CERTIFICATION

AOAC Research Institute
Performance Tested Methods^{SM}

Certificate No.
072202

The AOAC Research Institute hereby certifies the method known as:

AquaCHROM^{TM} ECC

manufactured by

CHROMagar
4 Place du 18 Juin 1940
75006 Paris, France

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*^{SM} Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*^{SM} certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Scott Coates
_____
Scott Coates, Senior Director
Signature for AOAC Research Institute

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SUBMITTING COMPANY
CHROMagar
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METHOD NAME
AquaCHROM™ ECC

CATALOG NUMBER
AQ056

INDEPENDENT LABORATORY
Q Laboratories
1930 Radcliff Drive
Cincinnati, OH 45204

APPLICABILITY OF METHOD
Analytes – *E. coli* and non-*E. coli* coliform bacteria.

Matrixes – (100 mL test portions) - Tap water, well water, lake water, and bottled water.

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.

REFERENCE METHODS

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)

Food and Drug Administration Bacteriological Analytical Manual Chapter 4: Enumeration of *Escherichia coli* and the Coliform Bacteria. July 2020BAM Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria (3)

ORIGINAL CERTIFICATION DATE
July 12, 2022

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2024.

METHOD MODIFICATION RECORD
NONE

SUMMARY OF MODIFICATION
NONE

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PRINCIPLE OF THE METHOD (1)

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.

DISCUSSION OF THE VALIDATION STUDY (1)

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 b-glucuronidase character allows the identification of *E. coli* but a small percentage of *E. coli* strains, such as *E. coli* serotype O157, is b-glucuronidase negative (7, 8). Those strains are detected as yellow in color with AquaCHROM ECC. The b-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.

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.

No.	Target strain	Source	Origin	Result
Table 2. AquaCHROM ECC Inclusivity Study Results for *E. coli* (1)				
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 b-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 (1)

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. *marcescens*	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.

No.	Non-target strains	Source	Origin	Result
	Table 4. AquaCHROM ECC Exclusivity Study Results (1)			
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 Result
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 7. AquaCHROM ECC Method Presumptive vs. Confirmed – POD Results (1)

Matrix[a]	cfu/100 mL[b]	N[c]	Presumptive result X[d]	POD_CP[e]	95% CI	Confirmed result 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 (1)

Matrix[a]	cfu/100 mL[b]	N[c]	AquaCHROM ECC			Reference method[f]			dPOD_C[h]	95% CI[i]
			X[d]	POD_C[e]	95% CI	X	POD_R[g]	95% CI		
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 (1)

Matrix	Cont. level[a]	n	AquaCHROM ECC		Reference Method[c]		DOM[d]	SE[e]	95% CI[f]		90% CI	
			Log_{10} Mean[b]	s_r	Log_{10} Mean	s_r			LCL[g]	UCL[h]	LCL	UCL
Tap Water[i] (100 mL)	Uninoculated	5	0.000	NA[k]	0.000	NA	NA	NA	NA	NA	NA	NA
C. *freundii* ATCC[j]	Low	5	0.897	0.412	0.977	0.102	-0.081	0.190	-0.518	0.357	-0.434	0.273
8090	Medium	5	1.740	0.066	1.737	0.036	0.003	0.034	-0.074	0.081	-0.059	0.066
& *E. coli* ATCC 25922	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)	Low	5	0.414	0.243	0.433	0.170	-0.019	0.133	-0.325	0.287	-0.266	0.228
Naturally	Medium	5	1.621	0.109	1.610	0.046	0.012	0.053	-0.110	0.134	-0.087	0.110
contaminated	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)	Low	5	0.859	0.120	0.709	0.135	0.151	0.081	-0.036	0.337	0.000	0.301
Naturally	Medium	5	1.734	0.049	1.731	0.032	0.004	0.026	-0.057	0.064	-0.045	0.052
contaminated	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.

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