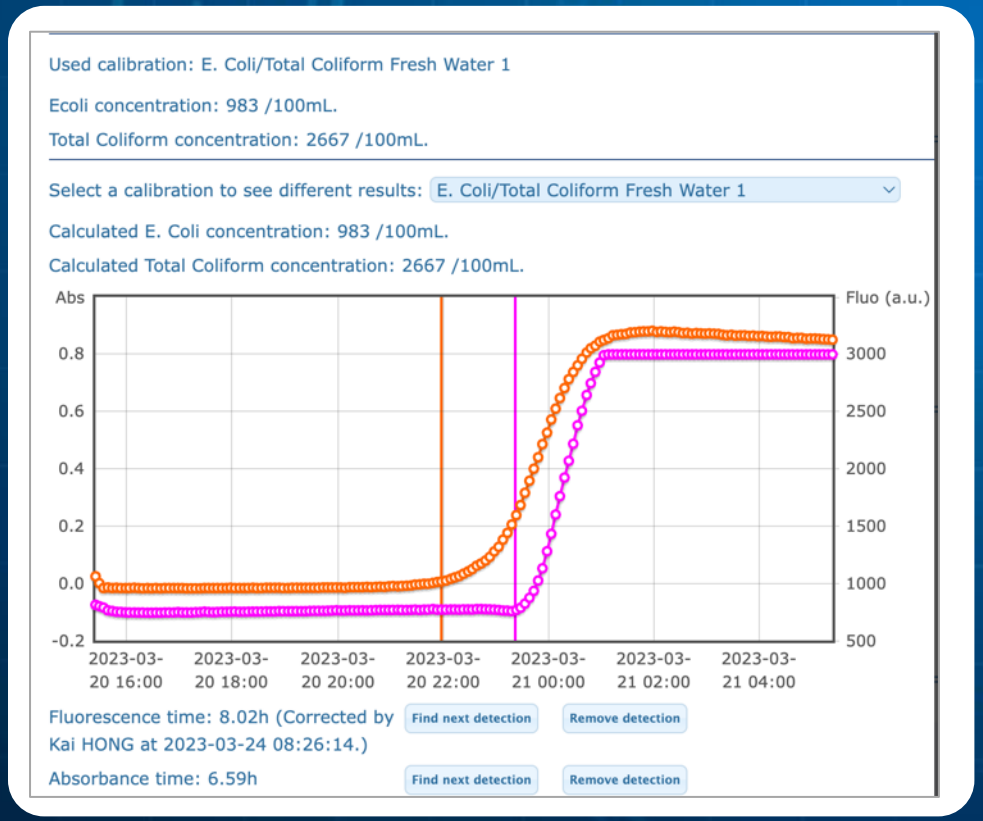
- Scalable worldwide IoT communication
- Real-time data visualization
- API-based SCADA integration
- Data as a Service (DaaS) option

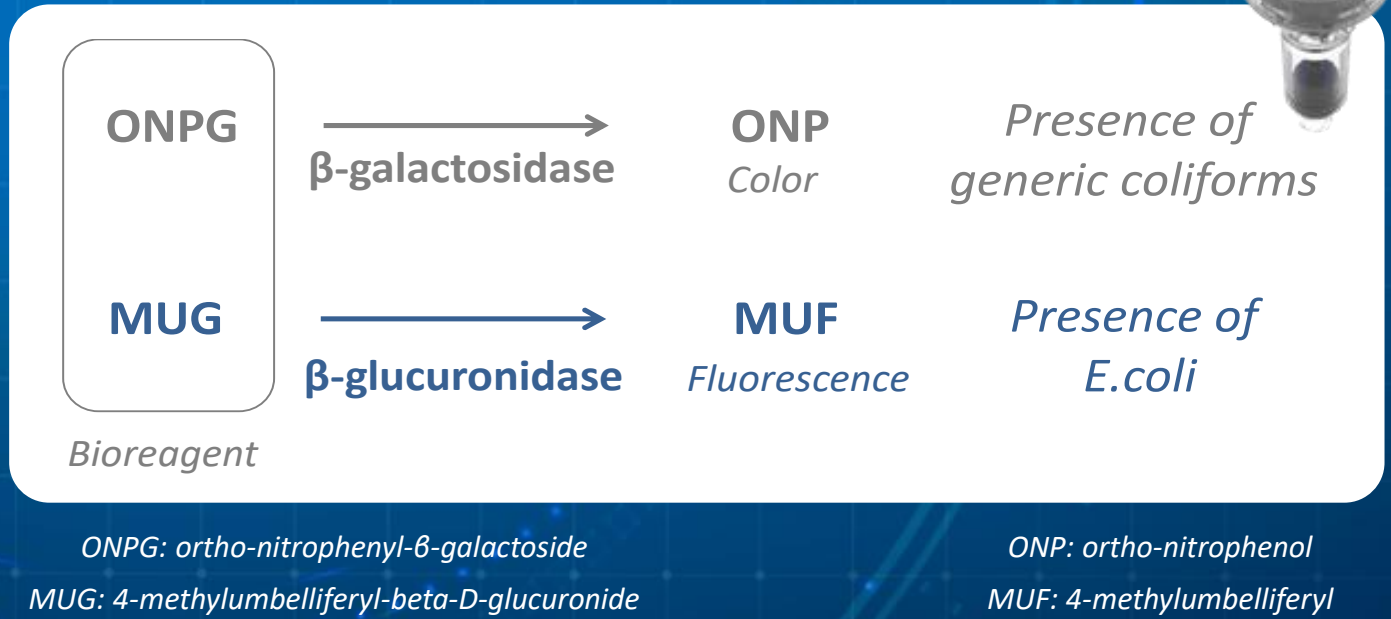


ALERT: Comprehensive viable, culturable *E. coli*

- Enzymatic reaction during **whole-sample** bacterial culture (incubation) in cartridge
- Time-resolved optical detection (real-time absorbance and fluorescence curves)
- Time-to-fluorescence linearly correlated with **log of total *E.coli*** in the sample
- Sensitive to **free and aggregate-bound *FIB***







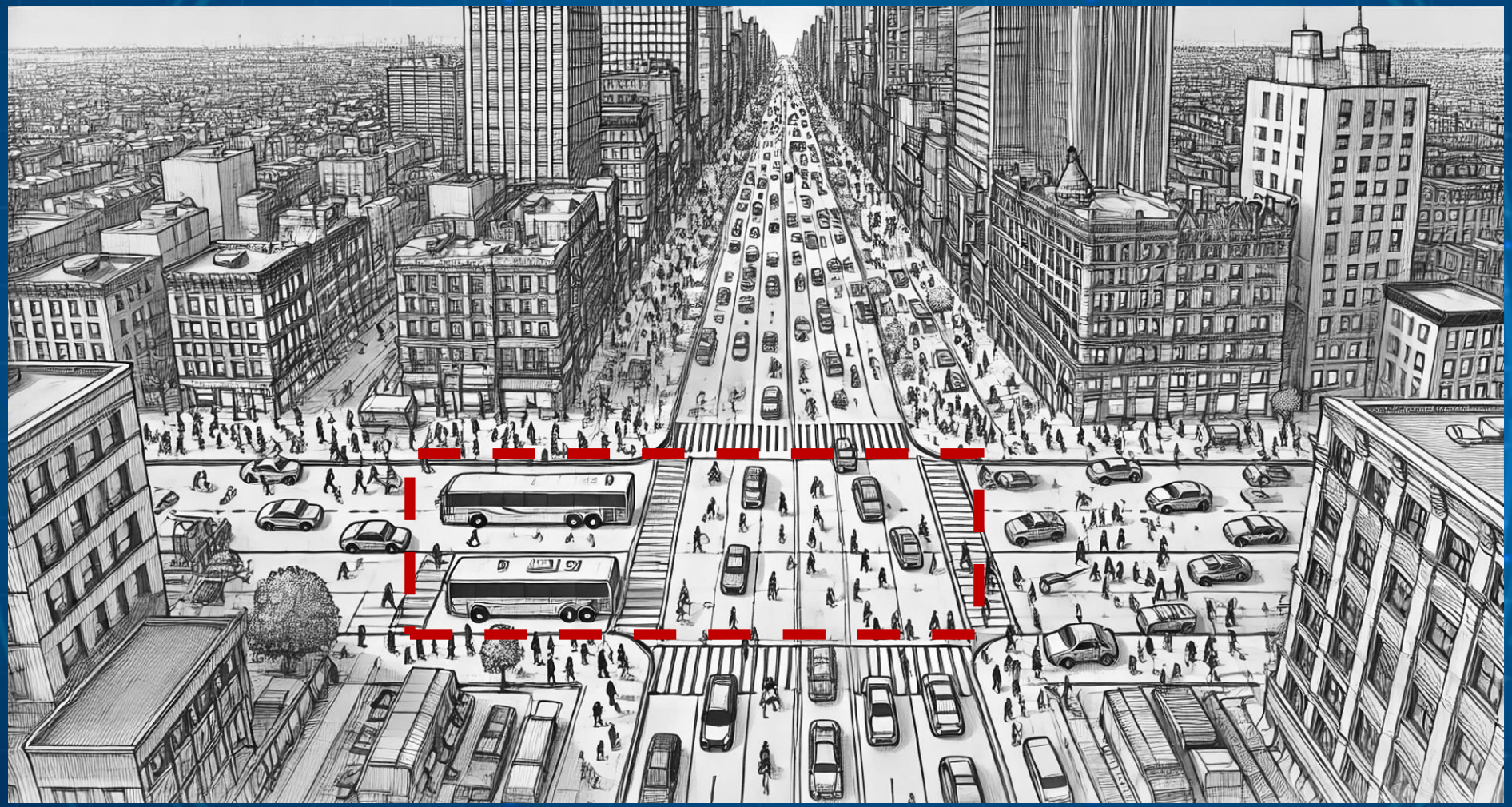
ONPG: ortho-nitrophenyl-β-galactoside

MUG: 4-methylumbelliferyl-beta-D-glucuronide

ONP: ortho-nitrophenol

MUF: 4-methylumbelliferyl

Laboratory MPN vs Planktonic vs Comprehensive *E.coli* counts



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Aggregates are a natural byproduct of raw sewage degradation

- Raw sewage (untreated fecal matter) is highly non-homogenous
- CSOs and stormwater can be responsible for significant pollution with untreated fecal matter
- Some treatment plants may release waters with significant suspended solids

- Aggregates are the end-product of the physical degradation process
- Small-size aggregates remain suspended in the water column, just like free *E.coli*
- Chemical micro-environment: resilience to disinfection processes (UV, chemical)
- Protection from environmental stressors
- Potent infection vectors for fecal pathogens

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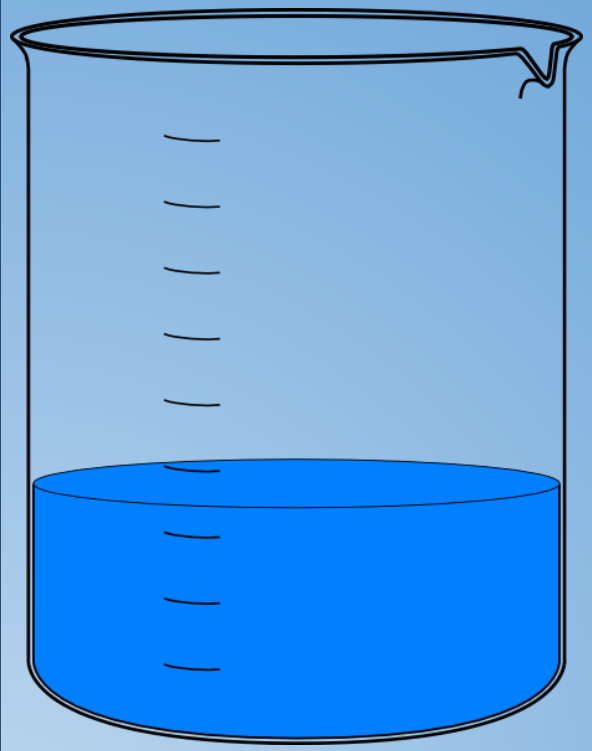
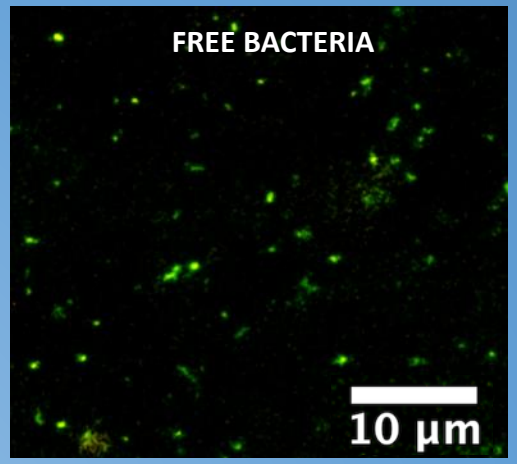
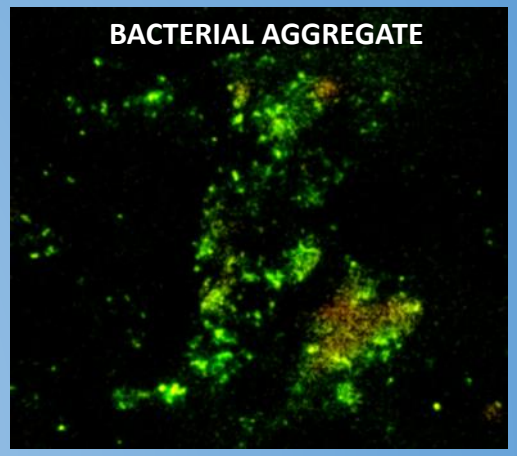
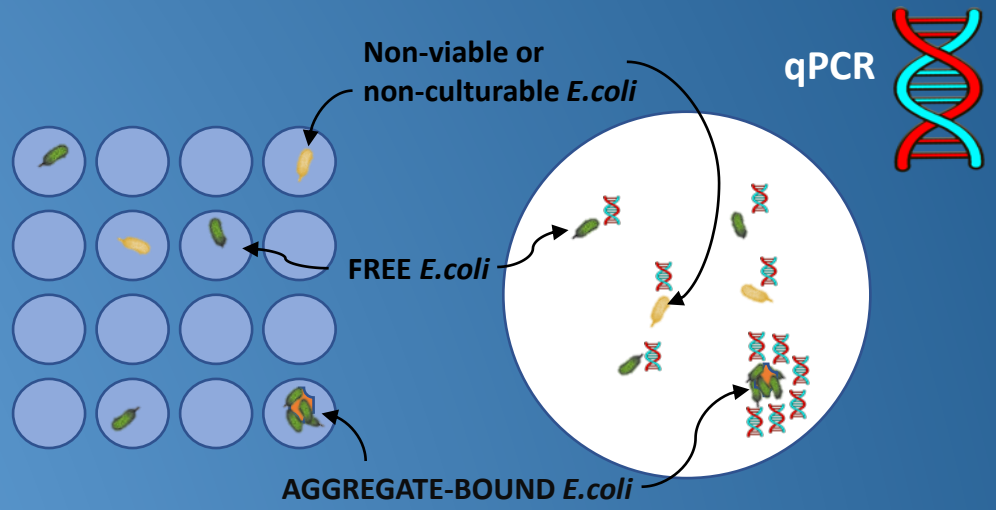
Current culture-based lab methods insensitive to aggregates
Molecular methods (qPCR) lack specificity to culturable cells

0.4µm Filtration + DNA Staining + Epifluorescence Microscopy

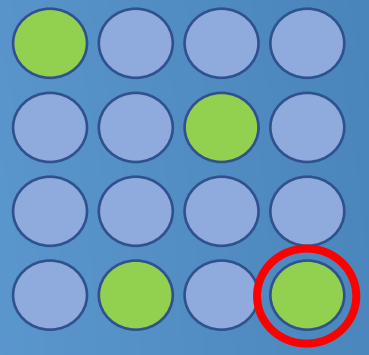






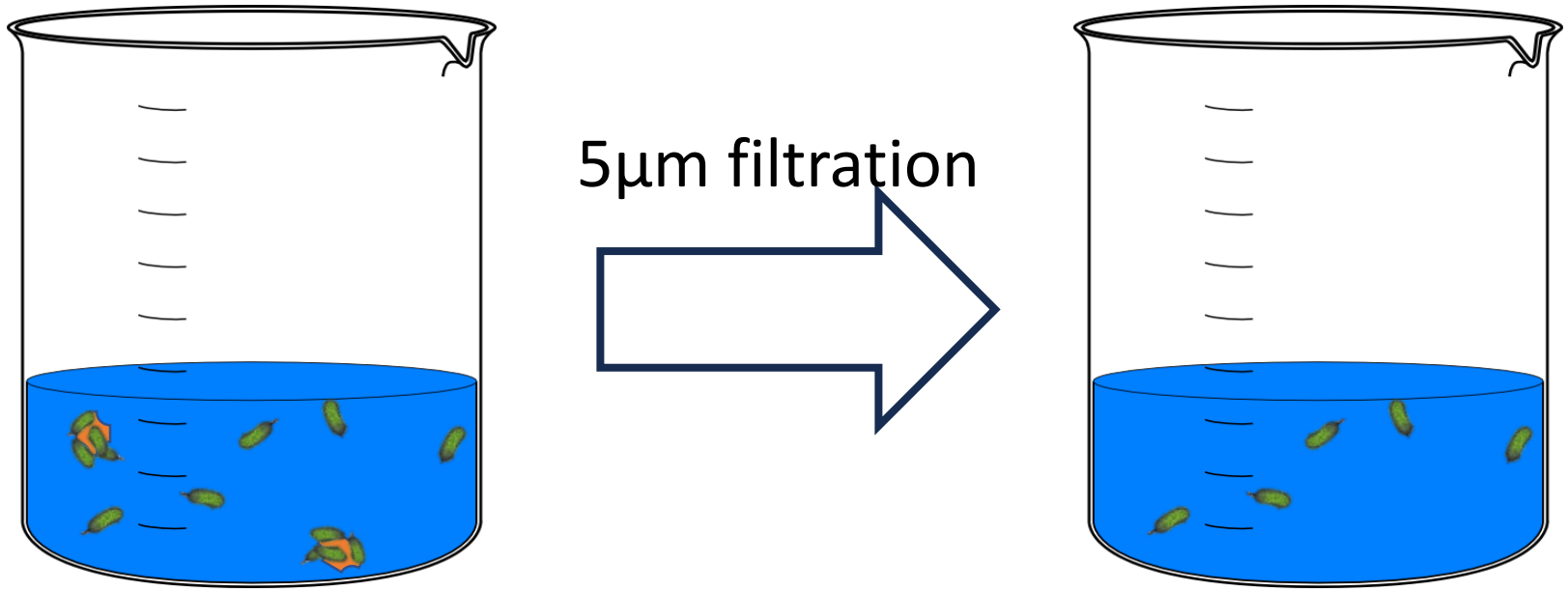


MPN methods (Colilert)



Membrane Filtration

Size fractionation study of aggregates



Raw MPN count: 7

Raw ALERT count: 13

5µm filtered MPN count: 5

5µm filtered ALERT count: 5

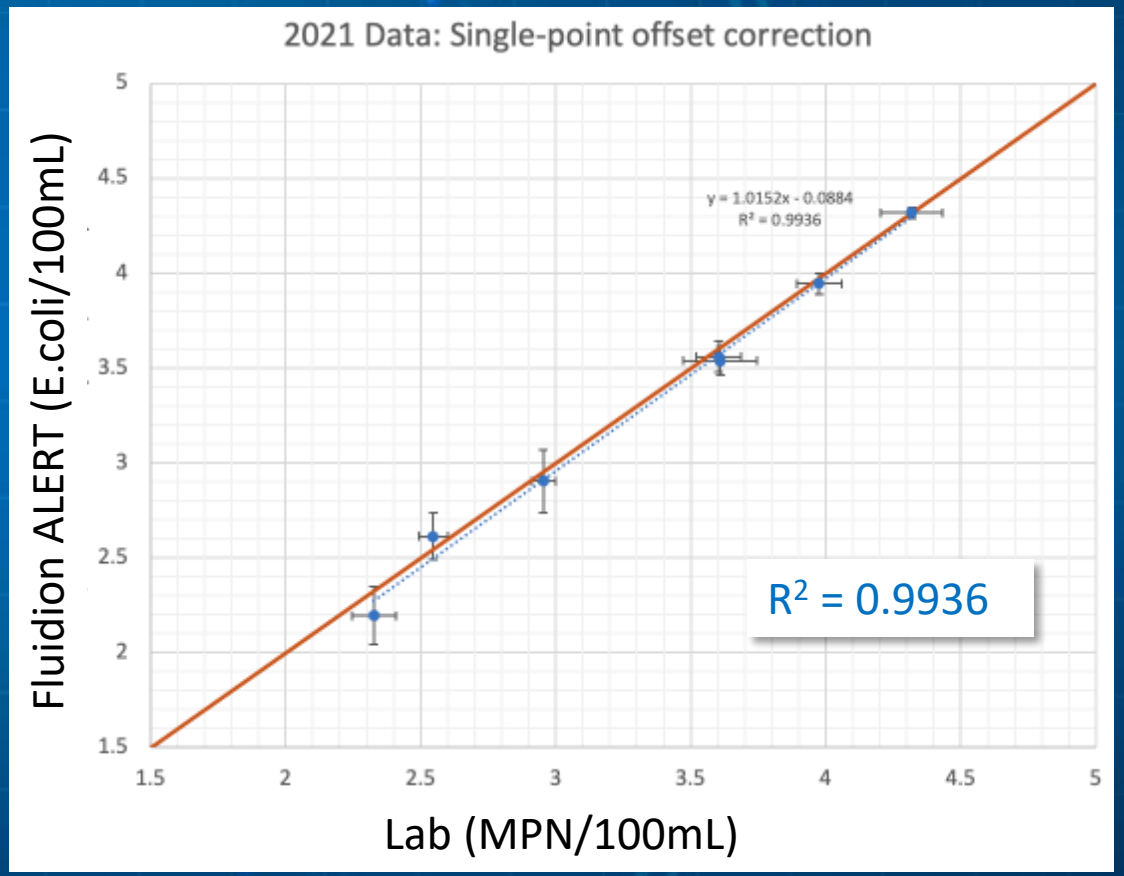
13-5=8 total FIB load on aggregates>5µm

7-5 = 2 aggregates >5µm

FIB per aggregate >5µm = (13-5)/(7-5)=4

ALERT repeatability study: agreement with MPN lab method (all aggregates removed by 5µm pre-filtration)

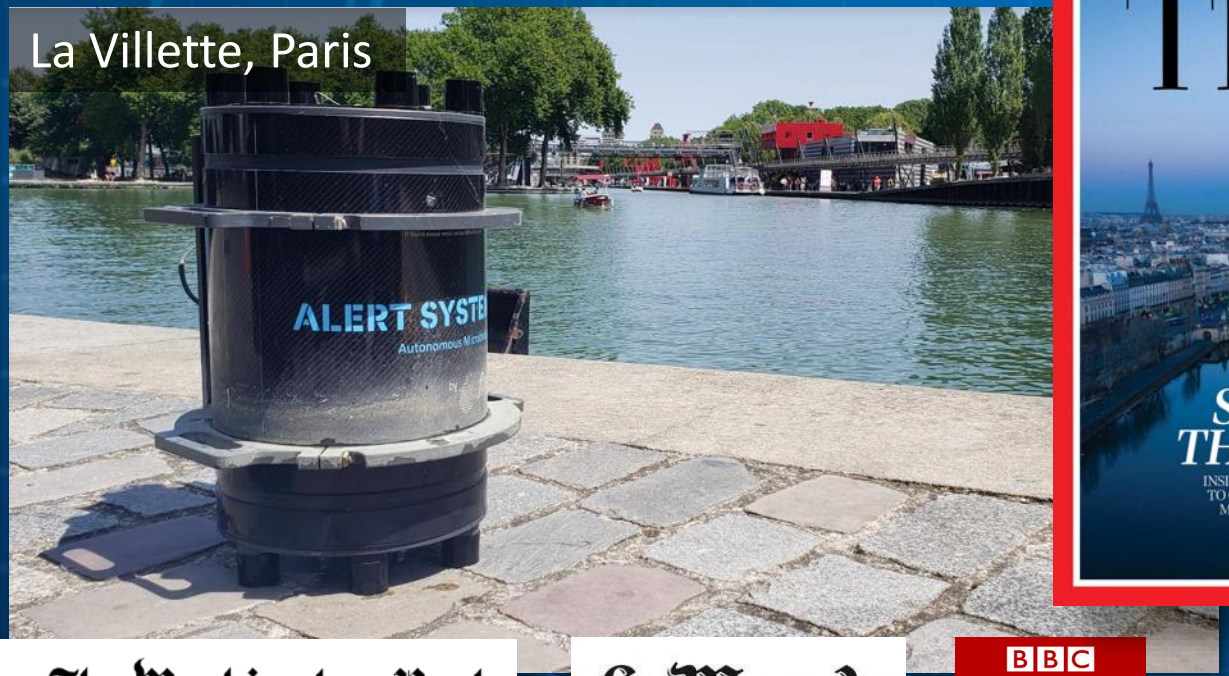
- Side-by-side testing against MPN method
- River water samples spiked with 5µm-filtered wastewater treatment plant effluent

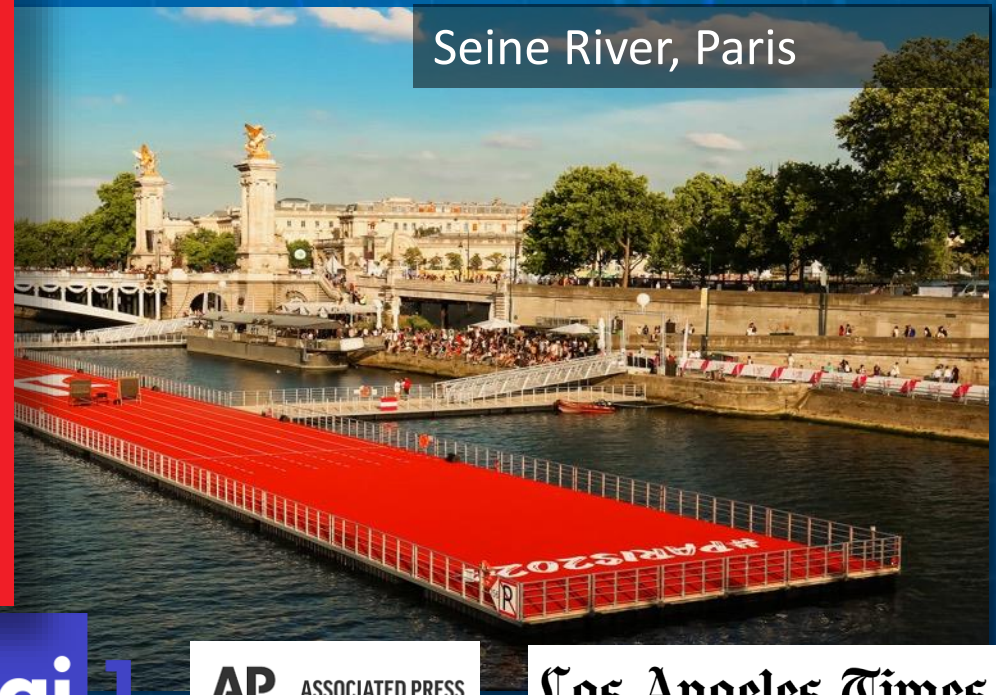


Presented at: Analytica Conference, 2022

ALERT: Monitoring Seine River and Paris bathing sites

2016	Fluidion partners with City of Paris for water quality monitoring
2017	Villette basin becomes first-ever approved Paris open-water swim site
2017-2023	High-frequency monitoring in preparation for Olympic Games
2025	Open-water swimming areas planned to be opened to the public





The Washington Post · Le Monde · BBC WORLD NEWS · NBC NEWS · Rai 1 · AP ASSOCIATED PRESS · Los Angeles Times

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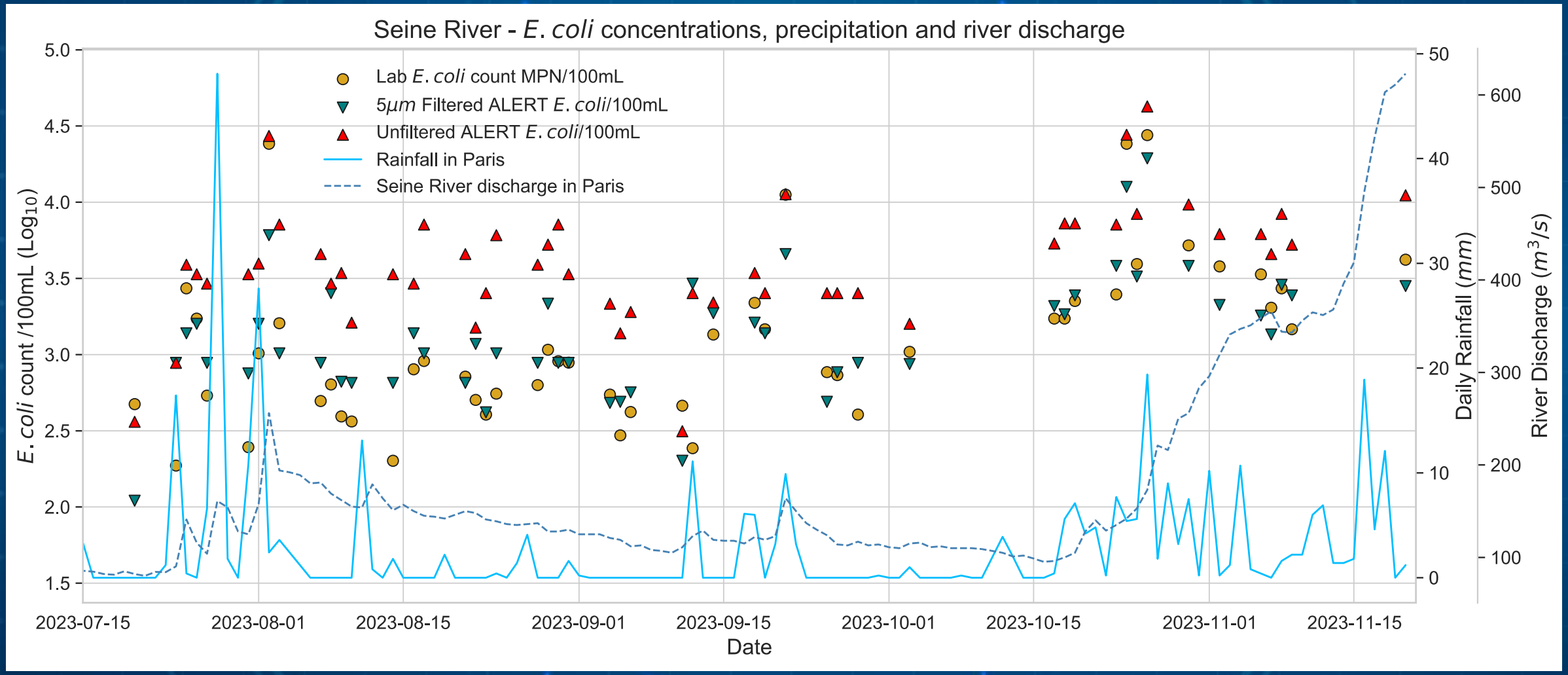
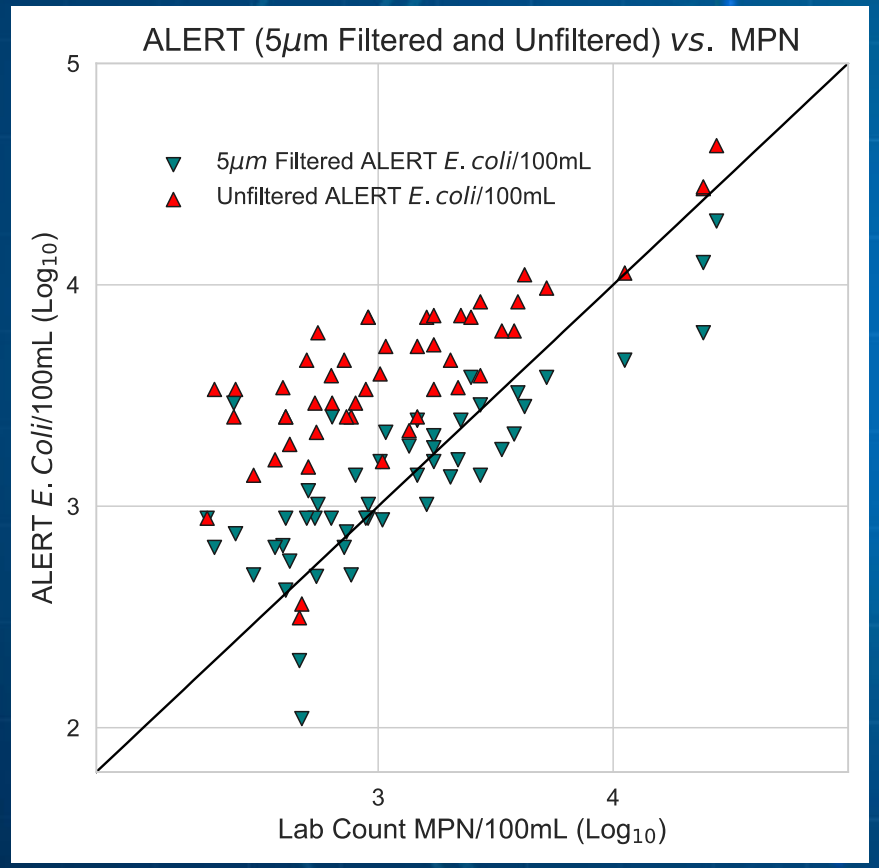
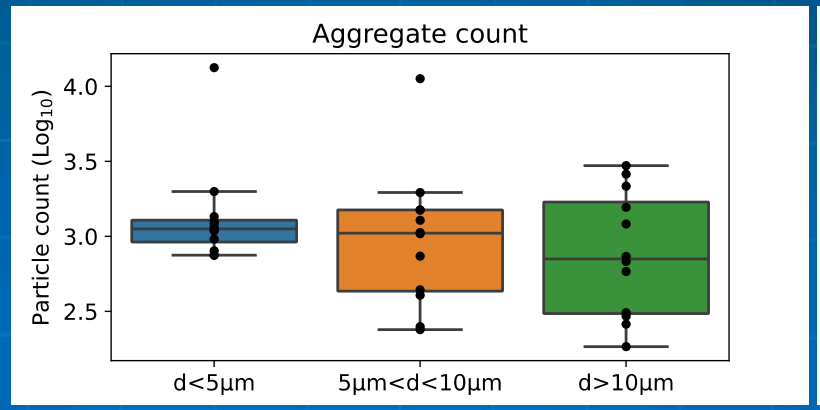
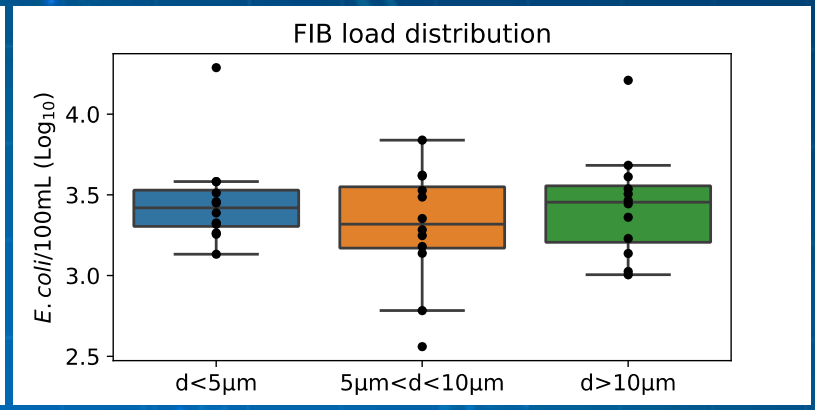
2023 *E. coli* concentrations in Seine river

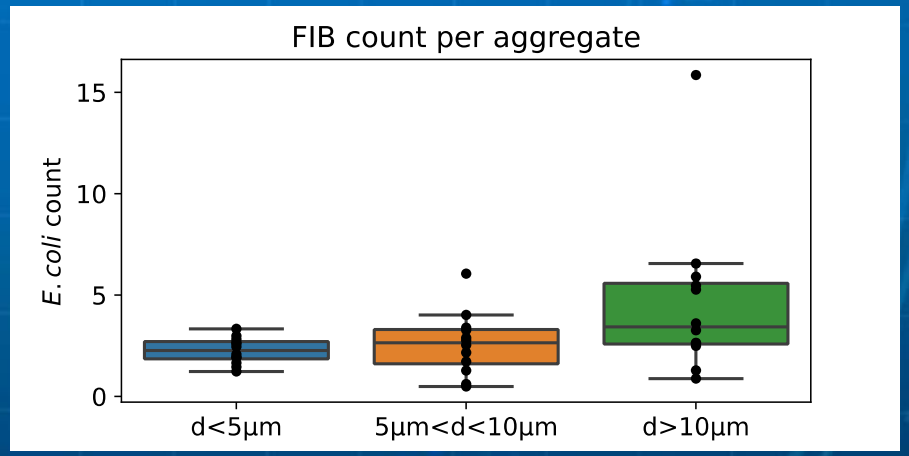


Size fractionation study of aggregates in Seine river (summer 2023 samples)









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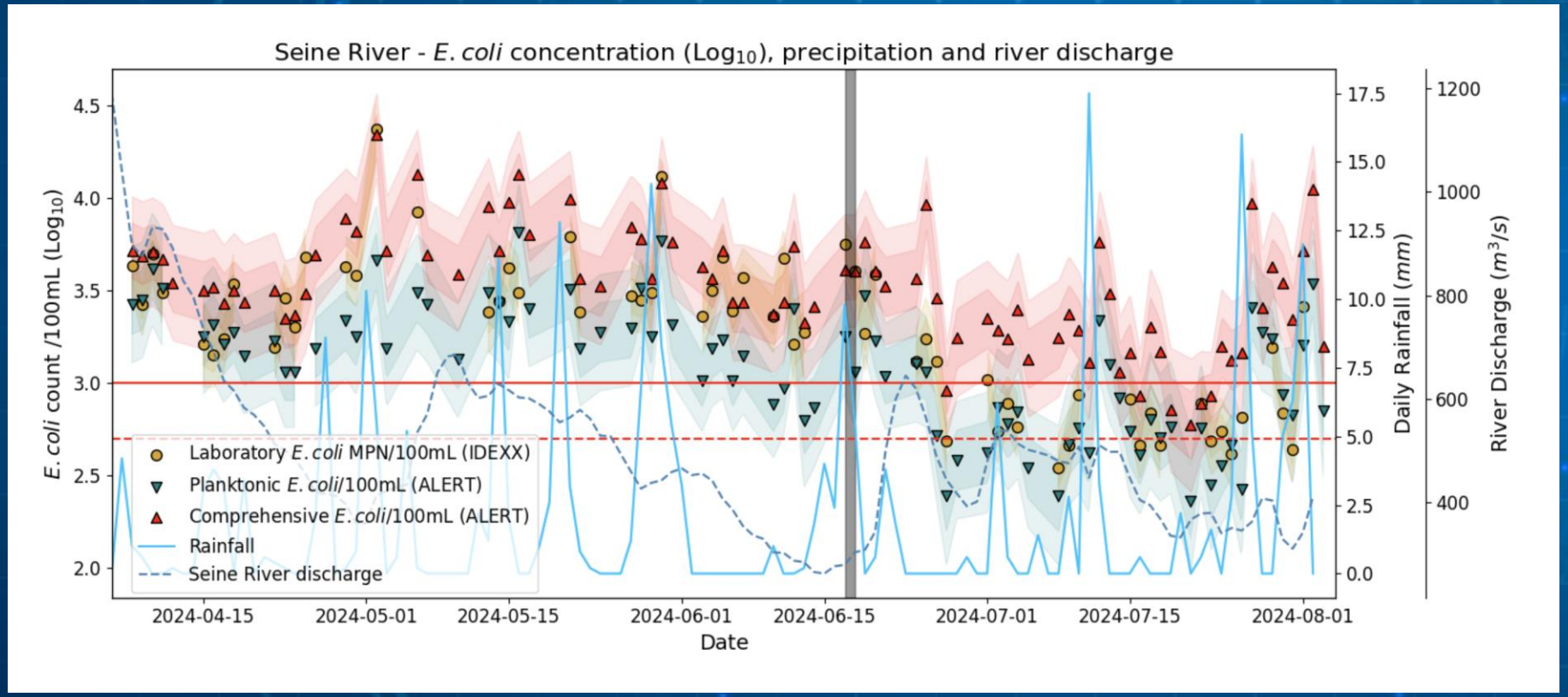
https://fluidion.com/open-data-initiative/2024-seine-water-quality

Fluidion's 2024 Olympics Open Data Initiative



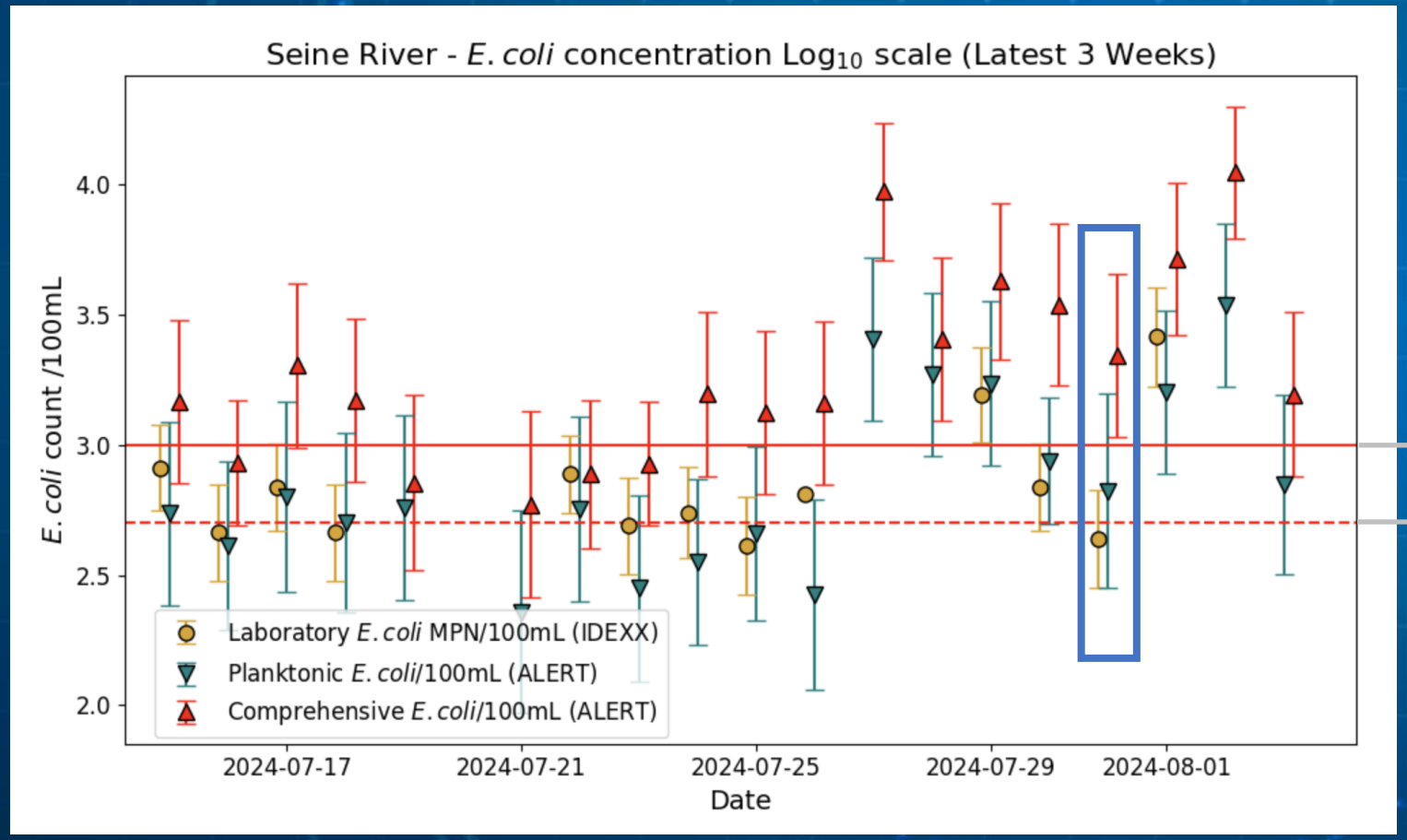
Sampling occurs at the Alexandre III bridge in Paris, the designated location for some of the 2024 Olympic aquatic events

https://fluidion.com/open-data-initiative/2024-seine-water-quality

Focus on the Latest 3 weeks of Seine River data

Culture-based E.Coli results:

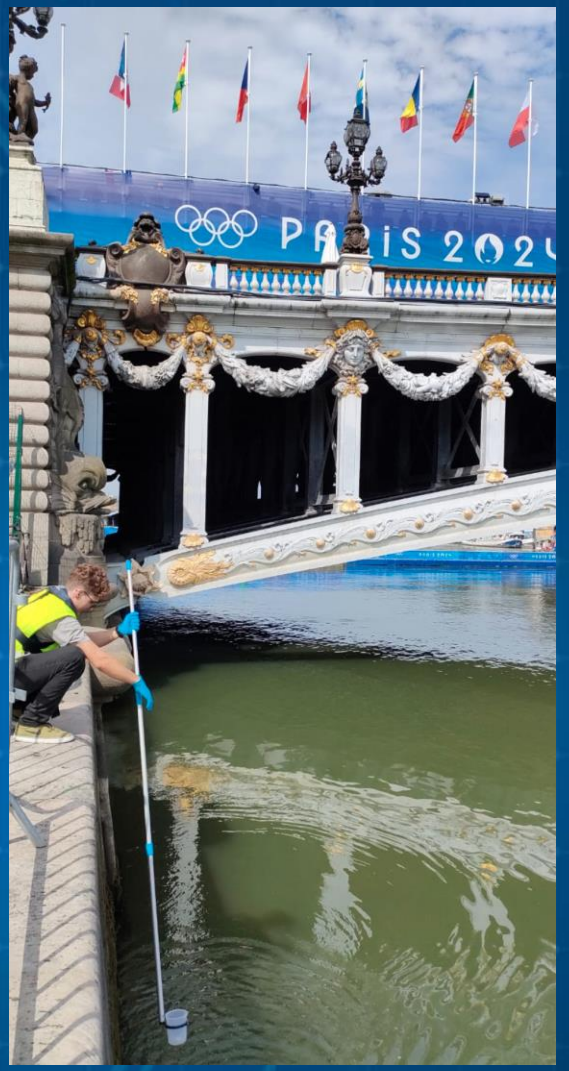
Comprehensive (ALERT-unfiltered) vs Planktonic (ALERT-filtered) vs Laboratory (IDEXX)



World Triathlon's guidelines for sufficient and good inland water quality

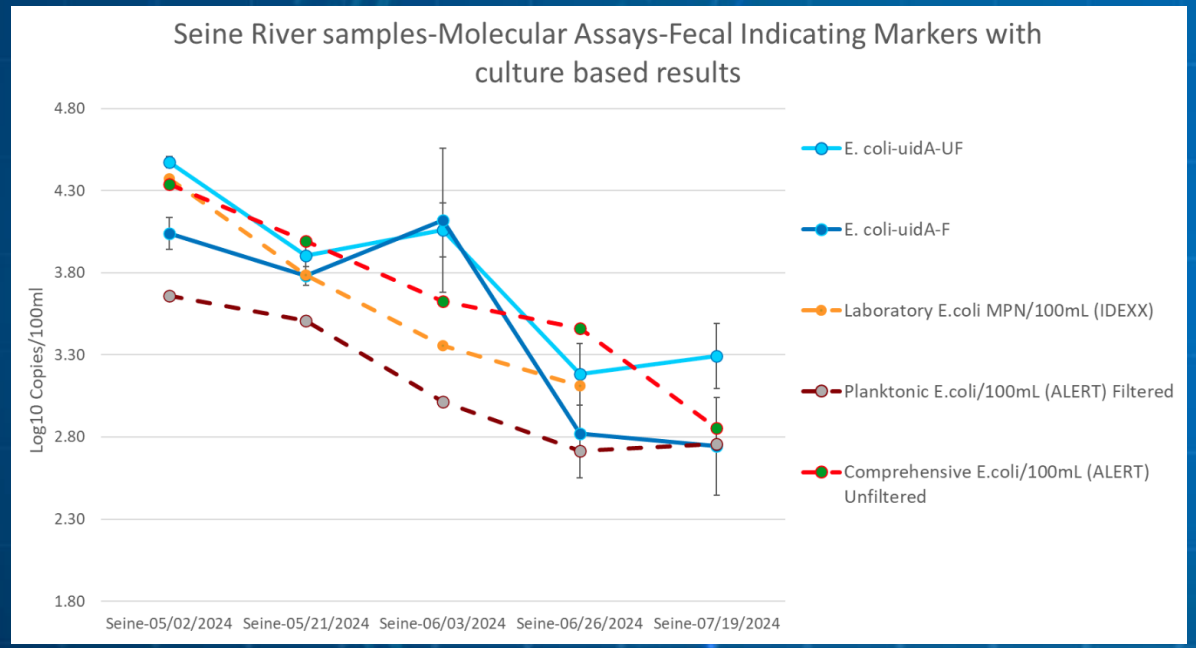
→ 1000 *E.coli* / 100mL

→ 500 *E.coli* / 100mL

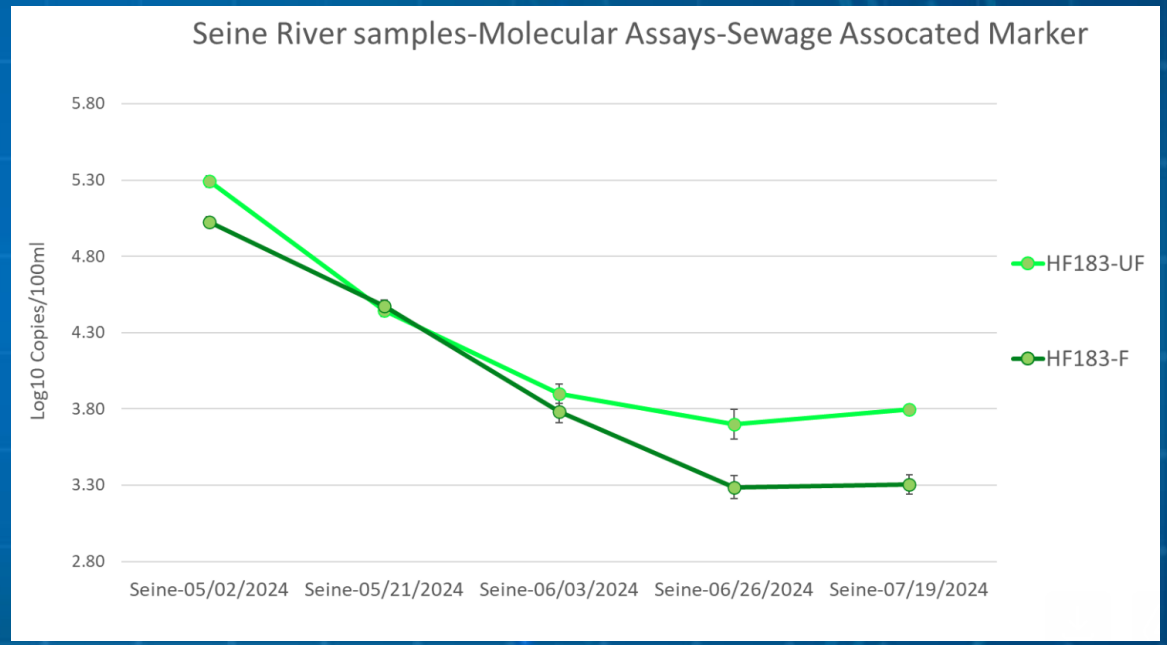


Seine River samples: Culture-based vs qPCR results

Fecal indicating marker: *E.coli*



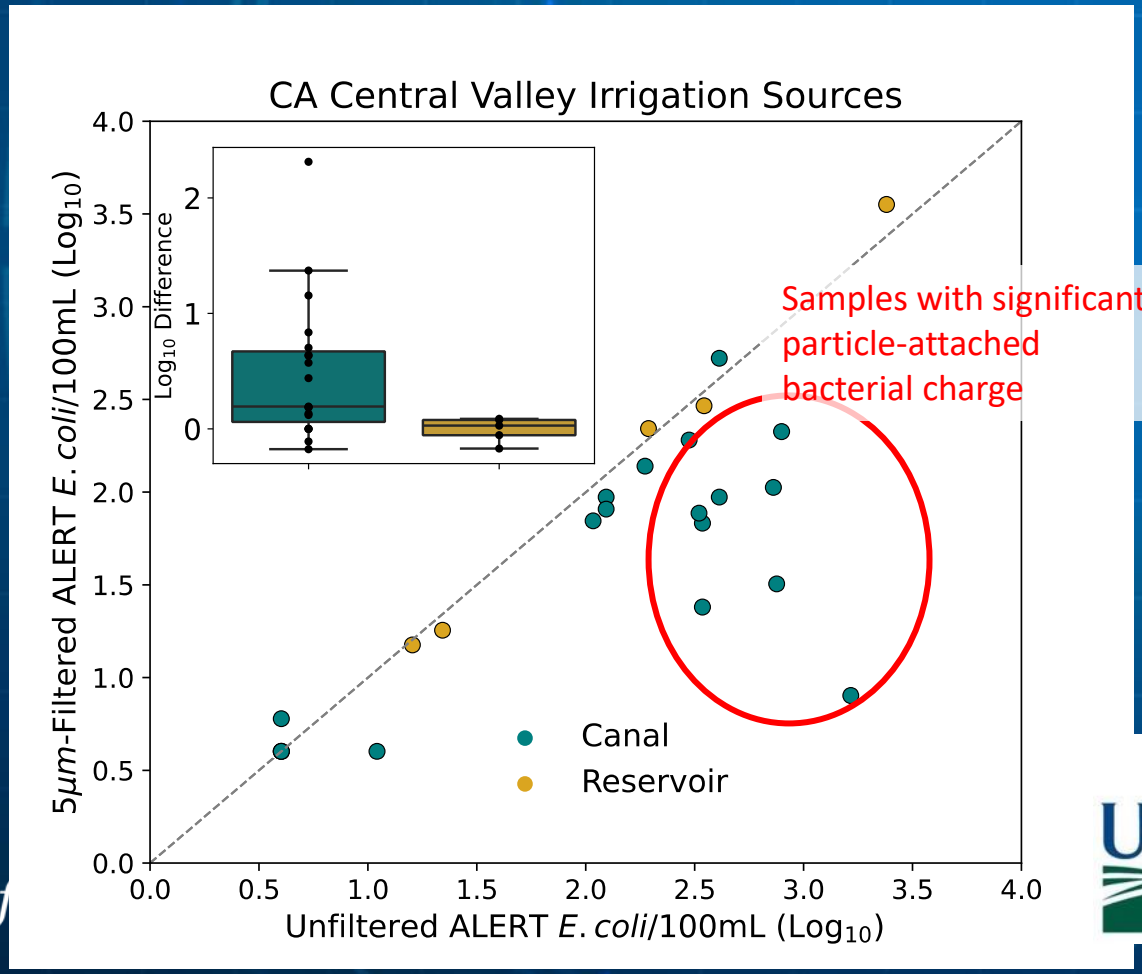
Sewage associated marker: HF183



- Molecular *E.coli* results (E.coli-uidA) follow the same trend as culture-based methods (ALERT & IDEXX)
- HF183 (sewage marker) shows similar effects of sample filtering (0.45um)

Agricultural irrigation samples (Yuma, AZ, USA)

- Working with Prof. Channah Rock (U. of Arizona) and Prof Trevor Suslow (U.C. Davis)
- Performing side-by-side analysis ALERT / Quantitray for Ag samples
- Goal: prevent *E.coli* outbreaks from leafy greens consumption



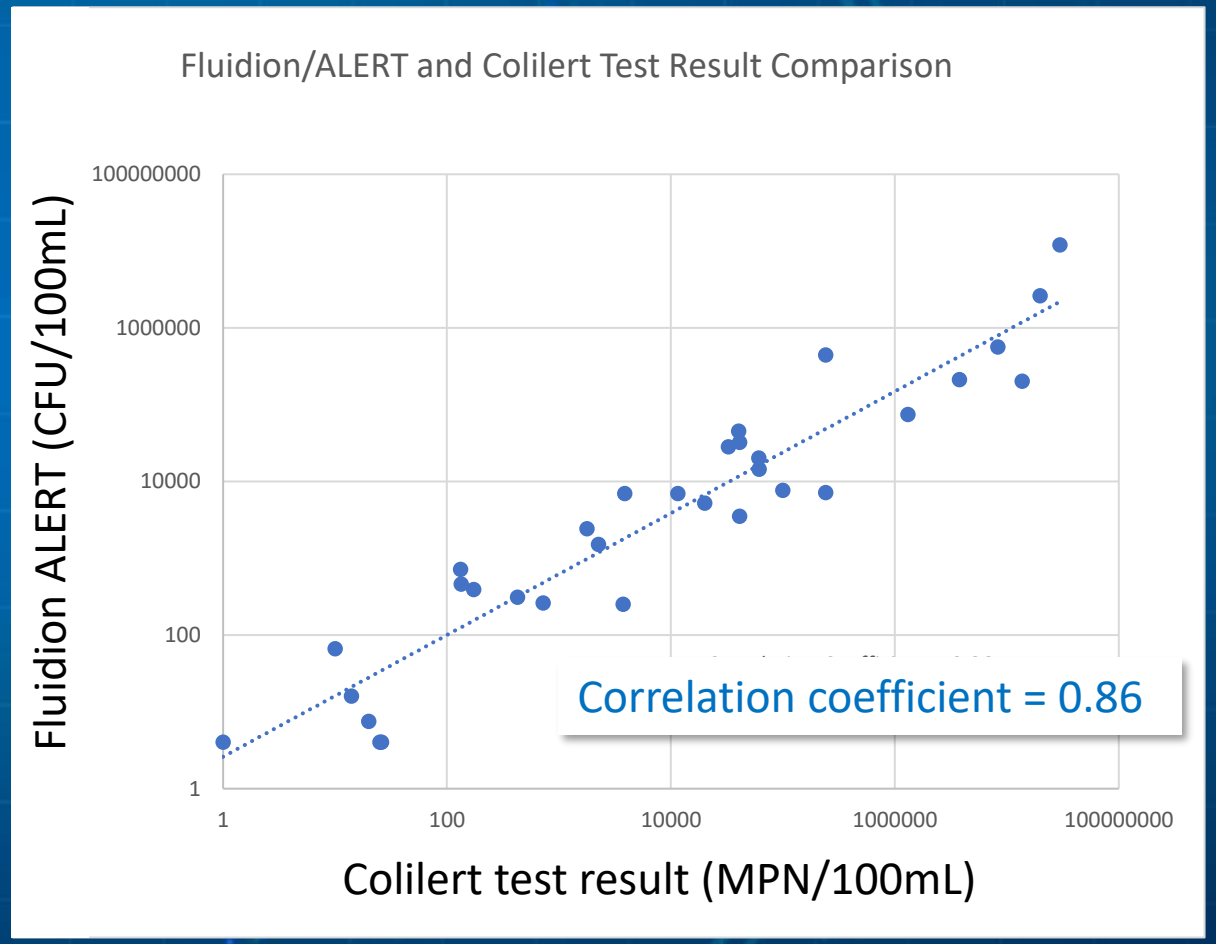
USDA NIFA

UC DAVIS UNIVERSITY OF CALIFORNIA

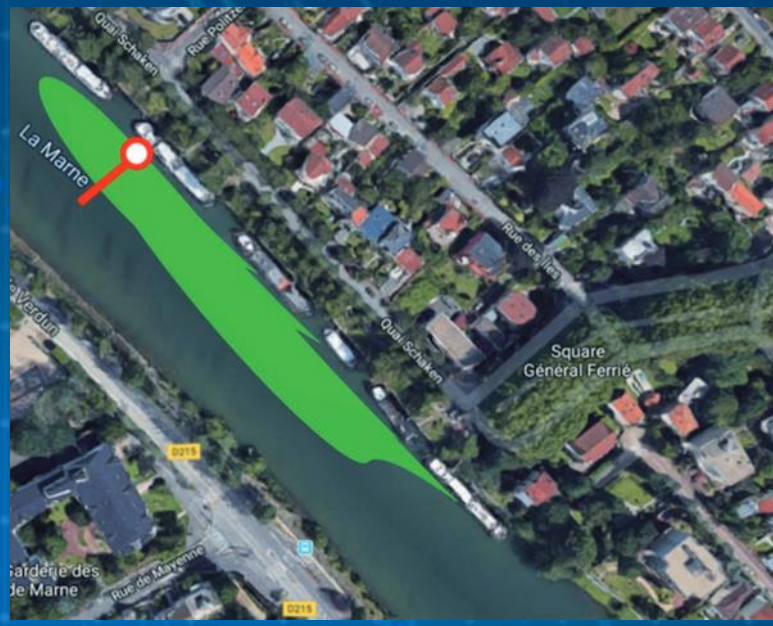
THE UNIVERSITY OF ARIZONA Environmental Science

Tijuana River: Identifying sewage dumping

- Excellent agreement with EPA-approved lab method over 8 LOG units
- Evaluated and validated by the San Diego RWQCB, investigative order issued



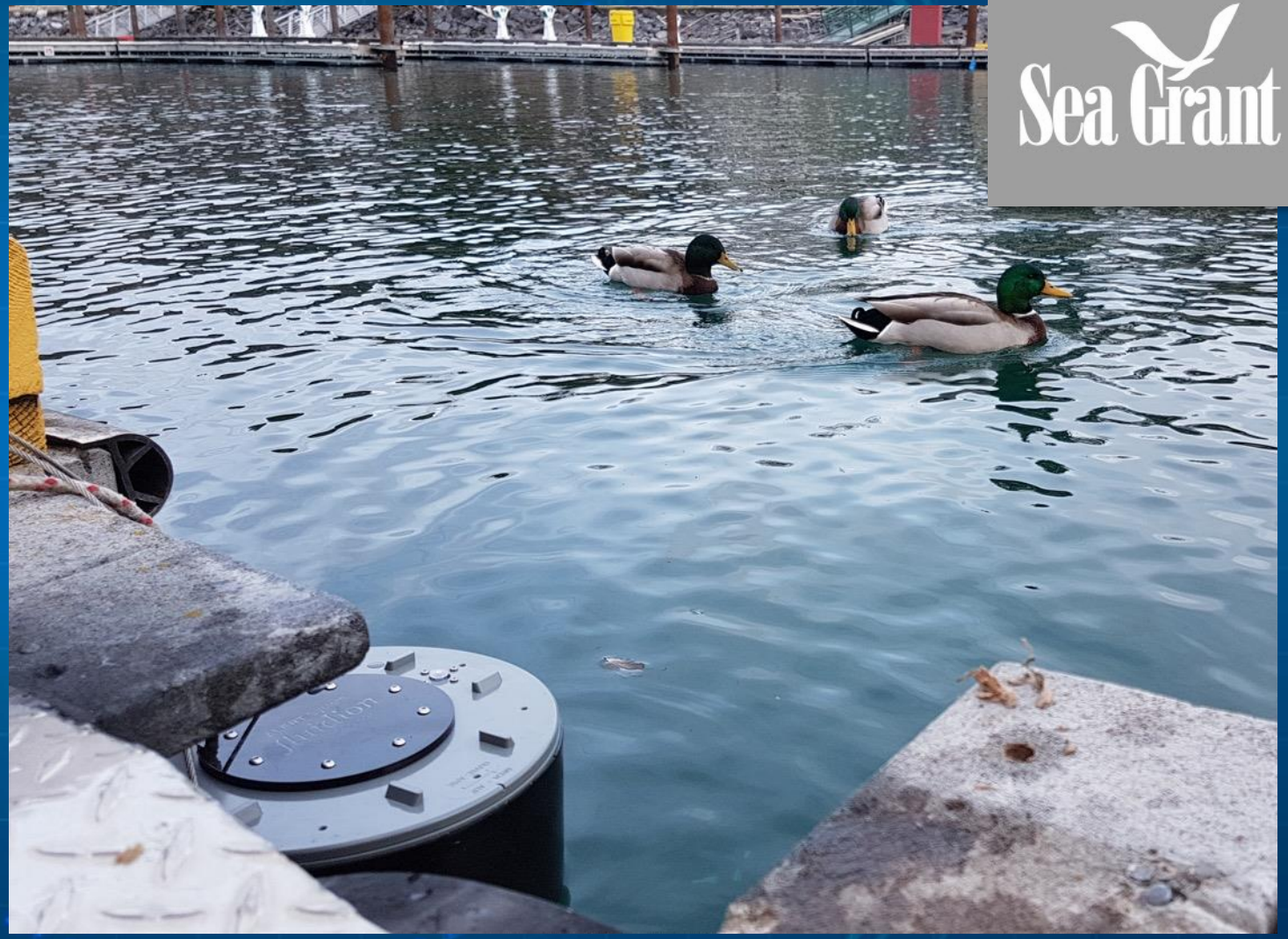
Measuring impact of discharges from live-in boats



Lake Chelan: Monitoring water safety

Lake Chelan Research Institute

Sea Grant



Monitoring urban lakes, and getting creative !

Deployment in SC

Photo Credits:
Michael Long, James Riddle, Woolpert

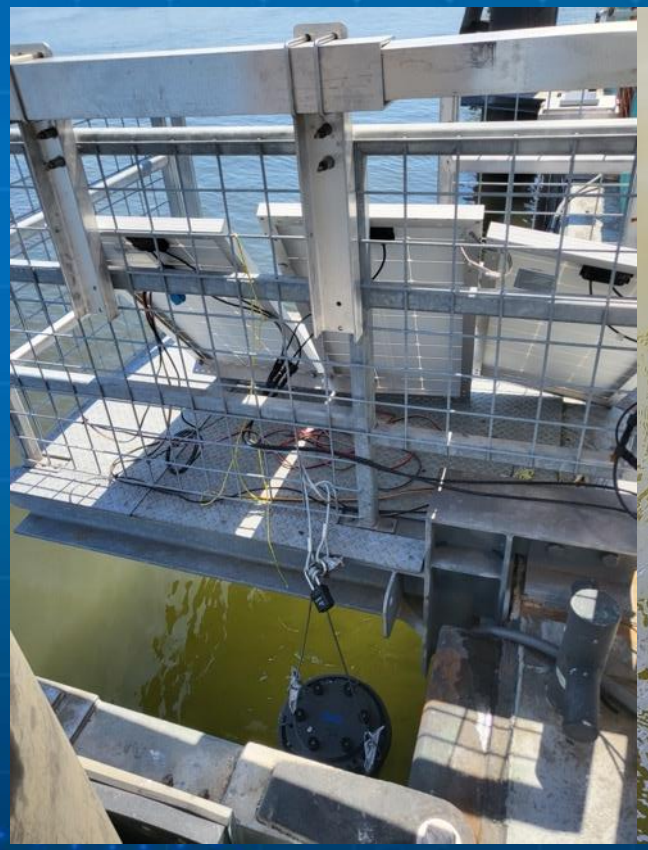
Photo Credits:
Kevin Dimzon, LASAN

LA Sanitation Watershed Protection

ALERT V2: high-frequency monitoring in urban rivers

Photo credits: DRBC
https://www.nj.gov/drbc/programs/quality/bacteria.html#3

Undergoing validation in the field in NJ, NY, PA, and MD



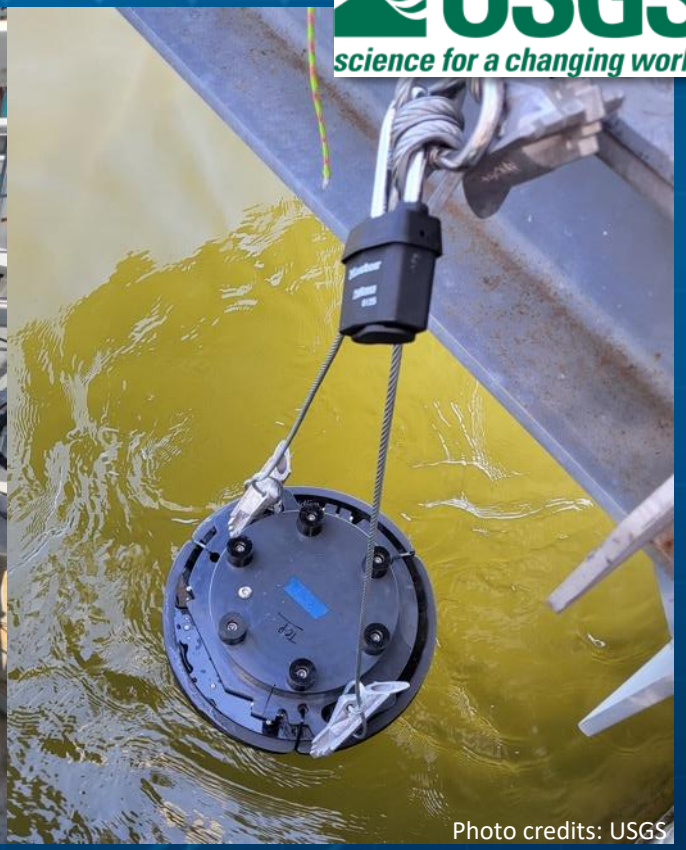


Photo credits: USGS

Thank you

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Water Intelligence

For further information:
Email: contact-us@fluidion.com
www.fluidion.com

Comparing FIB measurement methods: MPN,MF

MF

MPN

EPA approved

Specific to viable, culturable cells

Relatively simple lab work

Widespread

Lab-only, not automated

Insensitive to aggregates

Slow measurements

Expensive logistics, OPEX

MF and MPN count the number of *FIB*-containing entities

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Comparing FIB measurement methods: qPCR, ddPCR

MF, MPN		EPA approved Specific to viable, culturable cells Relatively simple lab work Widespread	Lab-only, not automated Insensitive to aggregates Slow measurements Expensive logistics, OPEX 
ddPCR, qPCR		EPA protocols in development **Sensitive to aggregates** Fast measurements (2-6 hrs)	Lab-only, not automated **Non-specific to culturable cells** Complex lab work Not widely available yet Expensive logistics, OPEX 

qPCR and ddPCR methods count the comprehensive number of *FIB* gene copies

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Comparing FIB measurement methods: proxies

MF, MPN		EPA approved Specific to viable, culturable cells Relatively simple lab work Widespread	Lab-only, not automated Insensitive to aggregates Slow measurements Expensive logistics, OPEX 
ddPCR, qPCR		EPA protocols in development Sensitive to aggregates Fast measurements (2-6 hrs)	Lab-only, not automated Non-specific to culturable cells Complex lab work Not widely available yet Expensive logistics, OPEX 
Enzymatic, Tryptophan		Fully automated, simple logistics Field-ready: In-situ Real-time measurements Possibly sensitive to aggregates	Measures a proxy, innacurate **No equivalence to cell count** **Non-specific to culturable cells** Not widely accepted 

Enzymatic and Tryptophan are only simple-to-measure proxies

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Comparing FIB measurement methods: ALERT

MF, MPN		EPA approved Specific to viable, culturable cells Relatively simple lab work Widespread	Lab-only, not automated Insensitive to aggregates Slow measurements Expensive logistics, OPEX 
ddPCR, qPCR		EPA protocols in development Sensitive to aggregates Fast measurements (2-6 hrs)	Lab-only, not automated Non-specific to culturable cells Complex lab work Not widely available yet Expensive logistics, OPEX 
Enzymatic, Tryptophan		Fully automated, simple logistics Field-ready: In-situ Real-time measurements Possibly sensitive to aggregates	Measures a proxy, innacurate No equivalence to cell count Non-specific to culturable cells Not widely accepted 
Fluidion ALERT		Passes site-specific EPA, WHO tests **Sensitive to aggregates** **Specific to viable, culturable cells** Field ready, Automated, Rapid (2-12 hrs)	Moderate CAPEX levels Periodic system maintenance (in-situ) 

fluidion Water Intelligence

ALERT: comprehensive count of viable and culturable *FIB*

Fluidion® ALERT: Accelerating Water Quality Monitoring

	Grab sample + Lab analysis	fluidion Water Intelligence ALERT
Time to Result	Very slow: Days to weeks	✓ Fast and online: Hours.
Sample Collection	Skilled personnel. Risks and human error	✓ Automated. Zero risk.
Logistics	Ship on ice. Limited holding time	✓ None: in the field, *in-situ*
Cost	Labor + Shipping + Lab analysis	✓ Cartridge or Vial
Accuracy	Blind to aggregate-bound bacteria	✓ Measures comprehensive *FIB*

Hunting down illicit connections…

When the team started work, the only way to track misconnections was by placing cages in drains to look for evidence of toilet paper. But the teams are now issued with **electronic 'Fluidion' testing devices** which measure telltale traces of wrongly connected facilities allowing more to be found and investigations to be quicker.

Bringing new technology to the task of tracking down these sources of pollution is **truly game changing**. **Each time a connection is rerouted, pollution is instantly cut improving bathing water quality and protecting wildlife and habitats.**

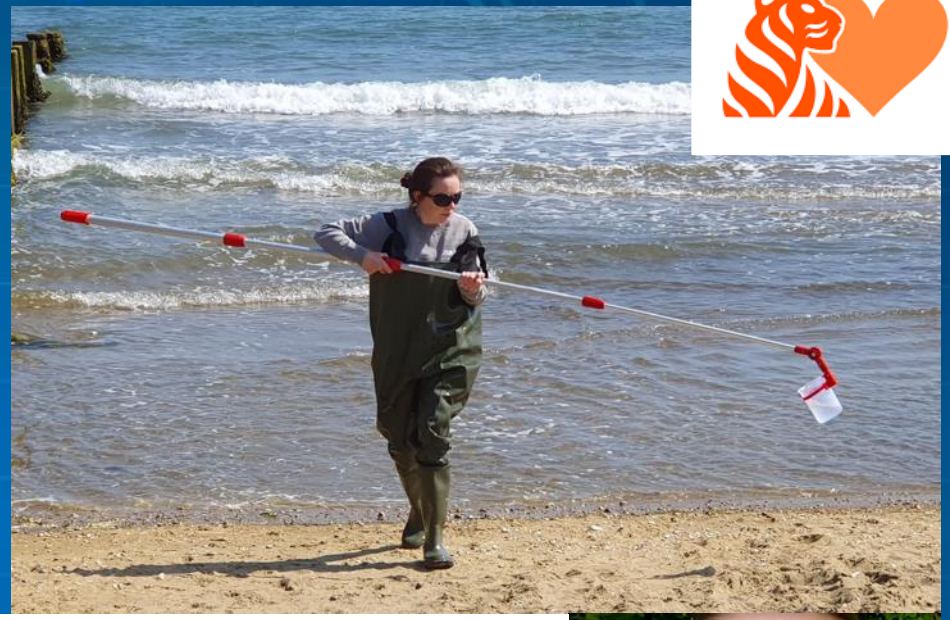
Rob Butson, Misconnections Manager, Southern Water

https://www.southernwater.co.uk/the-news-room/the-media-centre/2022/november/southern-water-hunts-down-wrongly-plumbed-homes-and-workplaces

…and evaluating impact where it matters

We've been undertaking a program of water testing for a number of months alongside Canterbury City Council using a **Fluidion kit that has been supplied by Southern Water**. **We test weekly and reactively to weather and Beachbuoy notifications of spills – at up to six sites from Herne Bay to Whitstable**

https://www.soswhitstable.com/water-testing



The **Fluidion ALERT Lab is very quick and easy to use**. Our small team of citizen scientists have been able to take almost **daily samples for 7 months**. We have the process down to less than half an hour! This is the first study taking such frequent samples in our area and we are learning a lot about the water quality at our local beach. The support from Fluidion has been great and they've answered our many questions quickly and thoroughly.

***Lisa Banfield**, Conservation and Research Officer, Wildheart Animal Sanctuary, Isle of Wight*