WATER QUALITY CONTROL NETWORK

PROCEEDINGS OF THE MEETING HELD IN OTTAWA, CANADA, 20–24 FEBRUARY 1989

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TABLE OF CONTENTS

FOREWORD

This publication complements the proceedings of the Global End-of-Project Meeting on Water Quality Control held in Banff, Canada, September 1988, also published by the International Development Research Centre (IDRC) under the title "Results of a Three Continent, Eight Country, IDRC-Sponsored Research Project on the Use of Simple, Inexpensive Microbial Water Quality Tests". Subsequent to the Banff meeting, the Centre brought together scientists from the eight countries participating in the global project and experts on environmental microbiology from the U.S. and Canada, to have a free exchange of ideas, review the progress of the work so far achieved, and develop strategies to address outstanding laboratory research issues and the field testing of selected microbiological techniques.

This report has been prepared from the discussions and presentations made during the week-long meeting. It is hoped that the information presented will stimulate new research initiatives in all countries where conventional bacteriological tests are expensive and too sophisticated in order to allow for the proper monitoring of drinking water sources.

The participants (listed in Annex 1) contributed freely with their ideas and jointly prepared the conclusions presented in this report. The extensive contribution made by Dr. P. Payment in summarizing the background technical material and preparing the evaluation of the microbiological tests is gratefully acknowledged. A special word of thanks is due to the support staff of the Health Sciences Division.

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SUMMARY

Water quality remains an important issue for developing countries, but the limited availability of funds as well as of technical facilities and trained staff seriously limits testing of water samples from areas that are not within range of large urban centers. Even in these urban centers, microbiological testing of distributed water is often limited to very few samples because of the high cost of standard tests. In order to provide developing countries and remote areas of industrialized countries with inexpensive and simple field tests for assessing their drinking water quality, researchers from several countries were involved in comparing the suitability of various alternative tests for laboratory and field use under local conditions. The Presence/Absence (P/A), the H_2S paper strip and the Coliphage tests were compared to the standard tests normally performed for water quality testing. The three tests appear to give good results even when incubation is done at ambient temperatures (20 to 35°C) and not at the standard 35°C. The standard tests for fecal contamination (m-FC, A-1 broth, etc...) were found of limited use because all require incubation at 44.5°C. The H_2S test was found to be the least expensive and easiest to perform. Its modification to a quantitative (mpn) test was recommended to permit classification of water sources. The coliphage test was found to be easy to perform for personnel with some form of laboratory training. The P/A test was found useful for treated waters where minimal contamination is expected. The study group suggested further research in the development of the H_2S quantitative test, the effect of incubation at ambient temperatures, the evaluation of physico-chemical water quality on the coliphage test, and the initiation of some field evaluation of these two tests.

1. INTRODUCTION

1.1 Objectives of Research Network

In response to the International Water Supply and Sanitation Decade, developing countries have made special efforts to increase the availability of drinking water to their rural and poor peri-urban populations. This multiplication of newly developed or rehabilitated drinking water sources worldwide has heightened the already neglected need for routine monitoring of microbiological water quality.

Because of the need for specialized personnel, and the high cost and sophistication of supplies and equipment associated with most microbiological water testing, water sources often remain unevaluated. Without appropriate monitoring technologies, water supply programs (source selection & protection, treatment, distribution, maintenance, and upgrading) cannot be implemented effectively.

The International Development Research Centre (IDRC) began in 1983 to support research in the development and testing of rapid, inexpensive and technically simple bacteriological water quality tests for use in the monitoring and classification of drinking water sources. To date, projects have been funded in Brazil, Chile, Egypt, Malaysia, Morocco, Peru, Singapore and Thailand.

Research activities have so far focused on the evaluation and adaptation of existing tests to the particular conditions and needs of the various countries. The tests that have been investigated include among others: the Presence/Absence (P/A), H_2S paper strip, A-1 broth, and Coliphage tests.

1.2 Purpose of Meeting

The objectives of the meeting were to review the results obtained during the last five years of research, examine information gaps, and develop a follow-up strategy to address outstanding issues regarding laboratory research and field testing of the various procedures. Experts on environmental microbiology, external to the network, were invited to the meeting to assist the Centre and participating research teams in the evaluation of the various testing procedures and provide advice on future directions of research. The list of participants is presented in Annex 1.

1.3 Background Information.

Water Quality and Health

Untreated water sources such as surface waters (streams, rivers, lakes, etc.) or unprotected open wells are the vehicle for waterborne bacterial diseases such as cholera and typhoid fevers. Disinfection of water dramatically reduces the incidence of these diseases. Untreated waters may also play a role in the transmission of water-washed viral enteric diseases such as hepatitis (hepatitis A virus and non-A non-B hepatitis agents), gastroenteritis (rotaviruses, Norwalk and Norwalk like viruses), as well as an unknown number of ill-defined diseases caused by the other enteric viruses (adenoviruses, astroviruses, coxsackieviruses and echoviruses). The fecal-oral route is probably the major route for transmission of these bacterial and viral diseases as well as of many parasitic diseases in poor sanitary conditions. An improvement of water quality and water usage for improving sanitary conditions should result in a decrease of waterborne as well as water-washed diseases (Feachem et al., 1983).

Microbiological Water Quality Indicators

The recognized bacterial indicators for assessing water quality are bacteria of the Enterobacteriacae family defined as the total coliform bacteria and the fecal coliform bacteria. The **coliform bacteria** are gram-negative rods, aerobic or facultative anaerobic, nonspore forming, rapid-lactose-fermenting with gas formation within 48 hours at 35°C. Some bacteria from this group are indigenous to soil and waters. This made necessary the use of a more stringent indicator for fecal contamination in many situations. The **fecal coliform bacteria** are members of the coliform group of bacteria usually (but not always) found in the feces of warm blooded animals: they have the characterisitics of the coliform group, but will also produce gas within 24 hours at 44.5°C. The presence of fecal coliform bacteria in water is indicative of contamination by fecal material and is therefore considered indicative of a health risk because many enteric pathogens (bacterial, viral and parasitic) are present in feces. Furthermore, the significance of the coliform group density has been established as an indication of the degree of pollution and thus the sanitary quality of water (Feachem et al., 1983).

Recently, the use of **Escherichia coli** has been suggested as the most sensitive indicator of fecal pollution. However, several researchers have reported that this bacteria could be indigenous in tropical humid climates. E. coli has been detected in pristine sites, on leaves and in the soil (Fujioka et al., 1988; Hazen, 1988). These findings will require a reassessment of the suitability of this bacteria and of the fecal coliform group as indicators of water quality in tropical environments.

Coliphages, viruses that infect and replicate in the **coliform bacteria**, have also been suggested as indicators of fecal pollution because they are found in the same environment (Dutka et al., 1987). These viruses can be indicative of the presence of coliform bacteria and they are relatively easy to enumerate. There are however, many types of coliphages: their taxonomy is difficult and their properties very different. Some of these viruses are more resistant to inactivation than the coliform bacteria and have been suggested as better indicators of water treatment processes (Havelaar, 1986). If E. coli is found to be indigenous in tropical climates, the usefulness of coliphages as indicators of fecal pollution and accompanying health risk would be put in doubt. They could however, remain indicators of water treatment (especially disinfection).

In untreated waters, the relative proportions of total coliforms: fecal coliforms: coliphages appear to be in the range of 100:10:1. In disinfected or conventionally treated waters, no specific ratios can be assigned because of the various degrees of resistance to inactivation of the organisms included in these groups. In general, coliphages appear to be more resistant to treatment than the fecal or total coliform bacteria.

Microbiological Water Quality Tests

The preferred methods for assessing water quality, using as indicators the members of the coliform group and the fecal coliform group, are the **membrane filtration (MF)** technique and the **multiple-tube fermentation** techniques (APHA, 1985). Both are quantitative methods with high levels of sensitivity and they can be used to evaluate all types of waters including treated drinking waters, recreational waters and untreated drinking waters. The membrane filtration technique is usually more expensive and requires more equipment than the multiple-tube fermentation technique.

For raw water supplies and recreational waters, the five-tube (MPN) procedure using **A-1 broth** (APHA, 1985) was found to be a reliable and sensitive test by the various research teams of the network. This test was also shown to be very selective for E. coli. Isolates collected and identified from positive A-1 broth MPN tubes were found to be E. coli 80 to 100% of the time. The test was developed in the mid-seventies as a means of testing shellfish waters. It is a relatively simple, quantitative, single medium test with an easily recognizable gas production endpoint.

For drinking waters of high quality (treated or untreated) the detection of minimal amounts of contamination by the coliform group bacteria is required by most water authorities. In order to achieve a very high level of sensitivity for water samples that are expected to be exempt of such bacterial contamination, simpler tests have been proposed. Most of them will achieve a

level of sensitivity of one coliform bacteria per 100 mL in only one bottle of medium. The best known of these tests are the **presence/ absence test (P/A)** described by Clark (1968) and the **H_2S** paper strip test (Manja et al., 1982). A positive result from either of these tests indicates the presence of one or more indicator bacteria, usually Enterobacteriaceae (coliforms, fecal streptococci, or species such as Staphylococci or Pseudomonas).

The last test evaluated was the **coliphage test** . This test was developed during the US-Vietnam war as a simple field-test for assessing drinking water quality. From extensive studies performed at the Atlanta Research Corporation (1979) and others reported in the literature recently, the coliphage test appears to be a reliable indicator of the presence of E. coli and other coliforms in various environmental and drinking waters. The test offers the advantage of being economical, simple to perform and fast (it can provide results within 6 hours of testing).

Table 1 summarizes the number and type of tests investigated by the various research teams of the network. This table also provides references to the standard testing procedures. Modifications to these procedures made by particular countries are decribed in the Proceedings of the Banff Meeting (Dutka and El-Shaarawi (1989).

TABLE 1

SUMMARY (BY COUNTRY) OF TESTS INVESTIGATED

	Test	Egypt	Morocco	Thailand	Singapore	Peru	Chile	Brazil	Canada	Malaysia
Coliforms	m-Endo LES (APHA 909A) 100 mL		X	X	X		X	X	X	
	5-tube MPN LST (APHA 908A) 55.5 mL	X	X	X	X	X	X	X	X	X
	P/A (APHA 908E) 100 mL	X	X			X	X	X	X	
	H2S paper strip (Manja et. al. 1982) 20 mL			X		X	X	X	X	
Fecal coliforms	m-FC (APHA 909C) 100 mL		X		X	X	X	X	X	X
	5-tube MPN A-1 broth (APHA 908C) 55.5 mL	X	X	X	X	X	X	X	X	X
Coliphage	APHA 919C 20 mL	X	X	X	X	X	X	X	X	X

2. RESEARCH RESULTS

2.1 General Observations

The following is a summary of observations and recommendations made during the meeting that concern the work so far achieved and the general direction of future research.

Approach and Purpose of Study

1. Total coliforms (as indicators of bacterial contamination) and fecal coliforms (as indicators of bacterial contamination of fecal origin) are the recognized indicators throughout the world for assessing the bacteriological quality of waters. These indicators have been proven very useful for many decades, and tests for their quantification are highly sensitive and reliable.

2. The intent behind this network's research thrust is not to replace accepted indicators and present standards, but to develop simpler and less expensive **tests** that will allow developing countries to collect basic information on water quality in urban and rural areas.

3. Information on bacteriological water quality is lacking worldwide because of the high cost and technical sophistication required to perform the present standard tests for total and fecal coliforms on a routine basis. This situation is unlikely to change in the future unless more appropriate tests are employed.

4. Developing countries must build data records on the microbiological quality of their drinking waters. This basic information is required for the correct management of water programs (allocation of funds, determination of maintenance & upgrading schedules, evaluation of water projects, identification of problems within water treatment and distribution, and planning of corrective actions). Also, without data, the concerned authorities (from central down to municipal governments) cannot be convinced to take any type of remedial or corrective action.

5. The principal characteristics of interest in the various tests investigated are: reliability, simplicity, low cost, and transportability to the field.

Water Quality Testing and Type of Water

Large urban centres in most countries have more or less well-equipped water microbiological laboratories under the authority of a regulatory agency that specifies water quality standards derived from world accepted standards of water quality. Some

form of decentralized regional laboratory network with a lower level of technical facilities is responsible for analyzing rural and urban water samples from very diversified sources: (a) untreated waters such as surface water, rain water collected from roofs, well water, groundwater; and, (b) treated waters after chlorination or after partial or full conventional treatment.

The objectives of testing waters from treated or untreated sources are different. **Untreated drinking water** sources must be first classified according to their bacterial content, in relation to fecal pollution and the ensuing potential presence of pathogens. Once found suitable as drinking water sources, they must be reevaluated on a regular basis to ensure their quality. **Treated drinking waters** must be tested to evaluate efficacy of the treatment in the removal of pathogens, and to indicate if recontamination by fecal material has occurred. The tests to be used for evaluating water quality must then be tailored to the need: the classification of **untreated waters** requires **quantitative tests**, while the assessment of **treated or well protected waters** requires very **sensitive tests** that can be **either qualitative (presence or absence) or quantitative.**

Data Analysis and Interpretation

1. Absence of indicator organisms should not be equated with absence of pathogens in waters, especially with respect to human viruses and parasites. In the North American experience, waters of acceptable bacteriological quality have been shown to contain Giardia cysts and viruses. In the U.S., proposed rules for drinking water are now based on treatment control (i.e. physicochemical processes such as disinfection and filtration) and not on indicators or pathogen detection.

2. Interpretation of correlations obtained between different tests should be made with caution, and must take into account differences in sample volumes, target indicators, and their relative concentrations and resistance in the various types of waters.

3. Risk assessment studies could be used in developing a classification scheme for drinking water sources. These studies could determine the levels of risk acceptable to a community with different concentration levels of indicators, and possible risk reductions achieved by different quality control measures.

4. The analysis of the data so far collected is incomplete. More work is needed for interpreting the generated data from each country and for integrating the data from the entire network. In particular, data should be analyzed to determine the reproducibility of the tests (precision) and their sensitivity. The effects of incubation temperature

and water quality (turbidity, bacterial content, pH, organic content, temperature, water type and composition), as well as the types of bacteria detected, should be examined more closely.

5. Common procedures for sampling, testing and laboratory quality control should be defined. Common methodologies for the interpretation of results need also to be determined and used in the design of future research protocols.

2.2 Evaluation of Microbiological Tests

Evaluation Parameters

The following parameters were used to guide the discussion and evaluation of the four tests investigated (P/A, A-1 broth, H_2S, and Coliphage): sensitivity, reliability, selectivity, suitability for field use, labour requirements, cost, incubation time and temperature, shelf life of reagents, and limitations of tests.

Summary of Results

The main observations made during the evaluation are presented below:

1. **Reliability:** Comparison of results from the different countries showed an overall good correlation between the standard coliform tests and the **P/A**, **H_2S** and **A-1 broth** tests. The reliability of these tests was considered high. Observed discrepancies (i.e. one test negative while one or more tests being positive, or vice-versa) could be assigned to different levels of sensitivity, due in turn to differences in the volumes of sample tested (i.e. 20 mL vs 100 mL) or the comparison between qualitative and quantitative tests (i.e. P/A or H_2S results compared to A-1 MPN, m-Endo or m-FC results).

   The **Coliphage** test was considered reliable in general, although many false-negative or false-positive combinations were observed when comparing results with those of standard coliform tests. These discrepancies could also be assigned to the differences in the volumes of samples tested, especially when considering the relative proportions of total coliforms: fecal coliforms: coliphages (100:10:1 respectively) that were apparent in untreated waters. Difference in resistance in the environment and/or to disinfection between the measured organisms could also affect results, but only a full scale research effort could bring possible answers to these questions. Such research was beyond the scope of this project.

2. **Sensitivity:** The level of sensitivity of the different tests was related to the largest volume of sample used. The sample volume for the **P/A** test was 100 ml, while the **H_2S** and **Coliphage** tests used 20 ml of sample (only in one project was a 100 ml sample used for coliphages). For these last two tests, studies to increase their sensitivity by increasing the sample volume were recommended.

3. **Selectivity:** Tests for **total coliform** bacteria were considered relatively selective; while some indigenous bacteria present in large numbers might modify the test results, this would not present a major problem for the classification of waters on the basis of their bacterial content. Tests for **fecal coliform** bacteria were considered relatively selective and 70 to 90% of the isolates were identified as E. Coli. It was noted however, that if E. Coli is found to be indigenous under tropical conditions, the value of this organism as indicator of fecal pollution from warm blooded animals will be considerably limited.
Both the **H_2S** and the **P/A** tests, because they detect all bacteria of the coliform group, would be less susceptible of being questioned if E.coli is found to be indigenous in tropical climates: fecal bacteria account for only 10% of the coliform bacteria and this group is used as indicator of general water quality and not of fecal pollution.

4. **Incubation Temperature:** Tests for fecal coliform bacteria (i.e. A-1 broth) require incubation at 44.5°C and thus require precise temperature control. On the other hand, tests for total coliform bacteria and coliphages were found to require less stringent temperature control: incubation from 20 to 35°C did not appear to modify significantly results obtained. This has important implications for field use as it may be possible to develop simple field incubators.

5. **Incubation Time:** The time required to obtain results is in general less for the rapid growing fecal coliform bacteria (24 hours) and coliphages (6 to 24 hours). The P/A and H_2S tests do require longer incubation periods, but they are designed for the detection of minimal contamination levels. Results could be obtained within 18 to 24 hours for heavy contamination, but incubation may be maintained for up to 5 days to insure detection of minimal bacterial contamination.

6. **Shelf Life of Reagents:** Liquid medium tests (A-1, P/A, and coliphage) have shorter life than tests using dried medium such as the H_2S test.

7. **Suitability for Field Use:** The H_2S, P/A and coliphage tests can be used in the field (without a temperature controlled incubator), or in a minimally equipped regional laboratory (with incubator). These tests require minimal sample handling

(i.e. no dilutions) and would be the easiest to transfer to personnel with minimal training. The suitability of the A-1 broth test for field conditions is somewhat limited because it requires handling of dilutions and incubation at 44.5°C. The H_2S test is the easiest to interpret (development of a black colour), followed by the P/A test (colour change from purple to yellow). The coliphage test requires counting plaques and differences in plaque size may complicate estimation. Sampling costs in rural areas can be far more significant than analytical testing costs. In the Moroccan experience, with the present network of regional labs, sampling costs are ten times larger than the costs of analysis. The possibility of performing the tests inside the communities is therefore very important. The Coliphage test with its field kit offers a significant advantage in this respect. The H_2S test is also well suited for field use. The use of dry reagents (paper strips) with a long shelf life make possible their preparation in a central location, with recycling of glassware (boiling of test tubes) on-site. This would make implementation simpler and less expensive.

8. **Cost:** Significant differences in the price of test components will be encountered in the various countries. The membrane filtration (m-FC) tests are essentially reserved for high level laboratories with adequate funds. The A-1 broth also appears to be expensive in many countries, while the H_2S and coliphage tests are less expensive. Bottles and other ancillary supplies do not appear to be of major concern and can be obtained locally at reasonable cost. The major costs associated with the coliphage test are the plastic plates and syringes used for volume measurement.

The results of the evaluation are summarized in Table 2.

TABLE 2

Comparison of Microbiological Tests

	Presence/Absence (P/A)	H2S paper strip	A-1 Broth test	Coliphage test
Organisms detected	Coliforms, Enterococci, Pseudomonas	Coliforms, Enterococci, Pseudomonas	Fecal coliforms	Coliphages
Type of water	Treated or minimally contaminated	Treated or minimally contaminated	Treated or untreated	Treated or untreated
Type of test	Qualitative (can be modified to be quantitative)	Qualitative (can be modified to be quantitative)	Quantitative	Quantitative
Sensitivity	++++(100 mL/bottle)	+++(20 mL/bottle)	+++(3 dilutions of 5 tubes of 10 mL)	+++ (1 to 5 plates of 20 mL)
Reliability	High	High	High	Very good
Selectivity	Coliform bacteria and some other	Coliform bacteria and some other	Fecal coliform bacteria, mainly E. coli	Bacteriophages of coliform bacteria
Incubation time	18-48 h or up to 5 days	18h - 5 days	24 h	6-24 h
Incubation temperature	Normally 35°C, but temperatures of 26 to 35°C have been used with success	Normally 35°C, but temperatures of 20 to 35°C have been used with success	3 h at 35°C and 21 h at 45.5°C	Normally 35°C, but temperatures of 20 to 35°C have been used with success
Suitability	Recommended if a minimally equipped community or regional laboratory is available (media preparation, incubator) minimum level of training and technical expertise required.	No equipment required if ambient temperatures are used for incubation. Minimal training and technical level required.	Recommended if a minimally equipped or regional laboratory with technical staff is available (need for stringent incubation temperature and confirmation procedures).	Recommended if a minimally equipped community or regional laboratory is available. Field kit available if ambient temperatures are used for incubation
Technical level required	Low	Low	Average	Average/Low
Shelf life of reagents	At least 6 months at room temperature	At least 7 months at room temperature (dryed paper strips)	3-4 months at room temperature if stored sealed and in the dark, more at lower temperatures	About 3 months at 4°C, less at higher temperatures
Cost	Medium/Low	Low	High	Medium
Research needs	Perhaps development of quantitative test	Development of quantitative test, increase sensitivity, effect of incubation temperature, interference by other bacteria	None	Increase sensitivity, verify bacterial host strain(s) evaluate effects of water quality and significance of plaque size, suitability as indicator, effect of incubation temperature

General Conclusions.

The general conclusions reached were as follows:

a. The H_2S and the Coliphage tests were found to be the most promising for field testing of remote water sources and their classification. The present limitations of these tests can be easily overcome. The H_2S test should be made quantitative and possible interference from non coliform bacteria examined. Both tests should be made more sensitive, and further evaluation of the effects of incubation temperature should be addressed. For the Coliphage test, the effects of water quality and significance of plaque size should be investigated, and the selectivity of bacterial host strain(s) verified. Its applicability to evaluate water treatment also merits further study.

b. Both P/A and A-1 broth tests are well established tests requiring no further laboratory research work, perhaps with the exception of making the P/A test quantitative. In its present form, this test is very well suited for the testing of treated or minimally contaminated waters in urban centres or even in remote areas of developed countries. The A-1 broth test is a highly reliable and quantitative test but does require proper incubation facilities and is more expensive than the other three tests.

3. RESEARCH NEEDS

3.1 General Issues

Need for Laboratory and Field Research

It was generally agreed that further laboratory based research is needed, in particular with respect to: the development of the H_2S quantitative test; the effects of incubation at ambient temperatures; and, the effect of physico-chemical water quality on the coliphage test.

The issue of field research was addressed several times during this meeting. Two types of field research were identified: laboratory testing of real water samples (collected in the field), and testing of field application (i.e. examining if tests can be performed in the field).

Concerns were expressed regarding the readiness of the tests for field application. The tests must be given all opportunity to succeed before promoting extended field testing to Ministries of Health.

The consensus was however, that some form of field testing should be initiated. Arguments in favour of this approach can be summarized as follows:

1. Evaluation of the results from the different countries led to the conclusion that the P/A, A-1 broth, H_2S and Coliphage tests could be used with good confidence as laboratory performed tests.

2. The main value of the tests lies in their ability (different for each test) to be performed on site.

3. It is important to involve the government (water authorities or Ministries of Health) at the research stage. This will ensure a better understanding by these authorities on the possibilities and limitations of the tests and classification scheme. In turn, their participation will help in securing their interest and will therefore facilitate future field testing on a larger scale and eventual adoption and implementation.

The involvement of regional laboratories and field staff from water authorities, in controlled field research activities, was suggested. On-site problems with the following items should be investigated: sample preparation, media storage, glassware preparation, sampling, reading and recording of test results, variation and effect of incubation temperatures, staff training and commitment, quality assurance aspects.

Presence of E. Coli in Tropical Climates

Recent reports on the presence of E. coli and related species in pristine waters may put in question the suitability of the fecal coliform bacteria and coliphages as indicators of fecal pollution and accompanying health risk. It was therefore considered important to look for these bacteria in the environment to see how prevalent they are in the various countries of the research network. The network offers an excellent forum for such testing as it includes countries from Asia, Africa and Latin America.

Quality Assurance

The lack of reference standards in microbiological work prevents a direct examination and comparison of the accuracy and precision of testing methods between countries. To compensate for this situation, all sources of errors in the laboratory should be looked at closely, and the development and use of precision curves in individual labs should be encouraged. With respect to the latter point, the information from triplicate (and even duplicate) testing of samples can be used to generate frequency distribution curves for assessing the reproducibility of results within labs. These curves can indicate problems with the testing methods or laboratory procedures.

In terms of minimizing laboratory errors, the main items to consider are listed below. More detailed information can be found in the references provided at the end of this section.

Organizational Structure: Clear definition of responsibilities and activities.

Laboratory Personnel: Training, motivation, communication, participation, comfort level and safety.

Laboratory Facilities: General upkeep, lab layout, room temperature, waste handling and disposal, possibility of cross-contamination.

Laboratory Equipment and Supplies: Procedures for standarization, maintenance and operation of equipment, regular preventitive maintenance, control of quality of labware and detergents.

Media and Reagents: Monitoring quality of distilled and de-ionized water, monitoring contamination of stock buffer solutions and culture media, proper labelling and storage.

Quality Control Procedures: Clear definition and schedule of procedures, verification of purity of host strains, positive and negative controls, reproducibility of tests within the lab.

Sampling: Sample collection, sample handling, type and sterility of sampling containers, elapsed time between sampling and analysis.

Useful References:

a) Microbiological Manual. EPA 1978. Part IV, Quality Assurance.

b) EPA - 670/9-75-006. Handbook for Evaluating Water Bacteriological Laboratories.

c) EPA - 570/9-82. Manual For the Certification of Laboratories Analyzing Drinking Waters, Criteria and Procedures.

d) APHA, 1985. Standard Methods For the Examination of Water and Wastewaters. 16th Edition. Sec. 902.

Common Testing Protocols

The need for common testing protocols was also considered important. The following items should be agreed upon for future research work:

1. Quality assurance procedures (including procedures for positive and negative controls in the field, verification of purity of host strains and their storage period).

2. Sampling (collection and handling).

3. Testing procedures according to the type of water (including test volumes, incubation time and monitoring of incubation temperatures).

4. Reading and interpretation of tests (eg. colour chart for H_2S test and plaque counting with variations in plaque size for coliphage test).

5. Additional water tests to be performed on samples (eg. turbidity, heterotrophic bacterial count, pH, organic content, cations, etc...).

Water Quality Classification

The development of a classification scheme for categorizing water sources according to the degree of contamination was considered an important issue yet to be addressed by the research network.

This classification scheme will be dependent on the purpose of testing. In this respect, it was agreed that the main purpose of the tests was their use in classifying **untreated drinking water** sources into broad levels of contamination, and evaluating the level of conformity of **treated drinking waters** to accepted quality standards (i.e. absence of total and fecal coliforms) in urban settings, in remote areas using treated waters but having no laboratory facilities, and even in areas with good laboratory support.

The classification scheme is to be complemented by sanitary surveys of the water sources and will eventually be incorporated into a computer software package for the management and analysis of data related to drinking water quality. This software package is primarily aimed at policy makers, planners, and program managers of departments of Public Health and Ministries of Health. It is being presently developed with support from IDRC (project: Water Quality Data Management (Malaysia/ Canada), 3-P-86-1051).

Time constraints did not permit a discussion on the details of a classification system nor on its relation to the Water Quality Data Management package.

3.2 Test-Specific Activities

A quantitative H_2S test and the Coliphage test were believed to be the most suitable for field testing of remote water sources in developing countries.

The H_2S test, which requires very little training, has long shelf life and is easy to interpret, has the potential as a test that could be applied at the community level. This test, qualitative as it is now or by simply increasing the number of 20 mL bottles used, could also be utilized at regional laboratories to lower the costs of analysis of treated water samples or to permit testing more samples for a better assessment of drinking water quality. The P/A test (needing no further laboratory research work) is also well suited for this type of situation.

Further studies on the Coliphage test were believed important for the following reasons:

i. The test relates more accurately to the presence of fecal coliforms than the H_2S test (i.e. it is more selective), and permits a more precise classification of waters when needed.

ii. For treated waters, measuring phages relative to the degree of treatment may prove to be a very useful tool. In the North American experience, samples showing no coliform bacteria are found many times to contain viruses and parasites. In drinking waters of developing countries, loads of these microorganisms are much heavier, and consequently the safety factors established by the use of bacterial indicators alone may be lower.

A number of outstanding research issues regarding the H_2S and Coliphage tests were discussed. These are summarized below.

H_2S Paper Strip Test.

1. **Incubation Temperature:** Temperatures between 20 to 35°C have been used with success in past projects. There is a need however to look more closely at the effects of varying temperatures on the test, increasing the study range to 10 - 40°C. The possibility of developing a temperature dependence curve for the interpretation of results or the determination of minimum incubation periods at different temperatures was also suggested. The study of inexpensive incubators for reducing variations in temperature during incubation was also considered important, although not a priority at the present time.

2. **Making the Test Semi-Quantitative:** For **treated waters** (or waters with expected low bacterial counts) the test can be made semi-quantitative by using 5 bottles of 20 mL (for a total sample volume of 100 mL). For **raw untreated waters** (or sources to be converted into drinking water sources) the test can be made quantitative by using several bottles at two dilutions to obtain an MPN count from computerized tables. The following combination was suggested: 2x20 mL and 4x5 mL. The latter volume (5 mL) was retained as the smallest volume measureable under field conditions by untrained personnel. On-site disposal of samples and recycling of test and dilution bottles should be also examined. Boiling of glassware for 30 minutes was suggested as being adequate. Non-disposable items were preferred to prevent environmental pollution from used testware.

3. **Preparation of Strips:** The possibility of using reagents directly dried into testing bottles was considered. It was pointed out however that colour development starts on the paper strip and serves as a background for reading purposes. This approach would also complicate logistics as it would make necessary the recycling of bottles (cleaning and preparation) from a centralized location. There is a need to examine different types of paper, and determine quality assurance and control procedures for the preparation of strips.

4. **Literature Search:** A literature review was considered necessary. This review should look into the availability of information regarding temperature effects, selectivity and sensitivity of test, H_2S producing bacteria in environment, and limitations of test. The literature search should be summarized by one or two persons and distributed among members of the working group.

5. **Positive and Negative Controls:** The use of positive and negative controls was proposed for routine quality control of the procedures in laboratories and the field. Filter paper disks impregnated with H_2S producing bacteria were suggested for use in positive controls, clean disks may be used for negative controls.

6. **Preparation and Testing of Printed Material and Audiovisual Aids:** The development and testing of a simple manual for training laboratory and field personnel was recommended. The manual should include a colour chart for the interpretation of test results. The preparation of a video training module was also suggested, although this could be done at a later time.

The researchers from the various developing countries also discussed their particular interests in pursuing research work on the above issues. Their research interests are summarized in Table 3.

TABLE 3

Selection matrix of research activities by country, H_2S test

Country	Objectives[1]					
	1	2	3	4	5	6
Egypt	x			x		
Malaysia				x		
Brazil		x		x	x	
Chile	x	x		x		x
Peru	x	x		x		
Thailand	x	x	x	x		
Morocco	x	x		x		
Singapore						

[1]Objective
1: Incubation Temperature
2: Making the Test Semi-Quantitative
3: Preparation of Strips
4: Literature Search
5: Positive and Negative Controls
6: Preparation and Testing of Printed Material and Audiovisual Aids

Coliphage Test

1. **Safety Factor:** To get a better grasp on health risk and the association of coliphages to fecal contamination, it was suggested that further research be done on the relationships between coliphage counts and those of selected waterborne bacterial pathogens (Salmonella typhosa, Shigella, Vibrio cholerae, Campylobacter, etc.) and even human enteric viruses (according to the relevance to and capabilities of individual countries).

   Researchers from Singapore and Brazil indicated an interest in looking at parasites and entero and rota viruses. Egypt was interested in looking at bacteria and parasites, and Malaysia at bacteria only.

2. **Presence of Coliphages in Pristine Waters in Tropical Settings:** It was also considered necessary to examine the presence of coliphages in addition to E. coli in the environment. Background levels in the environment of the other microorganisms to be tested in (1.) should also be determined.

3. **Enhancement of Sensitivity Versus Cost:** The sensitivity of the coliphage test is presently too low to allow meaningful statistical comparisons with coliform bacteria.

   Sensitivity could be related to sample volume and host strain. Increasing the sensitivity by increasing sample volume requires an examination of the increase in cost. The use of local host strains would impede comparison of results between countries.

   Standard testing protocols and quality assurance procedures are required. The latter should include periodic checks on the purity of the strain. Changes in sensitivity of host strain with length of storage should also be examined.
   The use of 100 ml samples for treated waters and 20 ml samples for raw waters was suggested.

4. **Water Quality Effects on Test:** The effects of pH, turbidity, bacterial content, organic content and cations on the coliphage test should be examined. Available data from past research projects should be analyzed more carefully.

5. **Water Type:** Data available for well, surface, rain and ground waters should be examined and compared to assess the limitations of the test according to water type. This analysis should take into consideration the effects of the various parameters enumerated in 4.

Further studies on well and rain waters should be carried out to clarify the reasons for the repeated false negative and positive results obtained in previous studies.

6. **Evaluation of Treatment Efficiency of Potable Water:** The applicability of Coliphages to assess the degree of treatment of existing facilities should be examined. This may include before and after treatment testing of coliphages, coliforms, parasites and possibly human enteric viruses.

7. **Plaque Size:** Standardization in plaque counting is necessary. One suggestion was to have no upper counting limit, count small plaques after six hours and count again at the end of the incubation period.
Means for control of bacterial growth in plaques should also be examined.

8. **Analysis of Existing Data Set:** The complete data set from all countries should be examined to establish the extent of information available. This analysis should determine the status of knowledge acquired, missing data required to answer concerns raised, and identify statistical approaches for the interpretation of data. Before a complete analysis of the integrated data set is performed, individual countries must analyze their own data according to commonly agreed procedures.

9. **Selectivity of Test:** The study of local bacterial host strains and specificity to coliphages should be examined.

The research interests expressed by the researchers are summarized in Table 4.

TABLE 4

Selection matrix of research activities by country, Coliphage Test

Country	Objectives[1]								
	1	2	3	4	5	6	7	8	9
Egypt	x	x							x
Malaysia	x	x	x		x	x			
Brazil	x	x	x	x	x		x		x
Chile		x		x	x	x			x
Peru		x	x	x					
Thailand		x	x	x	x				
Morocco		x	x	x	x	x			
Singapore	x	x					x		
Canada								x	

[1]Objective
1: Safety Factor
2: Presence of Coliphages in Pristine Waters in Tropical Settings
3: Enhancement of Sensitivity Versus Cost
4: Water Quality Effects on Test
5: Water Type
6: Evaluation of Treatment Efficiency of Potable Water
7: Plaque Size
8: Analysis of Existing Data Set
9: Selectivity of test

3.3 Additional Research Activities

Research Position Paper

There is a need to condense the information so far collected and clearly define the purpose of the research and suggested approaches. This material should be made available outside the working group to inform other scientists of the work being done, and promote future research on this topic.

The publication of a position paper in an international journal (such as the WHO bulletin or the Water Quality bulletin) or its presentation at an international conference was suggested. The draft of the position paper will be prepared by Dr. Payment (with the help of Drs. Fujioka, Gerba and Sattar), and will be circulated to the research teams from the eight countries and to Drs. Dutka and El-Shaarawi for their comments and review.

Statistical Data Analysis

The concensus was that the present statistical analysis of existing data was incomplete. Various researchers indicated that they had difficulties in finding statisticians in their countries with experience in the analysis of environmental microbiological data.

Dr. El-Shaarawi agreed to write a manual that would guide the various research teams in the interpretation of the data generated from each country. The manual will also address the design of research protocols from the statistical point of view, and means of assessing the reliability associated with each test and its scope of application.

REFERENCES

1. APHA, 1985. Standard methods for the examination of water and wastewaters. 16th Edition. American Public Health Association, Washington, D.C.

2. Atlantic Research Corporation 1979. Evaluation of Coliform Bacteria and Bacteriophage Relationships in Assessment of Water Quality. Final Technical Report, NSF Grant No. PFR78-19196, Dec. 1979. Division of Problem Focused Research Applications, NSF, Washington, D.C. 20550.

3. Clark, J.A. 1968. A presence/absence (P/A) test providing sensitive and inexpensive detection of coliform, faecal coliform and faecal streptococci in municipal drinking water supplies. Can. J. Microbiol. 14: 13-18.

4. Dutka, B.J., El-Shaarawi, A.H., Martins, M.T. and Sanchez, P.S. 1987. North and south American studies on the potential of coliphage as a water quality indicator. Wat. Res. 21: 1127-1135.

5. Dutka, B.J. and A.H. El-Shaarawi (editors), 1989. Results of a Three Continent, Eight Country IDRC Sponsored Research Project on the Use of Simple, Inexpensive Microbial Water Quality Tests. Proceedings of the Banff Meeting on Water Quality Control, Sept. 1988. Manuscript Report Series, IDRC, in print.

6. El-Abagy, M.M., Dutka, B.J. and Kamel, M. 1988. Incidence of coliphage in potable water supplies. Appl. Envir. Microbiol. 54: 1632-1633.

7. Feachem, R.G., Bradley, D.J. Garelick, H. and Mara D.D. 1983. Sanitation and Disease: Health aspects of excreta and wastewater management. World Bank Studies in Water Supply and Sanitation 3: 133-478. John Wiley and Sons, New York.

8. Fujioka, R.S., Tenno, K. and Kansako, S. 1988. Naturally occurring faecal coliform and faecal streptococci in Hawaii's freshwater streams. Toxicity Assessment 3: 613-630.

9. Havelaar, A.H. 1986. F - specific RNA bacteriophages as model viruses in water treatment processes. Rijkinstituut voor Volksgezonneid on Milieuhygiene, Bilthoven, The Netherlands.

10. Hazen, T.C. 1988. Faecal coliform as indicators in tropical waters: a review. Toxicity Assessment 3: 461-477.

11. Loh, C., Jangi, M.S., Ho, Y.C., Ngeow, Y.F., and Wang, C.W. 1988. Development of a field test kit for coliphage detection in natural waters. Toxicity Assessment 3: 451-460.

12. 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. 10: 797-801.

13. Ratto, A., El-Shaarawi, A.H., Dutka, B.J., Lopez, C., and Vega, C. 1988. Coliphage association with coliform indicators: a case study in Peru. Toxicity Assessment 3: 519-533.

14. Suan, S.T., Chuen, H.Y., and Sivaborvorn, K. 1988. Southeast Asian experiences with the coliphage test. Toxicity Assessment 3: 551-564.

ANNEX 1

WATER QUALITY CONTROL MEETING

FEBRUARY 20 - 24, 1989

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FEBRUARY 20 - 21, 1989

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