Fluidion[®] ALERT:

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

Dan Angelescu, David Wanless, Joyce Wong Fluidion US Inc.





Dan Angelescu CEO



Joyce Wong Principal Scientist

www.fluidion.com



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ENVIRONMENTAL MEASUREMENT SYMPOSIUM

> AUGUST 5-9, 2024 GARDEN GROVE, CA



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



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



- 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





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Powerful Cloud Data Analytics Interface Next-generation IoT real-time control and remote data

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- Scalable worldwide IoT communication
- Real-time data visualization

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- 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 <u>free and aggregate-bound FIB</u>





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





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



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



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

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



2023 E. coli concentrations in Seine river



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







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



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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













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



Fluidion/ALERT and Colilert Test Result Comparison





Measuring impact of discharges from live-in boats





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Lake Chelan: Monitoring water safety







Lake Chelan Research Institute

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



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ALERT V2: high-frequency monitoring in urban rivers



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







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

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Comparing FIB measurement methods: MPN, MF



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



Comparing FIB measurement methods: qPCR, ddPCR



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



Comparing FIB measurement methods: proxies

	gistics, OPEX
ddPCRqPCREPA protocols in developmentLab-only, notgPCRSensitive to aggregates Fast measurements (2-6 hrs)Complex lab Not widely av Expensive log	t automated to culturable cells work vailable yet gistics, OPEX
Enzymatic Tryptophan Fully automated, simple logistics Measures a propriation Field-ready: In-situ No equivalent Real-time measurements Non-specific t Possibly sensitive to aggregates Not widely act	roxy, innacurate <u>ice to cell count</u> <u>to culturable cells</u> ccepted

Enzymatic and Tryptophan are only simple-to-measure proxies



Comparing FIB measurement methods: ALERT

			EPA approved	Lab-only, not automated	
MF	MPN		Relatively simple lab work	Insensitive to aggregates Slow measurements	
		-7/	Widespread	Expensive logistics, OPEX	
			EPA protocols in development	Lab-only, not automated	
ddPCR	qPCR		Sensitive to aggregates	Complex lab work	
			Fast measurements (2-6 hrs)	Not widely available yet	
				Expensive logistics, OPEX	
			Fully automated, simple logistics	Measures a proxy, innacurate	
Enzymatic	Tryptophan		Field-ready: In-situ	No equivalence to cell count	
,			Real-time measurements Possibly sensitive to aggregates	Non-specific to culturable cells	
			Passes site-specific EPA, WHO tests		
Fluidio	on ALERT		Sensitive to aggregates	Moderate CAPEX levels	
			Field ready, Automated, Rapid (2-12 hrs)	(in-situ)	
fluidion	ALERT: con	nprehe	nsive count of viable and cu	ulturable FIB	

Fluidion[®] ALERT: Accelerating Water Quality Monitoring



Grab sample + Lab analysis



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





