A sensitive presence/absence test kit for detection of coliforms in drinking water
Ashish Tambi, Urmila Brighu and A. B. Gupta

ABSTRACT

Coliforms are the most widely accepted bacterial indicator of fecal pollution in water. Several commercially available portable kits make it possible to carry out on-site water quality testing, but are usually costly and often require technical expertise to operate. In developing countries like India, presence/absence test kits like the H₂S test kits are commonly used for routine microbiological water quality examination. H₂S test kits require an incubation time of more than 24 hours and often give false positive results. In this research work, we have developed a low-cost and sensitive test kit (ColiPAT) for the detection of coliforms including *Escherichia coli* in drinking water. The kit can detect very low contamination levels down to 2 coliforms/100 mL within 18 hours at 35 °C. The ColiPAT kit does not lose its sensitivity in the typical indoor temperature range of 27 °C to 35 °C. ColiPAT is also affordable and easy to perform so that local populace can conduct the test independently. This paper presents the results of sensitivity and specificity analysis of a ColiPAT kit and its comparison with the H₂S test kits as standardized using Colilert method.

Key words | Colilert, drinking water, *E. coli*, most probable number, presence/absence test kit, total coliforms

INTRODUCTION

Microbial contamination of drinking water is a major concern for public health in developing countries. About 1.1 billion people globally drink unsafe water, and the vast majority of water-borne disease in the world, such as diarrhea, is due to unsafe water, sanitation and hygiene (WHO 2004). It is practically not feasible to carry out routine monitoring of drinking water for the detection of pathogenic bacteria. Therefore, an accepted alternative is to detect indicator bacteria that always co-exist in association with pathogens of fecal origin (Edberg et al. 2000). Total coliforms and *Escherichia coli* are used worldwide as an indicator of fecal contamination of drinking water and recreational bathing water (Rompré et al. 2002). *E. coli* is the most suitable indicator of fresh fecal contamination for routine water quality monitoring in developing countries (Barrell et al. 2000; Skrabar et al. 2004).

Coliforms include members of the family Enterobacteriaceae, e.g., *Escherichia* spp., *Enterobacter* spp., *Klebsiella* spp., and *Citrobacter* spp. Coliform group members are aerobic and facultative anaerobic, Gram-negative, non-spore forming rod-shaped bacteria that ferment lactose with gas production within 48 h at 35 °C (APHA 1998). Coliforms are present in large numbers in feces of humans and other warm-blooded animals and therefore accepted as an indicator of fecal contamination. Thus, if fecal pollution has entered drinking water, it is likely that these bacteria will be present, even after significant dilution (Stevens et al. 2005). Some of the coliform bacteria have a soil origin but potable water is not a natural habitat for them (Rompré et al. 2002). Therefore, the presence of coliforms in drinking water is considered as a potential risk or indicative of microbiological water quality. Total coliform count in treated water may indicate treatment inadequacy, loss of disinfectant, breakthrough, and intrusion of contaminated water into a potable water supply (McFeters et al. 1986; Geldreich et al. 1992; Clark et al. 1996).
For qualitative and quantitative detection of total coliforms and E. coli, the multiple tube fermentation method was traditionally used (Hörman & Hänninen 2006). In recent years, enzymatic tests for the detection of total coliforms and E. coli such as Colilert® have gained popularity (Edberg & Edberg 1988; Brenner et al. 1993; Chuang et al. 2011). Polymerase chain reaction (PCR)-based methods are also available for direct detection of E. coli (Bej et al. 1991). Commercially available portable kits that make it possible to carry out on-site water quality testing are usually expensive and require technical expertise to operate. The presence/absence (P/A) test is an inexpensive procedure for a rapid qualitative determination of bacterial indicators in drinking water (Ramteke et al. 1994). The P/A test kits have the advantage where resources and time factors are a major constraint. Also, if a significant number of water samples are expected to be free of fecal contamination, then it could be a waste of resources to conduct a quantitative analysis of each sample. Some test kits meant primarily for quantitative analysis are also available in the P/A format such as Colilert, Aquagenx, etc., but these are relatively costly. The H2S test kit is the most commonly used P/A test in India, and can be used in the field without any skilled personnel (Manja et al. 1982; Tambekar et al. 2007). However, some drawbacks of the H2S test kits are false positive results, delayed incubation time up to 48 h and low sensitivity (Mosley & Sharp 2005). Therefore, there is a need to develop a new test kit for coliform detection that is sensitive, low-cost, and easy to use, especially in developing countries such as India.

The primary objective of this research work was to develop a sensitive and low-cost kit for identification of total coliforms and E. coli in drinking water. A simple kit named as ‘ColiPAT’ was developed at PHE Laboratory, MNIT, Jaipur (Rajasthan), India. The medium used in the ColiPAT kit is a slight modification of the MacConkey medium (MacConkey 1905), a selective medium for coliform detection. Secondary treated sewage predominantly consists of coliform bacteria including Klebsiella spp., Enterobacter spp., E. coli, and Citrobacter spp., which constitute about 75% of total microorganisms present in sewage-contaminated water (Kumar et al. 2012). For this purpose, the substrate used in the ColiPAT kit was especially chosen so that it can cater to a much larger population of these bacteria in order to enhance the sensitivity of the ColiPAT kit. The sensitivity of the ColiPAT kit was determined by comparing the results with the Colilert-18 h Quanti-tray method. The Colilert-18 h Quanti-tray is a standard test for accurate qualitative and quantitative determination of coliform bacteria in both chlorinated and non-chlorinated water samples (Fricker et al. 1997; Niemla et al. 2003). The sensitivity of the ColiPAT kit was also compared with the H2S kit (Hi-media K020), a commercially available P/A test kit for coliform detection, frequently used in developing countries such as India.

**MATERIALS AND METHODS**

**Preparation of P/A kit (ColiPAT)**

Keeping in mind the characteristics of coliforms, a microbiological growth medium specific to coliform bacteria was prepared in the laboratory. Analytical grade reagents of recognized companies were used in the preparation of medium for the ColiPAT kit. Components of the ColiPAT kit are lactose (carbon source), peptone (serves as proteins, vitamin, and nitrogen source), bromocresol purple (as a pH indicator), crystal violet (for inhibiting the growth of Gram-positive bacteria), and sodium chloride (for maintaining osmotic balance). Lactose and peptone were obtained from Merck Specialities Pvt. Ltd, Mumbai, India, whereas sodium chloride, crystal violet, and bromothylol purple were obtained from Thermo Fisher Scientific India Pvt. Ltd, Mumbai, India. In a sterile capped bottle, 5 mL of autoclaved medium was poured and labeled as the ‘ColiPAT kit’. The composition of the ColiPAT medium is shown in Table 1. The medium was prepared by modifying the MacConkey medium (MacConkey 1905) with the substitution of neutral red by bromocresol purple and bile salt by crystal violet. Bromocresol purple is more sensitive in recording pH variation in the medium as compared with neutral red. Neutral red has an inhibitory effect on the growth of some Gram-negative bacteria (Childs & Allen 1955). Therefore, it was substituted by bromocresol purple in the above medium. The presence of coliform bacteria in the sample using this kit is indicated by a change in color of the medium from purple to yellow.
Preparation of samples

Secondary treated sewage was used to prepare controlled 10-fold serially diluted samples. Sewage was obtained from STP of MNIT, Jaipur, which is based on a fixed film process termed Rotating Biological Contactor. These serially diluted samples were filled aseptically in sterile vials used for test analysis. All experiments were carried out in triplicate to assess method variability and to minimize handling errors. Care was taken in preparation and transfer of samples so that they did not contaminate by other means (APHA 1998).

Sensitivity and coliform detection limit analysis of ColiPAT kit

Colilert-18 h Quanti-tray/2000 is accepted as an efficient and standard method for quantitative analysis of coliform bacteria in water (Fricker et al. 1997; Niemla et al. 2003). Therefore, to determine the coliforms detection limit and sensitivity of the ColiPAT kit prepared in the laboratory, Colilert-18 h method was used. The IDEXX Quanti-tray uses a semi-automated quantification method based on the most probable number (MPN). The MPN method uses multiple qualitative (P/A) data points to generate a maximum probability coliform count per 100 mL value, given by standard MPN table. The Quanti-tray/2000 provides bacterial counts (of total coliform and *E. coli*) as low as 1 MPN/100 mL and up to 2,419 MPN/100 mL.

Coliforms detection limit of the ColiPAT kit was also compared with that of the *H₂S* test kit (HiMedia-K020). The *H₂S* test kit (HiMedia-K020) used in the present study simultaneously detects *Salmonella*, *Citrobacter*, and *E. coli*. The *H₂S* test identifies the presence of *H₂S*-producing bacteria, associated with fecal contamination in 20 mL of the water sample added to the *H₂S* test kit containing a paper strip soaked with the medium in a glass bottle. Blackening of media detects the presence of fecal coliform in *H₂S* kits (positive test) (HiMedia 2009).

Controlled 10-fold serially diluted samples were prepared using secondary treated sewage. A total of 100 mL of serially diluted samples was added to the Quanti-tray with Colilert medium, and 20 mL of the same sample was added into ColiPAT kits and *H₂S* kits (HiMedia-K020). All the kits were incubated at 35°C and room temperature (27°C) for 18 h, 24 h, and 36 h. The change in color of the medium was observed after incubation and the number of coliforms (quantitative) was determined from Colilert-Quanti-trays. ColiPAT kits with a change in color from purple to yellow were considered as positive for total coliforms and *E. coli*.

In the second phase of the experimental study, drinking water samples were analyzed using the ColiPAT kit and the Colilert-18 h test. The sensitivity (true positive rate) and specificity (true negative rate) of the ColiPAT kit were analyzed by comparing the results with the standard Colilert-18 h test results. About 125 drinking water samples were collected from various sources, such as handpump, borewell, dugwell, tanker supply systems, and source reservoir (municipal or community service provider). These samples were collected from the rural areas of Jaipur District (Rajasthan, India). In some cases where the direct supply water from the source was not available, samples were collected from individual households. All the samples were collected in the ColiPAT kits and also in sterile sample bottles. Colilert-18 h test was also conducted simultaneously on the same sample in the laboratory so as to determine the number of coliforms in the drinking water samples.

Streak plate method was also used to further confirm that the change in color from purple to yellow (positive test) in ColiPAT kits was due to the presence of coliforms only. The eosin methylene blue (EMB) agar medium was used for the streak plate method, which is a selective and differential medium for the isolation and differentiation of Gram-negative enteric bacilli (*Enterobacteriaceae* and several other Gram-negative rods). *E. coli* can be identified with EMB agar based on the occurrence of a metallic green sheen that appears on the surface of the bacterial colonies and other

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**Table 1 | Composition of the ColiPAT medium**

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone (peptic digest of animal tissue)</td>
<td>40 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>20 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>10 g</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.002 g</td>
</tr>
<tr>
<td>Bromocresol purple</td>
<td>0.02 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1,000 mL</td>
</tr>
<tr>
<td>Final pH 7.2 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>
Gram-negative bacteria appear as dark purple, dark centered mucoid colonies. A loopful of culture was taken from the positive ColiPAT kits and then streaked over the surface of EMB agar plate. Petri-plates were kept at 35 °C in an incubator and observed after 24 h of incubation.

**Sensitivity and specificity analysis of ColiPAT and H₂S kit using spread plate method**

Sensitivity and specificity of ColiPAT and H₂S kits were also assessed using the spread plate method. In this method, 1 mL of water sample was transferred to the center of an agar plate and spread evenly over the surface with a sterile bent glass-rod. Petri-plates were kept at 35 °C in an incubator and observed after 24 h of incubation for the presence of colonies of bacteria. The H₂S kit (HiMedia-K020) used in the present study detects *Salmonella*, *Citrobacter*, and *E. coli* (HiMedia 2009). Therefore, for this study, xylose lysine deoxycholate (XLD) agar medium, EMB agar medium, and Simmon’s citrate agar medium were used for enumeration of bacteria by the spread plate method. XLD agar medium was used for the identification and enumeration of *Salmonella* bacteria. EMB agar was used for the identification and enumeration of coliforms such as *E. coli*, *Enterobacter* spp., and *Klebsiella* spp. Simmon’s citrate agar medium was used for the identification of *Citrobacter* spp. All the dehydrated culture media were obtained from HiMedia Laboratories Pvt. Ltd, Mumbai, India.

A total of 20 mL of water sample was added to both ColiPAT and H₂S kits, which were incubated at 35 °C for 24-48 h. The change in the color of the medium in the two kits was corroborated by the results of the spread plate method.

**RESULTS AND DISCUSSION**

**Coliforms detection limit of ColiPAT kit and H₂S kit**

Coliforms detection limit of ColiPAT and H₂S kits was evaluated by detecting the P/A of coliform bacteria against the Colilert-18 h method. Table 2 summarizes the results for coliforms detection limit of ColiPAT and H₂S kit at different incubation temperatures and time periods. All tests were carried out in triplicate.

ColiPAT kit was able to detect coliform down to the level of 2 coliforms/100 mL within 18 h, whereas the H₂S kit was able to detect a count of 30 coliforms/100 mL after 36 h of incubation time. In another study, it was concluded that the sensitivity of the H₂S strip test for total coliform detection was ≥30 total coliform count/100 mL for 48 h of incubation (Kumar et al. 2012). Almost similar results were obtained in our study. These results showed that the ColiPAT kit has better coliforms detection limit as compared with the H₂S kit, even at room temperature.

**Sensitivity and specificity analysis of ColiPAT kit using Colilert-18 h method**

About 125 drinking water samples collected from various sources from rural areas of Jaipur were analyzed using the Colilert-18 h test and the ColiPAT kit. A total of 79 samples (63.20%) were found to be contaminated with coliform bacteria when samples were analyzed by the Colilert-18 h method. When the same samples were analyzed using the ColiPAT kit, 77 samples (61.6%) were found to contain coliform bacteria. Colonies of coliform bacteria were also obtained on the EMB agar plate when culture taken from all the positive ColiPAT kits was streaked. A comparison of the results obtained by analysis of water samples using two test kits is summarized in Table 3. The sensitivity (true positive rate) and specificity (true negative rate) of the ColiPAT kit compared with the standard Colilert-18 h test was found to be 97.47% and 100%, respectively. The results obtained from the Colilert-18 h method and ColiPAT kit was analyzed by applying the McNemar’s test for paired samples at 95% confidence interval (α = 0.05). The two-tailed p-value of the McNemar’s test was 0.48, indicating that the ColiPAT results and the Colilert-18 h results were not significantly different for coliform detection in drinking water.

**Sensitivity and specificity analysis of ColiPAT and H₂S kit using the spread plate method**

Analysis of 65 drinking water samples collected from various sources from rural areas of Jaipur was used to determine the
sensitivity and specificity of ColiPAT and H2S kits by the spread plate method. Out of 65 samples analyzed, 24 (36.92%) samples were found to be positive for coliforms by the spread plate method. The number of samples indicating positive result by ColiPAT and H2S kits was 24 (36.92%) and 30 (46.15%), respectively. The sensitivity and specificity of the H2S kit using the spread plate method were found to be 66.67% and 75%, respectively, whereas the sensitivity and specificity for the ColiPAT kit were 100%. These results indicate that the ColiPAT is more accurate for coliform detection in drinking water than the H2S kit.

**Cost of ColiPAT kit**

The ColiPAT kit proved to be a cost-effective method for coliform detection compared with the other P/A test kits (Colilert-18 h cost per sample: Rs. 750 (10.9 USD, retail price in Jaipur (Rajasthan), India). Aquagenx test kit cost per sample: Rs. 682.30 (10 USD, quotation received on 5 February 2016). The cost of testing with the ColiPAT kit would be around Rs 25–30 (<0.5 USD) per sample (including the cost of chemicals, bottle, sterilization, and packaging) whereas the cost of the H2S kit (HiMedia-K020) is Rs. 44 per sample (retail price in Jaipur (Rajasthan), India (0.64 USD)).

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**Table 2** | Coliforms detection limit of ColiPAT kit and H2S kit using Colilert-18 h method

<table>
<thead>
<tr>
<th>MPN index (Colilert-18 h)</th>
<th>ColiPAT kit</th>
<th>H2S kit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35°C</td>
<td>27°C</td>
</tr>
<tr>
<td></td>
<td>18 h</td>
<td>24 h</td>
</tr>
<tr>
<td>1,011.2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>960.6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>658.6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>313</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>259.5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>172.3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>121</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>107.7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>80.9</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>53.8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>50.5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14.6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

* + = positive; − = negative.

**Table 3** | Comparative analysis of Colilert-18 h and ColiPAT kit results

<table>
<thead>
<tr>
<th>MPN index</th>
<th>Colilert-18 h</th>
<th>ColiPAT Kit-18 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>0–2</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>2–20</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>20–60</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>60–100</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>100–300</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>300–600</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>600–900</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>900–1,200</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>125</td>
<td>79</td>
</tr>
</tbody>
</table>
CONCLUSION

In this study, we have developed a kit (ColiPAT) for detection of total coliforms and *E. coli* in water samples. The kit prepared in the laboratory at MNIT Jaipur for the qualitative analysis of total coliforms and *E. coli* in drinking water provides a simple and comparatively low-cost method. It is precise and rapid compared with other commercially available qualitative test kits such as the H₂S test kit. The effectiveness of the kit was confirmed with the Colilert-18 h method and spread plate method, the results of which were similar to the color change of the ColiPAT medium. When compared with the standard Colilert test, ColiPAT kit results were significantly similar to the Colilert-18 h method. The sensitivity and specificity of the ColiPAT kit were found to be 97.47% and 100%, respectively. It was observed that the kit was highly sensitive with a low detection limit of 2 coliforms/100 mL within 18 h at room temperature (27–35 °C). It was also concluded that the ColiPAT kit is better than other P/A test kits as it can detect coliforms within 18 h whereas the most commonly used H₂S test kit requires 24–48 h of incubation time. Therefore, ColiPAT kit is simple, sensitive, specific, inexpensive, and reliable for the screening of bacteriological quality of water where resources, manpower, and laboratory facilities are inadequate. The study suggests that the ColiPAT test kit is a more suitable alternative P/A test kit useful for field application in the monitoring of drinking water quality, particularly during outbreaks of water-borne infectious diseases.

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