AOAC® Independent Laboratory Evaluation of the CHROMagar AquaCHROM™ ECC for the Detection and Enumeration of Coliforms and Escherichia coli in Water Samples

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Introduction

AquaCHROM™ ECC is a chromogenic medium for the detection of *E. coli* and coliforms in water samples (ex. tap water, well water, lake water and bottled water). The product is composed of a powder medium and is supplied in pre-weighted doses. Each dose is for a 100 mL water sample. Species differentiation is based on the use of two chromogens instead of a chromogen and fluorogene, which eliminates the use of a UV lamp.
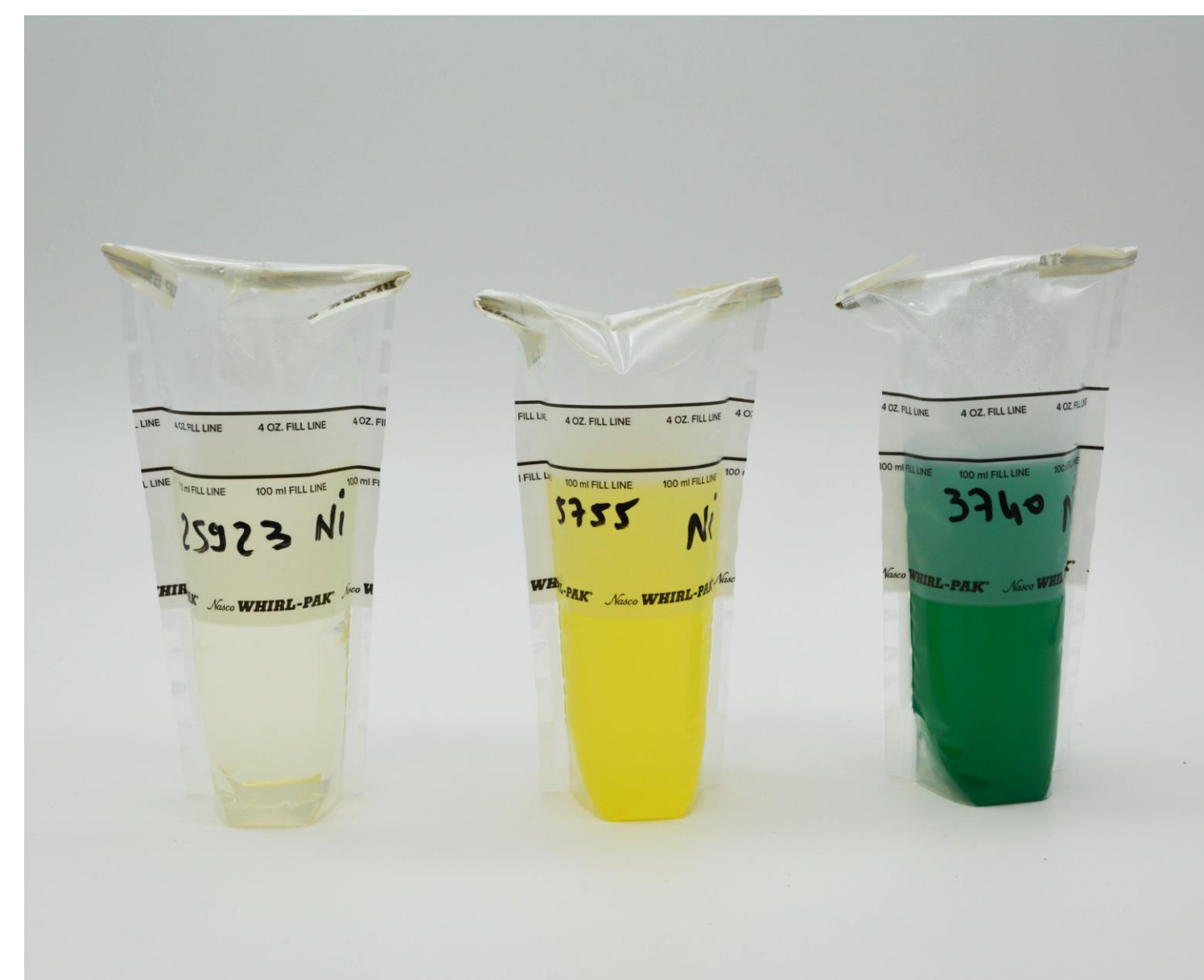
Fig 1. 100 mL water tests with AquaCHROM™ ECC after aerobic incubation at 37 °C for 24 h. *E. coli* results are green to bleu green, non-*E. coli* coliforms results are yellow and other bacteria are colorless or inhibited.

Purpose

The purpose of this AOAC® Independent Laboratory Study was to compare the alternative method to FDA/BAM Chapter 4 and EPA 1604 reference methods for the detection of coliforms and *E. coli* in water as part of the Performance Tested Method program.

Methods

The assay was compared to two reference methods: FDA/BAM Chapter 4: Enumeration of *Escherichia coli* and the Coliform Bacteria and EPA 1604: Total Coliforms and *Escherichia coli* in water by Membrane Filtration using a Simultaneous Detection Technique reference method. The assay was also compared to the AOAC OMA 991.15: Total Coliforms and *Escherichia coli* in water: Defined Substrate Technology (Colilert) Method following an unpaired study design.

Qualitative Results

Statistical analysis was conducted according to the Probability of Detection (POD) statistical model. No statistically significant difference was observed between the three methods.

Table 1. CHROMagar AquaCHROM ECC Method vs. EPA 1604 Reference Method, Tap Water

Matrix	N[b]	Candidate			Reference			dPOD[f]	95% CI[g]
		x[c]	POD[c][d]	95% CI	X	POD[R][e]	95% CI		
Tap Water (100 mL)	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37 0.19
	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.43, 0.43

Table 2. CHROMagar AquaCHROM ECC Method vs. EPA 1604 Reference Method, Well Water

Matrix	N[b]	Candidate			Reference			dPOD[f]	95% CI[g]
		x[c]	POD[c][d]	95% CI	X	POD[R][e]	95% CI		
Well Water (100 mL)	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
	20	13	0.65	0.43, 0.82	8	0.40	0.22, 0.61	0.25	-0.05 0.50
	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.43, 0.43

Table 3. CHROMagar AquaCHROM ECC Method vs. EPA 1604 Reference Method, Lake Water

Matrix	N[b]	Candidate			Reference			dPOD[f]	95% CI[g]
		x[c]	POD[c][d]	95% CI	X	POD[R][e]	95% CI		
Lake Water (100 mL)	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09 0.45
	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.43, 0.43

Table 4. CHROMagar AquaCHROM ECC Method vs. FDA/BAM Ch 4 Reference Method, Bottled Water

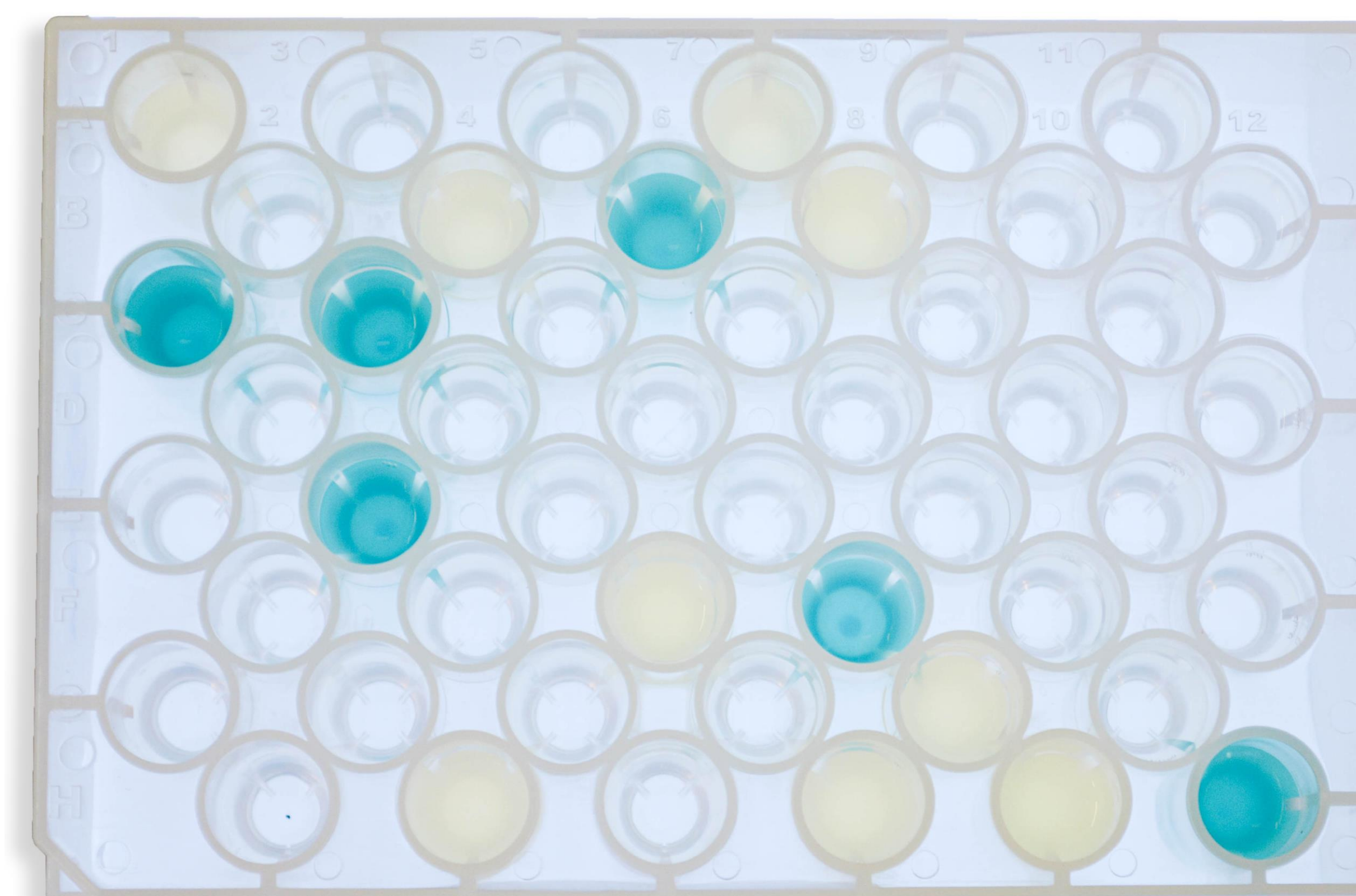| Matrix	N[b]	Candidate			Reference			dPOD[f]	95% CI[g]
		x[c]	POD[c][d]	95% CI	X	POD[R][e]	95% CI		
Bottled Water (100 mL)	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
	20	17	0.85	0.64, 0.95	15	0.75	0.53, 0.89	0.00	-0.15, 0.34
	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.43, 0.43

Fig 2. A 100 mL water test with AquaCHROM™ ECC MPN after aerobic incubation at 37 °C for 18 h. *E. coli* results (6 wells) are green to bleu green, non-*E. coli* coliforms results (9 wells) are yellow.

Quantitative Results

The 90% confidence interval of the bias between the two methods fell between -0.5 to 0.5 Log10 for each concentration indicating equivalence between the two methods

Table 5. Results of CHROMagar AquaCHROM ECC vs. Reference Method

Matrix	Cont. level[a]	n	AquaCHROM ECC			Reference Method[d]			DOM[e]	SE[f]	95% CI[g]		90% CI	
			Log10 Mean[b]	s_r	RSD_r[c]	Log10 Mean	s_r	RSD_r			LCL[h]	UCL[i]	LCL	UCL
Tap Water (100 mL)	Low	5	0.897	0.412	45.931	0.977	0.102	10.440	-0.081	0.190	-0.518	0.357	-0.434	0.273
	Medium	5	1.740	0.066	3.793	1.737	0.036	2.075	0.003	0.034	-0.074	0.081	-0.059	0.066
	High	5	2.008	0.134	6.673	2.024	0.026	1.285	-0.017	0.061	-0.157	0.124	-0.130	0.097
Well Water (100 mL)	Low	5	0.414	0.243	58.696	0.433	0.170	39.261	-0.019	0.133	-0.325	0.287	-0.266	0.228
	Medium	5	1.621	0.109	6.724	1.610	0.046	2.857	0.012	0.053	-0.110	0.134	-0.087	0.110
	High	5	1.983	0.048	2.421	1.940	0.028	1.443	-0.022	0.025	-0.079	0.036	-0.068	0.024
Lake Water (100 mL	Low	5	0.859	0.120	13.970	0.709	0.135	19.041	0.151	0.081	-0.036	0.337	0.000	0.301
	Medium	5	1.734	0.049	2.826	1.731	0.032	1.849	0.004	0.026	-0.057	0.064	-0.045	0.052
	High	5	2.052	0.096	4.678	2.001	0.019	0.950	0.051	0.044	-0.050	0.152	-0.030	0.132

Study Highlights

This novel assay allows for fast, reliable detection and enumeration of Coliforms and *Escherichia coli* with presumptive results obtained in as little as 18-24 hours.

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