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# An operational method for the real-time monitoring of *E. coli* numbers in bathing waters

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**Abstract**

The aim of this study was to investigate the potential application of the β-D-glucuronidase (GLUase) activity measurement for the routine detection and quantification of *E. coli* in marine bathing waters. GLUase activity was measured as the rate of hydrolysis of 4-methylumbelliferyl-β-D-glucuronide. Culturable *E. coli* were quantified by the most probable number (MPN) microplate method. Both methods were applied to a large set of seawater samples. Significant correlation was found between the log of GLUase activity and the log of culturable *E. coli*. The mean coefficient of variation (CV) of the GLUase activity was less than 15% at concentrations around the current standards of International regulations whereas the CV of the microplate method was around 30%. When samples were stored at 4 °C and 20 °C, the mean CV of the GLUase activity remained below 15% up to 6 hours after sample collection whereas the range of variation of the microplate method varied between 10 and 50%. We concluded that the GLUase activity is an operational, reproducible, simple, very rapid and low cost method for the real-time enumeration of *E. coli* in bathing waters and should be preferred to the microplate method. The GLUase activity method should be routinely applied to the rapid enumeration of *E. coli* in recreational waters and recommendations for its application were suggested to water quality managers.
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## 1. Introduction

The quality of waters in coastal areas is a subject of special interest during the summer season. In Europe, more and more bathing beaches are classified at the end of the high season based on a large set of criteria. One of the most important criteria is the microbiological quality of waters. Although the classification of beaches into quality classes was and remains useful, it does not preserve users of recreational waters from the risk of bacterial or pathogen-induced disease. The protection of bathers from possible health risks requires fast, sensitive, simple and quantitative methods for the real-time monitoring of fecal pollution. Rapid methods are also essential to water quality managers to determine the source of pollution when it occurs and more generally, to the operational management of recreational waters.

Fecal coliforms (FC) are used universally as microbiological indicators of water quality and are commonly used to determine the quality of bathing waters. For instance, bathing beaches in the European community (EC) have a guideline compliance limit of 100 FC/100 ml and a maximum allowable concentration of 2000 FC 100 $ml^{-1}$ (European Community Council Directive, 1975). Although FC have traditionally been regarded as good indicators of fecal contamination of

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waters, recent reviews suggested *Escherichia coli* to be a better indicator (Edberg et al., 2000; Fewtrell and Bartram, 2001). The detection of *E. coli* is actually commonly used in France as a current standard instead of FC. Until future changes of the EC regulations, *E. coli* are enumerated in bathing waters in France and their levels checked against the guidelines for FC, although *E. coli* represent only 80 to 95% of the FC counts. The choice of *E. coli* as an indicator organism of fecal pollution is already accepted by various world organizations (APHA, 1995; WHO, 2001). The compliance and maximum allowable values fixed by the future EC regulations should be 250 and 500 *E. coli* 100 $ml^{-1}$, respectively. These values are more related to those actually recommended by the US Environmental Protection Agency (US EPA, 1999).

The current culture-based test method (m-TEC agar) or the most probable number (MPN) microplate method take about 24–48 h to provide a result on *E. coli* numbers. Although culture-based tests to enumerate *E. coli* were improved in terms of specificity and rapidity by incorporating chromogenic and fluorogenic substrates (Manafi, 2000), they still require 18–24 h to complete. Thus, there is always a minimum one day delay between sample collection and analytical results. The closure of beaches based upon the data of the sample collected the previous day is not appropriate for an efficient protection of bathers. It also causes delay in reopening the beaches, which is not in the public's best interest. The need for a faster method that provides real-time results of *E. coli* concentrations has been widely recognized among responsible health departments. The desirable testing method should meet the following criteria: it should be fast, sensitive, simple, quantitative, portable and be well correlated with the current methods.

Several rapid assays have been developed for enumerating *E. coli*. They include a polymerase chain reaction (PCR)-based method (Iqbal et al., 1997), a fluorescent in situ hybridization (FISH) with a specific fluorescent rDNA 16S probe (Regnault et al., 2000), an immunofluorescence method (Zaccone et al., 1995), the detection of β-D-glucuronidase (an enzyme specific of *E. coli*) positive cells by solid phase cytometry (Van Poucke and Nelis, 2000) and more recently, an ATP bioluminescence assay associated with immunomagnetic separation (IMS) (Lee and Deininger, 2004). Most of these methods are specific enough but can only be performed in a well-equipped laboratory and require well-trained staff. The bioluminescence assay associated with IMS is fast and the equipment is portable but the IMS apparatus is expensive and the recovery of cells is never 100%. The PCR-based assays without any culture step have limitations in the quantification and discrimination of viable micro-organisms present at low concentrations (below 5 cells per ml) in the natural environment and are not yet appropriate for routine application. The FISH approach, although it is the most specific method, is time-consuming and it is often difficult or impossible to count cells in surface waters at concentrations below $10^{3-4}$ in 100 ml by epifluorescence microscopy (Garcia Armisen and Servais, 2004). Similar limitations apply to the immunofluorescence assay.

Some studies have proposed to estimate the β-D-glucuronidase (GLUase) activity of *E. coli* in rapid assays performed without any cultivation step as a surrogate of *E. coli*. Good correlations in log-log plot were generally found in natural waters between GLUase activity and FC or *E. coli* levels (Fiksdal et al., 1994; George et al., 2000, 2001, 2004; Farnleitner et al., 2001, 2002; Caruso et al., 2002). In these studies, GLUase activity was estimated by fluorometry as the production of fluorescent methylumbelliferone (MUF) resulting from the hydrolysis of the substrate 4-methylumbelliferyl-β-D-glucuronide (MUGlu). The aim of this study was to evaluate if the GLUase activity measurement can be routinely used for the monitoring of *E. coli* concentrations in coastal seawater samples in regards to the present regulations and to compare this method and its reproducibility to the normalized microplate method actually used in France for the routine monitoring of recreational waters during the summer season. The effect of sample storage at 4 °C and 20 °C between collection and microbiological analysis was also investigated. Preliminary results of the first application of GLUase activity measurement to the monitoring of bathing waters are also presented.

## 2. Methods

### 2.1. Samples collection

A total of 256 seawater samples were collected from different beaches along the French Catalan Mediterranean coast. Two beaches were analyzed daily (working days) from 15th of June to 1st of July 2004. Additional samples were sometimes collected near the release of treated wastewater in order to get samples within a wide range of *E. coli* concentrations. All samples were collected in sterile 2 l bottles, kept at 4 °C and analyzed within 2 h. When appropriate, samples were stored in the dark at room temperature (20 °C ± 3 °C) for storage experiments (storage time up to 24 h).

### 2.2. E. coli enumeration

A standardized miniaturized MPN method (ISO 9308-3) using microplates (Bio-Rad) was used for the enumeration of *E. coli*. In this method, based on the defined substrate approach (Edberg and Edberg, 1988), 200 µl of the several dilutions (1/2, 1/20, ...) of

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