Laboratory evaluation of portable water quality testing kits

Portable testing kit	Aquagenx CBT EC+TC MPN Kit
Manufacturer	Aquagenx, LLC PO Box 17181 Chapel Hill, NC 27516 USA Phone: +1-919-590-0343 Email: info@aquagenx.com Website: https://www.aquagenx.com/
Evaluation procedure	Independent laboratory evaluation, 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 the Aquagenx CBT EC+TC MPN Kit. 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 Aquagenx CBT EC+TC MPN kit successfully passed through Phase 1 testing and was challenged with different tests in Phase 2 testing:

- One minor false positive was found due to non-target bacteria (Aeromonas), 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. Across the four test waters (excluding sterilized blanks) and five natural water matrices, a total of 60 paired samples were tested.
- When incubated for 20 hours at 35 °C, in 83% of tests, the semi-quantitative risk class matched the expected value. Matches were lowest for natural water N3 (75%) and for the medium stock (1-10 CFU/100 mL; 60% matching). If used as a presence-absence test, the kit correctly identified the presence or absence of *E. coli* in 93% of cases with a threshold of 1 CFU/100 mL. With thresholds of 10 CFU/100 mL or 100 CFU/100 mL the kit matched expected results 93% and 97% of the time, respectively.
- When incubated for 48 hours at 25 °C, in 73% of tests, the semi-quantitative risk class matched the expected value. Matches were lowest for natural water N4 (50%) and for the medium risk stock (1-10 CFU/100 mL; 47% matching). If used as a presence-absence test, the kit correctly identified the presence or absence of *E. coli* in 90% of cases with a threshold of 1 CFU/100 mL. With thresholds of 10 CFU/100 mL or 100 CFU/100 mL the kit matched expected results 93% and 90% of the time, respectively.

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 CBT EC+TC MPN Kits and the reference method.

The CBT EC+TC MPN Kits 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 and 2).

Table 1: Overview of the regression analysis of CBT EC+TC MPN Kits experiments at 25°C.

Water matrix	Time (h)	Number of samples	Maximum value	Slope (raw)	Intercept (raw)	Slope (log)	Intercept (log)	Spearman's r
Lab water	20	21	<1	0	0.5	0	-0.30	0
	48	18	>400	0.81	1.74	0.91	0.1	0.930
N1	20	15	<1	0.00	0.50	0.00	-0.30	0.00
	48	11	>100	1.49	-2.00	0.81	-0.17	0.50
N2	20	15	<1	0.00	0.50	0.00	-0.30	0.00
	48	13	>100	0.29	7.51	0.96	-0.08	0.95
N3	20	15	<1	0.00	0.50	0.00	-0.30	0.00
	48	12	>100	0.92	1.85	0.82	0.17	0.72
N4	20	15	<1	0.00	0.50	0.00	-0.30	0.00
	48	12	>100	0.09	7.69	0.68	0.10	0.90
N5	20	15	<1	0.00	0.50	0.00	-0.30	0.00
	48	12	>100	2.53	1.4	1.28	0.2	0.96

Table 2: Overview of the regression analysis of CBT EC+TC MPN Kits experiments at 35°C.

Water matrix	Time (h)	Number of samples	Maximum value	Slope (raw)	Intercept (raw)	Slope (log)	Intercept (log)	Spearman's r
Lab water	20	18	>400	0.96	1.24	0.89	0.21	0.946
	48	18	>400	1.16	4.72	0.942	0.3	0.942
N1	20	12	>100	1.41	-0,27	1,07	-0,04	0,95
	48	11	>100	1.36	0.80	1.05	0.01	0.91
N2	20	13	>100	0,29	7,51	0,96	-0,08	0,95
	48	13	>100	0,29	7,51	0,96	-0,08	0,95
N3	20	12	>100	0,94	1,36	0,84	0,12	0,74
	48	12	>100	0,85	3,77	0,80	0,20	0,70
N4	20	12	>100	1.03	2,00	0,97	0,09	0,90
	48	11	>100	1.06	1,93	0,98	0,09	0,87
N5	20	13	>100	0,27	5,80	0,92	0,02	0,92
	48	12	>100	1.84	-0,88	1,10	0,02	0,90

The CBT EC+TC MPN Kit 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 CBT EC+TC MPN Kits 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.

Incubation temperature 25°C with an incubation time of 48 hours.

Across the four test waters (excluding sterilized blanks) and five natural water matrices, a total of 60 paired samples were tested. In 73% of these, the semi-quantitative risk class matched the expected value. Matches were lowest for natural water N4 (50%) and for the medium risk stock (1-10 CFU/100 mL; 47% matching).
If used as a presence-absence test, the kit correctly identified the presence or absence of E. coli in 90% of cases with a threshold of 1 CFU/100 mL. With thresholds of 10 CFU/100 mL or 100 CFU/100 mL the kit matched expected results 93% and 90% of the time, respectively.

Incubation temperature 35°C with an incubation time of 20 hours.

Across the four test waters (excluding sterilized blanks) and five natural water matrices, a total of 59 paired samples were tested. In 83% of these, the semi-quantitative risk class matched the expected value. Matches were lowest for natural water N3 (75%) and for the high risk stock (11-100 CFU/100 mL; 60% matching).
If used as a presence-absence test, the kit correctly identified the presence or absence of E. coli in 93% of cases with a threshold of 1 CFU/100 mL. With thresholds of 10 CFU/100 mL or 100 CFU/100 mL the kit matched expected results 92% and 95% of the time, respectively.

Abbreviations

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

Contents

Contents

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: CBT EC+TC MPN Kits

The Aquagenx CBT EC+TC MPN Kit simultaneously detects and quantifies E. coli (EC) and Total Coliform (TC) bacteria in a 100 mL sample

Principle and Interpretation:
The Aquagenx CBT EC+TC MPN Kit uses a proprietary powder growth medium with a glucose substrate called X-Gluc. When E. coli metabolize this substrate in Aquagenx's growth medium, the color of the water turns blue, indicating the presence of *E. coli.* The growth medium also contains a fluorogenic galactoside substrate called MUGal. If total coliforms are present, they metabolize this fluorogenic substrate and the sample fluoresces blue under a UV light (365 nm). Most Probable Number (MPN) test results are obtained by easy color match using the Aquagenx color-coded MPN Table. The total coliform group of bacteria includes E. coli, which is a fecal coliform as well as a thermotolerant coliform. Not all total coliforms are *E. coli*.

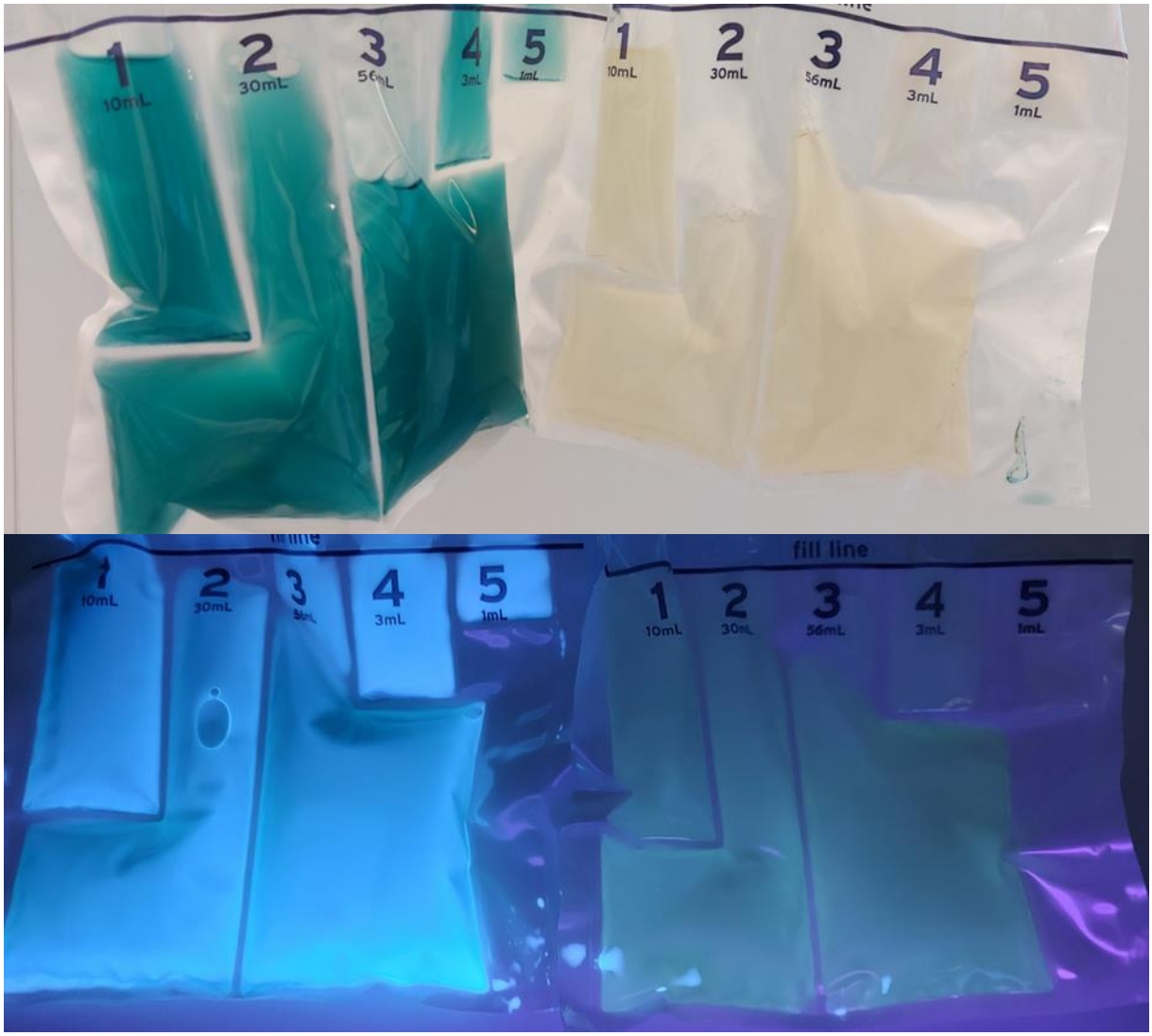


Figure 1 Positive and a blank sample with and without UV reaction.

3.2 Reference Method: IDEXX Quanti-Tray System

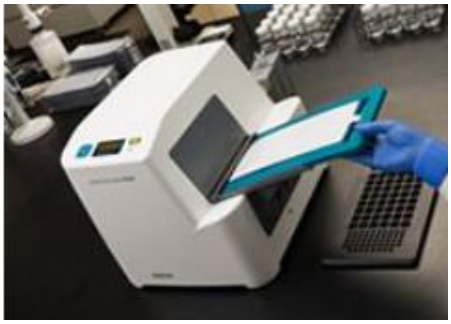
The Colilert Test uses proprietary Defined Substrate Technology (DST) to simultaneously detect coliforms and *E. coli*. Two nutrient-indicators, ONPG and MUG, are the major sources of carbon in Colilert and can be metabolized by the coliform enzyme β-galactosidase and the *E. coli* enzyme β-glucuronidase,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

Step 1
Add reagent to the sample.

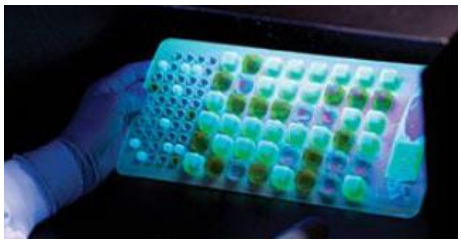
Step 2
Pour into Quanti-Tray/2000 (counts from 1–2,419).

Step 3
Seal in Quanti-Tray Sealer and place in 35°C ± 0.5°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/

4 Test protocol and criteria

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 3. 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*.

Table 3: Visual representation of the two-fold dilution, accounting for acceptable variance in the starting solution.

	Approximate *E. coli* concentration, cells/100 mL		
Stock	Lower acceptable limit	Target concentration	Upper acceptable limit
S1	300	1000	3000
S3	17	250	750
S5	19	64	188
S7	5	16	47
S9	1	4	12
S11	0.3	1	3
Blank	0	0	0

Two sets of the cultivated E. coli stocks were prepared, one was incubated at 25 °C and the other at 35 °C. Both sets were evaluated after 20 hours, and again after 48 hours.

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. Samples were incubated at 35 °C and evaluated after 20 and 48 hours

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. Samples were incubated at 35 °C and evaluated after 20 and 48 hours. 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 4.

Table 4: Criteria for the natural waters.

Natural water	Source	Turbidity	pH	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 $CaCO_3$
N5	GW or SW	Any	Any	

The natural waters were sterilised and then spiked with 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 5. The blank (A) was made by autoclaving the natural waters.

Table 5: Ten-fold dilution of effluent stock sollution in sterilised waste water, accounting for the acceptable variance in starting solution.

		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

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.

Two sets of natural water stocks were prepared; one was incubated at 25°C and the other at 35°C. Both sets were evaluated after 20 hours, and again after 48 hours. The reference method was incubated at 35°C and evaluated after 24 hours.

5 Results

5.1 Phase 1

Tests were performed by one technician. The stock dilutions were made the day of testing.

Results were compared to the reference method over a wide range of *E. coli* concentrations, under highly controlled conditions. In phase 1, the test was performed in 96 well plates instead of the supplied bags for the MPN.

For the purposes of this assessment only for phase 1, the media was converted into a quantitative Most Probable Number (MPN) test by mixing 100 mL of the sample with the appropriate amount of growth media and distributing the inoculated growth media into 96-well plates. The 96 well plates are covered with a plastic sheet so that no contamination can take place between the wells. After incubation, the number of positive wells was converted to an MPN value with 95% confidence intervals (see **Table 6** – **Table 9**).

Table 6: Results of the CFU testing using the reference method and trial method over multiple dillutions at 25°C after 20 hours.

	Reference method (CFU/100 mL)			Trial method (CFU/100 mL)		
Stock	1	2	3	1	2	3
S1	816.4	688.7	1046.2	< 1.0	< 1.0	< 1.0
S3	185	185	178.5	< 1.0	< 1.0	< 1.0
S5	48.1	42.2	45	< 1.0	< 1.0	< 1.0
S7	8.5	9.5	7.5	< 1.0	< 1.0	< 1.0
S9	1	3.1	< 1.0	< 1.0	< 1.0	< 1.0
S11	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Blank	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

Table 7: Results of the CFU testing using the reference method and trial method over multiple dillutions at 25°C after 48 hours.

	Reference method (CFU/100 mL)			Trial method (CFU/100 mL)		
Stock	1	2	3	1	2	3
S1	816.4	688.7	1046.2	> 400	> 400	> 400
S3	185	185	178.5	150	150	150
S5	48.1	42.2	45	43.6	40.5	37.5
S7	8.5	9.5	7.5	15.2	9.5	10.6
S9	1	3.1	< 1.0	1.0	1.0	2.0
S11	< 1.0	< 1.0	< 1.0	< 1.0	1.0	1.0
Blank	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

Table 8: Results of the CFU testing using the reference method and trial method over multiple dillutions at 35-°C after 20 hours.

	Reference method (CFU/100 mL)			Trial method (CFU/100 mL)		
Stock	1	2	3	1	2	3
S1	816.4	688.7	1046.2	> 400	> 400	> 400
S3	185	185	178.5	170	190	170
S5	48.1	42.2	45	43.6	40.5	42.0
S7	8.5	9.5	7.5	15.2	10.6	15.2
S9	1	3.1	< 1.0	2.0	2.0	1.0
S11	< 1.0	< 1.0	< 1.0	3.1	1.0	1.0
Blank	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

Table 9: Results of the CFU testing using the reference method and trial method over multiple dillutions at 35-°C after 48 hours.

	Reference method (CFU/100 mL)			Trial method (CFU/100 mL)		
Stock	1	2	3	1	2	3
S1	816.4	688.7	1046.2	> 400	> 400	> 400
S3	185	185	178.5	200	240	210
S5	48.1	42.2	45	45.2	55.3	59.0
S7	8.5	9.5	7.5	21.2	39.0	30.4
S9	1	3.1	< 1.0	3.1	3.1	1.0
S11	< 1.0	< 1.0	< 1.0	4.1	1.0	1.0
Blank	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

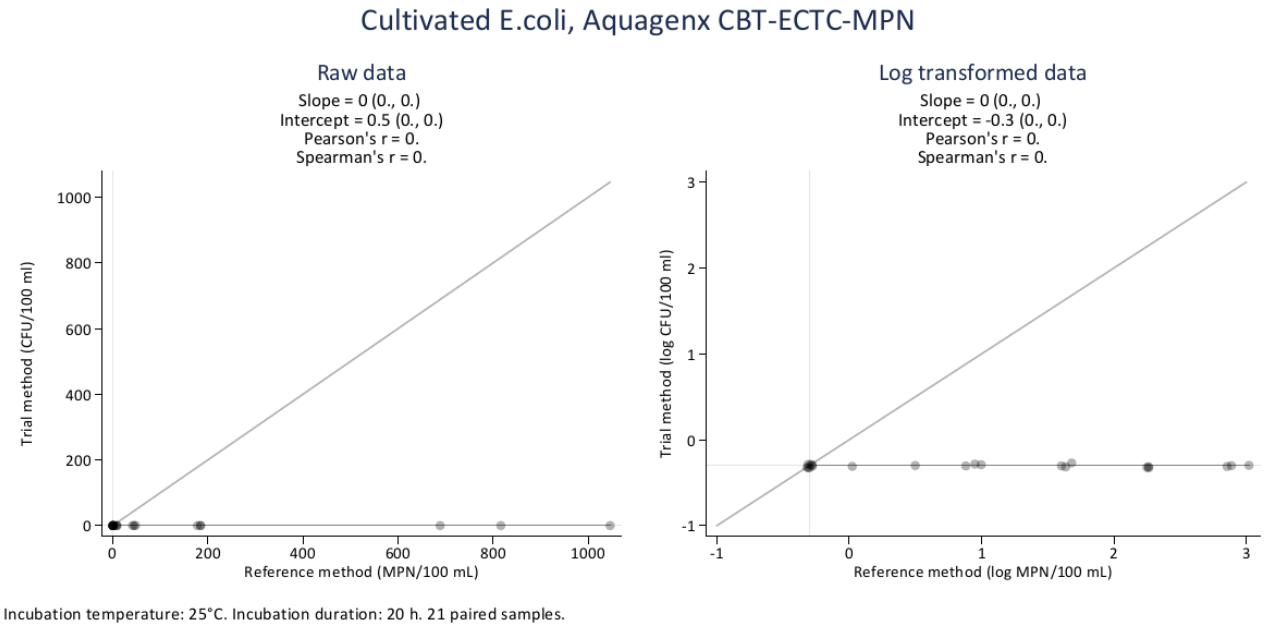


Figure 2 Statistical analysis of Phase 1 results after 20 hours at 25°C.

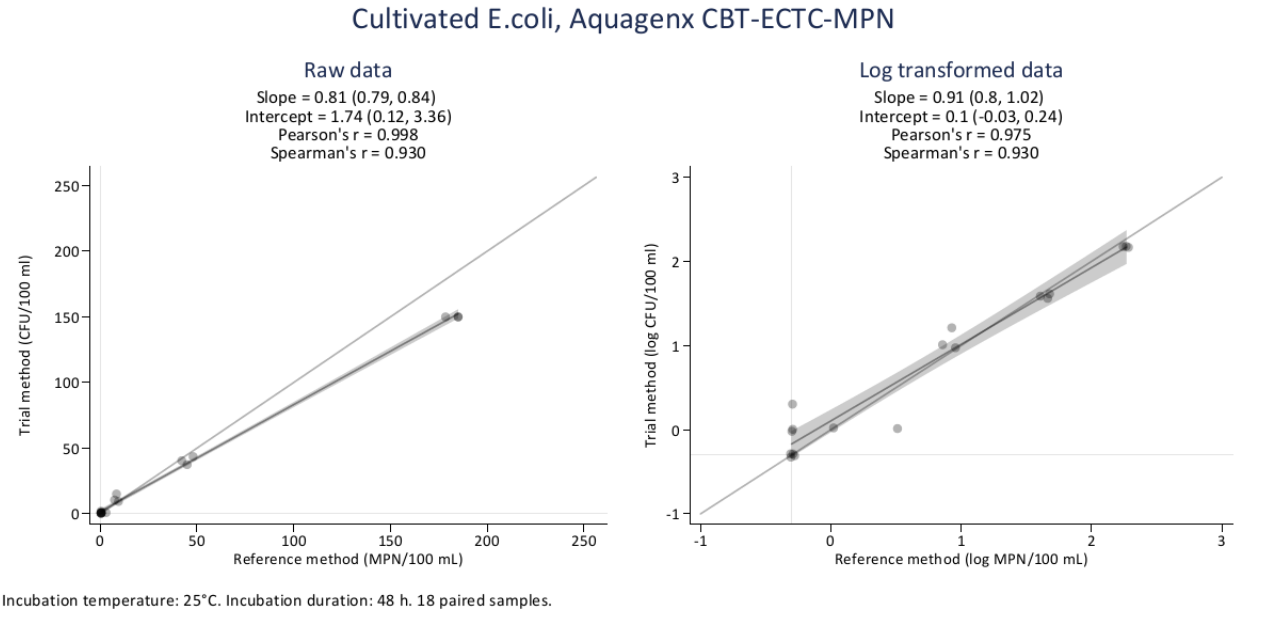


Figure 3 Statistical analysis of Phase 1 results after 48 hours at 25°C.

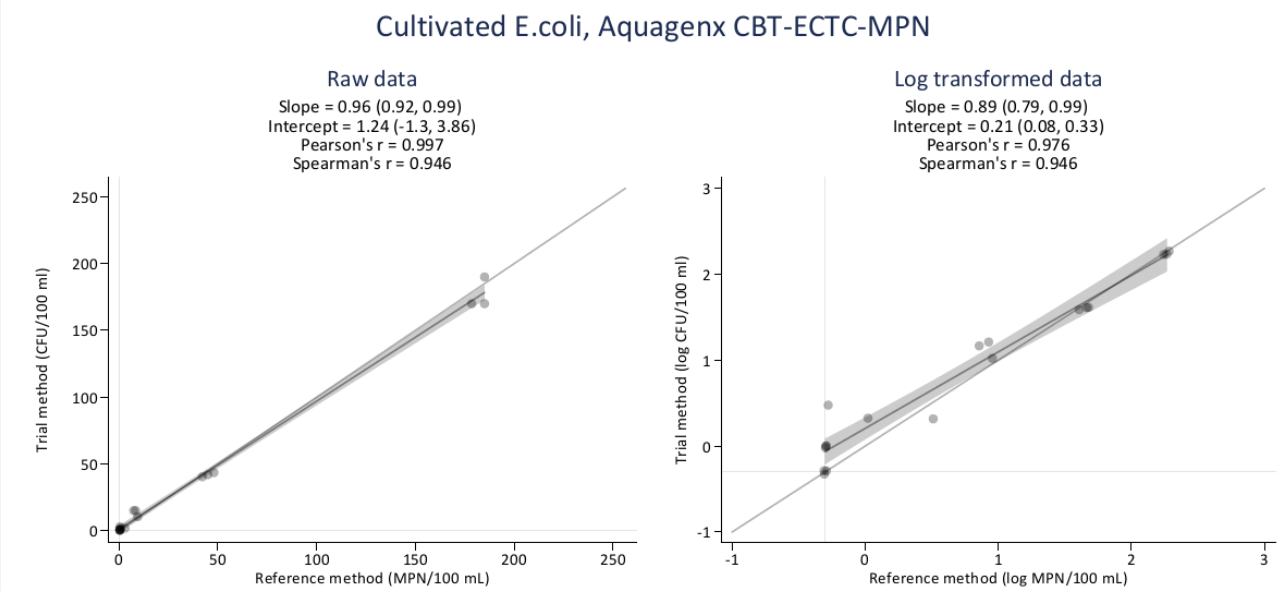


Figure 4 Statistical analysis of Phase 1 results after 20 hours at 35°C.

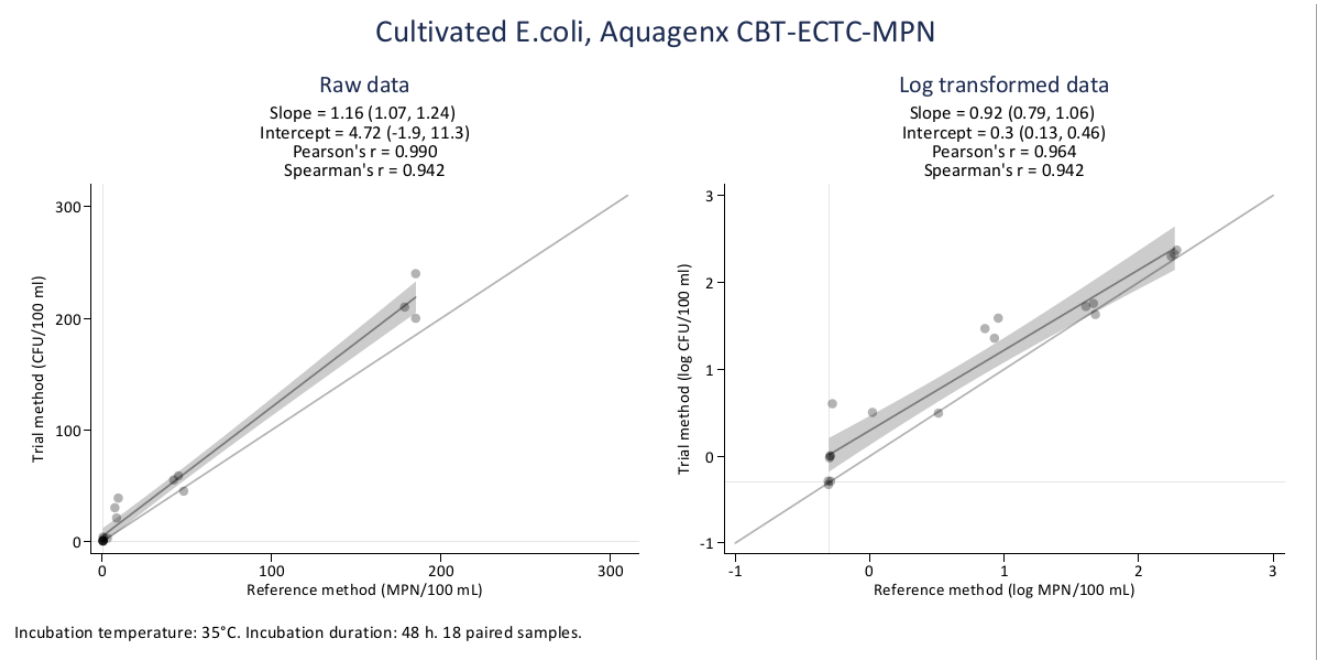


Figure 5 Statistical analysis of Phase 1 results after 48 hours at 35°C.

The Pearson's rank coefficient is higher (except for an incubation of 20 hours at a temperature of 25°C) than 0,9 and meets the criterion. WHO agreed to proceed to Phase 2 assessments with the CBT EC+TC MPN Kits.

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 10 and Table 11 below. The full list of numerical results can be found in Appendix 6.2.1.

Table 10: Results of the false positives test (incubation 25°C)

Non-target bacteria (100,000,000 CFU/100 mL)	Target bacteria (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.

Table 11: Results of the false positives test (incubation 35°C)

Non-target bacteria (100,000,000 CFU/100 mL)	Target bacteria (30 CFU/100 mL)	Test results
Aeromonas		positive
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 Tabel 12. The full list of numerical results can be found in Appendix 6.2.2.

Table 12: Results of the false negatives test **(for both incubation temperatures the same)**

Non-target bacteria (30,000 CFU/100 mL)	Target bacteria (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

5.2.3 Natural waters

pH, turbidity, and alkalinity of all natural water samples were tested and matched with the criteria from Table 4. Since autoclaving the water samples caused changes in the pH and turbidity, some samples were sterilised by filtering them through 0.22 µm filters in order to meet the (see below in Table 13).

Table 13: Selection of the natural water samples and their required and tested specifications.

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

5.2.4 Natural waters spiked with effluent.

In Table 14 -**Table 17**, 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 14: Results in CFU/100 mL of the natural waters spiked with effluent from the wastewater treatment plant for both the reference and trial method after 20 hours at 25°C.

		N1		N2		N3		N4		N5	
Stock	Replicate	Ref	Trial	Ref	Trial	Ref	Trial	Ref	Trial	Ref	Trial
S1	1	344.1	<1	191.8	<1	109.5	<1	410.6	<1	218.7	<1
	2	325.5	<1	167	<1	435.2	<1	365.4	<1	178.9	<1
	3	410.6	<1	119.8	<1	193.5	<1	547.5	<1	172.5	<1
S2	1	24.6	<1	18.9	<1	22.3	<1	47.1	<1	13.2	<1
	2	21.6	<1	18.7	<1	27.9	<1	39.9	<1	13.5	<1
	3	21.8	<1	23.1	<1	26.5	<1	47.1	<1	19.5	<1
S3	1	5.2	<1	3.1	<1	2	<1	1	<1	2	<1
	2	3.1	<1	3.1	<1	1	<1	5.2	<1	2	<1
	3	4.1	<1	3	<1	1	<1	6.3	<1	1	<1
S4	1	< 1	<1	< 1	<1	1	<1	2	<1	< 1	<1
	2	< 1	<1	< 1	<1	2	<1	< 1	<1	< 1	<1
	3	1	<1	< 1	<1	< 1	<1	< 1	<1	< 1	<1
A	1	< 1	<1	< 1	<1	< 1	<1	< 1	<1	< 1	<1
	2	< 1	<1	< 1	<1	< 1	<1	< 1	<1	< 1	<1
	3	< 1	<1	< 1	<1	< 1	<1	< 1	<1	< 1	<1

Table 15: Results in CFU/100 mL of the natural waters spiked with effluent from the wastewater treatment plant for both the reference and trial method after 48 hours at 25°C.

		N1		N2		N3		N4		N5	
Stock	Replicate	Ref	Trial	Ref	Trial	Ref	Trial	Ref	Trial	Ref	Trial
S1	1	344.1	>100	191.8	>100	109.5	>100	410.6	48.3	218.7	>100
	2	325.5	>100	167	48.3	435.2	>100	365.4	>100	178.9	>100
	3	410.6	>100	119.8	>100	193.5	>100	547.5	48.3	172.5	>100
S2	1	24.6	48.3	18.9	48.3	22.3	13.6	47.1	>100	13.2	48.3
	2	21.6	>100	18.7	32.6	27.9	13.6	39.9	48.3	13.5	48.3
	3	21.8	17.1	23.1	32.6	26.5	48.3	47.1	>100	19.5	32.6
S3	1	5.2	<1	3.1	1.2	2	4.7	1	13.6	2	13.6
	2	3.1	<1	3.1	1.5	1	13.6	5.2	13.6	2	3.4
	3	4.1	<1	3	<1	1	3.4	6.3	4.7	1	4.7
S4	1	< 1	<1	< 1	<1	1	<1	2	<1	< 1	<1
	2	< 1	1.2	< 1	<1	2	<1	< 1	<1	< 1	<1
	3	1	<1	< 1	<1	< 1	1.5	< 1	<1	< 1	<1
A	1	< 1	<1	< 1	<1	< 1	<1	< 1	<1	< 1	<1
	2	< 1	<1	< 1	<1	< 1	<1	< 1	<1	< 1	<1
	3	< 1	<1	< 1	<1	< 1	<1	< 1	<1	< 1	<1

Table 16: Results in CFU/100 mL of the natural waters spiked with effluent from the wastewater treatment plant for both the reference and trial method after 20 hours at 35°C.

		N1		N2		N3		N4		N5	
Stock	Replicate	Ref	Trial	Ref	Trial	Ref	Trial	Ref	Trial	Ref	Trial
S1	1	344.1	>100	191.8	>100	109.5	>100	410.6	>100	218.7	>100
	2	325.5	>100	167	48.3	435.2	>100	365.4	>100	178.9	>100
	3	410.6	>100	119.8	>100	193.5	>100	547.5	>100	172.5	48.3
S2	1	24.6	32.6	18.9	48.3	22.3	13.6	47.1	48.3	13.2	13.6
	2	21.6	13.6	18.7	32.6	27.9	13.6	39.9	48.3	13.5	13.6
	3	21.8	48.3	23.1	32.6	26.5	48.3	47.1	48.3	19.5	48.3
S3	1	5.2	13.6	3.1	1.2	2	1.5	1	13.6	2	9.1
	2	3.1	2.6	3.1	1.5	1	13.6	5.2	13.6	2	<1
	3	4.1	1.2	3	<1	1	1.5	6.3	4.7	1	1
S4	1	< 1	<1	< 1	<1	1	<1	2	<1	< 1	<1
	2	< 1	<1	< 1	<1	2	1.2	< 1	<1	< 1	<1
	3	1	<1	< 1	<1	< 1	1.5	< 1	<1	< 1	<1
A	1	< 1	<1	< 1	<1	< 1	<1	< 1	<1	< 1	<1
	2	< 1	<1	< 1	<1	< 1	<1	< 1	<1	< 1	<1
	3	< 1	<1	< 1	<1	< 1	<1	< 1	<1	< 1	<1

Table 17: Results in CFU/100 mL of the natural waters spiked with effluent from the wastewater treatment plant for both the reference and trial method after 48 hours at 35°C.

		N1		N2		N3		N4		N5	
Stock	Replicate	Ref	Trial	Ref	Trial	Ref	Trial	Ref	Trial	Ref	Trial
S1	1	344.1	>100	191.8	>100	109.5	>100	410.6	>100	218.7	>100
	2	325.5	>100	167	48.3	435.2	>100	365.4	>100	178.9	>100
	3	410.6	>100	119.8	>100	193.5	>100	547.5	>100	172.5	>100
S2	1	24.6	48.3	18.9	48.3	22.3	13.6	47.1	>100	13.2	13.6
	2	21.6	>100	18.7	32.6	27.9	13.6	39.9	48.3	13.5	13.6
	3	21.8	13.6	23.1	32.6	26.5	48.3	47.1	48.3	19.5	48.3
S3	1	5.2	13.6	3.1	1.2	2	1.5	1	13.6	2	9.1
	2	3.1	13.6	3.1	1.5	1	32.6	5.2	13.6	2	<1
	3	4.1	13.6	3	<1	1	3.4	6.3	4.7	1	1
S4	1	< 1	<1	< 1	<1	1	<1	2	<1	< 1	<1
	2	< 1	<1	< 1	<1	2	1.2	< 1	<1	< 1	<1
	3	1	<1	< 1	<1	< 1	1.5	< 1	<1	< 1	<1
A	1	< 1	<1	< 1	<1	< 1	<1	< 1	<1	< 1	<1
	2	< 1	<1	< 1	<1	< 1	<1	< 1	<1	< 1	<1
	3	< 1	<1	< 1	<1	< 1	<1	< 1	<1	< 1	<1

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 6 – Figure 25

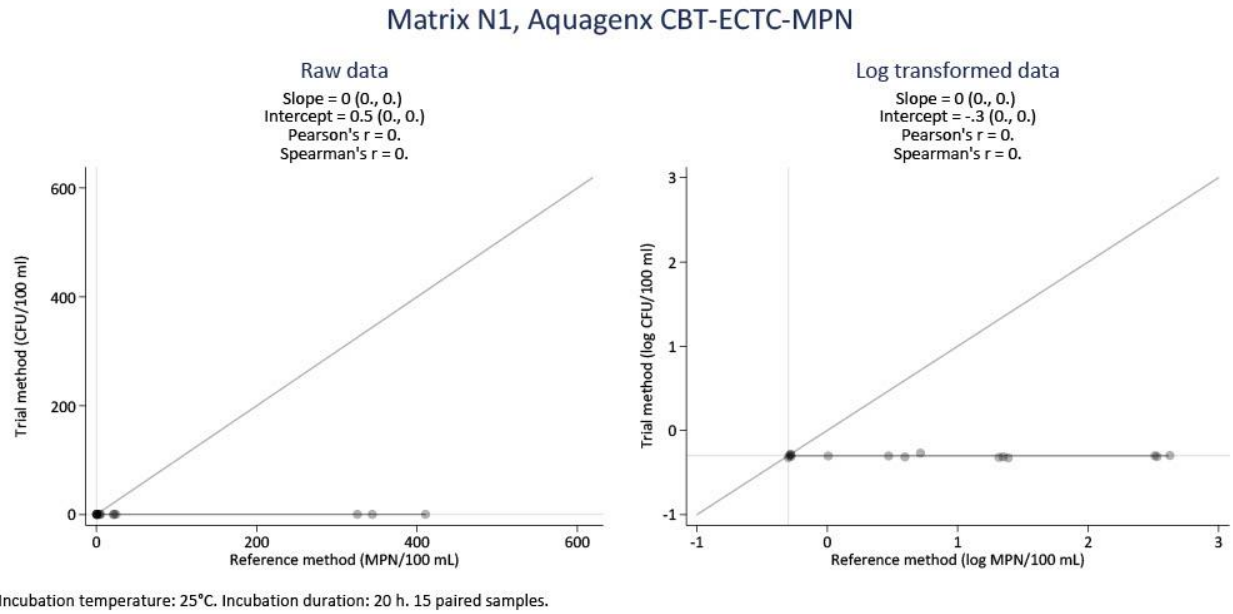


Figure 6: Statistical analysis Natural Matrix N1 after 20 hours at 25°C.

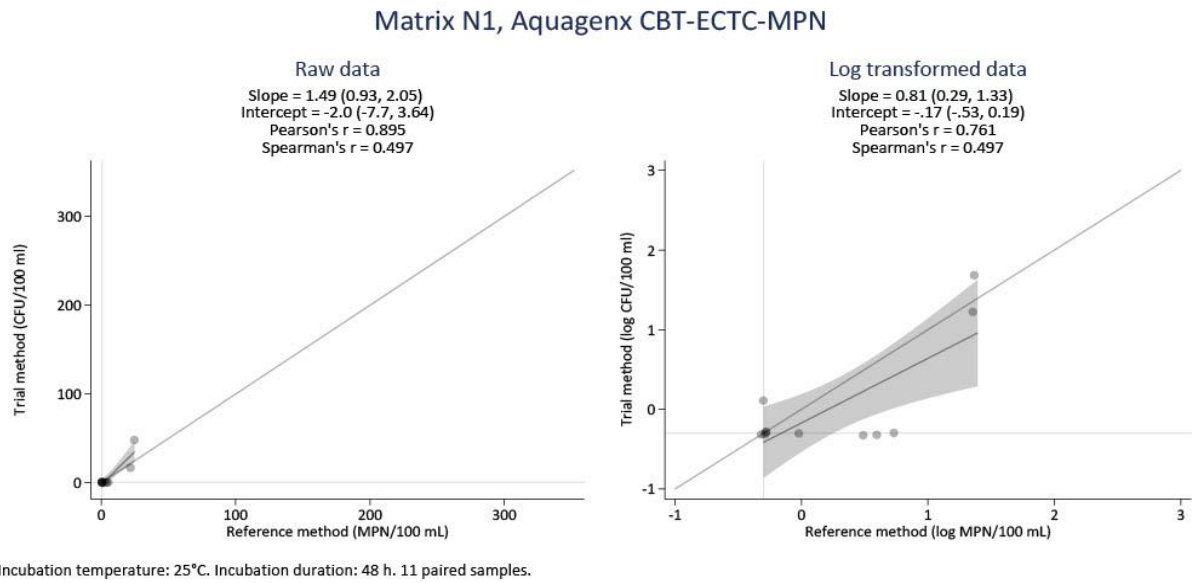


Figure 7: Statistical analysis Natural Matrix N1 after 48 hours at 25°C.

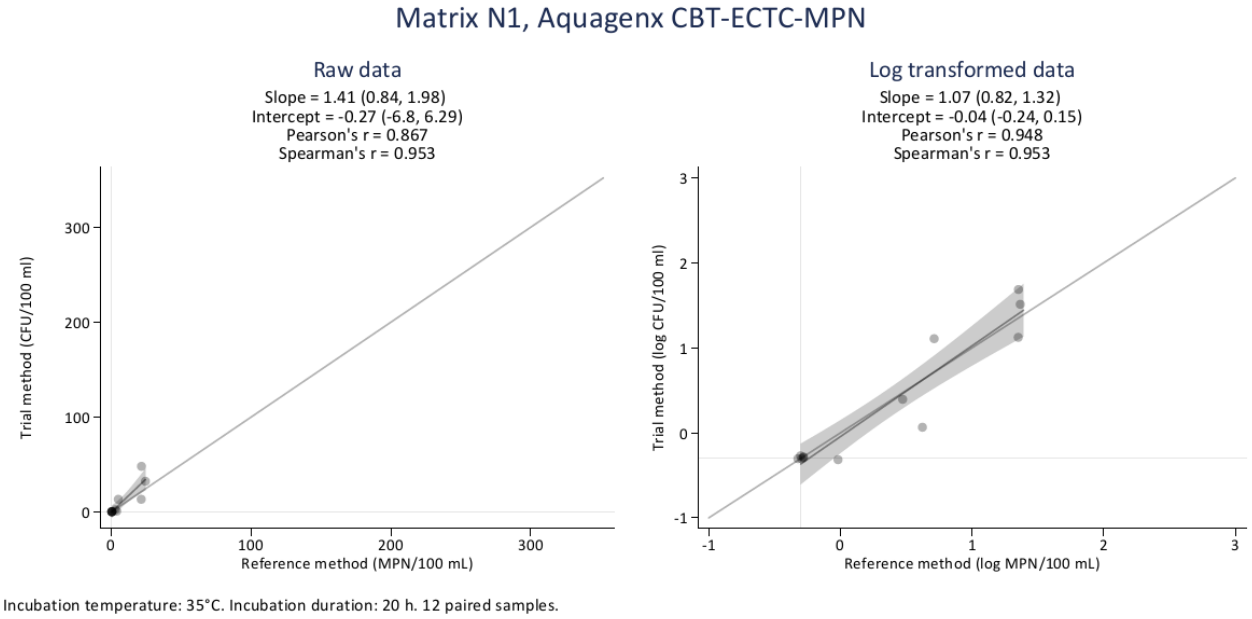


Figure 8: Statistical analysis Natural Matrix N1 after 20 hours at 35°C.

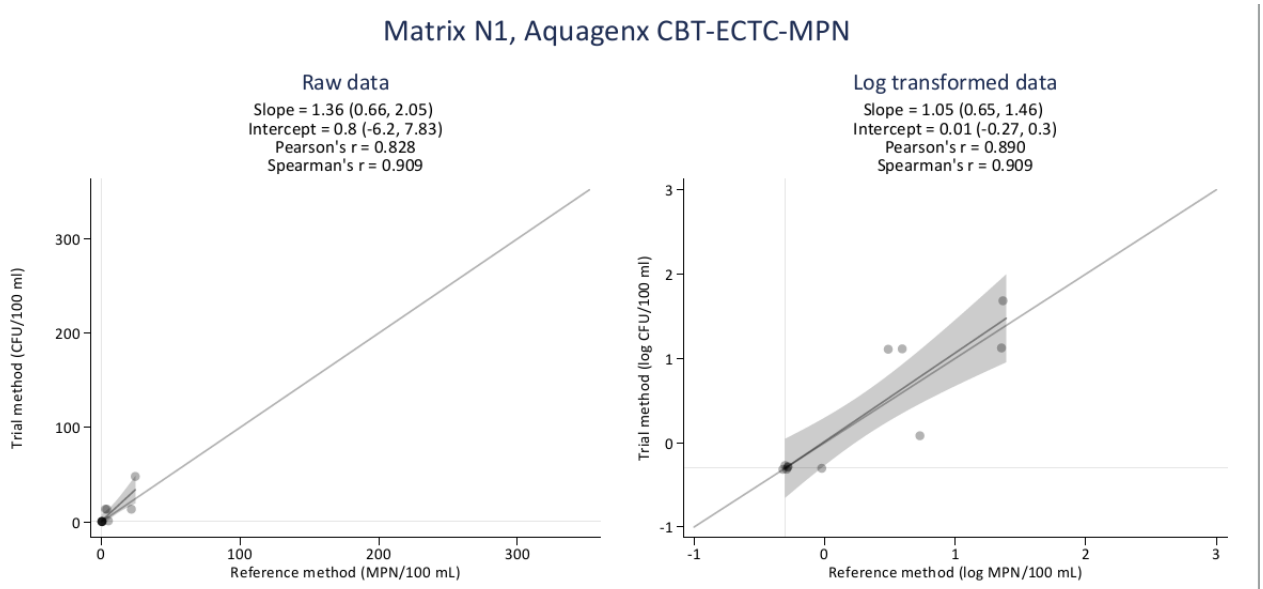


Figure 9: Statistical analysis Natural Matrix N1 after 48 hours at 35°C.

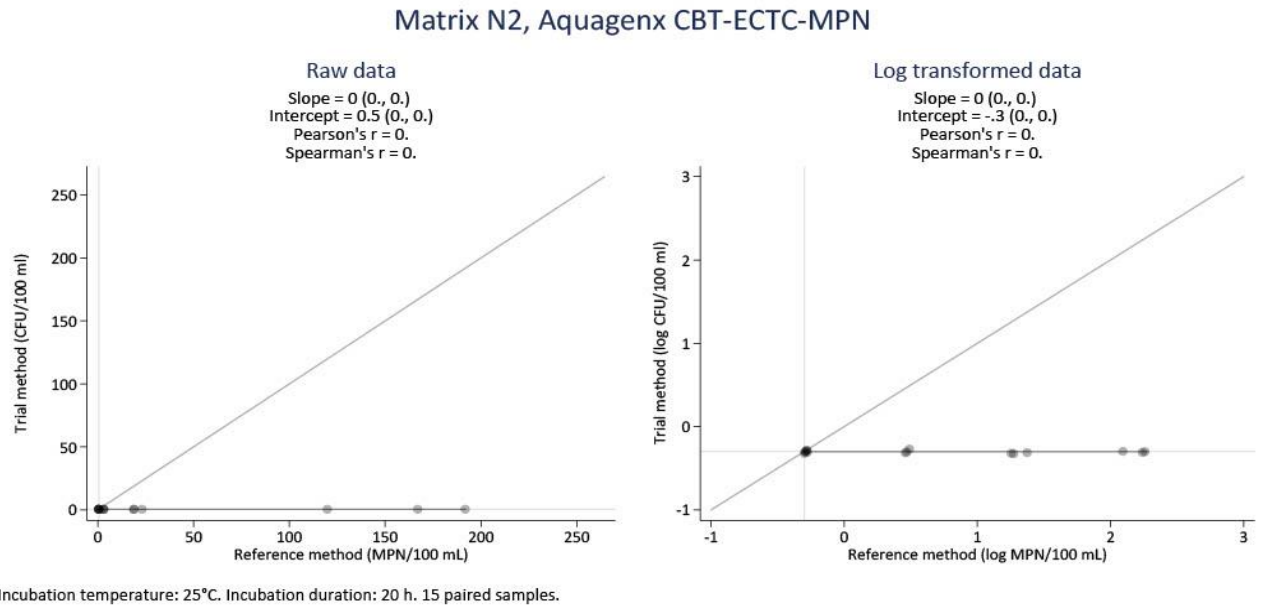


Figure 10: Statistical analysis Natural Matrix N2 after 20 hours at 25°C.

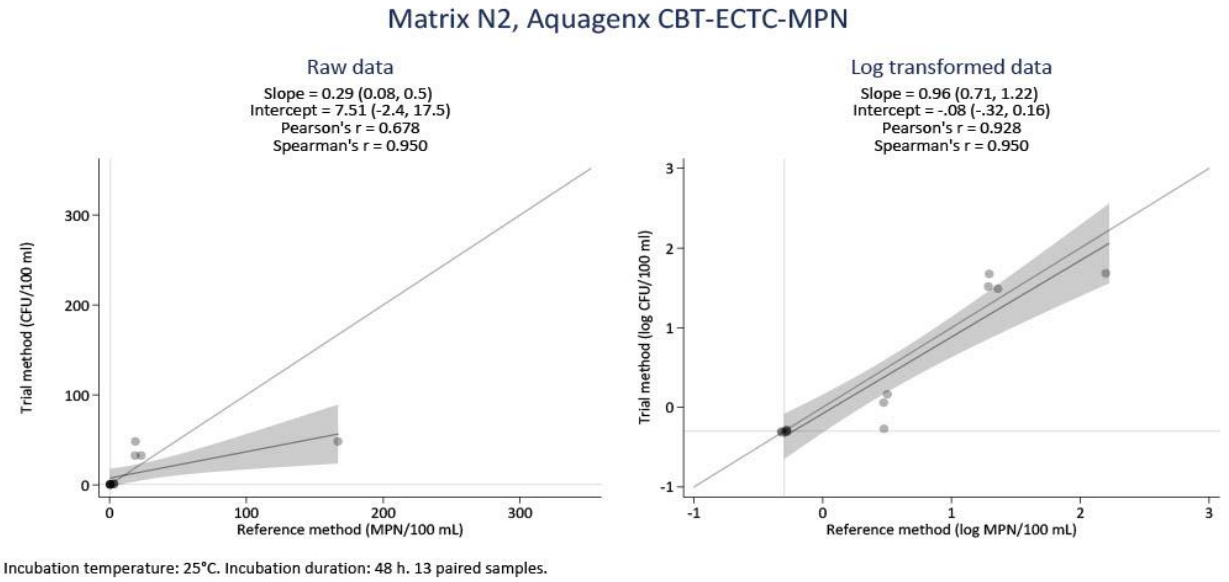


Figure 11: Statistical analysis Natural Matrix N2 after 48 hours at 25°C.

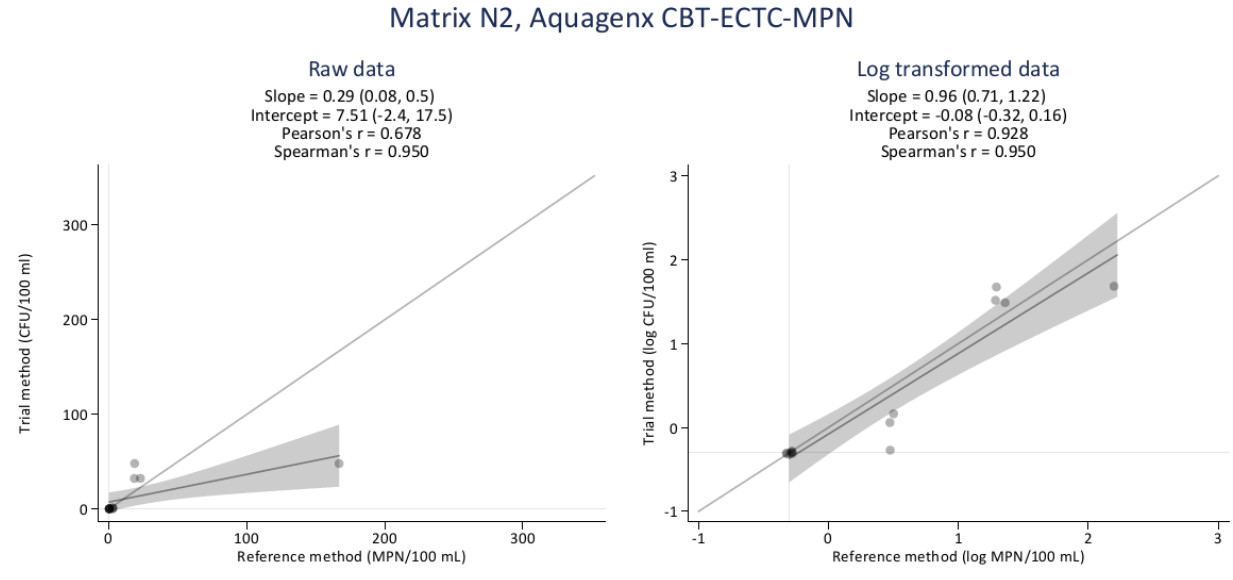


Figure 12: Statistical analysis Natural Matrix N2 after 20 hours at 35°C.

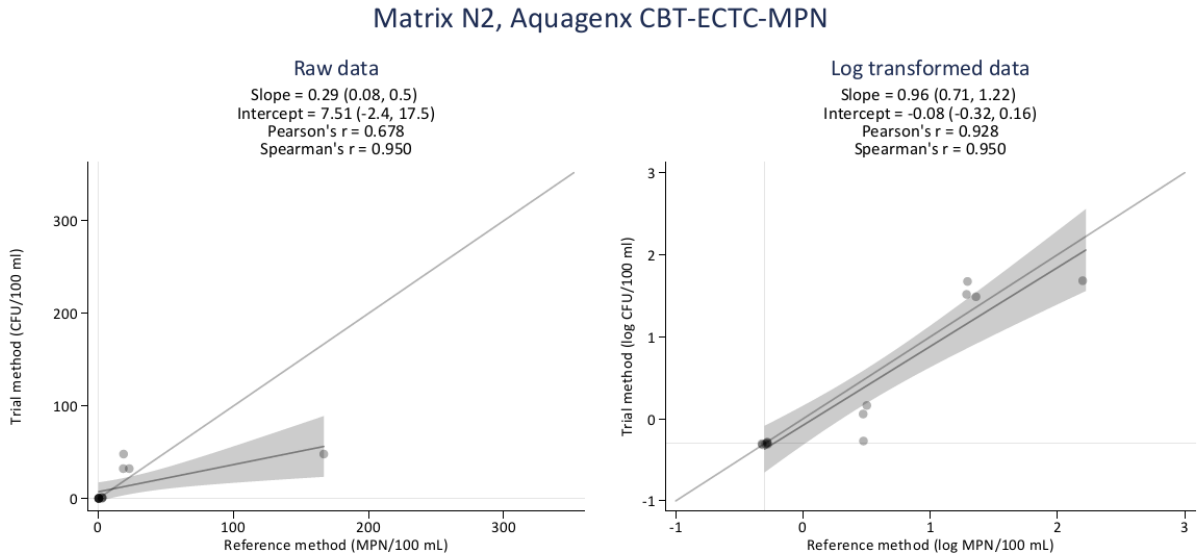


Figure 13: Statistical analysis Natural Matrix N2 after 48 hours at 35°C.

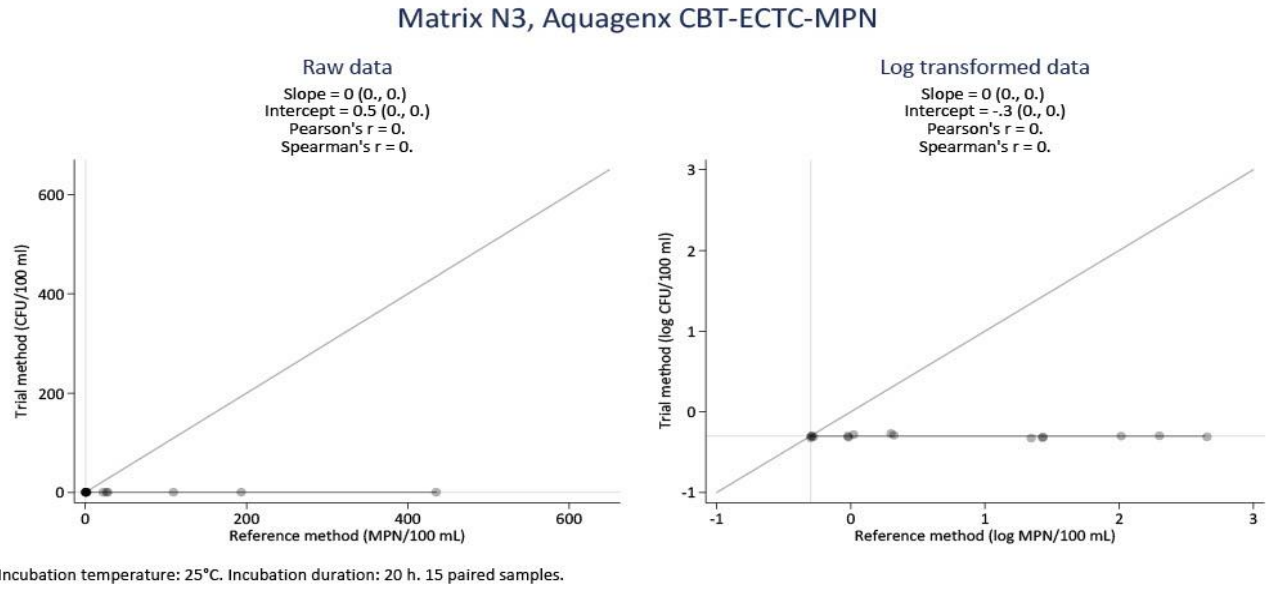


Figure 14: Statistical analysis Natural Matrix N3 after 20 hours at 25°C.

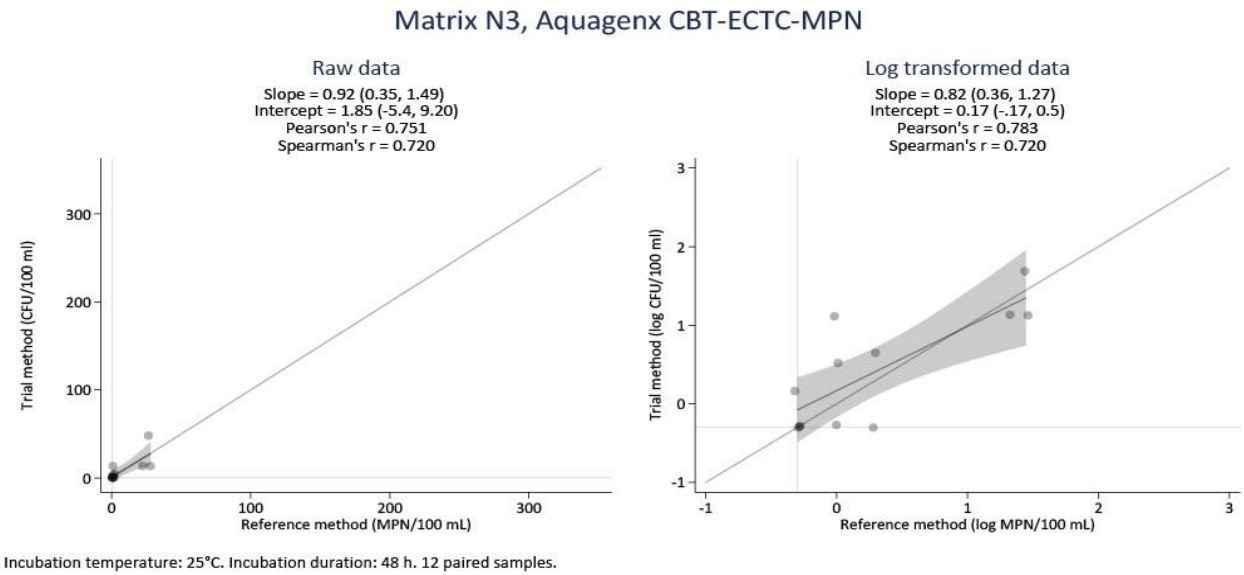


Figure 15: Statistical analysis Natural Matrix N3 after 48 hours at 25°C.

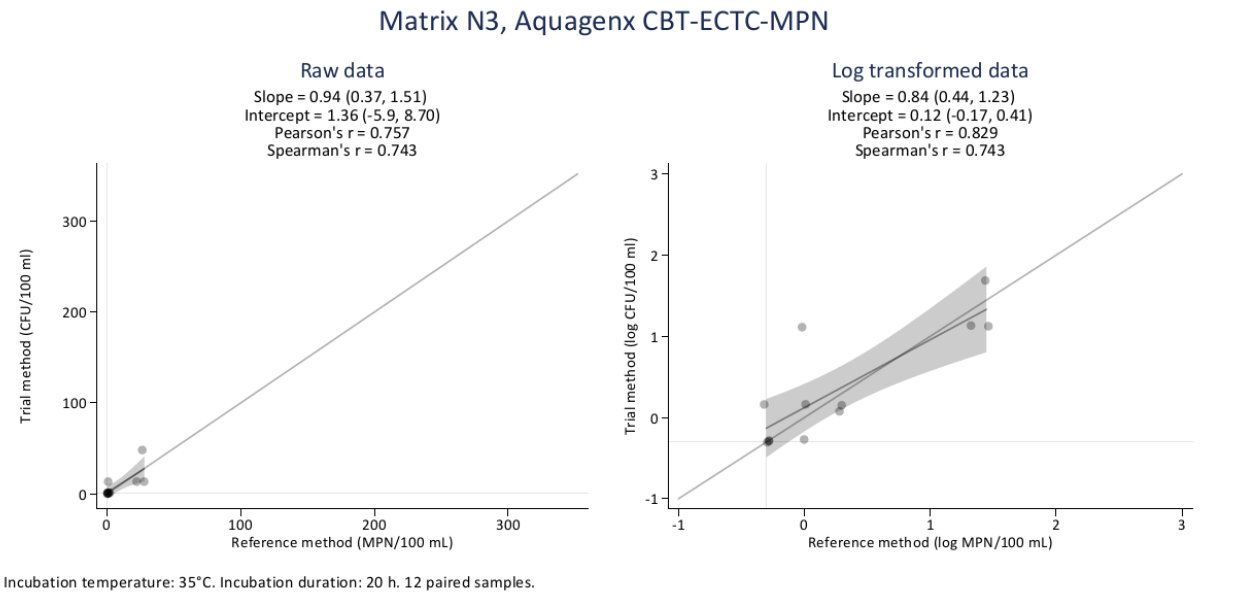
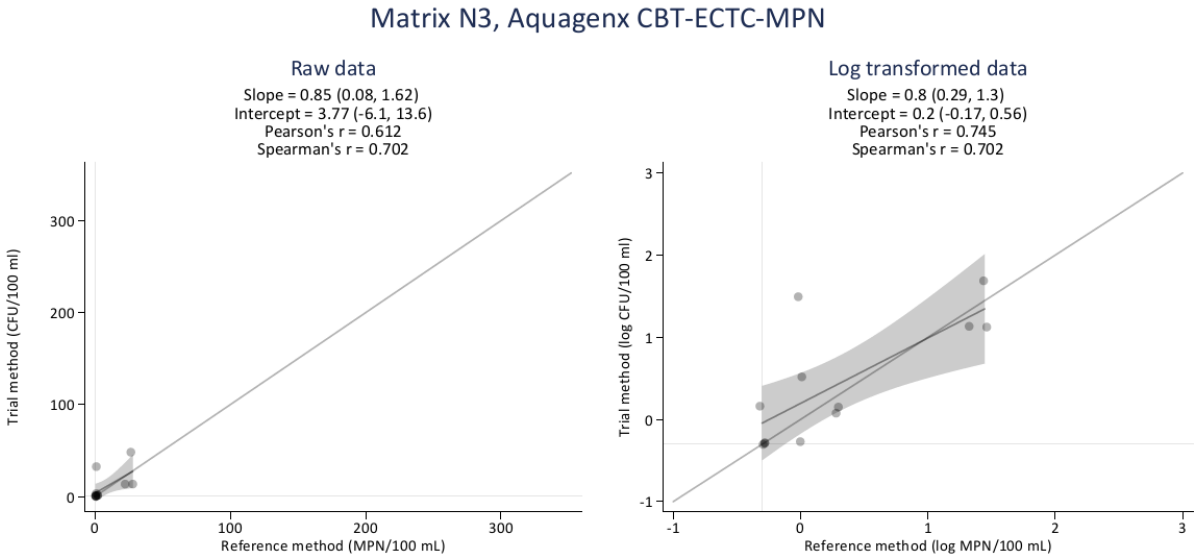
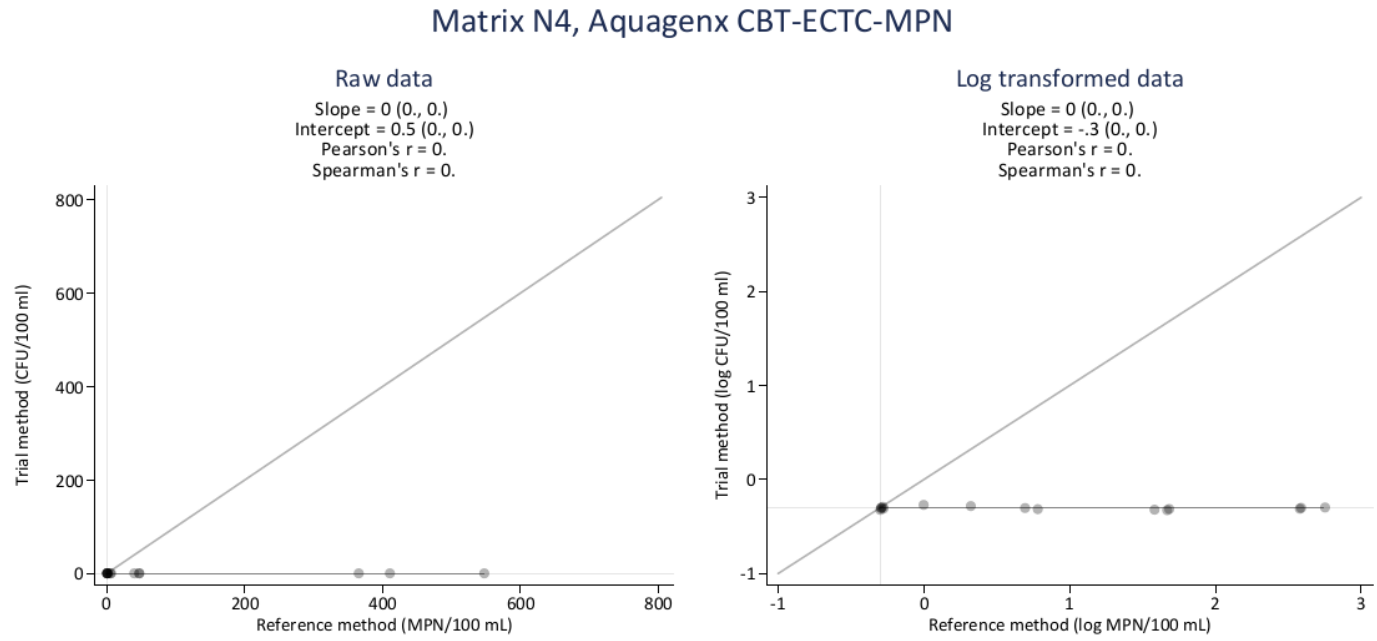


Figure 16: Statistical analysis Natural Matrix N3 after 20 hours at 35°C.



Incubation temperature: 35°C. Incubation duration: 48 h. 12 paired samples.

Figure 17: Statistical analysis Natural Matrix N3 after 48 hours at 35°C.



Incubation temperature: 25°C. Incubation duration: 20 h. 15 paired samples.

Figure 18: Statistical analysis Natural Matrix N4 after 20 hours at 25°C.

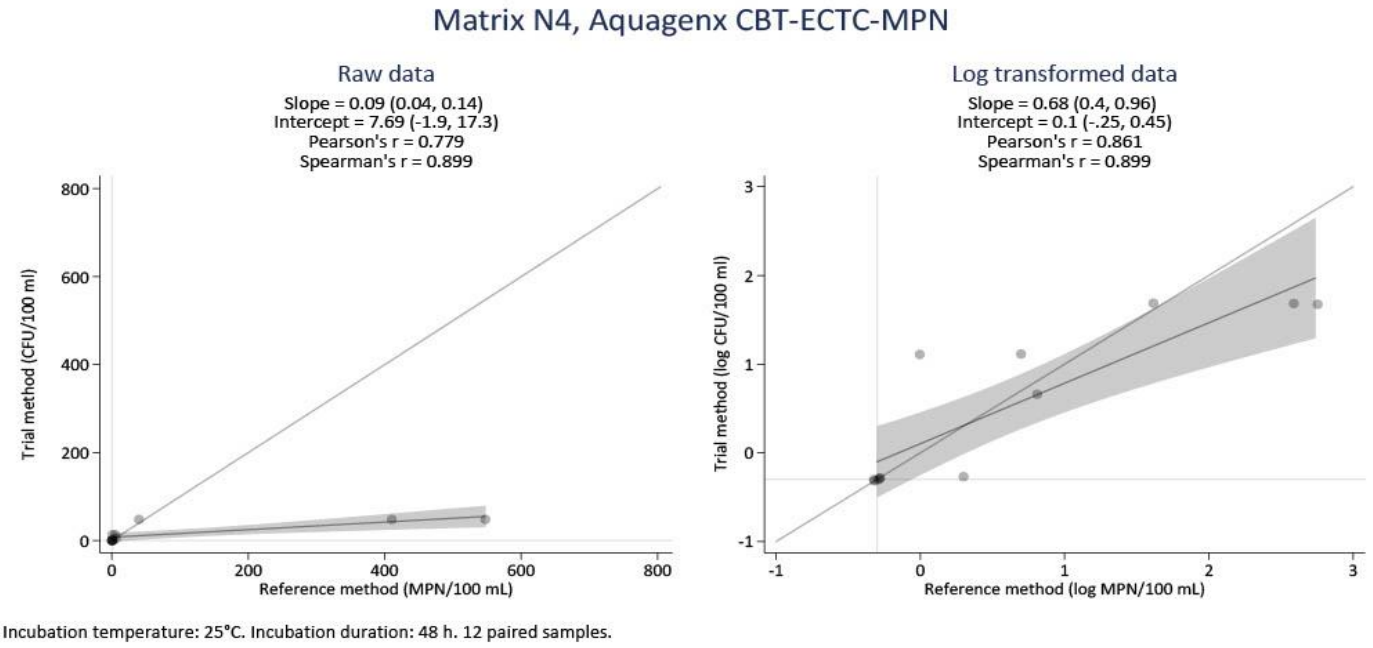


Figure 19: Statistical analysis Natural Matrix N4 after 48 hours at 25°C.

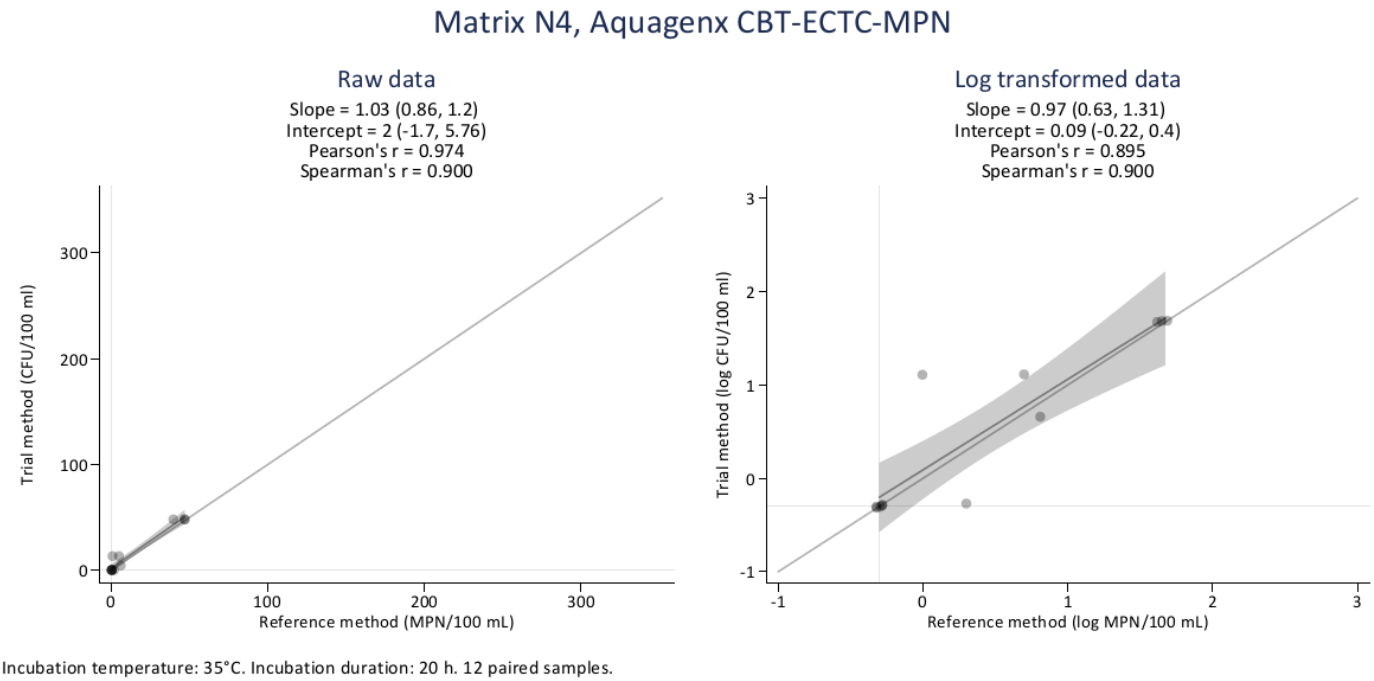


Figure 20: Statistical analysis Natural Matrix N4 after 20 hours at 35°C.

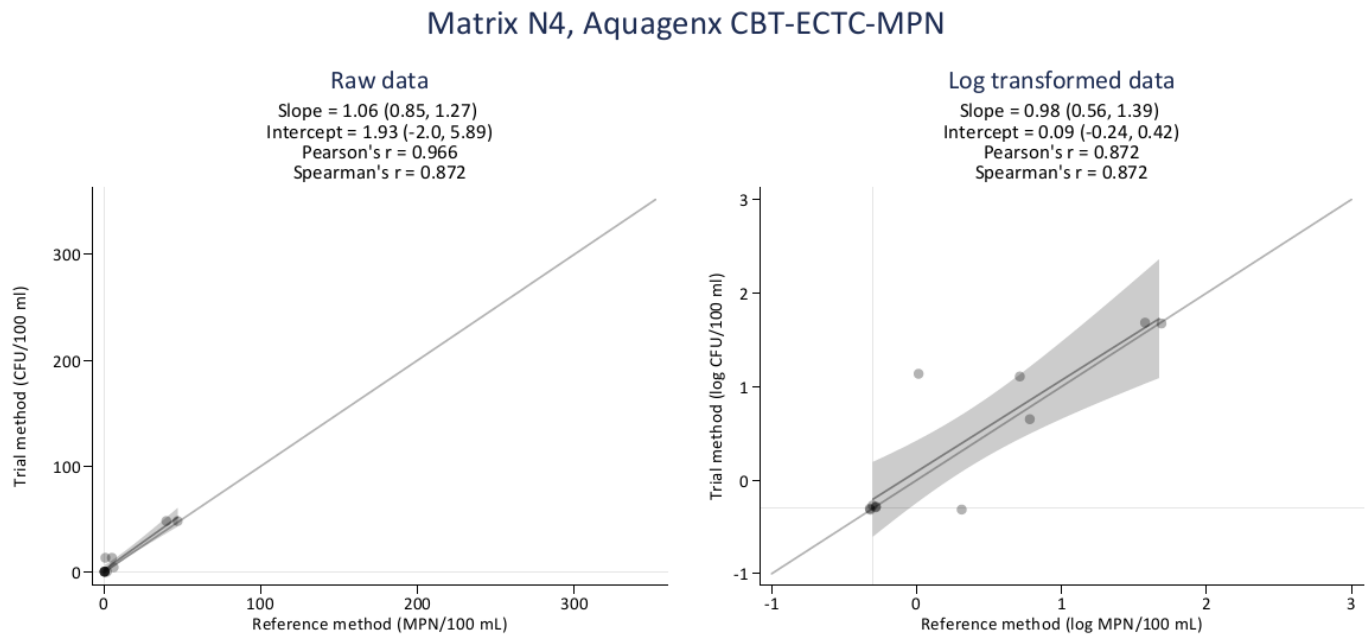


Figure 21: Statistical analysis Natural Matrix N4 after 48 hours at 35°C.

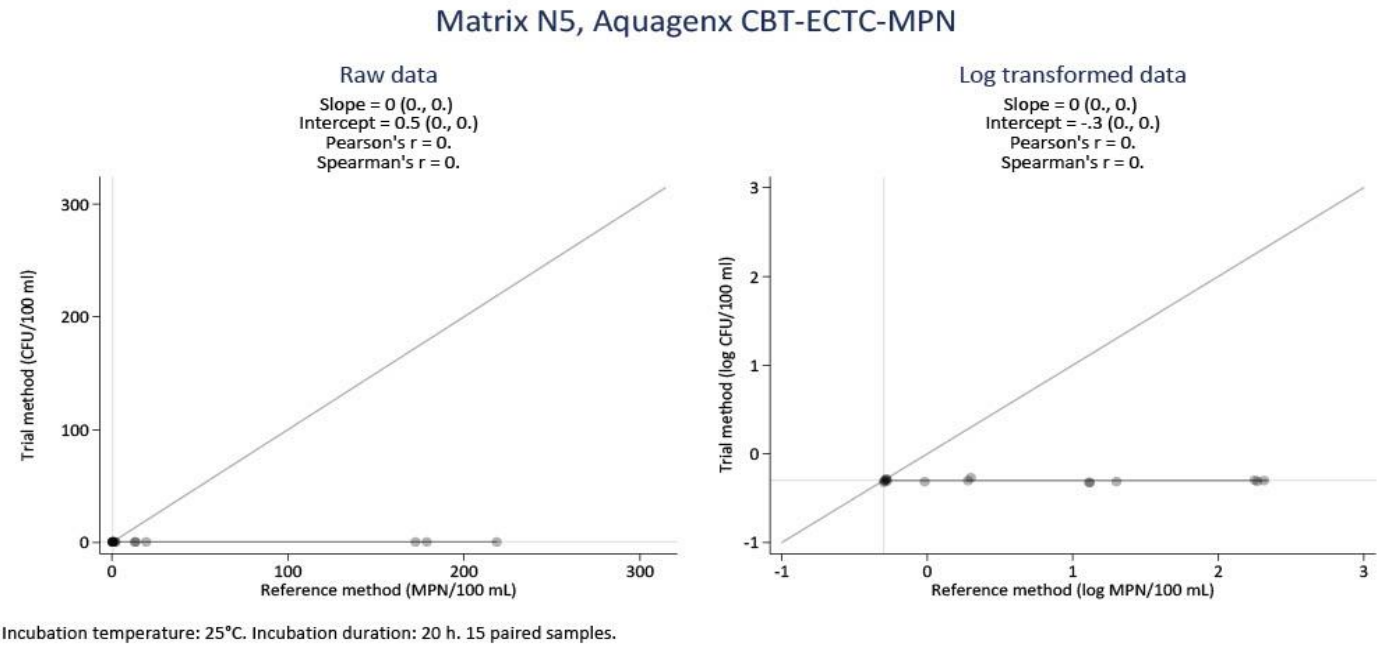


Figure 22: Statistical analysis Natural Matrix N5 after 20 hours at 25°C.

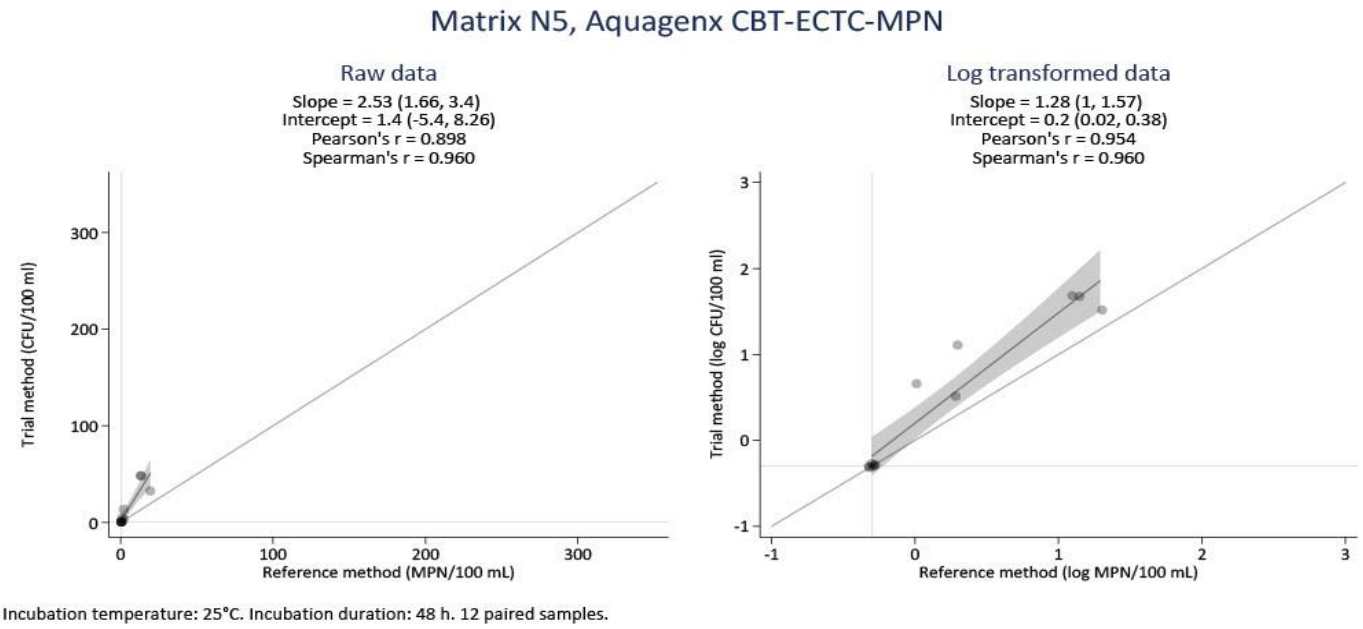


Figure 23: Statistical analysis Natural Matrix N5 after 48 hours at 25°C.

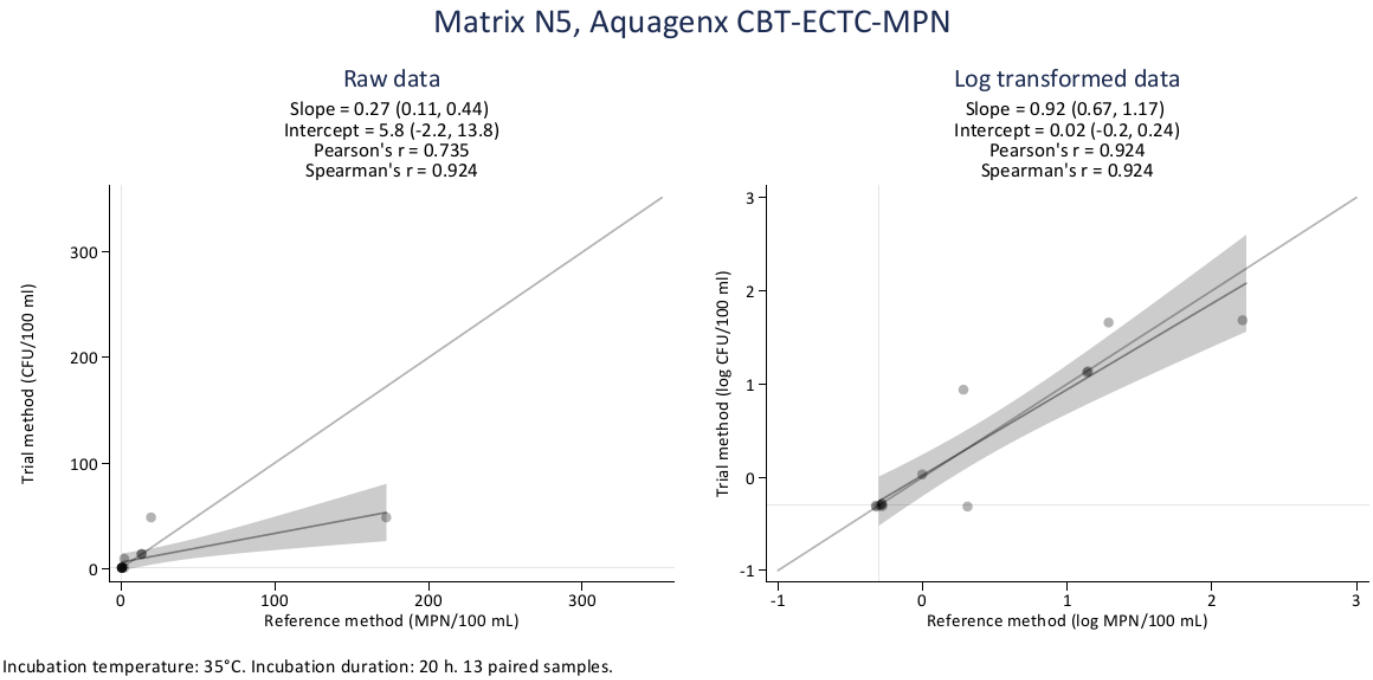
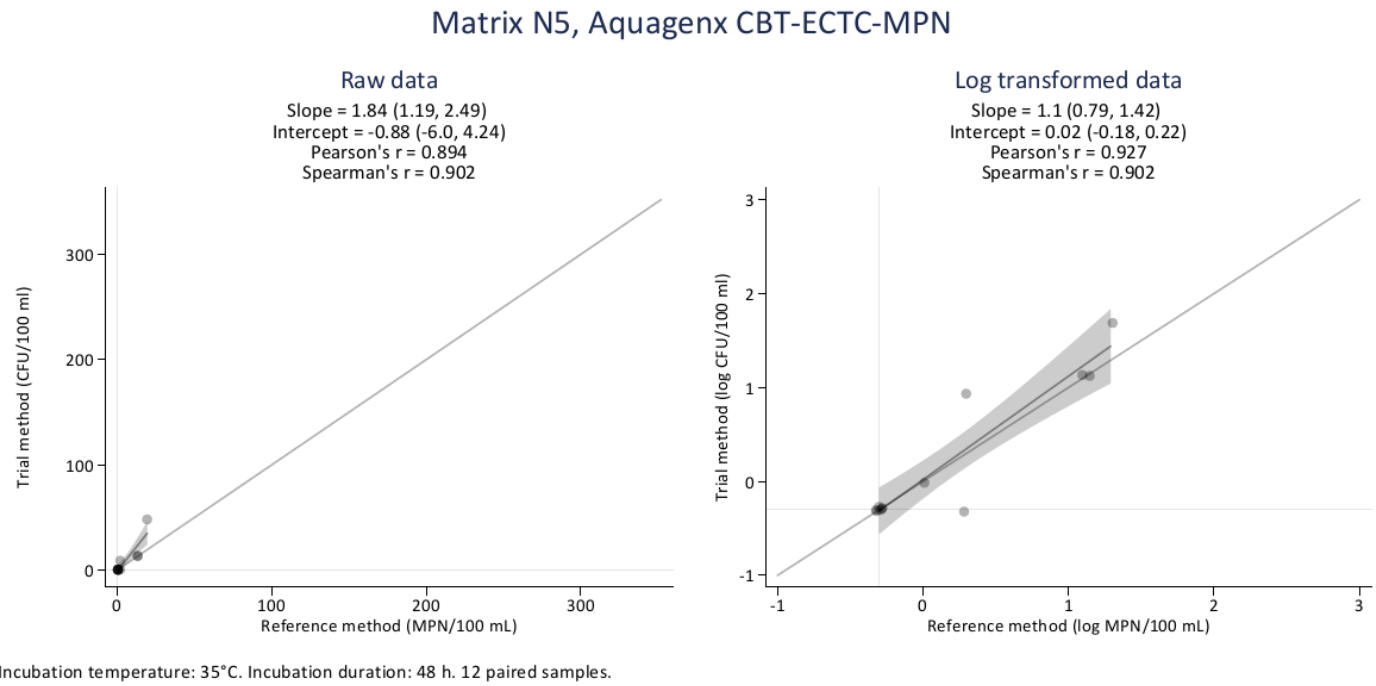


Figure 24: Statistical analysis Natural Matrix N5 after 20 hours at 35°C.



Incubation temperature: 35°C. Incubation duration: 48 h. 12 paired samples.

Figure 25: Statistical analysis Natural Matrix N5 after 48 hours at 35°C.

Interpretation and overview of results through linear regression on both raw data and log-transformed data is summarised below in Table 18 and Table 19.

Table 18: Overview of the regression analysis of the experiments at 25°C.

Water matrix	Time (h)	Number of samples	Maximum value	Slope (raw)	Intercept (raw)	Slope (log)	Intercept (log)	Spearman's r
Lab water	20	21	<1	0	0.5	0	-0.30	0
	48	18	>400	0.81	1.74	0.91	0.1	0.930
N1	20	15	<1	0.00	0.50	0.00	-0.30	0.00
	48	11	>100	1.49	-2.00	0.81	-0.17	0.50
N2	20	15	<1	0.00	0.50	0.00	-0.30	0.00
	48	13	>100	0.29	7.51	0.96	-0.08	0.95
N3	20	15	<1	0.00	0.50	0.00	-0.30	0.00
	48	12	>100	0.92	1.85	0.82	0.17	0.72
N4	20	15	<1	0.00	0.50	0.00	-0.30	0.00
	48	12	>100	0.09	7.69	0.68	0.10	0.90
N5	20	15	<1	0.00	0.50	0.00	-0.30	0.00
	48	12	>100	2.53	1.4	1.28	0.2	0.96

Table 19: Overview of the regression analysis of the experiments at 35°C.

Water matrix	Time (h)	Number of samples	Maximum value	Slope (raw)	Intercept (raw)	Slope (log)	Intercept (log)	Spearman's r
Lab water	20	18	>400	0.96	1.24	0.89	0.21	0.946
	48	18	>400	1.16	4.72	0.942	0.3	0.942
N1	20	12	>100	1.41	-0,27	1,07	-0,04	0,95
	48	11	>100	1.36	0.80	1.05	0.01	0.91
N2	20	13	>100	0,29	7,51	0,96	-0,08	0,95
	48	13	>100	0,29	7,51	0,96	-0,08	0,95
N3	20	12	>100	0,94	1,36	0,84	0,12	0,74
	48	12	>100	0,85	3,77	0,80	0,20	0,70
N4	20	12	>100	1.03	2,00	0,97	0,09	0,90
	48	11	>100	1.06	1,93	0,98	0,09	0,87
N5	20	13	>100	0,27	5,80	0,92	0,02	0,92
	48	12	>100	1.84	-0,88	1,10	0,02	0,90

The trial method was also assessed using the semi-quantitative risk classes defined in

Table 5. 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 Table 20 -Table 23 below presents a summary, showing the overall view of analyses gave the correct risk class.

Table 20: Results matching expected risk class after 20 hours at 25°C. (% results in risk class)

		Water Matrix					
Test Water	Risk Class	N1	N2	N3	N4	N5	Average
S1	>100 CFU/100 mL (very high risk)	0%	0%	0%	0%	0%	**0%**
S2	11-100 CFU/100 mL (high risk)	0%	0%	0%	0%	0%	**0%**
S3	1-10 CFU/100 mL (medium risk)	0%	0%	0%	0%	0%	**0%**
S4	<=1 CFU/100 mL* (low risk)	100%	100%	100%	100%	100%	**100%**
Average	n/a	**25%**	**25%**	**25%**	**25%**	**25%**	**25%**

Table 21: Results matching expected risk class after 48 hours at 25°C. (% results in risk class)

		Water Matrix					
Test Water	Risk Class	N1	N2	N3	N4	N5	Average
S1	>100 CFU/100 mL (very high risk)	100%	67%	100%	33%	100%	**80%**
S2	11-100 CFU/100 mL (high risk)	67%	100%	100%	33%	100%	**80%**
S3	1-10 CFU/100 mL (medium risk)	0%	67%	67%	33%	67%	**47%**
S4	<=1 CFU/100 mL* (low risk)	67%	100%	67%	100%	100%	**87%**
Average	n/a	**58%**	**83%**	**83%**	**50%**	**92%**	**73%**

Table 22: Results matching expected risk class after 20 hours at 35°C. (% results in risk class)

		Water Matrix					
Test Water	Risk Class	N1	N2	N3	N4	N5	Average
S1	>100 CFU/100 mL (very high risk)	100%	67%	100%	100%	67%	**87%**
S2	11-100 CFU/100 mL (high risk)	100%	100%	100%	100%	100%	**100%**
S3	1-10 CFU/100 mL (medium risk)	67%	67%	67%	33%	67%	**60%**
S4	<=1 CFU/100 mL* (low risk)	100%	100%	33%	100%	100%	**87%**
Average	n/a	**92%**	**83%**	**75%**	**83%**	**83%**	**83%**

Table 23: Results matching expected risk class after 48 hours at 35°C. (% results in risk class)

		Water Matrix					
Test Water	Risk Class	N1	N2	N3	N4	N5	Average
S1	>100 CFU/100 mL (very high risk)	100%	67%	100%	100%	100%	**93%**
S2	11-100 CFU/100 mL (high risk)	67%	100%	100%	67%	100%	**87%**
S3	1-10 CFU/100 mL (medium risk)	33%	67%	67%	33%	67%	**53%**
S4	<=1 CFU/100 mL* (low risk)	100%	100%	33%	100%	100%	**87%**
Average	n/a	**75%**	**83%**	**75%**	**75%**	**92%**	**80%**

Finally, the utility of the test to produce dichotomous presence/absence results was assessed at different thresholds.
Incubation temperature 25°C with an incubation time of 48 hours the threshold of 1 CFU/100 mL, 87% of tests were correctly classified. With thresholds of 10 and 100 CFU/100mL, the proportion of tests correctly classified were 47% and 80%, respectively.
Incubation temperature 35°C with an incubation time of 20 hours the threshold of 1 CFU/100 mL, 87% of tests were correctly classified. With thresholds of 10 and 100 CFU/100mL, the proportion of tests correctly classified were 60% and 100%, respectively.(see Table 24 - Table 27).

Table 24: Summary of presence/absence results after 20 hours at 25°C.

	Presence/absence cut-off		
Water matrix	1 CFU/100 mL	10 CFU/100 mL	100 CFU/100 mL
N1	25%	50%	75%
N2	25%	50%	75%
N3	25%	50%	75%
N4	25%	50%	75%
N5	25%	50%	75%
All	**25%**	**50%**	**75%**

Table 25: Summary of presence/absence results after 48 hours at 25°C.

	Presence/absence cut-off		
Water matrix	1 CFU/100 mL	10 CFU/100 mL	100 CFU/100 mL
N1	67%	100%	92%
N2	92%	100%	92%
N3	92%	92%	100%
N4	100%	83%	67%
N5	100%	92%	100%
All	**90%**	**93%**	**90%**

Table 26: Summary of presence/absence results after 20 hours at 35°C.

	Presence/absence cut-off		
Water matrix	1 CFU/100 mL	10 CFU/100 mL	100 CFU/100 mL
N1	100%	92%	100%
N2	92%	100%	92%
N3	83%	92%	100%
N4	100%	83%	100%
N5	92%	100%	92%
All	**93%**	**93%**	**97%**

Table 27: Summary of presence/absence results after 48 hours at 35°C.

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

5.3 Qualitative results

Lastly, a qualitative assessment of the CBT EC+TC MPN KITS 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 28.

Table 28: Scoring of the user friendliness of the CBT EC+TC MPN Kits test kits.

Subjects		Assessment	Explanation
User manual		Clear	In phase 1, the use of the clip to separate the compartments was not obvious
Execution test		Easy	
Interpretation results		Easy	
Contamination risk to:	Sample User	Medium Medium	Sample is transferred from one plastic bag to another plastic MPN bag. This could potentially cause contamination to the sample and the user. Some MPN bags were leaking
Dispose of materials with a high concentration of *E. coli*		Is described	Note: Is this disposal procedure sufficient to kill all the *E.coli* at high concentrations?

6 Appendix

6.1 Risk class matching

Table 29: Risk class matching expected risk class, Natural Matrix N1 after 20 hours at 25°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	<1	-3	0%	0%
		<1	-3	0%	
		<1	-3	0%	
S2	11-100 CFU/100 mL (high risk)	<1	-2	0%	0%
		<1	-2	0%	
		<1	-2	0%	
S3	1-10 CFU/100 mL (medium risk)	<1	-1	0%	0%
		<1	-1	0%	
		<1	-1	0%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	0	100%	
		<1	0	100%	
Average			**1.50**	**25%**	
Presence/Absence (1 CFU cut-off)				25%	
Presence/Absence (10 CFU cut-off)				50%	
Presence/Absence (100 CFU cut-off)				75%	

Table 30: Risk class matching expected risk class, Natural Matrix N1 after 48 hours at 25°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	>100	0	100%	100%
		>100	0	100%	
		>100	0	100%	
S2	11-100 CFU/100 mL (high risk)	48,3	0	100%	67%
		>100	1	0%	
		17,1	0	100%	
S3	1-10 CFU/100 mL (medium risk)	<1	-1	0%	0%
		<1	-1	0%	
		<1	-1	0%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	67%
		1,2	1	0%	
		<1	0	100%	
Average			**0.42**	**58%**	
Presence/Absence (1 CFU cut-off)				67%	
Presence/Absence (10 CFU cut-off)				100%	
Presence/Absence (100 CFU cut-off)				92%	

Table 31: Risk class matching expected risk class, Natural Matrix N1 after 20 hours at 35°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	>100	0	100%	100%
		>100	0	100%	
		>100	0	100%	
S2	11-100 CFU/100 mL (high risk)	32,6	0	100%	100%
		13,6	0	100%	
		48,3	0	100%	
S3	1-10 CFU/100 mL (medium risk)	13,6	1	0%	67%
		2,6	0	100%	
		1,2	0	100%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	0	100%	
		<1	0	100%	
Average			**0.08**	**92%**	
Presence/Absence (1 CFU cut-off)				100%	
Presence/Absence (10 CFU cut-off)				92%	
Presence/Absence (100 CFU cut-off)				100%	

Table 32: Risk class matching expected risk class, Natural Matrix N1 after 48 hours at 35°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	>100	0	100%	100%
		>100	0	100%	
		>100	0	100%	
S2	11-100 CFU/100 mL (high risk)	48,3	0	100%	67%
		>100	1	0%	
		13,6	0	100%	
S3	1-10 CFU/100 mL (medium risk)	1,2	0	100%	33%
		13,6	1	0%	
		13,6	1	0%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	0	100%	
		<1	0	100%	
Average			**0.25**	**75%**	
Presence/Absence (1 CFU cut-off)				100%	
Presence/Absence (10 CFU cut-off)				83%	
Presence/Absence (100 CFU cut-off)				92%	

Table 33: Risk class matching expected risk class, Natural Matrix N2 after 20 hours at 25°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	<1	-3	0%	0%
		<1	-3	0%	
		<1	-3	0%	
S2	11-100 CFU/100 mL (high risk)	<1	-2	0%	0%
		<1	-2	0%	
		<1	-2	0%	
S3	1-10 CFU/100 mL (medium risk)	<1	-1	0%	0%
		<1	-1	0%	
		<1	-1	0%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	0	100%	
		<1	0	100%	
Average			**1.50**	**25%**	
Presence/Absence (1 CFU cut-off)				25%	
Presence/Absence (10 CFU cut-off)				50%	
Presence/Absence (100 CFU cut-off)				75%	

Table 34: Risk class matching expected risk class, Natural Matrix N2 after 48 hours at 25°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	>100	0	100%	67%
		48,3	-1	0%	
		>100	0	100%	
S2	11-100 CFU/100 mL (high risk)	48,3	0	100%	100%
		32,6	0	100%	
		32,6	0	100%	
S3	1-10 CFU/100 mL (medium risk)	1,2	0	100%	67%
		1,5	0	100%	
		<1	-1	0%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		,<1	0	100%	
		<1	0	100%	
Average			**0.17**	**83%**	
Presence/Absence (1 CFU cut-off)				92%	
Presence/Absence (10 CFU cut-off)				100%	
Presence/Absence (100 CFU cut-off)				92%	

Table 35: Risk class matching expected risk class, Natural Matrix N2 after 20 hours at 35°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	>100	0	100%	67%
		48,3	-1	0%	
		>100	0	100%	
S2	11-100 CFU/100 mL (high risk)	48,3	0	100%	100%
		32,6	0	100%	
		32,6	0	100%	
S3	1-10 CFU/100 mL (medium risk)	1,2	0	100%	67%
		1,5	0	100%	
		<1	-1	0%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	0	100%	
		<1	0	100%	
Average			**0.17**	**83%**	
Presence/Absence (1 CFU cut-off)				92%	
Presence/Absence (10 CFU cut-off)				100%	
Presence/Absence (100 CFU cut-off)				92%	

Table 36: Risk class matching expected risk class, Natural Matrix N2 after 48 hours at 35°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	>100	0	100%	67%
		48,3	-1	0%	
		>100	0	100%	
S2	11-100 CFU/100 mL (high risk)	48,3	0	100%	100%
		32,6	0	100%	
		32,6	0	100%	
S3	1-10 CFU/100 mL (medium risk)	1,2	0	100%	67%
		1,5	0	100%	
		<1	-1	0%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	0	100%	
		<1	0	100%	
Average			**0.17**	**83%**	
Presence/Absence (1 CFU cut-off)				92%	
Presence/Absence (10 CFU cut-off)				100%	
Presence/Absence (100 CFU cut-off)				92%	

Table 37: Risk class matching expected risk class, Natural Matrix N3 after 20 hours at 25°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	<1	-3	0%	0%
		<1	-3	0%	
		<1	-3	0%	
S2	11-100 CFU/100 mL (high risk)	<1	-2	0%	0%
		<1	-2	0%	
		<1	-2	0%	
S3	1-10 CFU/100 mL (medium risk)	<1	-1	0%	0%
		<1	-1	0%	
		<1	-1	0%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	0	100%	
		<1	0	100%	
Average			**1.50**	**25%**	
Presence/Absence (1 CFU cut-off)				25%	
Presence/Absence (10 CFU cut-off)				50%	
Presence/Absence (100 CFU cut-off)				75%	

Table 38: Risk class matching expected risk class, Natural Matrix N3 after 48 hours at 25°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	>100	0	100%	100%
		>100	0	100%	
		>100	0	100%	
S2	11-100 CFU/100 mL (high risk)	13,6	0	100%	100%
		13,6	0	100%	
		48,3	0	100%	
S3	1-10 CFU/100 mL (medium risk)	4,7	0	100%	67%
		13,6	1	0%	
		3,4	0	100%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	67%
		<1	0	100%	
		1.5	1	0%	
Average			**0.17**	**83%**	
Presence/Absence (1 CFU cut-off)				92%	
Presence/Absence (10 CFU cut-off)				92%	
Presence/Absence (100 CFU cut-off)				92%	

Table 39: Risk class matching expected risk class, Natural Matrix N3 after 20 hours at 35°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	> 100	0	100%	100%
		> 100	0	100%	
		> 100	0	100%	
S2	11-100 CFU/100 mL (high risk)	13,6	0	100%	100%
		13,6	0	100%	
		48,3	0	100%	
S3	1-10 CFU/100 mL (medium risk)	1,5	0	100%	67%
		13,6	1	0%	
		1,5	0	100%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	33%
		1,2	1	0%	
		1,5	1	0%	
Average			**0.25**	**75%**	
Presence/Absence (1 CFU cut-off)				83%	
Presence/Absence (10 CFU cut-off)				92%	
Presence/Absence (100 CFU cut-off)				100%	

Table 40: Risk class matching expected risk class, Natural Matrix N3 after 48 hours at 35°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	> 100	0	100%	100%
		> 100	0	100%	
		> 100	0	100%	
S2	11-100 CFU/100 mL (high risk)	13,6	0	100%	100%
		13,6	0	100%	
		48,3	0	100%	
S3	1-10 CFU/100 mL (medium risk)	1,5	0	100%	67%
		32,6	1	0%	
		3,4	0	100%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	33%
		1,2	1	0%	
		1,5	1	0%	
Average			**0.25**	**75%**	
Presence/Absence (1 CFU cut-off)				83%	
Presence/Absence (10 CFU cut-off)				92%	
Presence/Absence (100 CFU cut-off)				100%	

Table 41: Risk class matching expected risk class, Natural Matrix N4 after 20 hours at 25°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	<1	-3	0%	0%
		<1	-3	0%	
		<1	-3	0%	
S2	11-100 CFU/100 mL (high risk)	<1	-2	0%	0%
		<1	-2	0%	
		<1	-2	0%	
S3	1-10 CFU/100 mL (medium risk)	<1	-1	0%	0%
		<1	-1	0%	
		<1	-1	0%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	0	100%	
		<1	0	100%	
Average			**1.50**	**25%**	
Presence/Absence (1 CFU cut-off)				25%	
Presence/Absence (10 CFU cut-off)				50%	
Presence/Absence (100 CFU cut-off)				75%	

Table 42: Risk class matching expected risk class, Natural Matrix N4 after 48 hours at 25°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	48,3	-1	0%	33%
		> 100	0	100%	
		48,3	-1	0%	
S2	11-100 CFU/100 mL (high risk)	> 100	1	0%	33%
		48,3	0	100%	
		> 100	1	0%	
S3	1-10 CFU/100 mL (medium risk)	13,6	1	0%	33%
		13,6	1	0%	
		4,7	0	100%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	0	100%	
		<1	0	100%	
Average			**0.50**	**50%**	
Presence/Absence (1 CFU cut-off)				100%	
Presence/Absence (10 CFU cut-off)				83%	
Presence/Absence (100 CFU cut-off)				67%	

Table 43: Risk class matching expected risk class, Natural Matrix N4 after 20 hours at 35°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	> 100	0	100%	100%
		> 100	0	100%	
		> 100	0	100%	
S2	11-100 CFU/100 mL (high risk)	48,3	0	100%	100%
		48,3	0	100%	
		48,3	0	100%	
S3	1-10 CFU/100 mL (medium risk)	13,6	1	0%	33%
		13,6	1	0%	
		4,7	0	100%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	0	100%	
		<1	0	100%	
Average			**0.17**	**83%**	
Presence/Absence (1 CFU cut-off)				100%	
Presence/Absence (10 CFU cut-off)				83%	
Presence/Absence (100 CFU cut-off)				100%	

Table 44: Risk class matching expected risk class, Natural Matrix N4 after 48 hours at 35°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	> 100	0	100%	100%
		> 100	0	100%	
		> 100	0	100%	
S2	11-100 CFU/100 mL (high risk)	> 100	1	0%	67%
		48,3	0	100%	
		48,3	0	100%	
S3	1-10 CFU/100 mL (medium risk)	13,6	1	0%	33%
		13,6	1	0%	
		4,7	0	100%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	0	100%	
		<1	0	100%	
Average			**0.25**	**75%**	
Presence/Absence (1 CFU cut-off)				100%	
Presence/Absence (10 CFU cut-off)				83%	
Presence/Absence (100 CFU cut-off)				92%	

Table 45: Risk class matching expected risk class, Natural Matrix N5 after 20 hours at 25°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	<1	-3	0%	0%
		<1	-3	0%	
		<1	-3	0%	
S2	11-100 CFU/100 mL (high risk)	<1	-2	0%	0%
		<1	-2	0%	
		<1	-2	0%	
S3	1-10 CFU/100 mL (medium risk)	<1	-1	0%	0%
		<1	-1	0%	
		<1	-1	0%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	**0**	**100%**	
		<1	**0**	**100%**	
Average			**1.50**	**25%**	
Presence/Absence (1 CFU cut-off)				25%	
Presence/Absence (10 CFU cut-off)				50%	
Presence/Absence (100 CFU cut-off)				75%	

Table 46: Risk class matching expected risk class, Natural Matrix N5 after 48 hours at 25°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	>100	0	100%	100%
		>100	0	100%	
		>100	0	100%	
S2	11-100 CFU/100 mL (high risk)	48,3	0	100%	100%
		48,3	0	100%	
		32,6	0	100%	
S3	1-10 CFU/100 mL (medium risk)	13,6	1	0%	67%
		3,4	0	100%	
		4,7	0	100%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	**0**	**100%**	
		<1	**0**	**100%**	
Average			**0.08**	**%**	
Presence/Absence (1 CFU cut-off)				100%	
Presence/Absence (10 CFU cut-off)				92%	
Presence/Absence (100 CFU cut-off)				100%	

Table 47: Risk class matching expected risk class, Natural Matrix N5 after 20 hours at 35°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	> 100	0	100%	67%
		> 100	0	100%	
		48,3	-1	0%	
S2	11-100 CFU/100 mL (high risk)	13,6	0	100%	100%
		13,6	0	100%	
		48,3	0	100%	
S3	1-10 CFU/100 mL (medium risk)	9,1	0	100%	67%
		<1	-1	0%	
		1	0	100%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	0	100%	
		<1	0	100%	
Average			**0.17**	**83%**	
Presence/Absence (1 CFU cut-off)				92%	
Presence/Absence (10 CFU cut-off)				100%	
Presence/Absence (100 CFU cut-off)				92%	

Table 48: Risk class matching expected risk class, Natural Matrix N5 after 48 hours at 35-37°C.

Test water	Risk class	CFU/100 mL	Risk class difference	Correct risk class	
				Single test	Triplicates
S1	>100 CFU/100 mL (very high risk)	> 100	0	100%	100%
		> 100	0	100%	
		>100	0	100%	
S2	11-100 CFU/100 mL (high risk)	13,6	0	100%	100%
		13,6	0	100%	
		48,3	0	100%	
S3	1-10 CFU/100 mL (medium risk)	9,1	0	100%	67%
		<1	-1	0%	
		1	0	100%	
S4	<=1 CFU/100 mL (low risk)	<1	0	100%	100%
		<1	0	100%	
		<1	0	100%	
Average			**0.08**	**92%**	
Presence/Absence (1 CFU cut-off)				92%	
Presence/Absence (10 CFU cut-off)				100%	
Presence/Absence (100 CFU cut-off)				100%	

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

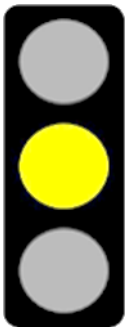
Incubation temperature:

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.

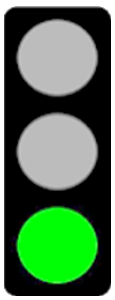
Natural waters:

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.

6.2.1 False positive due to non-target bacteria.

Table 49: Results of the false positives test at 25°C.

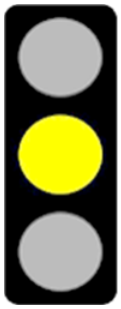
Non-target bacteria ($1*10^8$ CFU/100 mL)	Quantitative test results (CFU/100 mL) 16-24h	48h
Aeromonas	-	-
Citrobacter	-	-
Enterobacter	-	-
Klebsiella	-	-
Pseudomonas	-	-
E. coli *	>100	>100



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

Table 50: Results of the false positives test at 35°C.

Non-target bacteria ($1*10^8$ CFU/100 mL)	Quantitative test results (CFU/100 mL) 16-24h	48h
Aeromonas	1.5	1.5
Citrobacter	-	-
Enterobacter	-	-
Klebsiella	-	-
Pseudomonas	-	-
E. coli *	>100	>100



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

6.2.2 False negatives due to competition

Table 51: Results of the false negatives test at 25°C.

Non-target bacteria (30,000 CFU/100 mL)	Target bacteria (30 CFU/100 mL)	Quantitative test results (CFU/100 mL) 24h	48h
Aeromonas	*E. coli*	0	9.6
Citrobacter	*E. coli*	0	2.6
Enterobacter	*E. coli*	0	48.3
Klebsiella	*E. coli*	0	13.6
Pseudomonas	*E. coli*	0	48.3
	E. coli	0	13.6

Table 52: Results of the false negatives test at 35-37°C.

Non-target bacteria (30,000 CFU/100 mL)	Target bacteria (30 CFU/100 mL)	Quantitative test results (CFU/100 mL) 24h	48h
Aeromonas	*E. coli*	32.6	32.6
Citrobacter	*E. coli*	2.6	2.6
Enterobacter	*E. coli*	13.6	13.6
Klebsiella	*E. coli*	2.6	2.6
Pseudomonas	*E. coli*	48.3	48.3
	E. coli	13.6	13.6

6.2.3 Natural waters

Table 53: Results matching expected risk class, by water matrix after 20 hours at 25°C.

	Water Matrix				
Test Water	N1	N2	N3	N4	N5
S1	0%	0%	0%	0%	0%
S2	0%	0%	0%	0%	0%
S3	0%	0%	0%	0%	0%
S4	100%	100%	100%	100%	100%
Average	**25%**	**25%**	**25%**	**25%**	**25%**
Grand Average			**25%**		

Table 54: Results matching expected risk class, by water matrix after 48 hours at 25°C.

	Water Matrix				
Test Water	N1	N2	N3	N4	N5
S1	100%	67%	100%	33%	100%
S2	67%	100%	100%	33%	100%
S3	0%	67%	67%	33%	67%
S4	67%	100%	67%	100%	100%
Average	**58%**	**83%**	**83%**	**50%**	**92%**
Grand Average			**73%**		

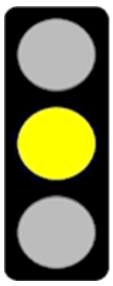


Table 55: Results matching expected risk class, by water matrix 20 hours at 35°C.

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

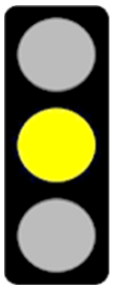


Table 56: Results matching expected risk class, by water matrix 48 hours at 35°C.

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

6.2.4 Manual

Aquagenx®

Aquagenx® CBT EC+TC (Compartment Bag Test)
Most Probable Number (MPN) Kit
Instructions for Use: Drinking Water

Overview
The Aquagenx CBT EC+TC MPN Kit simultaneously detects and quantifies *E. coli* (EC) and Total Coliform (TC) bacteria in a 100 mL sample. It uses a proprietary powder growth medium with a glucose substrate called X-Gluc. When *E. coli* metabolize this substrate in Aquagenx's growth medium, the color of the water turns blue, indicating the presence of *E. coli*. The growth medium also contains a fluorogenic galactoside substrate called MUGal. If total coliforms are present, they metabolize this fluorogenic substrate and the sample fluoresces blue under a UV light (365 nm). Most Probable Number (MPN) test results are obtained by easy color match using the Aquagenx color-coded MPN Table. The total coliform group of bacteria includes *E. coli*, which is a fecal coliform as well as a thermotolerant coliform. Not all total coliforms are *E. coli*.

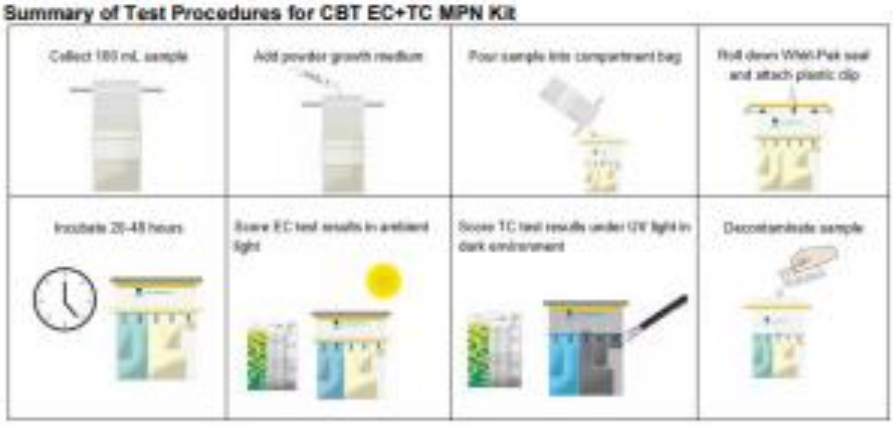
Instructions for testing surface and waste waters: https://www.aquagenx.com/dilutions-cbt-ecto/

How-to-use videos and product documents: https://www.aquagenx.com/product-documentation/

Shelf Life of Growth Medium
Aquagenx EC+TC powder growth medium is stable up to two-years after date of manufacture at 25° Celsius. Expiration date and lot number are printed on the medium packet.

Storage of Growth Medium
Recommended storage temperature is 10-25° Celsius. Growth medium can be stored in a refrigerator. Cold chain for Aquagenx EC+TC growth medium is not required.

Summary of Test Procedures for CBT EC+TC MPN Kit



How to Interpret Color-Change Test Results

Color of compartment in Compartment Bag	Yellow/Yellow Brown in ambient light and does not fluoresce blue under UV light	Yellow/Yellow Brown that ... fluoresces blue under UV light	Blue/Blue Green in ambient light	Blue/Blue Green that ... fluoresces blue under UV light
E. coli	Negative	Negative	Positive	Positive
Total Coliforms	Negative	Positive	Positive	Positive

1

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Basis of Aquagenx CBT Most Probable Number (MPN) Table
The Aquagenx CBT MPN Table (page 4) is based on the World Health Organization "Guidelines for Drinking Water Quality," 4th Edition. MPN of *E. coli* per 100 mL is estimated from the combination of positive (blue color) and negative (no blue color) compartments in the Aquagenx Compartment Bag. MPN of total coliforms per 100 mL is estimated from the combination of positive (blue fluorescence under UV light) and negative (no blue fluorescence under UV light) compartments in the Compartment Bag.

See **"Basis of Aquagenx MPN Table"**: https://www.aquagenx.com/product-documentation/

World Health Organization Guidelines for Drinking Water Quality, Table 5.4, Fourth Edition, 2017

E. coli classification (as decimal concentration/100)	Sanitary inspection risk score (susceptibility of supply to contamination from human and animal faeces) 0–2	3–5	6–8	9–10
< 1				
1–10				
11–100				
> 100				

Low risk: no action required	Intermediate risk: low action priority	High risk: higher action priority	Very high risk: urgent action required

PROCEDURAL NOTES

1. Prepare work area
- Sanitize work area with disinfectant cleaning solution, paper towels or wipes.

2. Collect 100 mL water sample with Whirl-Pak® Thio-Bag®
- Wearing disposable, thin plastic gloves is recommended. If you don't have gloves, do not touch inside of Thio-Bag with bare hands.
- White tablet in Thio-Bag is sodium thiosulfate, which neutralizes residual chlorine if present in sample. Do not remove.
- Fill Thio-Bag to 100 mL fill mark. Record sample details.

3. Add Aquagenx EC+TC growth medium to sample in Whirl-Pak Thio-Bag
- We recommend testing procedure begins within six hours of sample collection. Do not add growth medium to the Thio-Bag until you are ready to complete the entire testing procedure.
- Open growth medium packet with scissors and pour powder growth medium into Thio-Bag.
- Do not touch growth medium with bare fingers or hands.
- Roll down Whirl-Pak seal and close Thio-Bag shut.
- Dissolve medium in sample. Gently swirl the bag and squeeze clumps of powder until medium is dissolved.

4. Pour sample with dissolved medium from Thio-Bag into Aquagenx Compartment Bag
- Label Compartment Bag or attach barcode asset tag to Compartment Bag.
- Tear off perforated seam at top of bag.
- Rub top of bag and sides of bag together to open so sample can run into each compartment.
- Use white tabs at top of Compartment Bag to pull bag open. Do not touch inside of bag with bare fingers or hands.
- Slowly pour sample into bag while gently tilting and squeezing bag to distribute sample among five compartments.
- Fill evenly to the top of the fill line across all five compartments.

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5. Seal Compartment Bag shut

- Roll down Whirl-Pak seal at top of Compartment Bag and fasten shut.
- Attach plastic seal clip across Compartment Bag to prevent water from leaking out of compartments. Place U-shape part of clip across width of bag along the fill line and *below the compartment openings*. Place rod-shaped part of the clip on the opposite side of Compartment Bag and snap into U-shape to lock in place.

6. Incubation Period and Temperatures

- During the incubation period, CBTs can develop an odor. To help control odor, place CBTs in another sealed plastic bag or container during the incubation period.
- Ambient temperature incubation works at any temperatures between 25°- 44.5°C for detection of *E. coli* and/or total coliforms.
- Because the CBT works at variable temperatures, constant temperature control in an incubator is not required. However, at cooler temperatures, constant temperature incubation is recommended, if available.
- Note: over 40°C, some total coliforms will be inhibited, and the results may not be accurate for total coliform analysis.
- For regulatory compliance purposes, samples must be incubated at 35-37°C for 20-24 hours to detect and quantify *E. coli* and total coliforms.
- The CBT also can be used to detect and quantify thermotolerant (fecal) coliforms instead of total coliforms if the CBT samples are incubated at a temperature of 44.5°C (between 44-45 °C) throughout an incubation period of 20-24 hours. Strict temperature control is required for this procedure.

Recommended Incubation Periods at Ambient Temperature Conditions:

35-37°C: Incubate 20 hours
31-34°C: Incubate 24-30 hours
25-30°C: Incubate 40-48 hours

Below 25 C: Incubate in a portable incubator at 35-37°C for 24 hours or put in or near another heat source for up to 48 hours, depending on the temperature.
Over 40°C: Some total coliforms will be inhibited, and the results may not be accurate for total coliforms.

See "**Incubation Period Guidance**": https://www.aquagenx.com/product-documentation/

7. Score MPN test results

- Hold the Compartment Bag next to Aquagenx MPN Table on page 4 to score test results.
- ***E. coli* – view in ambient light:**
  - Yellow/yellow-brown compartment is negative for *E. coli* (absence).
  - Blue/blue-green compartment is positive for *E. coli* (presence). Positive compartments include any trace of blue/blue-green, such as one or more specks of blue/blue-green, or blue/blue-green sediment at bottom of a compartment.
- **Total Coliform - shine UV light (365 nm) on Compartment Bag in dark environment:**
  - Compartments that fluoresce blue are positive for total coliforms. These include any compartments that are yellow/yellow-brown in ambient light that fluoresce blue under UV light.
  - Compartments that are blue/blue-green in ambient light (positive for *E. coli*) are by definition also positive for total coliforms.
- Match color sequence of all five compartments to one of 32 color-coded rows in MPN Table to obtain MPN test results for *E. coli* and total coliforms.
- Record test results.

8. Decontaminate sample

- Add 4 mL of liquid bleach (NaOCl) or sufficient chlorine tablets (calcium hypochlorite or sodium dichloroisocyanurate) to compartment bag to provide about 200 milligrams of free chlorine.
- After 30 minutes, pour contents into a sink, toilet or hole in ground and safely dispose the empty compartment bag.
- Retain plastic seal clip for reuse.

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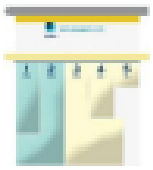
Aquagenx® CBT Most Probable Number (MPN) Table

Align the Compartment Bag so compartment #1 is on the left and compartment #5 is on the right. Match the color sequence of all five compartments to one of the 32 color-coded rows. Each compartment is scored according to the following criteria (also see color chart on page 1):

- Yellow compartment with and without UV light exposure is negative for *E. coli* and total coliforms
- Yellow compartment with blue fluorescence under UV light is positive for total coliforms
- Blue compartment in ambient light is positive for *E. coli* and by definition also is positive for total coliforms



Row Number	Compartment Number 1 10mL	2 30mL	3 66mL	4 3mL	5 1mL	MPN/100mL	Upper 95% Confidence Level/100mL	WHO Health Risk Category Based on MPN and Upper 95% Confidence Level
1						0.0	2.87	Low Risk/Safe
2						1.0	5.14	Intermediate Risk/ Probably Safe
3						1.0	4.74	
4						1.1	5.16	
5						1.2	5.64	
6						1.5	7.81	
7						2.0	6.32	
8						2.1	6.85	
9						2.1	6.64	
10						2.4	7.81	
11						2.4	8.12	
12						2.6	8.51	
13						3.2	8.38	
14						3.7	9.70	
15						3.1	11.36	Intermediate Risk/ Possibly Safe
16						3.2	11.82	
17						3.4	12.53	
18						3.9	10.43	
19						4.0	10.94	
20						4.7	22.75	
21						5.2	14.73	
22						5.4	12.93	
23						5.6	17.14	
24						5.8	16.87	
25						8.4	21.19	
26						9.1	37.04	
27						9.6	37.88	
28						13.6	83.06	High Risk/Possibly Unsafe
29						17.1	56.36	
30						32.6	145.55	High Risk/Probably Unsafe
31						48.3	351.91	
32						>100	9435.10	Unsafe
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