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# COMPARISON OF RAPID METHODS FOR ACTIVE BATHING WATER QUALITY MONITORING

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## 1 INTRODUCTION

In France, recreational water quality is at the junction of three categories of issues: medical, environmental, and economic. In the seventies, the European policy on water was established to limit the pollution of bathing sites in order to protect bathers from potential health risks.<sup>1</sup> This Directive considers the obligation for member states to follow the water quality of identified or designated bathing sites. A new European Directive for the management of bathing water quality was published in 2006.<sup>2</sup> It brings a legal framework to these problems and increases the responsibility of coastal towns in the monitoring of the sanitary risk of their bathing sites. This Directive also introduces the concept of bathing water active management and suggests the implementation of the following technical actions: (i) assess contamination vulnerability of the bathing area; and (ii) perform regular analyses of water for both indicators of faecal contamination i.e. *Escherichia coli* (*E. coli*) and intestinal enterococci.

The species *E. coli*, which belongs to the group of faecal coliforms, is found at high concentrations in the faeces of mammals, and is generally considered as not growing and reproducing in the aquatic environment. *E. coli* is recognized by US health departments as one of the best indicators of faecal pollution and the presence of potential pathogenic microorganisms.<sup>3,4</sup>

The detection of *E. coli* is commonly used in France as the current standard instead of faecal coliforms.<sup>2</sup> *E. coli* shows a specific enzymatic activity which is useful for its identification and enumeration.<sup>5,6,7</sup> Thus, approximately 97% of *E. coli* present will express the  $\beta$ -D-glucuronidase enzyme, in contrast to the majority of other coliform bacteria.<sup>8</sup> In Europe, the reference method usually used for *E. coli* enumeration in bathing waters is the "miniaturized method by inoculation in liquid medium". It is based on glucuronidase enzyme detection and is described by a standard procedure for surface water and waste water.<sup>9</sup> During the incubation (from 36 to 72 hours at 44°C), the target bacteria in the sample multiply and hydrolyze the substrate with a release of a fluorescent blue compound. This miniaturized method estimates the quantity of *E. coli* by the Most Probable Number (MPN) approach. Consequently, this method can only detect the viable and culturable

bacteria (VC) and cannot detect the viable but not cultivable cells (VBNC) or the dead cells.<sup>10</sup> It may also detect non *E. coli* bacteria which express  $\beta$ -D-glucuronidase.<sup>11,12,13</sup> The time needed to obtain a response (36-72h) with this method is a major disadvantage within the 2006 Directive cited above which requires real time detection. Indeed, Noble and Weisberg defined a “rapid” method as one which provides a result in “less than four hours”.<sup>14</sup> Thus, several rapid assays have been developed for enumerating *E. coli* such as molecular, impedance and indicator enzyme based methods.

Molecular-based methods include immunological methods, polymerase chain reaction (PCR) and *in-situ* hybridization (ISH) techniques.<sup>15,16,17</sup> These techniques are not easily applicable for the routine monitoring of bathing water quality due to their complex implementation, the need of an equipped laboratory with an experienced technician and cost. Moreover, ISH without a direct viable count step cannot discriminate between VC and VBNC cells.

Culture coupled with impedance or indicator enzyme-based microbiological methods are commonly used to detect *E. coli* in live shellfish or drinking water.<sup>18,19,20</sup> BioRad produces the XpIOrer technology based on an impedance measurement.<sup>21</sup> ENDETEC provides a technology based on an indicator enzyme measurement.<sup>19</sup> Each ENDETEC cartridge contains pre-measured amounts of growth media that support the enrichment of any target bacteria that are present in the sample. As target bacteria such as *E. coli* or total coliforms begin to multiply, they emit a specific enzyme that interacts with a proprietary chemical substrate in the cartridge, releasing fluorescent molecules. These fluorescent molecules rapidly move from the water matrix into a polymer optical sensor located within the cartridge, enabling automated detection by the instrument.<sup>22</sup> These two technologies are a possible alternative to the MPN method, with both using analysis of growth kinetics to estimate numbers of bacteria. These systems present disadvantages such as: (i) quite long delay to obtain a result (i.e. ~6 h when 2,000 *E. coli* are present in 100 mL); and (ii) high equipment cost.

Some recent studies showed that enzymatic based methods could be used to estimate rapidly *E. coli* contamination by measuring  $\beta$ -D-glucuronidase activity without any cultivation step.<sup>6,23,24,25,26</sup> Good correlations in log-log plots of  $\beta$ -D-glucuronidase activity versus *E. coli* numbers enumerated by culture-based methods were generally found for natural water samples. Based on this work, we developed the Coliplage® method which allows the estimation of *E. coli* contamination in bathing water in less than one hour with a detection limit of 100 bacteria per 100 mL.<sup>6</sup> The method is also inexpensive, and the equipment needed is limited to a spectrofluorimeter. Because of its simplicity, this method is accessible to all technicians and can be easily implemented in a laboratory close to the bathing area for active management.<sup>6,27</sup>

Since 2006, the Coliplage® method has received great interest for real time management of recreational water. Nevertheless, for some identified bathing water types, discrepancies were observed between the standard and Coliplage® methods.<sup>28,29,12,24,25</sup> Therefore, we evaluated alternative methods, including culture with impedance or enzymatic based detection and molecular (PCR) based methods, and their potential performance in bathing water management strategy. The aims of this chapter are: (i) to demonstrate that rapid enzymatic method such as Coliplage® are, in many cases in France, a good tool for active management of faecal contamination, complementary with the reference method (ISO 9308-3); and (ii) to propose, in cases where there are discrepancies, alternative methods to provide a complete bathing water quality assessment strategy.

## 2 MATERIALS AND METHODS

### 2.1 Annual bathing water quality monitoring

**2.1.1 Sample collection.** Samples used in this study were collected in rivers, the English Channel, the Atlantic Ocean and the Mediterranean Sea. Thus 3,431 recreational water samples were collected since 2006 from sites located on the French coastline and from other surface waters (lakes, rivers). All samples were collected in sterile plastic 0.5 L bottles, stored at 4°C, and analyzed within 2h. This sampling procedure is in accordance with the French health organization recommendations.

**2.1.2 MPN miniaturized method (reference method)** A standardized miniaturized MPN method (ISO 9308-3) using microplate (AES, FR) was used for the enumeration of *E. coli*. In this method, based on the defined substrate approach, 18 mL of sample was added to 18 mL of distilled water or saline buffer (depending on the reported salinity of the sample) to make the first dilution (1/2). A second dilution (1/20) was performed by adding 2 mL of the first dilution to 18 mL of saline buffer. Then 200 µl aliquots of different dilutions (1/2, 1/20) were added to microplate wells containing the substrate, 4-methylumbelliferyl-β-D-Glucuronide (MUG) such that 32 wells contained the 1/20 dilution and 64 wells the 1/2 dilution. The microplate was incubated for 36 to 72 h at 44 °C. The substrate MUG is hydrolyzed by β-D-glucuronidase, releasing the fluorescent compound 4-methylumbelliferone (MUF) that is detectable under ultraviolet light (e.g. 365 nm). *E. coli* abundance was based on the number of positive wells and a statistical analysis using Poisson's law (Most Probable number (MPN) per 100mL). Only the MPN result was considered for this study (without confidence limits). The detection limit of this method is 15 cultivable *E. coli* per 100 mL. The lower and upper limits for each MPN result were obtained from the statistical table provided by the manufacturer.

**2.1.3 Coliplage® method.** β-D-glucuronidase (GLUase) activity measurements were performed following the protocol proposed by Lebaron et al.,<sup>6</sup> but slightly modified. If turbidity was more than 30 NTU, a prefiltration step was performed using a Nalgene system. Water samples (100 mL) were first filtered through a 47 mm diameter, 20 µm pore-size nylon membrane (Millipore, USA) and then a 10 µm pore-size polycarbonate membrane (Millipore, USA). After that, water samples were filtered through a 47 mm diameter, 0.2 µm pore-size polycarbonate membrane (Millipore, USA). The membranes were placed in 100 ml sterile Erlenmeyer flasks containing 10 ml of sterile phosphate buffer (pH 6.9). The flasks were incubated in a water bath at 44 °C. Next, 2 ml of MUG stock solution (55 mg of MUG (Sigma, USA) and 20 µl of Triton X-100 (Sigma, USA) in 50 ml of sterile water) were added to each flask (final MUG concentration of 0.6 mmol L<sup>-1</sup>). Every 5 min for 20 min, a 2 ml aliquot was removed and added to a quartz cell and pH adjusted up to 10 by adding 40 µl of a 2N NaOH solution, (this pH corresponds to the fluorescence maximum for MUF).<sup>10</sup> Fluorescence intensities were measured with a fluorescence spectrophotometer (RF 1501, Shimadzu, JP) with an excitation wavelength of 362 nm and an emission wavelength of 445 nm. The spectrofluorimeter was calibrated using a set of MUF (SIGMA, USA) standard solutions from 0 to 500 nM. The production rate of MUF (picomoles of MUF liberated per minute for 100 mL of filtered sample), defined as the GLUase activity, was determined by least-squares linear regression when plotting MUF concentration versus incubation time. The GLUase activities were converted into *E. coli* concentrations by using a straight-line regression from a log-log plot of *E. coli*

concentrations estimated by the MPN method versus GLUase activity. Only the estimated value was considered for this study (without confidence rate). A specific straight-line regression was established for each bathing site. An example for marine water samples is presented by Lebaron et al.<sup>6</sup>

**2.1.4 The concordance rate evaluation.** Currently in France, the Mandatory Value (MV) for managing pollution is 2,000 *E. coli* per 100 mL, but ANSES (Agence Nationale de Sécurité Sanitaire de l'alimentation, de l'environnement et du travail) recommended two thresholds for better consistency between active management of faecal pollution and occurrence of illness: marine water = 1,000 *E. coli* /100 mL and inland water = 1800 *E. coli* /100 mL.<sup>30</sup> Coliplage® is a quality assessment tool, considered as a semi-quantitative method which allows an early evaluation of *E. coli* pollution in water. Coliplage® results must be interpreted as a signal compared to a defined threshold. Thus, results were expressed as an alert signal (red or green) compared to the MV and the ANSES thresholds. Conclusions can be assigned to three cases:

- Concordance case : Coliplage® signal and reference method result were the same compared to the defined threshold, pollution cases (both > MV or ANSES thresholds ) and non pollution cases (both < MV or ANSES thresholds))
- Overestimation case : Coliplage® signal > MV or ANSES thresholds > reference method result
- Underestimation cases : Coliplage® signal < MV or ANSES thresholds < reference method result

## 2.2 Evaluation of alternative methods

**2.2.1 Sample collection.** All samples (marine water) were collected in sterile plastic 0.5 L bottles, stored at 4°C, and analyzed within 2h. The sampling procedure is in accordance with the French health organization recommendations.

**2.2.2 Impedance-based method using BioRad system.** The protocol used was provided by the manufacturer BioRad (FR). Each sample (100 mL) was filtered through a 47 mm diameter, 0.45 µm pore-size membrane (Millipore, USA). The membrane was washed with 50 mL of sterile distilled water, then placed in the *E. coli* specific growth media cell (CheckN'Safe™). The cell containing the membrane was placed into the XplOrer64™ apparatus (44°C incubator and reader). The sample was incubated over 9 h (including 1 h preheating). The readings were automatic every 10 minutes after the 1 h preheating. The results were estimated directly by the software (for marine water), and reported as *E. coli* per 100 mL. The analyses were performed on 77 marine water samples collected in 2010.

**2.2.3 Enzymatic-based method using ENDETEC system.** The protocol used for *E. coli* was provided by the manufacturer ENDETEC (CA) and slightly modified. Each sample (100 mL) was filtered through a 47 mm diameter, 0.45 µm pore-size membrane (Millipore, USA). The membranes were washed with 50 mL of sterile distilled water and placed in the specific *E. coli* growth media cartridge. The cell containing the membrane was placed into the apparatus (41.5°C incubator and reader). The sample was incubated for up to 18 h (including 20 min of preheating). The ENDETEC system continuously monitors a fluorescence signal from the incubating cartridge and reports bacteria as “present” in the sample when the signal crosses a pre-defined threshold. The time when the sample becomes positive is the Time To Detect (TTD). If a sample is not positive when 18 hr is

reached, the sample is determined to be “absent” ( $< 1$  VC per 100 mL). The equivalent bacteria concentration (per 100 mL) was automatically calculated using the TTD value and a specific marine water calibration (provided by ENDETEC research department). The analyses were performed on 97 marine water samples collected in 2009 and 2010.

**2.2.4 Molecular-based method (qPCR).** The protocol used was provided by the manufacturer GeneSystems (FR). Analyses were performed on the GeneSystems apparatus GenExtract and GeneCycler (Recreational GeneDisc). Each sample (250 mL) was set for 30 min at room temperature or 20 min at 70°C. The sample was filtered through a 47 mm diameter, 0.4  $\mu$ m pore-size polycarbonate membrane. Bacteria were lysed by adding the manufacturer’s lysis buffer and DNA extraction and purification was performed by using the GenExtract. DNA was added to the Recreational GeneDisc, and amplification was performed with the GeneCycler. Data was analysed automatically by the software using a specific GeneSystems algorithm, and reported as the Genomic Unit (GU) per volume analysed. As proposed by GeneSystems, UG can be converted for bacteria estimation (per volume analysed) by the relation: 1 GU = 1 bacterium. The analyses were performed on 48 marine water samples collected in 2009.

### 3 RESULTS

#### 3.1 Comparison between the reference method and the Coliplage® method: a follow up for 5 years

Since 2006, more than 70 beaches have been monitored each summer. The bathing areas were located in the English Channel, the Atlantic Ocean, the Mediterranean Sea and various lakes and rivers in France. Different site classifications could be identified such as sandy beaches, proximity to a marina, proximity to a waste water discharge, etc. More than 12,000 Coliplage® results were collected in France including 3,428 samples analyzed with both Coliplage® and the reference method (MPN).

The results presented in Table 1 compared the two methods according to the 1976 council Directive MV and the two ANSES thresholds. Since 2006, the number of samples analysed with the two methods has been increased.

For the MV of 2,000 bacteria per 100 mL (A), the concordance rate remained stable each year, with an average concordance percentage around 92.95% for 5 years. The percentage of pollution cases detected by the both methods had a tendency to decrease since 2006 from 8.11% to 2.80%. Correspondingly, the percentage of non pollution cases slightly increased since 2006. Concerning the non-concordance cases, we can classify them into 2 categories: Coliplage® > MV > MPN, which represents overestimation of *E. coli* contamination by Coliplage® and Coliplage® < MV < MPN, which is underestimation. For the first category, the percentage of overestimation has slightly increased since 2006. Conversely, the second category has had a tendency to decrease.

Concerning the ANSES thresholds of 1,000 or 1800 bacteria per 100 mL (B), the concordance rate is close to 88% for the 5 years, and we observed a slight decrease of around 5% compared with the percentage obtained with MV at 2,000 bacteria per 100 mL. The rates of overestimation of *E. coli* levels by Coliplage® compared to reference method is still higher than underestimation whatever the threshold considered.

**Table 1** Concordance rates between the Coliplate® and reference (MPN) methods compared table A) the Council Directive MV = 2,000 bacteria per 100 mL and B) the ANSES thresholds = 1,000 or 1800 bacteria per 100 mL. n = 3,428.

A

Year	Numbers of analyses per year	Concordance		Non concordance	
		Pollution cases	Non pollution cases	Coliplate > MV > MPN	Coliplate < MV < MPN
2006	481	8,11%	83,99%	4,57%	3,33%
2007	380	6,58%	85,00%	5,26%	3,16%
2008	849	4,95%	87,99%	5,65%	1,41%
2009	749	2,80%	89,32%	6,28%	1,60%
2010	972	2,88%	91,67%	4,01%	1,44%
	<b>Weighted mean</b>	<b>4,52%</b>	<b>88,43%</b>	<b>5,13%</b>	<b>1,92%</b>
	<i>Confidence</i>	+/- 2,7%	+/- 3,7%	+/- 1,2%	+/- 1,1%
<b>TOTAL</b>		<b>92,95%</b>		<b>7,05%</b>	

B

Year	Numbers of analyses per year	Concordance		Non concordance	
		Pollution cases	Non pollution cases	Coliplate > threshold > MPN	Coliplate < threshold < MPN
2006	478	10,67%	74,27%	8,16%	6,90%
2007	380	11,58%	75,00%	6,05%	7,37%
2008	849	8,36%	81,27%	8,24%	2,12%
2009	749	4,14%	84,51%	8,81%	2,54%
2010	972	3,70%	84,57%	8,64%	3,09%
	<b>Weighted mean</b>	<b>6,80%</b>	<b>81,24%</b>	<b>8,23%</b>	<b>3,73%</b>
	<i>Confidence interval</i>	+/- 4,3	+/- 5,7	+/- 1,1	+/- 2,8
<b>TOTAL</b>		<b>88,04%</b>		<b>11,96%</b>	

### 3.2 Summary of alternative methods

The comparison of advantages and disadvantages of each selected method is presented in Table 2.

Some criteria were more important than others to classify methods of interest for active management, such as specificity (viability) and time to get a result. The position of each method regarding these two criteria is illustrated in Figure 1.

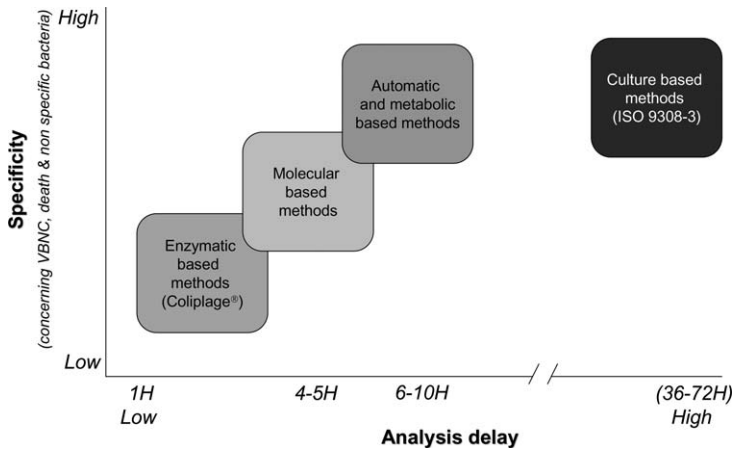
**Table 2** *E. coli* detection methods tested in this study.

	Culture based method	Enzymatic method (no culture)	PCR method (no culture)	Automatic culture and metabolic based method	
	ISO 9308-3	Coliplate®	Genesystems	ENDETEC	Biorad
Steps	- Sample dilution - Microplate loading - Incubation - Manual reading	- Filtration - 30 min for reading	- Filtration - Extraction & purification - Disc loading - Automatic reading	- Filtration - Incubation - Automatic reading	- Filtration - Incubation - Automatic reading
Delay (Analysis time)	36-72H	1 hour	4 hours	4-18 hours depending on bacteria concentration	4-10 hours depending on bacteria concentration
Specificity concerning					
Dead bacteria	Not Detected	Not Detected	Detected	Not Detected	Not Detected
ANC bacteria	Not Detected	Detected	Detected	Not Detected	Not Detected
Non specific bacteria	Not Detected	Few marine <i>Vibrio</i>	Not Detected	Not Detected	Not Detected
Sensibility	15 bacteria /100mL	100 bacteria /100mL	75 GU /100mL	1 bacterium /100mL	41 bacteria /100mL
Implementation	local lab	local lab	central lab	local lab	local lab
operator background	non experienced staff	non experienced staff	experienced staff	non experienced staff	non experienced staff
Cost					
appatus	3-5k€	10-15k€	30-40k€	11-15k€	50-70k€
consumables	10-15€	10-15€	10-20€	7-11€	10-15€
Number of samples per analysis	1	6	4	16	64
Limitations of use	Not identified	Presence of UV desinfected wastewater	Presence of UV desinfected wastewater turbid water	Not identified	Not identified

As mentioned in Table 2 and illustrated in Figure 1, concerning the time to result, the methods can be classified from most to least rapid as follows: Coliplate® > molecular based method (PCR) > automatic culture and metabolic based methods > culture based method (reference method).

Considering the viability of bacteria, culture and metabolic based methods appear to be the more adapted to detect only viable and cultivable bacteria, in agreement with the reference method. However, as reported in Table 2, the time to get a result is longer (4 to 10-18h) than specified in the 2006 Council Directive requirements (for active management). To summarize, the more the method is rapid the less it tends to be specific.





**Figure 1** Schematic representation of efficiency of each method for bathing water management.

### 3.3 *In situ* evaluation of alternative methods

The results of the three evaluation campaigns of *E. coli* alternative methods in bathing sites (marine water) are presented in Table 3.

**Table 3** Comparison of concordance rates (%) for different MV ( $A = 2,000$  and  $B = 1,000$  *E. coli*/ 100 mL), between the reference method (ISO 9308-3) and alternative methods during 3 different campaigns: Coliplage®/GeneSystems; Coliplage®/Impedance and Coliplage®/indicator enzyme (ENDETEC system).

A		Conclusions cases		
		Concordance	Overestimation	Underestimation
n=48	Coliplage®	68,75%	25,00%	6,25%
	PCR	62,50%	31,25%	6,25%
n=77	Coliplage®	81,82%	12,99%	5,19%
	Impedance	94,81%	2,60%	2,60%
n=97	Coliplage®	72,00%	23,00%	5,00%
	indicator enzyme	89,00%	6,00%	5,00%

B		Conclusions cases		
		Concordance	Overestimation	Underestimation
n = 48	Coliplage®	50,00%	43,75%	6,25%
	PCR	60,42%	29,17%	10,42%
n=77	Coliplage®	80,52%	14,29%	5,19%
	Impedance	97,40%	1,30%	1,30%
n=97	Coliplage®	58,00%	34,00%	8,00%
	indicator enzyme	90,00%	6,00%	4,00%

Whatever the analysis campaign or the mandatory value, we observed in Table 3 that Coliplage® presents some discrepancies (respectively 18.18% to 31.25% and 19.48% to 50% for the MV at 2,000 and the ANSES threshold at 1,000 *E. coli* / 100mL) compared to the reference method, especially over-estimation cases. This confirms the previous conclusions that application of Coliplage® is limited in certain bathing sites, and thus we can conclude that the samples used in this study are relevant for evaluating the efficiency of alternative methods in comparison to Coliplage®. Note that samples used in the impedance (BioRad) campaign have presented fewer discrepancies compared to samples used for the two other campaigns (qPCR and indicator enzyme (ENDETEC system)), so conclusions concerning the efficiency of this method could be affected.

For the MV of 2,000 *E. coli* per 100 mL (3A), the indicator enzyme based on the ENDETEC technology and impedance method, which were expected to have greater specificity, gave a higher concordance rate with the reference method compared to Coliplage®: 89% (+17%) for the indicator enzyme and up to 94% (+13%) for the impedance method. The PCR method was less effective, with a decrease of concordance to 62.5% (-6.3%) compared to the two previous methods and Coliplage®. In conclusion, the indicator enzyme and impedance methods seem to be the more accurate methods for this threshold.

For the ANSES threshold at 1,000 bacteria per 100 mL (3B), the indicator enzyme and impedance methods also gave a higher concordance rate with the reference method compared to Coliplage®: 90% (+32%) for the indicator enzyme and up to 97% (+17%) for the impedance method. The PCR method was less effective compared to the two previous methods, with a concordance of 60.4% (+10.4%). The most accurate method (for the ANSES threshold at 1,000 bacteria per 100 mL) seems to be the indicator enzyme method,

As presented in Tables 1 and 3, whatever the threshold (MV or ANSES) and method, concordance with the reference method was not perfect, and differences between results will always be present (over or under estimation). These results show that each method presents limitation, especially concerning specificity (VBNC, dead or non specific bacteria).

#### 4 DISCUSSION

In this article, we presented a Coliplage® data compilation collected over five years from more than 100 different bathing sites. We compared the reference MPN method used to detect *E. coli* in France with the Coliplage® method to assess the use of this rapid enzymatic method in the daily active management of recreational water.

Coliplage® has confirmed over 5 years that this enzymatic based method has a great potential to monitor faecal pollution in bathing water due to its rapidity (1 hour), simplicity and cost compared to the conventional method or other alternative methods. In more than 92% of measurements (Table 1), this method provided great concordance with the reference method, and even when the new ANSES threshold is considered, the percentage is close to 87%. Thus the Coliplage® method offers a useful tool for an active faecal pollution management by local authorities and to rapidly monitor the microbiological bathing water quality, as a complement to the reference method. However, we observed a slight number of samples (near 7%) which presented different conclusions between Coliplage® and the reference method. These discrepancies were described previously in the literature.<sup>12</sup> We can underline three major reasons to explain these slight differences.

For the cases of underestimation by Coliplage®, there is the potential presence of *E. coli* which does not express  $\beta$ -D-glucuronidase in natural water,<sup>31,32,25,33</sup> whereas in culture

conditions like the MPN method these bacteria will have enzymatic activity induced by the medium. We performed assays with high concentrations of *E. coli* from pure culture in BHI medium ( $10^6$  bacteria per mL) in Coliplate® and the reference method (data not shown), and no GLUase activity was measured by Coliplate®, while quantification with the reference method was observed. More experiments need to be performed to understand if this is caused by non-detection of the glucuronidase activity, or reduced induction of the enzyme in the target cells.

For the cases of over-estimation, the non-concordance can be attributed to the presence of VBNC cells and non specific cells. The VBNC state is defined as cells that will not grow on a favourable culture medium but keep some metabolic activity. This kind of state can be induced by stress from environmental parameters such temperature, salinity, UV, O<sub>2</sub>, predation, competition with other bacteria, and lack of nutrients in the environment.<sup>34,35,36</sup> These forms of stress are found in the natural environment, and in order to estimate more accurately the sanitary risk associated with bathing water, it is important to consider the presence of VBNC.<sup>37</sup> Indeed, the presence of VBNC cells attests to the presence of faecal pollution with a risk of associated pathogens (such as parasites and viruses) that is impossible to detect with culture-based methods including the reference method.<sup>7,38</sup> Coliplate® appears to be the most rapid and pertinent method which can detect VBNC. The over-estimation of GLUase activity was also previously described by many authors, suggesting that other members of *Enterobacteriaceae* including some *Shigella* and *Salmonella* stains, a few *Yersinia*, *Citrobacter*, *Edwardia* and *Hafnia* strains, and other non *Enterobacteriaceae* strains (e.g. a few marine *Vibrio* species) could potentially contribute to this enzymatic activity.<sup>39,13,12</sup>

To remedy this lack of robustness, we tried to identify one or more alternative methods to allow us to maintain a daily follow-up of the bathing water quality on all the French bathing sites. We identified three complementary methods: the molecular (PCR) method, the impedance method and the indicator enzyme method. As presented in this paper, it seems that if we want to have a higher correlation with the reference method, we need to use culture and metabolic methods (impedance or indicator enzyme), with a recommendation for the ENDETEC indicator enzyme method which had better efficiency compared to the impedance-based method, especially for the ANSES threshold. In fact, interfering bacteria are inhibited by the culture step, which increases selectivity. As a consequence, however, time-to-result is longer and may not meet the 2006 council Directive expectations. PCR could be an alternative, but difficulties in implementation and specificity of results seem to be the two parameters limiting its use for bathing water quality management.

## 5 CONCLUSIONS

It appears that with the 2006 Council Directive that reference method (ISO 9308-3) is not applicable for active (real-time) faecal pollution management due to its time-to-result (2 days). Alternative methods currently available could be good complementary tools for active faecal pollution management. Coliplate® due to its rapidity (1 hour), low cost and easy implementation, is the most accurate method to determine the microbiological water quality, and to allow an active management of the bathing area as a complement to reference method. However, Coliplate® and the reference method show some differences in less than 10% of cases. Concerning Coliplate®, discrepancies appeared on particular bathing sites such as close to a port, a UV disinfection water treatment plant discharge, or a marine pond (tidal water change). Concerning other alternative methods: i) PCR is a rapid

method (4 hours) but it overestimates *E. coli* contamination especially in sites which present recurrent dead and VBNC *E. coli* cells which contain DNA, ii) Automatic culture and metabolic based methods seem to be the more efficient compared to ISO 9308-3, with a higher concordance correlation (to 90%) compared to Coliplage®, especially in sites with interfering bacterial population (*i.e.* VBNC, dead or non specific). For the majority of bathing sites, Coliplage® is the most efficient method. For the sites with problems in terms of VBNC, dead cells and non specific bacteria, automatic and metabolic based methods could be recommended for the specific detection of *E. coli*.

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