

Address:
4 place du 18 juin 1940
75006 Paris – France



S.A.S au Capital de 165000€ R.C.S Paris B391 293 834
VAT n° : FR 27 391 293 834

CHROMagar@CHROMagar.com
Tel: +33 1 45 48 05 05

www.CHROMagar.com

Evaluation of AquaCHROM™ ECC

*Chromogenic Culture Broth for the Detection of Escherichia coli
and other Coliforms*

Laboratory:

CHROMagar™
4 place du 18 Juin 1940
75006 PARIS
FRANCE

This report contains 12 pages, including 6 pages of annexes

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1. Introduction

Worldwide, the biological quality of drinking water and food is based on detecting the presence/absence of *Escherichia coli* and coliforms (ECC), which are indicators of organic, environmental or fecal contamination. Indeed, the detection of these microorganisms represents a greater risk of the presence of more harmful pathogens and may lead to suspension of the water supply and foodstuff recalls.

There are several commercially available broth media to detect ECC, but all of them are traditionally based on the use of a chromogen/fluorogen combination. AquaCHROM™ ECC is a liquid-based chromogenic medium aimed to detect the presence of ECC in 100 mL water samples. The formulation of AquaCHROM™ ECC includes two different chromogens allowing *E. coli* detection under normal lighting conditions, without the need of ultra-violet light to perform quality control. Thus, water samples turn yellow when coliforms are present and develop a green coloration when *E. coli* is present.

In this study, we evaluated the specificity, sensitivity, limit of detection and the performances at room temperature incubation of AquaCHROM™ ECC. Furthermore, we determined the shelf life of the medium in powder.

2. Materials and methods

2.1. Bacteria tested

A total of 186 well-characterized bacteria strains of *E. coli* ($n = 70$), coliforms ($n = 29$) and non-coliforms ($n = 87$) were included.

Bacteria grouped as coliforms included *Citrobacter* ($n = 7$), *Cronobacter* ($n = 1$), *Enterobacter* ($n = 4$), *Serratia* ($n = 3$), *Yersinia* ($n = 3$), *Klebsiella* ($n = 6$), *Escherichia hermanii* ($n = 3$), and *Hafnia* ($n = 2$).

Non-coliform bacteria comprised *Salmonella* ($n = 14$), *Aeromonas* ($n = 4$), *Clostridium* ($n = 2$), *Clostridioides* ($n = 2$), *Enterococcus* ($n = 18$), *Listeria* ($n = 4$), *Staphylococcus* ($n = 12$), *Pseudomonas* ($n = 5$), *Proteus* ($n = 3$), *Shigella* ($n = 4$), *Streptococcus* ($n = 11$), *Vibrio* ($n = 4$), *Legionella* ($n = 2$), and *Acinetobacter* ($n = 2$).

2.2. Preparation of water tests

Inoculation of bacteria suspensions was carried out in 100 mL of sterile distilled water contained in Nasco Whirl-Pak® 4 oz. (118 mL) stand up bags properly labeled on their write-on strip. Cell count for each inoculum was performed on TSA plates. Ready to use pre-weighed doses of AquaCHROM™ ECC were added to the inoculated water samples. The bags were closed according to the manufacturer's instructions before incubation.

2.3. Evaluation of specificity and sensitivity

The whole of bacteria was used in this evaluation (See Annexes 1 to 3). *E. coli* and coliforms strains (inclusivity) were inoculated at about 10^2 CFU per 100 mL of sterile distilled water and non-coliforms strains (exclusivity) were inoculated at about 10^4 CFU per 100 mL of sterile distilled water. Incubation of TSA plates (cell counts) and bags was performed at 37 °C for up to 24 h, including results reading after 18 and 24 h of incubation.

2.4. Evaluation of limit of detection

In order to evaluate the limit of detection (LOD) of *E. coli* by AquaCHROM™ ECC testing, two strains, namely *E. coli* ATCC® 25922 and *E. coli* ATCC® 51446, were used. For the evaluation of the AquaCHROM™ ECC LOD for coliforms, *Hafnia* AR3962 and *E. cloacae* ATCC® 35030 strains were employed. We included a pair of *E. coli*/coliform mixtures obtaining a total of 9 different ratios for these strains. Triplicates of dilutions of bacteria suspensions and of ratios of *E. coli*/coliform were tested for each strain and mix of strains. Incubation of TSA plates (cell counts) and bags was performed at 37 °C for up to 36 or 48 h, including results reading after 18, 24, 36 and 48 h of incubation, accordingly.

2.5. Testing with incubation at room temperature

Bags inoculated with 10^2 CFU (per 100 mL of sterile distilled water) of *E. coli* ATCC® 25922, *E. coli* ATCC® 51446, *E. coli* AR4076 or *E. cloacae* AR3412 strains were incubated at room temperature, with results reading after 18, 24, and 33 h of incubation.

2.6. Determination of shelf life

Three batches of AquaCHROM™ ECC of different ages were tested. Visual and chemical features, as well as microbiological performance were registered, using *E. coli* ATCC® 25922, *E. coli* ATCC® 51446, *E. coli* AR3740, *E. coli* AR3857, *Salmonella* *abaetetuba* ATTC® 35640, *Klebsiella oxytoca* AR5755 (inclusivity), and *Staphylococcus aureus* ATCC® 25923 (exclusivity) inoculations. Incubation of TSA plates (cell counts) and bags was performed 24 h at 37 °C.

3. Results

3.1. Broth medium specificity and sensitivity

AquaCHROM™ ECC water tests turned yellow when coliforms were present and develop a green coloration when *E. coli* strains were present. The results for *E. coli*, were analyzed according to the glucuronidase character. Two groups were defined, “glucuronidase positive (Gluc+)” and “glucuronidase negative (Gluc-)”. For coliforms strains, two groups were defined, “common” (*Klebsiella*, *Enterobacter*, and *Citrobacter*) and “rare” (*Serratia*, *Hafnia*, *E. hermanii*, *Yersinia*, and *Cronobacter*).

Defined groups of bacteria strains tested for evaluation of specificity and sensitivity

Bacteria	Number of strains	Incubation at 37 °C for 24 h	
		Green	Yellow
<i>E. coli</i>	70		
Gluc+	60	58 (97 %)	
Gluc- / 0157	8		8 (100 %)
Coliforms	29		
Common	17		16 (94 %)
Rare	12 ¹		6 (86 %)

¹ Five out of 12 strains (3 *Yersinia*, 1 *E. hermanii* and 1 *Hafnia*) showed no growth at 37 °C.

Gram-positive and Gram-negative non-coliforms bacteria that did not show growth or were colourless at 37 °C were *Salmonella* (11 out of 14), *Clostridium*, *Clostridioides*, *Enterococcus*, *Listeria*, *Staphylococcus*, *Pseudomonas* (3 out of 5), *Proteus*, *Shigella* (2 out of 4), *Streptococcus*, *Vibrio*, *Legionella*, and *Acinetobacter*.

As for *Salmonella*, 3 out of 14 strains grew in green (1 did not grow). Two out of 4 *Shigella* strains grew in green (1 did not grow). Three *Aeromonas* strains developed yellow coloration (1 did not grow).

3.2. Limit of detection evaluation of the broth medium

When using pure strains, *E. coli* as well as coliforms were detected from 1 UFC per 100 mL of water (See Annex 4). Using *E. coli*/coliform mixtures incubated at 37 °C, *E. coli* was clearly detected in a ratio of 1:10⁴ after a 18 h incubation, and in a ratio of 1:10⁷ after a 24 h incubation (See Annex 5).

3.3. Performances of broth medium at room temperature

After a 33 h incubation at room temperature, the broth medium performances were obtained, including described readings of results.

Bacteria strains tested for evaluation of performances at room temperature

Strains	Incubation of AquaCHROM™ ECC batches at 37°C		
	18 h	24 h	33 h
<i>E. coli</i> ATCC® 25922	T	YG	BG
<i>E. coli</i> ATCC® 51446	T	Y/T	G
<i>E. coli</i> AR4076	T	Y	YG
<i>E. cloacae</i> AR3412	T	Y	Y

YG, yellow-green ; BG, blue-green ; G, green ; Y, yellow ; T, translucent.

3.4. Shelf life of powder product

For the three AquaCHROM™ ECC batches, the aspect of powder was white and homogeneous. The dissolved medium was homogeneous, colorless, transparent, pH $7,1 \pm 0,2$, and stayed sterile in a stand up bag after a 3 day incubation at 30°C .

After a 24 h incubation at 37°C , the broth medium performances were obtained.

Bacteria strains tested to determine the shelf life of AquaCHROM™ ECC batches

Strains	Incubation of AquaCHROM™ ECC batches at 37°C			TSA [§]
	# 062 (Exp. Date 2016)	# 061 (Exp. Date 2015)	# 059 (Exp. Date 2014)	
<i>E. coli</i> ATCC® 25922	BG+	BG+	BG++	68
<i>E. coli</i> ATCC® 51446	Y/G pp	Y/G pp	Y/G pp	81
<i>E. coli</i> AR3740	BG+	BG+/-	BG+	53
<i>E. coli</i> AR3857	BG+	BG+	BG+	68
<i>S. abaeituba</i> ATCC® 35640	B+	B+	B+	160
<i>K. oxytoca</i> AR5755	Y+	Y+	Y+	134
<i>S. aureus</i> ATCC® 25923	-	-	-	125

BG, blue-green ; Y, yellow ; B, blue ; G pp, green precipitate ; -, no growth ; +, color intensity.

[§]Cell counts on TSA

4. Conclusion

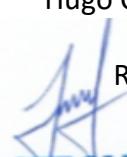
AquaCHROM™ ECC showed excellent specificity (96 % for Gluc+ *E. coli* strains and “common coliforms”), sensitivity (97 % for Gluc+ *E. coli* strains and 94 % for common coliforms), and LOD (1 UFC of *E. coli*/100 mL), displaying easy-to-read results, which are based on the combination of two chromogens. Some rare strains of *E. coli*, like *E. coli* O157, may display yellow coloration, some rare strains of *Salmonella* can give a dark blue coloration and colorless results can be obtained with some atypical strains of coliforms. These kind of cases can be considered as limitations of the broth medium.

The medium is suitable to be used in areas where no laboratory facilities are available, since there is no need of UV-light lamps and results can be obtained with an incubation at room temperature.

The product is supplied as ready-to-use powder doses that demonstrated a 2 year shelf life with a 15-30 °C storage in the dark.

Hugo CRUZ RAMOS

R&D Manager


CHROMagar
 4, place du 18 juin 1940
 75006 PARIS
 FRANCE

Annexes

Annex 1. *Escherichia coli* strains ($n = 70$) tested.

Strains		Incubation at 37°C		
		18 h	24 h	TSA [§]
<i>E. coli</i>	AR3740	G++	G++	63
<i>E. coli</i>	AR3741	G+	G++	95
<i>E. coli</i>	AR3859	G+	G++	115
<i>E. coli</i>	ATCC® 25922	G++	G+++	113
<i>E. coli</i>	AR3857	G+	G++	131
<i>E. coli</i>	AR3858	G+	G++	72
<i>E. coli</i>	AR3962	Gluc -	Y	106
<i>E. coli</i>	AR4076	YG	G+	58
<i>E. coli</i>	ATCC® 51446	Y	G++	70
<i>E. coli</i>	ATCC® 35150	O157	Y	40
<i>E. coli</i>	AR4094	O157	Y	108
<i>E. coli</i>	H7	O157	Y	70
<i>E. coli</i>	AR5168		G++	136
<i>E. coli</i>	AR4077		G++	147
<i>E. coli</i>	AR5030		G++	144
<i>E. coli</i>	AR5169		G+	105
<i>E. coli</i>	AR5306		G++	114
<i>E. coli</i>	AR5307		Y	72
<i>E. coli</i>	AR5180	Gluc -	G++	57
<i>E. coli</i>	AR5238		G++	80
<i>E. coli</i>	AR5387		G++	95
<i>E. coli</i>	AR5190		G++	68
<i>E. coli</i>	AR5189		Y	132
<i>E. coli</i>	AR5478	Gluc +	Y	60
<i>E. coli</i>	AR5477		Y	YG
<i>E. coli</i>	AR5690		G++	177
<i>E. coli</i>	AR5664		G++	231
<i>E. coli</i>	AR5665		YG	G+
<i>E. coli</i>	AR5666		YG	G+
<i>E. coli</i>	AR5191		Y	YG
<i>E. coli</i>	AR5192		G+	G++
<i>E. coli</i>	AR5689		Y	G+
<i>E. coli</i>	AR4524		G++	G+++
<i>E. coli</i>	AR4526		G++	G++
<i>E. coli</i>	AR4531		G++	G++
				160

Strains		Incubation at 37°C		
		18 h	24 h	TSA [§]
<i>E. coli</i>	AR4651		Y	G++
<i>E. coli</i>	AR4733		G++	G++
<i>E. coli</i>	AR4734		G++	G++
<i>E. coli</i>	AR4732		G++	G++
<i>E. coli</i>	AR5011		G++	G++
<i>E. coli</i>	AR5012		Y	G+
<i>E. coli</i>	AR5013		G+	G++
<i>E. coli</i>	AR5014		G++	G++
<i>E. coli</i>	AR5179		G++	G++
<i>E. coli</i>	AR5303		YG	G+
<i>E. coli</i>	AR5304		YG	YG
<i>E. coli</i>	AR5305		YG	G+
<i>E. coli</i>	AR5360		G+	G++
<i>E. coli</i>	AR5361		Y	YG
<i>E. coli</i>	AR5384		Y	G+
<i>E. coli</i>	AR5388		G++	G+++
<i>E. coli</i>	AR5389		G++	G+++
<i>E. coli</i>	AR5414		G+	G++
<i>E. coli</i>	AR5415		G+	G++
<i>E. coli</i>	AR5416		GY	G++
<i>E. coli</i>	AR5417		YG	G+
<i>E. coli</i>	AR5427	Gluc +	T++	Y
<i>E. coli</i>	AR5428		G++	G++
<i>E. coli</i>	AR5433		Y	G+
<i>E. coli</i>	AR5434		G++	G+++
<i>E. coli</i>	AR5435		G++	G++
<i>E. coli</i>	AR5436		G++	G++
<i>E. coli</i>	AR5438		G+	G++
<i>E. coli</i>	AR5440		G+	G++
<i>E. coli</i>	AR5442		G++	G++
<i>E. coli</i>	AR5458		Y	G++
<i>E. coli</i>	AR5510		YG	G+
<i>E. coli</i>	AR5514	O157	Y	Y
<i>E. coli</i>	AR5515	O157	Y	Y
<i>E. coli</i>	AR5519	O157	Y	Y
				58

G, green ; Y, yellow ; T, turbid ; YG, yellow-green ; +, intensity. [§] Cell counts on TSA

Annex 2. Bacteria strains grouped as coliforms ($n = 29$) tested.

Strains	Incubation at 37°C		
	18 h	24 h	TSA [§]
<i>C. freundii</i>	AR3870	Y	25
<i>C. freundii</i>	ATCC® 8090	Y	32
<i>C. freundii</i>	AR5663	G+	67
<i>C. freundii</i>	AR5662	Y	97
<i>Citrobacter</i>	AR3134	Y	111
<i>Citrobacter</i>	AR3378	Y	63
<i>Citrobacter</i>	AR3030	Y	89
<i>C. sakasakii</i>	AR5167	Y	65
<i>E. aerogenes</i>	AR5187	Y	119
<i>E. aerogenes</i>	ATCC® 13048	Y	174
<i>E. cloacae</i>	AR5480	Y	228
<i>E. cloacae</i>	ATCC® 35030	Y	105
<i>S. liquefaciens</i>	AR3964	-	63
<i>S. marcescens</i>	AR5568	Y	96
<i>S. phymuthica</i>	AR4048	-	23
<i>Y. enterocolitica</i>	AR5579	-	T++
<i>Y. enterocolitica</i>	AR5580	-	-
<i>Y. pseudotuberculosis</i>	AR5585	-	-
<i>K. oxytoca</i>	AR5204	Y	136
<i>K. oxytoca</i>	AR5236	Y	131
<i>K. oxytoca</i>	AR5755	Y	124
<i>K. pneumoniae</i>	AR5186	Y	129
<i>K. pneumoniae</i>	AR5251	Y	235
<i>K. pneumoniae</i>	ATCC® 13883	Y	127
<i>E. hermanii</i>	856.1	-	107
<i>E. hermanii</i>	AR5245	Y	64
<i>E. hermanii</i>	AR5341	Y	130
<i>Hafnia</i>	657.41	-	-
<i>Hafnia alvei</i>	AR3862	T++	117

G, green ; Y, yellow ; T, turbid ; -, no growth ; +, intensity. [§] Cell counts on TSA

Annex 3. Non-coliform strains ($n = 87$) tested.

Strains		Incubation at 37°C			TSA [§]
		18h	24h	TSA [§]	
<i>S. abaetetuba</i>	ATCC® 35640	B++	B++	149	
<i>S. arizona</i>	AR3910	Y/G	G++	125	
<i>S. dublin</i>	AR3580	T+	T++	122	
<i>S. typhi</i>	AR4052	T+	T++	103	
<i>S. typhi</i>	AR3104	-	T++	75	
<i>S. typhi</i>	AR3105	-	T++	152	
<i>S. typhimurium</i>	ATCC® 13311	-	-	195	
<i>S. typhimurium</i>	ATCC® 13311	T++	T++	175	
<i>S. typhimurium</i>	AR3015	T+	T++	76	
<i>S. worthington</i>	AR3911	Y/G	G++	166	
<i>Salmonella spp</i>	AR4053	T++	T++	53	
<i>Salmonella</i>	AR3011	-	T++	105	
<i>Salmonella</i>	AR3924	T++	T++	103	
<i>Salmonella</i>	AR3925	T++	T++	114	
<i>Aeromonas</i>	AR3881	-	-	29	
<i>Aeromonas</i>	AR3898	-	Y++	28	
<i>Aeromonas</i>	333	Y++	Y++	39	
<i>Aeromonas</i>	435	Y++	Y++	>200	
<i>C. difficile</i>	AR5681	-	-	10	
<i>C. difficile</i>	AR5682	-	-	1	
<i>C. perfringens</i>	AR5678	-	-	19	
<i>C. sordelii</i>	AR5679	-	-	14	
<i>E. avium</i>	AR5258	-	-	55	
<i>E. casseliflavus</i>	AR5111	-	Y Bkgd	28	
<i>E. durans</i>	AR5257	-	-	28	
<i>E. faecalis</i>	AR5312	-	-	26	
<i>E. faecalis</i>	AR5313	-	-	42	
<i>E. faecalis</i>	AR5316	-	-	29	
<i>E. gallinarum</i>	AR5112	-	-	26	
<i>E. gallinarum</i>	AR5266	-	-	26	
<i>E. gallinarum</i>	AR5218	-	-	6	
<i>E. hirae</i>	AR5687	-	-	29	
<i>Enterococcus</i>	AR5289	-	-	8	
<i>Enterococcus vanB</i>	AR5201	-	-	8	
<i>E. faecalis</i> (VRE)	AR5002	-	-	14	
<i>E. faecalis</i> (VRE)	AR5101	-	-	49	
<i>E. faecium</i> (VRE)	AR5102	-	-	19	
<i>E. faecium</i> (VRE)	AR5164	-	-	7	
<i>E. faecalis</i> (VSE)	ATCC® 29212	-	-	35	
<i>E. faecium</i> (VSE)	AR4437	-	-	23	
<i>L. innocua</i>	CIP8011	-	-	32	
<i>L. ivanovii</i>	ATCC® 19119	-	-	43	
<i>L. monocytogenes</i>	ATCC® 19115	-	-	36	
<i>L. monocytogenes</i>	AR4580	-	-	14	
Strains		Incubation at 37°C			
		18h	24h	TSA [§]	
<i>S. aureus</i> (MRSA)	ATCC® 43300	-	-	73	
<i>S. aureus</i> (MSSA)	ATCC® 25923	-	-	> 110	
<i>S. aureus</i>	AR3916	-	-	120	
<i>S. cohnii</i>	AR4909	-	-	30	
<i>S. haemolyticus</i>	ATCC® 29970	-	-	>120	
<i>S. intermedius</i>	AR5008	-	-	120	
<i>S. lentus</i>	ATCC® 700403	-	-	16	
<i>S. saprophyticus</i>	ATCC® 15305	-	-	> 100	
<i>S. simulans</i>	ATCC® 27851	-	-	31	
<i>S. warnerii</i>	ATCC® 49454	-	-	29	
<i>S. xylosus</i>	ATCC® 29971	-	-	25	
<i>S. epidermidis</i>	ATCC® 12228	-	-	> 60	
<i>P. aeruginosa</i>	SN27	-	-	39	
<i>P. aeruginosa</i>	AR5197	-	T++	13	
<i>P. aeruginosa</i>	AR5196	T+	T++	15	
<i>P. aeruginosa</i>	ATCC® 9027	-	-	21	
<i>P. aeruginosa</i>	ATCC® 10145	-	-	20	
<i>P. mirabilis</i>	AR5479	T++	T++	>120	
<i>P. mirabilis</i>	AR3022	T++	T++	>120	
<i>P. vulgaris</i>	AR4653	T++	T++	>90	
<i>S. boydii</i>	ATCC® 9207	Y/G	BG++	>140	
<i>S. dysenteriae</i>	ATCC® 13313	-	-	32	
<i>S. flexneri</i>	ATCC® 12022	T+	T++	>140	
<i>S. sonnei</i>	ATCC® 9250	Y/G	Y/G	69	
<i>S. agalactiae</i>	AR3938	-	-	29	
<i>S. agalactiae</i>	AR4186	-	-	33	
<i>S. bovis</i>	ATCC® 9809	-	-	51	
<i>S. dysgalactiae</i>	AR4143	-	-	31	
<i>S. equinus</i>	AR5688	-	-	14	
<i>S. oralis</i>	AR5649	-	-	10	
<i>S. uberis</i>	AR5495	-	-	67	
<i>S. uberis</i>	AR5496	-	-	39	
<i>Streptococcus</i> group A	AR5255	-	-	42	
<i>Streptococcus</i> group F	AR5408	-	-	4	
<i>Streptococcus</i> group G	AR5311	-	-	7	
<i>V. cholerae</i>	AR4482	-	-	8	
<i>V. cholerae</i>	AR4748	-	-	75	
<i>V. parahaEmolyticus</i>	AR4493	-	-	18	
<i>V. vulnificus</i>	AR4675	-	-	18	
<i>L. pneumophila</i>	AR4665	-	-	0	
<i>L. pneumophiliia</i>	AR4666	-	-	0	
<i>A. baumannii</i>	AR5624	T++	T++	11	
<i>A. hebeiensis</i>	AR5563	-	-	0	

G, green ; T, turbid ; Y, yellow ; Y/G, yellow-green ; B, blue ; BG, blue-green ; Bkgd, background; -, no growth ; +, intensity. [§] Cell counts on TSA

Annex 4. Strains tested for LOD evaluation.

Strains		Incubation at 37°C for 18 h			Incubation at 37°C for 24 h			Incubation at 37°C for 36 h			TSA [§]
		1	2	3	1	2	3	1	2	3	
<i>E. coli</i> ATCC® 25922	10 ⁻⁶	G++	G++	G++	G+++	G+++	G+++				178
	10 ⁻⁷	YG	G++	G++	G+++	G+++	G+++				
	10 ⁻⁸	YG	YG	-	G+++	G+++	-				
<i>E. coli</i> ATCC® 51446	10 ⁻⁶	-	Y+	Y++ G pp	-	Y G pp	G++	-	G++	G++	88
	10 ⁻⁷	Y++	Y+	Y++	G+	G+	G+	G++	G++	G++	
	10 ⁻⁸	Y++	-	Y++	Y/G	Y	G+	G++	G++	G++	
<i>Hafnia</i> AR3962	10 ⁻⁶	T++	T++	T++	Y++	Y++	Y++	Y++	Y++	Y++	89
	10 ⁻⁷	T+	T-/+	T+	Y++	Y++	Y++	Y++	Y++	Y++	
	10 ⁻⁸	-	-	-	G Bkgd	-	Y+	Y++	Y++	Y++	
<i>E. cloacae</i> ATCC® 35030	10 ⁻⁶	Y++	Y++	Y++	Y++	Y++	Y++				148
	10 ⁻⁷	Y++	Y++	Y++	Y++	Y++	Y++				
	10 ⁻⁸	Y++	Y++	Y Bkgd	Y++	Y++	Y++				

YG, yellow-green ; G, green ; Y, yellow ; T, translucent ; G pp, green precipitate ; Bkgd, background ; -, no growth ; +, color intensity. [§] Cell counts on TSA

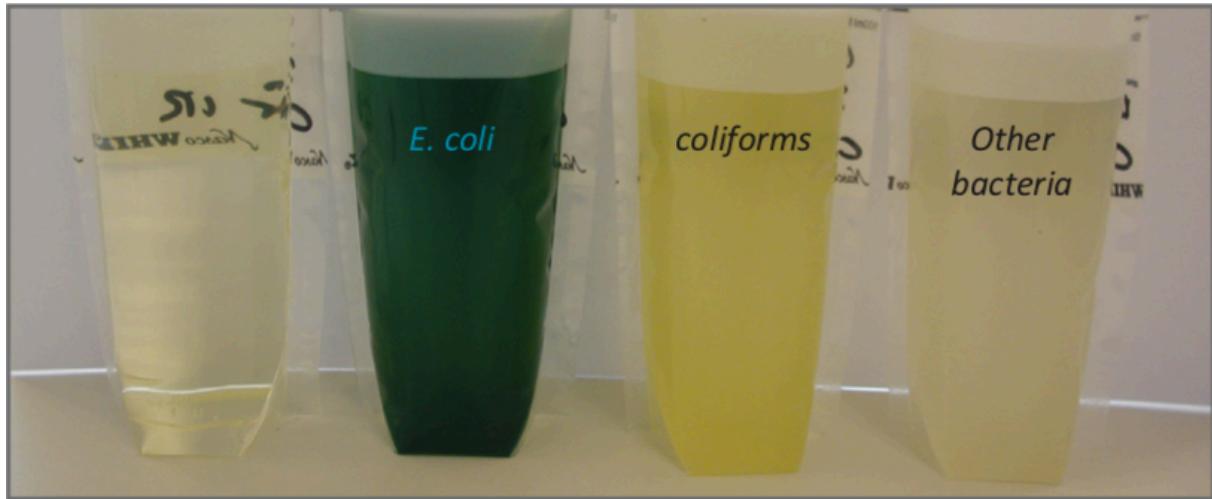
Annex 5. *E. coli*/coliform mixtures tested for LOD evaluation.

Mix of strains #1			Ratio	Incubation at 37°C for 18 h			Incubation at 37°C for 24 h			
				1	2	3	1	2	3	
<i>E. coli</i> ATCC® 25922	10 ⁻⁶	<i>E. cloacae</i> ATCC® 35030	10 ⁰	178 / 14 800 000	YG	YG	YG	G+	G+	G+
	10 ⁻⁷		10 ⁰	17,8 / 14 800 000	Y++	Y++	Y++	G++	G++	G++
	10 ⁻⁸		10 ⁰	1,78 / 14 800 000	Y++	Y++	Y++	G++	G++	G++
	10 ⁻⁶		10 ⁻²	178 / 148 000	G+	G+	G+	G++	G++	G++
	10 ⁻⁷		10 ⁻²	17,8 / 148 000	YG	YG	YG	G++	G++	G++
	10 ⁻⁸		10 ⁻²	1,78 / 148 000	Y++	Y++	Y/G pp	Y++	G+	G++
	10 ⁻⁶		10 ⁻⁴	178 / 14 800	G++	G++	G++	G++	G+	G++
	10 ⁻⁷		10 ⁻⁴	17,8 / 14 800	G+	G+	G+	G++	G++	G++
	10 ⁻⁸		10 ⁻⁴	1,78 / 14 800	Y/G pp	Y/G pp	Y/G pp	G+	G++	G++

Mix of strains #2			Ratio	Incubation at 37°C for 18 h			Incubation at 37°C for 24 h			Incubation at 37°C for 36 h			Incubation at 37°C for 48 h		
				1	2	3	1	2	3	1	2	3	1	2	3
<i>E. coli</i> ATCC® 51446	10 ⁻⁶	<i>E. cloacae</i> ATCC® 35030	10 ⁰	88 / 14 800 000	Y++	Y++	Y++	Y++	Y++	Y++	G++	G+++	Y++	G++	G+++
	10 ⁻⁷		10 ⁰	8,8 / 14 800 000	Y++	Y++	Y++	Y++	Y++	Y++	G++	G++	Y++	G++	Y++
	10 ⁻⁸		10 ⁰	0,88 / 14 800 000	Y++	Y++	Y++	Y++	G pp	Y++	YG	YG	Y++	G++	Y++
	10 ⁻⁶		10 ⁻²	88 / 148 000	Y++	Y++	Y++	G pp	G pp	Y++	G++	G+++	G++	G+++	G+++
	10 ⁻⁷		10 ⁻²	8,8 / 148 000	Y++	Y++	Y++	Y++	Y++	Y++	G++	G++	Y++	G++	Y++
	10 ⁻⁸		10 ⁻²	0,88 / 148 000	Y++	Y++	Y++	Y++	Y++	Y++	Y++	Y++	Y++	Y++	G+++
	10 ⁻⁶		10 ⁻⁴	88 / 14 800	Y++	Y++	Y++	G++	G pp	Y++	G+++	G+++	G+++	G+++	G+++
	10 ⁻⁷		10 ⁻⁴	8,8 / 14 800	Y++	Y++	Y++	Y++	G pp	Y++	Y++	G+++	Y++	G+++	Y++
	10 ⁻⁸		10 ⁻⁴	0,88 / 14 800	Y++	Y++	Y++	Y++	Y++	Y++	G+	Y++	G++	G++	G++

YG, yellow-green ; G, green ; Y, yellow ; G pp, green precipitate ; +, intensity

Annex 6. Typical appearance of AquaCHROM™ ECC tests.



The picture shown is not contractual.

Good performance of the broth medium can be tested, inoculating the following ATCC® strains:

Microorganism	Typical appearance
<i>E. coli</i> ATCC® 25922	→ Green blue
<i>C. freundii</i> ATCC® 8090	→ Yellow
<i>S. aureus</i> ATCC® 25923	→ Inhibited