Monitoring faecal contamination of the Thames estuary using a semiautomated early warning system

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Abstract The Colifast Early Warning System, based on measuring β-galactosidase activity (2 h method), was evaluated for monitoring the level of faecal contamination in the upper tidal Thames. Two trials were performed, one following heavy rain in November 2000, the next during a dry and sunny period in July 2001. In general the β-galactosidase activity and the two coliform reference methods (recovery following membrane filtration with membrane lauryl sulphate broth (MLSB) and Colilert™ Quantitray) were comparable. However, in several samples in July the β-galactosidase activity seemed to overestimate the number of culturable coliforms, suggesting that the rapid enzymatic method detected β-galactosidase produced by other bacterial sources, such as *Aeromonas* spp. or *Vibrio* spp., or nonculturable coliforms. The latter could be attributed to sunlight-induced injury. Nevertheless, the rapid method based on β-galactosidase activity gave an estimate of the level of culturable coliforms, which did not differ from both coliform reference methods by more than one log. Monitoring of β-galactosidase activity in river water samples using the Colifast Analyser may therefore be useful as an early warning indicator of faecal contamination.

Keywords Early warning; faecal contamination; β-galactosidase activity; rapid screening; river water

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Introduction

The monitoring of hygienic water quality generally depends upon the detection of indicator-bacteria, including coliform bacteria, *Escherichia coli* and enterococci as examples. Traditionally, detection of these indicators is by culture, which may take 18 to 48 h, thereby yielding information of value in recording a past event, but of little value as a decision-making tool for pro-active protection of public health. There is a recognised need for methods that permit rapid estimation of the microbiological quality of water, e.g. following a heavy rainfall or accidental pollution of recreational water or water supplies. Defined-substrate methodologies that utilise the detection of β-galactosidase and β-glucuronidase enzyme activities to indicate the presence of total coliforms and *E. coli*, respectively, are now widely used in public health microbiology (Edberg and Edberg, 1988). These methods are easy to perform, but they are in general based on the detection of a visual end product. The analysis time is therefore still between 18 to 24 h. Reduction in analysis time may be achieved by the use of sensitive instrumental techniques to detect released chemiluminescent (Van Poucke and Nelis, 1995), coloured (Apte *et al*., 1995) or fluorescent (Berg and Fiksdal, 1988) end products.

The patented Colifast® Analyser System combines the use of a coliform growth medium with a sensitive and automated laboratory analyser. The growth medium includes the substrate 4-methylumbelliferyl-β-D-galactopyranoside (MUGal), which fluoresces after hydrolysis by the enzyme β-galactosidase, with subsequent detection in the Colifast Analyser (CA). For detection of low levels of faecal contamination, an increase in coliform number by growth is required to obtain detectable fluorescence, i.e. the CA can be used for

detecting 1–10^2 coliforms/100 ml within 6–12 hours (Anglés D'Auriac *et al*., 2000, Tryland *et al*., 2001). For water samples with high levels of faecal contamination, fluorescence production caused by MUGal hydrolysis is detected after a few minutes or hours, prior to any growth (Eckner *et al*., 1999; Tryland *et al*., 2001).

The objective of the present work was to evaluate the Colifast Early Warning System for monitoring the level of faecal contamination in the upper tidal Thames. Since the Thames Estuary in general contains high levels of faecal contamination (coliform numbers > 500 cfu/100 ml), a rapid (2 h) method based on measurement of β-galactosidase activity, without cultivation, was selected. The β-galactosidase activity was evaluated as an indicator of faecal contamination by comparing it with traditional indicators, e.g. counts of total coliforms, *E. coli* and enterococci following membrane filtration. The relationship between β-galactosidase activity and coliform plate count was compared in two different trials, the first trial following heavy rain in November 2000, and the next trial during a dry and sunny period in July 2001.

Materials and methods

Collection of water samples

Water samples from different localities in the Thames Estuary were analysed in two different surveys; the first survey during and following heavy rain between 27–30 November 2000, and the next survey during a dry and sunny period between 2–5 July 2001. During the November 2000 survey, water samples taken at London Bridge, Charing Cross, Vauxhall Bridge, Cadogan Pier, Putney Bridge, Barnes Bridge and Kew Bridge were analysed daily. The same locations, plus samples taken at Erith, Woolwich Ferry and Greenwich, were analysed daily in the July 2001 survey. The water samples were analysed within 6 h of collection.

Reference methods

Total coliforms and *E. coli* were recovered and confirmed by standard methods using membrane filtration and membrane lauryl sulphate broth (MLSB) (Anon, 1994). In addition Colilert™ Quantitray was used for recovery of total coliforms and *E. coli* after 18–22 h incubation at 37°C. Enterococci were recovered by membrane filtration on Slanetz and Bartley medium after 44 ± 4 h incubation at 44 ± 1°C and confirmed by aesculin hydrolysis (Anon, 1994).

Colifast method

5 ml water samples were pipetted directly to 20 ml vials containing 5 ml Colifast 6 medium. The vials were incubated in the Colifast analyser at 37°C for 2.25 h (November trial) or 2.5 h (July trial). After 15 min pre-incubation, sub-samples were automatically taken every hour (November trial) or 45 min (July trial, a programmable variable), each result requiring 90 seconds to process. A report yielding the concentration of the fluorescent product, 4-methylumbelliferone (ppb MU), was generated. The results were calculated as the rate of production of the fluorescent product (ppb MU/h), reflecting the β-galactosidase activity of the water samples. The samples were analysed in duplicate.

Results and discussion

A good correlation was observed between the β-galactosidase activity, monitored by the CA and the coliform plate count on MLSB in the initial (November 2000) survey (Figure 1). The degree of correlation ($r^2 = 0.76$) was comparable to the correlation between the two coliform reference methods, Colilert™ Quantitray and plate count on MLSB ($r^2 = 0.72$). Similarly, the correlation between the β-galactosidase activity and Colilert™ Quantitray was $r^2 = 0.67$ (data not shown).

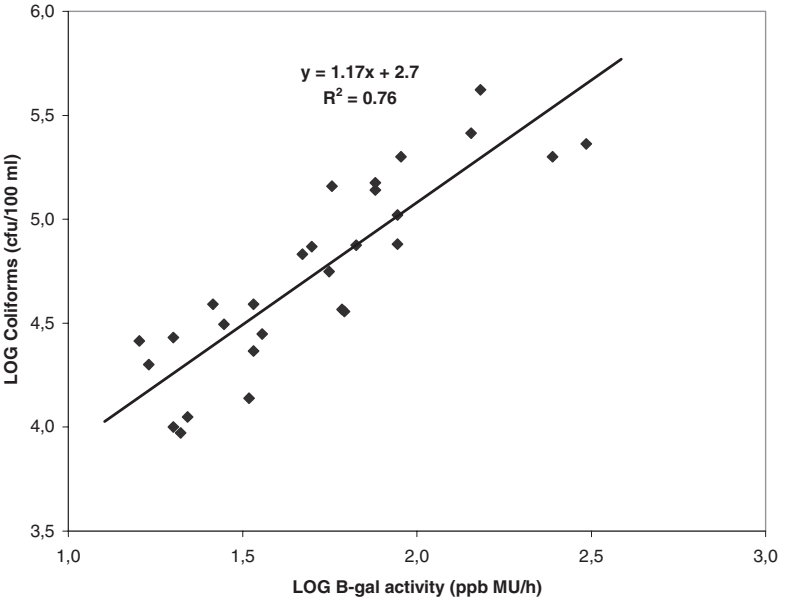

Figure 1 β-Galactosidase activity (measured by CA within 2 h) versus total coliforms (MLSB) of water samples from the upper tidal Thames, November 2000 survey

The Thames Estuary contained high levels of coliform bacteria during the November 2000 survey. This was reflected both by the total coliform numbers measured by MLSB and Colilert™ Quantitray and the Early Warning System based on β-galactosidase activity (Figure 2). Monitoring of β-galactosidase activity and use of the correlation curve (Figure 1) to convert the ppb MU/h to quantitative coliform number, may therefore be useful as a rapid predictive model for estimating the level of faecal contamination in river water samples. Rainfall, cold and cloudy weather was observed during the November 2000 survey, therefore a new survey was performed in July 2001 to investigate whether the initial model could be used during warm and sunny summer days. In general the β-galactosidase model obtained in November overestimated the coliform number in the July survey (Figure 2). However, in none of the samples did the result obtained by the β-galactosidase model differ from the results obtained for coliforms with either MLSB or Colilert™ Quantitray by more than one log; 61% of the samples differed by 0–0.5 log and 39% of the samples differed by 0.5–1 log. Besides, the three different methods in general showed similar trends along the Thames; high increases in faecal contamination obtained by one of the methods were in general reflected by the two other methods (Figure 2).

A few samples with high coliform numbers were detected in July, but in general the Thames contained fewer coliforms in July compared with November (Figure 2). This may be explained by less rainfall and thereby reduced intermittent discharges of faecal contamination from combined sewer overflows and surface water drains. In addition, the high temperature and sunshine may have resulted in an elevated die-off rate in July compared with in November (Barcina *et al.*, 1989; Davies-Colley *et al.*, 1994). The coliform number obtained by plate count on MLSB fluctuated widely during the July survey (Figure 2), indicating a variable die-off e.g. depending on local variations in water turbidity and exposure to the disinfecting sunlight. Coliform bacteria which may not be culturable after exposure to UV-light (Fiksdal and Tryland, 1999) or visible light (Pommepuy *et al.*, 1996) may retain β-galactosidase activity. This may explain why the β-galactosidase activity in general was less reduced from November to July compared with the coliform plate count (Figure 2). Coliform methods based on culture may underestimate the number of viable or active

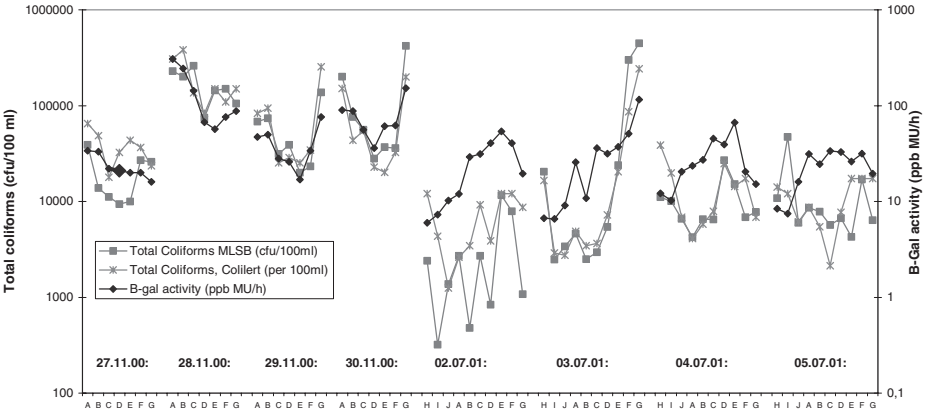

Figure 2 β-Galactosidase activity (measured by CA within 2 h) and total coliforms (measured by MLSB and Colilert™ Quantitray) of water samples taken along the Thames river (A: London Bridge, B: Charing Cross, C: Vauxhall Bridge, D: Cadogan Pier, E: Putney Bridge, F: Barnes Bridge, G: Kew Bridge, H: Erith, I: Woolwich Ferry and J: Greenwich) in the period 27.11.00–30.11.00 (November 2000 survey) and 02.07.01–05.07.01 (July 2001 survey)

coliforms (Barcina *et al*., 1989; Davies *et al*., 1995a). In this study a comparison of the results of the rapid method based on monitoring β-galactosidase activity, without culture, may indicate that the Thames contained relatively high levels of β-galactosidase-active but nonculturable bacteria during the July survey. Presumptive high levels of enzymatically-active but nonculturable coliforms has also been reported in the Seine River (George *et al*., 2001).

Another explanation of the relatively high β-galactosidase activity compared with coliform numbers obtained in July may be presence of a higher fraction of non-coliform activity. In addition to culturable coliforms and active but nonculturable coliforms, some non-coliform bacteria (Wallenfels and Weil, 1972; Davies *et al*., 1995b; Fiksdal *et al*., 1997), algae (Davies *et al*., 1994) or non-specific cell-free enzymes (Davies and Apte, 2000) may contribute to the total β-galactosidase activity of the water samples. *Vibrio* and *Aeromonas* contain species which produce β-galactosidase (Bryant *et al*., 1986) and have been shown to occur in significant numbers in Estuarine waters in the UK during the summer months but would be significantly reduced or absent during the winter months. Surveys carried out around the Kent waterways and coast have shown that there are significant numbers of Vibrios (>10^2/ml) in the summer months (Lee *et al*., 1982; West and Lee, 1982; Lee, personal communication). However, recently published papers indicate that predominantly sewage-derived organisms seem to be the main source of β-galactosidase activity of water samples with high faecal contamination (Tryland and Fiksdal, 1998; Davies and Apte, 2000; George *et al*., 2000).

Monitoring β-galactosidase activity using a rapid test format without culture is useful because of its rapidity and the capability of early warning if the faecal contamination is much higher than normal. If required, the Colifast Analyser can be used for verifying the presence of culturable coliforms within the same test, in an elongated (6–12 h) test format. The Colifast Analyser allows prolonged incubation of the samples and automatic subsampling to follow the additional fluorescence development associated with bacterial growth. A logarithmic increase in fluorescence indicates that new enzymes have been synthesised as a result of bacterial growth and induction in a selective medium. This is a stronger indication of presence of culturable coliforms than demonstrating β-galactosidase activity without growth. Prolonging the incubation and demonstration of a logarithmic

increase in fluorescence may therefore be useful as a confirmation step to verify presence of culturable coliforms (Tryland *et al*., 2001).

Despite the assumption that varying fractions of active but nonculturable coliforms and non-coliform activity may contribute to the measured β-galactosidase activity, e.g. depending on sunlight, a good correlation (in log-log plots) between β-galactosidase activity and faecal coliform plate counts of water samples has been reported in several studies (Fiksdal *et al*., 1994; Davies *et al*., 1996; George *et al*., 2000; Tryland *et al*., 2001). To prepare a general model for converting the β-galactosidase activity to quantitative coliform numbers in river water, the data from the November 2000 and the July 2001 study were collected in one figure (Figure 3A), showing a $r^2 = 0.5$. An approximately similar correlation was obtained between the β-galactosidase activity and the colony counts of *E. coli* (Figure 3B) and between the β-galactosidase activity and the colony counts of enterococci (Figure

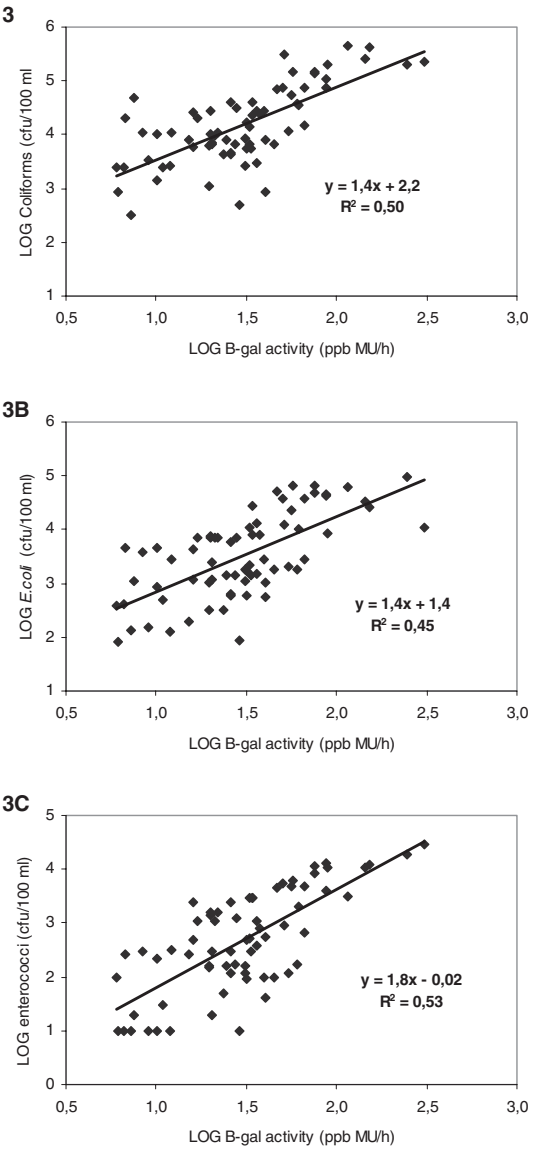

Figure 3 β-Galactosidase activity (measured by CA within 2 h) versus- total coliforms on MLSB (3A), *E. coli* on MLSB (3B) and enterococci (3C) of water samples from the Thames Estuary, November 2000 and July 2001

3C). Monitoring β-galactosidase activity and use of the correlation curves (Figure 3A–C) may be useful as a rapid predictive model for estimating the level of faecal contamination of river water samples. The study indicates that this may be done with a precision of approximately ± 1 log at these high levels.

Conclusion

A good correlation was observed between the β-galactosidase activity, monitored by the Colifast Analyser, and the coliform reference methods based on culture, in the trial performed in November 2000. In a similar trial performed during July 2001 the β-galactosidase activity in general seemed to overestimate the number of culturable coliforms, suggesting that the rapid enzymatic method were able to detect β-galactosidase-active but nonculturable coliforms, which could be attributed to sunlight-induced injury, or β-galactosidase produced by other bacterial sources such as *Vibrio* spp. or *Aeromonas* spp. Nevertheless, the rapid method based on β-galactosidase activity gave an estimate of the level of culturable coliforms, which did not differ from either coliform reference method by more than one log. The accuracy of the Early Warning Result (< 2 h) can be improved by extending the program to 6–12 h to differentiate the contribution of the culturable coliforms. Moreover, monitoring of β-galactosidase activity of water samples by using the Colifast analyser is semi-automated and simple (no need for time-consuming dilutions) and may therefore be useful as an early warning indicator of faecal contamination.

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