Wat. Res. Vol. 25, No. 10, pp. 1279–1283, 1991
Printed in Great Britain.

0043-1354/91 $3.00 + 0.00

COMPARISON OF THE PRESENCE–ABSENCE (P–A) TEST AND CONVENTIONAL METHODS FOR DETECTION OF BACTERIOLOGICAL WATER QUALITY INDICATORS

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(First received February 1990; accepted in revised form March 1991)

Abstract—Fifty samples of water, comprising sewage-polluted river water and artificially-contaminated spring water, were analyzed in order to compare the P–A test and the conventional membrane filter and multiple tube methods for the detection of total and fecal coliforms, fecal streptococci, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and sulfite reducer clostridia. The two presumptive media proposed for the P–A test, the MacConkey broth with tryptone (MacConkey–PA) and the lactose–lauryl tryptose–tryptone broth (STM–PA), were also compared. The P–A test using STM–PA medium showed better results than the MacConkey–PA when water with lower levels of contamination (artificially-contaminated spring water) was analyzed. For coliform detection the P–A test with 48 h of incubation showed better results than with an incubation period of 5 days. For the detection of other indicators such as *Ps. aeruginosa*, sulfite reducer clostridia, and fecal streptococci in water with low levels of contamination the incubation period should be extended, as very different results after 48 and 120 h incubation were obtained with percentages of positivity being respectively, for *Ps. aeruginosa*, 48%, 76%; for sulfite reducer clostridia, 16%, 48%; and, for fecal streptococci 24%, 92%. Similar results were obtained for sewage polluted river water. *Staphylococcus aureus* was not detected by the P–A test.

The P–A test using the STM–PA medium showed a good performance and is a promising tool for the evaluation of bacteriological quality of drinking water, especially in tropical climates where the coliform indicator may not be adequate. This test could overcome this problem, allowing the use of a multiple indicator approach.

Key words—presence–absence (P–A) test, pollution indicators, coliforms, fecal streptococci, sulfite reducer clostricia, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

INTRODUCTION

In order to evaluate the quality of drinking water two quantitative methods are traditionally used and accepted for the detection of fecal pollution indicators, the membrane filter and the multiple tube technique. In 1967, Clark proposed a qualitative method, the presence–absence (P–A) test, for the detection of total (TC) and fecal coliforms (FC) and fecal streptococci (FS).

The P–A test is a simplification of the multiple tube technique, since it uses a single bottle which contains the presumptive medium where 100 ml of the sample are inoculated (APHA, 1989). Through the utilization of specific confirmatory media, the P–A test could also be used for qualitative detection of sulfite reducer clostridia (SRC), *Pseudomonas aeruginosa* (Pa), *Staphylococcus aureus* (Sa), fecal streptococci (FS), *Aeromonas* sp., besides total and fecal coliforms (Ministry of Environment—Canada, 1983; APHA, 1989). This test requires less effort and is cheaper than the conventional methodology of membrane filter and multiple tube techniques (Clark, 1967; Geldreich, 1987; Martins and Pellizari, 1990).

Clark *et al.* (1982) suggested an alternative medium (STM–PA) for the presumptive step of the P–A test which consisted of lactose–lauryl tryptose–tryptone broth to replace the modified MacConkey broth formulation (MacConkey–PA). This medium is indicated in *Standard Methods* (APHA, 1989).

The P–A test was evaluated by Clark and Pagel (1977) Clark *et al.* (1982), Jacobs *et al.* (1986), Pipers *et al.* (1986) and Edberg *et al.* (1989). These authors compared this test with the membrane filter and/or multiple tube techniques only for the detection of coliforms.

Clark (1967, 1969, 1980), Clark and Vlassoff (1973), and Martins and Pellizari (1990), using the P–A test, detected, in addition to the coliforms, other microorganisms such as fecal streptococci, *Pseudomonas aeruginosa*, *Aeromonas* sp. and sulfite reducer clostridia (SRC).

The P–A test is usually proposed for the detection of Gram-positive and Gram-negative bacteria, but in most of the literature consulted, evaluation studies were based on coliform detection. According to Clark (1982), Gram-positive microorganisms such as fecal streptococci, *S. aureus* and *Clostridium perfringens*

were isolated by the P–A test using the alternative medium (STM–PA), although there is a need for further tests to determine whether this medium provides optimal conditions for their isolation.

Recently a problem has emerged concerning the reliability of coliforms as indicators of bacteriological water quality. For example, some researchers have reported the isolation of total coliforms and even *E. coli* from so-called pristine water in tropical climates (Hazen, 1988) or that fecal streptococci and fecal coliforms have been reported as naturally occurring in Hawaii's freshwater streams (Fujioka *et al.*, 1988).

To overcome this problem a multiple indicator approach should be used and this has been the concern of the FAO/WHO (1987). For the evaluation of natural mineral waters, their standards consider total coliforms, *E. coli*, group D streptococci, sulfite reducer clostridia and *Pseudomonas aeruginosa*, besides bacteria plate counts.

The purpose of this study was to study three aspects: (a) comparison of media for the P–A test; (b) evaluate the multiple test concept of the Clark P–A procedure; and (c) compare this concept to *Standard Methods* multiple tube and membrane filter tests for individual indicator systems.

MATERIALS AND METHODS

Samples

The P–A test is recommended for detecting the occurrence of coliforms and other indicators in drinking water. Since treated and bottled spring water contained very infrequent coliform positive results (Martins *et al.*, 1989) it was necessary to artificially contaminate sterilized spring water with pure cultures of *Escherichia coli*, *Enterobacter aerogenes*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Clostridium perfringens* and *Pseudomonas aeruginosa*, in low concentration.

In addition to these 25 special samples, another 25 samples were collected from the highly polluted Pirajussara River for the inclusion in this evaluation study.

Methodology

Membrane filter technique. For the detection of total coliforms (m-Endo Agar Les, Difco); fecal coliforms (mFC-broth, Difco); fecal streptococci (KF Agar, Difco); as described in APHA (1989). In order to enumerate *Staphylococcus aureus*, the sample was filtered through a 0.45 μm membrane filter (Millipore Corp.) which was incubated for 48 h at 35.0 + 0.5°C on Baird–Parker Agar (Difco) with egg yolk tellurite enrichment (Schwab *et al.*, 1984). The dark typical colonies were streaked on Mannitol Salt Agar (Difco) plates which were incubated at 35.0 + 0.5°C for 48 h. Suspected *S. aureus* colonies were tested in Gram stain, catalase and coagulase production. Baird–Parker agar was chosen because previous work performed by one of the authors showed that this medium gave better results than m-Staphylococcus broth (data not published).

Multiple tube procedure. For the detection of *P. aeruginosa* as described in APHA (1989); and of sulfite reducer clostridia, as described by the *Bacteriological Examination of Water Supplies* (1969).

The presence–absence test was performed as described by the Ministry of Environment—Canada (1983). Two media were tested simultaneously. The first one consisted of MacConkey broth with tryptone (MacConkey–PA) (Clark, 1967) and the other one (STM–PA) combined the components of lactose broth, lauryl tryptose broth and tryptone (Clark *et al.*, 1982; APHA, 1989).

Statistical analysis for evaluating significant differences among the media, the tests and the incubation periods used were based on the chi-square test.

RESULTS AND DISCUSSION

As described by the authors (Clark *et al.*, 1982) the P–A test was intended for drinking water monitoring. Thus the ideal sample selection should have been taken from public and private water supplies. This approach would have required hundreds of drinking water analyses in the hope of obtaining a significant number of positive samples containing coliform or other bacterial indicators. The only viable alternative was to artificially contaminate a bottled water with indicator organisms and use these as test samples. One might also question the use of pure cultures rather than contamination of the bottled water with polluted surface water, such as Pirajussara River. In this instance the decision to use pure cultures was justified because controlled densities of all indicators could then be added to the bottled water samples.

(1) Artificially inoculated samples

Based on the analysis of the total percentage of positive results obtained by P–A test and quantitative methods, with a 5-day incubation period (Table 1), it was observed that, for total coliforms, the conventional method, the MacConkey–PA and the STM–PA methods, showed the same results (100% of detection). These data are in agreement with Pipes *et al.* (1986) and Edberg *et al.* (1989) that did not find any significant difference between the two methodologies, although Clark (1980), Jacobs *et al.* (1986) and Martins and Pellizari (1990) reported a better detection of these indicators by the P–A test than the membrane filter technique (MF). There was agreement between the MF conventional method (96%) and the STM–PA test (92%) for the detection of fecal coliforms. However, the P–A test using the MacConkey medium showed the lowest efficacy (56%). This difference was statistically significant at the level of 5% when the chi-square test was applied.

The membrane filter method was efficient in the detection of *S. aureus* (88%), and this bacteria was not detected in the P–A test. For sulfite reducer clostridia it was observed that the quantitative method was more efficient (76%) than the MacConkey–PA (28%) and the STM–PA (44%) and these differences were statistically significant. The better performance of the multiple tube technique was due to the anaerobic conditions used in this methodology.

Fecal streptococci were better detected by the STM–PA (92%) whereas the MF technique presented only 60% positive results; also, the MacConkey–PA test did not detect this indicator. The different performance between the two P–A media could be explained by their composition, since the MacConkey–PA medium

Table 1. Percentage of positive results obtained from conventional methods and P–A test in 5 days of incubation of the P–A bottles for artificially contained samples

	Conventional methods	P–A	ALT-PA
TC	100%	100%	100%
FC	96%	56%	92%
Pa	64%	84%	76%
SRC	76%	28%	44%
FS	60%	0%	92%
Sa	88%	0%	0%

$n = 25$.
TC, total coliform; Pa, *Ps. aeruginosa*; Sa, *Staphylococcus aureus*; SRC, sulfite reducer clostridia; FC, fecal coliform; FS, fecal streptococci.

is more selective for the detection of Gram-negative bacteria and the STM–PA medium contains tryptose that enhances streptococci growth. The lower performance presented by the MF could be explained by the cultivation of this bacteria directly on the KF medium, which is more selective, and by the incubation period of only 48 h.

Sulfite reducer clostridia were detected in 28% of the samples by the MacConkey–PA test and in 44% of the samples by the STM–PA test, but these differences were not statistically significant.

Pseudomonas aeruginosa were found in 84% of the samples when the MacConkey–PA test was used; 76% with the STM–PA test, and 64% with the multiple tube technique. No significant differences were detected. The highest frequency of positive results for this microorganism occurred after the fourth day of incubation of the P–A bottle (Table 2). So, a longer incubation period may enhance the recovery of this microorganism, increasing the chances for its detection.

Table 2, which displays the percentages obtained for different daily incubation periods, presents remarkable information. For coliforms the incubation period of 48 h, as stated by APHA (1989), was enough. No significant differences were observed between the results after 48 and 120 h of incubation. Concerning the other indicators, SRC, FS and *Pseudomonas aeruginosa* were better detected by the STM–PA test after the fourth day of incubation, as the differences between the results after 48 and 120 h were statistically significant.

Table 3. Percentage of positive results obtained from quantitative methods and P–A test in 5 days of incubation of the P–A bottles for natural samples

	Conventional methods	P–A	ALT-PA
TC	100%	100%	100%
FC	100%	96%	100%
Pa	100%	28%	28%
SRC	100%	44%	100%
FS	100%	44%	100%
Sa	12%	0%	0%

$n = 25$.

(II) Natural samples

The percentage of positive results obtained in the conventional methods and for the two P–A media are displayed in Table 3. There was agreement among the methods for the detection of total and fecal coliforms. Both indicators were detected in 100% of the samples tested by the MF technique and the STM–PA test. The percentages found for the MacConkey–PA test were, respectively, 100% for total coliforms and 96% for fecal coliforms.

The multiple tube procedure was more sensitive (100%) for *Ps. aeruginosa* than the P–A test, and there was a similar response for both P–A test media (28%). Although the MPN recovered these bacteria in 100% of the studied samples, the polluted samples interfered with the results, as some positive results were found in the highest dilutions and some negative ones were observed in the lowest dilutions. Such results could be explained by possible competition between *Ps. aeruginosa* and other micoorganisms, which is minimized in the highest dilutions. This could explain the low percentage of detection of this indicator by the P–A test, and the decrease of positive results after the first day of incubation of P–A bottles, as 100 ml of the sample were inoculated directly in the presumptive media.

For the detection of SRC and FS, the STM–PA test and the conventional quantitative methods presented agreement and good efficacy, but the results were statistically different from those obtained by the MacConkey–PA test, which showed poor results.

A very low number of *S. aureus* was recovered by the MF technique and this indicator was not detected by the P–A test.

Table 2. Results obtained by qualitative and quantitative methods for artificially contaminated samples

	Quantitative method	Qualitative methods									
		24 h		48 h		72 h		96 h		120 h	
		Mac†	STM*	Mac	STM	Mac	STM	Mac	STM	Mac	STM
Indicator	Density range	% Positive samples									
TC	6–45	88	100	100	100	100	100	100	100	96	100
FC	<1–6	16	72	12	80	16	68	32	68	36	56
Pa	<2–50	40	40	56	48	64	60	76	72	76	76
SRC	<2–1700	16	8	16	16	20	32	20	36	28	48
FS	<1–5	0	20	0	24	0	32	0	76	0	92
Sa	<1–9	0	0	0	0	0	0	0	0	0	0

*STM = STM-PA; †Mac = MacConkey-PA; $n = 25$.

Table 4. Results obtained by qualitative and quantitative methods for natural samples, and for daily incubation periods for P-A test

Indicator	Quantitative methods: Density range/100 ml	Qualitative methods: 24 h Mac†	24 h STM*	48 h Mac	48 h STM	72 h Mac	72 h STM	96 h Mac	96 h STM	120 h Mac	120 h STM
		% Positive samples									
TC	($\times 10^5$) 70–520	92	76	80	88	12	88	8	84	0	96
FC	($\times 10^4$) 82–485	84	76	96	88	12	88	8	84	0	56
Pa	70–>1600 ($\times 10^4$)	20	24	4	8	4	4	4	0	0	0
SRC	34–>1600 ($\times 10^4$)	100	100	100	100	100	100	100	96	100	96
FS	35–100	44	100	4	100	4	100	4	88	4	100
Sa	<1–1	0	0	0	0	0	0	0	0	0	0

*STM = STM-PA; †Mac = MacConkey-PA; $n = 25$.

Analysing the data displayed in Table 4, no significant differences were detected among the results obtained after 48 and 120 h of incubation for all the indicators, except for FC where positive results showed a decrease after 48 h of incubation with statistically significant differences. So in waters where a high quantity of bacteria are expected, 48 h of incubation is enough.

CONCLUSION

Considering the overall results obtained in this study, the STM-PA medium showed a better performance than MacConkey-PA medium with the same incubation period.

The P-A test is not indicated for *S. aureus*, in opposition to the proposal of APHA (1989) and the Ministry of Environment—Canada (1983). More research on adequate methodology for the detection of this microorganism in water is necessary.

An incubation period of 48 h is enough for the detection of coliforms, but in waters expected to contain low levels of other indicators, such as SRC, FS and *Ps. aeruginosa*, an extended period of 5 days is advisable.

The main importance of the P-A test is the possibility of detecting indicators other than coliforms. It is a promising tool for monitoring the bacteriological quality of drinking water, mainly in tropical climates where coliforms alone could be considered inadequate due to the reports of its occurrence in pristine waters.

Acknowledgements—This research was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) and by IDRC (International Development Research Centre), Canada.

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