Mauritius Research Council

Portable Water Quality Assessment of Microorganism in Mauritius- Using PCR Technology

Final Report

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MAURITIUS RESEARCH COUNCIL

Potable Water Quality Assessment of Microorganisms in Mauritius - Using PCR Technology

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Executive Summary

1. *The principal aim of this study was to investigate the application of the Polymerase Chain Reaction (PCR) technique to detect indicator organisms in the potable water supplied by the Central Water Authority (CWA). Given the excellent tract record of PCR applications in agricultural, environmental and medical fields, it was hoped that the current method (Most Probable Number - MPN) utilised by the CWA which can take from three to four days to identify and confirm the presence of the indicator organisms, could be significantly improved.*

2. *Our results indicate that it is possible, using PCR, to identify and confirm the presence of indicator organisms namely coliforms and* E.coli *some four to five hours only after collection of water samples.*

3. *Bearing in mind the ultrasensitivity of PCR, we have investigated other modern approaches to support the PCR technique such that one would not have to depend totally on one series of results. Past experience has shown that in case of complication, scientific or otherwise, one should have a good alternative or alternatives.*

4. *We have therefore explored the Membrane Filtration technique (coupled with the utilisation of specifically designed media) to accurately identify the indicator organisms.*

5. *With a view to helping the CWA implement these methods we have already trained one their technical staff in the utilisation of these methods. In fact these methods can be coupled with the PCR to obtain results that significantly uplift the scientific value and level of information that in the near future could be very useful to the Ministries of Health, Agriculture, Tourism and Environment.*

6. *It is also important to note that the Membrane Filtration technique is currently recognised by Water Authorities in Europe and America. This technique enabled us to identify and confirm the presence of indicator organism within 24 hours after sample collection. This is also a very significant improvement over the MPN method. Nevertheless we have also looked into ways to improve the MPN method itself. More work is required in that direction before we can feel confident we can adopt the new alternatives we experimented with.*

7. *It is difficult at this point in time to compare the cost-effectiveness of all the methods explored because we bought all consumables in small quantities for a small scale research. As such, the methods described are likely to appear expensive compared to the MPN currently utilised y the CWA. Large scale purchasing would very significantly lower costs of both reagents and freight, thereby making several of the methods described accessible for the routine analysis of water samples.*

8. *In the light of the above, we make the following recommendations:*

8.1 *The Polymerase Chain Reaction (PCR) is a very sensitive and specific method that can be applied to routinely identify the presence of indicator organisms in water samples. However, the technical staff must be trained and committed. In addition the following should be borne in mind:*

(a) *If one is to adopt the PCR one should perhaps devote a specific area of the laboratory for PCR work. This should include a PCR work station where unnecessary adventitious contamination by bacterial organisms would be avoided.*

(b) *Costs can be reduced by large scale purchasing of reagents and other consumables.*

(c) *With some collaboration with the CWA, it should be possible to devise kits for the application of both the PCR and Membrane Filtration techniques so as to reduce the number of steps involved in these techniques. This, we believe, will significantly reduce the chances of error and contamination. Training of staff will also be facilitated.*

8.2 *The Membrane Filtration technique coupled with the New Medium which we have developed in our laboratory should also be utilised at the CWA as it opens new vistas for identifying more accurately the indicator organisms in a much shorter time than the multiple tube number method (MPN). This also can provide a back-up system for the PCR.*

9. *Having acquired considerable experience during the course of this project, we could therefore provide training courses to identify technical staff from the CWA and other agencies/departments for the proper and optimal use of PCR, Membrane Filtration, and the New Medium described in our Report.*

10. *Future Investigations*

We reiterate that the PCR is a very powerful, sensitive and specific technique with as yet unexplored possibilities that can be exploited to investigate pathogens (or other important organisms) including viruses that could be escaping our detection because currently, the only organisms being monitored by the CWA are coliforms and E.coli. *By extending the acquired expertise to investigate other pathogens or other organisms one would be better equipped and prepared for adequate preventive measures in health and other sectors to cope with the new exigencies arising from a fast developing country.*

PART I

STATE-OF-THE-ART

IMPORTANCE OF SAFE POTABLE WATER

1. Water is essential to life and too often we take it for granted and just open a tap without realising all the hard work required to bring drinking water to our home. It usually takes lots of efforts in terms of manpower, management and monitoring to come up with an effective potable water to consumers. Water is also the most valuable natural resource available to man; without it nothing can live or grow. Yet fresh water accounts for less than 1% of the total planetary water load. The rest is salty or locked up in ice caps and glaciers. Just this relatively small 1% keeps all the world's agricultural, manufacturing, community and personal household and sanitation needs operating. It is being increasingly recognised as a crucial ingredient in sustainable development and the demand on the world's finite fresh water resources is growing rapidly.

2. Human use of water has increased steadily over the centuries and it is unlikely that this trend will change given the continued growth of population and the ever-widening utilisation of water for domestic, agricultural, industrial

and recreational purposes. This situation has given rise to growing concern over the availability of adequate water supplies to accommodate the future needs of society. Overuse has resulted in the progressive deterioration of water quality. It is therefore crucial that the situation be remedied before it turns out to be too late!

3. This concern to produce water of the finest quality dates back to centuries. Several ancient civilisations realised that if they were to flourish, clean safe water had to be supplied to the population. There is much archaeological evidence to indicate that ancient people were concerned with their water supply. Wells were sufficient for small communities, and rivers provided enough water for civilisations, for example along the Tigris and Euphrates, the Nile, and the Indus rivers; but as populations grew, wells had to be dug deeper, and water had to be brought in from more distant sources. These ancient systems included storage reservoirs at water sources, canals and aqueducts for water-distribution systems. Highly advanced systems appeared about 2500BC and reached their peak in the system supplying ancient Rome. The water was distributed from large storage cisterns to public fountains and baths by an elaborate system of lead pipes. Romans, known for architectural feats built large aqueducts to carry sanitary water to their cities. It would seem that even people in the ancient civilisation were smart to realise that clean water meant less disease. Despite this long history of concern for clean water, and new technologies there are still reports

of outbreaks of water-associated diseases even in developed countries. Some of the diseases are associated with the presence of chemicals. Here we are only concerned with bacteriological contamination.

WATER-BORNE PATHOGENS

4. One of the most important safety concerns is keeping water free from organisms that cause diseases. These disease-causing organisms are called pathogens and are difficult to identify because there are so many different varieties. It would not therefore be practical to test for the presence of all of them individually. Instead, we test for representative micro-organisms called "indicator organisms". These are bacteria which although not typically pathogens, can alert us by their presence that water is likely to be contaminated by other more dangerous microorganisms (*E.coli*).

5. One of the most useful indicator organisms is the common *Escherichia coli* which itself is a member of the coliform family with rod-shaped bacteria found in abundance in the intestines of humans and other warm-blooded animals. This organism has been chosen for its inherent properties among which is the ease of detection by proven methods of analysis. Since the detection of *E.coli* indicates that there has been bacterial contamination of water by

faecal coliforms and other more dangerous pathogens, several methods have been used to monitor its presence in drinking water.

6. One of the most common methods to assess pathogen contamination in water is the "faecal coliform" bacterial assay. Faecal coliforms as mentioned are a class of bacteria that live only in the intestinal tract of warm-blooded animals. The more faecal coliforms present in water, the greater the danger that a gastrointestinal disease may be transmitted through drinking water. The presence of faecal coliform bacteria (essentially *E.coli)* are used as accurate indicators of contamination by faecal material from human or other warm-blooded animals. However one must not forget that bacteria are quite hardy organisms, for instance *E.coli* and other coliform bacteria that find themselves in the human gut must adjust to high temperature and the highly bactericidal (killing) effects of gastric acid, not to mention competition from other bacteria for food and space. Bile salts bathe them in powerful detergents. The constant downward squeeze of peristalsis (intestinal spasms) threatens to dislodge them. Many bacteria survive these conditions through sheer tenacity. Adhering tightly to the intestinal lining, they feed and reproduce, developing colonies that cling to inner surfaces of their human hosts like bits of wet tissue paper. Other bacteria make use of the adverse conditions to spur their growth. Many of the faecal coliforms or bacterial pathogens in food will be protected against this high acidity by the food itself, where they may well initiate disease.

7. This might be a description for saying that the bacteria are so resistant and persistent that the presence of even one indicator bacterium in a sample of water strongly indicates that the water may contain bacterial and/or other pathogens which might initiate diseases such as cholera, typhoid, jaundice, hepatitis A and diarrhoea among others.

8. One of the earliest, good and often cited reports on a waterborne disease is the one of John Snow, a 19th century English Physician. He traced a London recurrence of cholera outbreak to a public well, known as the Broad Street Pump, in Golden Square, which he determined was contaminated. The epidemy was simply controlled by avoiding using water from that pump! This was a noteworthy achievement, especially since it predated by several years the discovery of the role of bacteria cholera that he believed had moved westward from India over a period of centuries, reaching London and Paris in 1849. Such catastrophes have today been virtually eliminated by vastly improved sanitation of water and modern water-pollution control.

9. If such is the case, then why should we persist on trying to put up with large expenses to routinely monitor our water? The reason is that the situation has been changing dramatically during recent decades. Our sources of water supply, both surface and ground water, are being endangered by new chemicals or microbiological contaminants. To an alien coming from outer space it would seem unbelievable that man who has been able to set foot on the moon, and is currently probing planet Mars, eliminates his body wastes by dumping them into the public drinking water. Many believe that flushing toilet, considered as one of the prime signs of civilisation, contributes in the fouling of fresh water.

10. Today enormous quantities of industrial wastes are poured into rivers and streams, creating chemical soups. In many remote areas of Third World countries, water is contaminated with disease-producing bacteria. Others say the problem is not yet catastrophic. But if *yours* is the water that is contaminated, it *is* a catastrophe for you!

11. It has therefore become imperative, if not a must, to avert this crisis by looking for efficient methodologies to test or monitor the purity of our water regularly to ensure its quality so that corrective measures can be swiftly applied.

COMMON MONITORING TECHNIQUES

12. The two most commonly used techniques to monitor water quality include the **Most Probable Number (MPN)** and the **Membrane Filter (MF)** methods. Each one of these methods offer the ability of confirming the presence/absence of the "indicator" organism. The Central Water Authority in Mauritius currently uses the MPN method.

13. Briefly, the Most Probable Number technique is based on serial dilution of water sample. Various volumes of the samples of drinking water (taken from the source we wish to test) are inoculated into a series of tubes containing a liquid medium which is specially designed to allow only coliform bacteria to grow. Other bacteria do not grow due to inhibitors present in the medium. The media that receive one or more of the indicator organism will show growth and a characteristic colour change and turbidity. If the dilutions are chosen correctly, some of the serial dilutions (containing bacteria) will have some tubes with growth and some without. A statistical procedure based on these sets gives the number of bacteria in the original sample. This test is

sometimes referred to as "dilution to extinction". Although the MPN method is one of the most widely used methods it has several disadvantages, one of which is its being inaccurate, forcing the investigator to process a large number of samples so as to minimise error in bacterial estimation. However, the MPN can be used to analyse turbid samples. In addition to be tedious, this method is lengthy and requires between seventy-two and ninety-six hours for completion.

14. A more effective technique is perhaps the membrane filter method in which a known volume of water is filtered through gridded membrane-filter disk made of nitrocellulose designed with small enough pores to retain bacteria on its surface. The membrane is then placed upon a culture medium (solidified by agar) and then incubated overnight at 37°C (the ideal temperature permitting the recovery of indicator microorganisms). Bacterial colonies will then develop wherever bacteria are entrapped. Because the bacteria can be made to grow directly on the membrane (by laying it on nutrient media), an accurate colony count can be obtained.

PART II

RESULTS OF THIS STUDY

15. We have compared the two methods (i.e. Most Probable Number and Membrane Filter) for their efficiency at detecting and confirming the presence of the indicator organism (See Plate 1 and Table 1). Indeed, after a first detection of bacterial growth in dilution tubes or as a colony on the filters one must confirm if the presumed bacteria are really the "indicator organism". Confirmation tests are based on a series of chemical and biochemical tests specific for a given organism, for instance production of acid and fermentation abilities are biochemical clues that reveal the nature of the organism. After having determined and compiled a number of these clues, one can establish a physical, chemical and biochemical pattern which belongs specifically to a given organism which is thus clearly identified. All these tests are elaborate and time consuming. For instance in the case of the Most Probable Number method it took between seventy-two and ninety-six hours before an organism could be identified.

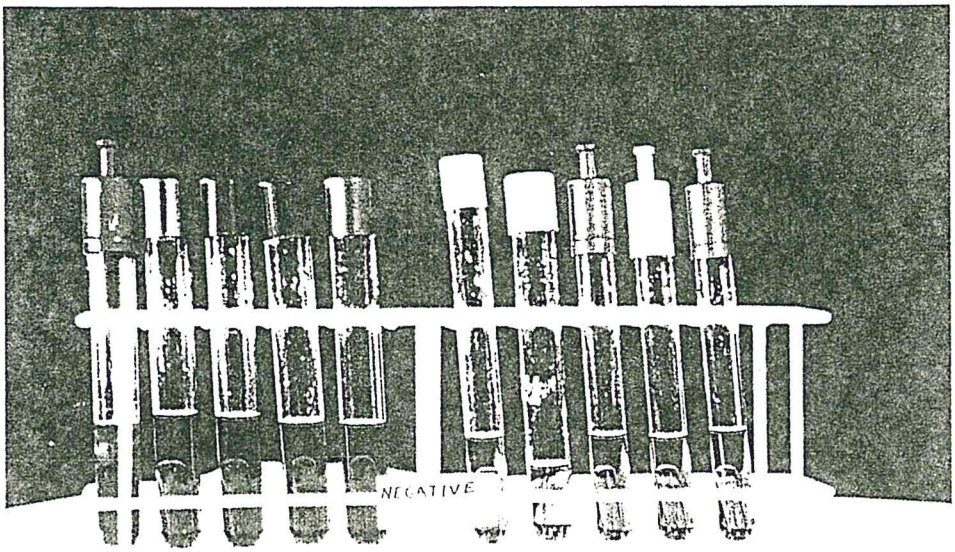


Plate 1(a): Negative reaction

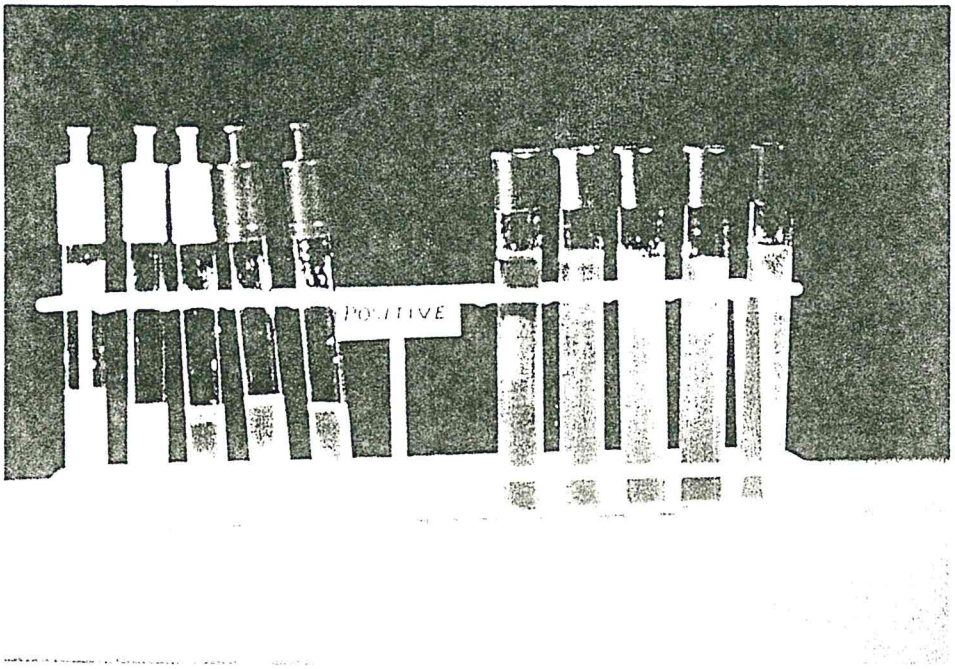


Plate 1(b): Positive reaction

Table 1: MPN of total coliform and *E.coli* per 100 ml of sample using multiple tube technique.

	Raw Water TC	*E.coli*	Filtered Water TC	*E.coli*	Treated Water TC	*E.coli*
1	3	0	35	1	0	0
2	8	8	8	8	0	0
3	11	3	8	2	0	0
4	25	25	3	1	0	0
5	50	3	160	0	0	0
6	8	8	5	0	0	0
7	35	3	25	0	0	0
8	180+	90	25	25	0	0
9	180+	160	180+	160	0	0
10	180+	160	160	90	0	0
11	180+	160	180+	160	0	0
12	180+	180+	180+	180+	0	0

16. The membrane filtration technique proved more effective and results were available within some twenty-four hours. The identification of organisms was greatly facilitated by using the API (Analytical Profile Index) system from Biomerieux. This system consists of a series of small cupules containing dried media which when rehydrated with a bacterial sample in solution provide a variety of specific media which upon bacterial growth will provide clues as to the identity of the bacterium. As the API system is a well established system that has won the support of many laboratories world-wide, it provided a fast and efficient system which when used in conjunction with the Membrane Filter technique helped to identify the indicator organism easily. It was even possible to identify several other bacteria found specially in untreated water.

SAMPLING

17. At this point it is important to mention that, in all our tests and using all methodologies, we used water samples from three sources: the Mare-aux-Vacoas reservoir (raw), sand filtered water (from La Marie treatment plant) and chlorinated tap water (treated) also at La Marie treatment plant. In this way we were able to observe the efficiency of the treatment by chlorine because sand filtration does not necessarily remove bacteria from the water.

18. In our hands, the Membrane Filter techniques proved to be quite efficient and reduced the confirmation time to a mere twenty-four hours. Although this represents a very significant progress, the method still depended quite heavily on traditional biochemical tests that required significant training to handle. We wanted to explore other available methods with a view to elaborating methodologies that could supplement or improve the Membrane Filter technique.

RAPID DETECTION TESTS

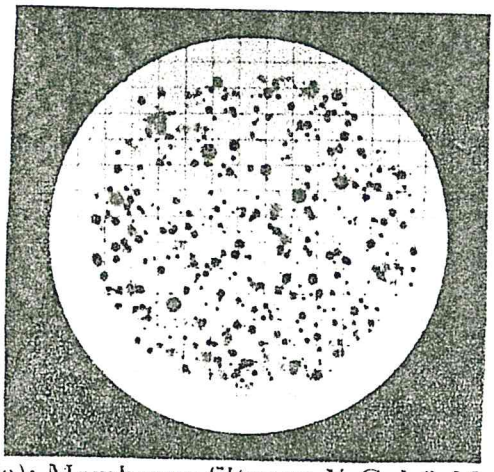
19. Very often, quicker results reporting presence/absence of coliforms is essential and desirable. Rapid detection tests have been developed for these situations. They vary in their acceptance by the regulatory bodies, but at least one, the Defined Substrate Technology (DST), is achieving broad use and regulatory standing.

DEFINED SUBSTRATE TECHNOLOGY

20. The Defined Substrate Technology method is a reagent system designed to enumerate specific target microbe(s) from a mixture of bacteria (See Plate 2). The system is designed to provide a simple and more rapid approach for differentiating indicator bacteria, both coliforms and *Escherichia coli*, on the

Plate 2

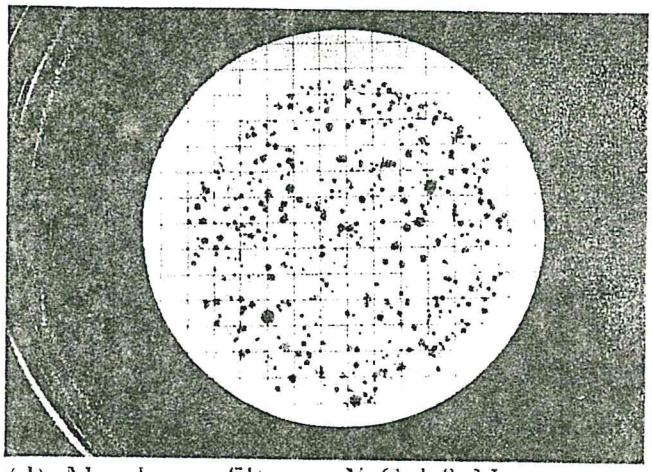
DEFINED SUBSTRATE MEDIA



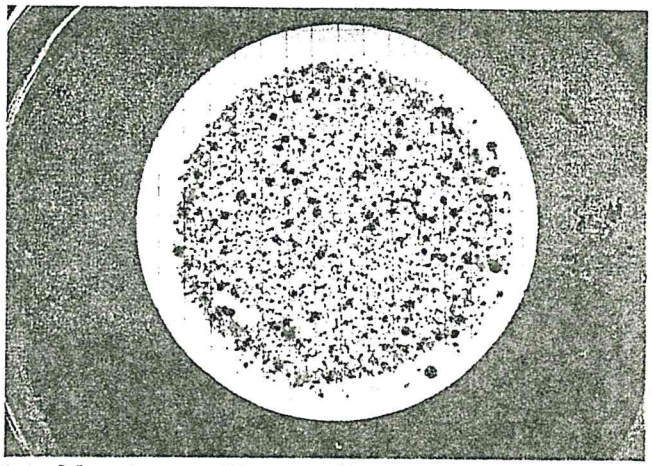
(a): Membrane filter on X-Gal & Mug agar when 200 µl of raw water was used (In ambient light).

(b): *E.coli* fluorescing under UV light

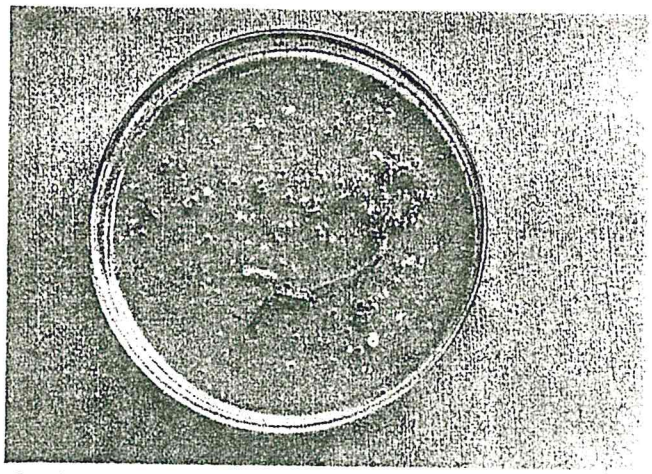
(c): Spread plate method using X-Gal & Mug agar



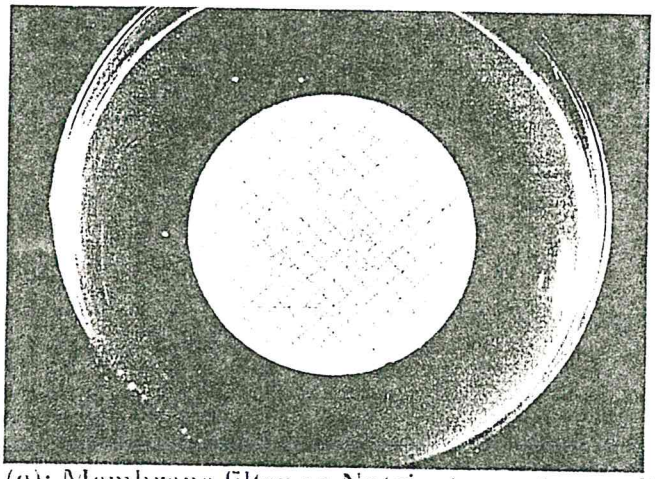
(d): Membrane filter on X-Gal & Mug agar when 200 µl of raw water was used. *E.coli* colonies are larger in size and deeper blue in colour



(e): Membrane filter on X-Gal & X-Glue agar when 5 ml of raw water was used



(f): Spread plate method using X-Gal & X-Glue agar



(g): Membrane filter on Nutrient agar (control) when 200 µl of raw water was used

same media. This makes identification far simpler, as different colonies assume clearly distinguishable features including colour differences. This is possible because substrates are added to the media as combined with chromogens which upon cleavage by a given enzyme. (Enzymes are biochemical catalysts that cause the modification of substrates. These catalysts can be very specific and will bring about the modification of a very specific substrate. In our case the enzyme will degrade a substrate). These targeted enzymes which are endogenous to bacteria, produce colour or fluorescence (emission of light). For instance one chromogen is hydrolysed by coliforms to produce a yellow colour whereas another one when cleaved specifically by *E.coli* produces a blue colour. We tried out various possible combinations of substrates to improve detection of coliforms and *E.coli* simultaneously on the basis of their enzyme activities. One such medium which turned out to be relatively sensitive contains two chromogens (ONPG and X-Gluc) combined with a fluorogen (MUG). MUG has been incorporated to enhance the detection of *E.coli* due to the production of a compound that fluoresces (shines) under the longwave UV light (302 nm).

THE ANALYTICAL PROFILE INDEX

21. We also confirmed (by biochemcial tests) that the blue colonies (obtained due to cleavage by bacterial enzyme) were in fact none other than *E.coli*

themselves. Confirmatory tests were carried out with the help of the miniaturised version of conventional procedures for the identification of bacteria. This involves incubating the presumptive colonies in miniaturised capsules of the API (Analytical Profile Index) strip containing a series of specific media standardised for Enterobacteriaceae, a family of bacteria living in man and other warm-blooded animals and other Gram-negative bacteria (bacteria that do not stain when exposed with a specific procedure involving crystal violet as opposed to Gram-positive bacteria whose cell walls can be stained by the same procedure. The Defined Substrate Technology tests coupled with both the API systems seem to be a very good, time efficient means to assess potability of water. It is important to note that on a routine basis with proper controls it should be necessary to do the confirmatory analysis (using API for instance). This would only be important in doubtful cases. The early signal obtained by the DST medium can therefore allow the largest saving to be made in the area of operational expenditure.

THE COLILERT METHOD

22. A variant of this test (the Colilert method - available commercially) uses chromogens (colour producing) and fluorogens (emits light) for detecting total coliforms and *Escherichia coli* in a single solution. The coliforms break down the chromogen with the help of their enzymes releasing the yellow coloured

indicator portion of the molecule. If *E.coli* is also present, the enzyme glucoronidase (specific to *E.coli*) hydrolyses the substrate that fluoresces under ultraviolet light. This permits separate and independent estimates of total coliform and *E.coli* counts in the same sample. We have used these DST methods and found them to be very sensitive and reliable. These methods are therefore highly recommended when presence or absence of indicator organisms is required.

THE POLYMERASE CHAIN REACTION (PCR)

23. While the MPN and MF methods for the detection of coliforms and *Escherichia coli* remain the mainstay of microbiological water quality determinations, there has been significant research into the development and evaluation of yet another technique which has proved very reliable in other fields of medicine, agriculture and biological science, namely the Polymerase Chain Reaction (PCR), which if it becomes applicable on a routine basis, holds the promise of really revolutionising the whole concept of water potability not only to detect and identify indicator organisms but also important pathogens could be specifically identified long before they become a health threat.

24. In fact this could be a case where using PCR could be a method to find the proverbial ***needle in a haystack and then turn the needle itself into haystacks!*** As the name implies - the reaction occurs step by step until the last one is reached, such that small units composing one or a few molecules are successively polymerised into a large molecule. Hence, this technique makes it possible to produce an enormous number of copies of one DNA (the genetic material - DeoxyriboNucleic Acid-which is a polymer of four bases joined together into repeating units) sequence from very tiny initial quantities of DNA - quantities so small that they are impossible to detect by routine methods.

25. In fact, the Polymerase Chain Reaction is a sensitive, specific and versatile tool, which has revolutionised molecular biology. It is a powerful tool which can be used to determine the presence or absence of certain DNA sequences and the technology is widely used in research. It is so sensitive that it has been suggested that if one millilitre of PCR products were evenly distributed in an Olympic Swimming Pool, then DNA could still be detected if another millilitre was removed and another PCR reaction performed. The method could therefore be very useful in detecting microorganisms in water where only a few cells are present.

26. In practice, the so-called Polymerase Chain Reaction (PCR) occurs, as the name implies, when a polymer, that is a molecule consisting of subunits linked together, is copied over and over again (chain reaction) if other subunits (primers) are made to latch on to it by a special procedure called hybridisation. The reaction itself is under the control of a specific enzyme called a polymerase. In this particular reaction the polymerase is isolated from heat resistant (up to 110°C) bacteria which therefore produce polymerases capable of resisting high temperatures. This is important because after each cycle of polymerisation, the reaction mixture is heated to above 90°C to separate the newly synthesised molecules from the templates and they themselves become templates and, with the help of primers, they are copied again. Therefore after a few cycles a geometric progression is generated, resulting in millions of copies of a particular region of DNA.

27. Given that all organisms have specific DNAs with regions very species-specific, one can design PCR experiments that copy only a very specific region of the DNA. The PCR product will therefore be very specific to the given organism. In principle if we generate PCR products from a known, highly specific region of the DNA of an organism, one could therefore identify the organism. To achieve this, of course, one would have to have proper primers which will very specifically hybridise to the targeted DNA. In the case of *E.coli*, the indicator organism we have been identifying by several

techniques, there exists a well identified DNA sequence that specifies the same glucuronidase enzyme mentioned earlier in the Defined Substrate Technology.

28 As mentioned earlier, all our tests were conducted on water samples from three sources namely raw water, sand filtered water and treated water. Water samples were filtered on the same filter membranes utilised in Membrane Filter techniques. DNA was extracted from the membranes and utilised in PCR mixes containing all necessary ingredients for a PCR reaction. We utilised different sets of primers and we were able to specifically generate specific DNA copies from coliforms and *E.coli*. After analysing the products of PCR using electrophoresis (that is forcing the various molecules to move in an agarose matrix in an electric field such that the smaller molecules move faster than the larger ones) in the presence of markers of known sizes and staining techniques, it was possible to identify the expected molecules generated when only *E.coli* DNA was present. The presence of *E.coli* DNA could have arisen from live *E.coli* or even those organisms that are unable to grow and divide. This is drastically different from all methods described earlier and they all depended on the *E.coli* to grow into a sizeable population or during the growth process to produce a biochemical reaction. The Polymerase Chain Reaction therefore can detect even dead or nonviable bacteria. ***This is very important in that the slightest faecal contamination can be revealed and authorities alerted. The other***

advantage of that method is that in only a few hours after the samples enter the laboratory one can complete the PCR and detect the presence of indicator organisms. We were routinely able to detect coliforms and E.coli in raw and sand-filtered water but during the study period we did not detect the presence of E.coli on treated tap water (See Fig. 1).

29. We should mention here that on account of its very sensitive nature, the PCR can be a two-edged sword introducing false positive (give a positive reaction in absence of *E.coli* DNA) and false negative (give a negative reaction in the presence of *E.coli* DNA). Therefore a certain amount of dexterity is required and proper controls were used during all PCR work. For instance, control reaction tubes were prepared as usual but the enzyme was omitted and in other similar tubes purified *E. coli* DNA (isolated from a known *E.coli* colony) was included in the reaction so that the band generated could be used as reference to compare with all samples utilised. ***These precautions are only a slight price to pay for a fast, specific, and sensitive technique.*** In fact, in a test experiment, we suspended an average-sized bacteria and serially diluted the suspension by 100-fold until we reached 100 million-fold such that there was only one individual viable bacterium. This was verified by growing samples from all dilutions on agar nutrient media (plating). We found that there were some 10^{8} bacteria living in an average-sized colony! In parallel experiments we used the dilution samples in PCR reactions using the

oligonucleotides specific to *E.coli*. ***We were able to detect the presence of E.coli DNA in dilutions of 1 000 million-fold representing a ten-fold sensitivity over the plating technique. The most logical explanation is that these are non-viable bacteria which are not able to form colonies but their DNA can nevertheless be amplified by PCR. This experiment illustrates the extreme sensitivity of the PCR (See Fig. 2).***

30. In conclusion, after comparing several modern approaches we found that the current Most Probable Number method used by the CWA could be significantly enhanced or replaced by the modern methods like Membrane Filtration (MF), and Defined Substrate Technology (DST). We found that the PCR method is extremely sensitive and fast and is well worth the precautions necessary to ensure its success. Using the PCR, one can envisage adapting it to detect a large number of pathogens that can help us avert epidemic situation. This sort of modern and efficient approach to monitor potability of water seems to be very appropriate for a country like Mauritius which would like to be viewed as a leading nation of the Indian Ocean Rim.

31. This study has the merit of having scrutinised all current techniques in water quality monitoring, pointing out their relative strengths and weaknesses, and comparing them with the high technological breakthrough of the novel technique, the Polymerase Chain Reaction technique which we consider to

be an indispensable tool in the hands of those whose responsibility is to ensure safe domestic water supply.

32. ***What we also consider as a breakthrough in this study is that we have been able to show that the Polymerase chain Reaction can reveal the presence of bacterium in several litres of water!***

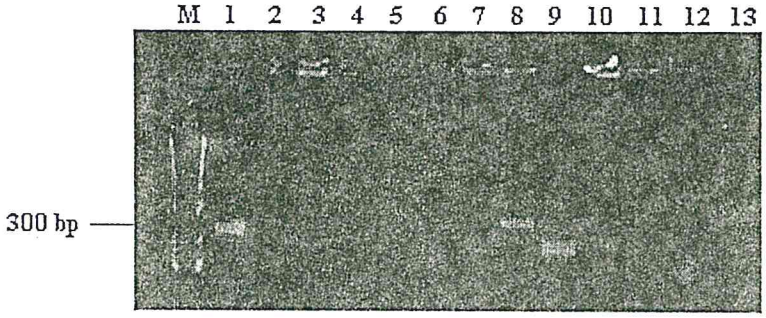


Fig. 1(a): Agarose Gel Electrophoresis of PCR Products. *Lanes 1-3: raw, filtered, & treated water using lac Z primers; Lanes 4-7: controls; Lane 8: E.coli with lac Z; Lanes 9-11: raw, filtered & treated water using uid A primers; Lane 12: E.coli with uid A primers.*

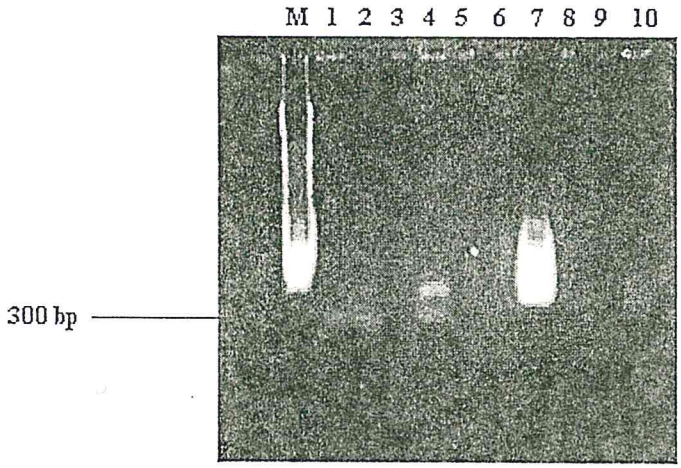


Fig. 1(b): Agarose Gel Electrophoresis of PCR products. *Lanes 1-3: raw, filtered & treated water with filters using lac Z primers; Lanes 4-6: raw, filtered & treated water with filters using uid A primers; Lane 7: raw water with no filters: lac Z; Lanes 8-9: filtered & treated water: uid A; Lane 10: E.coli: uid A*

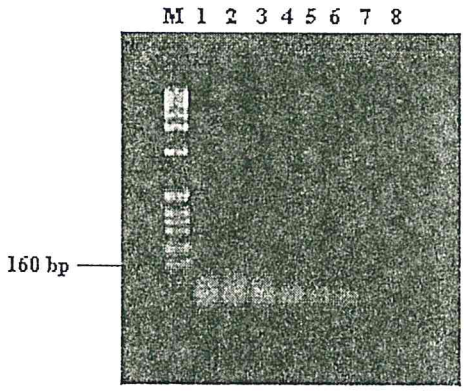


Fig. 2: PCR using DNA templates isolated from dilutions of an *E. coli* colony. *Lanes 1-7: show serial dilutions using Uid A primers. Lanes 1-6: 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10} respectively; Lane 8: control (Uid A primers and enzyme only).*

CONCLUSIONS

1. *We have examined several modern approaches to detect the presence of bacteria in water samples.*

2. *We have found that the current MPN method used by the CWA could be reinforced or replaced by the modern methods (Membrane filtration, DST, PCR & Colilert) which are faster, more precise and can yield more useful information.*

3. *We have clearly demonstrated that the PCR technique is a necessity in Mauritius now more than ever for reasons amply covered in the Report.*

4. *However we must stress that in the case of the PCR care must be exercised at all steps and a significant amount of training is required if one is to get the most out of this technique.*

5. *It is difficult to give exact costs as we were working only on a small project. Suffice it to say that the consumables for the Modern Methods (more particularly the PCR) are expensive but if done on a large scale we believe that one could lower costs to an affordable level.*

6. *There is also the possibility of organising reagents for various tests into kit forms so as to facilitate the training and routine utilisation of*

the various techniques. This would require further investigations and testing.

7. *We believe that this research study has not only provided means to improve the bacteriological monitoring of potable water but has also opened the gateway for more in-depth scientific investigation of the microorganisms in the Mauritian aquatic environment. This will certainly help the CWA in its efforts*

   (a) to give the best possible service to ever demanding customers,

   (b) to carry out country-wide monitoring of potable water quality in order to mitigate public uproars, and

   (c) to give consumer satisfaction.

   In the same breath, one must not forget the international exposure of Mauritius via tourism and the rapid globalisation process, hence the need for sustaining a very high standard of potable water quality.

8. *We have analysed treated (chlorinated) water leaving the La Marie Reservoir and certify that the treatment is effective as no coliform/E.coli has been detected during our survey period in the water samples, even by using the finest technique to date (i.e. PCR).*

9. *We have in this study rather than just investigate the applicability of PCR alone, considerably eased the task (and techniques) of potential user groups by providing them with tested, improved methodologies*

and tools. We have already done the 'trial and error' part that can be viewed as a sort of technical feasibility study in that we identified critical parameters for the proper running of the methods we described, the precautions necessary and how to optimise from the technology.

10. Mauritius is now poised to play a leading role and be viewed as a model for several countries in the region (for example, Indian Ocean Rim Initiative and SADC) that wish to implement the methods described to monitor the bacteriological content of potable water in their areas.