

## ON SITE BACTERIOLOGICAL TESTING OF WATER IN REMOTE ABORIGINAL COMMUNITIES

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Bacteriological water quality in remote communities is difficult to test because of distance to health laboratories and the need to collect samples under strict a-septic conditions and to bring the samples to the laboratory within 24 hours. This paper provides details on an investigation currently under way into the use of self contained bacteriological testing kits in remote Aboriginal communities.

### BACKGROUND

The Water Authority of Western Australia has responsibility for the monitoring of water quality for Aboriginal communities in remote areas of the State, such as the Pilbara, Western Desert and Kimberley regions. At present, water quality monitoring is achieved by collecting a sample from a source on a regular basis, usually once a month, and chartering an aircraft to deliver this sample to a central testing laboratory, located at the Queen Elizabeth II Medical Centre, Perth. Chartering is necessary because the sample must arrive at the laboratory within 24 hours to be valid.

Only a limited number of communities are tested at present. In the Pilbara, the communities of Jigalong, Yandearra and Warralong are sampled monthly and Punmu is sampled every quarter. The following communities are not sampled at all. Milyakirri, Well 33, Parnngurr, Kuta Kuta, Camp 61, Billanooka, Walgun, Robertson Range, Pundawarrie, Ngurrawana, Gurradunja and the Wapet road bores on the way out to Punmu. The 'Strelley Nomad' communities of Mijijimaya, Callawa, Coongan, Carlindi, Lalla Rookh and Strelley are also not monitored, as the Strelley Nomads do not want the Water Authority to be involved with their communities. No detailed information is available as yet for the Kimberley, but an estimated 100 small communities have no water quality monitoring at present. It should be noted that most of the communities which are not tested are very small (30 or less people) and can be totally uninhabited for several months.

Total and faecal Coliform counts (tested for at 37 and 44 degrees centigrade respectively) and detection for amoebae are performed for all samples, and samples showing high background growth counts are examined for salmonella.

The current procedures for analysis of water samples from remote areas detailed above have various drawbacks:

- \* a large number of small communities which do not get tested
- \* it is difficult to guarantee that samples arrive within the necessary time period. If samples are delayed, another sample

must be collected. This does not protect the community effectively and is costly

- \* the system has little flexibility, and can break down in emergency situations, such as when there is a marked increase in water related illness, flooding etc

- \* Water Authority personnel often do not have sufficient time to carry out other tasks on site such as routine maintenance, checking of facilities etc, because aircraft must leave promptly to ensure delivery within 24 hours

- \* it typically takes 5 or 6 days to analyse a sample and get the result to an inspector using the current system. Thus, if contamination is discovered, it is likely that much of the community will have been exposed before any warning can be given or remedial action taken

- \* high costs. In particular charter of an aircraft once a month costs about \$16000 per annum

Use of a portable, self contained testing kit by local members of the community or eg community nurses would address these shortfalls, allowing more effective water quality monitoring at significantly reduced cost.

The objectives of the study are:

- \* to test the technical applicability and accuracy of various commercially available kits

- \* to undertake a pilot training scheme in kit usage. This is targetted at members of local communities, but other people, such as community health nurses, may be trained as well

- \* produce a cost / benefit analysis, comparing the current situation with projected scenario if kits were introduced

- \* produce a draft training manual and, if additional funds can be found, a training video on the kit which is found to be the most appropriate for use in remote Aboriginal communities

Training in kit usage will form an advanced module in the Environmental Health Worker (EHW) Training Programme, which is taught at Pundulmurra College, Port Hedland.

#### WATER TESTING KITS

A range of different types of proprietary water testing kits are available. These are based

on two basic methods:

- \* the membrane filtration (MF) technique

- \* the 'most probable number' (MPN) or multiple tube technique

These are described below, together with a range of advantages and disadvantages related to each method, which have either been found in the literature or identified during the project.

#### Membrane Filtration Technique

A minimum volume of 10 ml of the sample is introduced aseptically into a sterile or properly disinfected filtration assembly containing a sterile membrane filter. A vacuum is applied and the sample is drawn through the membrane filter. All indicator organisms are retained on or within the filter which

is then transferred to a suitable culture medium in a petri dish. Following a period of resuscitation, during which the bacteria acclimate to their new conditions, the petri dish is transferred to an incubator at the appropriate temperature where it is incubated for a standard time period. The bacteria multiply during incubation, producing visually identifiable colonies. These colonies are counted, and results are expressed in terms of colony forming units (cfu) per 100 ml of sample.

The filtration unit and associated equipment, such as sampling cup and measuring cylinder, must be sterilised after each sample is processed. This is generally achieved in the field by burning methanol in the absence of oxygen to produce formaldehyde, a potent, gaseous sterilising agent. Equipment is designed to allow oxygen free burning of methanol.

Principal advantages of the membrane filtration technique are:

- \* it is the technique used by central laboratories, and thus direct comparison of results obtained is possible
- \* a large number of samples can be processed routinely, giving more representative samples and the opportunity to run samples in duplicate or even triplicate
- \* cost for consumables per test is lower than for the MPN technique
- \* results are obtained in approximately 24 hours, as compared with 48 hours or more using the standard MPN technique. Note however, that certain advances have been made on the standard MPN technique which produce a result in 24 hours. These are described below
- \* the equipment used is reasonably compact, portable and durable

Drawbacks of MF techniques include:

- \* the physical manipulations required to produce a result increase the chances of contamination from external sources
- \* if sterilisation of filtration equipment is not performed properly, carry-over from previous sample may occur
- \* the two factors cited above make training needs for effective kit usage significant
- \* sterilisation in the field can be time consuming if several samples must be processed ( fifteen minutes is required for each sample )
- \* MF techniques may be interfered with by:
  - \* high turbidity waters, caused by clay, algae etc which can interfere with filtration and produce a deposit on the membrane that could interfere with bacterial growth
  - \* the presence of a relatively high non coliform count. This can interfere with the determination of coliforms
  - \* toxic substances in the test water that may be absorbed and concentrated on the membrane and affect the growth of the coliforms

Two proprietary kits using MF techniques are being trialled, produced by the companies of Delagua and Millipore respectively.

#### Delagua

The Delagua Water Testing Kit was developed on behalf of the charity organisation Oxfam by the Robens Institute, based at the University of Surrey, England.

The kit is extremely compact, being roughly the size of briefcase, and contains:

- \* a combined measuring funnel and filtration unit
- \* an incubator which can run off its own rechargeable power source, a car battery or mains electricity (via a transformer). The incubator can process sixteen samples at one time. Operating temperature is 44 degrees Centigrade, allowing enumeration of faecal coliforms only
- \* a kit for determining chlorine residual and pH of samples
- \* a simple kit for determining water turbidity
- \* sufficient storage compartments for all consumables and a reasonable set of spare parts

The kit was developed primarily for use in developing countries, and employs resterilisable petri dishes and culture media bottles. The incubator is designed specifically for the aluminium petri dishes. These are rather smaller than the one@use plastic petri dishes used by the Health Department laboratories, and thus changing to use of one@use plastic petri dishes is not straightforward. The need to employ resterilised petri dishes increases training needs and the possibility of getting a wrong result due to contamination.

#### Millipore

Filtration apparatus and incubator are contained in two separate briefcase size units. The equipment is of extremely high quality and robust construction. This is reflected in the cost of equipment, which is roughly double that of the Delagua kit. Methanol sterilisation is considerably easier to perform and more likely to be effective using the Millipore system. This is because the methanol is introduced onto a 'wick' built into the filter base, rather than simply squirting an estimated volume of methanol into the equipment, as is the case with the Delagua kit.

An incubator is available which can process samples at 37 and 44 degrees centigrade simultaneously, allowing enumeration of both total and faecal coliforms. The incubator requires an external 12 volt power source, such as a car battery. The total bulk of equipment required to perform on@site tests with the Millipore system (filtration unit, incubator and transformer/power source) is considerably greater than the Delagua kit.

### Other options using MF techniques

Certain other possibilities have been identified using MF techniques.

#### Delayed Incubation

It is possible to treat the filtered membrane with a special 'holding' media which preserves bacteria for up to 72 hours. This has the advantage that samples have more time for transport to the central laboratory, but has the disadvantage that the community has to wait longer for a result to be produced. Delayed incubation techniques are being tested in conjunction with other methods.

#### Millipore 'Milliflex' system

This system combines a measuring funnel, membrane filter holder and the filter itself in a 'one use' plastic unit which is supplied in a sterile container. After filtration, the measuring funnel is sheared off from the membrane filter housing and thrown away. The filter is housed such that it can be transferred to a petri dish without the need for forceps, significantly reducing the chance of external contamination. The researcher is currently pursuing the possibility of trialling this method in the field.

### Most Probable Number Technique

In the standard MPN method different amounts of water are added to tubes containing a suitable culture medium. This requires the use of sterile glass pipettes and test tubes. The bacteria present in the water reproduce and, from the number of tubes inoculated and the number with a positive reaction, the most probable number (MPN) of bacteria present in the source water can be determined statistically. A double incubation lasting a total of 48 hours is required to test for faecal coliforms. Incubation must occur for 24 hours at 37 degrees and for 24 hours at 44 degrees. The complexity and longer time period needed to get a result meant that this method was originally excluded from investigation. However, a research of the literature showed that an improved MPN technique is now available. This is marketed by Palintest, and is known as the Colilert system.

#### Colilert

Sterile, pre-packed, sealed test tubes are available which contain all necessary reagents. 10 ml of the sample water is added to each of either five or ten tubes, depending on the result accuracy required. The test tubes are incubated for 24 hours at 37 degrees centigrade. Total coliforms are established by colour change in test tubes under normal light. Faecal coliforms are checked by assessing fluorescence of test tube contents under UV light. Results can only be quoted in terms of a 'most probable number', that is, precise enumeration is not possible. A portable UV torch is available for use with the kit. No specifically designed incubator to carry out this test has

yet been identified, although the Australian representative of Palintest, Crown Scientific, have kindly provided a large incubator to allow testing of the method.

Principal advantages of the Colilert system are:

- \* it is very simple to use. leading to :
  - \* reduced need for training
  - \* less possibility of wrong result due to user error or contaminated equipment
- \* significantly less time needed to process a sample, taking into account the need for methanol flaming using MF techniques
- \* both faecal and total coliforms can be tested for using one temperature, allowing simpler and probably more compact incubator design

The drawbacks of the Colilert system include:

- \* test for test, it is much more bulky than MF methods. An estimated maximum of six samples could be processed at one time in an incubator small enough to be considered portable
- \* incubation temperature of 37 degrees cannot be guaranteed in Australian outback conditions. The sensitivity of the test to higher temperatures is to be investigated
- \* the glass test tubes, although reasonably durable and well packed, would be susceptible to breakage during transport along dirt roads
- \* positive samples take on an appealing yellow colour. Disposal of test tubes and contents would need careful training and policing to avoid eg children trying a sip of cultured bacteria

#### Other Options for Field Testing

A method is described in the literature whereby water is screened for faecal pollution by detection of production of hydrogen sulphide. The presence of coliforms in water is associated with organisms which produce hydrogen sulphide.

The method is extremely simple, and requires a minimum of equipment:

- \* medium is prepared in a central laboratory, and 1 ml of the medium absorbed onto eg folded tissue paper. This is placed in a 20 ml bottle, sterilised, dried and sealed
- \* bottles are then transported into the field, for storage at convenient distribution points
- \* water is tested by filling the bottle, and allowing it to stand at ambient temperature reported at 30 @37 deg. C
- \* faecal pollution is indicated if the contents of the bottle turn black within 12 to 18 hours

This method is not thought to be a useful replacement for areas where more accurate determination is possible, however, in extremely remote locations, or where a large group of people are gathered for a short time ( for instance at a law meeting) or

where a trained individual is simply not present, this test may be of particular use.

Tests are currently under way in the central laboratories in Perth to ascertain the detection level for faecal pollution using the method, and whether temperatures in excess of 40 degrees centigrade affect results.

#### PRELIMINARY FINDINGS

A range of findings have already been described above. Other important findings to date are described briefly below. The majority of work to date has involved the Delagua kit as equipment and consumables for other kits have only recently become available. The study is due for completion in December 1991 and is at present on schedule.

#### Parameters tested on-site.

It is not practical to assess for amoebae and probably salmonella on-site, and thus portable testing kits should generally be viewed as an augmentation rather than a substitution to current sampling procedures. However, NH&MRC guidelines on Desirable Quality for Drinking Water in Australia point out that:

"...it is far more important to examine numerous samples by means of a simple test than occasional samples by a more complicated test."

It is also important to remember that a large number of emerging communities have no monitoring at all at present.

#### Training

EHW workers have been trained in usage of the Delagua kit. Major aspects requiring attention are techniques of sterilising reusable equipment, how to count colonies after culturing, and the need to time various steps in the experimental procedure, as EHW's do not commonly have access to a watch or clock. At this stage, it appears logical to avoid the need for training in resterilisation techniques by use of the Millipore kit or development of 'one use' petri dishes etc for the Delagua kit.

Counting of colonies is not simple to teach. The character of cultures can vary significantly in size, definition and colour making it difficult to specify a teaching package which will allow EHW's to gain the skill within a reasonable time. However, training exercises to date indicate that the basic skills could be acquired in a classroom situation and then refined over a couple of months of working in the field. It is possible to gain a 'feel' for interpreting colony counts.

It is important to keep in mind that training in remedial action if a source is found to be contaminated is also important, such as shot dosing with chlorine tablets.

### Timing of procedures.

The sensitivity of timing methanol sterilisation has been investigated for the Delagua kit, and incubation time has been investigated for both the Delagua kit and the Colilert kit.

Notably, the study has found that no carryover occurs between consecutive samples even if methanol flaming is totally omitted, providing the equipment is dried between filtration runs. There is thus considerable margin for error in methanol sterilisation technique.

Incubation time can be varied between 14 and 20 hours using the Delagua kit without significantly affecting colony counts. Any less than this and colonies may be too small to identify reliably, any longer and petri dishes tend to dry out, which makes enumeration much harder.'

The Colilert system requires a minimum of 24 hours incubation to be sure of a result. Incubation for longer periods may indicate that water is more polluted than it actually is. A nominal maximum of 28 hours incubation is recommended.

### Contamination Carryover.

The chances of carryover or contamination have been investigated using the Delagua kit due to introduction of faecal contamination to:

- \* underside of petri dish lids
- \* absorbent pads
- \* reverse side of filter membranes
- \* culture medium

With the exception of the underside of the petri dishes, no false positives have been recorded. When introduced to the underside of the petri dish, one sample from four produced a single colony. This is encouraging, in that there is apparently a large margin for error in aseptic technique without producing false positives.

### False Negatives

The Delagua kit has produced a small number of false negative results, while being compared with the Colilert system. This means that a water known to be unfit to drink appeared to be safe. The reason for this is still being pursued, but the fact that this is occurring is obviously a cause for concern. The researcher has established that the false negatives are **not** due to excess methanol remaining within the filtration apparatus after sterilisation.



## Possible Uses.

Specific uses for portable water testing kits identified to date include:

- \* **water quality monitoring at bush gatherings** - this is not practical to undertake using current methods, but is simple with a portable kit
- \* **water quality monitoring for very small, very isolated or sporadically populated communities** - This is not done at present, and, realistically, it is unlikely to be practical to perform without using a portable kit of some description
- \* **education** - to give visual reinforcement to general health and hygiene education
- \* **equipment monitoring at commissioning** - portable kits allow UV sterilisation units to be tested while fitters etc are still on@site. This is not possible using current methods.

## CONCLUSION

It can be said that there is a reasonable range of equipment available on the market for the testing of water quality in remote areas. The current situation whereby bottles need to be flown to Perth within 24 hours makes adequate monitoring a difficult, if not impossible task. Use of perhaps two types of proprietary kits with minor modifications by a trained person based in the community would considerably improve the frequency, standard and coverage of water quality monitoring in remote areas. The kit to be used will vary depending on facilities available, such as a power source, the training level and skill of the user, and the accuracy of result required in a given situation.

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