YRIRP REPORT

RAPID TEST METHODS FOR E. COLI AND ENTEROCOCCI

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KEY MESSAGE

For the bacterial concentrations likely to be encountered in EPA sampling, B2P products and testing methods (Watercheck and Coliquick) do not provide a significant advantage over current methods.

SCOPE AND PURPOSE

This study was designed to trial B2P bacterial testing products as rapid test methods for *E. coli* and coliforms in fresh, estuarine and marine waters.

The purpose of this study was to determine whether the B2P rapid test method could enhance the current Yarra River recreational water quality sampling program through faster reporting on the Yarra Watch website.

INTRODUCTION

The Yarra River is a major waterway, a natural feature of Melbourne's landscape. It has shaped Melbourne's development and growth, supports industry and tourism, and is highly valued as an environmental and recreational asset.

In 2006 EPA Victoria developed the Yarra River Investigation and Response Program (YRIRP). YRIRP aimed at increasing understanding of water quality in the Yarra and reducing pollutant inputs, particularly from industrial and commercial sources.

Over the four years of YRIRP (2006 to 2010) the program focused on scientific analysis and investigations, social research, knowledge transfer and partnerships with other statutory enforcement bodies, all aimed at improving the amenity of the Yarra River.

RESEARCH BACKGROUND

E. coli and enterococci are commonly used as indicators of faecal contamination. The current methods used to enumerate these indicator bacteria have a turnaround time of approximately 24 hours. This means that results of samples taken for recreational water quality assessment give an indication of previous conditions, which may not necessarily reflect the current situation.

EPA uses *E. coli* concentrations to give an indication of recreational water quality in the Yarra. Water samples are collected at selected sites along the Yarra every Wednesday morning and transported to the ALS Water Resources Group laboratories at Scoresby. Results are received on Thursday afternoon, approximately 24 hours after sampling.

These results are reported on the Yarra Watch website, which is used by community groups such as rowing clubs to assess potential human health risk associated with recreational use of the Yarra.

EPA also collects samples for *E. coli* and/or enterococci counts when there is a pollution incident with potential faecal contamination. The results from these samples may be used in prosecutions or as part of ongoing monitoring programs.

B2P Testing (http://www.b2ptesting.com.au) has designed a rapid (one to 14 hours, depending on concentration) method for determining faecal coliform and *E. coli* concentrations. Testing does not require specialised skills or laboratory equipment. The advantages of the B2P products (Watercheck and Coliquick) are the potential for use in the field for pollution response sampling and the possibility of faster results.

Traditional methods for *E. coli* and enterococci counts

Most-probable-number (MPN) multiple-tube fermentation is a traditional technique that was widely used for measuring coliform and *E. coli* concentrations. The mechanism is based on the lactose fermentation ability of coliforms and *E. coli*, which can be separated by different formulations of the growth medium. The examination of replicates and dilutions gives an estimated mean density of the microbial indicator; the quantity of microbial indicator in the samples can be estimated by using a probability table.

Membrane filtration (MF) is the alternative traditional method used for enumerating *E. coli* and enterococci. The MF method provides a direct count of bacteria in water, based on the development of colonies on the surface of the membrane filter (Levin et al. 1975). Specific media are chosen to make the microbial indicator colonies identifiable through unique growth features.

Both of these techniques (MF and MPN) have the disadvantages of long incubation time (up to 96 hours,

including the confirmation step), interference by heterotrophic plate count bacteria and difficulties in interpreting the results (American Public Health Association 1986).

Current methods for *E. coli* and enterococci counts

Enzyme detection methods (EDM) are relatively new approaches for detecting and enumerating bacterial indicators. They are based on the presence of specific enzymes in the target microbial indicator.

Bacteria are cultured on substrates that become coloured or fluorescent due to the reaction of a specific enzyme. This effect can help detect or count the bacterial indicator.

EDM can be used for measuring *E. coli*, coliforms and enterococci in water. This method is specific, sensitive and rapid (Manafi 1998).

Colilert and Enterolert

Colilert, used to detect coliforms and *E. coli*, and Enterolert, used to detect enterococci, were developed by IDEXX. They have proven to be sensitive and reliable in detecting and enumerating coliforms, *E. coli* and enterococci (Olson 1991, Manafi 1998, Palmer 1992).

Both of the methods are enzyme-based and enumerate bacteria using MPN. Generally, 18 to 24 hours are needed for a result. Colilert and Enterolert are widely accepted as standard methods.

B2P Testing (Watercheck™ and Coliquick™)

Coliquik and Watercheck were recently developed by B2P Testing for the detection and quantification of coliforms and *E. coli* using EDM methods. Test results can be obtained within 12 hours, depending on bacterial levels in the sample.

The concentration of coliforms can be estimated by the time taken for the colour of the sample to change; the longer it takes for the colour to change, the lower the concentration of coliforms or *E. coli* (Fig. 1). After incubation, blue/purple denotes no coliforms in the sample, pink denotes the presence of coliforms and white denotes *E. coli*.

METHODS USED TO COMPARE COLILERT WITH COLIQUIK AND WATERCHECK

Water samples were collected from a number of sites along the Yarra River and Port Phillip Bay.

Freshwater samples were collected from Heidelberg, Kew and Springthorp wetland.

Estuarine samples were collected from the Yarra River at Southbank.

Marine samples were collected from St Kilda Beach and Port Melbourne Beach.

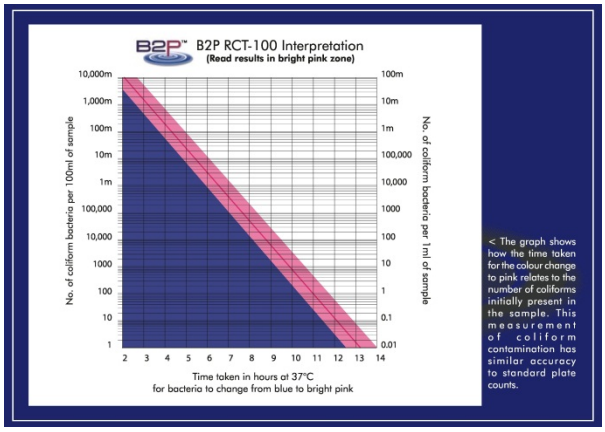

Figure 1: Chart used to determine *E. coli* concentrations using Coliquik and Watercheck, based on time taken for colour change.

All water samples were collected on the same day, beginning at approximately 8:00 am. The sampling location at each site was randomly selected. Two litres of water were collected at each site in a laboratory-cleaned 2 L glass jar containing sodium thiosulphate. The samples were placed on ice and brought back to the EPA Centre for Environmental Sciences.

Several subsamples were taken from each 2 L water sample:

- Five for analysis using Coliquik.
- Six for analysis using Watercheck (including Watercheck, RCT-T and RCT-S).
- A single 500 mL sample for Colilert analysis by a commercial laboratory.

Care was taken to ensure all analyses were carried out at the same time, and within standard holding times, in order to ensure results were comparable.

Samples were placed in the Coliquik (10 mL) and Watercheck (100 mL) containers, following the manufacturer's instructions.

A range of Watercheck-style sample containers were tested (Watercheck, RCT-T and RCT-S).

Following the recommendation of B2P Testing's representative, Watercheck was not used for undiluted saline samples; samples from St Kilda Beach, Port Melbourne and Southbank were analysed using a 10 per cent dilution.

To provide a control, all analyses were repeated using deionised water.

The majority of samples were placed in a laboratory incubator set at 37 °C and checked every 10 minutes to determine whether there was any colour change.

Four sample-filled Watercheck containers were placed in the incubator designed by B2P (the Micro Magic). This system also incubates the sample at 37 °C, and monitors the progress of the test.

RESULTS

All B2P samples were incubated for a maximum of 20 hours. Colour changes were monitored every 10 minutes for the first 9.5 hours, at which time they were assessed for the presence of a colour change. At this stage, an estimate was made of the total coliforms present in the sample, assessing the colour against the calibration curve provided by the manufacturer (Figure 1). All samples were re-examined after 20 hours. A summary of the results is presented in Table 1.

A number of B2P samples returned no results. In the majority of these cases there had been some leakage of the sample, clogging of membranes or damage to the testing apparatus.

Calibration curves were not available for some test bottles (RCT-S and RCT-T).

There was also some difficulty in interpreting some samples, including significant difficulty in reading colour changes when individual membranes had patches of a range of colours, or bottles had turbid or variably coloured contents.

DISCUSSION

The B2P rapid testing methods produced bacterial counts in a range similar to those measured using the more conventional testing methods (Table 1).

The speed of the test was dependant on bacterial concentration in the sample; the lower the concentration, the longer the incubation period.

Typically, samples from rivers and beaches have relatively low bacterial concentrations, so it is unlikely that the time needed to incubate samples using the B2P methods would be significantly shorter than conventional methods.

Unless samples are collected very early in the morning, final results are unlikely to be available for reporting on the same day as sampling.

FURTHER DIRECTION

Rapid alternative field-based methods for determining *E. coli* and enterococci levels in recreational waters remain a goal for organisations assessing and reporting on potential public health risk.

EPA will continue to investigate developments in bacterial testing that may provide significant advances in the field.

CONCLUSION

Under the conditions used in this trial, the B2P bacterial testing procedure did not produce results that would significantly enhance the Yarra Watch recreational water quality program.

The results produced using the alternative methods were similar to, but not directly comparable with, the standard bacterial testing methods.

Time frames for reporting were not short enough to ensure same-day reporting of recreational water quality on the Yarra Watch website.

Table 1: Summary of B2P and conventional testing results

Sampling site	Conventional testing results		B2P testing results, Coliquik and Watercheck combined			
	Total coliforms (org/100 mL)	*E. coli* (org/100 mL)	Total number of valid results per site	Number of samples with bacteria detected after 20 hours incubation		Range of total coliforms estimated (org/100 mL) after 9.5 hours incubation
				Coliforms	*E. coli*	
Yarra River at Heidelberg (freshwater)	1800	145	9	0	9	<360 to >1500
Yarra River at Kew (freshwater)	1400	183	11	0	11	<500 to >1500
Yarra River at Southbank (estuarine)	3300	223	9	0	9	<200 >1500
St Kilda Beach (marine)	110	10	10	0	9	<1500 to <15000
Port Melbourne (marine)	31	<10	7	4	2	<90 to <1500
Springthorpe wetland (freshwater)	360	41	4	0	4	<80 to >1500
Distilled water	0	0	4	0	0	

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