Validation of AquaCHROM™ ECC for the Detection and Enumeration of Coliforms and *Escherichia coli* in Water Samples

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Abstract

Background: The AquaCHROM™ ECC method from CHROMagar™ is intended for the detection and enumeration of *Escherichia coli* and coliform bacteria in 100 mL water samples after 18–24 h of incubation at 35–37°C.

Objective: To validate the AquaCHROM ECC method for qualitative and quantitative detection of *E. coli* and non-*E. coli* coliforms with different water matrixes.

Methods: Inclusivity/exclusivity studies were conducted. AquaCHROM ECC was compared to U.S. cultural reference methods in unpaired matrix studies for detection of *E. coli* and coliforms in tap water, well water, lake water, and bottled water, and for enumeration in tap water, well water, and lake water. Three production lots of AquaCHROM ECC were tested for product consistency and stability. Variations in incubation time and temperature were evaluated in robustness testing.

Results: Inclusivity/exclusivity results demonstrated expected performance with the exception of three strains of *Salmonella enterica*, two species of *Shigella* and one strain of *Aeromonas*, which turned the media blue instead of yellow. Results from the matrix studies demonstrated that the candidate and the reference methods can be considered not statistically different for detection of *E. coli* and coliforms and statistically equivalent for enumeration of *E. coli* and coliforms. Production of the AquaCHROM powder was proven to be consistent with a shelf life of 24 months. Variation in temperature did not affect the method performance. Shortening the incubation time is not recommended.

Conclusions: AquaCHROM ECC is an effective method for the detection and enumeration of *E. coli* and coliforms in 100 mL water samples for the water matrixes evaluated.

Highlight: The AquaCHROM ECC method is a quick, one-step method for the recovery and enumeration of *E. coli* and other coliform bacteria in 100 mL water samples. It is a non-agar based chromogenic medium which provides a clear result without the use of a UV-lamp.

General Information

Coliform bacteria are a category of rod-shaped, non-spore forming Gram negative bacteria. These organisms can be motile or non-motile and can ferment lactose with the production of acid and gas. While coliform bacteria are quite common and normally harmless, coliform contamination in food or beverage products could pose a health risk. Coliform bacteria are commonly found in soil and vegetation, but when coliforms are found in the food and/or water supply, this can be an indication of fecal contamination. This raises the question of pathogen contamination occurring through a similar process. Many coliforms including *Escherichia coli*, a subgroup of coliforms, can be found in the human digestive tract. While some strains of *E. coli* are harmless, other strains can cause serious illness. If *E. coli* contamination is detected, it is possible that other pathogens could be present (1).

Principle of the Method

The AquaCHROM™ ECC is a chromogenic medium for the detection and/or enumeration of *E. coli* and coliforms in water samples. Coliforms are *Enterobacteriaceae* able to ferment lactose and are present in human and warm-blooded animals' intestinal flora, in the soil and water. This method is intended for laboratory use and field testing, it should be used by personnel following good laboratory practices.

The product is composed of a powder medium and is supplied in ready-to-use, pre-weighed doses. Each dose is for a 100 mL water sample. The product is stored at 15–30 °C. For presence absence testing, the pre-weighed dose is added to a sterile transparent vessel containing a 100 mL water sample and then incubated at 35–37 °C for 18–24 h. *E. coli* results are green to blue-green, and non-*E. coli* coliform results are yellow. If a mixture of *E. coli* and non-*E. coli* coliforms are present, the medium will appear green. The product can also be used for MPN analysis. For this method, the 100 mL water sample is poured into a dispenser, and then the dose of AquaCHROM ECC is added. After shaking to dissolve the AquaCHROM ECC powder, the 100 mL sample is dispensed into the wells of a 48-well Deep well sample plate. The plate is incubated at 35–37°C for 18–24 h. *E. coli* results are green, and non-*E. coli* coliform results are yellow. If a mixture of *E. coli* and non-*E. coli* coliforms are

present, the medium will appear green. The wells are counted based on color, and then compared to the AquaCHROM ECC MPN Table.

Scope of Method

(a) *Analytes.—E. coli* and non-*E. coli* coliform bacteria.

(b) *Matrixes.*—Tap water, well water, lake water, and bottled water.

(c) *Summary of Validated Performance Claims.*—The AquaCHROM ECC method is comparable to the U.S. Environmental Protection Agency (EPA) Method 1604 (2002), *Total Coliforms and Escherichia coli in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)* (2) for detection of *E. coli* and non-*E. coli* coliform bacteria in tap water, well water, and lake water and to the U.S. Food and Drug Administration Bacteriological Analytical Manual (FDA/BAM) Chapter 4: *Enumeration of Escherichia coli and the Coliform Bacteria* (3) for bottled water. In addition, the AquaCHROM ECC method is equivalent to EPA 1604 for enumeration of *E. coli* and non-*E. coli* coliform bacteria in tap water, well water, and lake water.

Definitions

(a) *Probability of Detection (POD).*—The proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent.

(b) *Difference of probabilities of detection (dPOD).*—Difference of probabilities of detection is the difference between any two POD values. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5 % level.

(c) *Difference of Means.*—Difference in Log_{10} of the average results between the candidate and reference method for one level of contamination.

(d) *Repeatability.*—Precision where independent test results are obtained with the same method on equivalent test items in the same laboratory by the same operator using the same equipment within a short interval of time.

(e) *Confidence interval (CI).*—A confidence interval displays the probability that a parameter will fall between a pair of values around the mean. Confidence intervals are calculated at the 90 % and 95 % levels.

(f) *Statistical equivalence*.—The acceptance criterion for statistical equivalence is that the 90 % CI on the bias between the methods falls within -0.5, 0.5.

(g) *Most Probable Number (MPN).*—An estimate of the level of viable microbial contamination of a sample based on probability statistics.

Materials and Method

Test Kit Information

(a) *Kit name.*—AquaCHROM™ ECC.

(b) *Catalog number.*—AQ056.

(c) *Ordering information.*—http://www.chromagar.com/.

Test Kit Components

(a) *AquaCHROM ECC.*—Box, 100 vials.

(b) *AquaCHROM ECC MPN 48-Well Plate.*

(c) *AquaCHROM ECC MPN Table.*

Additional Supplies and Reagents

(a) *Sterile polypropylene bottles*.—Capable of holding 100 mL of water.

(b) *Whirl-Pak® Stand-up Bags.*—100 mL per sample bag.

Apparatus

(a) *Incubators.*—Capable of maintaining 35–37°C.

Safety Precautions

Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials. Wear appropriate protective equipment which includes but is not limited to protective eyewear, face shield, clothing/laboratory coat, and gloves. Biological samples such as enrichments have the potential to transmit infectious diseases including Biological Safety Level 2 (BSL 2) organisms. After use, all containers and any other contaminated materials must be sterilized or disposed of by appropriate internal procedures and in accordance with local legislations.

General Preparation

(a) Use aseptic techniques.

(b) Clean the workstations with a disinfectant of choice before and after use. (Sodium hypochlorite solution, phenol solution, quaternary ammonium solution, etc.).

(c) Wear personal protective equipment (PPE).

Sample Preparation

For detection, add a pre-weighed dose of AquaCHROM ECC to each sterile transparent polypropylene bottle or Whirl-Pak® Stand-up bag containing a 100 mL water sample. Close the containers, shake, and incubate at 35–37°C for 18–24 h. After incubation, determine the results based on the color of the liquid.

For enumeration, add a pre-weighed dose of AquaCHROM ECC to each sterile transparent polypropylene bottle containing a 100 mL water sample. Close the container and shake. Pour the 100 mL water sample into the wells of a 48-well Deep well plate (approximately 2 mL per well). Incubate

at 35–37°C for 18–24 h. After incubation, determine the results based on the color of the liquid in each well.

Analysis

Presence of *E. coli* will turn the water sample green to blue-green. If the water turns yellow, non-*E. coli* coliform bacteria are present. The MPN can be determined by referring to the AquaCHROM ECC MPN Table (Table 1).

Confirmation

If desired, AquaCHROM ECC enriched samples can be streaked onto either MI agar (tap water, well water and lake water) or onto m-Endo medium or LES Endo Agar (bottled water) for confirmation. On MI agar, blue colonies under normal/ambient light are *E. coli*. When exposed to longwave ultraviolet light (366 nm), *E. coli* will fluoresce blue/green, while blue/white fluorescence indicates coliforms other than *E. coli*. On m-Endo medium, colonies will be pink to dark red with a green metallic surface sheen. Further confirmation can be conducted as needed, as outlined in the appropriate reference method (EPA 1604 or FDA/BAM Ch. 4).

Validation Study

This validation study was conducted under the AOAC Research Institute *Performance Tested Method*[SM] (PTM) Program and the *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces,* Appendix J (4). Method developer studies were conducted in the laboratory of CHROMagar (Paris, France) which included the inclusivity/exclusivity study, product consistency and stability studies, and robustness testing. The independent laboratory study was conducted by Q Laboratories (Cincinnati, OH) which included matrix studies comparing the AquaCHROM ECC method to the EPA 1604 reference method for tap water, well water, and lake water for detection and enumeration of *E. coli* and coliform bacteria, and

to the FDA/BAM Ch. 4 reference method for bottled water for detection of *E. coli* and coliform bacteria.

Reference Cultures

Microorganisms used in this study were obtained from the American Type Culture Collection (ATCC®; Manassas, VA), the National Collection of Type Cultures (NCTC; Public Health England, Salisbury, UK), the Collection Institut Pasteur (CIP; Paris, France), the German Collection of Microorganisms and Cell Cultures GmbH (DSM; DSMZ; Leibniz Institute, Germany), the Q Laboratories Culture Collection (QL; Cincinnati, OH), and the CHROMagar Strain Collection (AR; Paris, France).

Method Developer Studies

Inclusivity and exclusivity study methodology.—The inclusivity and exclusivity study examined the ability of the AquaCHROM ECC method to detect a variety of *E. coli* and coliforms target species and to distinguish those from non-target species. For inclusivity, 59 and 51 different isolates of *E. coli* and coliforms, respectively, were selected (Tables 2 and 3). For each strain, a 100 mL water sample was inoculated with approximately 10^2 cfu/100 mL. For exclusivity, 87 isolates of related non-coliform *Enterobacteriaceae* strains and other strains relevant to the matrices were selected (Table 4). For each strain, a 100 mL water sample was inoculated with approximately 10^4 cfu/100 mL. The inclusivity and exclusivity isolates were tested in a randomized blind coded fashion so that analyst did not know the identity of the test samples. One vial of AquaCHROM ECC powder was added to each 100 mL water sample. Inoculated media were incubated at 36 ± 1°C for 18–24 h.

Inclusivity/exclusivity study results.—Of the *E. coli* inclusivity strains tested, 58 were detected as green in color, one *E. coli* strain serotype O157 was detected as yellow in color and there was no undetected strain (Table 2). Of the coliform inclusivity strains tested, 49 were detected as yellow in color, one isolate was detected as green in color, namely *Citrobacter freundii* AR5663, and one was not detected. The strain not detected was *Hafnia* sp. (Table 3). Of the exclusivity strains tested, 81

were not detected by AquaCHROM ECC, and 6 were detected (Table 4). The strains detected were *Aeromonas* sp. (as yellow in color), *Salmonella enterica* subsp. *enterica* (serovar Abaetetuba) (as greenish blue in color), *Shigella sonnei* (as greenish yellow in color), *Shigella boydii*, *Salmonella enterica* subsp. *enterica* (serovar Worthington), *Salmonella enterica* subsp. *arizonae* (as green in color).

Product consistency and stability studies methodology.—This study examined the lot-to-lot variability and product stability. Selected lots that are near the expiration date, near the middle of the expiration period, and recently manufactured were tested. One target coliform isolate (*E. coli* ATCC 8739 at 1–5 cfu/100 mL, source: feces) and one non-target isolate (*Staphylococcus aureus* subsp. *aureus* ATCC 25923 at 10^4 cfu/100 mL, source: clinical isolate) were used for the study. Ten portions of each isolate (*E. coli* and *S. aureus*) for each production lot of AquaCHROM ECC were tested. One vial of AquaCHROM ECC powder was added to each 100 mL test portion. Samples were blind-coded and randomized to that the analyst did not know the identity of the test samples. Inoculated media were incubated at 36 ± 1°C for 18–24 h.

Product consistency and stability studies results.—The POD statistical analysis was used to compare the performance of the different lots and storage time points of AquaCHROM ECC (5). No growth was observed with all the test portions inoculated with the non-target isolate. While eight out of 10 target isolate portions were positive in the oldest lot, and 10 out of 10 were positive in the newer lots, the POD analysis between the AquaCHROM ECC lots indicated that there was no significant statistical difference, with 95 % confidence, between the lots. A summary of POD analyses is presented in Table 5.

Robustness Study methodology.—Incubation temperature and incubation time were varied above and below the nominal test condition (36 ± 1°C for 18–24 h) using a factorial design to evaluate the ability of the AquaCHROM ECC method to remain unaffected by small variations. In addition, testing was conducted at 25°C to simulate room temperature conditions. One target coliform isolate (*E. coli* ATCC 8739 at 1–5 cfu/100 mL) and one non-target isolate (*S. aureus* subsp.

aureus ATCC 25923 at 10^4 cfu/100 mL) were used to inoculate 100 mL sterile distilled water test portions. Ten portions of each isolate (*E. coli* and *S. aureus*) were tested. One production lot of AquaCHROM ECC was used for this study. One vial of AquaCHROM ECC powder was added to each 100 mL test portion. Samples were blind-coded and randomized to that the analyst did not know the identity of the test samples.

Robustness Results.—The POD statistical analysis was used to compare the different treatment combinations (time and temperature of enrichment) to the nominal growth conditions. No growth was observed with the test portions inoculated with the non-target isolate. The analysis indicated a significant difference at the 16 h incubation time, especially at 34°C. Therefore, test portions should always be incubated at least 18 h. The study also showed that the AquaCHROM ECC can be incubated at 25°C (room temperature) for an extended enrichment time (36 h) and still give consistent results when compared to the nominal test condition. A summary of POD analyses is presented in Table 6.

Independent Laboratory Studies

The full matrix study was performed by the independent laboratory. The AquaCHROM ECC method was compared to the EPA 1604 reference method in qualitative and quantitative study designs for tap water, well water, and lake water, and to the FDA/BAM Chapter 4 reference method in a qualitative study design for bottled water.

The study outline consisted of a matrix study for qualitative analysis following an unpaired study design at three levels of contamination (0 cfu/100 mL), (0.2–2 cfu/100 mL), (5–10 cfu/100 mL) and a matrix study for quantitative analysis following an unpaired study design at four levels of contamination (0 cfu/100 mL), (1–50 cfu/100 mL), (51–100 cfu/100 mL), (101–150 cfu/100 mL).

Preparing Levels for Natural Contamination.—Well water and lake water were found to have natural coliform contamination after screening; however, the levels were too high for the levels specified in the protocol. A portion of each contaminated water matrix was filter sterilized to reduce

the amount of natural contamination. The filtered material for each water type was used to dilute the contaminated water in order to create the appropriate test levels required for the study.

Organism Preparation and Inoculation for Artificial Contamination.—Natural contamination of coliforms or *E. coli* were not found in tap water or bottled water matrixes; therefore, artificial contamination was conducted. The waters were artificially contaminated as follows: *E. coli* ATCC 25922 (source: clinical isolate) was used to inoculate tap water (qualitative & high level of quantitative), *Citrobacter freundii* ATCC 8090 (source: unknown) was used to inoculate tap water (low and medium levels of quantitative), and *E. coli* QL 41411.1 was used to inoculate bottled water.

All cultures were propagated on Tryptic Soy Agar with 5% Sheep Blood (SBA) from a stock culture stored at -70°C. The SBA was incubated at 35 ± 1°C for 24 ± 2 h before transferring a single colony to Brain Heart Infusion (BHI) broth and incubating at 35 ± 1°C for 24 ± 2 h. Using BHI broth as the diluent, the cultures were diluted to the proper contamination levels.

EPA 1604 Reference Method.—For tap water, well water, and lake water, each 100 mL test portion was filtered using sterile, white, gridded, 47 mm diameter, 0.45 µm pore size filters for enumeration of bacteria. The filter was then transferred to MI Agar and incubated at 35 ± 0.5°C for 24 h.

After incubation, colonies were inspected for the presence of blue color and for fluorescence under longwave ultraviolet light (UV), 366 nm. Total coliforms are those bacteria that produce fluorescent colonies upon exposure to longwave UV light after culturing on MI agar. The fluorescent colonies can be blue-white (coliforms other than *E. coli*) or blue-green (*E. coli*). Non-fluorescent blue colonies, which rarely occur, were added to the total count because the fluorescence can be masked by the blue color.

FDA/BAM Chapter 4, Enumeration of Escherichia coli and the Coliform Bacteria.—For bottled water analysis, each 100 mL test portion was filtered using sterile, white, gridded, 47 mm diameter, 0.45 µm pore size filters (or equivalent, as specified by the manufacturer) for enumeration of bacteria. The filter was then transferred to m-Endo medium and incubated at 35 ± 0.5°C for 22–24 h.

After incubation, pink to dark red colonies with green metallic surface sheens were counted. The sheens varied from pinpoint to complete coverage of the colonies. Use of a low power, dissecting microscope was used to examine filters.

AquaCHROM ECC detection method.—For each water matrix, a 100 mL test portion was placed into a transparent vessel. A pre-weighed dose (1 vial) of AquaCHROM ECC was added to each portion. The vessel was closed and shaken until dissolution. It was then incubated at 35–37°C for 18–24 h. Presence of *E. coli* will turn the water sample green to blue-green. If the water turns yellow, non-*E. coli* coliform bacteria are present.

AquaCHROM ECC MPN Method.—For tap water, well water and lake water matrixes, each 100 mL test portion was poured into a dispenser. A pre-weighed dose (1 vial) of AquaCHROM ECC was added to each portion. The vessel was closed and shaken until dissolution. The 100 mL portion was poured into the wells of a 48-well Deep well plate (approximately 2 mL per well). The plates were then incubated at 35–37°C for 18–24 h. The presumptive results were read and recorded at 18 h and at 24 h. Presence of *E. coli* will turn the water sample green to blue-green. If the water turns yellow, non-*E. coli* coliform bacteria are present. The MPN was determined by referring to the AquaCHROM ECC MPN Table (Table 1).

Qualitative Results.—Differences between the POD values were calculated for the AquaCHROM ECC presumptive and confirmed results (Table 7), as well as for the AquaCHROM ECC confirmed and reference method results (Table 8). All presumptive positive results confirmed positive, and there were no presumptive negative results that confirmed positive. For the reference method results (EPA 1604 and FDA/BAM Ch.4), any colonies seen on the reference method plates indicated a positive result for the reference method test portion. The positive colony counts on the reference method plates were averaged to determine the cfu/100 mL for each contamination level. The 0 cfu/100 mL level was not tested for well water and lake water since natural contamination was present. The AquaCHROM results were all “green” for the well water portions and all “yellow” for the lake water portions. This corresponded with results obtained by the Bruker MALDI Biotyper Method (6), as *E.*

coli was isolated and confirmed from each positive well water test portion, and *Enterobacter bugandensis* and *Enterobacter asburiae* were isolated and confirmed from the lake water test portions. Although there were some differences seen between the AquaCHROM ECC results and the reference methods results, the POD analysis between the methods indicated that there was no significant difference, with 95 % confidence. A summary of POD analyses is presented in Tables 7 and 8.

Quantitative Results.—Statistical analysis was conducted for each *E. coli* and non-*E. coli* coliform contamination level for tap water, well water and lake water matrixes. Logarithmic transformation of the counts (cfu/100 mL) was performed and the difference of means, with 90 and 95% confidence intervals, between the candidate method and the reference method was determined for each matrix and each contamination level. The difference of means and confidence intervals were calculated using the Least Cost Formulations Quantitative Analysis for Micro Methods v1.2 (Virginia Beach, VA) worksheet, supplied by the AOAC Research Institute. The 90 % confidence interval of the bias between the two methods fell between -0.5 to 0.5 Log_{10} for each concentration indicating equivalence between the two methods (7).

Repeatability standard deviation (s_r) was calculated for AquaCHROM ECC MPN method and the reference method for tap water, well water, and lake water. A Grubbs outlier test was performed for the AquaCHROM ECC MPN Method and the reference method to determine if any outliers were present, and none were detected. The 90% CIs on the difference in results between the AquaCHROM ECC and the reference method for each level of each matrix fell within the -0.5, 0.5 range, indicating that the methods were statistically equivalent for tap water, well water and lake water. A summary of the study data are presented in Table 9. Figures 1–3 display graphs comparing the Log_{10} values of the candidate method and the reference method.

Discussion

In the inclusivity study, all *E. coli* strains tested were positive green to blue-green, with the exception of *E. coli* O157, which is expected. The efficacy of the β-glucuronidase character allows the identification of *E. coli* but a small percentage of *E. coli* strains, such as *E. coli* serotype O157, is β-glucuronidase negative (7, 8). Those strains are detected as yellow in color with AquaCHROM ECC. The β-glucuronidase phenotype in other *Enterobacteriaceae* is rare, one *C. freundii* isolate was found positive in green color. A few false positive results were detected, including 3 strains of *Salmonella enterica*, 2 species of *Shigella* and 1 strain of *Aeromonas*. One strain of *Hafnia* sp. was found to be false negative.

In this study, AquaCHROM ECC showed lot-to-lot consistency and stability. The method allows the user to obtain results within 36 h at room temperature, i.e., 25°C, in this case there would not be need of an incubator.

The AquaCHROM ECC method evaluated in this study showed no statistical difference in detection of *E. coli* and coliform bacteria compared to EPA 1604 (tap water, well water, and lake water) and FDA/BAM Ch. 4 (bottled water) and was statistically equivalent for enumeration of *E. coli* and coliform bacteria to the EPA 1604 for tap water, well water, and lake water in 100 mL water samples.

The method allows the user to obtain accurate results within 24 h in the matrixes evaluated for the presence of coliforms in water samples incubated at 35–37°C. The non-agar-based medium was easy to interpret based on a color change to green (*E. coli*) or yellow (non-*E. coli* coliforms) that can be read under normal lighting conditions. The AquaCHROM ECC method required no additional media or Petri dishes to perform, creating an easier workflow by eliminating all the confirmation steps needed for the reference method. The independent laboratory analyst stated how straightforward and easy the method was to perform. One item of note, during the matrix study at the independent laboratory the polypropylene dispenser bottles provided by the client did not hold up well to repeated autoclave decontamination cycles (121°C at 15 psi for 60 min) between uses and had to be discarded. Those bottles might have been tightly closed during the autoclave

decontamination cycles leading to their deformation. It is therefore recommended to loosely close the bottles when autoclaved for decontamination to ensure multiple use.

Conclusion

The data from this study supports the product claim that the AquaCHROM ECC is an effective method for the detection of *E. coli* and coliforms in 100 mL water samples for all matrixes evaluated (tap water, well water, lake water, and bottled water) and for enumeration of *E. coli* and coliforms in 100 mL water samples in tap water, well water, and lake water. The method simple to use, requires no filtration or UV light, and allows the end user to acquire accurate results within 24 h of incubation. This is a significantly shorter timeframe than the EPA 1604 and FDA/BAM Chapter 4 methods, and will allow laboratories to process samples faster, while still obtaining statistically equivalent results.

Acknowledgments

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Conflict of Interest

All authors declare no conflict of interest.

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Table 1: AquaCHROM ECC MPN Table[a]

Positive wells	MPN	Lower 95 %	Upper 95 %
0	0	0	3.8
1	1.1	0.14	7.8
2	2.1	0.52	8.8
3	3.2	1	10
4	4.4	1.6	12
5	5.5	2.2	13
6	6.7	2.9	15
7	7.9	3.7	17
8	9.1	4.5	19
9	10	5.3	20
10	12	6.2	22
11	13	7.1	24
12	14	8.1	26
13	16	9	28
14	17	10	30
15	19	11	31
16	20	12	34
17	22	13	36
18	24	15	38
19	25	16	40
20	27	17	42
21	29	18	45
22	31	20	47
23	33	21	50
24	35	23	53
25	37	24	55
26	39	26	58
27	41	28	61
28	44	30	65
29	46	32	68
30	49	34	72
31	52	36	76
32	55	38	80
33	58	40	84
34	62	43	89
35	65	45	94
36	69	48	99
37	74	51	110
38	78	55	110
39	84	58	120
40	90	62	130
41	96	67	140
42	100	72	150
43	110	78	160
44	120	85	180
45	140	93	210
46	160	100	250
47	190	120	320
48	>190	120	-

[a]Based on BAM Appendix 2 (10).

Table 2. AquaCHROM ECC Inclusivity Study Results for *E. coli*

No.	Target strain	Source	Origin	Result
1	*E. coli*	ATCC[a] 8739	Feces	Positive, green
2	*E. coli*	ATCC 11775	Urine	Positive, green
3	*E. coli*	ATCC 25922	Clinical isolate, USA	Positive, green
4	*E. coli* O157:H7	ATCC 35150	Feces	Negative, yellow[b]
5	*E. coli*	ATCC 35218	Canine	Positive, green
6	*E. coli*	ATCC 51446	Clinical isolate, France	Positive, green
7	*E. coli*	CIP[c] 52.168	Child, feces	Positive, green
8	*E. coli*	CIP 52.172	Feces	Positive, green
9	*E. coli*	CIP 103982	Clermont-Ferrand, France	Positive, green
10	*E. coli*	CIP 107196	Human	Positive, green
11	*E. coli*	NCTC[d] 13846	Human blood culture	Positive, green
12	*E. coli*	NCTC 13476	Not available	Positive, green
13	*E. coli*	DSM[e] 1103	Clinical isolate	Positive, green
14	*E. coli*	DSM 22312	Urinary tract infections	Positive, green
15	*E. coli*	AR[f]3740	Clinical isolate, France	Positive, green
16	*E. coli*	AR3857	Clinical isolate, France	Positive, green
17	*E. coli*	AR3858	Clinical isolate, France	Positive, green
18	*E. coli*	AR3859	Clinical isolate, France	Positive, green
19	*E. coli*	AR4076	Clinical isolate, France	Positive, blue green
20	*E. coli*	AR4077	Clinical isolate, France	Positive, green
21	*E. coli*	AR4524	Foodborne, Japan	Positive, green
22	*E. coli*	AR4526	Not available	Positive, green
23	*E. coli*	AR4531	Not available	Positive, green
24	*E. coli*	AR4732	Foodborne, Switzerland	Positive, green
25	*E. coli*	AR4733	Foodborne, Switzerland	Positive, green
26	*E. coli*	AR4734	Foodborne, Switzerland	Positive, green
27	*E. coli*	AR5011	Clinical isolate	Positive, green
28	*E. coli*	AR5012	Clinical isolate	Positive, blue green
29	*E. coli*	AR5013	Clinical isolate	Positive, green
30	*E. coli*	AR5014	Clinical isolate	Positive, green
31	*E. coli*	AR5030	Foodborne	Positive, green
32	*E. coli*	AR5179	Clinical isolate, France	Positive, green
33	*E. coli*	AR5189	Clinical isolate, France	Positive, green
34	*E. coli*	AR5190	Clinical isolate, France	Positive, green
35	*E. coli*	AR5238	Clinical isolate, France	Positive, green
36	*E. coli*	AR5303	Foodborne, Japan	Positive, blue green
37	*E. coli*	AR5305	Foodborne, Japan	Positive, blue green
38	*E. coli*	AR5306	Foodborne, Japan	Positive, green
39	*E. coli*	AR5360	Foodborne	Positive, green
40	*E. coli*	AR5387	Foodborne	Positive, green
41	*E. coli*	AR5388	Foodborne	Positive, green

42	*E. coli*	AR5389	Foodborne	Positive, green
43	*E. coli*	AR5414	Clinical isolate	Positive, green
44	*E. coli*	AR5415	Clinical isolate	Positive, green
45	*E. coli*	AR5416	Clinical isolate	Positive, green
46	*E. coli*	AR5417	Clinical isolate	Positive, blue green
47	*E. coli*	AR5428	Foodborne	Positive, green
48	*E. coli*	AR5433	Foodborne, France	Positive, blue green
49	*E. coli*	AR5434	Foodborne, France	Positive, green
50	*E. coli*	AR5435	Foodborne, France	Positive, green
51	*E. coli*	AR5436	Foodborne, France	Positive, green
52	*E. coli*	AR5438	Foodborne, France	Positive, green
53	*E. coli*	AR5440	Foodborne, France	Positive, green
54	*E. coli*	AR5442	Foodborne, France	Positive, green
55	*E. coli*	AR5458	Clinical isolate, Germany	Positive, green
56	*E. coli*	AR5510	Clinical isolate, France	Positive, blue green
57	*E. coli*	AR5664	Clinical isolate, France	Positive, green
58	*E. coli*	AR5665	Clinical isolate, France	Positive, blue green
59	*E. coli*	AR5666	Clinical isolate, France	Positive, blue green

[a]ATCC = American Type Culture Collection, Manassas, VA.

[b]*E. coli* serotype O157 are β-glucuronidase negative being detected as yellow with AquaCHROM ECC.

[c]CIP = Collection Institut Pasteur, Paris, France.

[d]NCTC = National Collection of Type Cultures, Public Health England, Salisbury, UK.

[e]DSM = DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Leibniz Institute, Germany.

[f]AR = CHROMagar Strain Collection, Paris, France.

Table 3. AquaCHROM ECC Inclusivity Study Results for non-*E. coli* Coliform Bacteria

No.	Target strain	Source	Origin	Result
1	*Citrobacter freundii*	ATCC[a] 8090	Not available	Positive, yellow
2	*Cronobacter muytjensii*	ATCC 51329 (formerly *Enterobacter sakazakii*)	Not available	Positive, yellow
3	*Enterobacter cloacae* subsp. *cloacae*	ATCC 13047	Spinal fluid	Positive, yellow
4	*E. cloacae* subsp. *cloacae*	ATCC 35030	Not available	Positive, yellow
5	*Klebsiella aerogenes*	ATCC 13048 *(formerly Aerobacter aerogenes)*	Sputum	Positive, yellow
6	*K. pneumoniae*	ATCC BAA-1705	Urine	Positive, yellow
7	*K. pneumoniae* subsp. *Pneumoniae*	ATCC 13883	Not available	Positive, yellow
8	*K. pneumoniae* subsp. *Pneumoniae*	ATCC 700603	Urine	Positive, yellow
9	*K. variicola*	ATCC 31488	Soil	Positive, yellow
10	*Serratia marcescens* subsp. m*arcescens*	ATCC 13880	Pond water	Positive, yellow
11	*K. pneumoniae*	NCTC[b] 13438	Blood, urine	Positive, yellow
12	*Citrobacter amalonaticus*	AR[c]6391	Clinical isolate, France	Positive, yellow
13	*C. farmeri*	AR6390	Clinical isolate, France	Positive, yellow
14	*C. freundii*	AR3870	Not available	Positive, yellow
15	*C. freundii*	AR5662	Clinical isolate, France	Positive, yellow
16	*C. freundii*	AR5663	Clinical isolate, France	Positive, green
17	*C. freundii*	AR6662	Foodborne, France	Positive, yellow
18	*C. koseri*	AR6387	Clinical isolate, France	Positive, yellow
19	*C. sedlakii*	AR6389	Clinical isolate, France	Positive, yellow
20	*Citribacter* sp.	AR3030	Not available	Positive, yellow
21	*Citrobacter* sp.	AR3134	Human Feces	Positive, yellow
22	*Citrobacter* sp.	AR3378	Foodborne, France	Positive, yellow
23	*Enterobacter aerogenes*	AR5187	Clinical isolate, France	Positive, yellow
24	*E. aerogenes*	AR6081	Foodborne, Israel	Positive, yellow
25	*E. agglomerans*	AR5646	Laboratory isolate, France	Positive, yellow
26	*E. amnigenus*	AR6110	Human Feces	Positive, yellow
27	*E. asburiae*	AR6392	Clinical isolate, France	Positive, yellow
28	*E. cloacae*	AR5339	Foodborne, Japan	Positive, yellow
29	*E. cloacae*	AR5480	Clinical isolate, Japan	Positive, yellow
30	*E. cloacae*	AR6002	Clinical isolate, France	Positive, yellow
31	*Enterobacter spp.*	AR5965	Human Feces	Positive, yellow
32	*Escherichia hermannii*	AR5245	Human Feces	Positive, yellow
33	*E. hermannii*	AR5341	Foodborne, Japan	Positive, yellow
34	*Hafnia* sp.	AR5850	Not available	No growth
35	*H. alvei*	AR3862	Human Feces	Positive, yellow
36	*H. alvei*	AR5331	Foodborne, Japan	Positive, yellow
37	*Klebsiella oxytoca*	AR5204	Clinical isolate, France	Positive, yellow
38	*K. oxytoca*	AR5236	Human Feces	Positive, yellow
39	*K. oxytoca*	AR5755	Not available	Positive, yellow

40	*K. oxytoca*	AR6655	Foodborne, France	Positive, yellow
41	*K. oxytoca*	AR5755	Not available	Positive, yellow
42	*K. pneumoniae*	AR5186	Not available	Positive, yellow
43	*K. pneumoniae*	AR5251	Clinical isolate, France	Positive, yellow
44	*K. pneumoniae*	AR5995	Clinical isolate, France	Positive, yellow
45	*K. pneumoniae*	AR6663	Foodborne, France	Positive, yellow
46	*Serratia liquefaciens*	AR3964	Foodborne, France	Positive, yellow
47	*S. liquefaciens*	AR4046	Clinical isolate, France	Positive, yellow
48	*S. liquefaciens*	AR6146	Chicken	Positive, yellow
49	*S. marcescens*	AR5568	Clinical isolate, France	Positive, yellow
50	*S. plymuthica*	AR5492	Raw milk	Positive, yellow
51	*S. rubidaea*	AR6664	Sweet bell pepper	Positive, yellow

[a]ATCC = American Type Culture Collection, Manassas, VA.
[b]NCTC = National Collection of Type Cultures, Porton Down, Salisbury, UK.
[c]AR = CHROMagar Strain Collection, Paris, France.

Table 4. AquaCHROM ECC Exclusivity Study Results

No.	Non-target strains	Source	Origin	Result
1	*Clostridium perfringens*	ATCC[a] 13124	Not available	No growth
2	*Enterococcus casseliflavus*	ATCC 700327	Not available	No growth, yellowish
3	*E. gallinarum*	ATCC 49573	Chicken intestine	No growth
4	*E. hirae*	ATCC 8043	Not available	No growth
5	*E. faecalis*	ATCC 29212	Urine	No growth
6	*E. faecalis*	ATCC 51299	Peritoneal fluid	No growth
7	*Listeria ivanovii* subsp. *ivanovii*	ATCC 19119	Sheep	No growth
8	*L. monocytogenes*	ATCC 19115	Not available	No growth
9	*Macrococcus caseolyticus*	ATCC 35662 (formerly *S. cohnii subsp. cohnii*)	Not available	No growth
10	*Paeniclostridium sordellii*	ATCC 9714 (formerly *Clostridium sordellii*)	Not available	No growth
11	*Pseudomonas aeruginosa*	ATCC 9027	Not available	No growth
12	*P. aeruginosa*	ATCC 10145	Not available	No growth
13	*Proteus vulgaris*	ATCC 6380	Not available	Growth, uncolored
14	*Salmonella enterica* subsp. *enterica* (serovar Abaetetuba)	ATCC 35640	Creek water	Positive, greenish blue
15	*S. enterica* subsp. *enterica* (serovar Typhimurium)	ATCC 13311	Feces, food poisoning	Growth, uncolored
16	*Shigella boydii*	ATCC 9207	Not available	Positive, green
17	*S. dysenteriae*	ATCC 13313	Foreign seaman	No growth
18	*S. flexneri*	ATCC 12022	Not available	Growth, uncolored
19	*S. sonnei*	ATCC 9290	Not available	Positive, greenish yellow
20	*Staphylococcus aureus* subsp. *aureus*	ATCC 43300	Clinical isolate, US	No growth
21	*S. aureus* subsp. *aureus*	ATCC 25923	Clinical isolate, US	No growth

22	*S. epidermidis*	ATCC 12228	Not available	No growth
23	*S. haemolyticus*	ATCC 29970	Skin	No growth
24	*S. lentus*	ATCC 700403	Not available	No growth
25	*S. saprophyticus* subsp. *saprophyticus*	ATCC 15305	Urine	No growth
26	*S. simulans*	ATCC 27851	Skin	No growth
27	*S. warneri*	ATCC 49454	Not available	No growth
28	*S. xylosus*	ATCC 29971	Skin	No growth
29	*Streptococcus agalactiae*	ATCC 13813	Not available	No growth
30	*S. gallolyticus*	ATCC 9809 (formerly *Streptococcus bovis*)	Not available	No growth
31	*S. dysgalactiae* subsp. *dysgalactiae*	ATCC 27957	Bovine udder infection	No growth
32	*Yersinia enterocolitica* subsp. *enterocolitica*	ATCC 23715	Blood, petechiae, anterior eye chamber	No growth
33	*Y. pseudotuberculosis*	ATCC 29833	Turkey	No growth
34	*Listeria innocua*	CIP[b] 80.11T	Bovine, brain	No growth
35	*Streptococcus equinus*	CIP 102504T	Not available	No growth
36	*S. uberis*	CIP 103219T	Not available	No growth
37	*S. uberis*	CIP 105450	Bovine udder infection	No growth
38	*Yersinia enterocolitica palearctica*	CIP 101776	Blood	Growth, uncolored
39	*Acinetobacter baumannii*	AR[c]5624	Clinical isolate, France	Growth, uncolored
40	*Acinetobacter* sp.	AR5563	Clinical isolate, France	No growth
41	*Aeromonas* sp.	AR3881	Foodborne	No growth
42	*Aeromonas sp.*	AR3898	Not available	Positive, yellow
43	*Clostridioides difficile*	AR5681	Not available	No growth
44	*C. difficile*	AR5682	Not available	No growth
45	*Enterococcus avium*	AR5258	Clinical isolate, France	No growth
46	*E. durans*	AR5257	Not available	No growth
47	*E. faecalis*	AR5289	Clinical isolate, France	No growth
48	*E. faecalis*	AR5313	Clinical isolate, France	No growth
49	*E. faecalis*	AR5316	Clinical isolate, France	No growth
50	*Enterococcus* sp.	AR5201	Clinical isolate, France	No growth
51	*Enterococcus* sp.	AR5312	Clinical isolate, France	No growth
52	*E. gallinarum*	AR5266	Not available	No growth
53	*E. gallinarum*	AR5218	Not available	No growth
54	*E. faecalis*	AR5101	Clinical isolate, France	No growth
55	*E. faecium*	AR5102	Clinical isolate, France	No growth
56	*E. faecium*	AR5164	Clinical isolate, France	No growth
57	*E. faecium*	AR4437	Foodborne	No growth
58	*Listeria monocytogenes*	AR4580	Clinical isolate, France	No growth
59	*Legionella pneumophila*	AR4665	Not available	No growth

60	*L. pneumophila*	AR4666	Not available	No growth
61	*P. aeruginosa*	AR5196	Clinical isolate, France	Growth, uncolored
62	*P. aeruginosa*	AR5197	Clinical isolate, France	Growth, uncolored
63	*P. aeruginosa*	AR5847	Not available	No growth
64	*Proteus mirabilis*	AR5479	Clinical isolate, Finland	Growth, uncolored
65	*P. mirabilis*	AR3022	Not available	Growth, uncolored
66	*Salmonella enterica* subsp*. arizonae*	AR3910	Not available	Positive, green
67	*S. enterica* subsp*. enterica* (serovar Dublin)	AR3580	Clinical isolate, France	Growth, uncolored
68	*S. enterica* subsp*. enterica* (serovar Typhi)	AR4052	Foodborne	Growth, uncolored
69	*S. enterica* subsp*. enterica* (serovar Typhi)	AR3104	Not available	Growth, uncolored
70	*S. enterica* subsp*. enterica* (serovar Typhi)	AR3105	Not available	Growth, uncolored
71	*S. enterica* subsp*. enterica* (serovar Typhimurium)	AR3015	Not available	Growth, uncolored
72	*S. enterica* subsp*. enterica* (serovar Worthington)	AR3911	Not available	Positive, green
73	*Salmonella sp.*	AR4053	Foodborne	Growth, uncolored
74	*Salmonella sp.*	AR3011	Not available	Growth, uncolored
75	*Salmonella sp.*	AR3924	Not available	Growth, uncolored
76	*Salmonella sp.*	AR3925	Not available	Growth, uncolored
77	*S. aureus*	AR3916	Not available	No growth
78	*S. intermedius*	AR5008	Clinical isolate, France	No growth
79	*Streptococcus agalactiae*	AR4186	Clinical isolate, France	No growth
80	*S. oralis*	AR5649	Clinical isolate, France	No growth
81	*S. pyogenes*	AR5255	Clinical isolate, France	No growth
82	*Streptococcus sp.*	AR5408	Clinical isolate, France	No growth
83	*Streptococcus sp.*	AR5311	Clinical isolate, France	No growth
84	*Vibrio cholerae*	AR4482	Foodborne, Japan	No growth
85	*V. cholerae*	AR4748	Foodborne, Japan	No growth
86	*V. parahaemolyticus*	AR4493	Foodborne, Japan	No growth
87	*V. vulnificus*	AR4675	Foodborne, Japan	No growth

[a]ATCC = American Type Culture Collection, Manassas, VA.
[b]CIP = Collection Institut Pasteur, Paris, France.
[c]AR = CHROMagar Strain Collection, Paris, France.

Table 5. Product Consistency (lot-to-lot) and Stability of AquaCHROM ECC – POD Comparison

Lot No.	Lot age, months	N[a]	x[b]	POD_A[c]	95% CI	Lot No.	Lot age, months	N	x	POC_B[d]	95% CI[g]	$dPODC_{AB}$[e]	95% CI[f]
E. coli ATCC[g] 8739 (target)													
P002172[h]	12	10	10	1.0	0.72, 1.00	P002383[i]	8	10	10	1.0	0.72, 1.00	0.0	-0.28, 0.28
P001789[j]	27	10	8	0.8	0.49, 0.94	P002383	8	10	10	1.0	0.72, 1.00	-0.2	-0.51, 0.11
P001789	27	10	8	0.8	0.49, 0.94	P002172	12	10	10	1.0	0.72, 1.00	-0.2	-0.51, 0.11
Staphylococcus aureus ATCC 25923 (non-target)													
P002172	12	10	0	0.0	0.0, 0.28	P002383	8	10	0	0.0	0.0, 0.28	0.0	-0.28, 0.28
P001789	27	10	0	0.0	0.0, 0.28	P002383	8	10	0	0.0	0.0, 0.28	0.0	-0.28, 0.28
P001789	27	10	0	0.0	0.0, 0.28	P002172	12	10	0	0.0	0.0, 0.28	0.0	-0.28, 0.28

[a]N = Number of test portions.

[b]x = Number of positive test portions.

[c]POD_A = Positive outcomes divided by the total number of trials first member of pair.

[d]POD_B = Positive outcomes divided by the total number of trials second member of pair.

[e]$dPOD_{AB}$ = Difference in POD between the lots.

[f]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

[g]ATCC = American Type Culture collection, Manassas, VA.

[h]Lot P001789 was produced October 17, 2019.

[i]Lot P002172 was produced January 13, 2021.

[j]Lot P002383 was produced May 7, 2021.

Table 6. Robustness study of AquaCHROM ECC – POD Comparison

Test Condition[a]	Test Parameters			Test Condition Results			Nominal Condition[e] Results				
	Incubation time	Incubation temperature	N[b]	x[c]	POD_T[d]	95% CI	x	POD_N[f]	95% CI	$dPOD_{TN}$[g]	95% CI[h]
E. coli ATCC[i] 8739 (target)											
1	16h	34°C	10	0	0.0	0.0, 0.28	9	0.9	0.60, 1.00	-0.9	-1.0, -0.49
2	16 h	38°C	10	6	0.6	0.31, 0.83	9	0.9	0.60, 1.00	-0.3	-0.60, 0.08
3	26 h	34°C	10	8	0.8	0.49, 0.94	9	0.9	0.60, 1.00	-0.1	-0.43, 0.24
4	26 h	38°C	10	8	0.8	0.49, 0.94	9	0.9	0.60, 1.00	-0.1	-0.43, 0.24
5	36 h	25°C	10	9	0.9	0.60, 1.00	9	0.9	0.60, 1.00	0.0	-0.32, 0.32
Staphylococcus aureus ATCC 25923 (non-target)											
1	16h	34°C	10	0	0.0	0.0, 0.28	0	0.0	0.0, 0.28	0.0	-0.28, 0.28
2	16 h	38°C	10	0	0.0	0.0, 0.28	0	0.0	0.0, 0.28	0.0	-0.28, 0.28
3	26 h	34°C	10	0	0.0	0.0, 0.28	0	0.0	0.0, 0.28	0.0	-0.28, 0.28
4	26 h	38°C	10	0	0.0	0.0, 0.28	0	0.0	0.0, 0.28	0.0	-0.28, 0.28
5	36 h	25°C	10	0	0.0	0.0, 0.28	0	0.0	0.0, 0.28	0.0	-0.28, 0.28

[a]Each test condition is being compared to the nominal test condition. Note: Test conditions 1–5 (36 h at 25°C; 16 h at 34°C; 26 h at 34°C; 16 h at 38°C; and 26 h at 38°C) were compared to the nominal condition in different experiments.

[b]N = Number of test portions per condition.

[c]x = Number of positive test portions per condition.

[d]POD_T = Positive outcomes divided by the total number of trials per condition.

[e]Nominal condition = 36°C for 18–24 h.

[f]POD_N = Positive outcomes divided by the total number of trials per nominal condition.

[g]$dPOD_{TN}$ = Difference in POD between the test condition and nominal condition.

[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

[i]ATCC = American Type Culture collection, Manassas, VA.

Table 7. AquaCHROM ECC Method Presumptive vs. Confirmed – POD Results

			Presumptive result			Confirmed result				
Matrix[a]	cfu/100 mL[b]	N[c]	X[d]	POD_{CP}[e]	95% CI	X	POD_{CC}[f]	95% CI	$dPOD_{CP}$[g]	95% CI[h]
Tap Water	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
(100 mL)	1.3	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
E. coli ATCC[i] 25922	6.2	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Well Water	0.5	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.13, 0.13
(100 mL)	2.8	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Lake Water	0.7	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.13, 0.13
(100 mL)	4	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Bottled Water	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
(100 mL)	1.8	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0.00	-0.13, 0.13
E. coli QL[j] 41411.1	6.6	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[a]Matrix = Well water and lake water were naturally contaminated. Tap water and bottled were artificially contaminated.

[b]cfu/100 mL = Colony counts based on the reference method plate results. Counts were averaged based on the number of replicate portions tested.

[c]Number of test portions.

[d]x = Number of positive test portions.

[e]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.

[f]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.

[g]$dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values.

[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

[i]ATCC = American Type Culture collection, Manassas, VA.

[j]QL = Q Laboratories Culture Collection, Cincinnati, OH.

Table 8. AquaCHROM ECC Method vs. Reference Method – POD Results

			AquaCHROM ECC			Reference method[f]				
Matrix[a]	cfu/100 mL[b]	N[c]	X[d]	POD_C[e]	95% CI	X	POD_R[g]	95% CI	$dPOD_C$[h]	95% CI[i]
Tap Water	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
(100 mL)	1.3	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37 0.19
E. coli ATCC[j] 25922	6.2	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Well Water	0.5	20	13	0.65	0.43, 0.82	8	0.40	0.22, 0.61	0.25	-0.05 0.50
(100 mL)	2.8	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Lake Water	0.7	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09 0.45
(100 mL)	4	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Bottled Water	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
(100 mL)	1.8	20	17	0.85	0.64, 0.95	15	0.75	0.53, 0.89	0.10	-0.15, 0.34
E. coli QL[k] 41411.1	6.6	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]Matrix = Well water and lake water were naturally contaminated. Tap water and bottled were artificially contaminated.

[b]cfu/100 mL = Colony counts based on the reference method plate results. Counts were averaged based on the number of replicate portions tested.

[c]N = Number of test portions.

[d]x = Number of positive test portions.

[e]POD_C = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.

[f]Reference method = EPA 1604 for tap water, well water, and lake water; BAM Ch.4 for bottled water

[g]POD_R = Reference method confirmed positive outcomes divided by the total number of trials.

[h]$dPOD_C$= Difference between the confirmed candidate method result and reference method result POD values.

[i]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

[j]ATCC = American Type Culture collection, Manassas, VA.

[k]QL = Q Laboratories Culture Collection, Cincinnati, OH.

Table 9. Results of AquaCHROM ECC vs. Reference Method

			AquaCHROM ECC		Reference Method[c]				95% CI[f]		90% CI	
Matrix	Cont. level[a]	n	Log_{10} Mean[b]	s_r	Log_{10} Mean	s_r	DOM[d]	SE[e]	LCL[g]	UCL[h]	LCL	UCL
Tap Water[i] (100 mL) C. *freundii* ATCC[j] 8090 & *E. coli* ATCC 25922	Uninoculated	5	0.000	NA[k]	0.000	NA	NA	NA	NA	NA	NA	NA
	Low	5	0.897	0.412	0.977	0.102	-0.081	0.190	-0.518	0.357	-0.434	0.273
	Medium	5	1.740	0.066	1.737	0.036	0.003	0.034	-0.074	0.081	-0.059	0.066
	High	5	2.008	0.134	2.024	0.026	-0.017	0.061	-0.157	0.124	-0.130	0.097
Well Water (100 mL) Naturally contaminated	Low	5	0.414	0.243	0.433	0.170	-0.019	0.133	-0.325	0.287	-0.266	0.228
	Medium	5	1.621	0.109	1.610	0.046	0.012	0.053	-0.110	0.134	-0.087	0.110
	High	5	1.983	0.048	1.940	0.028	-0.022	0.025	-0.079	0.036	-0.068	0.024
Lake Water (100 mL Naturally contaminated	Low	5	0.859	0.120	0.709	0.135	0.151	0.081	-0.036	0.337	0.000	0.301
	Medium	5	1.734	0.049	1.731	0.032	0.004	0.026	-0.057	0.064	-0.045	0.052
	High	5	2.052	0.096	2.001	0.019	0.051	0.044	-0.050	0.152	-0.030	0.132

[a]Tap Water has an uninoculated level that yielded no recovered growth for all five replicates. Well and lake water were naturally contaminated and therefore have no uninoculated level.
[b]Mean of five replicate portions, after logarithmic transformation: Log_{10}[CFU/g + (0.1)f]. There were no differences in results between the 18 and 24 h timepoints.
[c]Reference method is EPA 1604.
[d]DOM = Difference of means; $mean_{cand}$ - $mean_{ref.}$
[e]SE = Standard Error of DOM.
[f]CI = Confidence interval for DOM.
[g]LCL = Lower confidence limit for DOM.
[h]UCL = Upper confidence limit for DOM.
[i]Tap water was inoculated with C. freundii (ATCC 8090) at the low and medium levels and with *E. coli* (ATCC 25922) at the high level.
[j]ATCC = American Type Culture Collection, Manassas, VA.
[k]NA = Not applicable.

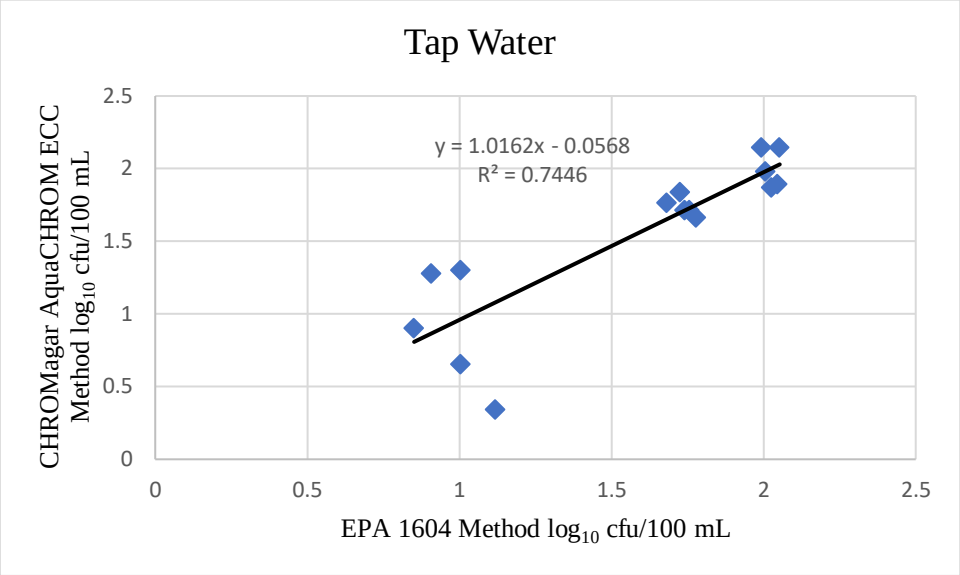

Figure 1: Method Comparison Results of AquaCHROM ECC vs. EPA 1604 for Tap Water

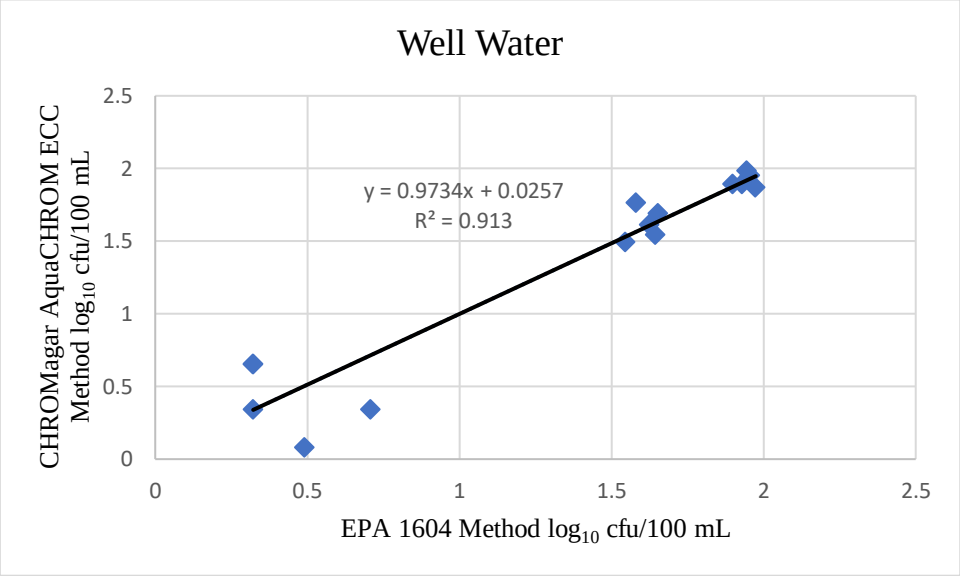

Figure 2: Method Comparison Results of AquaCHROM ECC vs. EPA 1604 for Well Water

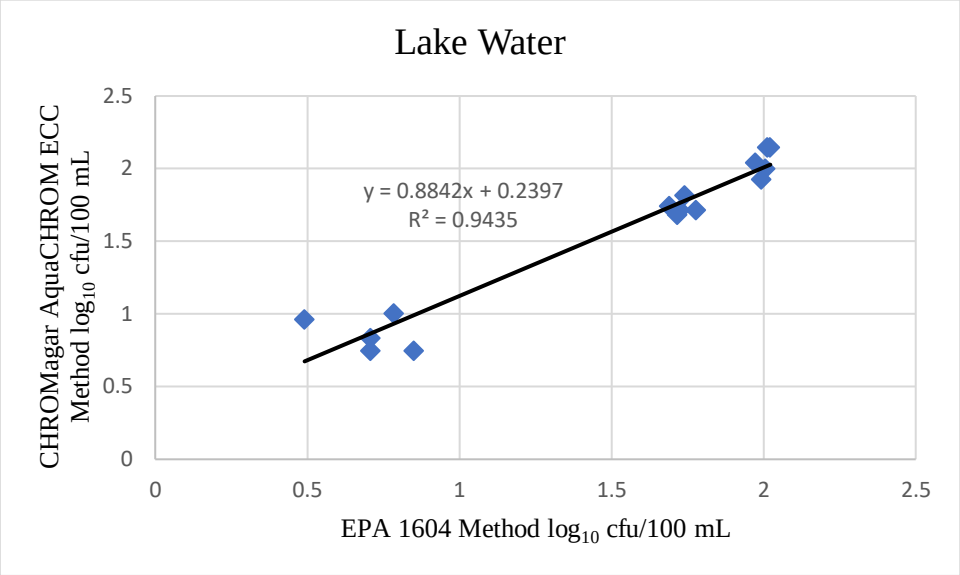

Figure 3: Method Comparison Results of AquaCHROM ECC vs. EPA 1604 for Lake Water