

EVALUATION OF FOUR SIMPLE, INEXPENSIVE
MICROBIAL WATER QUALITY TESTING PROCEDURES:
A REVIEW

by

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ABSTRACT

This review describes the development of the seven country, three continent International Development Research Centre (IDRC) project for evaluating simple, inexpensive microbiological water testing procedures. The tests evaluated and compared were the coliphage test, A-1 broth (MPN) test and the P/A and H₂S paper strip potable water tests. A summary of the observations and conclusions of these studies is presented.

RÉSUMÉ

Ce compte rendu présente les réalisations d'un projet du Centre de recherches pour le développement international (CRDI) ayant pour but d'évaluer certains essais microbiologiques simples et peu coûteux servant à déterminer la qualité de l'eau. Sept pays appartenant à trois continents différents participent à ce projet. Les essais qui ont été évalués et comparés sont l'essai de coliphage, l'essai de culture en bouillon A-1 (M.P.N.), l'essai P/A et l'essai d'eau potable sur papier indicateur à H_2S . Le compte rendu présente un résumé des observations et des conclusions de ces études.

MANAGEMENT PERSPECTIVE

It is accepted that the primary purpose of water supply programs is to deliver potable water which is safe, adequate and accessible to all. However, in developing countries and in rural and northern Canada, this task is not easy. Limited resources must be directed towards achieving an optimum balance between all three objectives.

An important consideration in the development and maintenance of safe water supplies is the setting of realistic standards and the use of appropriate monitoring technology for assessing bacteriological water quality. Bacteriological water quality tests presently being used have several disadvantages for routine use in developing countries and isolated communities in Northern Canada. Firstly, they are not easily portable for use in rural areas. They require either trained technicians, sophisticated laboratory equipment or expensive supplies, most of which are not readily available in developing countries.

To overcome these problems, simplified, inexpensive, reliable microbiological water quality tests are required. Testing procedures which fulfill the above requirements would make it possible for countries to monitor and classify their raw water supplies and potable waters with a minimum of input in terms of resources and expertise.

This report describes the international research studies to achieve these goals with the following four tests: coliphage, P/A, H₂S paper strip and A-1 broth and the application of results to the Canadian scene.

PERSPECTIVES DE GESTION

Tout le monde s'entend pour reconnaître que le but principal des programmes d'approvisionnement en eau consiste à distribuer de l'eau potable de bonne qualité, accessible à tous et en quantité suffisante. Mais il n'est pas facile de réaliser cette tâche dans les pays en voie de développement et dans les régions rurales et septentrionales du Canada. Les ressources limitées de ces régions doivent être canalisées de façon à atteindre le mieux possible ces trois objectifs.

La mise en place de normes réalistes et l'utilisation d'une technologie appropriée de contrôle continu de la qualité bactériologique de l'eau constituent un aspect important du développement et du maintien de sources sûres d'approvisionnement en eau. Les essais de qualité bactériologique de l'eau présentement utilisés ont plusieurs défauts en ce qui a trait à leur utilisation comme essais courants dans les pays en voie de développement et dans les communautés isolées du nord du Canada. Avant tout, ils sont difficiles à transporter dans les régions rurales. Aussi, des techniciens spécialisés, des instruments de laboratoire sophistiqués ou des produits coûteux sont nécessaires pour effectuer ces essais, mais la plupart de ces éléments ne sont pas facilement disponibles dans les pays en voie de développement.

Pour surmonter ces problèmes, il faut disposer d'essais de qualité microbiologique de l'eau plus simples, peu coûteux et fiables. Les essais qui satisfont à ces exigences permettraient aux pays intéressés de contrôler de façon continue et de classer leurs eaux non traitées et leurs approvisionnements d'eau potable avec un minimum de ressources et d'interventions de spécialistes.

Ce rapport présente les recherches internationales qui ont poursuivi ces objectifs en mettant à l'épreuve les quatre essais suivants : essai de coliphage, essai P/A, essai de culture en bouillon A-1 et essai d'eau potable sur papier indicateur à H_2S . L'application des résultats dans le contexte canadien est aussi présentée.

INTRODUCTION

In keeping with the goals of the United Nation's Water Decade Program, developing countries are concentrating on providing adequate quantities of drinking water to their populations. Unfortunately, little attention is being given to protecting and monitoring water quality. If this trend continues, the long term effect would be a continued high incidence of water-borne diseases.

It is accepted that the primary purpose of water supply programs is to deliver potable water which is safe, adequate and accessible to all. However, in developing countries this task is not easy. Limited resources must be directed towards achieving an optimum balance between all three objectives. Moreover, when a water source is developed, it must be maintained. Water systems which fall into disrepair because of the lack of maintenance, waste scarce resources and defeat the purpose of the program.

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 bacteriological water quality. In the developed and developing countries there are no simple solutions to the above considerations. Realizing that it is nearly impossible for rural water resources to meet established standards, Ministries of Health tend to allocate too few resources to water quality control programs.

However, if a classification scheme could be established based on microbiological properties, then these drinking water sources could at least be categorized and prioritized according to the levels of contamination or perceived relative health risk and perhaps the degree of sanitary protection available or used for the source waters. To implement such a scheme would require the monitoring of all potable water sources and the potable waters on a routine basis. One of the immediate impediments of this goal is the lack of appropriate inexpensive simple technology and laboratory facilities.

Bacteriological water quality tests presently being used have several disadvantages for routine use in developing countries. Firstly, they are not easily portable for use in rural areas. They require either trained technicians, sophisticated laboratory equipment or expensive supplies, most of which are not readily available in developing countries. In some instances, the long incubation time required for some tests before results can be obtained is a hindrance. These limitations seriously inhibit the effectiveness of most water quality control programs.

To overcome these problems, simplified, inexpensive, reliable microbiological water quality tests are required. In some instances, rapidity of results would be of immense benefit, if this could be achieved economically. Testing procedures which fulfill the above requirements would make it possible for countries to monitor and classify their raw water supplies and potable waters with a minimum of input in terms of resources and expertise. Consequently, priorities

and goals could be established for maintaining and improving drinking water quality, thereby protecting public health.

In 1983, an International Development Research Centre (IDRC)-sponsored project identification seminar was held in Singapore to discuss the problem and the options for developing such tests. From this seminar emerged the concept and protocols for an IDRC funded seven country, three continent research study on the feasibility of using several nontraditional microbiological procedures for estimating water quality and using these data along with sanitary surveys of the raw and potable water sources to characterize and categorize these waters.

Initially the project goals were to evaluate the use of a bacteriophage commonly called coliphage, and an enzyme, common to Enterobacteriaceae (beta-galactosidase) as techniques to classify raw potable water sources.

TESTS

Bacteriophages are virus-like entities that invade bacterial cells. Guelin in 1948 was the first researcher to properly apprise the potential of bacteriophages as indicators of fecal pollution. Since Guelin's recognition of the potential of bacteriophage to act as indicator systems, there have been several research reports indicating the potential of bacteriophage/coliphage to act as indicators of microbiological water quality (Besco, 1963; Amin-zade and Poultof,

1964; Kenard and Valentine, 1974; Scarpino, 1975; Wentzel et al., 1982; Grabow et al., 1984; Kennedy et al., 1985). The most detailed and intensive studies on growth and recovery of coliphage can be found in the Atlanta Research Report of 1979 by Scott et al. In an earlier major review of coliphages by Scarpino (1975) he stated "correlations appear to exist in fresh and marine waters between fecal bacterial pathogens, such as Salmonella and Shigella species and fecal indicator such as Escherichia coli and their bacteriophage". Then in 1984, Grabow et al. reported "coliphage counts could give a useful estimate of numbers of other microorganisms in sewage polluted waters" and in their studies "evidence is presented, that though counts of coliphages may not always directly correlate with those of enteric viruses, coliphages meet the basic requirements of an indicator for the virological safety of water". Based on the incidence and behaviour of coliphages and enteric viruses in raw water sources, various treatment and disinfection processes, and final supplies, Grabow et al. (1984a) have proposed a coliphage limit of 0/100 mL for drinking water, including supplies directly from wastewater.

From the studies performed at the Atlanta Research Corporation (1979) and others reported in the recent literature, it would appear that in the various environmental and drinking waters tested that the coliphage procedure is a reliable indicator of E. coli and coliforms. There is also sufficient evidence to suggest that the coliphage test has many advantages over traditional bacteriological and virological tests in that the procedure is economical, simple to perform and can provide results within six hours of testing.

The correlation/relationship studies between coliphage and bacteria and the inferences drawn from these which are reported above and in the literature may be considered by many to be inappropriate, as there are no direct consistent numerical relationships between coliforms, fecal coliforms, E. coli and the degree of hazard as related to the incidence and infectivity rate of waterborne Salmonella, Shigella, Vibrio cholerae and viruses (Dutka, 1973). Also, there are no consistent and obvious numerical relationships in receiving waters and drinking waters between fecal coliforms, E. coli, Salmonella, Shigella, Vibrio cholerae, viruses and coprostanol, the absolute indicator of fecal contamination (Dutka and El-Shaarawi, 1975).

In all uses of indicator organisms, a concept is used that usually works and is protective (and possibly over protective) of users of potable and natural waters. The authors of a recent international study (Dutka et al., 1987) believed that due to increasing stresses on water supplies, rising analytical costs, decreasing budgets, frequency of natural disasters which require immediate responses, e.g., volcanic eruptions, earthquakes, frost upheaval of pipes, etc., the need exists to develop cheaper, simpler and quicker indicator systems which will reflect both bacterial and viral contamination from sewage.

Testing for the inducible bacterial enzyme beta-galactosidase was the second procedure considered at the Singapore meeting. It was believed that there was a potential for the development of a simple

test, using this microbial enzyme, to screen for hazardous or indicator bacteria in raw and potable waters. To evaluate this potential, it was proposed (by the Thailand researchers) to add a chemical analog of lactose, one of the substrates for beta galactosidase, to the water samples, in expectation that any target bacterial cells present would be induced to produce large quantities of this enzyme. It was believed that even if small numbers of Enterobacteriaceae were present, the beta galactosidase produced would be detectable by a sample colorimetric assay.

This simple two pronged research proposal escalated as a better awareness of the needs of developing countries in the field of water bacteriology came into focus. It soon became evident that laboratories and trained staff were at a premium, funding for water quality testing was rapidly disappearing due to rising costs and that the majority of rural populations rarely if ever had their water supplies tested. Thus there was an obvious need for simple, inexpensive reliable bacteriological tests which could be performed in urban centres as well as in isolated rural communities and under field conditions.

As the number of laboratories involved in this IDRC program increased from the original site in Bangkok to encompass the S.E. Asian sites of Kuala Lumpur and Singapore and then Lima, Santiago and Sao Paulo in South America to finally Cairo and Rabat in North Africa, the complexity of the research program also increased.

Initially the fecal coliform Most Probable Number (MPN) 15 tube test using A-1 broth was added to the system to evaluate raw waters as well as a variety of potable water supplies. The A-1 broth test is a simple 24 hour single medium test with an easily recognizable endpoint-gas production, after 22-24 hours incubation at 44.5°C. The results obtained by this test were compared to traditional fecal coliform and total coliform procedures used in each country. The A-1 broth test was also used to study the relationship between E. coli, fecal coliform and coliphage.

It was recognized that potable water supplies were not being adequately controlled in many developing countries for a variety of reasons, two of which were the cost factor and the lack of trained personnel. To try to help solve this problem, two extremely simple, inexpensive and reliable procedures were proposed for evaluation in some of the laboratories, the Presence/Absence (P/A) test (Clark, 1968) and the H₂S paper strip test (Manja et al., 1982). Both of these tests are single bottle tests to which potable water is added and then incubated at 35°C for up to 5 days. Later research results would show that 26°-35°C incubation produced similar results.

The P/A test can be performed using various amounts of media so that a rough quantitative measure may be made. In the routine test, 50 mL of media is placed into a screw capped bottle and autoclaved. Then 100 mL of potable water is added, the capped bottle is shaken, then incubated. If the colour of the media changes from red to yellow, a positive result is recorded indicating the potential

presence of one or more indicator bacteria (coliforms, E. coli, Pseudomonas aeruginosa, staphylococci or streptococci). Isolation and identification procedures may be carried out on positive samples for confirmation, if desired. In the research studies, positive P/A tests were confirmed by isolate identification.

The H₂S paper strip procedure is equally simple. Filter paper or tissue paper sheets (75-80 cm²) are impregnated with a simple chemical mixture (1 mL per 75-80 cm²) which are placed into screw capped bottles and dried under sterile conditions at 50°C. Similar to the P/A test routine quantitative estimates may be made by using different volumes of water and more or fewer cm² of impregnated paper strips (not fully tested yet). In the routine tests, 20 mL of potable water are added to the bottle and incubated at 35°C for up to 5 days. A blackening of the paper strip (usually within 24 hrs) indicates the presence of an indicator bacteria, usually Enterobacteriaceae. As with the P/A test positive results were confirmed by isolate identification.

As new countries were brought on stream, the research projects became more complex. By the time the seventh country became involved in the IDRC coliphage project, samples being collected and tested were: bottled drinking water, with and without gas, potable tap water, drinking water reservoirs and distribution lines, city wells and rural wells, as well as potable water storage vessels ranging from huge standpipes to local earthenware jars with stored rain water. A variety of drinking water source waters were also sampled, usually

small lakes or ponds, rivers and ditches. Data from these waters will at a later date be combined with sanitary surveys in order to establish a priority ranking of water supply sources.

The four tests, being evaluated by this seven country network, were controlled by and compared to a variety of procedures used locally on a routine and research basis. The control tests varied from routine membrane filtration procedures using total coliform and fecal coliform media to the use of square gridded membrane filters with special and traditional coliform/fecal coliform media. Various MPN media and procedures (10 tube and 5 tube series) were used to estimate total coliform and fecal coliform densities. Other variables in these studies included testing local E. coli host strains for the coliphage test, evaluating temperature ranges for the P/A, H₂S paper strip and coliphage tests as well as studying the relationship between coliphage, indicator bacteria and human enteric viruses in various waters. A great effort was also expended on isolating and identifying the bacteria from each of the enumeration procedures. This effort was very important in establishing the selectivity and reliability of the various media and procedures being evaluated and compared.

GENERAL OBSERVATIONS

1. The coliphage test was found by the laboratories to be a simple, inexpensive and easy to perform test with the E. coli host ATCC 13706 being the most sensitive in the waters tested.

2. Several laboratories reported that they believed that the coliphage method (with 8 hours incubation) could be used as an indicator of fecal contamination.
3. The majority of the laboratories found that there was a good statistical relationship between coliphage and fecal coliform populations in river and lake waters. However, in some well waters, turbid river waters and rain waters the strong statistical relationship between indicator bacteria (total-fecal coliforms) and coliphage broke down, and this appears to be an area requiring more research to clarify the reasons for this relationship breakdown.
4. In one study dealing with a fresh water river, a freshwater lake and marine beaches it was concluded (a) that within location fecal coliforms and coliphage are positively correlated, (b) coliphage values can be indicated or predicted by using fecal coliform MPN, fecal streptococci and E. coli data, (c) it would be feasible to propose a water quality guideline of 20 coliphage/100 mL for recreational waters (d) fecal coliform or coliphage counts in marine waters are not always predictable of the presence of Salmonella and enteroviruses and (e) in marine waters where pathogens are found, coliphage-pathogen ratios are smaller than fecal coliform-pathogen ratios.
5. Laboratories on three continents studying potable water (bottled with and without gas, and treated tap water) found that it was not uncommon to find coliform free potable water containing coliphage. Thus the finding of coliphage in these drinking

waters with and without coliform presence, suggests that enteroviruses can also survive the normal treatment and disinfection process accorded these potable water samples (Havelaar, 1986). Another implication of the data from these studies is that coliform-free potable waters are not necessarily pathogen-free potable waters. Therefore we suggest, based on these studies, that coliphage tests be included as part of any potable-water testing schemes.

6. The general conclusion from these studies is that the coliphage test has an advantage over traditional microbiological tests, in that it can be read after 6 hours incubation. The procedure is very economical and easy to perform and its sensitivity can easily be increased by testing more 5 mL aliquots or by using a coliphage MPN technique or even by using a large petri dish and testing 100 mL of sample.
7. In all laboratories where the A-1 broth test for fecal coliforms was evaluated it was usually found to be the most sensitive technique and produced higher fecal coliform counts than any of the other media and procedures that were compared. In the rare instance when the A-1 test was found to only produce equivalent results to traditional local fecal coliform population estimation techniques, it was found that the A-1 test had a cost and time advantage over the other fecal coliform estimation tests.
8. Isolates collected and identified from positive A-1 broth MPN tubes were found to be E. coli, 80-100% of the time. Thus the

A-1 broth procedure can provide laboratories with an approximation of the E. coli concentration in raw water supplies and recreational waters.

9. It was concluded by several countries that the A-1 broth technique should be considered as the preferred bacteriological test for the examination of raw water supplies and recreational waters and the A-1 test combined with the coliphage test would make an excellent screening program for health hazards in raw water supplies (Castillo, 1988).
10. The presence/absence (P/A) test was found by all laboratories using the procedure, to be the most sensitive and cost effective means of testing potable water supplies (bottled and tap) for bacteriological contamination. In many instances, potable waters which were negative by traditional MF and MPN coliform estimation techniques, would be found positive by the P/A test.
11. The P/A test was found to be a very portable test in that bottles of media could be transported anywhere without refrigeration and the sample could be collected and tested by untrained personnel. This factor coupled with its low cost and minimal storage requirements makes it the ideal test for potable water safety anywhere in the world. The P/A test can be made partially quantitative by preparing smaller volumes of media, e.g., 5 mL, 12.5 mL and 25 mL and thus 10 mL, 25 mL and 50 mL of water sample could be tested.
12. In summation from this three continent study the superiority of the sensitivity of the P/A test for monitoring potable water

samples can easily be seen. This test is relatively inexpensive, simple to perform, and is recommended without reservation for all routine water quality analyses. We believe this procedure can enhance water quality testing procedures, especially when cost is a factor (Castillo, 1988).

13. The H₂S paper strip technique for testing potable water supplies was found, by the majority of laboratories, to be equivalent to or slightly less sensitive than their traditional/routine potable water testing procedure for coliforms.
14. Several of the laboratories indicated that the paper strip procedure would be ideal for testing rural and isolated drinking water supplies as well as local urban potable waters. The medium (impregnated paper strip) has an unlimited shelf life and the test procedure requires no training for collecting the sample and interpreting the results.
15. Since the H₂S paper strip technique is based on the testing of only 20 mL of water samples while the other MF, MPN and P/A tests use either 55.5 mL or 100 mL of sample, there is a possibility that with research into media concentrations versus sample volume, the H₂S paper strip could be used to test 100 mL of sample, thus perhaps making it compatible to the P/A test in sensitivity.
16. In summation, the H₂S paper strip test is probably the best and simplest method to test remote water supplies, as well as for use in city and town laboratories. It is believed that the P/A and

H₂S paper strip techniques combined with the coliphage test, would provide an excellent assessment of the safety of potable waters from bacterial and virus contamination.

ADDENDUM

In 1988, these simple, inexpensive microbiological water quality assessing procedures described above were used by a remote Canadian Indian Band to test and monitor their recreational and drinking water supplies. This pilot project has proven to be a major success story with the Band members realizing that they now have the ability to control the quality of their own water supplies.

It is expected in 1989 that this pilot project will be expanded, at the request of other Indian Bands. Staff from the federal Departments of Environment and National Health and Welfare are monitoring and assisting this technology transfer.

REFERENCES

- Amin-zade, A.M. and Y.G. Poultof. 1964. Results of the use of the phage titer rise reaction for the indication of Salmonella typhi in the waters of open-water reservoirs. Zh. Mikrobiol. Epidemiol. Immunobiol. 41:93-96.

- Atlantic Research Corporation. 1979. Evaluation of coliform bacteria and bacteriophage relationships in assessment of water quality. Final Technical Report, NSF Grant No. PFR78-19196 December, 1979. Division of Problem Focused Research Applications, NSF, Washington, D.C. 20550.
- Bosco, G. 1963. Enterophages in coastal sea waters - considerations on the general epidemiological importance of their discovery in surface waters. *Nuovi Ann. Iq. Microbiol.* 14:8-20.
- Castillo, G., R. Thiers, B.J. Dutka and A.H. El-Shaarawi. 1988. Coliphage association with coliform indicators: A case study in chile. *Tox. Assessment* 3:535-550.
- Clark, J.A. 1968. A presence-absence (P/A) test providing sensitive and inexpensive detection of coliforms, fecal coliforms and fecal streptococci in municipal drinking water supplies. *Can. J. Microbiol.* 14:13-18.
- Dutka, B.J. 1973. Coliforms are an inadequate index of water quality. *J. Environ. Hlth.* 36:39-46.
- Dutka, B.J. and A.H. El-Shaarawi. 1975. Relationship between various bacterial populations and coprostonal and cholesterol. *Can. J. Microbiol.* 21:1386-1398.
- Grabow, W.O.K., P. Coubrough, E.M. Nupen and B.M. Bateman. 1984. Evaluation of coliphages as indicators of the virological quality of sewage polluted water. *Water SA* 10, 7-14.

- Grabow, W.O.K., P. Coubrough, C. Hilner and B.W. Bateman. 1984a. Inactivation of hepatitis A virus, other enteric viruses and indicator organisms in water by chlorination. *Water Sci. Technol.* 17:657-664.
- Guelin, A. 1948. Etude des bacteriophages Typhiques. Vi-dans les eaux. *Ann. Inst. Pasteur, Paris* 75, 485-489.
- Havelaar, A.H. 1986. F-specific RNA bacteriophages as model viruses in water treatment processes. Rijkinstituut voor Volksgezondheid on Milieuhygiene, Bilthoven, The Netherlands.
- Kenard, R.P. and R.S. Valentine. 1974. Rapid determination of the presence of enteric bacteria in water. *Appl. Microbiol.* 27:484-487.
- Kennedy, J.E., G. Bitton and J.L. Oblinger. 1985. Comparison of selective media for assay of coliphages in sewage effluent and lake water. *App. and Environ. Microbiol.* 49:33-36.
- Manja, K.S., M.S. Maurya and K.M. Rao. 1982. A simple field test for the detection of faecal pollution in drinking water. *Bull. W.H.O.* 10:797-801.
- Scarpino, P.B. 1975. Human enteric viruses and bacteriophages as indicators of sewage pollution. In: *Discharge of Sewage from Sea Outfalls*. A.L.H. Gameson, Ed. Pergamon Press, Oxford, pp 49.
- Wentzel, R.S., P.E. O'Neal and J.F. Kitchens. 1982. Evaluation of coliphage detection as a rapid indicator of water quality. *Appl. and Environ. Microbiol.* 43:430-434.