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On-site Bacteriological Test for Potable Water

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Summary

Bacteriological water quality from remote communities in Western Australia is difficult to test because of the distance to health laboratories, the need for aseptic sampling conditions, the lack of skilled workers and the high temperatures involved when transporting samples. Kits have been developed that can test for bacteria at the point of sampling. These have the advantage that they are cheaper, less complicated to operate, easier to interpret and take less time to complete than the standard laboratory procedures. The Colilert and Colisure kits were tested, with the aim to produce a video and explanatory brochure that can be used to train people in remote communities. It was concluded that both kits are adequate for the purpose of a portable testing kit, and that the Presence/Absence format was more suitable for use by the Environmental Health Worker.

Introduction

Routine testing of water quality is a public health requirement, and the National Guidelines for Drinking Water Quality in Australia recommend frequent sampling for microbial constituents, at least once a month, and preferably once a fortnight (NHMRC, 1987). Despite the acceptance of these guidelines, the majority of remote Aboriginal communities in Western Australia do not at present have any form of water testing. Of the 250 (approximately) communities in Western Australia, only 48 of them are funded to receive water testing and maintenance. Those communities receiving funding are tested every four to eight weeks, but the site for testing is immediately after the storage tank, which means that the sampled water may not be of the same quality as the drinking water. Those communities not receiving funding cannot get their water tested due to the high transportation costs, and the lack of access to a laboratory.

To improve this situation, research has focussed on the development and adaptation of portable water testing kits for use in remote Aboriginal communities. These would have the advantage of being faster, cheaper and able to be used by trained Aboriginal personnel. Turner and Mathew (1991) compared the Colilert, DelAgua, Millipore One Use Unit and Millipore Dipslides for their applicability for use in remote Aboriginal communities. They recommended the Colilert Presence/Absence as it is a one-step test that is easy to use, easy to interpret and was found to be as sensitive and reliable as the methods used by the State Health Laboratories of Western Australia. National evaluations by Edberg *et al.* (1988, 1989) and further evaluations by Clark and El-Shaarawi (1993), McFeters *et al.* (1993) and Covert *et al.* (1992) have supported the conclusion that the Colilert is equal in performance to the standard methods employed for the differentiation of total coliforms from water.

The choice of Colilert over the other portable test kits was primarily due to its ease in operation and interpretation. The Colilert is an MPN method designed for the differentiation and enumeration of total coliforms and *E. coli* from water in 24 hours. It consists of a dry blended reagent dispensed in a sterile five-tube MPN, six-tube MPN, ten-tube MPN or a 100ml P/A (Colilert promotional brochure). The water sample is added to the tubes, which are then incubated for 24 hours at 37^0C. Tubes receiving one or more of the total coliform organisms will show a colour change from clear to yellow, and the most probable number of organisms in the sample can then be estimated. Tubes receiving *E. coli* can be identified by fluorescence under a long-wave ultraviolet (U.V.). lamp.

It was not until a late stage in the study that an additional portable test became available; the Colisure. The Colisure is an MPN test that is very similar to the Colilert, dispensed in either a five tube MPN (20ml in each tube) or a 100ml P/A. After incubation for 24 hours at 37^0C, a colour change from yellow to red indicates the presence of one or more coliform organisms, and the presence of *E. coli* is identified by fluorescence under a long-wave U.V. lamp.

Unlike the Colilert, the Colisure P/A test has the reagent pre-dispensed in a sterile 100ml bottle. As the Colilert P/A test was recommended over the MPN for use in remote Aboriginal communities due to the reduced avenues for contamination, this aspect of the Colisure P/A was seen as an advantage over the Colilert P/A. The purpose of this study, then, was to evaluate the Colisure P/A to determine its potential as a field testing kit. The reliability and sensitivity of the Colisure P/A for the differentiation of total coliforms and *E. coli* from water was compared to that of the Colilert and to the methods employed by the State Health Laboratories of Western Australia (SHL).

In addition to this, the performance of the Colisure and Colilert P/A tests was compared to the results obtained by the methods used by the SHL after the sample water had been delayed for 21 hours. This is to simulate the field situation, where the water may be analysed immediately by the Colilert or Colisure, but transported to the SHL, thus involving a "delay" from the time of sampling to the processing time.

Aims:

1. To compare the reliability and sensitivity of the Colisure P/A test to differentiate total coliforms and *E. coli* from water, to that of the methods used by the State Health Laboratories of Western Australia.

2. To compare the reliability and sensitivity of the Colisure P/A test to differentiate total coliforms and *E. coli* from water, to that of the Colilert P/A.

3. To investigate the effect of a 21 hour delay on the results produced by the State Health Laboratories of Western Australia for the differentiation of total coliforms and *E. coli* from water, as compared to the immediate results from the Colisure P/A and/or the Colilert P/A.

4. To identify a portable test for the differentiation of total coliforms and *E. coli* from water that is appropriate for use in remote Aboriginal communities. Considerations for such a test are:
   - the reliability and sensitivity of the test, as compared to current procedures
   - the feasibility for correct operation of the test by personnel relatively untrained in bacterial analysis, and under conditions experienced in open-air sampling
   - the difficulty level for correct interpretation of the test
   - the amount and specificity of equipment needed to operate the test

Materials and Methods

Sample location and collection.

Approximately 30 samples of final effluent were collected from Subiaco Waste Water Treatment Plant. Final effluent from this source has received primary and secondary treatment only, and is collected daily for monitoring by the Plant. Samples were collected in either 100 or 500ml sterile polyethylene sampling bottles, which were transported immediately to the laboratory and either diluted and then analysed within 24 hours, or kept undiluted at 4^OC until

used. (All undiluted samples were used within 48 hours, with the exception of samples 7 & 8, which were used after storage at 4°C for 6 days.)

The effluent was diluted with either sterile or nonsterile deionised water to a range of concentrations. Nonsterile water contained no coliforms, and neither water contained chlorine. After vigorous stirring for approximately 30 seconds, the water was analysed for total coliforms and *E. coli* (at 37° C). All thirty samples that were analysed for total coliforms and *E. coli* with the Colisure P/A were laboratory simulations (see also Table one). Twenty of the thirty samples for the analysis of total coliforms and *E. coli* with the Colilert P/A were laboratory simulations, with the remaining ten samples consisting of field samples.

Standard quantitative methods were performed on all laboratory samples by the SHL as per regular samples. Each sample was processed twice; once immediately after dilution, (denoted SHL "immediate" processing), and once after a delay of 21 hours (denoted SHL "delayed" processing). The delayed samples were not chilled in order to simulate the worst case scenario of transportation from remote areas to the laboratory.

The Colilert method was tested in the field for a week. (The Colisure method was not able to be tested at this time as the product was unavailable for that trip.) Water samples were taken from: Punmu Reticulation, Punmu Bore, Tuu Tuu Bore, Yanderra Bore, Walgun, Jigalong Reticulation, Robertson Range, Number 7/85, Billinooka, Warralong Bore and Warralong Reticulation. These field samples formed ten of the thirty samples analysed for total coliforms and *E. coli* (see also Table one). SHL methods for the field samples involved taking 200ml of the water in a standard SHL sampling bottle, chilling and transportation to Perth for analysis (the sampling bottle contained sodium thiosulphate to neutralise residual chlorine). The bacterial analysis performed in the laboratory was for total coliforms and *E. coli*. Since the samples were sent to Perth for standard analysis, there was a delay in time from when the sample was taken and when the sample was processed by the SHL methods. These samples will be denoted "field" to distinguish from the laboratory samples.

Table 1: Outline of Sampling Regime

Test	Sample Number	Place of Sample	Time of analysis
Colisure P/A	1 to 30	laboratory	immediate
Colilert P/A	1 to 10; 21 to 30	laboratory	immediate
Colilert P/A-field	11 to 20	field	immediate
SHL-immediate	1 to 30	laboratory	immediate
SHL-delay	1 to 30	laboratory	21hr, unchilled delay
SHL-field	11 to 20	field	undefined, chilled delay

Colilert (CL) Testing.

100ml Colilert P/A tubes of defined substrate were purchased from **Crown Scientific.** Diluted samples were shaken, then a 100 ml sample together with a P/A substrate were added to a 200 ml State Health Laboratory sampling bottle. The substrate was dissolved through agitation. The test was then incubated for 24 hours at 37°C. The presence of total coliforms or thermotolerant coliforms was indicated by the development of a yellow colour from the colourless solution. The presence of *E. coli* was indicated by fluorescence under a long-wavelength (366nm) UV light.

Colisure (CS) Testing.

100ml Colisure P/A bottles with defined substrates were purchased from **Millipore**. Diluted samples were shaken, then a 100 ml sample was added to the P/A bottle. The substrate was dissolved through agitation. Similarly to the Colilert and following the same procedure, the test was then incubated for 24 hours at 37°C. The presence of total coliforms or thermotolerant coliforms was indicated by the development of a red colour from the yellow solution. The presence of *E. coli* was indicated by fluorescence under a long-wavelength (366nm) UV light.

Methods Employed by the SHL

Total Coliform Count. 100 ml of sample water was filtered through a 0.45mm membrane and placed on membrane enriched lauryl sulphate agar for an initial incubation of 4-6 hours at 30°C, and then 14 hours at 37°C. Positive (yellow) colonies were inoculated into Durham's (gas) tubes with tryptone lactose formate broth and incubated for 48 hours at both 37°C and 44°C. Gas production in the tubes incubated at 37°C confirmed the presence of total coliforms.

E. coli Count. Similar to the total coliform count, except that all incubation is carried out at 44°C instead of 37°C. 100 ml of sample water was filtered through a 0.45mm membrane and placed on membrane enriched laurel sulphate agar for an initial incubation of 4-6 hours at 30°C, and then 14 hours at 44°C. Positive (yellow) colonies were inoculated into Durham's (gas) tubes with tryptone lactose formate broth and incubated for 48 hours at both 37°C and 44°C. Gas production in the tubes incubated at 44°C confirmed the presence of thermotolerant coliforms. Kovak's reagent was added to the tubes positive for thermotolerant coliforms. A positive indole reaction confirmed a presumptive *E. coli*..

Test Design

Enough sample water was collected each day for four analyses to be performed; Colilert P/A test for total coliforms and *E. coli*, Colisure P/A test for total coliforms and *E. coli*, SHL standard methods for total coliforms and *E. coli* and a 21 hour delayed SHL analysis for total coliforms and *E. coli* (see table two).

Table 2: Summary of Test Design

Test	Sample volume	Time	Analysis
Colilert P/A	100ml	immediate	total coliform, *E. coli*
Colisure P/A	100ml	immediate	total coliform, *E. coli*
SHL immediate	200ml	immediate	total coliform, *E. coli*
SHL delayed	200ml	21 hour delay	total coliform, *E. coli*

Statistical Analyses.

Quantitative MF data was transformed into P/A data for ease of comparison. Samples which had total coliforms or *E. coli* in numbers ≥1/100ml were considered to be P/A positive, and samples with bacteria in numbers ≤1/100ml were considered to be P/A negative (Lewis and Mak, 1989). The data were arranged in 2x2 contingency tables of positive and negative results for each test, and analysed by several statistical methods. Firstly, the index of agreement was calculated; this gives the level of agreement between the tests, but does not evaluate the amount of agreement that could have been due to chance. The index of agreement ranges between 0 and 1, with 0 representing no agreement and 1 representing complete agreement. The Kappa

statistic is a "chance-corrected" adjustment to the index of agreement, that ranges between -1 and +1; +1 indicates complete agreement, 0 indicates no agreement and -1 indicates less agreement than expected due to chance (Edberg *et al.*, 1989). The D statistic evaluates the homogeneity between tests containing binary data, and approximates a chi-square distribution (Clark and El-Shaarawi, 1993). The Pearson's chi-square tests for independence of association between tests, and the McNemar's chi-square analyses the proportion of results that are not in agreement, to determine if one combination of results is found in greater proportion to the other than would be expected due to chance alone (Bishop *et al.*, 1975).

Results

A total of 40 samples were taken for bacterial analysis, although no more than 30 were analysed in tandem. Results from the statistical analyses are shown in table three.

Colisure - SHL comparison

The level of agreement between the Colisure and the SHL-immediate and delayed methods was very high, ranging from 0.67 to 0.93 for differentiation of both total coliforms and *E. coli*. The Kappa statistic, however, indicated that the level of agreement between the Colisure and the SHL-delayed methods for the differentiation of *E. coli* was much lower than for the others, with a value of 0.34 compared to 0.60 - 0.84. The D statistic indicated that there was homogeneity between the Colisure P/A and the SHL-immediate and delayed methods, with none of the values significant at p=0.01 or p=0.05 level. Likewise, Pearson's chi-square indicated that none of the tests had a significantly different detection rate for total coliforms or *E. coli* , and McNemar's chi-square showed that the rate of false-positives and false-negatives was the same for both tests.

Table 3: Assessment of the agreement between the Colilert, Colisure and SHL methods for the differentiation of total coliforms and *E. coli* . Results for *E. coli* are in italics.

Type of Test	index of agreement	Kappa statistic	D statistic	Pearson's chi	McNemar's chi
Colisure/ SHL immediate	0.93, *0.80*	0.84, *0.60*	0.31, *0.06*	0.73, *0.41*	0.50, *0.17*
Colisure/ SHL delayed	0.83, *0.67*	0.63, *0.34*	0.28, *1.62*	0.40, *0.13*	0.00, *0.90*
Colilert/ SHL field	0.86, *0.86*	0.73, *0.73*	0.27, *0.27*	0.54, *0.54*	0.25, *0.25*
Colilert/ SHL delayed	0.84, *0.74*	0.68, *0.48*	0.10, *0.90*	0.47, *0.80*	0.00, *0.80*
Colilert/ Colisure	0.90, *0.95*	0.79, *0.90*	0.40, *0.10*	0.66, *0.81*	0.50, *0.00*

Colilert - SHL comparison

The results for the Colilert test are very similar to those of the Colisure. The agreement levels ranged from 0.74 to 0.86, and the Kappa statistic indicated that the performance was not as close for the differentiation of *E. coli* by the Colilert and the SHL-delayed methods, with a value of 0.48 compared to 0.68 - 0.73. The D statistic showed homogeneity between the tests at a significance level of p=0.05 or p=0.01. The Pearson's chi-square indicated that there was no significant difference in detection levels between the Colilert and the SHL-immediate and delayed methods, and the McNemar's chi-square showed no difference in rates of false-positives and false-negatives between the tests.

Colisure - Colilert comparison

The level of agreement between the Colilert and the Colisure is very high, with a value of 0.90 for total coliform differentiation, and a value of 0.95 for *E. coli* differentiation. The Kappa statistic supports this, with values of 0.79 for the former comparison, and 0.90 for the latter. The D statistic indicates homogeneity between the two tests at a significance level of p=0.05 or p=0.01. The Pearson's chi-square shows no significant difference between the detection levels of the two tests, and the McNemar's chi-square indicates that there is no significant difference in the rate of false-positives and false-negatives between the two tests.

Discussion

The purpose of this study was to compare the Colisure P/A test with the Colilert P/A test and the SHL methods, with the aim of identifying the most appropriate test for total coliform and *E. coli* differentiation in remote Aboriginal communities. A laboratory simulation was performed, with each of the tests analysing water that had been spiked with waste-water effluent. It was evident that all three tests perform equally well in terms of reliability and sensitivity. Although the results aren't presented above, the Colilert and the Colisure P/A tests were able to demonstrate the presence of 1 total coliform or *E. coli* bacteria per 100ml, as identified by the SHL methods.

It is interesting to note that the only differences in agreement occurred when the P/A tests were compared to the SHL-delayed methods for the differentiation of *E. coli* . Although these differences were not significant, it suggests that a delay in processing the sample may change the numbers of the bacteria within the sample. This has important implications for water testing in remote communities, as routine testing requires the sample to be transported to the laboratory, thus incurring a delay between sampling time and testing. Turner and Mathew (1991) looked at this problem in greater detail, by investigating the change in bacterial populations under three different storage conditions, and by looking at the temperature change of the sample water if it is stored for transporting as recommended. They found that under the "worst case scenario" (bottles not chilled prior to sampling, stored in esky at 42^{o}C with ice block , ice block replaced after 6 hours), growth of bacteria was likely to occur. Under the "best case scenario" (bottles chilled, stored in esky at 22^{o}C with ice block , ice block replaced after 6 hours), bacterial numbers appeared to reduce. When the temperature of the water stored under the best conditions was examined over a 24 hour period, they found that the temperature did not fall to the recommended 4^{o}C, and was greater than 10^{o}C for the majority of the transporting time. This indicates that the current procedures for transporting sample water are inadequate, and, particularly under the extreme conditions found in the Northwest of Western Australia, that the sample water may not represent the water the community is drinking. The Colilert and the Colisure, however, do not have these limitations. As they are one-step, portable tests, they may be performed at the sampling site, thus reducing the need for transportation of the sample.

A further advantage of the Colilert and the Colisure is their substrate specificity. The Colilert and the Colisure are derived from technology originally designed to identify microbes on the basis of their constituent enzymes (Edberg *et al.*, 1988), whereby the nutrient portion of the test is simultaneously the indicator portion. Basing differentiation of the total coliforms and *E. coli* on enzymic reactions has the advantage of being specific -- only those bacteria possessing the enzymes for growth will produce the colour change -- and more rapid than the traditional culturing methods (Feng and Hartman, 1982). Furthermore, Covert *et al.* (1989) and Edberg *et al.* (1988, 1989) found that the performance of the Colilert was not affected adversely by the presence of heterotrophic bacteria in excess of 500 per ml.

The Colilert and the Colisure also met the additional criteria necessary for recommendation for use in remote Aboriginal communities. The tests are very simple to operate and interpret; the

sample is poured into the bottle containing the reagent, and a colour change indicates the presence of indicator bacteria. This is an important consideration, as the people most likely to use the tests will have minimal training in bacterial analysis. Membrane filtration requires the differentiation of one type of culture from another, which can be difficult as the character of cultures can vary significantly in size, definition and colour (Turner and Mathew, 1991), and the presence of other bacteria can overlap or mask the growth of the coliforms.

A related criteria is the avenues for contamination. The tests can be operated in the open air in high winds with a low risk of contamination, compared to the risk involved with membrane filtration under the same conditions. The Colilert and the Colisure operate by simply pouring the sample into a sterile bottle containing the reagent, hence the term "one-step operation". Membrane filtration, however, involves placing a membrane in the filter, filtering the sample, and then placing the membrane onto the nutrient medium. The possibility of contamination is greatly increased by the number of manipulations and delicate tasks required by the membrane filtration technique. As mentioned earlier, the Colisure comes with the reagent pre-dispensed in the sterile sampling bottle, further reducing the avenues for contamination.

The Colilert and the Colisure require much less time to process the sample (24 hours), compared to the SHL methods (68 hours). This is as a direct result of the specificity of the enzyme reactions the Colilert and Colisure are based upon. If the travelling time of the sample to the laboratory, and the time taken to send the results back to the community is taken into account, the Colilert and the Colisure are nearly five times faster in analysing community water than the current procedures.

Finally, the Colilert and the Colisure can differentiate total coliforms and *E. coli* at a single temperature. This is advantageous in remote communities as it reduces the number of samples that need to be taken and does not require more than one incubator. This, in turn, reduces the cost of the analysis.

Conclusions

1. The Colisure P/A is as reliable and sensitive for the differentiation of total coliforms and *E. coli* from water as the methods used by the State Health Laboratories of Western Australia.

2. The Colisure P/A is as reliable and sensitive for the differentiation of total coliforms and *E. coli* from water as the Colilert P/A.

3. The Colilert P/A and the Colisure P/A are appropriate portable water testing kits for use in remote Aboriginal communities, as they meet the following criteria:

   * they are as reliable and sensitive as the methods used by the SHL of Western Australia;
   * they are easy to operate and provide the lowest risk of external contamination;
   * the results from the Colilert and Colisure are easy to interpret;
   * only one temperature is needed to differentiate both total coliforms and *E. coli* , and thus only one incubator is necessary.

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