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SUGGESTED MICROBIOLOGICAL WATER QUALITY TESTS FOR DEVELOPING COUNTRIES AND RURAL AND ISOLATED NORTH AMERICAN COMMUNITIES

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ABSTRACT

In developing countries and in the rural and northern parts of North America, the deliverance of potable water which is safe, adequate and accessible to all is not an easy task. Limited resources must be directed toward achieving an optimum balance between these needs. An important consideration in the development and maintenance of safe water supplies is the use of appropriate monitoring technology for assessing microbiological water quality. Presently used microbiological water quality tests have several disadvantages for routine monitoring programs in developing countries and rural isolated communities in North America. Firstly, they are not easily portable for use in isolated areas. They require trained technicians, sophisticated laboratory equipment and expensive supplies, most of which are not readily available in developing countries.

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

Controlled studies on the use of A-1 broth MPN test for fecal coliforms in raw and recreational waters and the Presence/Absence (P/A), H_2S paper strip test and coliphage count tests for predicting

potential hazards in potable waters were carried out on four continents and in nine countries. The results of these studies indicated:

1. In all countries 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 and *E. coli* counts that any of the other media and procedures that were compared.
2. In comparison with traditional coliform membrane filter and MPN multiple tube tests, the superiority of the sensitivity of the P/A test for monitoring potable water samples was readily observed. The H_2S paper strip technique was shown to be equally sensitive as traditional bacterial water quality indicator systems for use in potable water testing, and
3. These studies revealed that coliphage were present in many drinking water samples with and without the presence of other indicator bacteria. Implications of finding coliphage in bacterial-free potable waters are that human enteric viruses which have similar disinfection sensitivities as coliphages, may also be able to survive the normal treatment and disinfection processes accorded the potable waters tested (bottled and tap) in this study.

RÉSUMÉ

Dans les pays en développement et dans les régions rurales et septentrionales de l'Amérique du Nord, il n'est pas facile d'assurer un approvisionnement en eau potable à la fois salubre, adéquat et accessible à tous. Un équilibre optimal doit être atteint entre ces besoins et ce, au moyen de ressources limitées. L'utilisation d'une technique d'analyse microbiologique de l'eau appropriée est prioritaire afin de garantir et de maintenir l'innocuité des approvisionnements en eau. Les techniques actuelles présentent plusieurs inconvénients pour ce qui est de la surveillance régulière dans les pays en développement et dans les collectivités rurales isolées de l'Amérique du Nord. Ainsi, elles ne sont pas facilement applicables dans les régions isolées. En outre, elles nécessitent un personnel qualifié, un équipement de laboratoire sophistiqué et un matériel coûteux, lesquels font généralement défaut dans les pays en développement.

Des méthodes d'analyse microbiologique de l'eau fiables et simples sont donc requises pour régler ces problèmes. L'utilisation de méthodes d'analyse adaptées aux besoins susmentionnés devrait permettre aux pays et aux agglomérations isolées de surveiller et de classer les eaux brutes, les sources d'eau potable et les eaux utilisées à des fins récréatives avec un minimum de ressources et de savoir-faire.

Des études contrôlées sur l'emploi de la méthode du nombre le plus probable (NPP) sur un milieu de type bouillon A-1 pour dénombrer

les coliformes dans les eaux brutes et les eaux utilisées à des fins récréatives, le test de présence/absence (P/A), la méthode de la bande de papier filtre pour mesurer la production de H_2S et la méthode de numération des coliphages ont été faites pour évaluer les risques potentiels en matière d'approvisionnement en eau potable dans neuf pays répartis sur quatre continents. Ces études ont donné les résultats suivants:

1. Dans tous les pays où la méthode du NPP sur bouillon A-1 a été évaluée, les chercheurs ont généralement remarqué que cette technique était la plus sensible et qu'elle permettait des numérations de coliformes fécaux et d'<u>E. coli</u> plus élevées que tout autre milieu et technique;

2. Comparativement à la méthode classique de la membrane filtrante et à la technique de fermentation en tubes multiples ou du nombre le plus probable (NPP), le test P/A utilisé pour analyser les échantillons d'eau potable s'est révélé le plus sensible. Le méthode de la bande de papier filtre pour mesurer la production de H_2S s'est avéré aussi sensible que les autres méthodes traditionnelles indicatrices de la qualité bactériologique de l'eau potable;

3. Maints échantillons d'eau potable contenaient des coliphages, en l'absence ou non d'autres bactéries indicatrices. La présence de coliphages dans des échantillons d'eau potable non contaminée par les bactéries signifie que les entérovirus humains, qui sont aussi résistants que les coliphages aux procédés de désinfection,

peuvent survivre au traitement et à la désinfection, comme ce fut le cas des échantillons d'eau potable (embouteillée et du robinet) analysés dans la présente étude.

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 areas of Canada and the United States, 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 North America. Firstly, they are not easily portable for use in rural areas. They require trained technicians, sophisticated laboratory equipment or expensive supplies, most of which are not readily available in developing countries and difficult to install in remote communites.

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 application and verification of the coliphage, P/A, H_2S paper strip and A-1 broth tests which fulfill all the above requirements.

PERSPECTIVES GESTION

Il est généralement admis que les programmes d'alimentation en eau doivent viser avant tout à assurer un approvisionnement en eau potable à la fois salubre, adéquat et accessible à tous. Toutefois, dans les pays en développement et dans les régions rurales et septentrionales du Canada et des États-Unis, cette tâche est ardue. Un équilibre optimal doit être atteint entre ces trois objectifs et ce, avec des ressources limitées.

Afin de garantir et de maintenir l'innocuité des approvisionnements en eau, il est essentiel d'établir des normes réalistes et d'utiliser les techniques de surveillance appropriées pour l'analyse bactériologique de l'eau. Les méthodes actuelles présentent plusieurs inconvénients pour ce qui est de leur utilisation régulière dans les pays en développement et dans les collectivités isolées de l'Amérique du Nord. En premier lieu, elles ne sont pas facilement applicables dans les régions rurales. En outre, elles requièrent un personnel qualifié, un équipement de laboratoire sophistiqué ou du matériel coûteux, lesquels font habituellement défaut dans les pays en dévelolppement et sont difficiles à obtenir dans les régions éloignées.

Des méthodes d'analyse microbiologique simples, peu coûteuses et fiables sont donc requises pour résoudre ces problèmes. L'utilisation de techniques répondant aux exigences susmentionnées permettrait aux divers pays de surveiller et de classer les eaux brutes et de consommation avec un minimum de ressources et de savoir-faire.

INTRODUCTION

In accordance with the goals of the United Nation's Water Decade Program, developing countries are concentrating on the provision of 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.

In a recent (1989) workshop on drinking water sponsored by the World Health Organization, Western Pacific Region, it was reported that "water quality control was one of a series of integrated public health measures needed to reduce infant mortality and improve life expectancy. The combined effects of increased water supply quality and quantity have been shown to produce an average of 25% reduction in water-related diseases in recent studies. Water quality alone accounts for an 18% average reduction of disease when supplies are significantly improved. Together these several factors were shown to produce an average of 37% reduction in diseases, whilst sanitation may add a further 22% reduction."

Unfortunately, everywhere it is the same; all countries want the best water quality monitoring technology even though they might not be able to afford it. This is the problem facing the majority of scientists, technologists, engineers, and managers in developing countries. They would all like to have the best procedures, the newest instrumentation and the newest media which have become familiar to them through the scientific literature, and often through their visits to the developed countries. Then as some of this new water quality technology reaches laboratories in developing countries, there is often a slow realization, or in some cases, a quick awareness that this new technology is very costly. The cost of chemicals, specialized media, repairs to specialized instruments, is not so slowly draining their often meager budgets. There is often the realization that local technical expertise to repair specialized instruments is frequently not available, and if available, the parts are not available. Then gradually, but eventually, the laboratories revert back to their own traditional, secure, safe procedures that they learned years ago and have used for years.

One of the consequences of this venture into "modern" technology is the depletion of funds which had been allocated to maintain the service. Instead of three samples a week being processed, perhaps one is now able to be done. In rural areas where one sample was collected every week or two, now samples are collected once a month and even once every six months.

Not all countries or areas within developing countries and even developed countries go through the above procedures. In many cases, the funds or technology are not available to provide even the simplest form of bacteriological water quality monitoring.

These universal problems, which we somehow choose not to be aware of, make it imperative that we try to promote basic simple affordable procedures which have always worked, but are not "flashy" or avidly discussed on the symposium circuit.

The primary purpose of all 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 and often almost impossible. Limited resources must be directed towards achieving an optimum balance between accessible, adequate and safe water. 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 water supply 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 based on one or two simple microbiological screening tests could be established, 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 needed to protect these 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 to 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 trained technicians, sophisticated laboratory equipment or expensive supplies, most of which are not readily available in developing countries and not easily established in remote communities of North America. 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, rapid results would be of immense benefit, if this could be achieved economically; and economically in developing countries usually means labour intensive with little investment in supplies and equipment. 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.

Through my involvement in a nine country, four continent study (funded by the International Development Research Centre) on the use of simple inexpensive, reliable microbiological procedures to monitor drinking water, drinking water source water and recreational water quality, I observed the utility and sensitivity of these types of tests. They have also been proven through the peer reviewed literature, and all they need is to be applied.

Proposed Microbiological Tests

The prime candidate procedure for the bacteriological evaluation of drinking water source waters and recreational waters is the five tube Most Probable Number (MPN) test using A-1 broth (APHA, 1985). This procedure was found to be very simple to perform such that a nontechnical person, to whom a pipette was a sophisticated instrument, was able to be trained to carry out this procedure in four hours. Also, this procedure was found to be relatively inexpensive as all the glassware can be recycled, and only the one medium had to be purchased or made by following formula directions. In all instances where this procedure (A-1 broth) was compared to locally used fecal coliform estimation procedures, the A-1 broth was invariably more sensitive (Martins et al., 1989; Castillo et al., 1988; Ratto et al., 1988). The only drawback for the world wide use of this procedure is the requirement for incubation at 44.5°C, a not easily-achieved temperature in developing countries and in the isolated rural areas of developed countries.

Another candidate procedure that is easy to perform, inexpensive to run and may provide an added margin of safety in water quality testing, is the bacteriophage/coliphage indicator system. 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; Kenard and Valentine, 1974; Scarpino, 1975; Wentsel et al., 1982; Grabow et al., 1984; Kennedy et al., 1985; Petrovicova, 1988). 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 behavoiur 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.

Simkova and Cervenka in 1981 reported that their data was supportive of the concept that coliphages could act as indicators of enteroviruses in some water systems. Then in 1988, Petrovicova et al. concluded their studies on enteroviruses and coliphage in different water systems with "presence of coliphages in drinking water is an indication for aimed virological examination". In the same paper they report that since 1987, examination of water quality by the coliphage test has been adopted by the Hygiene Services and Water Supply Authorities in Slovakia.

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 (ASTM, 1982) 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.

As the coliphage test may be indicative of both bacteriological and possible virological hazards, this procedure has great potential for universal application, especially as all countries are faced with increasing stresses on water supplies, rising analytical costs, decreasing budgets. There is also the continuing need to respond to natural disasters which require immediate responses, e.g., volcanic eruptions, earthquakes, frost upheavals of pipe lines, etc., and the coliphage test is one of the means by which the safety of potable water supplies can be evaluated, simply, quickly (6 hours) and inexpensively.

During the author's involvement in the IDRC (Ottawa) international water quality programme, it was necessary to visit many of the developing countries to observe first hand the water quality problem. During these visits, 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 H_2S paper strip test (Maja 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 aeruoginosa, staphylococci or streptococci). Isolation and identification procedures may be carried out on positive samples for confirmation, if desired.

The H_2S paper strip procedure is equally simple. Filter paper or tissue paper sheets (75-80 cm^2) are impregnated with a simple chemical mixture (1 mL per 75-80 cm^2) 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 cm^2 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 hours) indicates the presence of an indicator bacteria, usually in our experience, a member of the Enterobacteriaceae.

These four microbiological water quality tests were evaluated on four continents in nine countries on raw drinking water source water, recreational waters, untreated drinking waters, treated drinking waters and bottled potable waters, and were controlled by and compared to a variety of procedures used locally on a routine and research basis; many of the procedures used were those found in APHA Standard Methods (1985). 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 E. coli host strains for the coliphage test (Sim et al., 1988; Martins et al., 1989), evaluating temperature ranges for the P/A, H_2S paper strip and coliphage tests (Castillo et al., 1988; Ratto et al., 1988) 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 (Castillo et al., 1988; Ratto et al., 1988; Martins et al., 1989). This effort was very important in establishing the selectivity and reliability of the various media and procedures being evaluated and compared.

GENERAL OBSERVATIONS AND CONCLUSIONS

1. 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.
2. Isolates collected and identified from positive A-1 broth MPN tubes were found to be E. coli, 78-94% 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.

3. 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 et al., 1988).
4. The coliphage test was found by all the laboratories involved in the study to be a simple, inexpensive and easy to perform test with the E. coli host ATCC 13706 being the most sensitive in all the waters tested.
5. Several laboratories reported that they believed that the coliphage method (with 8 hours incubation) could be used as an indicator of fecal contamination.
6. 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.

7. In one study dealing with a fresh water river, a freshwater lake and marine beaches, it was concluded (a) that, in the same sampling area, 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 (Dutka et al., 1987), (d) fecal coliform or coliphage counts in marine waters are not always predictive 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.

8. 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 processes 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, based on these studies, it is recommended that coliphage tests be included as part of any potable-water testing scheme. This recommendation is also supported by the findings of Petrovicova et al. (1988) and Simkova and Cervenka (1981).

9. 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 incubation temperatures between 26-35°C do not seem to effect the test's sensitivity. The sensitivity can easily be increased by testing more 5 mL aliquots, e.g., 6 or 8, or by using a coliphage MPN technique or even by using a large petri dish and testing 100 mL of sample.
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 can be tested.
12. In summation, from this four continent study, the superiority of the sensitivity of the P/A test over traditional coliform estimation techniques for monitoring potable water samples was readily

observed. This test is relatively inexpensive, simple to perform, and is recommended without reservation for all routine water quality analyses. This procedure, it is believed, can enhance water quality testing procedures, especially when cost is a factor (Castillo et al., 1988).

13. The H_2S 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 H_2S paper strip technique is based on the testing of only 20 mL of water sample 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 H_2S paper strip could be used to test 100 mL of sample, thus perhaps making it comparable to the P/A test in sensitivity.

16. In summation, the H_2S paper strip test, while not as sensitive as the P/A test in the 20 mL aliquot format used, is probably the best and simplest method to test remote water supplies. It is

believed that either the P/A or volume modified H_2S paper strip techniques, combined with the coliphage test, would provide an excellent assessment of the safety of potable waters from bacterial and possibly viral contamination.

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