Evaluation of Colilert-18® as an alternative method for monitoring total coliforms and *Escherichia coli* in some faecally polluted river waters

Amanda S. Brand and Jo M. Barnes

ABSTRACT

The increase in numbers and contamination levels of faecally polluted water has resulted in shifts worldwide towards methods which enumerate faecal indicator bacteria faster. Rapid methods enable more timely remedial and preventative actions which protect the health of water users. However, especially in the developing world, straightforward methods are also preferred as they reduce the requirement for highly qualified analysts. This study investigates the feasibility of using the rapid, semi-automated enzyme substrate test Colilert-18® instead of multiple-tube fermentation (MTF) in total coliform and *Escherichia coli* enumeration for South African river water, as one example of a surface water source carrying considerable faecal pollution, which needs monitoring. Spearman rank correlation coefficients (ρ) of 0.83 and 0.86 were obtained for total coliforms and *E. coli* respectively, indicating Colilert-18® performed acceptably in the pollution ranges encountered. A Bland–Altman plot further revealed that Colilert-18® showed no significant difference ($p > 0.05$) from MTF values below 100,000 *E. coli* most probable number/100 mL (estimated true value). Above this level Colilert-18® was found to progressively underestimate *E. coli*. This inadequacy of Colilert-18® was considered acceptable from a health risk assessment viewpoint as such high counts should have sounded the alarm for preventative and corrective action irrespective of method inaccuracy.

Key words | faecal pollution monitoring, health risk assessment, method comparison, polluted environmental waters

Amanda S. Brand (corresponding author)
Jo M. Barnes
Division of Community Health,
Faculty of Medicine and Health Sciences,
University of Stellenbosch,
South Africa
E-mail: *anibrand85@gmail.com*

INTRODUCTION

The determination of the pollution levels of environmental waters is a crucial component of the ability to assess the risk that such waters may hold for a variety of water users (Percival *et al.* 2000). It also determines the speed with which appropriate remedial action can be instituted when significant pollution incidents occur. The ability to keep track of the microbiological quality of surface water in rural areas where significant volumes of water are abstracted for the irrigation of edible crops and even used as the domestic water supply is heavily dependent on reliable and rapid methods for the assessment of *Escherichia coli*, the indicator organism of choice for such determinations (Edberg *et al.* 2000). In recognition of this global need, the World Health Organization (WHO), in its Water Quality and Health Strategy 2013–2020 report (WHO 2013), undertook to promote the use of rapid water quality tests in the interest of protecting public health.

In South Africa, rivers are the most inexpensive and readily available source of irrigation as well as raw drinking water. In cities and larger towns, the raw drinking water is purified at water treatment plants, but in rural and informal urban settings such water is often used for drinking and other domestic purposes in its untreated state. Unfortunately, the microbiological quality of South African river water is

doi: 10.2166/washdev.2014.040

poor due to failing urban sanitation systems and fast expanding urban areas of informal housing with no or poor sanitation draining into the surface water via formal and informal storm water channels (Govender *et al.* 2011a, 2011b). The deteriorating microbiological quality of river water together with water usage practices, particularly in vulnerable low-income areas, increase the risk of spreading diseases should inadequate monitoring or underestimated microbiological risks become widespread (Barnes 2012). Moreover, the pollution levels in rivers in the area are highly variable since they are affected by point-source and diffuse pollution, rising and falling water levels due to rainfall variation, changing temperatures and effluent discharge (Barnes 2012).

In South Africa, the extent of faecal contamination in water is determined through the enumeration of total coliforms and/or *E. coli*. For this purpose, the multiple-tube fermentation (MTF) method (Standard Methods 9221B) (APHA AWWA & WEF 2012) is often used and is still the only method recognised for forensic purposes in South African litigation. MTF is typically used in many developing countries (Macy *et al.* 2005). The method is, however, highly labour intensive and requires large amounts of laboratory glassware and a high degree of technical skill (Elmund *et al.* 1999). These requirements are often lacking in the developing world (Mannapperuma *et al.* 2011), and in South Africa there is an acute shortage of efficient commercial microbiological laboratories. These factors have a detrimental impact on the ability to effectively monitor water sources and produce quality results on which management decisions are based. In addition, MTF results are only available two (WHO 2001) to four (Maheux *et al.* 2008) days after initial analysis. Such time lapses cause a considerable delay before corrective or preventative action can be taken. This has resulted in a need for more rapid enumeration methods for water, especially in the event of an emergency (IWA 2000).

The enzyme substrate test method using the combined Colilert-18® and QuantiTray products has become a possible alternative to the traditional MTF method after the development of the QuantiTray enabled the enumeration of total coliforms and *E. coli*. Apart from the advantage of being less sensitive to human errors, particularly those related to several manual steps, because of improved automation, the greatest benefit of the method is that results are available within 18–22 h (Wohlsen *et al.* 2008). This considerable saving in time could potentially result in large risk reduction, as water users could be alerted of pollution in the water within 18 h.

For Colilert-18® to be considered a feasible alternative, the method should show acceptable performance when compared with the MTF method. This comparison would represent the most rigorous test for Colilert-18® as MTF is considered the 'gold standard' for the enumeration of total coliforms and *E. coli*. In addition, differences between local pollution scenarios result in compositional variation between water sources. This, in turn, requires that a comparative study be done specifically for water from a specific pollution profile. Any results obtained during such a study would only be applicable to such water. For this reason, the necessity for determining whether Colilert-18® could reliably replace MTF in the analysis of locally obtained environmental water with a high likelihood of faecal pollution was identified, as no such study has been undertaken in South African waters before. Therefore, the first objective of this study was to determine whether Colilert-18® is a reliable substitute for MTF when enumerating total coliforms and *E. coli* from faecally polluted river water in South Africa. The second objective was to determine the total coliform and *E. coli* concentration ranges at which Colilert-18® substituted MTF reliably, given that the method proved to be an acceptable alternative.

METHODS

Sample sites

Four rivers in the Western Cape province of South Africa were sampled during this study. The first is the Plankenburg River in Stellenbosch, which flows past the Kayamandi informal settlement and an industrial area before its confluence with the Eerste River. This river has been reported by several other studies using the MTF method as carrying high levels of faecal contamination (Barnes 2003; Paulse *et al.* 2009). The Eerste River was also sampled before its confluence with the Plankenburg River. This river originates in the Jonkershoek Valley on the outskirts of Stellenbosch and flows through the town, receiving considerable sewage pollution through storm water ingress from residential

areas. The river also supplements the raw drinking water supply for Stellenbosch (River Health Programme 2005) and is an important source of irrigation water (DWAF 2004) for fruits and vegetables grown along its banks.

Samples of the Lourens River in Somerset West and the upper Berg River outside Franschhoek were also drawn. The Berg River is a large river and an important irrigation source for numerous agricultural activities in the area. These activities include the production of export fruits and vegetables consumed raw which contribute a farm gate value of around $124 million or €91 million (Louw 2008) to the South African economy. However, Paulse *et al.* (2007) reported that faecal coliform levels in this river occasionally exceed the WHO's guideline for the irrigation of crops likely to be eaten raw (WHO 1989) and that other pathogens of importance for human and animal health were present in the water (Paulse *et al.* 2012).

Sampling procedure

A total of 54 samples were drawn at three-week intervals in the period between May 2009 and May 2010 to ensure equal sample distribution over all seasons. The proportional distribution of these samples across rivers was 51.9% ($n = 28$) for the upper Berg, 29.6% ($n = 16$) for the Plankenburg, 14.8% (8) for the Eerste and 3.7% ($n = 2$) for the Lourens River.

Sampling in sterilised 1 L glass bottles was done according to the guidelines set out by the South African Bureau of Standards, which incorporates the standard methods set out by the American Public Health Association American Water Works Association & Water Environment Federation (1996, 2012). All samples were transported on ice and brought to the analysing laboratory within 90 min.

Microbiological analyses

The enumeration of total coliforms and *E. coli* was done according to the enzyme substrate test Colilert-18® (Standard Methods 9223B) (APHA AWWA & WEF 2012) and the MTF method (Standard Methods 9221B) (APHA AWWA & WEF 2012) using aliquots of the same water sample to enable a fair comparison. For Colilert-18® analyses, samples were serially diluted tenfold up to 10^{-4} by using 90 mL portioned sterile saline (0.85% w/v NaCl in distilled water). One Colilert-18® reagent (IDEXX, Westbrook, Maine, USA) was added per 100 mL of undiluted sample or serial dilution. Samples were gently mixed to dissolve the reagent before being poured into Quanti-Tray 2000 trays (IDEXX). Trays were sealed and incubated at 35 ± 0.5 °C for 18 h in accordance with the manufacturer's instructions (IDEXX Laboratories Inc. 2013). Enumeration of total coliforms and *E. coli* was subsequently done by looking up the number positive wells in the Colilert-18® MPN table.

For the MTF method, water samples and tenfold sterile saline (0.85% w/v NaCl in distilled water) dilutions up to 10^{-7} were analysed using lauryl sulphate tryptose broth for 48 h at 35 ± 0.5 °C, followed by brilliant green lactose bile broth for 24 h at 35 ± 0.5 °C, *E. coli* (EC) broth with 4-methylumbelliferyl-β-D-glucuronide (MUG) for 24 h at 44.5 ± 0.5 °C, and Levine eosin methylene blue (L-EMB) agar for 24 h at 35 ± 0.5 °C using Standard Methods 9221B (APHA AWWA & WEF 2012). All media were obtained from Oxoid, Basingstoke, UK. The enumeration of total coliforms and *E. coli* were then done using the De Mans MPN table as published in the joint publication of the APHA AWWA & WEF (1996, 2012).

Statistical comparison of methods

The very nature of rivers result in highly variable pollution loads, often due to point and non-point source pollution events. In microbiological risk assessment, the outliers occurring during such events are important as they are indicative of high health risks. Therefore, the data were not transformed and the Spearman rank correlation coefficients (ρ) (Estelberger & Reibnegger 1995) between MTF and Colilert-18® enumerations were calculated for both total coliforms and *E. coli* using the entire four-river data set. This correlation coefficient was used as it is more robust against outlier data (Bin Abdullah 1990) than the Pearson correlation.

To determine the performance of Colilert-18® as compared to MTF in the analysis of water with increasing pollution levels, Bland–Altman (Dewitte *et al.* 2002) scatter plots were constructed for both total coliforms and *E. coli*. This statistical analysis is accepted as a useful methodology for determining whether one method can be substituted with

an alternative method (Ludbrook 2010) at varying concentrations of the target parameter and is considered superior to correlation coefficients for this purpose (Bland & Altman 1990). These scatter plots used the arithmetic mean between two corresponding Colilert-18® and MTF counts as the estimated true pollution level.

RESULTS AND DISCUSSION

The descriptive statistics for the two methods for the entire total coliforms and *E. coli* data set are given under Table 1.

Enumeration of total coliforms

The Spearman ρ between MPN and Colilert-18® values for the enumeration of total coliforms was calculated as 0.83. The Bland–Altman scatter plot comparing Colilert-18® and MPN values for total coliforms at varying pollution levels is given as Figure 1. Estimated true pollution levels (taken as the arithmetic mean of Colilert-18® and MTF enumerations) are indicated on the *x*-axis.

The findings reported in this study are similar to those reported by Noble *et al.* (2004), who obtained a Pearson correlation coefficient of 0.91 between Colilert-18® and MTF for the enumeration of total coliforms. However, that work was done on coastal waters, which differ from river water in composition and microbiological population. The presence of marine vibrios in the coastal water may have resulted in large numbers of false positives, both in Colilert-18 and MTF. Several species of *Vibrio* have been identified for their production of beta-galactosidase (Davies *et al.* 1995; Adin *et al.* 2008). These false positives may have led to an artificial increase in the method correlation. Additionally, the use of Pearson correlation coefficients in the study by Noble *et al.* (2004) may further explain the differences, as the Pearson correlation measures linear relationships while the Spearman rank correlation coefficient measures monotonic relationships and is consequently less influenced by data outliers (Bin Abdullah 1990).

In contrast, our results do not agree well with the findings by Al-Turki & El-Ziney (2009), who obtained a Spearman ρ of 0.59 when comparing Colilert-18® with MTF in the enumeration of total coliforms from Saudi Arabian drinking water. Again, the differences between the two types of water sources would contribute to these discrepancies. Drinking water is typically of a good microbiological quality due to stringent international regulatory standards (WHO 2001). In contrast, the pooled results of four rivers used in this study included three rivers which were shown to carry heavy faecal pollution loads. The variation across samples in the total coliform data presented in this study may have resulted in an increased correlation for the two methods, as high total coliform concentrations greatly influence the fit of the regression line during method comparison. This finding further confirms the need for Bland–Altman analysis to determine the method agreement at varying pollution levels.

For the pollution range most often encountered during this study, Colilert-18® performed reasonably well against MTF in the analysis of total coliforms. The Bland–Altman scatter plot in Figure 1 indicates that Colilert-18® enumerations agreed well with MTF enumerations when coliform counts were between 0 to approximately 100,000 MPN/100 mL. However, when the estimated true pollution level (*x*-axis) increased above 100,000 MPN/100 mL,

Table 1 | Descriptive statistics for the total coliforms and *E. coli* paired data set as enumerated by MTF and Colilert-18® from samples obtained from four rivers

	Total coliforms ($n = 54$)		*E. coli* ($n = 54$)	
Descriptive statistics	**MTF (MPN/100 mL)**	**Colilert-18® (MPN/100 mL)**	**MTF (MPN/100 mL)**	**Colilert-18® (MPN/100 mL)**
Highest count	1,700,000.00	2,419,600.00	1,300,000.00	173,290.00
Lowest count	130.00	203.00	8.00	20.00
Arithmetic mean	69,488.88	166,164.93	36,905.06	9,040.56
Median	6,700.00	19,863.00	790.00	921.00
Standard deviation	236,323.12	437,854.45	177,206.49	25,666.44

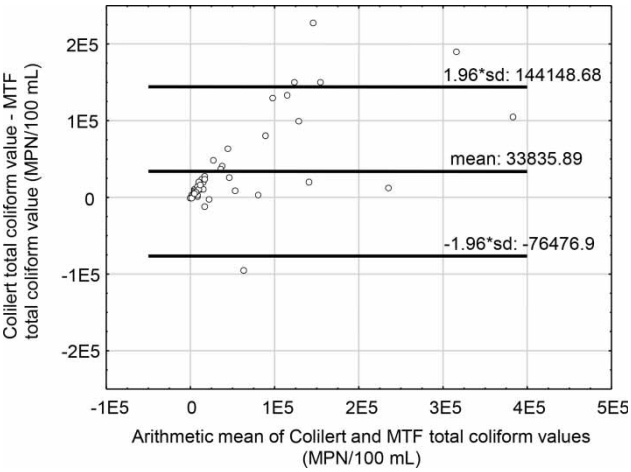

Figure 1 | Bland–Altman scatterplot illustrating the agreement of Colilert-18® with MTF in the enumeration of total coliforms at varying estimated true pollution levels (as the mean of Colilert-18® and MTF values).

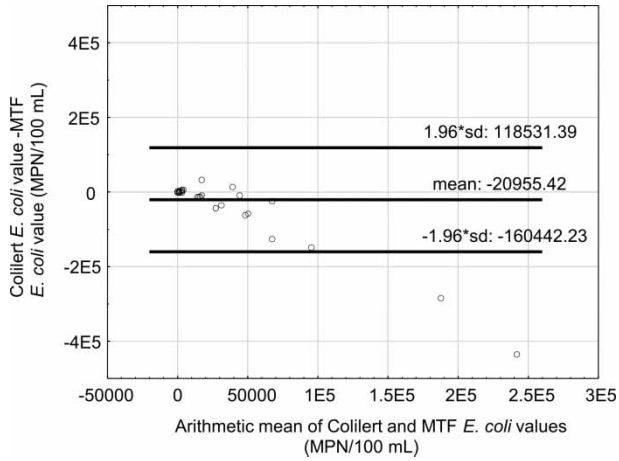

Figure 2 | Bland–Altman scatterplot illustrating the agreement of Colilert-18® with MTF in the enumeration of *E. coli* at varying estimated true pollution levels (as the mean of Colilert-18® and MTF values).

Colilert-18® enumerations became increasingly higher than MTF enumerations. For example, a total coliform concentration of around 300,000 MPN/100 mL resulted in a Colilert-18® enumeration that is nearly 200,000 MPN/100 mL higher than the MTF enumeration. It was, however, only in these very high pollution ranges where Colilert-18® showed progressively and significantly higher results.

Enumeration of *E. coli*

The Spearman ρ for *E. coli* enumeration was 0.86 between the two methods. The Bland–Altman scatter plot for *E. coli* at varying pollution levels is given as Figure 2. Estimated true pollution levels as the arithmetic mean of Colilert-18® and MTF counts are shown on the x-axis.

A good correlation between Colilert-18® and MTF *E. coli* counts was obtained in this study. Similar correlations of 0.79 (Noble *et al.* 2004) and 0.80 (Al-Turki & El-Ziney 2009) were found in other studies, despite the differences in water samples. It should be noted, however, that the study by Noble *et al.* (2004) used MTF results for thermotolerant coliforms and calculated the Pearson correlation. In addition, the salinity of seawater has been reported as a causative agent of sublethal injury in *E. coli*, particularly in EC broth (Anderson *et al.* 1979). This aspect would have adversely affected the correlation found by Noble *et al.* (2004) as the long incubation times of MTF may have aided in bacterial recovery to a greater extent. Nevertheless, the similarity of these results suggests that Colilert-18® compares favourably with MTF in the enumeration of *E. coli* in a wide range of water types. Therefore, Colilert-18® may be a feasible alternative method for monitoring in a variety of water resources.

The Bland–Altman scatter plot for *E. coli* counts (Figure 2) indicates that Colilert-18® enumerations agreed well with MTF enumerations when *E. coli* counts are between 0 and approximately 50,000 MPN/100 mL. Of the 54 samples compared in the present study, 52 (96%) occurred within 1.96 standard deviations for the pollution levels encountered indicating that these values did not differ statistically at the 95% confidence level. In contrast with Figure 1, however, this scatter plot shows that at higher estimated true *E. coli* levels (x-axis); Colilert-18® *E. coli* counts were increasingly lower than those of MTF. Figure 2 shows that an estimated true *E. coli* count of around 180,000 MPN/100 mL resulted in a Colilert-18® enumeration that was nearly 300,000 MPN/100 mL lower than the MTF enumeration. Consequently, as the faecal pollution level of the river increased the Colilert-18® enumeration of *E. coli* progressively underestimated the expected value. It was, however, only in these very high pollution ranges where Colilert-18® showed significant differences.

These results indicate that Colilert-18® can be used instead of MTF in the analysis of river water from the

study area. This is only applicable to *E. coli* counts below 100,000 MPN/100 mL, where no significant difference exist between the two methods ($p > 0.05$). However, as is the case for total coliforms, this *E. coli* level is sufficiently high for the water to be considered a health risk regardless of the accuracy of the enumeration. This is especially true in the case of irrigation water used on minimally processed crops, where it is recommended that thermotolerant coliform levels do not exceed 1000 MPN/100 mL (WHO 1989).

Colilert-18® as an alternative to MTF for river water

Statistical comparison of Colilert-18® with MTF has indicated that this method can perform satisfactorily for the monitoring of South African river water. With the Colilert-18® method, laboratory turnaround time is decreased from 2–4 days to 18 h. This dramatic decrease can reduce early warning response time when disastrous sewage spills occur in the river. These events typically occur either as runoff from formal or informal residential areas during rain events, discharges of untreated sewage, or overflowing sewage treatment works. The method can enable warnings to be issued more rapidly to agricultural producers, to cease irrigating until the danger has passed, and to other water users, to avoid contact with the water as far as possible, when faecal pollution levels are high. The resultant health risks of such a spill could therefore be reduced. For the purpose of risk surveillance, conventional enumeration by MTF is not suitable as 2–4 days is far too long.

The lack of competent microbiological laboratories in South Africa makes the surveillance of water resources by MTF highly problematic in many areas. If the technical skill to conduct the analysis is not available, municipal authorities are unable to perform their duties in terms of risk assessment and cannot act proactively. The lower technical requirements for conducting Colilert-18® analyses is an additional benefit of the method, as more analysts will be competent to perform and interpret *E. coli* analyses by Colilert-18® when compared to MTF.

In terms of costs, Colilert-18 also compares favourably against MTF. The consumables required for one undiluted sample at the time of analysis was around $4.80 or €3.50 with Colilert-18, while the consumables for the same analysis was $14.60 or €10.70 with MTF (without the cost of *E. coli* confirmation using L-EMB agar). For subsequent dilutions, the price per analysis for Colilert-18 remained the same while the cost per analysis with MTF dropped to $4.90 or €3.60 (due to the absence of double strength tubes in subsequent dilutions).

The levels of faecal pollution encountered in the rivers used for this comparative study give cause for great concern, with a highest estimated true *E. coli* count of 240,000 MPN/100 mL obtained. Such levels of *E. coli* in river water pose serious health risks for both direct and indirect water users as *E. coli* indicates faecal contamination and the likely presence of enteric pathogens associated with this type of contamination (Krumperman 1983). These disquieting results add weight to the call for rapid and uncomplicated but reliable enumeration methods that are easily accessible to enable improved surveillance of faecal pollution in river water. Such rapid techniques could potentially improve the prevention of outbreaks of disease through faster preventative and corrective measures.

CONCLUSIONS

The results of this study lead to the following important conclusions:

- The levels of *E. coli* obtained during the study indicated that the four rivers had poor microbiological quality. This further highlights the need for rapid *E. coli* enumeration to reduce the loss of life and livelihoods through quicker preventative and corrective measures;
- Spearman rank correlation coefficients indicated that Colilert-18® is an acceptable alternative to MTF for the enumeration of total coliforms and *E. coli* from faecally polluted river water;
- Colilert-18® begins to produce inaccurate enumeration results above a certain range for total coliforms and *E. coli*. However, these levels are so high (100,000 and 50,000 MPN/100 mL respectively) that preventative and corrective action should be implemented regardless of the margin of error inherent to the method;
- Colilert-18® holds two important advantages, which could potentially decrease the burden of disease due to faecally contaminated water. First, it enables risk

assessment and, if necessary, action within 18 h of analysis. Second, it allows laboratory analysts with lower technical skill levels to produce reliable results. The latter is extremely important for the developing world, where individuals with high levels of technical skill are rare and, consequently, extremely expensive to employ; and

- Colilert-18® also holds an economic advantage for laboratories using this method, as the overall cost of consumables per analysis is considerably lower than for MTF. In addition, the reduced operating time quite possibly represents the largest indirect cost reduction in the form of salaries and/or wages of analysts.

ACKNOWLEDGEMENTS

This study was part of the ongoing solicited research project K5/1773 funded by the Water Research Commission (WRC) and co-funded with the South African Department of Agriculture, Forestry and Fisheries (DAFF). The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at are those of the authors and are not necessarily attributed to the funding organisations. Professor Martin Kidd at the Centre for Statistical Consultation, Stellenbosch University is gratefully acknowledged for his assistance with the performing and interpretation of statistical analyses.

REFERENCES

Adin, D. M., Visick, K. L. & Stabb, E. V. 2008 Identification of a cellobiose utilization gene cluster with cryptic β-galactosidase activity in *Vibrio fischeri*. *Appl. Environ. Microbiol.* **74**, 4059–4069.

Al-Turki, A. & El-Ziney, M. G. 2009 Evaluation of commercial Colilert-18 Quantitray® Method by ISO techniques for enumeration and quantification of total coliforms and *Escherichia coli* in drinking-water of Buraidah, Saudi Arabia. *J. Appl. Sci.* **9**, 3357–3363.

American Public Health Association, American Water Works Association & Water Environment Federation 1996 *Standard Methods for the Examination of Water and Wastewater*. 19th edn., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.

American Public Health Association, American Water Works Association & Water Environment Federation 2012 *Standard Methods for the Examination of Water and Wastewater*. 22nd edn., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.

Anderson, I. C., Rhodes, M. & Kator, H. 1979 Sublethal stress in *Escherichia coli*: a function of salinity. *Appl. Environ. Microbiol.* **38**, 1147–1152.

Barnes, J. M. 2003 The impact of water pollution from formal and informal urban developments along the Plankenburg River on water quality and health risk. PhD dissertation. Stellenbosch University, South Africa.

Barnes, J. M. 2012 Water Supply and Quality. In: *Sustainable Stellenbosch – opening dialogues* (M. Swilling, B. Sebitosi & R. Odendaal eds.). SUNMeDIA, Stellenbosch, South Africa, pp. 204–214.

Bin Abdullah, M. 1990 On a robust correlation coefficient. *J. R. Stat. Soc: Series D (The Statistician)* **39**, 455–460.

Bland, J. M. & Altman, D. G. 1990 A note on the use of the intraclass correlation coefficient in the evaluation of agreement between two methods of measurement. *Comput. Biol. Med.* **20**, 337–340.

Davies, C. M., Apte, S. C. & Peterson, S. M. 1995 Possible interference of lactose-fermenting marine vibrios in coliform beta-D-galactosidase assays. *J. Appl. Bacteriol.* **78**, 387–393.

Dewitte, K., Fierens, C., Stöckl, D. & Thienpont, L. M. 2002 Application of the Bland–Altman plot for the interpretation of method-comparison studies: a critical investigation of its practice. *Clin. Chem.* **48**, 799–801.

DWAF (Department of Water Affairs and Forestry) 2004 Berg water management area: internal strategic perspective. DWAF Report No. PWMA 19/000/00/0304. Department of Water Affairs and Forestry, Pretoria, South Africa.

Edberg, S. C., Rice, E. W., Karlin, R. J. & Allen, M. J. 2000 Escherichia coli: the best biological drinking water indicator for public health protection. *J. Appl. Microbiol.* **88**, 106S–116S.

Elmund, G. K., Allen, M. J. & Rice, E. W. 1999 Comparison of *Escherichia coli*, total coliform, and fecal coliform populations as indicators of wastewater treatment efficiency. *Water Environ. Res.* **71**, 332–339.

Estelberger, W. & Reibnegger, G. 1995 The rank correlation coefficient: an additional aid in the interpretation of laboratory data. *Clin. Chim. Acta* **239**, 203–207.

Govender, T., Barnes, J. M. & Pieper, C. H. 2011a Housing conditions, sanitation status and associated health risks in four subsidized low-cost housing settlements in Cape Town, South Africa. *Habitat Int.* **35**, 335–342.

Govender, T., Barnes, J. M. & Pieper, C. H. 2011b Low-cost housing settlements in Cape Town – environmental water pollution from failing sanitation and poor housing contributing to diarrhoeal disease. *Am. J. Public Health* **101**, e4–e9.

IDEXX Laboratories, Inc. 2013 *Colilert-18 Test Kit*. http://www.idexx.com/resource-library/water/colilert-18-procedure-en.pdf (accessed 13 September 2013).

IWA (International Water Association) 2000 *Rapid Microbiological Monitoring Methods: The Status Quo*. IWA Publishing, London.

Krumperman, P. H. 1983 Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of faecal contamination of foods. *Appl. Environ. Microbiol.* **46**, 165–170.

Louw, D. B. 2008 Possible impact of water pollution in the Berg River irrigation region. Report compiled for the Upper Berg River Irrigation Board. Optimal Agricultural Business Systems, Paarl, South Africa.

Ludbrook, J. 2010 Confidence in Altman–Bland plots: a critical review of the method differences. *Clin. Exp. Pharmacol. Physiol.* **37**, 143–149.

Macy, J. T., Dunne, E. F., Angoran-Benie, Y. H., Kamelan-Tano, Y., Kouadio, L., Djai, K. A. & Luby, S. P. 2005 Comparison of two methods for evaluating the quality of stored drinking water in Abidjan, Côte d'Ivoire, and review of other comparisons in the literature. *J. Water Health* **3**, 221–228.

Maheux, A. F., Huppé, V., Boissinot, M., Picard, F. J., Bissonnette, L., Bernier, J.-L. T. & Bergeron, M. G. 2008 Analytical limits of four β-glucuronidase and β-galactosidase-based commercial culture methods used to detect *Escherichia coli* and total coliforms. *J. Microbiol. Meth.* **75**, 506–514.

Mannapperuma, W. M. G. C. K., Abayasekara, C. L., Herath, G. B. B., Werellagama, D. R. I. B. & Heinonen-Tanski, H. 2011 Comparison of bacteriological methods for detecting and enumerating total coliforms and *Escherichia coli* in water. *Res. J. Microbiol.* **6**, 851–861.

Noble, R. T., Leecaster, M. K., McGee, C. D., Weisberg, S. B. & Ritter, K. 2004 Comparison of bacterial indicator analysis methods in stormwater-affected coastal waters. *Water Res.* **38**, 1183–1188.

Paulse, A. N., Jackson, V. A. & Khan, W. 2007 Comparison of enumeration techniques for the investigation of bacterial pollution in the Berg River, Western Cape, South Africa. *Water SA* **33**, 165–174.

Paulse, A. N., Jackson, V. A. & Khan, W. 2009 Comparison of microbial contamination at various sites along the Plankenburg and Diep Rivers, Western Cape, South Africa. *Water SA* **35**, 469–478.

Paulse, A. N., Jackson, V. A., Khan, S. & Khan, W. 2012 Isolation and identification of bacterial pollutants from the Berg and Plankenburg Rivers in the Western Cape, South Africa. *Water SA* **38**, 819–824.

Percival, S. L., Walker, J. & Hunter, P. R. 2000 *Microbiological Aspects of Biofilms in Drinking Water*. CRC Press, Boca Raton.

River Health Programme 2005 *Greater Cape Town's rivers*. State of rivers report of the Department of Water Affairs and Forestry. Department of Water and Forestry, Pretoria.

WHO (World Health Organization) 1989 Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture. Report of a WHO Scientific Group (WHO Technical Report Series No. 778). WHO Press, Geneva.

WHO (World Health Organization) 2001 *Water Quality: Guidelines, Standards and Health*. IWA Publishing, London.

WHO (World Health Organization) 2013 *Water quality and Health Strategy 2013–2020*. WHO Press, Geneva.

Wohlsen, T., Bayliss, J., Bates, J., Gray, B., Johnson, S. & Schneider, P. 2008 An evaluation of membrane faecal coliform agar and Colilert-18 for the enumeration of *E. coli* bacteria in surface water samples. *J. Water Supply Res. T.* **57**, 569–576.

First received 20 February 2014; accepted in revised form 1 June 2014. Available online 8 July 2014