MICA Advance

E. coli

SCIENTIFIC REPORT

DIAMIDEX

CONTENTS

I. Introduction

MICA Advance *E. coli* is a simple and rapid solution that detects *Escherichia coli* in drinking water (bottled waters and mains waters) in 6 hours.

MICA Advance *E. coli* detects presence or absence of this microorganism and give an estimation of the number of *E. coli* in the sample in colony-forming units.

As the standard method, MICA Advance *E. coli* does not allow the detection of the serotype O157:H7 (detection as coliform bacteria, false-negative result for *E. coli*) and gave false positive result for beta-glucuronidase positive microorganisms other than *E. coli* such as some *Shigella sonnei* strains.

II. Principle and procedure

a. Principle

The principle of MICA Advance *E. coli* solution procedure is based on the standard method ISO 9308-1 and uses membrane filtration method. The advantages are that MICA Advance *E. coli* has a shorter incubation time which include a revelation step and the enumeration is done automatically.

b. Procedure

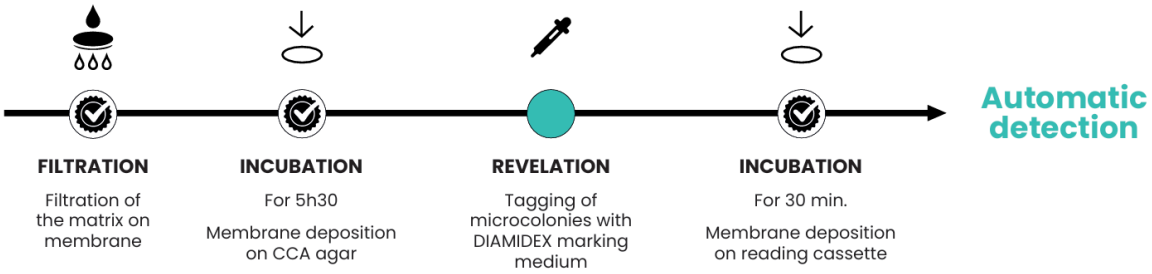


Figure 1: MICA Advance *E. coli* drinking water process based on membrane filtration

c. Comparison with standard method

ISO 9308-1:2014 is a standard method used for enumeration of *E. coli* for waters with low bacterial numbers like drinking water. This method is particularly accurate and suitable for microbial analyses in drinking waters such as bottled waters and mains waters.

The culture media used in this standard method and addressed by MICA solution is chromogenic coliform agar (CCA). For the standard method, incubation conditions are 37°C compared to 40°C with MICA Advance method. ISO 9308-1 allows detection of *E. coli* and also coliforms whereas MICA Advance method is specific for *E. coli*. We used higher incubation temperature which increases the specificity of our method. The time to result is 21±3h for standard method compared to 6h for MICA Advance method.

	ISO 9308-1:2014	MICA Advance *E. coli*
Target samples	Drinking waters (bottled and mains water)	
Target microorganisms	*E. coli* and other coliforms	Specific to *E. coli*
Temperature	37°C	40°C
Incubation	21h	6h

Figure 2 : ISO 9308-1:2014 VS MICA Advance *E. coli*

III. Performances

The results shown below were acquired using 3 references of 0.45µm sterile MCE membranes (non-gridded Sartorius 11306-47-ACN, non-gridded Whatmann 10401670, gridded Merck Millipore HAWG047S6) and 3 references of chromogenic coliform agar (Condalab 2080.0, Oxoid CM1205B, ChromAgar EF342).

1. Inclusivity/exclusivity

a. Selected strains

For inclusivity and exclusivity, we tested strains representative of those found in drinking water samples:

- For inclusivity, 27 *E. coli* strains were tested: 3 *E. coli* strains from the ATCC collection specific for water testing were selected. To complete the data set, 24 environmental strains isolated from real matrices from customers or prospects around the world were included. The bacterial load was 30-50 bacteria/membrane.
- For exclusivity, 29 collection strains were tested (16 coliforms and 13 contaminants). The species selected in the exclusivity data set are well documented and representative of the strains in drinking water samples [(1,2)]. The bacterial load was 150 bacteria/membrane and 10^3 bacteria/membrane for coliforms and contaminants respectively.

Collection strains come from Leibniz Institute in Germany (DSMZ), the Biological Resources Center of the Pasteur Institute in France (CIP) and American Type Culture Collection (ATCC).

For inclusivity and exclusivity testing, all strains were diluted in 0.9% sterile NaCl, filtered and analyzed with MICA Advance *E. coli* method developed by Diamidex and the results were compared to those obtained with the standards. The strains were stored in 20% glycerol stocks prepared from stationary phase cultures to imitate stressful environmental conditions.

b. Inclusivity

	Number of strains tested	Origin	MICA *E. coli* 6h	ISO 9308-1:2014 21h
E. coli	27	***E. coli*** ATCC 11775 ***E. coli*** ATCC 8739 ***E. coli*** ATCC 25922 ***E. coli*** environmental strains (x24)	100% positive	100% Positive

Conclusion: MICA Advance *E. coli* detects 100% of the *E. coli* strains tested in 6 hours as the standard method.

c. Exclusivity

	Number of strains tested	Origin	MICA *E. coli* 6h	ISO 9308-1:2014 21h
Contaminants	13	***Acinetobacter baumannii*** ATCC 19606 ***Acinetobacter calcoaceticus lwoffi*** CIP 70.17 ***Acinetobacter haemolyticus*** CIP 64.3T ***Acinetobacter radioresistens*** CIP 64.3T ***Aeromonas allosaccharophila*** DSM 11576 ***Aeromonas hydrophila*** CIP 103697 ***Proteus mirabilis*** ATCC 29906 ***Providencia rettgeri*** ATCC 29944 ***Providencia rettgeri*** CIP 103182T ***Providencia stuartii*** ATCC 29914 ***Pseudomonas aeruginosa*** ATCC 9027 ***Salmonella enteritidis*** CIP 82.97 ***Salmonella typhimurium*** CIP 58.58	Negative	Negative
Coliforms	16	***Citrobacter freundii*** ATCC 8090 ***Citrobacter koseri*** CIP 82.87T ***Citrobacter youngae*** ATCC 29935 ***Enterobacter aerogenes*** ATCC 35028 ***Hafnia alvei*** B8.66 ***Klebsiella oxytoca*** ATCC 13182 ***Klebsiella pneumoniae*** ATCC 13883 ***Lelliottia amnigena*** B3.48 ***Moellerella wisconsensis*** DSM 5076 ***Pantoea agglomerans*** CIP 57.51T ***Raoultella planticola*** CIP 100751T ***Serratia marcescens*** CIP 103235T	Negative	Negative
		Cronobacter sakazakii ATCC 29544 **Klebsiella variicola** ATCC 31488 **Kluyvera sp.** DSM 30112 **Leclercia sp.** DSM 22903	Positive*	

Conclusion : MICA Advance *E. coli* has a specificity of 86% for collection strains.

*MICA Advance *E. coli* method identified four strains as false positives due to non-specific reaction and rapid growth. Other enzymatic methods, such as Colilert-18, can produce false-positive results due to other coliform strains like *Klebsiella oxytoca*[3].

2. Limit of detection

a. LOD determination method

The relative detection level using the 50% cut-off point known as LOD_{50} was used to estimate the limit of detection. This calculation is performed using the Spearman-Kärber method (calculated using formula 3.3 S_0 + X_0 of the first level with 50% positive results). LOD_{50} can also be interpreted as the level of contamination that would theoretically correspond to a recovery rate of 50%.

b. MICA Advance *E. coli* LOD

To estimate the LOD_{50}, an *E. coli* environmental strains collected from The General Association of Analysis and Testing Laboratories (AGLAE, France) was spiked in tap water with 4 different contamination levels. For each level, 6 membranes were analyzed. The LOD_{50}

Spiking level	Analyzed samples	MICA *E. coli* positive results	Standard deviation (S_0)	Biais (X_0)
0.25 CFU/ 100mL	6	0	0	0
0.5 CFU/ 100mL	6	3	0.548	0.5
0.75 CFU/ 100mL	6	4	0.516	1
2.2 CFU/ 100mL	6	6	1.52	2.5

Using the Spearman-Kärber method, **the relative level of detection LOD_{50} of the MICA Advance *E. coli* method is 2,3 CFU/ 100 mL.**

c. LOD performance comparison with other methods

The LOD_{50} of the following solution was calculated using the same method as for MICA Advance *E. coli* :

Solution	**LOD_{50} (CFU/100mL)**	**Reference**
Compass cc Agar	3.48	Afnor validation Certificate BKR 23/08-06-12 by microsept[4]
Rapid'E. coli 2	6	Afnor validation Certificate BRD 07/20-03-11 by upscience[5]
Colilert-18	1.7	Afnor validation Certificate 33/01-11-09 by microsept[6]

MICA Advance *E. coli* **LOD performance is better or equivalent of other solutions** for *E. coli* detection in drinking waters.

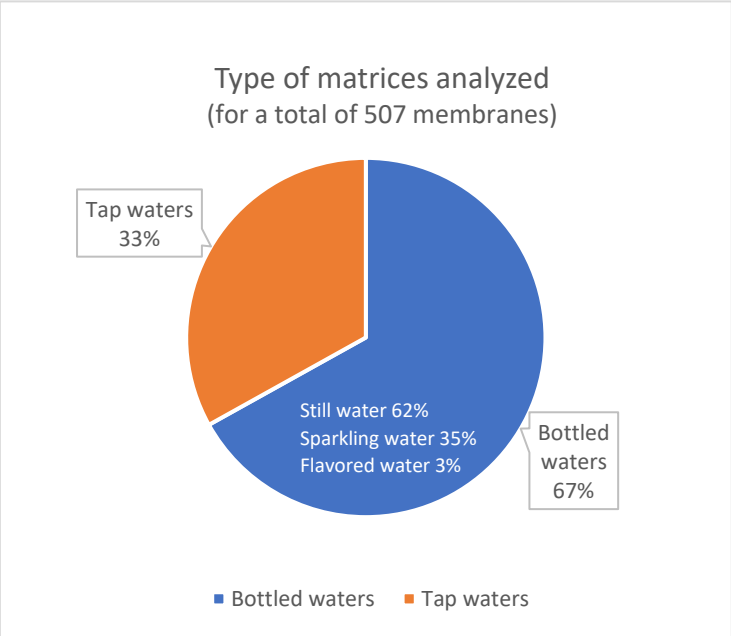
3. Real matrices

We tested real matrices are from bottled waters and mains waters.

As naturally contaminated real matrices are difficult to obtain, we artificially contaminated drinking water with surface water to obtain positive samples of real matrices.

250 mL and 100 mL of sample were analyzed for bottled waters and mains waters respectively.

a. Listing of tested matrices



For bottled waters, 24 different brands were tested (15 of still water, 9 of sparkling waters including one flavored sparkling water). Waters came from Europe (France, Norway, Italy, …) and around the world (Fiji Islands, Iceland, ..).

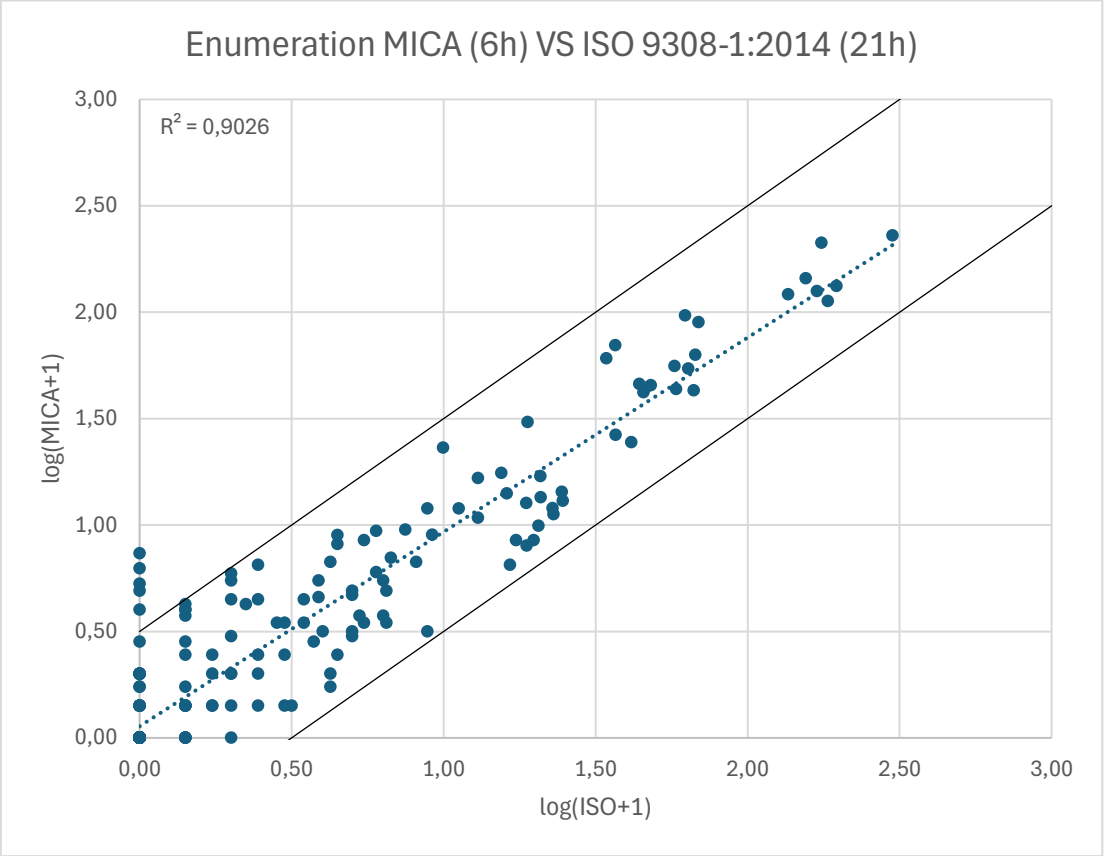
Performances	
Sensitivity	95%
Specificity	86%*
Positive predictive value	83%
Negative predictive value	96%

* Enzymatic method Colilert-18 shows a reported specificity of 72–98% for water samples[7-8-9] depending on studies.

b. Enumeration results for real matrices (non-contaminated and contaminated)

An acceptability threshold with a criterion of ± 0.5 log deviation was used in the validation of the microbiological method to establish equivalence with the various standards addressed. This criterion is found in ISO 16140-2:2016 – Validation of alternative methods establishing the requirements for comparing an alternative method with a reference method. It indicates that the acceptable difference between the two methods is generally ± 0.5 log (i.e. a factor of 3 in number of microorganisms). This

acceptability threshold is also present in the standard NF EN ISO 13843:2017 – Validation of microbiological methods for water.



Conclusion: Enumeration results for MICA Advance *E. coli* solution are comparable to those obtained with standard method.

Annex I : bibliographic references

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