Early warning of faecal contamination of water: a dual mode, automated system for high- (<1 hour) and low-levels (6–11 hours)

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Abstract There is a recognised need for methods that permit rapid estimation of the sanitary quality of water e.g. during raw water monitoring and emergencies involving water treatment failure or main breaks in a distribution network. In this study, two models for predicting the level of faecal contamination of water were studied. The first format, based on measurement of β-galactosidase activity by the automated Colifast analyser, detected faecal contamination of high levels, corresponding to >15 thermotolerant coliforms (FC)/5 mL, in 1–3 h, in a format that allowed for semi-quantification of results. By setting up a cut-off level, the system could be used as an operational tool identifying random increases in faecal contamination during routine raw water monitoring. A second Presence–Absence format was dependent upon the growth of low levels of FC with subsequent detection in the Colifast analyser. 95% of water samples containing 1–15 FC/sample volume showed positive detection after 11 h.

Keywords Rapid technique, early warning, β-galactosidase activity, coliform bacteria

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Introduction

The hygienic monitoring of water quality generally depends upon the detection of indicators of pathogenic microbes including thermotolerant coliforms (FC) or *E. coli,* and faecal streptococci or Enterococcus spp as examples. Traditionally, visible growth of these indicators after 18–48 h is the endpoint, thereby yielding information of value in recording trends, but is of little value as a decision-making tool for protecting public health. During recent years Colifast AS has developed methods that have significantly reduced the detection time of total coliforms (TC) and FC (Eckner *et al.,* 1999). The patented Colifast® analyser system combines a selective coliform growth medium with an automated laboratory analyser. The growth medium includes 4-methylumbelliferyl-β-D-galactopyranoside, which fluoresces after hydrolysis by the enzyme β-galactosidase, with subsequent detection in the Colifast analyser. β-galactosidase is produced by coliform bacteria but is also found in some non-coliform strains (Wallenfels and Weil, 1972; Van Poucke and Nelis, 1997; Tryland and Fiksdal, 1998).

The objective of the present work was to use the automated Colifast technology for establishing and testing a dual model for estimating the level of faecal contamination of water samples: (1) a rapid format based on initial measurement of β-galactosidase activity and (2) a Presence-Absence (PA) format based on verifying β-galactosidase induction by coliforms growing in the selective medium. The purpose for both modes, was to provide a simple, rapid and robust tool for the user, such as a water treatment plant operator, to supplement microbiological information to other physical and chemical monitors and make operational decisions.

Materials and methods

Collection of water samples

Two types of experimental data were collected: (a) in a laboratory setting (LS) to develop the models, instrument and methods, using river and brackish water from the Oslo, Norway

region and (b) in a field setting (FS) from source-water rivers for the City of Gothenburg, Sweden. The laboratory experiments involved environmental waters from six different sources, eight independent tests yielded 79 data points for total coliforms (TC) and 53 for faecal coliforms FC. In the field study, water samples from four different sources in the Gothenburg region were analysed 2–3 times per week over 10 months yielding 222 data points.

Reference methods

FC and TC were recovered from water samples by membrane filtration (0.45 μm) on m-FC agar (24 h at 44–44.5ºC) and m-Endo agar (24 h at 37ºC) respectively.

Colifast method

Water samples (5 mL) were mixed with 5 mL Colifast medium in 20 mL vials and incubated in the Colifast analyser at 44ºC and/or 37ºC for 10–13 h (LS) or 2 h (FS). Sub-samples were automatically taken every 1–1.5 h (LS) and 25 min (FS, a programmable variable) each result requiring 90 s to process. A report yielding the concentration of the fluorescent product 4-methylumbelliferone (ppb MU) was generated. In the field study the rate of production of MU (ppb MU/h) reflecting β-galactosidase activity prior to any growth, was monitored.

Results and discussion

Water samples with high levels of faecal contamination, corresponding to >15 FC/5 mL, showed a linear fluorescence development (constant β-galactosidase activity) during the first 5–6 h of incubation in the Colifast analyser (Figure 1). After 5–7 h a logarithmic increase in fluorescence was observed indicating that new enzymes had been synthesised as a result of bacterial growth and enzyme induction. Water samples with low levels of faecal contamination, corresponding to <15 FC/5 mL, showed no or low fluorescence development (in general <3 ppb MU/h) in the first 6 h of incubation but a logarithmic increase was observed after 7–11 h (Figure 1, Table 1).

The applicability of using β-galactosidase activity (measured on CA within 2 h) as a predictive model for estimating the level of faecal contamination of water was tested at Gothenburg Water and Sewage Works. A correlation between log β-galactosidase activity and log FC was generated for all four water sources (Figure 2). The agreement between β-

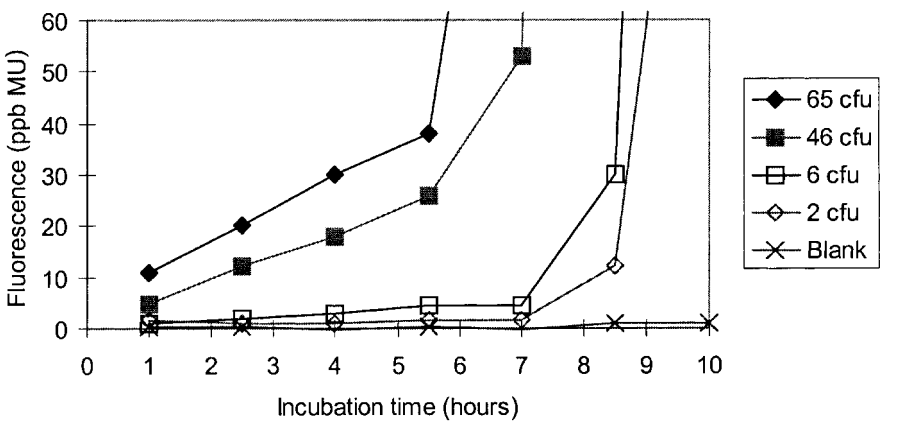

Figure 1 Fluorescence development of environmental water samples mixed with Colifast medium and incubated in the Colifast analyser (37°C, 10 h). Four typical samples are plotted (two coastal water samples containing 65 and 2 TC/vial and two river water samples containing 46 and 6 TC/vial)

Table 1 Detection of thermotolerant coliforms in Colifast medium incubated at 44.5°C

Plate count m-FC agar, 44.5°C		% of samples showing logarithmic increase in fluorescence at different incubation times: 7h	8h	9h	10h	11h
1–15 cfu/vial	(40 samples)	43%	83%	88%	93%	95%
16–400 cfu/vial	(13 samples)	100%				

Table 2 Detection of total coliforms in Colifast medium incubated at 37°C

Plate count m-FC agar, 37°C		% of samples showing logarithmic increase in fluorescence at different incubation times: 7h	8h	9h	10h	11h
1–50 cfu/vial	(67 samples)	37%	67%	88%	90%	94%
51–400 cfu/vial	(12 samples)	100%				

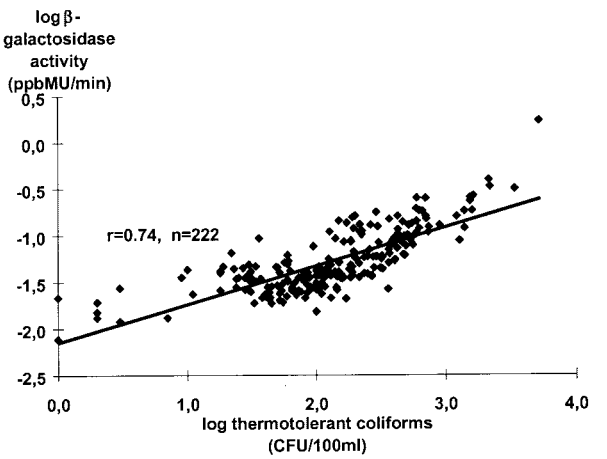

Figure 2 β-galactosidase activity (measured by CA within 2 h) versus thermotolerant coliforms (m-FC) of water samples from four different sources in the Gothenburg region, analysed 2–3 times per week over 10 months

galactosidase activity and FC counts was even better when considering strategic sampling points individually over time (Figure 3). By establishing a cut-off level, the system could be used as an operational tool identifying random increases in faecal contamination during routine raw water monitoring. Consequently, the waterworks operators were able to take appropriate action to avert problems within the travel time of the raw water to the intake of the plant.

A good correlation (in log-log plots) between β-galactosidase activity and faecal coliform plate counts of water samples has also been shown by others (Fiksdal *et al.*, 1994; Davies and Apte, 1996; George *et al.*, 2000). Recently publications have indicated that predominantly sewage-derived organisms, e.g. culturable coliforms and active but non-culturable coliforms, are the main source of β-galactosidase activity of environmental water samples (Tryland and Fiksdal, 1998, George *et al.*, 2000) although β-galactosidases may also be found in some non-coliform strains. Monitoring of β-galactosidase activity may, therefore, be useful as a general indicator of faecal contamination, of water with high level of faecal contamination, defined in this study as >15 FC/5 mL.

For detection of low levels of faecal contamination, defined as 1–15 FC/5 mL, an increase in coliform number by growth was required to obtain detectable fluorescence

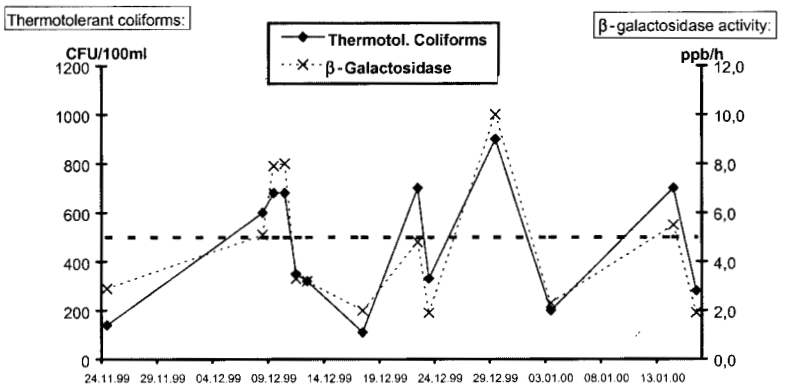

Figure 3 β-galactosidase activity (measured by CA within 2 h) and thermotolerant coliforms (m-FC) from one raw water sampling point (Lärjeholm, Gothenburg) over time

(Figure 1). Extended incubation and demonstration of a logarithmic increase in fluorescence may also be useful as a confirmation step to verify presence of culturable coliforms in water samples showing initial β-galactosidase activity. Logarithmic increase in fluorescence indicated bacterial growth and enzyme induction in a selective medium and was a better indication of presence of culturable coliforms than only β-galactosidase activity.

All samples showing >15 FC/vial and >50 TC/vial showed logarithmic increase in fluorescence within 7 h when incubated in Colifast medium at 44.5ºC and 37ºC, respectively (Tables 1 and 2). Generally, the results confirmed earlier work (Eckner *et al.*, 1999) in that low levels of FC and TC (<15 FC/vial and <50 TC/vial) were detected in 7–11 h (Tables 1 and 2).

Conclusions

The primary objective of the study, developing a simple early warning tool for water plant operators, was met: a <2 h test for raw water screening and a 6–11 h test for PA monitoring.

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