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Laboratory evaluation of portable water quality testing kits

WHO UNICEF



Portable testing kit	Fluidion ALERT LAB	
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Evaluation procedure	Independent laboratory evaluatio	n, Phase 1 and Phase 2
JMP report issue date	October, 2022	

Summary

This report summarizes the results of an independent laboratory assessment of a portable water quality testing kit called Fluidion ALERT LAB. The evaluation was carried out at KWR Research laboratory, with support from the WHO/UNICEF Joint Monitoring Programme for Water Supply, Sanitation and Hygiene (JMP) following a protocol established by WHO. The Fluidion ALERT LAB successfully passed through Phase 1 testing and proceeded to the Phase assessment, including analysis of five natural water matrices labelled N1-N5, with the following results:

- No false positives were found due to non-target bacteria, and no false negatives were found due to competition
- The portable kit results were compared in triplicate against a reference method using five different natural water matrices, and four different levels of *E. coli* contamination. This report presents the results quantitatively and qualitatively.
- Across the four test waters (excluding sterilized blanks) and five natural water matrices, a total of 60 paired samples were tested. In 87% of these, the semi-quantitative risk class matched the expected value. Matches were lowest for natural water N1 (75%) and for the medium risk stock (1-10 CFU/100 mL; 53% matching), possibly due to the smaller sample volume used (40 mL).
- When used as a presence/absence test, the Fluidion ALERT LAB correctly classified 93% of samples using a 1 CFU/100 mL cut-off, 95% of samples using a 10 CFU/100 mL cut-off, and 98% of samples using a 100 CFU/100 mL cut-off.
- In most cases where Fluidion ALERT LAB misclassified results, it showed a positive bias. It therefore overestimated, rather than underestimated, the risk.

Executive Summary

The primary concern regarding drinking water quality is that contamination of drinking water could lead to disease. A large number of pathogens can cause water-borne disease. The majority of these pathogens are fecal in origin, but it is not practical to test drinking water for all potential pathogens. Instead, measurement of fecal indicators is preferred. There is widespread agreement that *Escherichia coli* (*E. coli*) is the best currently available indicator of fecal contamination in drinking water.

A large number of test kits are available to quantify the presence of *E. coli* in water. The objective of this project has been to test and compare a range of kits against a certified reference method, which was chosen to be the IDEXX Quantitray 2000 method using Colilert medium. This report summarizes a set of laboratory assessments of different waters with different compositions and levels of contamination and presents the results of both the Fluidion ALERT LAB and the reference method.

The Fluidion ALERT LAB was compared to the reference method using cultivated *E. coli* in laboratory water with a phosphate-buffered saline matrix, as well as using wastewater treatment plant effluent diluted in five different sterilized natural waters (N1-N5). Results were interpreted graphically and through linear regression on both raw data and log-transformed data (see Table 1).

Water matrix	Number of paired samples	Maximum value	Slope (raw)	Intercept (raw)	Slope (log)	Intercept (log)	Spearman's r
Lab water	39	491	0.28	10.88	0.79	-0.03	0.961
N1	15	1168	2.66	5.11	1.01	0.41	0.924
N2	15	272	1.48	-0.81	0.90	0.22	0.909
N3	15	839	2.19	53.70	1.10	0.27	0.898
N4	15	442	1.84	3.72	0.88	0.48	0.907
N5	15	516	1.80	5.93	0.98	0.28	0.960

Table 1: Overview of the regression analysis for Fluidion ALERT LAB.

The Fluidion ALERT LAB was also assessed for false positives by using concentrated stocks of six non-target bacteria (*Aeromonas, Citrobacter, Enterobacter, Klebsiella, Pseudomonas aeruginosa* and *Serratia*); and for false negatives by using the same non-target bacteria spiked with low levels of *E. coli*. The Fluidion ALERT LAB did not report any false positive values in the absence of *E. coli* and was able to detect *E. coli* in the presence of each of the non-target bacteria.

Across the four test waters (excluding sterilized blanks) and five natural water matrices, a total of 60 paired samples were tested. In 87% of these, the semi-quantitative risk class matched the expected value. Matches were lowest for natural water N1 (75%) and for the medium risk stock (1-10 CFU/100 mL; 53% matching). The relative lack of concordance for the medium risk stock is to be expected as the Fluidion ALERT LAB uses a 40 mL volume for analysis, rather than 100 mL.

When used as a presence/absence test, the Fluidion ALERT LAB correctly classified 93% of samples using a 1 CFU/100 mL cut-off, 95% of samples using a 10 CFU/100 mL cut-off, and 98% of samples using a 100 CFU/100 mL cut-off.

Abbreviations

Colony Forming Unit	CFU
Defined Substrate Technology	DST
Ground water	GW
Lower Quantification Limit	LQL
Surface water	SW
Upper Quantification Limit	UQL

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1 Background information

WHO and UNICEF both support national counterparts in monitoring and surveillance of drinking water quality in a variety of settings. In many countries where WHO and UNICEF work, logistical challenges mean that testing drinking water quality in laboratories is often not feasible, due to long distances and travel times required to transport samples. This has led to an interest in portable water quality testing kits, especially for measures of faecal contamination. Both WHO and UNICEF regularly procure portable water quality testing kits for national counterparts and share an interest in ensuring that the equipment procured can produce results that are reliable and match within reasonable margins the results from standard reference methods. In addition, both organizations wish to catalyse the continuous improvement of existing portable water quality testing products, and the development of innovative new products which might allow more efficient, accurate, or low-cost testing of drinking water quality in the field.

2 Rapid Water Quality Testing project

UNICEF, in collaboration with WHO, has developed a Rapid Water Quality Testing project to catalyse the continuous improvement of existing portable water quality testing products, and the development of innovative new products which might allow more efficient, accurate, or low-cost testing of drinking water quality in the field. The project has produced a Target Product Profile to describe the desired characteristics of a field test kit, and UNICEF has requested WHO to provide technical guidance on how to assess the performance of innovative products that result from the Rapid Water Quality Testing project.

There are a number of standards and methods used for measurement of microbiological quality of water, and many of the field test kits purport to follow these standards and methods. However, it can be difficult to conduct assessments with field kits out of a controlled laboratory environment, and some commercially available products, or innovative products recently developed, may in practice not meet all requirements.

In the absence of a clear procedure for assessing field test kits, the WHO Water, Sanitation and Hygiene team developed a template protocol for conducting such an assessment in a laboratory setting. This protocol has been reviewed by an independent technical advisory committee convened by WHO and UNICEF to support the Rapid Water Quality Testing project. The current protocol is focused on culture-based methods of measuring the faecal indicator bacterium *Escherichia coli* (*E. coli*).

The protocol consists of a first phase screening to determine if the assay under evaluation produces results comparable to the reference method over a range of *E. coli* concentrations, under highly controlled conditions. Assays that have passed Phase 1 assessments can proceed to the Phase of 2 of the assessment, which will examine the performance of the test under more challenging conditions (competition from non-target bacteria, use of different natural water matrices and wild *E. coli* strains, and variable temperature incubation if claimed by the manufacturer).

3 Products

3.1 Trial Method: Fluidion ALERT LAB

The Fluidion ALERT LAB is a remotely controllable, fully portable, and autonomous analyser for the measurement of *E. coli* and other bacteria. Suitable for source water and environmental monitoring at a field location, in a moving vehicle, or in a lab. It can perform up to six measurements at the same time using a 12V power source or battery.



Figure 1: FLUIDION ALTER LAB test kit.

Fluidion ALERT LAB is based on a modified real-time Defined Substrate Technology (DST) method, integrated into an automated instrument capable of performing bacterial quantification in the field. The device is capable of automating the incubation at 37°C, performing real-time multispectral optical analysis (absorbance/fluorescence), turbidity correction, signal analysis, and bacterial quantification of culturable *E. coli* with a time-to-result ranging between 2 and 12 hours. The technology tests 40 mL samples, and reportedly detects all bacteria present in solution, including those aggregated into clusters which might be interpreted as bacteria with standard methods.

Manufacturer information: FLUIDION SAS Bio&D, Et 1 C.C-ial Echat 94, Avenue du Général de Gaulle 94000 Créteil France Phone: +33 182 390 290 Email: contact@fluidion.com

The Fluidion ALERT LABs with the following serial numbers were used during this validation study.

A52200005632 A52200005633 A52200005634

3.2 Reference Method: IDEXX Quanti-Tray System

The Colilert Test uses proprietary Defined Substrate Technology (DST) to simultaneously detect coliforms and *E. coli*. Two nutrient-indicators, ONPG and MUG, are the major sources of carbon in Colilert and can be metabolized by the coliform enzyme β -galactosidase and the *E. coli* enzyme β -glucuronidase, respectively.

Step 1 Add reagent to the sample.

Step 2 Pour into Quanti-Tray/2000 (counts from 1–2,419).

Step 3

Seal in Quanti-Tray Sealer and place in $35^{\circ}C \pm 0.5^{\circ}C$ incubator for 24 hours.

(temperature requirement may be different per regulatory requirements in other countries)

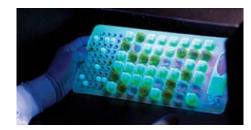
Step 4

Yellow wells = total coliforms Yellow/fluorescent wells = *E. coli* Count positive wells and refer to MPN table

More information: https://www.idexx.co.uk/en-gb/water/water-products-services/colilert/







Test protocol and criteria 4

4.1 Phase 1

The first phase aimed to determine if the assay under evaluation produced results comparable to the reference method. This was done under highly controlled conditions over a range of E. coli concentrations.

A stock solution of a known lab strain of E. coli (ATCC 25922) with a concentration of approximately 1000 viable and culturable E. coli cells per 100 mL, was prepared (acceptable range: 300 - 3000 cells/100 mL). This was measured and confirmed using the IDEXX Quantitray method in a background of sterile phosphate buffered saline (pH 7.4 ± 0.2). This stock solution was then serially diluted using two-fold dilution with a sterile phosphate buffered saline, for details see Table 2. The resulting stock solutions spanned a range of concentrations which were expected to yield positive results, ranging from zero to above most detection limits, with several critical range stock concentrations in between.

As a blank (A), a sample of stock solution 1 was autoclaved to eliminate any viable and culturable E. coli.

	Approximate <i>E. coli</i> concentration, cells/100 mL						
Stock	Lower acceptable limit	Target concentration	Upper acceptable limit				
S1	300	1000	3000				
S2	150	500	1500				
S3	17	250	750				
S4	38	125	375				
S5	19	64	188				
S6	9	32	94				
S7	5	16	47				
S8	2	8	23				
S9	1	4	12				
S10	0.6	2	6				
S11	0.3	1	3				
S12	0.1	0.5	1.5				
Α	0	0	0				

The results of the essay under evaluation and the results of the reference method were plotted against each other using a log transformed linear regression of both datasets. Within a given stock, the triplicate samples from the essay under evaluation were "paired" with the triplicate analyses made with the reference method during sample processing (before the incubation period).

Samples below the minimum detection limit were fixed at 50% of the detection limit. Linear regression was made on the datapoints that were within the quantification range, or below the minimum detection limit, for both assays.

An assay proceeded to the Phase 2 assessment if the Spearman's rank coefficient was at least 0.90, and if the blanks did not show positive results. It was originally intended that tests with a regression slope (before log transformation) significantly different from 1.0 would be excluded from Phase 2 assessment. However, a large number of trial assays had regression slopes significantly different from unity, so this condition was relaxed.

4.2 Phase 2

4.2.1 False Positives due to non-target bacteria

Some tests could potentially generate positive results in the absence of *E. coli* through the growth of non-target organisms. Cultures of six non-target bacteria (*Aeromonas, Citrobacter, Enterobacter, Klebsiella, Pseudomonas aeruginosa* and *Serratia*) that could potentially cause false positives, were made with a target concentration of 100,000,000 viable and culturable cells/100 mL (acceptable range: 30,000,000 – 300,000,000 cells/100 mL). These cultures were tested using the trial assay without any addition of *E. coli*. Any positive results were considered a false positive. Single tests instead of triplicates were done, and the reference method was not challenged with the non-target organisms.

4.2.2 False negative due to competition

The same six cultures of non-target organisms were mixed 1:100 with *E. coli* Stock 1, resulting in an approximate concentration of 30 CFU/100 mL *E. coli* and 30,000 CFU/100 mL of the non-target organism. The resulting stock was tested using the trial kit. Any negative results were considered to indicate that in the presence of competing bacteria, *E. coli* might not be detected by the trial method.

As for the False Positive experiments, the reference method was not tested and only single tests instead of triplicates were done.

4.2.3 Natural waters

The water matrix, as well as the strain of *E. coli* used, may affect the performance of the trial method. To assess this possibility, five different natural waters were selected. These included at least two surface water (SW) and two groundwater (GW) sources. Full list of requirements for the natural waters can be found in Table 3.

Natural water	Source	Turbidity	рН	Alkalinity
N1	GW or SW	> 10	Any	
N2	GW or SW	< 10	< 6.5	At least one of the
N3	GW or SW	< 10	> 8.0	waters should have a
N4	GW or SW	Any	6.5 – 8.0	low <50 mg/L CaCO₃
N5	GW or SW	Any	Any	

 Table 3: Criteria for the natural waters.

The natural waters were sterilised and then spiked with unfiltered effluent from a wastewater treatment plant to reach a target concentration of 300 *E. coli* per 100 mL (acceptable range: 100 – 1000 cells/100 mL). Pre-testing of the effluent was required to determine the concentration in order to properly dilute it into the natural waters. The stock solutions of effluent in natural water were serially diluted using ten-fold dilutions with the sterilised natural waters three times. The resulting stock solutions spanned a range of concentrations which would be expected to yield at least one stock in each of the risk classes listed below in Table 4. The blank (A) was made by autoclaving the natural waters.

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		Approximate E. coli concentration, cells/100 mL				
Stock	Risk class	Lower acceptable limit	Target concentration	Upper acceptable limit		
N*S1	Very high	100	300	1000		
N*S2	High	10	30	100		
N*S3	Medium	1	3	10		
N*S4	Low	0.1	0.3	1		
N*A	Not applicable	0	0	0		

Table 4: Ten-fold dilution of effluent stock sollution in sterilised waste water, accounting for the acceptable variance in starting solution.

All natural water stocks were tested in triplicate with the trial method, using three different sets of equipment per triplicate: 5 water stocks (N1-5) * 5 dilution stocks (N*S1-A) * 3 replicates using different equipment, for a total of 75 analyses in all (60 stocks and 15 blanks). The same was done for the reference method.

Samples below the minimum detection limit were fixed at 50% of the detection limit. Linear regression was made on the datapoints that were within the quantification range, or below the detection limit, for both assays. Statistical tests were made as in Phase 1.

5 Results

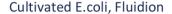
5.1 Phase 1

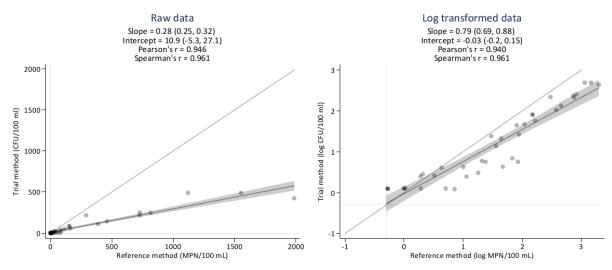
Tests were performed by one technician. The stock dilutions were made the day of testing. For the FLUIDION ALTER LABs it was not possible to analyse all the stocks on the same day due to time restraints. This means that the blanks were measured the day before the test. The next day, the stock solutions made with *E. coli* suspension, and stock 1-6 were analysed. The day after (within 24 hours), stocks 7-12 were tested. This is reflected in the results of the reference method.

Results were compared to the reference method over a wide range of *E. coli* concentrations, under highly controlled conditions (see Table 5).

	Reference method (CFU/100 mL)			Trial	method (CFU/100	mL)
Stock	1	2	3	A52200005632	A52200005633	A52200005634
S1	1553	1120	1986	491	491	425
S2	727	727	816	217	251	247
S3	291	461	387	217	143	111
S4	152	162	155	85	56	83
S5	114	82	86	49	43	25
S6	29	46	35	25	22	13
S7	47	64	81	4	7	6
S8	22	17	23	6	3	6
S9	7.5	12.1	9.8	< 2.5	2.5	4
S10	3.1	5.2	4.1	2.5	< 2.5	4
S11	1	2	2	< 2.5	2.5	< 2.5
S12	1	1	2	< 2.5	< 2.5	3
Blank	< 1.0	< 1.0	< 1.0	< 2.5	< 2.5	< 2.5

Table 5: Results of the CFU testing using the reference method and trial method over multiple dillutions and different sets of equipment.





Incubation temperature: Built in. Incubation duration: Built in. 39 paired samples.

Figure 1: Statistical analysis Cultivated E.coli.

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The Spearman's rank coefficient = 0.961 and meets the criterion. The slope of the un-transformed regression was significantly less than unity. However, because the same was true of many other trial assays, and because the Fluidion ALERT LAB is very different from other commercially available assays, WHO and UNICEF agreed to proceed to Phase 2 assessments with the Fluidion ALERT LAB.

5.2 Phase 2

5.2.1 False positive due to non-target bacteria.

The results of the false positives test can be found in Table 6 below. The full list of numerical results can be found in Appendix 6.2.1.

Non-target bacteria	Target bacteria	
(100,000,000 CFU/100 mL)	(30 CFU/100 mL)	Test results
Aeromonas		negative
Citrobacter		negative
Enterobacter		negative
Klebsiella		negative
Pseudomonas		negative
	E. coli *	positive

* E. coli has been analysed as a positive control to ensure growth conditions.

5.2.2 False negatives due to competition

The results of the false positives test can be found below in Table 7. The full list of numerical results can be found in Appendix 6.2.2.

Non-target bacteria	Target bacteria	
(30,000 CFU/100 mL)	(30 CFU/100 mL)	Test results
Aeromonas	E. coli	positive
Citrobacter	E. coli	positive
Enterobacter	E. coli	positive
Klebsiella	E. coli	positive
Pseudomonas	E. coli	positive
	E. coli	positive

Table 7: Results of the false negatives test.

5.2.3 Natural waters

pH, turbidity, and alkalinity of all natural water samples were tested and matched with the criteria from Table 3. Since autoclaving the water samples caused changes in the pH and turbidity, some natural waters were sterilized by filtration through 0.22 μ m filters in order to meet the target specifications (see below in Table 8).

Waters	Sample point coding	Matrix	Sterilization	Specifications	Required	Tested
N1	Supply channel after	SW	Autoclave	рН	any	8.4
	Bethune polder pumping			Turbidity (FTU)	> 10	89
	station			Alkalinity (mg/L)	any	210
N2	Pumping station	GW	Filtration	рН	< 6.5	6.2
	Archemberg joint raw		0.22 μm	Turbidity (FTU)	< 10	< 0.1
	groundwater			Alkalinity (mg/L)	any	18
N3	Surface water intake point	SW	Autoclave	рН	> 8	8.3
	on the Petrusplaat			Turbidity (FTU)	< 10	3.4
				Alkalinity (mg/L)	any	50
N4	Pumping station Nijmegen	GW	Filtration	рН	6.5 - 8.0	7.5
	joint raw ground water		0.22 μm	Turbidity (FTU)	any	< 0.1
				Alkalinity (mg/L)	any	55
N5	Pumping station Vessum	GW	Filtration	рН	any	6.6
	joint raw ground water		0.22 μm	Turbidity (FTU)	any	5.7
				Alkalinity (mg/L)	< 50	22

Table 8: Selection of the natural water samples and their required and tested specifications.

5.2.4 Natural waters spiked with effluent.

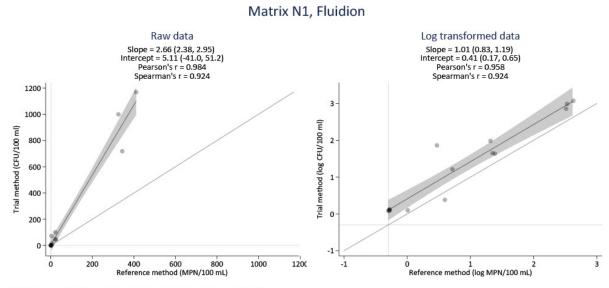
In Table 9, the results for the measurement of colony forming units using both the reference and the trial method can be found. This was done for all the natural water sample with different effluent concentrations. A total of 15 paired samples were analysed for each natural water, for a grand total of 75 paired samples, including 15 blanks. No *E. coli* was detected in any of the blank samples, using either the trial or reference method.

 Table 9: Results in CFU/100 mL of the natural waters spiked with effluent from the wastewater treatment plant for both the reference and trial method.

		N	1	N	2	N	3	N	4	N	15
Stock	Replicate	Ref	Trial								
	1	344.1	718	191.8	233	109.5	839	135.4	272	204.6	516
S1	2	325.5	999	167	272	435.2	718	195.6	442	290.9	516
	3	410.6	1168	119.8	228	193.5	839	214.3	318	325.5	516
	1	24.6	46	18.9	13	22.3	74	13.5	53	28.8	45
S2	2	21.6	101	18.7	18	27.9	74	26	62	23.8	53
	3	21.8	46	23.1	28	26.5	53	30.9	13	21.3	62
	1	5.2	15	3.1	< 2.5	2	2.5	1	24	4.1	4
S 3	2	3.1	73	3.1	< 2.5	1	2.5	3.1	9	2	< 2.5
	3	4.1	2.5	3	4	1	< 2.5	< 1	2.5	3.1	2.5
	1	< 1	< 2.5	< 1	< 2.5	1	< 2.5	< 1	< 2.5	< 1	< 2.5
S4	2	< 1	< 2.5	< 1	< 2.5	2	< 2.5	< 1	< 2.5	< 1	< 2.5
	3	1	< 2.5	< 1	< 2.5	< 1	< 2.5	1	< 2.5	< 1	< 2.5
	1	< 1	< 2.5	< 1	< 2.5	< 1	< 2.5	< 1	< 2.5	< 1	< 2.5
Α	2	< 1	< 2.5	< 1	< 2.5	< 1	< 2.5	< 1	< 2.5	< 1	< 2.5
	3	< 1	< 2.5	< 1	< 2.5	< 1	< 2.5	< 1	< 2.5	< 1	< 2.5

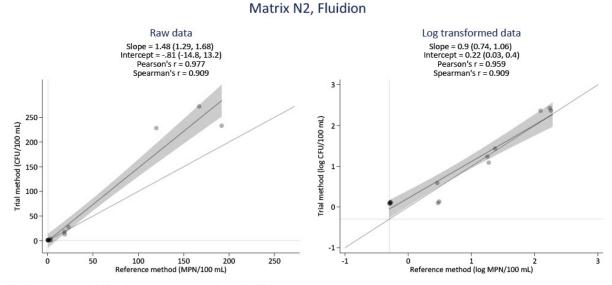
5.2.5 Statistical analysis Natural Waters.

Graphical interpretation and overview of results on both raw data and log-transformed data for all five natural water matrices can be found below in Figure 2 to Figure 6.



Incubation temperature: Built in. Incubation duration: Built in. 15 paired samples.

Figure 2: Statistical analysis Natural Matrix N1.

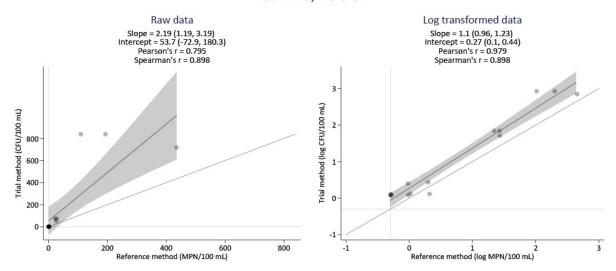


Incubation temperature: Built in. Incubation duration: Built in. 15 paired samples.

Figure 3: Statistical analysis Natural Matrix N2.

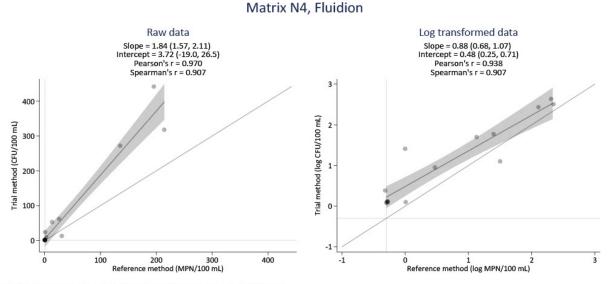
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Matrix N3, Fluidion



Incubation temperature: Built in. Incubation duration: Built in. 15 paired samples.

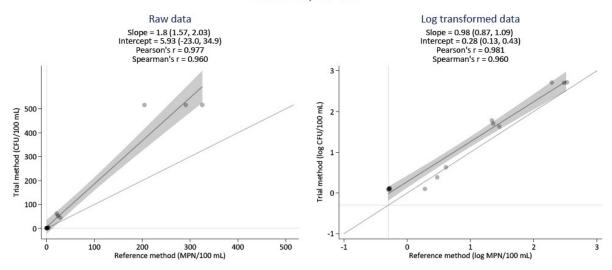
Figure 4: Statistical analysis Natural Matrix N3.



Incubation temperature: Built in. Incubation duration: Built in. 15 paired samples.

Figure 5: Statistical analysis Natural Matrix N4.

Matrix N5, Fluidion



Incubation temperature: Built in. Incubation duration: Built in. 15 paired samples.

Table 10. Overview of the regression encloses for Eluidian ALERT LAR

Figure 6: Statistical analysis Natural Matrix N5.

Interpretation and overview of results through linear regression on both raw data and log-transformed data is summarised below in Table 10.

Water	Number of	Maximum	Slope	Intercept	Slope	Intercept	Spearman's
matrix	paired samples	value	(raw data)	(raw data)	(log trans)	(log trans)	r
Lab water	39	491	0.28	10.88	0.79	-0.03	0.961
N1	15	1168	2.66	5.11	1.01	0.41	0.924
N2	15	272	1.48	-0.81	0.90	0.22	0.909
N3	15	839	2.19	53.70	1.10	0.27	0.898
N4	15	442	1.84	3.72	0.88	0.48	0.907
N5	15	516	1.80	5.93	0.98	0.28	0.960

The trial method was also assessed using the semi-quantitative risk classes defined in Table 4. An analysis was considered to correctly match the risk class if stock 1 yielded a result above 100 CFU/100 mL, if stock 2 yielded a result of at least 11 and no more than 100 CFU/100 mL, if stock 3 yielded a result of at least 1 and no more than 10 CFU/100 mL, and if stock 4 had either no detectable *E. coli* or a maximum of 1 CFU/100 mL. Detailed tables for each natural water matrix are shown in 6.1, and Table 11 below presents a summary, showing the overall, 87% of analyses gave the correct risk class.

				Water Matrix	<		
Test Water	Risk Class	N1	N2	N3	N4	N5	Average
C1	>100 CFU/100 mL	1000/	100%	1000/	1000/	100%	1000/
S1	(very high risk)	100%	100%	100%	100%	100%	100%
S2	11-100 CFU/100 mL	67%	100%	100%	100%	100%	93%
32	(high risk)	0770	100%	100%	100%	100%	93%
S3	1-10 CFU/100 mL	33%	33%	67%	67%	67%	53%
33	(medium risk)	5570	3370	0770	0770	0776	22%
S4	<=1 CFU/100 mL*	100%	100%	100%	100%	100%	100%
34	(low risk)	100%	100%	100%	100%	100%	100%
rand Average							87%

Table 11: Results matching expected risk class. (% results in risk class)

Finally, the utility of the test to produce dichotomous presence/absence results was assessed at different thresholds. With a threshold of 1 CFU/100 mL, 98% of tests were correctly classified. With thresholds of 10 and 100 CFU/100mL, the proportion of tests correctly classified were 95% and 93%, respectively (see Table 12).

		Presence/absence cut-off	
Water matrix	1 CFU/100 mL	10 CFU/100 mL	100 CFU/100 mL
N1	100%	83%	92%
N2	83%	100%	100%
N3	92%	100%	100%
N4	100%	92%	100%
N5	92%	100%	100%
All	93%	95%	98%

 Table 12: Summary of presence/absence results for Fluidion ALERT LAB.

5.3 Qualitative results

Lastly, a qualitative assessment of the FLUIDION test kits was made with reference to categories ranging from the ease of use to the safety of the user and environment. Summary of these results can be found below in Table 13.

Subjects		Assessment	Explanation
User manual			
Contact with a remote sever		Variable	If you use google to go to the page of FLUIDION it will be better. It is important to first bring the device into contact with the site before taking the sample.
Execution test		Easy	
Interpretation results		Easy	Results are clearly visible, per sample, visible on the site of FLUIDION.
Contamination risk to:	Sample	Low	
	User	Low	
Dispose of materials with			The manual recommends disposing of used samples
a high concentration		Easy	following the guidelines of the country of operation,
of <i>E. coli</i>			or adding liquid bleach to disinfect the samples.

Table 13: Scoring of the user friendliness of the FLUIDION test kits.

6 Appendix

6.1 Risk class matching

			Risk class	Correct	risk class
Test water	Risk class	CFU/mL	difference	Single test	Triplicates
		718	0	100%	
S1	>100 CFU/100 mL	999	0	100%	100%
	(very high risk)	1168	0	100%	
	11 100 0511/100	46	0	100%	
S2 (high rick)	101	1	0%	67%	
	(high risk)	46	0	100%	
	1.10.0511/100.001	15	1	0%	
S 3	1-10 CFU/100 mL	73	1	0%	33%
	(medium risk)	2.5	0	100%	
		< 2.5	0	100%	
S4	<=1 CFU/100 mL	< 2.5	0	100%	100%
	(low risk)	< 2.5	0	100%	
Average			0.25	75	5%
Presence/Abs	sence (1 CFU cut-off)			10	0%
Presence/Abs	sence (10 CFU cut-off)			83	3%
Presence/Abs	sence (100 CFU cut-off)			92	2%

 Table 14: Risk class matching expected risk class, Natural Water N1.

Table 15: Risk class matching expected risk class, Natural Water N2.

			Risk class	Correct	risk class
Test water	Risk class	CFU/mL	difference	Single test	Triplicates
		233	0	100%	
S1	>100 CFU/100 mL	272	0	100%	100%
(very high r	(very high fisk)	228	0	100%	
	11 100 0511 /100	13	0	100%	
S2 (high right)		18	0	100%	100%
	(high risk)	28	0	100%	
	1 10 0511/100	< 2.5	-1	0%	
S3	1-10 CFU/100 mL	< 2.5	-1	0%	33%
	(medium risk)	4	0	100%	
	1 CELL/100 ml	< 2.5	0	100%	
S4	<=1 CFU/100 mL	< 2.5	0	100%	100%
	(low risk)	< 2.5	0	100%	
Average			0.17	83	3%
Presence/Abs	sence (1 CFU cut-off)			83	3%
Presence/Abs	sence (10 CFU cut-off)			10	0%
Presence/Abs	sence (100 CFU cut-off)			10	0%

			Risk class	Correct	risk class
Test water	Risk class	CFU/mL	difference	Single test	Triplicates
		839	0	100%	
S1	>100 CFU/100 mL	718	0	100%	100%
	(very high risk)	839	0	100%	
11_100 CEU/100 ml	11 100 0511/100	74	0	100%	
S2 11-100 CFU/100 mL (high risk)	74	0	100%	100%	
	(nign risk)	53	0	100%	
	1 10 0511/1000	2.5	0	100%	
S 3	1-10 CFU/100 mL	2.5	0	100%	67%
	(medium risk)	< 2.5	-1	0%	
		< 2.5	0	100%	
S4	<=1 CFU/100 mL	< 2.5	0	100%	100%
	(low risk)	< 2.5	0	100%	
Average			0.08	92	2%
Presence/Abs	sence (1 CFU cut-off)			92	2%
Presence/Abs	sence (10 CFU cut-off)			10	0%
resence/Abs	sence (100 CFU cut-off)			10	0%

Table 16: Risk class matching expected risk class, Natural Water N3.

Table 17: Risk class matching expected risk class, Natural Water N4.

			Risk class	Correct	risk class
Test water	Risk class	CFU/mL	difference	Single test	Triplicates
	100.0511/100.1	272	0	100%	
S1	>100 CFU/100 mL	442	0	100%	100%
	(very high risk)	318	0	100%	
	11 100 CELL/100 ml	53	0	100%	
S2 (high rick)	62	0	100%	100%	
	(high risk)	13	0	100%	
	1 10 0511/100	24	1	0%	
S 3	1-10 CFU/100 mL (medium risk)	9	0	100%	67%
	(mealum risk)	2.5	0	100%	
	1 CELL/100 mil	< 2.5	0	100%	
S4	<=1 CFU/100 mL (low risk)	< 2.5	0	100%	100%
	(IOW TISK)	< 2.5	0	100%	
Average			0.08	92	2%
Presence/Abs	sence (1 CFU cut-off)			10	0%
Presence/Abs	sence (10 CFU cut-off)			92	2%
Presence/Abs	sence (100 CFU cut-off)			10	0%

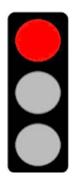
			Risk class	Correct	risk class
Test water	Risk class	CFU/mL	difference	Single test	Triplicates
		516	0	100%	
S1	>100 CFU/100 mL	516	0	100%	100%
	(very high risk)	516	0	100%	
	11 100 CELL/100 mil	45	0	100%	
S2 (high right)	53	0	100%	100%	
	(high risk)	62	0	100%	
		4	0	100%	
S3	1-10 CFU/100 mL	< 2.5	-1	0%	67%
	(medium risk)	2.5	0	100%	
	1.0511/1.00	< 2.5	0	100%	
S4	<=1 CFU/100 mL	< 2.5	0	100%	100%
	(low risk)	< 2.5	0	100%	
Average			0.08	92	2%
Presence/Abs	sence (1 CFU cut-off)			92	2%
Presence/Abs	sence (10 CFU cut-off)			10	0%
vresence/Abs	sence (100 CFU cut-off)			10	0%

Table 18: Risk class matching expected risk class, Natural Water N5.

6.2 Traffic light assessment scheme.

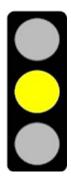
In order to assist with the interpretation of the Phase 2 results, the following 'traffic light' assessment scheme is used, in which results are considered to be 'green' if the results meet the statements listed in the kit's manual, 'yellow' if there is some disparity between results and the expected results, or there is a potential risk of infection to the user, and 'red' if the results deviate significantly from the expected results. The detailed assessment scheme is described below.

Results do not meet the guidelines listed in the kit's manual.



False positives:
Two or more tests are positive
False negatives:
Two or more tests are negative
Incubation temperature:
A score is given to each temperature and the score deviates by a factor of more than 2.
Natural waters:
The results match the expected risk class less than 50% of the time in at least one natural water matrices, or less than 80% of the time in at least three natural water matrices

Disparity between results and the kit's guidelines compared to the potential risk to the user.



False positives:
If only one test is positive. Risk of infection to the user is minimal.
False negatives:
One test is negative
Incubation temperature:
A score is given to each temperature. If the score does not deviate by a factor of more than 2, the results stay in the same risk class.
Natural waters:
The results match the expected risk class at least 50% of the time in all five natural water matrices, and at least 80% of the time in at least three natural water matrices.

Results meet the guidelines listed in the kit's manual.

False positives:

None of the tests are positive. False negative:

All the tests are positive.

Incubation temperature:

Incubation results matches the temperature range in the kit's manual.

Natural waters:

The results match the expected risk class at least 80% of the time in all five natural water matrices, and at least 90% of the time in at least three natural water matrices.

Non-target bacteria		Quantitative test results (CFU/100 mL)					
(1*10 ⁸ CFU/100 mL)		A5220005632	A5220005633	A5220005634			
Aeromonas		< 2.5	< 2.5	< 2.5			
Citrobacter		< 2.5	< 2.5	< 2.5			
Enterobacter		< 2.5	< 2.5	< 2.5			
Klebsiella		< 2.5	< 2.5	< 2.5			
Pseudomonas		< 2.5	< 2.5	< 2.5			
	E. coli *	1.35*10 ⁷	1.37E*10 ⁷	1.37E*10 ⁷			

6.2.1 False positive due to non-target bacteria.

 Table 19: Results of the false positives test

* E. coli has been analysed as a positive control to ensure growth conditions.

6.2.2 False negatives due to competition Table 20: Results of the false negatives test.

Non-target bacteria Target bacteria Quantitative test results (CFU/100 mL					
(30,000 CFU/100 mL)	(30 CFU/100 mL)	A5220005632	A5220005633	A5220005634	
Aeromonas	E. coli	9	11	11	
Citrobacter	E. coli	39	50	59	
Enterobacter*	E. coli	324	85	549	
Klebsiella*	E. coli	247	98	191	
Pseudomonas	E. coli	38	29	43	
	E. coli	33	25	25	

*Results are much higher. The number of *E. coli* added are approximately 30 CFU/100 mL.

6.2.3 Natural waters

Table 21: Results matching expected risk class, by water matrix.

	Water Matrix					
Test Water	N1	N2	N3	N4	N5	
S1	100%	100%	100%	100%	100%	
S2	67%	100%	100%	100%	100%	
S3	33%	33%	67%	67%	67%	
S4	100%	100%	100%	100%	100%	
Average	75%	83%	92%	92%	92%	
Grand Average			87%			

Note that this yellow light is driven by three non-concordant samples with water matrix N1 (table 14). One of these results was extremely close to the threshold: if the second replicate for test water S2 had read "100" rather than "101" it would have been within the target risk class, and the overall average for the N1 water matrix would have been 83%, resulting in a green light assessment. The yellow light rating should therefore be considered in context.

Fluidion

6.3 Manual







System Overview	3	
Operation and Maintenance	7	
Web Interface	18	
Command Portal	21	
Data Management Interface	25	

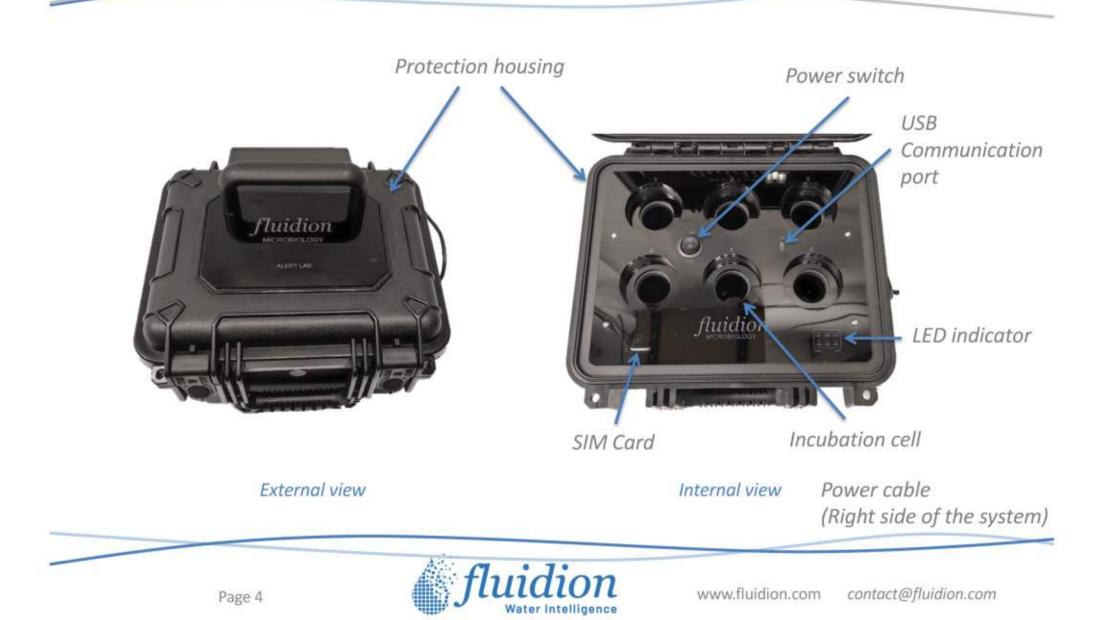




System Overview



System Configuration



System Configuration





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Copyright fluidion 2022, all rights reserved. Specifications Overview

TECHNICAL SPECIFICATIONS

Dimensions	Dimensions 26x24x22 cm		6
Weight	3 kg	Response time	2h-14 h
Measurement	On-demand	Communication	GSM, USB, secure web
trigger	Un-aemana	Communication	interface
Measurement range	4 CFU - 1x10 ⁶ CFU/100 mL	Antenna	Internal
Materials	PMMA, PP, Acetal, SST 316L, glass	Alert notifications	email (optional)



Operation and Maintenance





Safety Precautions





Wear gloves to protect yourself and avoid contamination of the sample



Wear safety glasses whenever handling broken glass. Decontaminate hands / gloves prior to touching eyes.



All waste produced by microbiology methods must be discarded following the

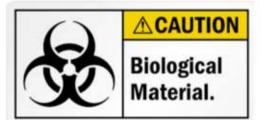
local biohazard waste management regulation.



Never short the power connector or allow water to enter the system.



Read and follow the instructions of all MSDS sheets for the reagents used







System Power



Use only the power supply adapter or Li-ion battery provided!

Connect the power adapter located right side of the system.

Turn ON: Set power switch to ON position

Turn OFF: Set power switch to OFF position



Battery holder

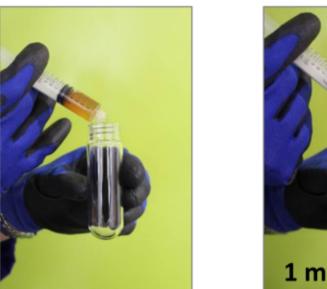


Page 10



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Bioreagent filling – fresh water







Inject 1 mL of ALERT Bioreagent E.coli or Entero in each sterile vial using syringe provided.

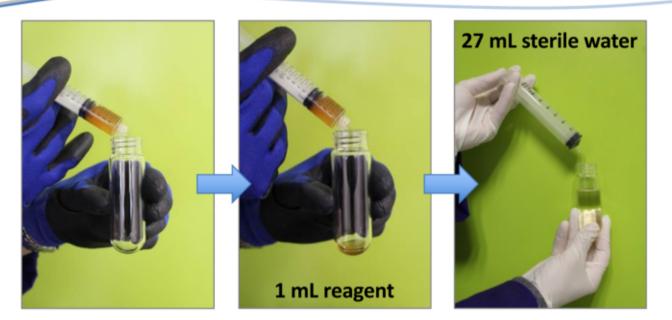
Add 25 mL of sample.

Ensure cap has rubber gasket correctly installed (only for Pyrex round bottom vials) and close the vial. Stir gently to homogenize the solution.





Bioreagent filling – sea water





Inject 1 mL of ALERT Bioreagent E.coli or Entero in each sterile vial using syringe provided.

Add 27 mL of sterile water.

Add 9 mL of sample.

Ensure cap has rubber gasket correctly installed (only for Pyrex round bottom vials) and close the vial.

Stir gently to homogenize the solution.



Bioreagent filling – powder reagent



Step 2: Add one "snap-pack" of powder bioreagent to sample



Step 3: Mix



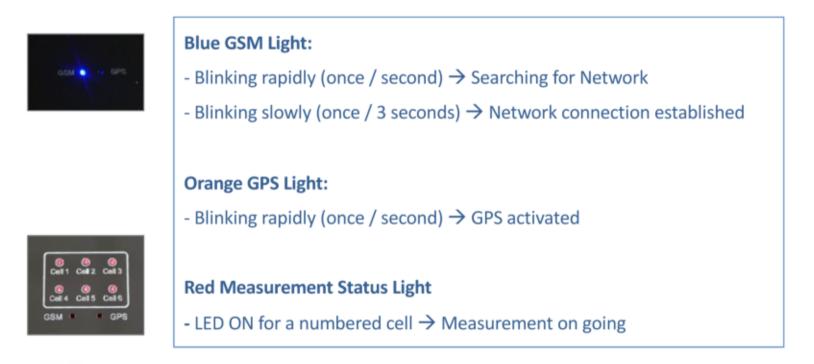


Add 40ml of sample to single-use plastic vial Add one pack of powder bioreagent to sample Mix until dissolved Insert in device and start the analysis



LED indicator status

When the system is powered on, LEDs indicate the system operation status.



Best Practice:

Send "**PING**" command to the system to make sure communication is working before starting a measurement (see "**Command Portal**" section).



Operating Procedure

To operate the system, you must be an authorized user and Login through the Secure Fluidion website (see *"Command Portal" section*)

Note: After setting the power switch to the ON position, the system will take 1-2 minutes to establish connection to the mobile network.

1 - Login to the Command Portal and select the device you wish to control using the dropdown menu.

2 - Click the « **PING** » button to get the device status and wait for the device response. Verify if the date, incubation time and other parameters are correct.

3 - To start an individual measurement, insert the sample vial in the cell X and click the « **START MEASUREMENT** » button. Select the cell(s) to be activated and click the « **CONFIRM** » button. (X=number between 1 and 6)

(An optional text label can be added to each measurement for easy identification)

For advanced detail see the « **Command Portal** » section.



Reusable glass vial cleaning



Ensure working area is clean to avoid any external contamination. Never reuse single-use plastic vials.

Disinfect vials with bleach solution (**10%**), clean using brush and autoclave following regular microbiological cleaning procedures.

Clean white plastic caps with bleach solution. Do not autoclave.

Dispose of the sample according to local biological waste regulations.









Sample disposal – single-use plastic vials

The contents of the plastic tube should be disposed properly as per the guidelines in the country of operation. Typically, this liquid solution is classified as hazardous biological laboratory waste and needs to be treated accordingly.

In the event that certified waste disposal services are not available in the place of operation, a small amount (minimum 4mL) of commercially available freshly-prepared liquid bleach solution (typically 3-6% sodium hypochlorite), should be added to each plastic vial which contains bio-waste solution. The solution with bleach should be allowed to stand for a minimum of 15 minutes contact time, to ensure that all bacteria within the solution have been deactivated. This solution should then be safe to dispose; example: by flushing down a toilet.

The plastic vials should never be re-used.







Web Interface



Data Account

www.secure-fluidion.com

luidion Buiche intelligence	Theme Lager
Fluidion Online Device Management	
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Timing Sompling Sensing Wireless telemetry	fluidion
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Device management	This not a robot
User management	Login
Maintenance	Forgot your password?

Data Account

Data Management Inte	erface Command Porta	al	
fluidion	Data Management Interface System: A5221005635 (A5221005635)		
Texm Texm Provent Provent	System: AS221005655 (AS221005655) System: Status	Recent measurements 2022-02-02 12/05/27 (Europe/Pars), Cell 1 - Latel - E. Cell: c4 /200m.(Pinished, 14.0 hears - Total Cellform: 54 /100m.(Pinished, 1 - Cell: c4 /100m.(Pinished, 1 - L. Cell: c4 /100m.(Pinished, 1 - E. Cell: c4 /100m.(Pinished, 1 - Cell cellform: 54 /100m.(Pinished, 1 - Cell cellform: 54 /100m.(Pinished, 1 - Cell cellform: 54 /100m.(Cellished, 1 - Cell: cellform: 54 /100m.(Cellished, 1 - Cell: cellform: 54 /100m.(Cellished, 1 - Cell: cellished) - Cell: cellished	() 4.0 hours) () 4.0 hours)
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Water Intelligence



Command Portal



Command Portal (Quick Commands)

Data Management Interface		Data Managen	nent interfa	ce		
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4 5 5 Wat autor Autor A	2-02-03 14:19:35 urementi Coll/Total Coliform Fresh ar 1 orki	Recent measurements 2022-02-02 17:05:27 (Europe/Paris). Co - Label: - E. Coli: ≤4/100mL(Finished, 14.0 hr - Total Coliform: ≤4/100mL(Finished - Get report 2022-02-02 17:05:27 (Europe/Paris). Co - Label: - E. Coli: ≤4/100mL(Finished, 14.0 hr - Total Coliform: ≤4/100mL(Finished - Get report 2022-02-02 17:05:27 (Europe/Paris). Co - Label: - Label:	ours) d, 14.0 hours) sll: 4. ours) d, 14.0 hours)			
anced Commands V	Total; 71 rec	ords Page: 1/3 ~<< < > >>	,	Please	I send a A5221005635 nd to system: select the cells that you wish to S	tart Measurement.
Command	Operator Omar Bach Rais	Created at 2022-02-02 17:02:51	Status	Cell #	Measurement Label (Optional) Sample 1	
FING 5:N: A5221009635 DATETIME: 2022-02-02 10:02:57 (Europ 8AT: 12.71 51G: 29 INC TIPE: 14 MEASURE: E.COLI ONGOING: OPS: ON V5:46;			C HARANGE	2 🖬 3 🖬 4 🗆 5 🗆 Select a	Sample 2 Sample 3	
	Omar Bach Rais	2022-02-01 15:51:48	Finished			Confirm Cancel
Automatic: 2022-02-02 12-02.13 START MEASUREMENT 1,2,3,4,5,6	ALCINE MOULD FRAME	AREA OF ALL FRIDE TO	1 201001010	-		1



Command Portal (Advanced Commands)

Data Management Interface				
System: A5221005635 (A522100563	(5)		υ.	
Available of Commands	6 2-03 14:19-38 ments Tabai Culture Fresh 1	Recent measurements 2022-03-02 17:05:27 (Europe/Paris). (- Lobel: - E. Colit: s4 /100mL(Finished, 14.0 1 - Total Coliform: s4 /100mL(Finished, 14.0 1 - Colit: s4 /100mL(Finished, 14.0 1 - Lobel: - E. Colit: s4 /100mL(Finished, 14.0 1 - Total Coliform: s4 /100mL(Finished, 14.0 1 - Total Coliform: s4 /100mL(Finished, 14.0 1 - Cot: seport 2022-02-02 17:05:27 (Europe/Paris). s - Label:	hears) hod, 14.0 hours) Call: 4. hours) hed, 14.0 hours)	This will send a A5221005635 command to system: Please select the cells that you wish to Stop Measurement. Cell # 1 2 2 2 3 2 4 0 5 0 6 0 Select all 0
e in the command:	Download data Cet result Ge	t CPS CPS On CPS Off Set measuremen	na type	Confirm Cancel
pe in the command: Your message	new certificate at the same time. Pr Total: 73 record Operator Omar Bach Raie	ease make sure the previous upload are finishe ease make sure the previous upload are finishe provide at 2022-03-02 \$7;02:65	$\overline{}$	Confirm Cancel Measurement Type: Please select the measurement type:



Command Portal (Command Responses)

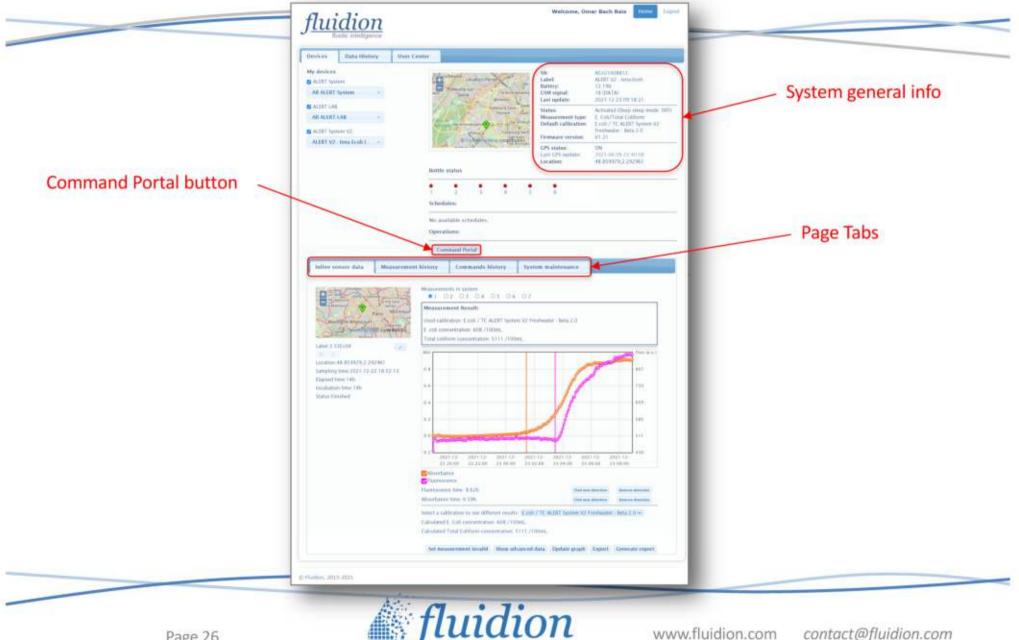
ata Management Interface			
stem: A5221005635 (A5221005635)	×	set measurement ecoli	
stem Status	Recent measurements	MEASUREMENT SET: E.COLI;	
1 2 3 Last synce	2022-02-02 17:05:27 (Europe/Paris), Cell: 3.	Received at: 18 Jun, 2020 15:36:56	
(seal) 4 5 6 Kealy 4 5 6 Kealy 4 5 6 Kealy 5 6 Kealy 5 6 Kealy 6 Kealy 6 Kealy 6 Kealy 6 Kealy 6 Kealy 6 Kealy 7 0222-02-03 14:19:35 Measurementi E. Coll/Total Coliform Fresh Water 1	 Label: E. Coli: ≤4 /100mL(Finished, 14.0 hours) Total Coliform: ≤4 /100mL(Finished, 14.0 hours) Get report 		
(near) (kasty) (heaty) fluidion (heaty) Grange Last communications 2022-02-03 14137:58	2022-02-02 17:05:27 (Europe/Paris). Cell: 4. - Label: . - E. Coli: <4 /100mL(Finished, 14.0 hours)	STOP MEASUREMENT 1,2,3,4,5,6	
	 Total Coliform: ≤4 /100mL(Finished, 14.0 hours) 	MEASUREMENT 1,2,3,4,5,6 HAS BEEN STOPPED;	
	- Get report 2022-02-02 17:05:27 (Europe/Paris), Cell: 5. - Label:	Received at: 18 Jun, 2020 14:22:25	
lick Commands			
Ivanced Commands 🗸		download data	
Totz	I: 71 records Page: 1/3 << < > >>	DATA DOWNLOAD SUCCESSFUL;	
Command Operator	Created at Status	Received at: 2 Jun, 2020 14:25:04	
PING Omar Bach Rais	2022-02-02 17:02:51 Finished		
5/N: A5221005635 DATETIME: 2022-02-02 10:02:57 (Europe/Paris)			
8A7: 12.71 5JG: 29	GET GPS		
INC TIME: 14 MEASURE: E.COLI	LAT: 48.796889		
ONGOING: GPS: ON	LONG: 2.448735		
V5.46;	SAT VIEWED: 13		
Austral at 2022-02-02 17:55:15 START MEASUREMENT 1,2,3,4,5,6 Omar Bach Rais		GET RESULT	
2022/02/01 15:52:45 MEASUREMENT 1,2,3,4,5,6 STARTED; Humined at: 2022 43 41 15:54.03	SIGNAL[0-55]: 27;	Cell 1 Label:	
	Received at: 8 Jun, 2020 11:56:30	Started: 2020-06-18 14:02:37 (3.2h)	
	Google Maps %	Calibration: E. Coli/Total Coliform Fresh Water 1	
		Total Coliform: ≤6.58×10 ⁵ /100mL (In progress)	
		E. Coll: ≤5.48×10 ⁶ /100mL (In progress)	



Data Management Interface



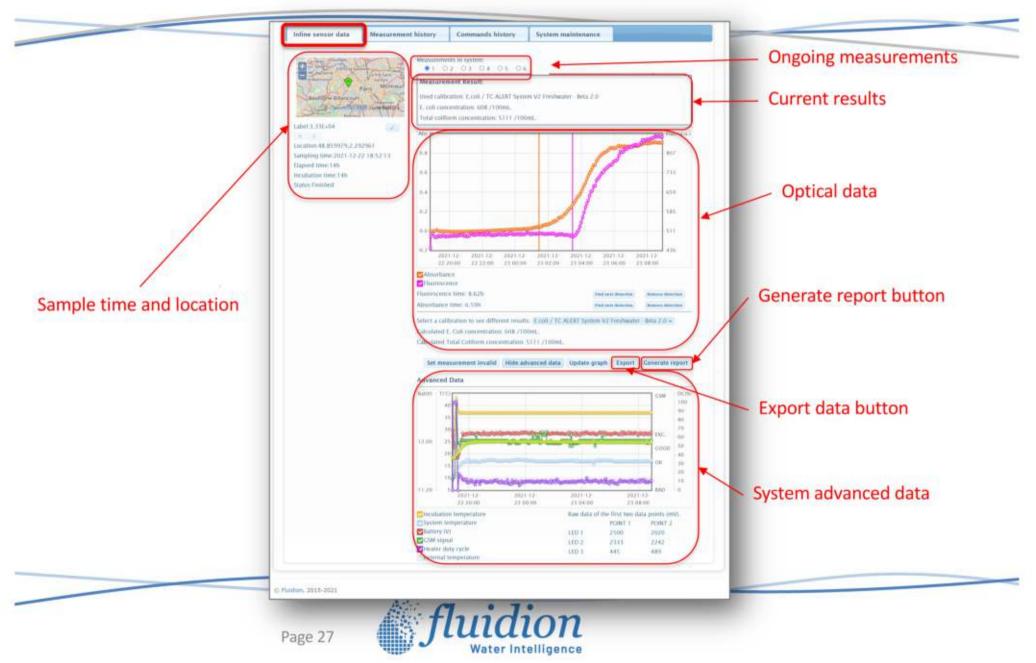
Data Management Interface (Front Page)



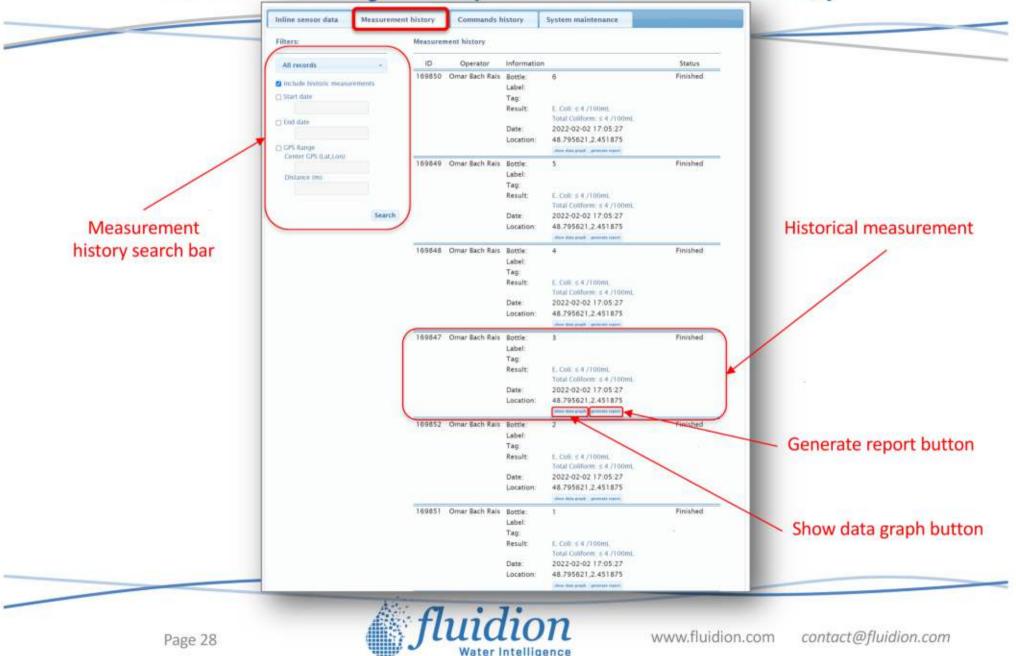
Water Intelligence

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Data Interface (Ongoing Measurements)



Data Interface (Measurement History)



Data Interface (Commands History)

Master Comn	nands:					
						Send Quick View
Log of comm	ands:				uf.	
220377 START I	MEASUREMENT 1,2,3,4,5,6	Omar Bach Rais	2022-02-01 15:51:48	2022-02-01 15:51:48	Kesponse processed. Finished.	2022-02-01 15:53:12
220370	PING	Omar Bach Rais	2022-02-01 15:45:44	2022-02-01 15:45:44	Response processed. Finished.	2022-02-01 15:46:08
220369	INIT	System	2022-02-01 15:45:22	2022-02-01 15:45:22	Response processed. Finished.	2022-02-01 15:45:42
220355	PING	Omar Bach Rais	2022-02-01 12:32:55	2022-02-01 12:32:55	Response processed. Finished.	2022-02-01 12:33:24
220311 START	MEASUREMENT 1,2,3,4,5,6	Patrick EA	2022-01-31 16:20:49	2022-01-31 16:20:49	Response processed. Finished.	2022-01-31 16:22:31
					Response processed	•
Start Date		End Date		Search		



Data Interface (System Maintenance)

nline sensor data	Measurement history	Commands history	System maintenance	J		
Device parameters						
Device GPS: 48.79607,	2.449939					
Device ALERT : E. Coll	Alert 🗹 🛛 Total Coliform Alert 🗹					
Measurement Type: E	Coli/Total Coliform 🛩					
Default Calibration:	Coli/Total Coliform Fresh Water	rl v				
Tags:						
				Edit	Save	Cancel



