



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
072202

The AOAC Research Institute hereby certifies the method known as:

AquaCHROMTM ECC

manufactured by

CHROMagar

29 Avenue George Sand

93210 La Plaine

Saint-Denis, France

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*SM Program. 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.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date

October 23, 2024

Expiration Date

December 31, 2026

METHOD NAME
AquaCHROM™ ECC

CATALOG NUMBER
AQ056

ORIGINAL CERTIFICATION DATE
July 12, 2022

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 in compliance with 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.

CERTIFIED CLAIM STATEMENT: The AquaCHROM ECC method is certified for the detection and/or enumeration of *E. coli* and coliforms within the scope of Tables 1 and 2.

Table 1. Method Performance Claims

Matrix	Test Portion	Enrichment Conditions				Reference method ^b	Claim ^c
		Broth	Volume ^a	Temperature	Time		
Qualitative							
Tap water	100 mL	AquaCHROM ECC	100 mL	36 ± 1°C	18–24 h	EPA 1604	NSDD
Well water	100 mL	AquaCHROM ECC	100 mL	36 ± 1°C	18–24 h	EPA 1604	NSDD
Lake water	100 mL	AquaCHROM ECC	100 mL	36 ± 1°C	18–24 h	EPA 1604	NSDD
Bottled water	100 mL	AquaCHROM ECC	100 mL	36 ± 1°C	18–24 h	BAM Ch. 4	NSDD
Quantitative							
Tap water	100 mL	AquaCHROM ECC	2 mL/well	36 ± 1°C	18–24 h	EPA 1604	Eq
Well water	100 mL	AquaCHROM ECC	2 mL/well	36 ± 1°C	18–24 h	EPA 1604	Eq
Lake water	100 mL	AquaCHROM ECC	2 mL/well	36 ± 1°C	18–24 h	EPA 1604	Eq

^a A pre-weighed dose of AquaCHROM powder is added to 100 mL test portion. For quantitative analysis, 100 mL test portions are dispensed into a 48-well Deep well plate, approximately 2 mL/well.

^b EPA 1604: Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium); and BAM CH. 4: numeration of *Escherichia coli* and the Coliform Bacteria.

^c NSDD = No statistical difference detected using SLV study design from OMA Appendix J (2012). The SLV qualitative method comparison study design from OMA Appendix J (2012) is not intended to demonstrate statistical equivalence. Expert opinion is that the method is appropriate for its intended use. Eq = Equivalence of candidate and reference method demonstrated by 90% confidence interval on the difference of means contained entirely within -0.5 to 0.5 log₁₀.

Table 2. Method Selectivity

Organism	Enrichment		Inclusivity Strains		Exclusivity Species	
	Broth	Temp., °C	No. Tested ^a	No. Positive ^b	No. Tested ^c	No. Positive ^d
<i>Escherichia coli</i>	AquaCHROM ECC	36 ± 1°C	59	58	87	5
Coliforms	AquaCHROM ECC	36 ± 1°C	51	50	87	1

^a *E. coli* inclusivity comprising 58 generic *E. coli* strains plus one *E. coli* O157:H7 strain. Coliform inclusivity comprising *Citrobacter*, *Cronobacter*, *Enterobacter* *Escherichia* (non-*coli*), *Hafnia*, *Klebsiella*, and *Serratia* species.

^b *E. coli* O157:H7 was negative for *E. coli* but positive Coliform. One *Hafnia* species did not grow.

^c 87 *E. coli* and coliform exclusivity strains comprising 50 different species.

^d Five strains gave positive results for *E. coli* (green). Those strains were *Salmonella enterica* subsp. *arizonae*, *Salmonella enterica* subsp. *enterica* (serovar Abaetetuba), *Salmonella enterica* subsp. *enterica* (serovar Worthington), *Shigella boydii*, *Shigella sonnei*. One strain gave a positive result for coliform (yellow). That strain was an *Aeromonas* species.

Table 3. Method History

No.	Date	Summary	Supporting Data
1	March 2024	Original Certification.	Certification Report (link pending)