Use of rapid enzymatic assays to study the distribution of faecal coliforms in the Seine river (France)

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Abstract In 1997 and 1998 faecal contamination of the Seine river and its estuary was studied for the first time by rapid enzymatic methods, based on the presence of the β-D-glucuronidase enzyme in E. coli, in parallel with traditional plate counts of faecal coliforms on specific culture medium. Our study focused on a 450 km stretch of the river, including the Parisian area, and presenting highly variable levels of faecal pollution. Both methods showed that wastewater outfalls of the Parisian area and the presence of a maximum turbidity zone (at the mouth of the estuary) had a strong impact on the abundance of faecal coliforms in the river. Downstream from the Parisian outfalls, β-D-glucuronidase activity measurements decreased 5–6× less rapidly than plate counts suggesting that rapid enzymatic assays could detect enzymatically-active but non-culturable bacteria.

Keywords Enzymatic methods; β-D-glucuronidase; faecal coliforms; Seine river and estuary

Introduction
Faecal coliforms are one of the major groups of faecal indicators stated in the European standards regarding microbiological quality of surface waters (ECC, 1975). Conventional methods for assessment of microbiological quality are based on their enumeration in liquid or on solid culture media. These methods are time-consuming and are unable to immediately detect any faecal pollution because they require at least 18 h incubation. Moreover, the presence of active but non-culturable bacteria (Colwell et al., 1985; Grimes and Colwell, 1986) in natural waters, probably prevent plate count techniques from detecting all the indicator bacteria in such environments. In the 1990s, several rapid enzymatic tests were developed based on the presence of specific enzymes in coliforms (β-D-galactosidase and β-D-glucuronidase in total coliforms and E. coli respectively) without any cultivation step (Apte and Batley, 1994; Fiksdal et al., 1994; George et al., 2000). These rapid methods, which allow the detection of coliforms in 30 min to 1 h, were used for the first time in this study to assess the range of faecal contamination in the River Seine (NW France). They were applied in parallel with reference plate counts during several sampling campaigns carried out on the River Seine and its estuary in 1997 and 1998.

Material and methods
Site description and sampling
The Seine catchment in France is characterised by a high population density (>500/km² in the urban area of Paris), intensive industrial activity and agriculture. The average annual flow rate of the River Seine at the Poses Dam (200 km downstream of Paris) is 410 m³/sec with high flows in winter and low flows in summer. In the Paris area, the river receives: (a) two major tributaries (the Marne and Oise rivers); and (b) the effluents of the 10 million inhabitants of Paris and its suburbs (treated mainly in the Seine Amont and Seine Aval wastewater treatment plants, respectively 1×10⁶ and 7.5×10⁶ p.e. capacity) (Figure 1). Downstream from the Seine and Oise confluences, the Seine runs over 100 km without receiving any important tributary or effluents discharge until the Poses Dam which
constitutes an obstacle to tidal movements upstream. Downstream of Poses, in the estuary, the river receives the treated effluents of the Rouen WWTP ($4.5 \times 10^5$ p.e. capacity). At the mouth of the estuary, the combination of tidal currents and a gradient of salinity results in a zone of maximum turbidity. Four sampling campaigns were conducted in May 1997, July 1997, March 1998 and September 1998. Samples were collected at various stations between 100 km upstream from Paris to Honfleur or the Seine Bay (Figure 1). The Seine discharge at Poses Dam for the four campaigns was 230–440 m$^3$/sec. Water samples, taken in the river with a plastic bucket from bridges halfway to the banks (except in Rouen and downstream where they were collected from a boat at 1–1.5 m depth) were analysed within 4 h.

**Enumeration of culturable faecal coliforms (FC) by plate count**

FC were enumerated by membrane filtration (0.45 µm, 47 mm cellulose nitrate filters, Sartorius) and incubation at 44ºC (24 h) on lactose agar with Tergitol and TTC (AFN, 1994).

**β-D-glucuronidase (GLUase) assay**

The protocol was derived from the method proposed by Fiksdal *et al.* (1994) and its optimisation for freshwaters (George *et al.*, 2000). Briefly, bacterial cells were retained on a 0.2 µm filter after filtration of 100 mL sample and incubated at an optimised pH of 6.9 and temperature of 44ºC after addition of the fluorogenic substrate MuGlu (final concentration: 150 mg/L). The fluorescence intensity due to the substrate hydrolysis and release of the fluorescent product MUF was measured by spectrofluorometry at regular time intervals. The total production rate of MUF (pmol MUF/ min for 100 mL of sample filtered) was determined by least-squares linear regression when plotting MUF concentration versus incubation time. The autohydrolysis rate of the substrate in the same assay conditions, but in the absence of bacteria, was subtracted from the total production rate of MUF and the remaining production rate corresponded to the release of MUF by bacterial enzymatic activity.

![Figure 1](https://example.com/figure1.jpg)

**Figure 1** The River Seine from 100 km upstream of Paris to the estuary. (● Sample stations; ▼ WWTP outfalls; pK = kilometre unit used by the Service de la Navigation de la Seine, which is set to zero at "Pont d’Austerlitz" in downtown Paris and increases to the estuary.)
Results and discussion
Spatial distribution of coliforms in the Seine river
As culturable FC enumerations and GLUase activity measurements showed similar longitudinal profiles for the four sampling campaigns, only one of these (March 1998) is presented here (Figure 2) and discussed subsequently.

FC abundance and GLUase activity increased drastically when the River Seine flowed through Paris and its suburbs, particularly downstream of its two main WWTP outfalls (Figure 2). Indeed, the coliform abundance in the raw wastewater was so high (10^6–10^8 FC/100 mL) that, even if current treatments in Parisian WWTP efficiently reduce the microbiological pollution, treated outfalls obviously damage the microbiological quality of the receiving water. Downstream from the Seine Aval WWTP, a spectacular decrease of coliform abundance and GLUase activity was observed until the Poses Dam. In the estuary, FC concentrations and GLUase activity increased to a lesser extent when the water flowed through the Rouen agglomeration with a small increase downstream from the Rouen WWTP outfall. Culturable FC abundance and activity increased again in the estuarine maximum turbidity zone (MTZ) where high suspended matter concentrations protected bacteria from nutrient scarcity and solar bactericidal effect. In the Seine Bay, FC abundance and activity decreased abruptly due to the dilution of freshwater with seawater (Figure 2). Compared to the European Standards regarding microbiological quality of bathing waters (guideline 100 FC/100 mL; mandatory: 2,000 FC/100 mL), the River Seine from Paris to the estuary constituted a highly polluted system despite numerous efforts to purify urban outfalls from the dense populations living nearby.

Decrease of culturable FC and GLUase activity downstream from the Seine Aval WWTP outfall
At the Poses Dam, 150 km downstream from the Seine Aval WWTP effluent discharge, the culturable FC abundance had decreased 5.5× more than the GLUase activity (mean of the four campaigns). This could be explained by the progressive loss of culturability of coliforms when they are carried by the water downstream whilst conserving their enzymatic activity. This transition to an "active but non-culturable state" would be the result of a nutritional stress encountered by coliforms when they are discharged with the WWTP effluents into river waters less rich in organic matter. This hypothesis was tested by George et al. (2000) in laboratory experiments where cultured *E. coli* cells were discharged into sterile

![Figure 2](image.png)

**Figure 2**  Culturable FC abundance and glucuronidase (GLUase) activity in the Seine (March 1998)
(SAm/SAv WWTP = Seine Amont/Seine Aval wastewater treatment plant. MTZ = maximum turbidity zone)
river water. They showed that enzymatic methods could detect GLUase activity of bacteria that had become non-culturable after nutritional and light stresses.

**Conclusion**

This paper presents the first application of rapid enzymatic methods to study the distribution of coliforms in the River Seine and its estuary. Both enzymatic methods and plate counts reflected the various levels of faecal contamination of the river. The higher decrease of culturable FC abundance than glucuronidase activity measurements on a 150 km stretch downstream from the Parisian wastewater outfalls suggested that rapid enzymatic methods were able to detect enzymatically-active but non-culturable coliforms in the River Seine.

**References**


