

A simple field test for the detection of faecal pollution in drinking water*

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A comprehensive field investigation in several parts of India has revealed that the presence of coliforms in drinking water is associated with hydrogen sulfide-producing organisms. This paper describes a simple, rapid, and inexpensive field test for the screening of drinking water for faecal pollution, based on the detection of hydrogen sulfide. The new test showed good agreement with the standard most probable number (MPN) test. It proved highly successful in the field when it was used to detect faecal pollution and to monitor water quality during an outbreak of water-borne hepatitis A infection in the city of Gwalior. The test is reliable and simple to perform, and will be especially useful for screening rural water supplies and for large-scale screening of urban water supplies where resources, time, manpower, and laboratory facilities are limited.

Water-borne infections are the most common cause of infectious disease in the developing countries, often resulting from the lack of a protected water supply or from a faulty water supply system. The conventional assay used in bacteriological quality testing of water is the enumeration of the most probable number (MPN) of coliforms per 100 ml of water (1). This test needs the services of a qualified technician, laboratory facilities, and takes 72 h to produce the result. There is thus a need for a simple, reliable field test for use by village public health workers. Such a test would have particular significance in view of the current international drinking water supply and sanitation decade (1981-90).

Most of the work on improved methods of analysis has been carried out in advanced research laboratories (2-5), and has been based on expensive instrumental aids. A ready-to-use water testing device to count coliforms is available^a but is reliable only for grossly polluted water (6) and is expensive. Initial attempts to adapt the technique of lactose fermentation to a one-tube assay to detect coliforms were unsuccessful largely because of the difficulty in handling a liquid medium in the field. The test is also difficult to apply in acidic water because of its pH dependence.

Allen & Geldreich (7) have proposed an improved

technique for detection of bacteria and have suggested that other bacterial parameters should be investigated in a comprehensive field survey of ground water supplies. We have observed that the presence of coliforms in drinking water is consistently associated with organisms that produce hydrogen sulfide (H₂S). Furthermore, enteric bacteria such as *Salmonella*, *Proteus*, *Citrobacter*, and some strains of *Klebsiella* also produce H₂S. This communication presents a very simple method for assessment of contamination in drinking water based on the detection of H₂S-producing organisms, and reports its efficacy and use during an epidemic of hepatitis A infection.

MATERIALS AND METHODS

Preparation of the medium and the test

The concentrated medium used in the test contained 20 g of peptone, 1.5 g of dipotassium hydrogen-phosphate, 0.75 g of ferric ammonium citrate, 1 g of sodium thiosulfate, 1 ml of Teepol, and 50 ml of water. Aliquots of 1 ml of the concentrated medium were absorbed onto folded tissue paper (80 cm²), which was placed in a McCartney bottle, sterilized, and dried at 50 °C under sterile conditions. The water samples to be screened for faecal pollution were placed in the bottles, up to a pre-calibrated mark (20 ml) and allowed to stand at ambient temperature (30-37 °C). Faecal pollution is indicated if the contents of the bottle turn black within 12-18 h; in this case, the water was graded as unfit for consumption.

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Comparison of the new test and the MPN test

The performance of this test was compared with the standard MPN test in public health laboratories in Madras, Trivandrum, Calcutta, Patna, and Gwalior, located at a distance of 2000 km, 3000 km, 1600 km, 1000 km, and 10 km respectively from this laboratory (Fig. 1). The medium was prepared by the authors, as described, and test bottles were sent to the various laboratories. Drinking water samples received for routine MPN assay were tested simultaneously by the new method. Water samples with a coliform count of 10 or more per 100 ml (8-9), as assessed by the MPN method, and those turning black in the new test were graded as unsatisfactory.



Fig. 1. Map of India showing locations of laboratories where drinking water samples were examined.

Isolation and identification of *H₂S*-producing organisms

The water samples giving a positive result in the new test were cultured on nutrient agar and EMB agar.^b The various colonies were then subcultured into the *H₂S*-sensitive medium, and the organisms producing *H₂S* were identified by standard methods, as described by Edwards & Ewing (10).

Application of the test in the field

In February 1979, there was an epidemic of hepatitis A infection in the city of Gwalior. The city covers an area of 350 km² and has a population of 550 000.

^b Himedia, Bombay, India.

Although it is an endemic area for viral A hepatitis, the incidence is generally less than 10 cases per month. On 12 February, there were an estimated 5000 cases of infective hepatitis in the city, although few of these cases were reported to the hospitals. Water samples from 54 distribution points in various parts of the city were collected on the same day in the specially prepared bottles, transported to the laboratory, and stored overnight. The bottles were examined the next day, and in the samples giving a positive result, the presence of *Escherichia coli* was confirmed by the Eijkman test (9). The test was used routinely to screen the quality of the city water supply until the disease was brought under control in March 1979.

RESULTS

In all, 699 water samples were tested by both the MPN and the new test methods. Both tests gave positive results in 298 samples, and negative results in 293 samples (Table 1). Statistical analysis by McNemar's test (11) showed no significant difference in the performance of the two tests. A detailed coliform count is not available for the samples analysed in the laboratories in Calcutta and Madras. However, the results for the 434 samples analysed in the other laboratories indicated that all samples found to be grossly polluted in the MPN test (>40 coliforms per 100 ml) were graded as unsuitable in the new test (Table 2). In addition, in 21 of 24 samples that were MPN-negative but new-test positive, the mean coliform concentration was 6 ± 3 per 100 ml of water. The remaining 3 samples had no detectable coliforms but showed some turbidity in lactose broth. The 16 samples that were MPN-positive but new-test negative contained an average of 22 ± 9 coliforms per 100 ml of water.

H₂S-producing organisms isolated from drinking water

Of 72 *H₂S*-positive cultures received in this laboratory, 37 were investigated for the identification of *H₂S*-producing organisms. *Citrobacter freundii* were found in 23 samples, *Salmonella* species in 6 samples, *Proteus mirabilis* in 2, *Arizona* in two, *Klebsiella* in 1, and *H₂S*-producing variants of *E. coli* in 3. Only one strain of *H₂S*-producing organism was isolated from each of the 37 samples.

Monitoring of water quality during an outbreak of viral A hepatitis

Of the 54 water samples collected from the various distribution points in the city of Gwalior on 12 February 1979, 17 turned black on exposure to the

Table 1. Comparison of the results of the new test and the MPN test in water samples received in laboratories in different regions of India

Location of laboratory	No. of samples tested	MPN suitable		MPN unsuitable		Agreement between tests (%)
		NT ^a suitable	NT unsuitable	NT suitable	NT unsuitable	
Gwalior 1	135	58	5	4	68	93.4
Madras	111	35	9	4	63	88.2
Calcutta	124	53	6	19	46	79.8
Trivandrum	120	50	6	9	55	87.5
Gwalior 2	128	80	12	3	33	88.3
Patna	51	17	1	0	33	98.2
	669	293	39 ^b	39 ^b	298	88.34

^a NT = New test.

^b There is no difference in the efficiency of the two tests since exactly half of all disputed positives fell into each of the two test groups.

Table 2. Coliform counts in water samples received in laboratories in India

No. of coliforms per 100 ml of water	No. of samples	NT-positive	NT-negative
≤ 10	230	24	206
11-20	44	37	7
21-40	34	25	9
41-80	27	27	0
81-160	10	10	0
161-320	19	19	0
> 320	70	70	0
Total	434	212	222

new-test medium, indicating faecal pollution. The Eijkman test confirmed the presence of *E. coli* in these samples. It was found that most cases of viral A hepatitis occurred in the areas with a polluted water supply (Table 3). The populations in the areas with polluted or unpolluted water supply were comparable, but 10 times more cases of hepatitis were reported to the hospitals located in the polluted area.

The whole city of Gwalior has a chlorinated water supply system, but because of an acute water shortage, some tubewells had been dug in the city and the raw tubewell water connected to the treated-water pipeline. This raw water was the source of faecal pollution that caused the outbreak of hepatitis. All the tubewells were therefore disconnected and water samples taken from the distribution points were rechecked for faecal pollution. All 20 samples collected 8 days after the disconnection were suitable for drinking, and the disease was brought under control in March 1979.

Table 3. Distribution of reported cases of hepatitis A infection in Gwalior

Area	No. of hospitals	Population	No. of cases reported in February 1979	No. of cases reported in March 1979
With polluted water supply	9	300 000	313	82
With unpolluted water supply	8	250 000	31	18

DISCUSSION

It is evident from the data presented in this paper that H₂S-producing organisms are consistently associated with the presence of coliforms in water. Enteric bacteria such as *Citrobacter*, *Salmonella*, *Proteus*, and certain species of *Klebsiella* also produce H₂S. Further, Magalhaes & Veras (12) have reported H₂S-producing variants of *E. coli* of faecal origin. The predominant H₂S-producing bacteria found in polluted drinking water in the present study were *Citrobacter freundii*, *Salmonella* spp., and H₂S-producing variants of *E. coli*.

Swaroop (13) has indicated the difficulty involved in judging the exact number of coliforms in a sample of water by the standard MPN method. Thus, a coliform count of 10 per 100 ml of water means that the "true" value lies between 2 and 23. Our new test disagreed with the MPN results only in the samples with a low level of pollution. Of the 24 samples that were

graded as satisfactory in the MPN and unsatisfactory by the new test, only 3 did not have detectable coliforms. Even in these three samples, it is possible that the coliforms were suppressed by other bacteria (14, 15). In the samples that were negative by the new test and positive in the MPN test, the coliform counts ranged between 12 and 40 with an average of 22 ± 9 coliforms per 100 ml of water. All the samples with more than 40 coliforms were graded as polluted by the new test. The disagreement between the tests in samples with a low level of pollution may be related to a lack of precision in the MPN method; exactly half of all disputed positives fell into each of the two test groups.

The new test for detection of faecal pollution was found to be reliable, simple to perform, and required few facilities. Use of the method to study pollution of the urban water supply system in Gwalior demonstrated its practical application in an emergency.

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RÉSUMÉ

ÉPREUVE SIMPLE À EFFECTUER SUR LE TERRAIN POUR LA DÉTECTION DE LA POLLUTION FÉCALE DE L'EAU DE BOISSON

Une épreuve simple et peu coûteuse, réalisable sur le terrain en vue de la détection de la pollution fécale de l'eau de boisson, a été mise au point après une étude dans différentes parties de l'Inde. L'enquête a révélé que des microorganismes producteurs d'hydrogène sulfuré sont régulièrement présents dans de l'eau de boisson contenant 10 coliformes ou plus pour 100 ml. D'après cette observation, un nouveau milieu d'épreuve prêt à l'utilisation a été préparé et expédié à des laboratoires de santé publique à Patna, Calcutta, Madras, Trivandrum et Gwalior, afin que soit établie une comparaison avec la méthode classique du nombre le plus probable. Les résultats obtenus sur 669 échan-

tillons d'eau échantillonnés dans ces laboratoires ont montré qu'il y avait une bonne concordance entre ces deux épreuves. Les principaux microorganismes producteurs d'hydrogène sulfuré, présents dans l'eau de boisson, étaient *Citrobacter freundii*, *Salmonella* spp., des variants d'*Escherichia coli* produisant H₂S, *Proteus mirabilis*, *Arizona* spp., et *Klebsiella* spp. L'épreuve a également été utilisée pour détecter et surveiller la pollution fécale de l'eau de boisson au cours d'une épidémie d'hépatite virale A transmise par l'eau dans la ville de Gwalior. L'exécution de l'épreuve dans les conditions du terrain s'est révélée hautement fiable.

REFERENCES

1. *Bacteriological examination of water supplies*. London, Her Majesty's Stationery Office, 1969 (Report No. 71).
2. BACHRACH, U. & BACHRACH, Z. *Applied microbiology*, **28**: 169-171 (1974).
3. NEWMAN, J. S. & O'BRIEN, R. T. *Applied microbiology*, **30**: 584-588 (1975).
4. TRINEL, P. F. ET AL. *Applied and environmental microbiology*, **39**: 976 (1980).

5. WARREN, L. S. ET AL. *Applied and environmental microbiology*, **35**: 136-141 (1978).
 6. HEDBERG, M. & CONNOR, D. A. *Applied microbiology*, **30**: 881-883 (1975).
 7. ALLEN, M. J. & GELDREICH, E. E. *Ground water*, **13**: 45-52 (1975).
 8. *Manual of standards of quality for drinking water supplies*. New Delhi, Indian Council of Medical Research, 1975, pp. 16-17 (Special Report Series, No. 44).
 9. CRUICKSHANK, R. *Medical microbiology*, 10th ed., Livingstone, 1965, pp. 967-969.
 10. EDWARDS, P. R. & EWING, W. H. *Identification of Enterobacteriaceae*, Minnesota, Burgess Publishing Company, 1962.
 11. CONOVER, W. J. *Practical nonparametric statistics*. New York, John Wiley & Sons, 1971.
 12. MAGALHAES, M. & VERAS, A. *Revista do Instituto de Medicina Tropical de São Paulo*, **19**: 355-359 (1977).
 13. SWAROOP, S. *Indian journal of medical research*, **39**: 107 (1951).
 14. HUTCHINSON, D. ET AL. *Journal of bacteriology*, **45**: 29 (1943).
 15. WEAVER, R. H. & BOITER, T. *Transactions of the New York Academy of Sciences*, **13**: 183-188 (1951).
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