

ENVIRONMENTAL LABORATORIES: TESTING THE WATERS

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PROCEEDINGS

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ASSESSMENT OF THE EFFICIENCY OF DRINKING WATER TREATMENT USING THE COLIPHAGE, TOTAL COLIFORM AND H₂S PAPER STRIP TESTS

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ABSTRACT

Two potable water quality monitoring tests, the H₂S paper strip test and coliphage test, were evaluated on Chilean raw potable source waters and on drinking waters after receiving conventional treatment to render these waters safe for drinking. Turbidity, total coliform MPN and residual chlorine measurements were also made on these water samples. Based on 108 samples compared in this study, it was found that the H₂S paper strip test provided consumers of these waters with equivalent to, or greater protection than, the total coliform MPN test.

In treated water, a highly significant relationship ($p < 0.00001$) between total coliform and coliphage was observed as well as between total coliform, H₂S paper strip and coliphage ($p < 0.002$). These studies provide evidence that the H₂S paper strip test and the coliphage tests are viable indicators of potable water quality and water treatment in two medium sized cities in Chile.

INTRODUCTION

Safe potable water is a luxury that is generally unavailable to the majority of rural and suburban populations of developing and underdeveloped countries (WHO 1981). In the urban areas of these countries the potable water supplies are often contaminated due to inefficient and dilapidated systems, old and decaying distribution systems, sporadic water flows which increase back siphoning and inadequate and non-sterile storage systems. Thus water-borne infections is the most common cause of infectious disease in these countries (Manja et al., 1982), in spite of the Nation's Water Decade Program (Gupta and Chauduri, 1990).

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Important considerations in the development and maintenance of safe water supplies are the setting of realistic standards and use of appropriate monitoring technology for assessing microbiological water quality. Bacteriological water quality tests which are used and often devised in developed countries with temperate climatic zones have several disadvantages for routine use in developing and underdeveloped countries. Firstly, these tests are not easily portable in rural areas. Also, they require trained technicians, sophisticated laboratory equipment or expensive supplies, most of which are not readily available in developing countries and in isolated communities in developed countries (Dutka et al., 1990).

To overcome these problems, simplified inexpensive reliable microbiological water quality tests are required. In 1983, the International Development Research Centre (IDRC), Ottawa, Canada, sponsored a seminar in Singapore to discuss the problems and options for developing such tests. As a result of this meeting, the IDRC funded research on four continents, and in nine countries to develop and evaluate these tests (Dutka & El Shaarawi, 1990).

Two of the tests, the H_2S paper strip test and the coliphage test were used in an IDRC sponsored study to evaluate the efficiency of several Chilean potable water treatment systems. The APHA Standard Methods (1989) total coliform test was used to provide traditional coliform information from these potable water treatment systems. The results of this study are described. Regarding the parameters discussed in this paper, current Chilean regulations specify drinking waters not to exceed 5 NTU turbidity, 5 CFU (or MPN)/100 mL total coliforms, and 0.5 mg/L free residual chlorine (NCh 409.Of84, Instituto Nacional de Normalización).

METHODS

Samples

Fifty-four water samples were collected from various raw water sources which after treatment would serve as potable water. These samples were collected from rivers, channels and groundwater, within two separated medium sized cities in Chile. The northern city was in a normal temperate zone while the southern was greatly influenced by prolonged rainy seasons and colder weather.

Another 54 samples were collected, from the respective water treatment plants after the water had received their normal treatment. In most cases the water was treated using flocculation, sedimentation, filtration and chlorination processing in conventional drinking water plants.

Disinfection by chlorination was done with either hypochlorite or Cl_2 gas, using automatic chlorinators which provided a dose of 0.8 - 1 mg/L free residual chlorine. In these plants there were 0.5 - 1 hour contact time before the water was released. Samples from groundwater received only chlorination treatment. All disinfected samples were dechlorinated using thiosulphate, as per APHA Standard Methods, 1989.

Microbiological test

Coliphage and total coliform (TC) MPN tests (5 tubes, 3 dilutions) were performed as described in the 1989 edition of APHA Standard Methods. The H_2S paper strip technique (Manja et al., 1982) was originally developed to test 20 mL of drinking water. For this study the testing procedure was modified to test 100 mL of water by increasing the media concentration on paper

strip (Castillo et al, 1994). Details on types of organisms found in H₂S paper strip positive samples and their relationship to the blackness are described in Castillo 1992, and Castillo et al., 1994.

RESULTS AND DISCUSSION

All total coliform and coliphage tests performed on the raw water samples were positive for total coliform and coliphage. The maximum, minimum and geometric mean of the MPN total coliform were 92000, 110 and 4477/100 mL, while the maximum, minimum and mean for coliphages were 7500, 10 and 386 PFU/100mL.

Of 54 raw water samples tested with the H₂S paper strip test, only one sample was negative. This negative sample had a turbidity reading of 6.5 NTU, a coliphage count of 1265 plaque forming units/100 mL and a total coliform density of 3300/100 mL.

Most of (42/54) treated drinking water samples were simultaneously negative for total coliform, coliphage and the H₂S paper strip tests. From these waters, the maximum, minimum and mean of MPN total coliform were 1600, < 2.0, and 2.0/100 mL.; maximum, minimum and mean coliphage were 25, > 5, and 5 PFU/mL. Of all the samples, 50/54 were negative for coliphage, 48/54 negative for total coliform, and 45/54 negative for the H₂S paper strip test. Maximum, minimum and mean free residual chlorine were respectively, 1.0, 0.0, and 0.5 mg/L. Turbidity of the treated waters complied with Chilean regulations, as all samples were below 5 NTU.

Table I presents all the data (12/54) from treated drinking water samples where at least one of the three indicator test produced a positive finding. It can be seen that only 4 treated samples (4/54) were positive for coliphage and two of these, #36 and #46, were negative for both total coliform and the H₂S paper strip test. These samples also had a free residual chlorine (DPD colorimetric) of 0.2 and 0.4 mg/L and turbidity of 2.4 and 0.4 NTU, respectively. Free residual chlorine and the relationship of the degree of difference in turbidity measurements, before and after treatment, appear to have no bearing on whether or not coliphage will be found in treated waters. Samples #53, #37, and #36, #33 are excellent examples of this statement.

Perhaps the nature of the flocculating material or the condition and nature of the sand filters are more important in coliphage removal, and those factors are not appropriately addressed by the free residual chlorine and turbidity measurements. The free residual contact time may also have been overestimated due to plant operational problems. Another consideration is that due to flow and retention logistics, the exact same body of water was not sampled before and after treatment, as processes are assumed to be in steady state.

Comparing total coliform and H₂S paper strip responses on a presence/absence basis, it can be seen in Table I that both tests were positive in 6 samples and negative in 2. The H₂S test was positive in three samples where the total coliform test was negative (310, 51 and 53), while the coliform test was positive in only one sample (TC 2/100 mL) where the H₂S paper strip test was negative.

From the data presented in Table I, it can be seen that the H₂S paper strip test compared to the total coliform MPN provides consumers with equivalent or greater protection from

TABLE I. Results of treated and raw water when treated water presented positive results for coliphage, total coliform (TC) or H₂S paper strip

#Sample	Cl mg/L*		Turbidity NTU		Coliphage PFU/100 mL		TC MPN/100 mL		H ₂ S paper strip	
	Treated		Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
33	0.5		74.0	0.3	780	20	> 1600	1600	+	+
36	0.2		23.0	2.4	25	5	1600	<2	-	+
46	0.4		10.0	0.4	15	5	35000	<2	-	+
49	0.0		3.1	2.5	145	25	920	2	+	+
31	0.5		71.0	0.5	880	<5	> 1600	540	+	+
37	0.0		30.0	1.0	25	<5	3500	170	+	+
38	0.3		40.0	0.3	10	<5	400	95	+	+
48	0.0		5.5	2.0	25	<5	350	49	+	+
54	0.6		3.0	2.0	155	<5	490	2	-	+
10	0.8		8.2	0.9	260	<5	7900	<2	+	+
51	0.0		3.8	4.5	40	<5	350	<2	+	+
53	0.0		8.4	4.1	790	<5	> 16000	<2	+	+
max.	0.8		74.0	4.5	880	25	35000	1600		
min.	0.0		3.0	0.3	10	<5	350	<2		
mean	0.3		23.3	1.7	263	5	4209	205		

* free residual chlorine

† black colour - no black colour

contaminated potable water. The greater protection is provided, we believe, from the very frequent finding of *Clostridium* spp. in the same samples with various *Enterobacteriaceae* (Castillo et al., 1994). This conclusion is consistent with other reports from tropical and subtropical countries (Hazbun and Parker, 1983, Manja et al., 1982, Castillo et al., 1988, Ratto et al., 1988, Kromoredjo and Fujioka, 1991). Thus, these accumulated studies support the belief that the H₂S paper strip test in tropical and subtropical potable waters provides consumer safety at about a 20% of the cost of the TC procedure.

The importance of the positive coliphage tests in treated potable water with free residual chlorine values from 0.0 to 0.5 mg/L, and turbidity values of 0.3 - 2.5 NTU is that these findings raise the question of which factors in the potable water treatment process are critical for removing coliphage and other viruses. The knowledge that coliphage and enteroviruses have more or less similar sensitivities to free residual chlorine, suggests that enteroviruses if present in these waters would also survive the same treatment process (Stetler, 1984; Simkova and Cerveka, 1981; Kott et al., 1974).

Using correlation matrices (Pearson), relationships between physical-chemical and microbiological variables were evaluated. Table II and Table III show relationships found in raw and treated water.

TABLE II. Correlation (Pearson) between physical-chemical and microbiological variables in raw water samples

	Total Coliform	Turbidity	Coliphages
Coliphage	Turbidity - 0.050 ^R		
	Turbidity 0.0623	0.4062***	
	H ₂ S paper trip test - 0.2641	0.2718 ^a	- 0.2182

Legend: Sample size = 54

R = Coefficient

p = Significance level

p < 0.0001 = *****

p < 0.001 = ****

p < 0.01 = ***

p < 0.02 = **

p < 0.05 = *

A different pattern of the results in raw and treated water was observed when the correlation matrix was applied. In raw water, a highly significant correlation between coliphage and turbidity at the level of significance ($p < 0.01$) was observed. No correlation between coliphage and total coliform were observed as confirmed by other authors. This corroborates the findings of Martins et al. (1989) and disagrees with Isbister et al. (1983), who noted a correlation in North American temperate waters. In treated water a highly significant ($p < 0.00001$) relationship between total coliform and coliphage was seen, and also between total coliform, H₂S and coliphage ($p < 0.002$). This suggests that any of these indicators can be used to assess drinking water quality.

TABLE III. Correlation matrix (Pearson) between physical-chemical and microbiological variables in treated water

	Total Coliform	Turbidity	Residual Chlorine	Coliphages
Turbidity	-0.1698 ^R			
Residual chlorine	-0.0225	-0.4574 ^{****}		
Coliphages	0.5470 ^{*****}	0.1624	-0.2055	
H ₂ S paper strep test	0.4731 ^{****}	0.3215 ^{**}	-0.4621 ^{****}	0.4210 ^{***}

Legend: Sample size = 54

R = Coefficient

p = Significance level

p < 0.00001 = *****

p < 0.002 = ***

p < 0.001 = ****

p < 0.02 = **

Significant correlationship (or association):

Negative: Residual chlorine versus turbidity, and residual chlorine versus H₂S paper strip test.

Positive: Turbidity versus H₂S paper strip test, coliphages versus H₂S paper strip tests; coliphages versus total coliform, and H₂S paper strip tests; coliphages versus total coliform, and H₂S paper strip test versus.

It was also noticed that there was a significant negative ($p < 0.001$) correlation between residual chlorine and the H₂S paper strip test showing the high sensitivity of this test to assess chlorination efficacy. Chlorine was also negatively associated with turbidity, supporting the observation that turbidity interferes with chlorination efficiency (Lee and Walker, 1970).

In conclusion, these studies have provided evidence that both the H₂S paper strip test and coliphage test are viable indicators of potable water quality and potable water treatment. Also finding that 22% of the treated drinking water samples were still contaminated, strongly supports the opening sentence of this paper "safe potable water is a luxury that is a generally unavailable to the majority of people in developing and underdeveloped countries".

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REFERENCES

American Public Health Association (1989) Standard Methods for the examination of water and wastewater. 17th ed. APHA; AWWA; WPCF. Washington, D.C.

Castillo, G.(1992) IDRC project water quality control (Brazil-Chile) phase II. Mid-Term technical report CF:3-P-90-0100. International Development Research Centre, Ottawa, Canada.

Castillo, G., Duarte, R., Ruiz, Z., Marucic, M.T., Honorato, B., Mercado, R., Coloma, V., Lorca, V., Martins, M.T. and Dutka, B.J.(1994) Evaluation of disinfected and untreated drinking water supplies in Chile by the H₂S paper strip test. Wat. Res. 28:1765-1770

- Castillo, G., Thiers, R., Dutka, B.J. and El-Shaarawi, A.H. (1988) Coliphage association with coliform indicators: A case study in Chile. J. Tox. Assess. 3(5):535-550.
- Dutka, B.J. and El-Shaarawi, A.H. (1990). Use a simple unexpensive microbial water quality tests: Results of a three continent, eight country research project. IDRC Report IDRC-MR247e, Jan. 1990.
- Gupta, B.J. and Chauduri, M. (1992) Domestic water purification for developing countries. J. Water SRT-Aqua 41:290-298.
- Hazbun, J.A. and Parker, M. (1983) Simplified test for the detection of faecal pollution in drinking water. Third National Rural Water Supply and Sanitation Workshop, Solomon Islands, June 6-17.
- Isbister, J.B., Simmons, J.A., Scott, W.M. and Kitchens, J.F. (1983) A simplified method for coliphage detection in natural water. Act. Microbiol. Pol. 32:197-206.
- Kott, Y., Roze, N., Sperber, S. and Betzer, N. (1974) Bacteriophages as viral pollution indicators. Wat. Res. 8:165-171.
- Kromoredjo, P. and Fujioka, R. (1991) Evaluating Three Simple Methods to Assess the Microbial Quality of Drinking Water in Indonesia. Environ. Tox. & Wat. Qual. J. 6:259-270
- Lee, W.C. and Walker, D.H. (1970) Effect of solids. In Proceedings of the National Specialty Conference on Disinfection. July 8-10, 1970 University of Mass., Amherst. American Society of Civil Engineers, New York, N.Y.
- Manja, K.S., Maurya, M.S. and Rao, K.M. (1982) A simple field test for the detection of faecal pollution in drinking water. Bull. W.H.O. 60:797-801.
- Martins, M.T., El-Shaarawi, A.H., Dutka, B.J., Pellizari, V.H., Alfredo, G., Ribeiro, G. and Matsuto, E.F. (1989) Coliphage association with coliform indicators: A case study in Brazil. Tox. Assess. J. 4(2):329-338.
- Ratto, A., El-Shaarawi, A.H., Dutka, B.J., López, C. and Vega, C. (1988) Coliphage association with coliform indicators: A case study in Perú. Tox. Assess. J. 3(5):519-533.
- Simkova, A. and Cervenka, J. (1981) Coliphage as ecological indicators of enteroviruses in various water systems. Bull. W.H.O. 59:611-618.
- Stetler, R.E. (1984) Coliphage as indicators of enteroviruses. Appl. Environ. Microbiol. 48:668-670.
- World Health Organization. (1981). Global strategy for health for all by the year 2000. W.H.O., Geneve, Switzerland.