Appropriate Microbial Indicator Tests for Drinking Water in Developing Countries and Assessment of Ceramic Water Filters

by

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B.A.Sc., Civil and Environmental Engineering
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ABSTRACT

Indicator organisms such as coliforms and *E.coli* frequently replaced pathogens in the monitoring of microbial quality of drinking water. Tests for indicator organisms are typically easy to perform and results can be obtained quickly. Many studies have concluded that total coliform is not an appropriate indicator in tropical environments. Instead, *E.coli* is a better indicator of recent fecal contamination and *E.coli* is proposed as the indicator organism of choice for routine water monitoring in developing countries.

Two Presence/Absence (P/A) tests were studied and compared to Membrane Filtration (MF). The P/A-Total Coliform test is useful in evaluating disinfected water supplies. The P/A-H_2S-producing bacteria test is simple, inexpensive, and suitable for monitoring microbial quality of drinking water in the rural areas. The MF test allows the enumeration of indicator organisms and can be used to assess the microbial removal efficiencies of point-of-use water filters. Different culture media for various indicator organisms were compared based on cost, ease of result interpretation, and medium preparation. The author concluded that m-ColiBlue24® be used for total coliform detection, m-FC with rosalic acid for fecal coliform detection, and either EC with MUG or m-ColiBlue24® for *E.coli* detection.

For point-of-use water treatment, the author also fabricated a ceramic disk filter in collaboration with Hari Govinda Prajapati, a local pottery maker in Thimi, Nepal. The manufacturing process was documented and design improvements were recommended. Two of these ceramic filters were brought back to MIT and evaluated. Two other Indian TERAFIL terracotta ceramic filters were also tested in the laboratories in Nepal and MIT. Both TERAFIL filters consistently removed 85% turbidity and produced water with less than 1.0 NTU. Total coliform, fecal coliform, and *E.coli* removal rates exceeded 95% with one exception. However, the two TERAFIL filters have very different maximum flow rates of 2 and 7 L/hr. The Thimi ceramic filters have similar turbidity and microbial removal rates. However, they have significantly lower flow rates of 0.3 L/hr. Despite the high microbial removal rates, some form of household disinfection is necessary for these filters if zero coliform count is to be achieved.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µm	Micrometer
BCP	Bromocresol Purple
BGLB	Brilliant Green Lactose Bile
cm	Centimeter
CFU	Colony Forming Unit
Chromocult®	Agar for simultaneous detection of Total Coliform and *E.coli*
CRW	Charles River Water
DST	Defined Substrate Technology
E.coli	Escherichia Coli
EC	Escherichia Coli
ENPHO	Environment and Public Health Organization
FC	Fecal Coliform
g	Gram
H_2S	Hydrogen Sulfide
HPC	Heterotrophic Plate Count
IBDG	Indoxyl-β-D-glucuronide
ID	Infective Dose
LT	Lauryl Tryptose
MAC	Maximum Acceptable Concentration
MF	Membrane Filtration
MF-*E.coli*	*E.coli* Membrane Filtration Test
MF-FC	Fecal Coliform Membrane Filtration Test
MF-TC	Total Coliform Membrane Filtration Test
MI	Agar for simultaneous detection of Total Coliform and *E.coli*
MPN	Most Probable Number
MTF	Multiple Tube Fermentation
MUG	4-methyl-umbelliferyl-β-D-glucuronide
m-ColiBlue24®	Medium for simultaneous detection of Total Coliform and *E.coli*
m-Endo	Medium for detection of Total Coliform
m-FC	Medium for detection of Fecal Coliform
m-TEC	Medium for detection of *E.coli*
mg	Milligram
ml	Milliliter
NGO	Non-government Organization
NRs	Nepali Rupee (US$1 = NRs 75)
ONPG	o-nitrophenyl-β-D-galactopyranoside
P/A	Presence/Absence
P/A-H_2S	Hydrogen Sulfide Producing Bacteria Presence/Absence Test
P/A-TC	Total Coliform Presence/Absence Test
POU	Point-Of-Use
Rs	Indian Rupee (US$1 = Rs 45)
TC	Total Coliform
TSA	Tryptic Soy Agar
TTC	Triphenyltetrazoliumchloride
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
X-Glu/BCIG	5-bromo-4-chloro-3-indolyl-β-D-glucuronide

Chapter 1 : INTRODUCTION

1.1 Motivation for Microbial Indicator Study

Indicator organisms are often used in place of disease causing pathogens because their presence is indicative of pathogen presence and indicator organisms are easier to detect. Another reason for using simple indicator tests is that pollution is often irregular. It is better to monitor drinking water frequently by means of a simple test than occasionally using more complicated direct pathogen detection tests. Indicator organisms, however, are not universal. Many studies have shown that while traditional indicators may have worked for developed countries in temperate climates, they are not necessarily appropriate for developing countries in tropical environments. There is a need to investigate the suitability of these indicators for their use in tropical environments for the detection of recent fecal contamination in drinking water supplies. Extensive research has already been carried out in this area. These indicators have different characteristics and their significance to the microbial quality of drinking water can vary depending on the monitoring region. After the most appropriate indicator organisms are identified, the methods for their detection are assessed and compared.

There is a wide variety of methods available for testing the microbial quality of drinking water through indicator organisms. The two most common methods that are studied in detail in this thesis are the Presence/Absence (P/A) test and Membrane Filtration (MF) test. The P/A test is a simple method to identify the presence or absence of the indicator organism and is often indicated by a color change. While the P/A test may be adequate for detecting the presence of indicator organisms, it is unable to assess the extent of contamination in the water sample. The ability to enumerate indicator organisms is particularly important when assessing the performance of a water treatment device such as a water filter. It allows the researcher to calculate microbial removal efficiency by finding out how much of the indicator organisms are removed by the filter. However, the MF test is more elaborate in terms of its equipment and incubation requirements compared to the P/A test. There are also many kinds of culture media to choose from for the MF test. In this thesis, based on the author's research, the most appropriate indicator test for monitoring the microbial quality of drinking water and assessment of filter efficiency will be proposed. Specifically, the best culture medium to use for each indicator

organism during MF is proposed based on the selection criteria: costs, ease of result interpretation, and ease of preparation.

1.2 Filtration as Point-Of-Use Water Treatment

Since the quality of the water supply is often variable and cannot be adequately controlled for millions of people in developing countries, one viable approach could be the implementation of simple, low-cost point-of-use (POU) treatment systems to ensure the provision of safe water for consumption. Point-of-use treatment systems refer to the treatment of water at the household level as opposed to centralized, larger capacity municipal or private systems that carry out treatment of water for a larger population. While an advanced large-scale water treatment system is able to supply many households at any one time, a simple and affordable household water treatment system will be able to reach even the most rural areas of developing countries such as Nepal, therefore reducing their dependency on unsafe drinking water supplies. A good POU system should also satisfy the criteria of requiring minimum training and being easily and cheaply maintained.

Filtration is potentially an appropriate POU treatment process because filters are usually easy and small enough to be used in individual households. Currently in Nepal, the most commonly available point-of-use water treatment system is the ceramic candle filter. This filter can be easily purchased from market-places in Kathmandu Valley. The middle to upper class population in Kathmandu and other urban areas can often afford to boil and filter their water before drinking (Sagara, 2000). Both processes together – boiling and filtering - ensure that the water is sufficiently treated before it is consumed. However, boiling water requires the burning of fuel, which is a valuable and limited resource that may not be affordable for the rural community, and which may also contribute to further deforestation in Nepal. Moreover, there are performance issues with the candle filter such as inadequate water flow rates and ineffective microbial removal from the raw water (Sagara, 2000). It is recommended by Sagara that "the (candle) filter system must be used combined with a disinfection process." This disinfection process could refer to boiling (as already carried out by the better-off community), chlorination, solar disinfection etc. Unfortunately, the taste of residual chlorine in the drinking water may be unacceptable to some of the local population. If chlorine is to be applied, the residual chlorine

concentration has to be high enough to achieve the required disinfection, and low enough to maintain a palatable taste to the water.

Currently, other studies are being conducted by the MIT Nepal Water Project and other MIT Masters of Engineering projects to study the effectiveness of filtration as a POU treatment method of drinking water. One study involves the application of colloidal silver on a ceramic filter developed by an organization called "Potters for Peace" (Rivera, 2001). Colloidal silver has a disinfecting effect and depending on the applied concentration, it is possible to kill or inactivate microorganisms in water to achieve safe drinking water guidelines. Another filter under study is the BioSand water filter which uses a thin microbiological film in the top layer sand to remove harmful microorganisms from the water (Lee, 2001; Lukacs, 2002). In this thesis, laboratory studies were conducted both in MIT and Nepal, on an Indian TERAFIL terracotta ceramic filter. In collaboration with a local Nepal ceramic cooperative, the author also manufactured and brought back two ceramic filters for testing at MIT.

1.3 Field Studies in Kathmandu, Nepal

In January 2002, the author visited Nepal and stayed in the capital city, Kathmandu, for three weeks. He was hosted and worked in the laboratory of Environment and Public Health Organization (ENPHO), a Non-Government Organization (NGO) in Nepal whose mission is monitoring and improving local drinking water supply, wastewater treatment, solid waste disposal, and air quality monitoring. ENPHO has a well-equipped water quality testing laboratory which the author used during his stay. He carried out microbial tests on 15 different drinking water sources in the Kathmandu Valley (primarily in the city of Kathmandu and Patan). He also assessed the performance of an Indian TERAFIL ceramic water filter in the lab. Finally, the author was also making a terracotta ceramic filter disk in a nearby town, Thimi. Next, a brief background on the water supply and contamination situation in Nepal is presented.

Nepal, officially known as The Kingdom of Nepal, is a landlocked country in southern Asia, bordered on the north by the Himalayas and the Tibet region of China and bounded by India to the east, south, and west (See Figure 1-1). There are three distinct geographic regions in Nepal: the plains to the south, the central foothills, and the Himalayas to the north. The plains region,

also called the Terai districts, contain an abundant source of groundwater resources for irrigation and drinking purposes. The low water table is generally found between 3 to 18 meters below ground (Shrestha, 2000). The central foothills are densely populated and most of Nepal's major cities including the capital Kathmandu, and tourist attraction center of Pokhara are located there. The northern mountainous region contains the highest peak in the world, Mount Everest.

Figure 1-1: Map of Nepal.

Although Nepal is rich in freshwater resources, they are unevenly distributed and the water infrastructure is poorly developed. Forty-three percent of the rural population has access to safe water (WHO, 2001). More than 4 million people living in the rural areas do not have access to safe water. Although 90% of the urban population is served with piped water supply, many water supply systems provide water for only a few hours each day (Shrestha, 2000). From the author's personal experience in Kathmandu, a significant number of the urban households still depend heavily on traditional and communal water supplies e.g. public taps and wells, for their water needs. Forty percent of the piped supplied water is estimated to be lost due to leakage in distribution pipes and the bypassing of the water meter by consumers (Shrestha, 2000). The greatest water demand comes from industry and hotels, leaving little for residential use. Only 20% of the rural population, compared to 75% of the urban population, has access to adequate sanitation (UNICEF, 2000). See Table 1-1 for a summary of these indicators of Nepal.

Table 1-1: Summary of indicators for Nepal.

Indicators	**Nepal**
Total Population	23.9 million
Urban Population Rural Population	12% 88%
Annual GNP per capita	US$230 (42% lives below poverty line)

Life Expectancy	58 years
Infant Mortality	77 per 1,000 live births
Literacy Rate	22% (women) 57% (man)
Access to safe water	43% (rural)
Piped Water Supply	90% (urban)
Water Loss in Urban Distribution Pipes	40%
Average Per Capita Water Availability	52 Liters per day
Access to Adequate Sanitation	75% (urban) 20% (rural)

The problems of clean drinking water and lack of proper sanitation are closely related. Pathogen-laden human and animal wastes, food and garbage pile up near homes and tubewells and drain into waterways, contaminating the water sources. For example, surface water, such as rivers in the Kathmandu Valley, is polluted by industrial effluent, dumping of untreated waste, and sewage from residential areas (NepalNet, 1999). Seepage from poorly maintained septic tanks also contribute to the groundwater contamination. Leakage from sewer pipes, which often run parallel to the water supply pipes, can also contaminate the supply pipes through cracks. The city water is often inadequately treated due to the lack of maintenance. In addition, since piped water is available for only a few hours a day, residents store water in storage tanks and own privately dug wells so that water is available for use throughout the day. These containers are seldom washed and properly maintained, therefore contaminating water that could be clean originally (Rijal et al., 2000). Water obtained from the wells do not usually undergo any form of treatment before consumption, therefore they are unsafe for drinking.

Despite an increase in access to water supply from 46% in 1991 to about 80% in 2000, there is another problem with the lack of proper sanitation and hygiene practiced among the residents. Overall latrine coverage in Nepal is only 27% in 2000 (UNICEF, 2001). This also translates into the discharge of at least 1,500 tonnes of feces onto the fields and waterways everyday (UNICEF, 2001). The combined effect of inadequate access to a safe water supply, poor environmental sanitation, and personal hygiene has adversely affected the quality of life and health conditions of the Nepali people. Sanitation-related diseases account for 72% of total ailments and diarrhea continue to be one of the leading causes of childhood deaths in Nepal (ADB, 2000). Other

common waterborne diseases in Kathmandu include gastroenteritis, typhoid and jaundice (Shrestha, 2000).

1.4 Study Objectives

There are three objectives to this study after an assessment of a number of options and they are:

1. To propose the most appropriate indicator organisms and their corresponding microbial tests for the monitoring of drinking water quality in Nepal and other developing countries;
2. To propose the most appropriate microbial indicator tests for assessing the performance of point-of-use water filter systems;
3. To assess the effectiveness of two different types of ceramic water filters as POU treatment solutions.

Chapter 2 : WATERBORNE PATHOGENS AND DISEASES

2.1 Introduction to Waterborne Diseases

"Infectious diseases caused by pathogenic bacteria, viruses, and protozoa or by parasites are the most common and widespread health risk associated with drinking water." (WHO, 1993a)

The wide variety of waterborne diseases and their public health impact is an important concern with far-reaching implications. 3.4 million people, mostly children, die annually from water-related diseases. Out of this number, 2.2 million people die from diarrheal diseases (including cholera) (WHO, 2000). Waterborne diseases are typically caused by enteric pathogens which are mainly excreted in feces by infected individuals, and ingested by others in the form of fecally-contaminated water or food. These pathogenic organisms include many types of bacteria, viruses, protozoa and helminths, which differ widely in size classification, structure and composition. Pathogenic organisms are highly infectious and disease-causing. They are responsible for many thousands of diseases and deaths each year (See Table 2-1 for waterborne disease outbreaks in United States[1]), especially in tropical regions with poor sanitation. In the following discussion, only the human pathogens potentially transmitted in drinking water are considered.

Table 2-1: Waterborne disease outbreaks in the United States, 1980 to 1996 (AWWA, 1999).

Disease	Number of Outbreaks	Cases of Illness
Gastroenteritis, undefined	183	55,562
Giardiasis	84	10,262
Chemical poisoning	46	3,097
Shigellosis	19	3,864
Gastroenteritis, Norwalk virus	15	9,437
Campylobacteriosis	15	2,480
Hepatitis A	13	412
Cryptosporidiosis	10	419,939*
Salmonellosis	5	1,845
Gastroenteritis, *E. coli* O157:H7	3	278
Yersiniosis	2	103
Cholera	2	28
Gastroenteritis, rotavirus	1	1,761

[1] U.S. statistics for outbreaks and specific waterborne diseases are given instead of developing world statistics because developing countries statistics are generally lacking.

Typhoid fever	1	60
Gastroenteritis, Plesiomonas	1	60
Amoebiasis	1	4
Cyclosporiasis	1	21
TOTAL	**402**	**509,213**

*Includes 403,000 cases from a single outbreak of Cryptosporidiosis.

2.2 Significance of Pathogens in Drinking Water Supplies

According to WHO[2], not all potential waterborne human pathogens are of equal public health significance. Some of them present a serious risk of disease whenever they are consumed in drinking water and are given high priority for health significance. Examples include strains of *Escherichia coli, Salmonella, Shigella, Vibrio cholerae, Yersinia enterocolitica,* and *Campylobacter jejuni*. On the other hand, some organisms may cause disease "opportunistically". These organisms cause infection mainly among people with impaired natural defense mechanisms. These people include the very old, the very young, immuno-compromised people, and patients in hospitals. Examples of these organisms include *Pseudomonas, Klebsiella,* and *Legionella* (WHO, 1996).

For pathogens of fecal origin, drinking water is the main **route of transmission**. Unhygienic practices during the handling of food, utensils and clothing also play an important role. Humans are typically the main carriers of large populations of these bacteria, protozoa, and viruses (WHO, 1996). Pathogens originating from human sources, often from human feces, are called "enteric" (of intestinal origin) pathogens. An example is *E.coli* O157:H7. The intestine of many domestic and wild animals, their meat, milk and dairy products, are sources of the bacteria *Yersinia enterocolitica* and *Campylobacter* (WHO, 1996). The **persistence** of a pathogen in water also affects their transmission to humans. A more persistent pathogen that can survive longer outside the host body is more likely to be transmitted to other people. The **infective dose** (ID) of the pathogen determines the number of organisms needed to produce an infection in humans. The ID_{50} is the dose required to produce a clinically detectable infection in 50% of the subjects (Refer to Table A1 in Appendix A).

[2] Throughout this thesis, the focus of which is developing countries generally and Nepal specifically, WHO Guidelines will be given as the benchmark on international grounds for microbiological water quality. U.S. EPA standards are also provided for the purpose of comparison.

There are many other causes of waterborne disease outbreaks. They include treatment deficiencies and the consumption of contaminated groundwater (Refer to Table 2-2 for some causes of waterborne outbreaks). Therefore, improvements in the quality and availability of water, sanitation facilities, and general hygiene education will all contribute to the reduction of morbidity and mortality rates due to waterborne diseases (Munasinghe, 1990).

Table 2-2: Causes of waterborne disease outbreaks in USA, 1981-1990 (Craun, 1992).

Cause of Outbreak	Community	Non-community	Other
Untreated groundwater	15	43	19
Inadequate disinfection of groundwater	17	32	
Ingestion of contaminated water while swimming			41
Inadequate disinfection of surface water	35	9	
Distribution deficiencies	30	3	3
Filtration deficiencies	16	1	
Unknown	7	3	1
Untreated surface water	2	4	3
Miscellaneous	2	2	3
TOTAL	**124**	**97**	**70**

2.3 Four Main Classes of Pathogens

There are 4 main classes of pathogenic organisms related to waterborne diseases. They are bacteria, viruses, protozoa, and helminths.

2.3.1 Bacteria (Prokaryotic)

Bacteria are single-celled prokaryotes (without nucleus) with sizes ranging from 0.3 to 100 micrometers (μm) in length (Metcalf and Eddy, 1991). Many of these pathogenic bacteria belong to the family Enterobacteriaceae (See Figure 2-1 for a classification table created by the author of typical waterborne pathogens). They include the human pathogen, *Salmonella typhi* which is typically present in all kinds of food grown in fecally polluted environments. Another type of bacteria in this family, *Yersinia enterocolitica* (certain strains) causes acute gastroenteritis with diarrhea. *Y. enterocolitica* are present in sewage and fecally contaminated surface water. A special feature of *Y. enterocolitica* is their ability to grow even at low temperatures of 4ºC. Therefore, these organisms can survive for long periods in water habitats (WHO, 1996). *Shigella*, also part of Enterobacteriaceae, causes dysentery in humans and is

usually transmitted through direct contact. Other bacteria species of significance but not part of this family include the following: *Vibrio cholerae,* specifically the serogroup O1, causes cholera, an acute intestinal disease with massive diarrhea, vomiting, dehydration, possibly leading to death. Some other pathogenic bacteria include *Campylobacter* and opportunistic pathogens such as *Legionella pneumophila* and *Aeromonas* (Refer to Tables A1 and A2 in Appendix A).

Escherichia coli, which is commonly used to indicate fecal contamination, causes bacterial infections of the intestines where the major symptom is diarrhea. It typically has a length of 3 μm and width of 1 μm. *E.coli* are characterized by their ability to produce potent "enterotoxins". Enterotoxins are similar to hormones which act on the small intestine, causing massive secretion of fluids which lead to the symptoms of diarrhea (Madigan et al., 2000). For example, the *E.coli* O157:H7 produces a potent enterotoxin that causes both hemorrhagic diarrhea and kidney failure. These diseases can cause death if untreated.

2.3.2 Viruses (Noncellular)

Unlike other pathogens, viruses are not cells. Viruses are minute particles containing nucleic acid surrounded by protein and other macromolecules. They lack many of the cell attributes such as metabolic abilities and reproduction pathways (Madigan et al., 2000). Viruses are smaller than bacteria, ranging in size from 0.02 to 0.3 μm. Viruses are known to infect virtually all cells. The pathogenic pathway starts with the attachment of the virion (a virus particle) to a host cell. The virion then penetrates and replicate within the cell, altering the host biosynthetic machinery with its own nucleic acid synthesis (Madigan et al., 2000).

Most pathogenic waterborne viruses are enteric viruses which multiply and infect the gastrointestinal tract of humans and animals before they are excreted in their feces. People infected with any of the enteric viruses, particularly the Hepatitis A virus, will become ill. Infectious hepatitis may cause diarrhea and jaundice and result in liver damage. Other disease-causing viruses include *rotaviruses* causing gastroenteritis primarily in children, *polioviruses* causing polio, and *adenoviruses* causing acute gastroenteritis (Refer to Table A1 and A2 in Appendix A). Waterborne transmission via the fecal-oral route has been demonstrated for Hepatitis A and E viruses, rotaviruses and Norwalk virus (AWWA, 1999).

Figure 2-1: Waterborne pathogen classifications.

2.3.3 Protozoan Parasites (Eukaryotic)

Protozoa are unicellular eukaryotic microorganisms that lack cell walls. Protozoa usually obtain their food by ingesting other organisms or organic particles (Madigan et al., 2000). Large numbers of protozoa can infect human by staying as parasites in the intestines of humans. The most common protozoal diseases are diarrhea and dysentery. *Giardia lamblia* causes an acute form of gastroenteritis. The cyst form is 8 to 12 μm long by 7 to 10 μm wide, and is infectious to people by the fecal-oral route of transmission. Their germination in the gastrointestinal tract brings about the symptoms of giardiasis: diarrhea, nausea, vomiting, and fatigue. These cysts can survive up to 77 days in water less than 10ºC and are highly resistant to chlorine disinfection, although they will be inactivated when subjected to temperatures of 54ºC and above for 5 minutes. Risk analysis, using a probabilistic model, suggests that if *Giardia lamblia* can be reduced to 0.7 to 70 cysts per 100 liters of drinking water, the annual risk of infection will be less than one per 10,000 population (AWWA, 1999).

Another important protozoan, the *Cryptosporidium* species, also causes diarrhea. Specifically, *C. parvum* is the major species causing the disease. Human beings are the reservoir for these infectious protozoans and one infected human can excrete 10^9 oocysts a day. *C. parvum* oocysts are 4 to 6 μm in size and spherical in shape. Similar to *Giardia* cysts, *C. parvum* oocysts can survive for several months in water at 4ºC and are highly resistant to chlorine. *C. parvum* also has a low infective dose. The disease was produced in two primates when they were given a dose of only 10 oocysts (Miller et al., 1990).

2.3.4 Helminths (Eukaryotic)

Helminths are intestinal worms that do not multiply in the human host. For example, hookworms live in the soil and can infect humans by penetrating their skin. With a heavy worm infection, the symptoms are anaemia, digestive disorder and abdominal pain. The guinea worm measures 0.5 to 25 millimeters (mm) in length, and their eggs are usually transmitted through contaminated drinking water supplies in rural areas (AWWA, 1999). These worms cause a condition called “dracunculiasis” and the worms emerge from blisters in a few weeks. Normally, the wound heals rapidly without treatment. Sometimes, the wound may become infected and affect joints and tendons, causing significant disability (Hunter, 1997).

2.4 Indicator Organisms of Drinking Water

The probability that a person will be infected by a pathogen cannot be deduced from the pathogen concentration alone. This is because different humans respond differently to the pathogens. As a result, there is no real lower limit for acceptable levels of pathogens in water. Instead, it follows that "safe" drinking water intended for human consumption should contain none of these pathogens.

To determine if a given water supply is safe, the source needs to be protected and monitored regularly. There are two broad approaches to water quality monitoring for pathogen detection. The first approach is direct detection of the pathogen itself, for example, the protozoan *Cryptosporidium parvum*. While it will be more accurate and precise if specific disease-causing pathogens are detected directly for the determination of water quality, there are several problems with this approach. First, it would be practically impossible to test for each of the wide variety of pathogens that may be present in polluted water. Second, even though most of these pathogens can now be directly detected, the methods are often difficult, relatively expensive, and time-consuming (WHO, 1996). Instead, water monitoring for microbiological quality is primarily based on a second approach, which is to test for "indicator organisms" (See Figure 2-2 for a classification table created by the author of typical indicator organisms). The indicator organism should fulfill the following criteria (Stetler, 1994):

1) An indicator should always be present when pathogens are present;
2) Indicators and pathogens should have similar persistence and growth characteristics;
3) Indicators and pathogens should occur in a constant ratio so that counts of the indicators give a good estimate of the numbers of pathogens present;
4) Indicator concentrations should far exceed pathogen concentration at the source of pollution;
5) The indicator should not be pathogenic and should be easy to quantify;
6) Tests for the indicator should be applicable to all types of water;
7) The test should detect only the indicator organisms thus not giving false-positive reactions.

Figure 2-2: Indicator organism classifications.

Another reason for using simple indicator tests is that pollution is often intermittent and/or undetectable. It is often better to monitor drinking water frequently by means of a simple test than to monitor infrequently using a longer and more complicated direct pathogen detection test.

While these indicator bacteria or viruses are not necessarily pathogenic themselves, some of them have the same fecal source as the pathogenic bacteria and can therefore indicate fecal contamination of water (WHO, 1993a). One example which fulfils many of the above criteria is the indicator organism *E.coli*. Therefore, it may be sufficient to get an indication of the presence of pathogens of fecal origin with the detection and enumeration of *E.coli*. Such a substitution is especially valuable when resources for microbiological examination are limited as in Nepal or other developing countries.

2.4.1 Coliform Organisms (Total Coliform)

"Coliform bacteria" are metabolically defined as gram-negative, rod-shaped bacteria capable of growth in the presence of bile salts and able to ferment lactose at an optimum 35ºC, with the production of acid, gas, and aldehyde within 24 to 48hours (WHO, 1993). They are also oxidase-negative, non-spore-forming and display β-galactosidase activity. In U.S., coliform bacteria have been recognized by the EPA Safe Drinking Water Act since 1989 as a suitable microbial indicator of drinking water quality (USEPA, 2001). The main reason is because they are easy to detect and enumerate in water and are representative enough for determining microbial contamination of drinking water. However, for developing countries in tropical climates, WHO states that,

> *Total coliform bacteria are not acceptable indicators of the sanitary quality of rural water supplies, particularly in tropical areas…. It is recognized that, in the great majority of rural water supplies in developing countries, fecal contamination is widespread (WHO, 1996).*

Therefore, the use of Total Coliform (TC) as a microbiological indicator of water quality in developing countries is not appropriate. A better indicator of recent fecal contamination is required (See Chapter 3.3 for a more in-depth discussion).

Coliform bacteria traditionally include the genera *Escherichia, Citrobacter, Enterobacter* and *Klebsiella*. Modern taxonomical methods also include lactose-fermenting bacteria, such as *Enterobacter cloacae* and *Citrobacter freundii*, which can be found in **both** feces and the environment (WHO, 1993a). The inclusion of both non-fecal bacteria and lactose-fermenting bacteria limits the applicability of this group as an indicator of fecal contamination or pathogens in drinking water. However, the coliform test is still useful for monitoring the microbial quality of treated pipe water supplies despite its lack of specificity to fecal contamination (Gleeson & Glay, 1997). If in doubt, especially when coliform organisms are detected in the absence of thermotolerant coliform and *E.coli*, further analysis for other indicator organisms should be undertaken to determine if fecal contamination is present. For total coliform (TC), an incubating temperature of 35ºC for 24 hours is used during bacteria culture. Under the WHO Guidelines, no samples are allowed to contain any coliform per 100 milliliters (ml) of treated water sample in the distribution sample. For large water supplies, coliforms must not be present in 95% of samples taken throughout any 12-month period. Under the Total Coliform Rule by EPA, a violation is triggered if 1 sample tests coliform-positive in a system collecting fewer than 40 samples per month. If more than 40 samples are collected per month, not more than 5% of all samples can test positive.

2.4.2 Thermotolerant Coliform Bacteria

This group of bacteria comprises the bacteria genus *Escherichia*, and to a lesser extent, *Klebsiella*, *Enterobacter,* and *Citrobacter*. They are defined as a group of coliform organisms that are able to ferment lactose at 44 to 45ºC. Sometimes, this group is also called Fecal Coliform (FC) to specify coliforms of fecal origin. This is not appropriate since thermotolerant coliforms other than fecal coliforms may also originate from organically enriched water such as industrial effluents, from decaying plant materials and soils, or on vegetation in a tropical rainforest (WHO, 1996). Of these organisms, only *E.coli* is specifically of fecal origin. However, concentrations of thermotolerant coliforms are usually directly related to that of *E.coli* and thus can be used as a surrogate test for *E.coli*. When a sample is tests positive for thermotolerant coliforms, it is usually subjected to further confirmed tests for *E.coli.* Positive results for both indicators are a strong indication of recent fecal contamination (WHO, 1996). Since thermotolerant coliforms can be readily detected by simple, single-step methods, it often

plays an important secondary role as an indicator of the efficiency of individual water-treatment processes in removing fecal bacteria (WHO, 1996). The WHO Drinking Water Guidelines state that zero thermotolerant coliform or *E.coli* may be found per 100 ml of drinking sample. This group of indicator organisms is currently not listed in the EPA drinking water standards.

2.4.3 Escherichia coli (E.coli)

Escherichia coli is a specific subset of the thermotolerant coliform bacteria which possess the enzymes β-galactosidase and β-glucuronidase that hydrolyzes 4-methyl-umbelliferyl-β-D-glucuronide (MUG). They are found abundantly in human feces (as much as 10^9 per gram (g) of fresh feces) and warm-blooded animals. Ninety-five percent of all coliform found in human feces can be *E.coli* (Waite, 1985). Sewage, treated effluents, all natural water and soils that are subject to recent fecal contamination from humans or wild animals will contain *E.coli*. Usually, *E.coli* cannot multiply in any natural water environment and they are, therefore, used as specific indicators for fecal contamination (WHO, 1996) (See Chapter 3.4 for a counter argument). Therefore, while the presence of both thermotolerant coliforms and *E.coli* is not able to distinguish between human and animal contamination, nonetheless, they are better indicators than TC for the presence of recent fecal contamination. Both WHO Guidelines and EPA standards require zero *E.coli* to be found per 100 ml of drinking water sample.

2.4.4 Fecal Streptococci

Most of the species under the genus *Streptococcus* are of fecal origin and can be generally regarded as specific indicators of human fecal pollution (WHO, 1993a). However, certain species may be isolated from the feces of animals. Fecal streptococci seldom multiply in polluted water and they are more persistent than coliform and *E.coli* bacteria. Therefore, they are generally useful as additional indicators of treatment efficiency (WHO, 1996). This indicator organism is commonly tested with *E.coli* for evidence of recent fecal contamination.

2.4.5 Sulfite-Reducing Clostridia

Sulfite-reducing clostridia are gram-positive, anaerobic, spore-forming bacteria. Clostridial spores can resist treatment and disinfection processes better than most pathogens, including

viruses. One of the members, *Clostridium perfringens,* like *E.coli,* is normally present in feces, but in much smaller numbers. However, they are not exclusively of fecal origin and can be found in other environmental sources (WHO, 1996). Clostridial spores can survive in water much longer and resist disinfection better than other coliform groups (AWWA, 1999). However, they are not recommended for routine monitoring of distribution systems because they tend to accumulate and are detected long after pollution has occurred, thus giving rise to false alarms.

2.4.6 Hydrogen Sulfide-Producing Bacteria

Another related group of bacteria called the hydrogen-sulfide producing bacteria include *Citrobacter freundii, Salmonella typhimurium, Proteus vulgaris,* strains of *Klebsiella* (Manja et al., 1982; Grant and Ziel, 1996), genuses *Edwardsiella* and *Arizona* (Madigan et al., 2000). A common sulfate-reducing (to hydrogen sulfide) anaerobic bacteria, *Desulfovibrio,* is commonly found in aquatic habitat containing abundant organic material and sufficient levels of sulfate (Madigan et al., 2000). Together with the previous genre, these bacteria have since at least 1980s (Manja et al., 1982) been isolated and detected using Presence/Absence (P/A) and Most Probable Number (MPN) tests. The significance of testing for this group of bacteria is because of their strong fecal origin correlation to FC (Manja et al., 1982; Grant and Ziel, 1996).

2.4.7 Bacteriophages

Bacteriophages (phages) are viruses that infect and replicate in specific bacteria. The ability to identify phages (coliphages) of *E.coli*, also detects fecal contamination. This is because the presence of coliphages also indicates the presence of *E.coli.* The significance of coliphages as indicators of sewage contamination, and their greater persistence compared to bacterial indicators make them useful as additional indicators of treatment efficiency. A current method of coliphage detection is through the culture of *E.coli* in a Tryptic Soy Agar (TSA) medium (Stetler, 1994).

2.4.8 Protozoan Parasites

Cysts of the *Giardia* and *Cryptosporidium* species are exceptionally resistant to traditional disinfection by chlorination and are not readily detectable. Since their response to disinfection processes differ extensively from the other bacteria indicators, quality control of these organisms are generally based on specifications for raw water quality and the removal efficiencies during treatment processes rather than testing for their presence (WHO, 1996). *Cryptosporidium* is detected using microscopic staining methods and immunofluroscence microscopy through the injection of fluorescently labeled antibodies (Fayer et al., 2000).

2.4.9 Heterotrophic Bacteria

Heterotrophic bacteria are members of a large group of bacteria that use organic carbon for energy and growth. Many laboratories measure heterotrophic bacteria by the heterotrophic plate count (HPC). The presence of heterotrophic bacteria does not indicate the likelihood of pathogen presence. However, a sudden increase in HPC may suggest a problem with treatment or water disinfection (AWWA, 1999).

2.4.10 Human Viruses

Occurrence of human viruses in water environments may differ extensively from fecal indicators because viruses are excreted only by infected individuals while coliform bacteria are excreted by almost all warm-blooded animals. Generally, the number of viruses is lower by several orders of magnitude. Furthermore, tests for viruses are relatively expensive, complicated and time-consuming. Therefore, the best control of viruses, as also with protozoan parasites, is to use a water source that is known to be free of fecal contamination and to ensure a sufficient residual level of disinfectant in storage and distribution system (WHO, 1993a).

Refer to Table A3 for WHO Drinking Water Bacteriological Guidelines and Table A4 for EPA National Primary Drinking Water Standards, in Appendix A.

Chapter 3 : SUITABILITY OF COLIFORMS AS INDICATORS

3.1 Introduction to the Coliform Indicator

"In general, the coliform test has proved a practical measurement of treatment effectiveness, although there is much debate concerning the adequacy of the coliform index and its ability to determine the potability of drinking water." (Gleeson and Gray, 1997)

The above statement summarizes the essence of this chapter. As discussed in the previous chapter, WHO and EPA use coliform as the main indicator in their drinking water guidelines and standards (See Table A3 and A4 in Appendix A). Recognizing the limitations of only using the TC indicator, WHO adopted the use of thermotolerant coliforms and *E.coli* as additional indicators. EPA took this one step further and recognized other microbes such as *Giardia lamblia, Cryptosporidium* (protozoa), and enteric viruses to indicate recent fecal contamination. Informed by the debate concerning the adequacy of the coliform index and the limitations recognized by WHO and EPA, this chapter investigates why the coliform group is not an appropriate indicator of drinking water quality, especially for developing countries in tropical regions.

The coliform indicator (which is also referred to the "coliform index") was first introduced in the late 1880s (Gleeson and Gray, 1997). The approach is based on the assumption that there is a quantifiable relationship between the concentration of coliform indicators and the **potential** health risks involved. In 1901, the first edition of the *Standard Methods for the Examination of Water and Wastewater* was published in the U.S. Today, in developed countries such as the U.S. and the U.K., the water industry realized they could not guarantee that the drinking water they supplied would be free from all pathogens, however meticulously they adhered to the accepted practices of using coliforms and other indicator organisms. Waterborne diseases are now known to be caused by a much broader spectrum of organisms than just enteric bacteria, including viruses and protozoa, some of which are more resistant to conventional water treatment. Viruses and protozoa are often more difficult to isolate, takes a longer time to detect, and most importantly, they are not associated with the coliform indicators. Another group, the opportunistic pathogens, which can put immuno-compromised, people in particular, at a much

higher risk than healthy people, is also not included in the coliform index. In addition to the problems encountered with the use of coliform indicator organisms, there are also other limitations with the detection methods currently determined by Standard Methods. This prompted the following recommendations by water quality monitoring experts: 1) alternative methods of detection, and 2) establishment of alternative indicator systems. However, before the next chapter looks into the shortcomings of existing detection methods, an in-depth examination of the coliform group and why they are unsuitable as indicators is carried out.

3.2 Why Coliforms are Chosen as Indicators

Besides the criteria discussed previously in regard to the choice of indicator organisms, there are numerous reasons for their use. Waterborne pathogens such as *Vibrio cholerae* and *Salmonella* spp. usually die very quickly and are present in very low numbers. These characteristics make their isolation and detection difficult and impractical. Furthermore, the water will most likely have been consumed by the user by the time the pathogen is detected. The value of frequent monitoring of a water supply using simple tests is greater than occasional monitoring using a complicated test or series of tests (London Department of the Environment, 1994). This is because the appearance of pathogens are often intermittent, of short duration, and the organisms are readily attenuated and few in number (Bonde, 1977).

Criteria for indicator organisms have been discussed in Chapter 2.4. The rationale for the use of indicator organisms can be crudely illustrated mathematically:

$$[\text{indicator}] \alpha \text{ fecal contamination } \alpha [\text{pathogen}] \equiv \text{disease occurrence}$$

This shows the indirect relationship between the concentration of indicator organisms and pathogen population. It has been established that when a certain population of pathogens is present in humans, they can cause diseases. Figure 3-1 and Figure 3-2 show the direct relationship between disease risk and viruses, *Salmonella*, and coliforms. When the concentration of the pathogens or coliforms increases, the risk of illness also increases proportionately. Studies have also shown that most of these waterborne pathogens originate from fecal sources (Olson and Nagy, 1984). Therefore, if the indicator organism can accurately indicate the extent of recent fecal contamination, by implication, it is a good indicator of

pathogen concentration and the incidence of waterborne disease (Pipes, 1982), even if it is not pathogenic on its own. In reality, no organisms or groups of organisms fulfill all the criteria, although the coliform group fulfils most of them.

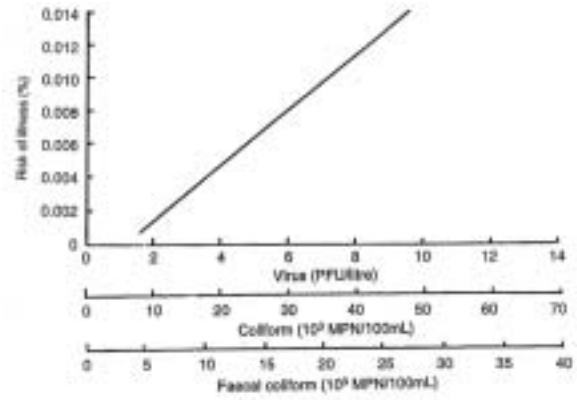

Figure 3-1: Relationship between disease risk and viruses, coliforms and FC (Olson and Nagy, 1984).

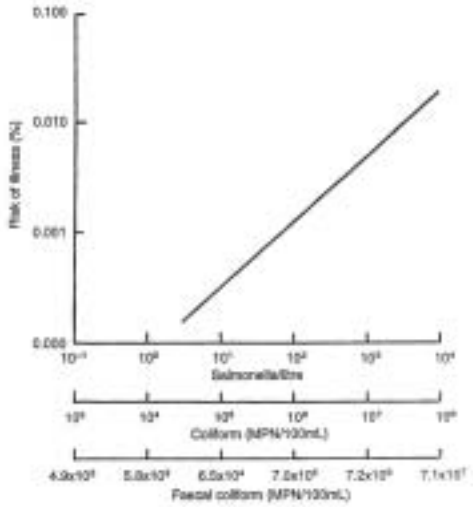

Figure 3-2: Relationship between disease risk and Salmonella, coliforms and FC (Olson and Nagy, 1984).

It is important to note that the definition of the coliform group has been based on methods of detection, and not systematic bacteriology. According to the WHO and EPA, coliforms are described as lactose fermenting bacteria with the production of acid and gas. A more recent definition by WHO and EPA also states that a coliform must possess the β-galactosidase gene. (Refer to Table 3-1 for a sample breakdown of coliform bacteria identified with the LES ENDO agar). The thermotolerant coliform group is a subset of coliform that is capable of fermenting lactose at 44ºC. Thermotolerant coliforms should not be called fecal coliforms as has already been mentioned because some non-fecal organisms are also capable of growth at 44ºC, such as non-fecal *Klebsiella* spp. (Madigan et al., 2000). With recent advances in recovery techniques, coliforms are increasingly recovered as naturally occurring in non-fecally contaminated environments, in both temperate and tropic climates. *E.coli,* on the other hand, is considered to be the true FC as other thermotolerant coliforms can be found in non-fecally contaminated waters too. Therefore, the TC test should only be taken as a **presumptive test**. If it tests positive, the sample should be examined for thermotolerant coliforms and *E.coli*, as a **confirmed test** (Lisle, 1993).

Table 3-1: Identification of coliforms isolated from drinking water on LES ENDO agar (Mates and Shaffer, 1989).

	No. of strains	Lauryl Tryptose Broth	Brilliant Green Broth	EC Broth	% of Strains
E.coli MUG +ve	36	36	36	36	23
E.coli MUG –ve	1	1	1	1	0.5
Enterobacter spp.	6	6	6	0	4
Klebsiella spp.	9	9	9	0	6
Citrobacter spp.	85	85	85	0	53
Oxidase positive organisms	23	0	0	0	14
Total	160	137	137	37	100

3.2.1 Presumptive and Confirmed Tests

The first step or presumptive test essentially serves to revive the TC. The selected presumptive medium facilitates the growth of the coliforms, but also allows some non-coliforms to grow. Because of this additional non-coliform growth, there is a relatively high percentage of false-positive results associated with the presumptive test (Lisle, 1993). Therefore, an additional step called the confirmed test should be carried out to confirm the presence of the TC isolated in the presumptive test. In the confirmed test, the TC is extracted from positive presumptive tests. The broth used in the confirmed test is more selective for TC (because it inhibits non-coliforms) than the presumptive test broths, thereby minimizing false positives (Lisle, 1993). The TC is not inoculated directly in the confirmed tests because they are "stressed" and need time to get their systems revived to grow and multiply at an optimal capacity. The presumptive step allows the coliforms to adjust to the media with a minimal loss of viability while increasing their numbers. If the TC is able to survive the presumptive test, they will be more likely to tolerate the more selective ingredients of the confirmed test broth (Lisle, 1993).

In the U.S., FC or *E.coli* counts are used to assess the microbiological quality of surface waters because of their public health implications. For treated drinking water, TC is usually enumerated since it is assumed that waters designated for human consumption should not contain any micro-organisms (Cabelli, 1978). It is assumed that when the broader class TC is absent, FC and *E.coli* are also absent. The following section discusses why these drinking water standards can be unrealistic for use in tropical developing countries.

3.3 Why Coliforms are Unsuitable Indicators

The coliform concept was developed and preserved until this day,

> *"based on decisions and assumptions which were largely correct in the light of knowledge available at the time." (Waite, 1985)*

It was developed more than a century ago and therefore reflects the disease profile of that time and not of the 21st century. In addition, there are several deficiencies associated with their use in water quality assessment (Gleeson and Gray, 1997).

3.3.1 Coliforms are Not Accurate Indicators of Pathogens and Waterborne Diseases

The most important reason why coliforms are not good indicators is because they are not necessarily indicative of the presence of pathogens (bacteria, protozoa, and viruses) and hence of a health threat. A comparative study of community and non-community water systems by Craun, Batik and Pipes (1983) showed that it is possible to find coliforms in systems for which there are no reported outbreaks and to have outbreaks in systems for which there are no positive coliform results (Refer to Table 3-2).

Table 3-2: Non-community water systems: comparison of coliform monitoring results prior to and after an outbreak (Craun, Batik and Pipes, 1983).

	Coliform results		
	Positive result	Negative result	Total
Non-community system experiencing an outbreak	8	8	16
Non-community system not experiencing an outbreak	343	455	798
Total	351	463	814

Coliforms such as the non-fecal *Klebsiella, Citrobacter* or *Enterobacter* have been found present in the distribution system where no waterborne disease outbreak occurred (Geldreich and Rice, 1987), although no *E.coli* or positive FC tests were observed. The conclusion drawn from this research was that the incidence of coliform was due to colonization within the distribution system and not due to fecal contamination.

While the coliform index recognizes that there is no absolute correlation between coliforms and bacterial pathogens, afterall, the underlying principle of the index is that its presence in waters indicates the **potential** presence of pathogens (Townsend, 1992). There have been reports of where *Vibrio* sp. (Kaper et al., 1979) and *Salmonella* sp. (Dutka and Bell, 1973; Morinigo et al., 1990) have been recovered from waters containing few or no coliforms or FC. This may be due to coliforms having a faster die off rate than *Salmonella* sp. (Borrego et al., 1990) and also, *Salmonella typhi* has been reported to be more resistant to chlorination than coliforms (Dutka, 1973). This lack of reliability of the coliform indicator has prompted the need to replace it with the direct detection of pathogens.

It is accepted that coliform bacteria do not reflect the concentration of enteric viruses in natural waters (Geldenhuys and Pretorius, 1989; Metcalf, 1978). Viruses can persist longer and remain infectious at lower temperatures for many months, unlike coliform bacteria. Protozoan cysts such as *Crpytosporidium* oocysts and *Giardia* cysts are also more resistant to chlorination than coliforms (Metcalf and Eddy, 1991). Data produced by Rose, Darbin and Gerba (1988) revealed no association between coliform bacteria and either *Crpytosporidium* oocysts or *Giardia* cysts (Refer to Table 3-3).

Table 3-3: Correlation coefficients for coliform bacteria, turbidity and protozoa in a watershed. (Rose, Darbin and Gerba, 1988).

	TC	**FC**	***Cryptosporidium***	**Giardia**
Turbidity	0.277	0.288	0.242	0.284
TC		0.709	0.154	0.018
FC			0.291	0.102
Cryptosporidium				0.778

3.3.2 Coliforms Should Not Re-Grow in the Environment

An ideal indicator organism (See Chapter 2.4) should not be able to proliferate to a greater extent than enteric pathogens in the aquatic environment (Feacham et al., 1983). Studies have shown that TC is capable of regrowth even in chlorinated sewage (Shuval et al., 1973). High coliform counts have also been reported in enriched waters receiving pulp and paper mill effluents, sugar beet wastes and domestic sewage (Geldreich, 1970; Dutka, 1973; Pipes, 1982) (See Figure 3-3, Figure 3-4, and Figure 3-5). These graphs show the increase in coliform and *E.coli* survival in effluent and environmental lake waters after several days. Regrowth of coliform bacteria has

also been found in drinking water distribution systems (Olson and Nagy, 1984). This is often the result of the lack of residual disinfection i.e. inadequate treatment leaving the treatment plant, and recovery of injured coliforms.

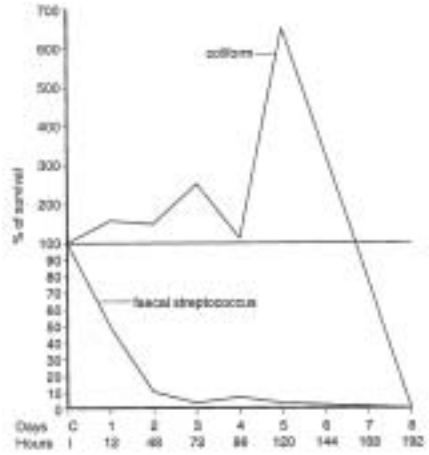

Figure 3-3: Study of the survival and multiplication of coliforms and faecal streptococci in relatively unpolluted lake waters (Dutka, 1973).

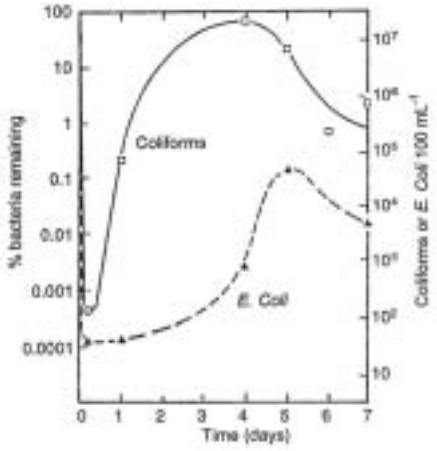

Figure 3-4: Regrowth of coliforms and *E.coli* in sewage effluent after inactivation with 5mg/L chlorine (Shuval, Cohen and Kolodney, 1973).

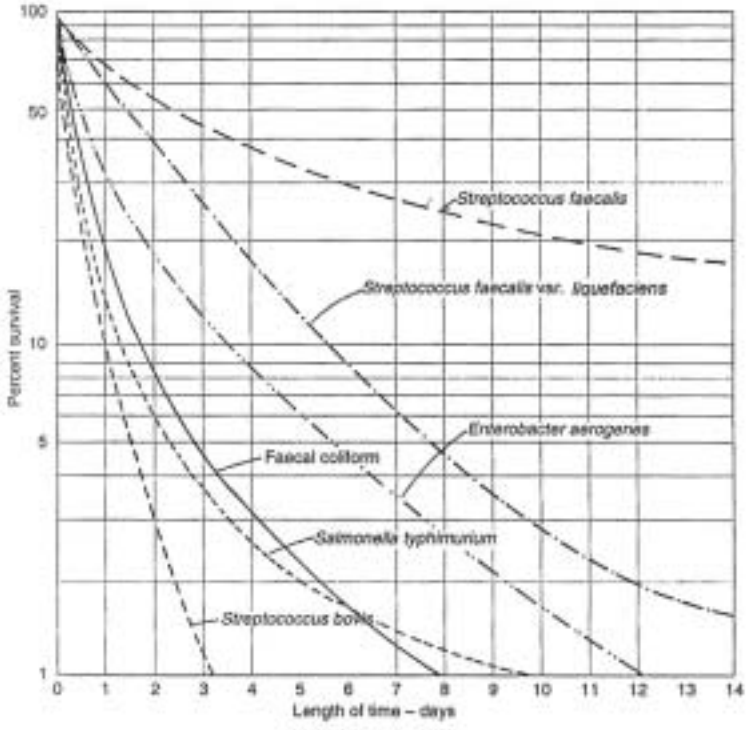

Figure 3-5: Persistence of selected enteric bacteria in storm water stored at 20°C (Geldreich, 1970).

The growth of bacteria on pipe surfaces is controlled by the availability of assimilable organic carbon in the water. These coliforms originate from biofilms on the pipe walls and are able to

coexist with chlorine residuals under certain circumstances (Geldreich, 1996). For example, *E.coli* is 2,400 times more resistant to chlorine when attached to a surface than as free cells in water (Le Chevallier et al., 1988). Le Chevallier et al. also discovered that up to 20 milligrams (mg) per liter of free chlorine was required to control biofilm. (Chlorine has a maximum allowed concentration of 5 mg per liter in drinking water (WHO, 1993b).) Waters that contain high turbidity often reported high coliform counts for two reasons: 1) the suspended particles protect the organisms such that chlorine is unable to come in contact with them (Le Chevallier et al., 1981), 2) turbidity, interferes with coliform detection by the Membrane Filtration (MF) technique.

The presence of high background bacteria growth can suppress the growth of coliform. These antagonists include strains of *Pseudomonas, Sarcina, Micrococcus, Flavobacterium, Bacillus,* and *Actinomyces* as well as some yeasts (Hutchinson et al., 1943). It is observed that chlorinated waters containing high numbers of antagonists have low coliform counts (Refer to Table 3-4). As much as 57% of the coliform counts can be underestimated under such suppressive conditions (Le Chevallier at al., 1981).

Table 3-4: Relationship between percentage of coliform antagonists and the presence of coliforms (Le Chevallier, Seidler and Evans, 1980).

Sample	No.	No. with Coliforms	Occurrence (%)
Distribution			
> 20%	16	3	19
< 20%	7	4	57
Raw Water			
> 20%	0	0	-
< 20%	11	11	100

3.3.3 High Probability of False Positive and False Negative Results with Coliform Tests

False positive and false negative results with the TC tests can also take place. The Aeromonads species is able to mimic the Enterobacteriaceae and produce acid and gas at 37ºC like the coliforms thus inflating TC counts (Waite, 1985). These organisms will give rise to positive presumptive coliform tests and therefore confirmed tests should be followed up. In a particular study by Grabow and Du Preez (1979), they found 40 to 58% of TC consisted of *Aeromonas*

hydrophila. However, these organisms do not give false positive problems with *E.coli* and thermotolerant coliform tests. In the case of false negative results with TC, Leclerc et al. (1976) showed that 20% of coliforms can be non-lactose fermenting. These coliforms will therefore not show up in the routine coliform counts, resulting in false negative results. A study of coliform recovery by MF showed 47 to 61% of colonies are anaerogenic[3], or late or non-lactose fermenting coliforms (Waite, 1985; Dutka, 1973).

3.4 Inappropriate Use of Coliforms as Fecal Indicators in Tropical Environments

At present, it is widely considered that the coliform index is highly inadequate for detecting fecal contamination in tropical conditions (Gleeson and Gray, 1997). A number of authors have reported the frequent presence of naturally occurring coliforms in unpolluted tropical sites, as well as the ability of enteric coliforms to survive for considerable lengths of time outside the intestine (Bermundez and Hazen, 1988; Carrillo et al., 1985; Rivera et al., 1988; Santiago-Mercado and Hazen, 1987), thus implying that coliforms are naturally occurring in tropical waters. A large proportion of these coliform species are also thermotolerant (Santiago-Mercado and Hazen, 1987). The following authors found these relationships as shown in Table 3-5:

Table 3-5: Relationships between different indicators as extracted from different literature sources.

Tropical waters	**Sources**
E.coli/TC = 14.5%	(Lamka, Le Chevallier and Seidler, 1980)
Thermotolerant coliform/TC = 10-75%	(Lamka, Le Chevallier and Seidler, 1980)
Therefore, *E.coli*/Thermotolerant coliform = 19-100%	(simple derivation)
Temperate waters	
E.coli/Thermotolerant coliform = 90%	(Ramteke et al., 1992)

These proportions show that there is no benefits in using FC as opposed to TC in evaluating tropical waters as both groups give equally inaccurate results. Therefore, we recommend that *E.coli* replace TC as the preferred indicator for use in tropical countries. *E.coli*, which can represent up to 95% of the Enterobacteriaceae found in feces (Waite, 1985), can be considered exclusively fecal in origin (WHO, 1993a). However, a paper by Solo-Gabriele et al. (1999)

[3] Anaerogenic means "fails to produce gas when fermenting lactose".

showed that *E.coli* is able to multiply in the tidally-influenced areas of Florida, thus challenging the use of *E.coli* as a suitable indicator of water quality in these areas. WHO recommends the detection of fecal streptococci and sulfite-reducing clostridia as confirmed tests for the fecal origin of the contamination (WHO, 1993a). (Refer to Table 3-6 for a more general breakdown of bacteria found in human feces.)

Table 3-6: Number of indicator bacteria commonly found in human feces (Wet Weight) (Feacham et al., 1983).

Indicator	**Cells/g feces (w/w)**
Bacteroides spp.	10^7 - 10^{11}
Bifidobacterium spp.	10^7 - 10^{11}
Clostridium perfringens	10^3 - 10^{10}
Coliforms	
Fecal	10^6 - 10^9
Non-fecal	10^7 - 10^9
Fecal streptococci	10^5 - 10^8

3.5 Proposed Drinking Water Monitoring Methodologies in Tropical Developing Countries

There has been a long tradition of legislation, policy and technology being directly transferred from developed to developing countries such as Nepal without proper consideration to their applicability. In case of applying drinking water quality guidelines or standards to developing countries, there is little justification to apply the same high standards of zero TC per 100 ml sample for drinking water in developing countries. Moreover, the use of coliform index as an indicator of drinking water quality is still strongly debatable, especially in tropical conditions. The coliform index accepts the fact of a small but allowable risk of enteric infection and that all risk from enteric pathogens cannot be realistically eliminated. It is difficult with the current epidemiological knowledge to assess risk to health presented by any particular concentration of pathogens in water, not to mention the indirect relationship with indicator organisms. This is because the risk varies significantly depending on the infectivity and invasiveness of the pathogen and on the innate and acquired immunity of the individuals consuming the water (WHO, 1993a). There is also a need to accept the fact that it is not feasible to have a single indicator for all locations. Therefore, these universal pollution indices should be interpreted with caution (Gleeson and Gray, 1997).

Is it sensible for developing countries to try to mitigate or eliminate the substantial waterborne disease risks and meeting the same high standards as developed countries when those standards are inaccurate and misleading? Is it a good use of financial and human resources? Will the incurred opportunity cost be too great and unattainable?

To answer the above question, the following solutions are suggested:

3.5.1 Encourage incremental improvements

This solution serves to encourage an incremental improvement in water quality at the most affordable cost to the local community. This will serve as the first step towards providing safe drinking water supplies especially in the rural areas which have greater difficulty in achieving these drinking water standards. For example, if the existing water quality is 100 TC per 100 ml, incentives can be provided when the quality improves by 50% to 50 CFU[4] per 100 ml. The improvement in quality can be achieved from the increased use of point-of-use treatment options and/or disinfection.

3.5.2 Improve sanitary surveys

Besides encouraging incremental improvements in treatment of drinking water supplies, better sanitary surveys could be carried out. The sanitary surveys seek to investigate the possible sources and routes of pollution. Take the case of tubewells as an example. During a tubewell maintenance survey, the researcher will study the construction practices, usage patterns, and maintenance program (if any) of the tubewells in a certain village or district to determine possible sources of contamination (Gao, 2002). He or she might also evaluate water use practices, latrine availability, hand-washing practices as possible causes. Corrective measures can then be carried out to isolate the source of pollution through the education of users and formation of maintenance groups (Gao, 2002).

[4] CFU stands for Colony Forming Unit, which is assumed to grow from one single bacterium. See Chapter 6.2.

3.5.3 Re-evaluate "acceptable risk" used in determining water quality guidelines

Risk assessment involves the evaluation of the risks posed by all the bacterial, viral, and parasitic pathogens in the water supply. In order to come up with the acceptable risk, appropriate epidemiological studies are fundamental. These studies should also pay attention to opportunistic pathogens which can put immuno-compromised people, in particular, at a much higher risk than healthy people. In addition, since financial resources are limited for developing countries, a cost-benefit approach could be used to determine the acceptable risk. Focus should also be placed on the incremental benefits achievable with incremental improvements in the water quality. This is recognized by WHO which specifies that the national surveillance agency should set medium-term goals for the progressive improvement of water supplies (WHO, 1993a). This will enable the decision-maker to increase the value of his/her expenditure since he/she will ensure that the maximum benefits are gained per dollar spent on the improvements in water monitoring and treatment.

3.5.4 *E.coli* as proposed indicator but with revised standards

As discussed throughout Chapter 3, *E.coli* is the most suitable indicator of recent fecal contamination and is proposed as the indicator organism of choice for routine water quality monitoring in developing countries like Nepal. Simple, yet frequently administered tests could be used to monitor drinking water quality using *E.coli*. These tests should be affordable, easy to perform and understand so that most middle and lower-class consumers can conduct the tests independently. An example is the use of P/A test to detect the presence of *E.coli*. However, it is also important to adjust the sensitivity of these test kits such that they are not over-sensitive and give too many false-positive results. A suggestion is to design for a detection level that coincides with the previously established idea of acceptable risk. In addition, the guideline values recommended should be considered as a future goal, not an immediate requirement. Very often, in order to meet the guideline values, the elimination of the contamination sources can only be achieved with corresponding improved sanitation practices. Unless other sources of risk are adequately controlled, it will be difficult to reduce waterborne diseases with only the improvement of drinking water supplies.

However, in circumstances when there may be a very small concentration of *E.coli,* FC is the next most appropriate indicator to use. Both P/A and enumeration methods such as MF can be used. In particular, the P/A-H_2S test which is a good and simple indicator test for fecal contamination can also be used (See Chapter 5).

3.5.5 Implement alternative indicators and detection methods

Finally, alternative indicator systems, detection technology or even direct pathogen enumeration can be recommended. This is a more universal solution which can also be applied to developed countries. At present, the inability to detect indicators or pathogens within a few hours of sample processing is a major limitation in water quality assessment. Very often, by the time the outbreak is detected, the water is already consumed by the users. Future developments involving PCR and gene probe technology for the direct detection of pathogens may remove the need for indicators altogether (Gleeson and Gray, 1997).

Chapter 4 : PRESENCE/ABSENCE INDICATOR TEST

4.1 P/A Test for Coliform Indicator

The Presence/Absence (P/A) test for the coliform group is a simple modification of the multiple-tube procedure. The P/A technique was first developed by Dr. James A. Clark[5] in 1968 to provide 'a more economical device for coliform analyses' (Clark, 1968). This test has been used in Canada since 1969. The test provides information on TC being present or absent in a 100 ml drinking water sample, a larger sample size than the multiple-tube enumeration method which uses 20 ml volumes. As only a single 100 ml vessel is used in Clark's P/A test, there is no information about the number of coliforms in the sample.

Traditionally, methods of analysis such as MF and multiple-tube fermentation were developed primarily to identify both the presence and numbers of TC bacteria in order to determine the degree of pollution. However, questions were raised as to the necessity of enumerating coliform bacteria when studies showed that these organisms were irregularly distributed throughout municipal water systems (Pipes and Christian, 1984). Instead, the frequency of occurrence of coliform-positive samples was considered more representative of the overall microbiological water quality (Clark, 1990). This orientation formed the basis of the WHO guidelines and EPA standards of using P/A tests to assess microbial contamination. Instead of stating a Maximum Acceptable Concentration (MAC) of coliforms as with other water contaminants, both WHO guidelines and EPA standards state that no coliforms should be detected by either P/A or other enumeration methods in 5% of all drinking water samples (See Table A4 in Appendix A).

4.2 P/A Test for Total Coliform and E.coli

Lauryl Typtose (LT) Broth with 4-methylumbelliferyl-β-D-glucuronide (MUG) is selected as the P/A medium for the simultaneous detection of Total Coliform (TC) and *E.coli* presence. Specifically in this thesis, the HACH LT/BCP (BCP stands for bromocresol purple) with MUG broth is

Figure 4-1: HACH LT/BCP 20ml glass ampule.

[5] Dr. James A. Clark, Laboratory Services Branch, Ontario Ministry of the Environment, Rexdale, Ontario, Canada

used. HACH P/A broth with MUG comes pre-packaged in disposable glass ampules. Each ampule contains 20 ml of 6X strength sample medium for 100 ml of water sample. Other commonly available products may contain 50ml of 3X strength sample medium. Figure 4-1 shows the HACH 20 ml LT/BCP glass ampule.

4.3 Water Sampling and Testing Methodology

The general sampling and testing methodology used by the author in the research is repeated both in the MIT lab and at the ENPHO lab in Kathmandu. It can be summarized in Figure 4-2 below.

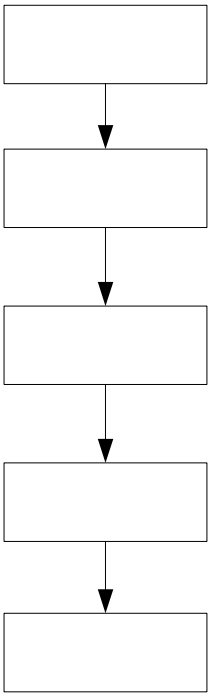

Figure 4-2: General sampling and testing methodology of the author.

Sterile conditions were always ensured by the author during all the stages of sampling and testing. In Kathmandu, water samples were carefully collected in sterile, 300 ml transparent plastic Whirl Paks. These plastic paks had sodium thiosulfate tablets to remove any residual chlorine that could exist in the water sample. These bags were then kept in a cooler box and brought back to the labs and the samples were tested within 6 hours of collection. The testing table top was wiped with alcohol to ensure a sterile working environment. The exteriors of the

sterilized sampling bottles were also first wiped with alcohol before they are used to contain the samples.

4.4 Sampling Procedures for P/A-Total Coliform Test

Instruments/Reagents used: 100ml glass sampling bottle, candle, lighter, alcohol, UV lamp, HACH Lauryl Typtose with Bromocresol Purple (LT/BCP) Broth with MUG reagent for 100 ml sample (See Figure 4-3 for the test equipment and supplies used.).

Figure 4-3: P/A equipment and supplies for TC test.

Procedures:

- Sterilize sampling bottle in air oven at 170°C for 1 hour and allow it to cool.
- Pour 100 ml sample into bottle.
- Break broth bottle and pour into sample bottle. Mix.
- Incubate sample at 35°C.
- Take P/A-TC reading at 24 and 48 hours.
    - Murky Yellow = Positive, Purple = Negative.

Purple: Absence of TC

Dark Yellow with little gas: Presence of TC

Bright Yellow with a lot of gas: More Definite Presence of TC

Figure 4-4: Different reactions with the P/A broth when TC are absent or present in various concentrations after 48 hours.

- Take *E.Coli* P/A reading with UV lamp at 24 and 48 hours.
    - Fluoresce = Positive, No fluoresce = Negative.

Figure 4-5: Fluorescence of the P/A broth after 48 hours in the top most of the 3 bottles when *E.coli* is present in the water sample.

4.5 Identification of Total Coliforms with Varying Reactions

When coliforms ferment lactose, they produce acids that change the bromocresol purple indicator to yellow. Turbidity is also produced in the broth. Gas is produced by the coliforms during fermentation and with the correct setup, captured in inverted tubes. In 1983, the use of inverted tubes in P/A was discontinued to save labor (Clark, 1990). Instead, the degree of foaming was observed after each P/A bottle is gently swirled to release dissolved gas. (Notice the foam formed at the sample surface in the third bottle of Figure 4-4.)

It should be noted, however, that acid reactions occur more frequently than gas and foam formation, because many indicator bacteria can ferment lactose without producing gas. Clark, Burger, and Sabatinos (1982) carried out a study and showed a confirmation rate of 54% when strong acid is formed. Non-coliform bacteria, such as *Aeromonas* spp., were also recovered from P/A tests with acid reactions. In fact, *Aeromonas* spp. was isolated 28% of the time, coliforms 10%, and fecal streptococci 1% (Clark et al., 1982).

Gas and foam formation, although produced less frequently, were more predictive for TC. In the 1982 study by Clark et al., the production of >10% gas in the inverted tubes resulted in 94% confirmation rate for TC. When no inverted tubes were used, a rate of 98% was found if foaming is vigorous enough to cover the surface of the medium (Refer to Table 4-1 for a more detailed breakdown). Jacobs et al. (1986) also showed similar confirmation results as shown in Table 4-2, when 94% of coliforms were confirmed with strong or slight acid, and gas production.

Table 4-1: Frequency of reactions in P/A bottles and their confirmation rate for TC (Clark, 1990).

Type of Reaction	Detailed Reaction	TC Confirmed (1982)	TC Confirmed (1985)
Acid reactions	Bright yellow, strong acid	54%	76%
	Dark yellow, medium acid	41%	42%
	Slight yellow, weak acid	53%	33%
Gas reactions	Inverted tube with >10% gas	94%	99%
	Inverted tube with 10% gas	85%	86%
	Inverted tube with <10% gas	62%	67%
Foam reactions	Surface foam layer	-	98%
	Slight foam at edge of medium	-	74%

Table 4-2: Confirmation efficiencies of TC with P/A, MPN, MF techniques (Jacobs et al., 1986).

Test	Description of Test results	TC Confirmed (1986)
P/A	Strong acid, gas	97%
	Slight acid, gas	69%
	Strong or slight acid, gas	94%
	Strong or slight acid, no gas	0%
MPN	Tubes with gas	93%
MF	Metallic-sheened colonies	93%

The amount of time required for a P/A test to show a positive result after inoculation with a sample was shown to be partly related to the numbers of TC in the sample. Table 4-3 shows that the rapidity of the P/A reaction correlated well with the rising TC counts that consists of FC (Clark, 1990). Within 24 hours, 95%[6] of these samples produced positive reactions. TC combinations had 77% showing presumptive positives after 48 hours and 90% within 72 hours. Anaerogenic coliforms took more time and needed 96 hours for 90% of these organisms to be detected (Clark, 1990). While these results suggest that the standard 48 hours incubation period may not be long enough for a thorough detection of TC, the author did not have problems achieving positive results within 48 hours during his sampling routines.

Table 4-3: Effect of increasing coliform numbers on indicator bacteria combinations and on the response time to produce a presumptive positive P/A result (Clark, 1990).

Indicator bacteria found in P-A test	Total coliform MF counts				Time required to produce a presumptive positive P-A result				
	<1	1–10	11–100	>100	24h	48h	72h	96h	120h
FC/FS/PSA/CL			6	7	13				
FC/PSA/CL		2	5	9	15	1			
FC/FS/CL	2	3	34	39	74	4			
FC/FS/PSA		3	32	134	169				
FC/PSA		9	35	20	61	3			
FC/CL	12	14	57	19	92	8	2		
FC/FS	18	69	303	302	672	19	1		
FC	136	170	255	105	614	43	9		
TC/FS/PSA/CL		1	1		1	1			
TC/PSA/CL		3	3		5	1			
TC/FS/CL	2	3	8	5	18				
TC/FS/PSA		3	6	5	12	2			
TC/PSA	10	11	16	6	26	10	7		
TC/CL	23	30	39	19	80	28	3		
TC/FS	33	44	76	35	86	88	10	3	1
TC	644	401	302	91	522	532	200	114	70
AC/FS/CL		1			1				
AC/PSA	2	6			4	1	1		
AC/CL	8	5		1	3	10	1		
AC/FS	10	8	2	1	3	10	5	2	1
AC	335	116	51	15	52	165	139	92	55

*FC = Fecal coliforms
TC = Total coliforms
AC = Anaerogenic coliforms
FS = Fecal streptococci
PSA = *Pseudomonas aeruginosa*
CL = *Clostridium perfringens*

[6] Looking at 2nd column on time required to produce a presumptive positive result, take the sum of all FC combinations that produced positive in 24 hours, divided by sum of all FC combinations that produced positive at all times = 1710/1800 = 0.95.

The LT/BCP P/A test serves as a presumptive test for TC according to EPA standards (WHO does not specify that confirmed tests should be conducted). The positive presumptive test is then further subjected to a confirmed test which uses the same methodology but a different Brilliant Green Lactose Bile (BGLB) broth. Gas production within 48 hours confirms the presence of coliform bacteria. The confirmed test is not used directly for several reasons. The selective nature of the confirmed test broth is more inhibitory to TC. When the coliforms are first introduced into the sampling bottles, they are usually "stressed". That is why the presumptive step is so important. It allows the coliforms to acclimatize to the media with a minimal loss of viability while they can multiply at an optimal capacity (Lisle, 1993). If they survive the presumptive test, these coliforms will be more capable of tolerating the more selective ingredients of the confirmed test broth (See detailed discussion on Section 3.2.1). However, for developing countries, the presumptive test is considered to be sufficient because of time and economical constraints.

4.6 Indicator Organisms Isolated from P/A-Total Coliform Test

As discussed in Section 2.4.1, the coliform group consists mainly of the genera *Enterobacter, Klebsiella, Citrobacter,* and *Escherichia* from the family *Enterobacteriaceae*. In 1982, Clark et al. categorized the genera isolated from samples taken from raw water, drinking water, and water from new mains using the P/A test. The results are summarized in Table 4-4.

Table 4-4: Distribution of organisms isolated from raw, drinking, and water from new mains by P/A tests (Clark, Burger and Sabatinos, 1982).

Identification	**Raw Water**	**Drinking Water**	**Water from New Mains**
Enterobacter cloacae	18%	26%	22%
E. agglomerans	3%	6%	3%
E. aerogenes	3%	3%	3%
E. hafniae	1%	<1%	<1%
Klebsiella pneumoniae	8%	8%	10%
K. oxytoca	3%	5%	6%
Escherichia coli	40%	19%	12%
Citrobacter freundii	6%	6%	23%
Serratia spp.	1%	2%	1%
Proteus spp.	1%	1%	<1%
Aeromonas hydrophila	9%	17%	17%
Others – oxidase positive	2%	1%	<1%
Others – oxidase negative	5%	6%	3%
Number of cultures	3036	7442	1036

Escherichia coli was the only species to show a significant decline in frequency following water treatment (Clark, 1990). While the 9 to 17% of *Aeromonas hydrophila* present (or the 20% found by Katamay, 1990) was not as significant as 40 to 58% found by Grabow and Du Preez (1979), these non-coliforms has been proven to show a significant possibility of producing false positives with the P/A test.

4.7 Sensitivity of P/A-Total Coliform Test

Many studies have found the P/A test produces more positive results when compared to MF and MPN tests for the detection of coliform bacteria (Clark, 1968; Jacobs et al., 1986; Fujioka et al., 1986; Caldwell and Monta, 1988). For example, a comparison by Jacobs et al. (1986) showed the P/A method detected 88%, MPN method 82%, and MF method 64% of the TC present in the samples. These results show that the P/A method is much more sensitive than the MF and only slightly more sensitive than the MPN. According to IDRC (1998), the detection limit of the P/A-TC method is as low as 1 indicator bacteria per 100 ml volume of sample.

The author also conducted both P/A and MF detection tests on TC during his study of the efficiency of ceramic filters and the microbial quality of the drinking water in Kathmandu. The tests were carried out under laboratory conditions in both MIT and ENPHO laboratories. The HACH LT/BCP broth was used for the P/A test while both m-Endo and m-ColiBlue24® broth from Millipore were used for the MF test to enumerate TC. A positive P/A result was recorded when the broth changes from purple to murky yellow in 48 hours at incubation temperatures at 35ºC. MF results were taken after incubation at 35ºC for 24 hours. A total of 61 water samples with coliform counts from 0 to greater than 10,000 per 100 ml were analyzed. A histogram of the frequency of positive and negative tests versus the number of TC enumerated is plotted in Figure 4-6.

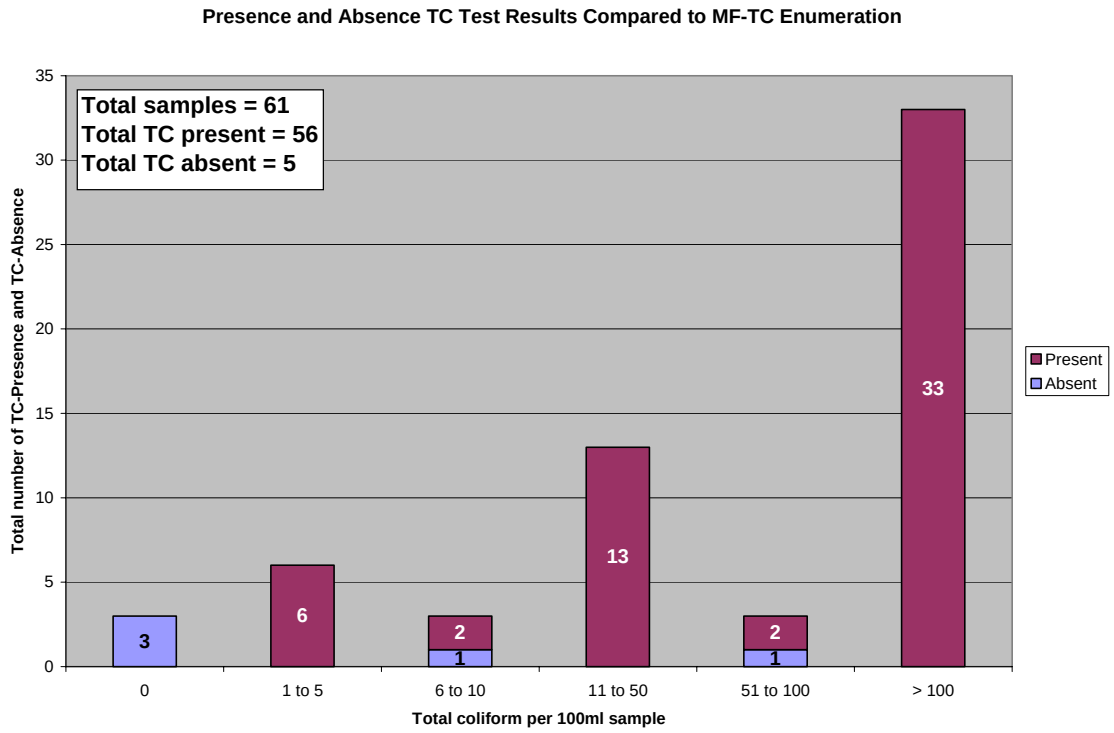

Figure 4-6: Presence and Absence TC results compared to MF-TC test enumeration.

Of the 61 samples, only 5 samples showed "Absence" results with the HACH LT/BCP P/A test. Three samples correctly showed "Absence" when no TC is detected by the MF test. Other than the other two samples which showed "Absence" results but gave 9 and 60 coliforms per 100 ml with the MF test, all other 56 samples showed "Presence" at ≥1 CFU per 100 ml. If we assume a minimum detection limit of 1 CFU per 100 ml for the P/A test, this means that there is a 97% (59 of 61 tests) agreement[7] between the P/A and MF tests.

Several explanations are provided by Jacobs et al. (1986) for the greater sensitivity of the P/A method over the MPN and MF methods. The coliforms have a lower survival and revival rate on a membrane filter compared to survival in broth. Also, the m-Endo broth used for the MF test could be overly selective which may be inhibitory to stressed coliforms.

[7] Assuming we define "Absence" in MF test as zero CFU/100ml and "Presence" as ≥1CFU/100ml, the level of agreement is defined as the number of P/A outcomes which is consistent to the MF outcomes.

4.8 Summary of P/A-Total Coliform Test

- With a 100 ml sampling volume, the test has a detection limit of 1 TC CFU per 100 ml.
- P/A-TC test is a useful and simple test that can be carried out both in the field and lab to indicate the presence of TC. However, it has been established in Chapter 3.4 that TC is not an appropriate indicator for determining fecal contamination.
- The P/A-TC test, however, can still be used as an indicator of treatment efficiencies in treatment facilities. When a presence result is obtained in the treated water samples, it will indicate that some deficiencies have occurred during the treatment process. In the context of assessing point-of-use water filters, the P/A-TC test can also be used in cases where disinfection is used. This test can however be too sensitive for assessing filters without disinfection as the TC counts in the filtered samples without disinfection are expected to be much greater than 1 CFU per 100 ml.

Chapter 5 : ANOTHER PRESENCE/ABSENCE INDICATOR TEST

5.1 P/A Test for H_2S-producing Bacteria

The P/A test for H_2S-producing bacteria was first developed by Manja et al. in 1982 as a simple field test for the detection of fecal pollution in drinking water. Their investigation revealed that the presence of coliforms in drinking water is associated with hydrogen-sulfide producing organisms. Several other studies (Kromoredjo and Fujioka, 1991; Rijal and Fujioka, 1995; Grant and Ziel, 1996; Pillai et al., 1999) also showed good correlation of the H_2S test with FC. The author's studies also showed better agreement between H_2S-producing bacteria and FC.

5.2 Rationale for Developing the H_2S Test

The H_2S test was developed as a simple, rapid, and inexpensive field test to replace the standard Most Probable Number (MPN) test to screen for fecal contamination in drinking water, especially in rural and remote areas where incubation equipment is not readily available (Manja et al., 1982). The H_2S method could be used in the temperature range of 15 to 44ºC to produce results within 24 to 48 hours, although temperatures between 28 to 37ºC gave faster results (Pillai et al., 1999). This flexibility in temperature requirement lessens or possibly removes the need for expensive incubating equipment which is often unaffordable in the poorer rural areas. At a cost of US$0.05 to US$0.23 per test (IDRC, 1998), or US$0.60 for the HACH PathoScreen (20 ml sample), it is also less inexpensive compared to US$1.41 for the HACH LT/BCP TC test. The H_2S test is very easy to carry out and requires minimum training and laboratory setup. The results show up distinctly and are easy to interpret. Most importantly, the occurrence of H_2S bacteria correlated very well for TC, FC and to some extent, *E.coli* (See later discussions).

5.3 Preparation of H_2S Test Medium

The detailed preparation of the medium and test can be found in the paper by Manja et al. (1982) and IDRC (1998). In summary, strips of filter paper impregnated with the culture medium are dried under sterile conditions at 50ºC. The paper is then stored in sterile sampling bottles which can be stored up to 6 months (IDRC, 1998). The ingredients listed below are dissolved by stirring into distilled water (IDRC, 1998):

- 40.0g bacteriological peptone;
- 3.0g dipotassium hydrogen phosphate;
- 1.5g ferric ammonium citrate;
- 2.0g sodium thiosulphate;
- 2.0ml Teepol (or 0.2g sodium lauryl sulfate);
- 100.0ml water, distilled or boiled tap.

The original test by Manja et al. is designed for 20 ml sample volume. The test can be modified for 100 ml sample volume for better sensitivity when a sixfold concentrated culture medium is used (Grant and Ziel, 1996). HACH also produces a pre-packaged powdered medium called the PathoScreen P/A Medium for either 20 ml or 100 ml samples. Figure 5-1 shows the media pillows for 20 ml volumes.

Figure 5-1: HACH PathoScreen P/A media pillow and box.

5.4 Sampling Procedures for H_2S Test

Instruments used: 20 ml glass sampling bottle, candle, lighter, alcohol, nail-clipper, HACH PathoScreen Medium for 20ml sample (See Figure 5-2 for the test equipment and supplies used).

Figure 5-2: P/A test equipment and supplies for H_2S bacteria test. 100 ml sample bottle shown in picture.

Procedures **:**

- Sterilize sampling bottle in air oven at 170°C for 1 hour and allowed to cool.
- Pour 20 ml sample into bottle and allowed to stand for about 15 minutes.
- Cut medium sachet and pour into sample bottle. Mix.
- Place sample at 35°C (See later discussion on effect of temperature on incubation duration).
- Take H_2S Bacteria P/A reading at 24 and 48 hours.
  - Black → Positive, Yellow → Negative.

Yellow: H_2S bacteria absent

Black: H_2S bacteria present

Figure 5-3: Absence and presence results of the H_2S test after 24 or 48 hours.

5.5 Association of H_2S-producing Bacteria with Coliforms and Fecal Contamination

One important question to ask about this H_2S test is: "What indicator organisms is this method testing for?" According to Kromoredjo and Fujioka (1991), hydrogen-sulfide producing bacteria have been found together with fecal contamination and TC. Therefore, HACH developed the PathoScreen medium (based on the original H_2S test) to specifically screen for these H_2S-producing organisms to indicate specific fecal contamination.

Based on several studies (Manja et al., 1982; Kromoredjo and Fujioka, 1991; Rijal and Fujioka, 1995; Grant and Ziel, 1996; Pillai et al., 1999), the isolated H_2S-producing bacteria include *Citrobacter freundii, Salmonella typhimurium, Proteus mirabilis, Proteus vulgaris, Clostridium perfringens,* and some species of *Arizona, Klebsiella, Edwardsiella*. It also detects some variants

of H_2S-producing *E.coli*. As previously mentioned, the H_2S test is not a specific test for coliforms, since *Clostridium perfringens, Proteus* spp., *Arizona* spp., and *Edwardsiella* spp. are non-coliforms. At the same time, most of the indicator organisms (coliform and *E.coli*) do not produce H_2S. While this classification may appear to challenge the relevance of this test, several studies have shown good correlation between the presence of H_2S-producing bacteria and coliforms.

The following Table 5-1 summarizes the agreement of positive H_2S tests with various indicator tests across five different studies.

Table 5-1: Agreement of positive H_2S tests with various indicator tests – A cross comparison between studies.

Authors	**Correlation in % with Positive H_2S Test**				
Manja et al., 1982	**H_2S + samples**	**MPN-TC**			
H_2S test (12-18hrs)	332	88.3%			
Kromoredjo and Fujioka, 1991	**H_2S + samples**	**Colilert**	**LT/MUG**		
H_2S test (12-15hrs)	46	81.8%	85.7%		
H_2S test (18-24hrs)	46	100%	104.8%*		
Grant and Ziel, 1996	**H_2S + samples**	**LT/MUG**	**A-1/ MUG**	**m-FC**	**m-7 hour**	**m-*C. perfringens***
H_2S test (24hrs, 30ºC)	90	92.2% (98.9% with MUG)	94.4%	90.0%	92.2%	80.0%
Castillo et al., 1997	**H_2S + samples**	**LT/MUG**	**MPN-TC**	**m-Endo**	**FC-MPN**	
H_2S test (24hrs, 35ºC)	30	55.6%	75.0%	73.2%	111.1%	
Ratto et al., 1997	**H_2S + samples**	**MPN-TC**	**m-FC**			
H_2S test (24hrs, 35ºC)	14	87.5%	140.0%			

*Agreement % refers to the proportion of H_2S test positives to the other indicator tests. When it is greater than 100%, it means that there are more H_2S test positives than that indicator test. Grant and Ziel used 100 ml sampling volume which has a greater detection sensitivity.

	Refers to tests for TC.
	Refers to tests for FC.
	Refers to test for non-coliform *Clostridium perfringens.*

In the study by Manja et al., they carried out a single agreement study between the positive H_2S tests and TC using MPN. 88.3% of all positive MPN-TC tests also showed positive H_2S tests.

Similar agreement rates of 75.0% and 87.5% were achieved by Castillo et al. and Ratto et al. respectively. When the H_2S test is compared to the LT/MUG test, a range between 55.6 to 104.8% agreement was found. The "greater than 100%" means that more samples produced positive results with the H_2S test than the control test. Agreement rates were 81.8-100% for the Colilert® test carried out by Kromoredjo and Fujioka. Finally, when the H_2S test is compared to the MF test using m-Endo broth, 73.2% agreement is obtained. These numbers show that the H_2S test is a relatively good (>70% on average) surrogate for the standard tests used to identify TC.

When the H_2S test is compared with standard tests to identify FC, the agreement rates ranged from 90 to 94.4% by Grant and Ziel, 111.1% by Castillo et al., and 140% by Ratto et al. Grant and Ziel also found an 80% agreement with *Clostridium perfringens* which are known to be of strong fecal origin. These numbers show that the H_2S test is a very good surrogate (>90% correlation) for the standard test to identify FC. From the previous studies cited above, it appears that the H_2S test is a more sensitive test than other FC tests. The H_2S test is more likely to overestimate the presence of FC than TC. This is also partly due to the greater specificity of the FC group.

Figure 5-4 shows a simple illustration of the relationship between the three groups of indicator organisms developed by the author of this thesis. Rijal and Fujioka (1995) compared the lab-made H_2S test with total and FC tests and obtained similar results: "When total coliform as well as other fecal indicator bacterial counts dropped to zero so did the H_2S bacteria: When the concentrations of TC by the MPN method increased, so did the concentrations of H_2S-producing bacteria." They also found that the rise and fall of H_2S bacteria matched that of FC. However, it was also noticed that the H_2S MPN counts always equaled or exceeded the FC MPN counts in their tests. This showed that FC counts can be overestimated with the H_2S test.

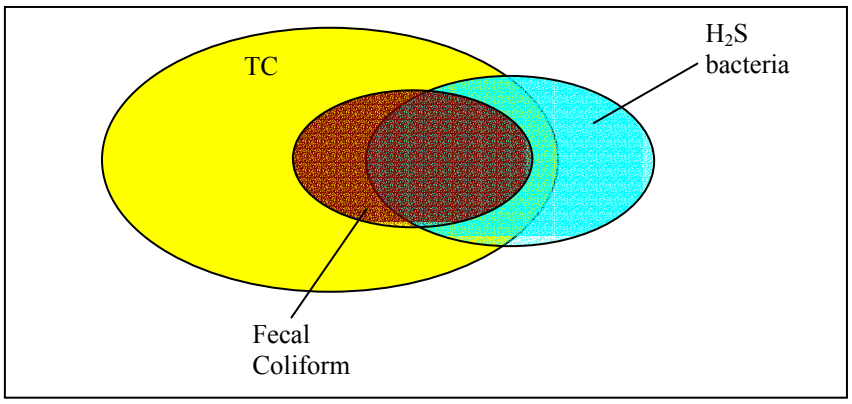

Figure 5-4: Illustration of the relationships between TC, FC, and H_2S bacteria.

5.6 Indicator Organisms Isolated from H_2S Test

In the study by Manja et al., the following H_2S-producing organisms were isolated from drinking water (Refer to Table 5-2).

Table 5-2: H_2S-producing bacteria isolated from drinking water samples (Manja et al., 1982)

Isolated H_2S-Producing Organism	Results	Percentages
Citrobacter freundii	23	62%
Salmonella species	6	16%
Proteus mirabilis	2	5.5%
Arizona species	2	5.5%
Klebsiella species	1	3%
H_2S-producing *E.coli*	3	8%
TOTAL NUMBER OF SAMPLES	37	100%

Although not listed on Table 5-2, the presence of the non-coliform *Clostridium perfringens* will also produce a positive H_2S test (Pillai et al., 1995; Grant and Ziel, 1996). This specie is a H_2S producer and is monitored in some countries as an indicator of water quality (Fujioka and Shizumura, 1985; Sorensen et al., 1989). The H_2S-producing characteristic is also shared by the majority of *Salmonella* spp. (Gawthorne et al., 1996). In fact, *Salmonella* spp. has been found in tropical waters where traditional coliform bacteria are absent (Jimenez et al., 1989; Townsend, 1992). Thirty percent of all *Salmonella* isolations from water occurred in the absence of indicator bacteria in Western Australia (Peterson and Schorsch, 1980). They suggested that these *Salmonella* spp. originated from feces of birds and reptiles which did not contain coliform bacteria. At the same time, since 92% of salmonellae produce H_2S, the H_2S test can be used to

indicate the presence of salmonellae and hence serve as additional indicators to the coliform test (Jay and Davey, 1989; Gawthorne et al., 1996).

It is also found that there is little interference by non-H_2S-producing bacteria in the test. When 10^4 cells of known non-H_2S-producing *E.coli*, *Enterobacter cloacae,* or *Klebsiella pneumonia* were inoculated, no blackening of the medium occurred even after 48 hours of incubation (Grant and Ziel, 1996).

5.7 Sensitivity of H_2S Test

The sensitivity of the H_2S test refers to the minimum number of coliform forming units (CFU) required to produce a positive result per 100 ml of sample. Manja et al. tested with 20 ml samples and found that it takes 8 to 9 TC CFU per 100 ml to produce a positive result. On the other hand, Pillai et al. determined a sensitivity level as low as 1 TC CFU per 100 ml of coliform bacteria. Grant and Ziel tested using 100 ml samples and estimated a sensitivity of about 5 TC CFU per 100 ml. More specifically, they found that in every 100 ml sample, as little as 1 *Salmonella typhimurium,* 2 *Citrobactor freundii,* 2 *Proteus vulgaris*, will produce a positive result with the H_2S test within 40 hours. Therefore, it is suggested that only one or two cells of H_2S-producing bacteria is required to produce a positive reaction with the H_2S test.

Manja et al. also determined that the best agreement between the H_2S test and Standard Methods occurred when total population exceeded 40 CFU per 100 ml. This means that the chances that both methods will produce similar results are greater when the TC count is greater than 40 CFU per 100 ml. Kromoredjo and Fujioka found the best agreement to occur at greater than 16 TC CFU per 100 ml. These findings indicate that when the H_2S test is used to enumerate low bacteria counts (less than 16 TC CFU per 100ml) by the MPN method, one can obtain significantly different (even lesser) coliform counts. For example, in the case when non-coliform *Clostridium perfringens* are present in the sample, it is possible to obtain higher counts from the H_2S test than from the TC test.

Similar to Chapter 4, the author also conducted the H_2S test together with MF tests on TC, FC, and *E.coli.* Assuming a detection limit of 1 CFU per 20 ml, the detection limit is 5 CFU per 100

ml. The HACH PathoScreen medium for 20 ml sample was used for the P/A-H_2S test; both m-Endo and m-ColiBlue24® broth from Millipore were used with the MF test to enumerate TC; m-FC and EC (Escherichia Coli) broth were used for FC; EC with MUG broth was used for *E.coli*. Assuming a detection limit of 1 H_2S bacteria per 20 ml sample, the detection limit is 5 H_2S bacteria per 100 ml sample. A positive P/A-H_2S test was recorded when the sample changed from clear yellow to black in 24 hours at incubation temperatures between 20 and 35ºC (depending on test conditions). MF results were taken after incubation at 35ºC for TC, and 44.5ºC for FC and *E.coli* for 24 hours. A total of 61, 34, and 37 water samples with counts from 0 to greater than 10,000 per 100 ml were taken for TC, FC, and *E.coli* respectively. Histograms of the frequency of positive and negative tests versus the number of these indicator organisms enumerated are plotted in Figure 5-5, Figure 5-6, and Figure 5-7.

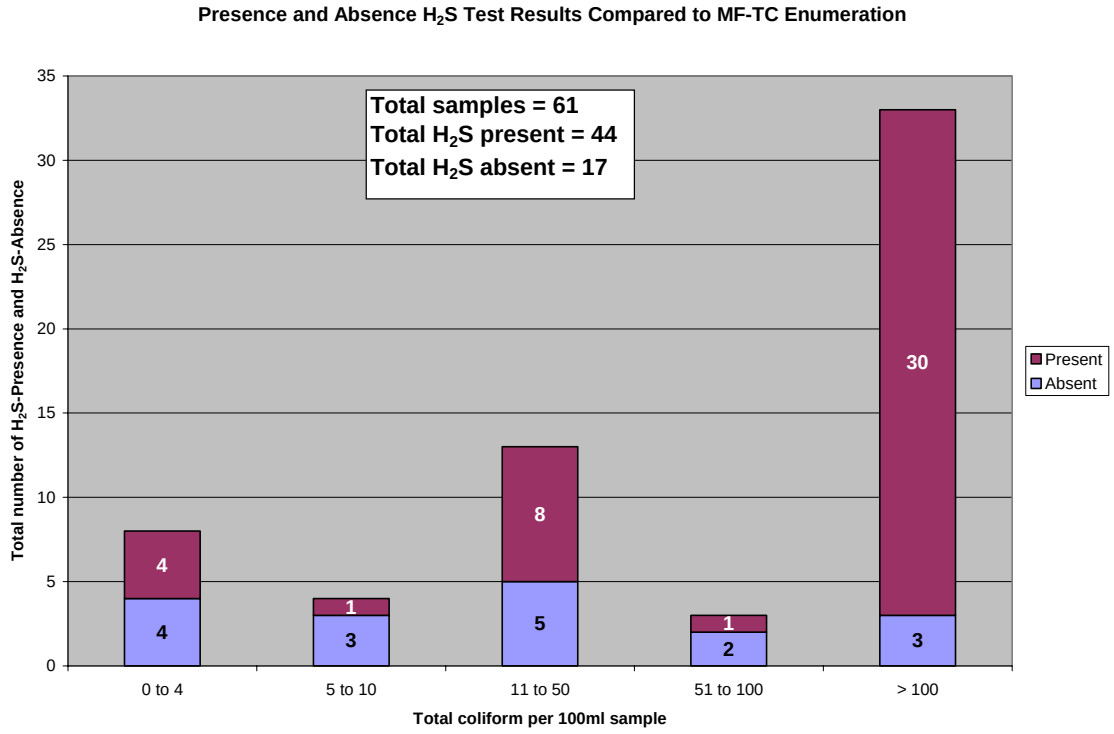

Figure 5-5: Presence and absence H_2S results compared to MF-TC test enumeration.

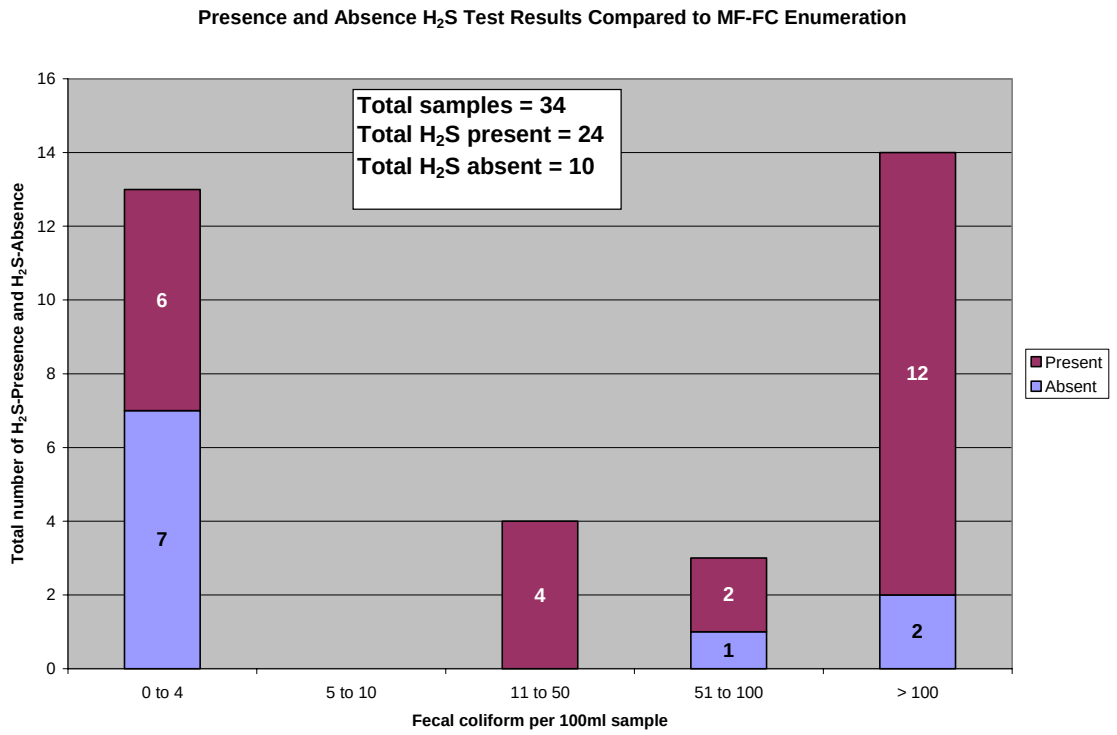

Figure 5-6: Presence and absence H_2S results compared to MF-FC test enumeration.

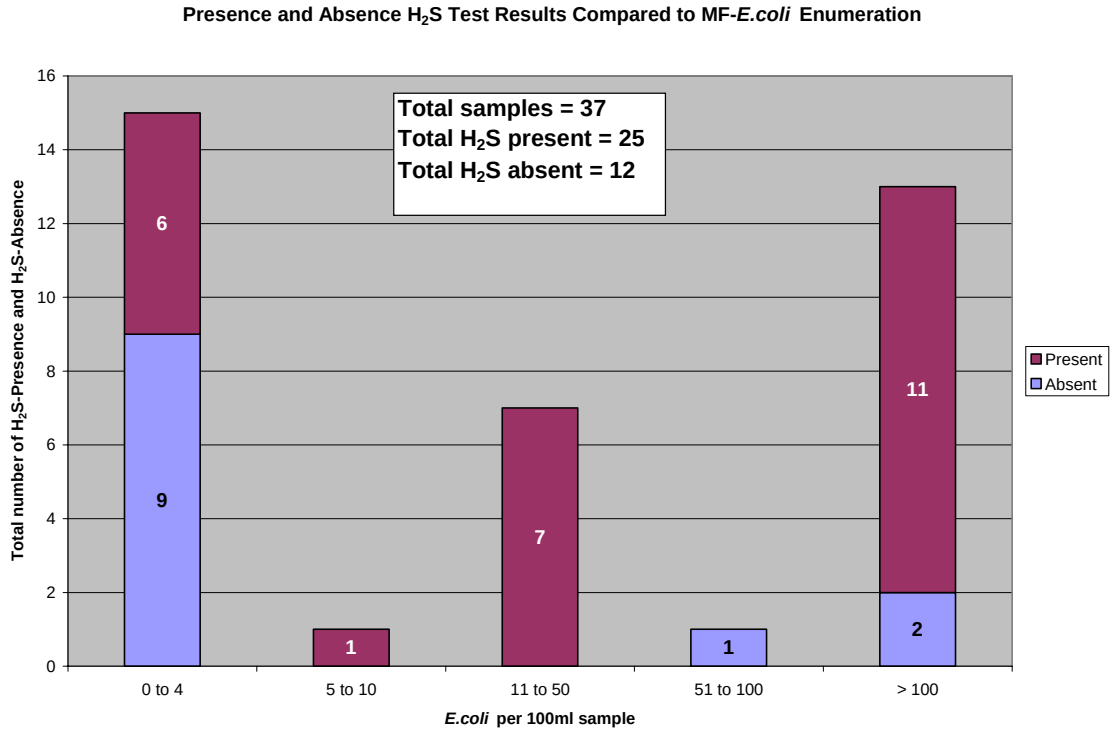

Figure 5-7: Presence and absence H_2S results compared to MF-*E.coli* enumeration.

Of the 61 samples tested for TC, 4 samples showed "Absence" when no TC is detected by the MF test. Forty samples showed "Presence" at greater than 5 CFU per 100 ml. This means that there is a 72% (44 of 61 tests) agreement[8] between the H_2S and MF-TC test, when a detection limit of 5 CFU per 100 ml for the H_2S test is assumed. Similarly, the agreement between the H_2S and MF-FC test is 74% (25 of 34 tests). Finally, the agreement between the H_2S and MF-*E.coli* test is 76% (28 of 37 tests). These agreements show that among the three indicator organisms, the H_2S test best indicates the presence of FC.

From the charts, it is also noted that the proportion of false negatives is greater when the H_2S test is used to indicate the presence of TC than FC or *E.coli.* This can be seen from the greater proportion of "Absence" results when compared to TC than FC or *E.coli* when there is at least 5 CFU. When TC is detected by MF, there are still significant samples showing an "Absence" with the H_2S test. This means that the H_2S test is likely to underestimate the presence of TC with the larger number of false negatives at high TC counts. Twenty-two percent (13 of 58) gives false positives with the TC test at counts greater than 5 CFU per 100ml. On the other hand, only 14% (3 of 21) gives false negatives with the FC test. For *E.coli,* the false negative rate is only 9% (3 of 32). Since *E.coli* produces the lowest rate of false negatives, their presence are most accurately indicated by the H_2S test.

5.8 Effect of Incubation Temperature on H_2S Test

Pillai et al. (1999) studied the effect of temperature on the incubation period required to produce a positive result with the H_2S test using FC. They found that although the method could be used between 20 to 44ºC, temperatures between 28 to 37ºC produced faster results. When the FC concentration was lowered, a corresponding increase in incubation period required was observed. They also noticed that the black color developed only slightly at the bottom during the lower concentrations compared to the whole bottle turning black at higher concentrations. This is also verified by the author of this thesis when he carried out both H_2S and MF-TC test on water samples, as shown in Figure 5-8.

[8] Assuming we define "Absence" in MF test as zero CFU/100ml and "Presence" as ≥5 CFU/100ml, the level of agreement is defined as the number of P/A outcomes which is consistent to the MF outcomes.

Figure 5-8: Left sample was incubated at 35ºC for 24 hours and some black color can be seen at the bottom. MF results show 9 TC per 100ml. Right sample showed a positive H_2S Test with TC exceeding 600 CFU per 100ml.

Pillai et al. demonstrated a trend of shorter incubation periods with increasing incubation temperature. Only 36 hours are required at 37 and 44ºC while 48 hours are required between 22-28ºC when FC counts are greater than 400 CFU per 100ml. When FC counts are as low as 11 CFU, it took 90 hours at 37ºC to show a positive result. No positive results were shown at other incubating temperatures. Figure 5-9 compiles the results of the effect on temperature and FC concentration on incubation period.

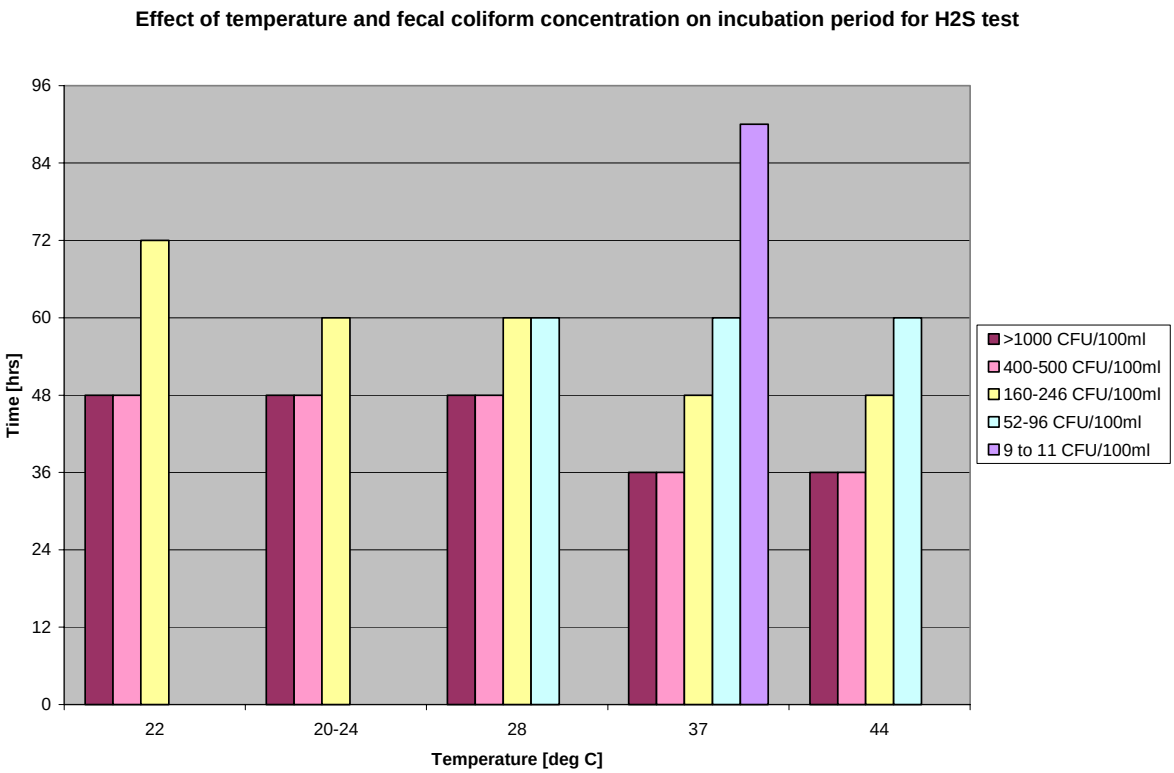

Figure 5-9: Effects of temperature and FC concentration on incubation period (Pillai et al., 1999).

Pillai et al. also found out that the addition of L-cystine improved the detection rate. From their tests, only 18 hours of incubation was required at 37ºC irrespective of the FC concentration. However, at lower and higher temperatures, the incubation period increased as the growth of the H_2S producers slowed down at these other non-optimum incubation temperatures.

Gao (2002) also conducted similar studies when she used P/A-H_2S tests to detect fecal contamination in the waters of tubewells in Butwal, Nepal. For each tubewell, she collected two samples. One was incubated at 37ºC and the other was left at ambient temperature between 15 to 25ºC. Her unpublished results show that compared to all the incubated samples that produced presence results within 24 hours, all the non-incubated samples also produced presence results within 72 hours. Of these presence samples, about 60% of the corresponding non-incubated samples produced presence results within 48 hours.

These studies demonstrated the versatility of the H_2S test in terms of its incubation requirements. For example, the test can still be carried out at less than optimum temperatures and obtain the same result but with a longer incubation period. This is very useful for assessing drinking water quality in households who do not have access to expensive incubators. The test can be easily administered and the results evaluated without specialized training and equipment. More importantly, this test is cheaper than the standard coliform P/A test.

5.9 Summary of H_2S Test

- With a 100 ml sampling volume, the test has an approximate detection limit of 5 H_2S bacteria CFU per 100 ml.
- The H_2S test agrees best with the presence of *E.coli* and also produces the lowest rate of false negatives with *E.coli*. It also performs reasonably well when compared with FC.
- The P/A-H_2S test is a simple and versatile test that can be carried out in the field within a broad range of incubation temperatures (or no incubation at all depending on ambient temperature). Therefore, this test is recommended for the routine monitoring of water for recent fecal contamination in the field where technical expertise and incubation equipment are not readily available.

Chapter 6 : MEMBRANE FILTRATION INDICATOR TEST

6.1 Methods of Microbial Enumeration

The Membrane Filtration (MF) technique was developed to offer the bacteriologist a quicker and easier method over the Multiple Tube Fermentation (MTF) technique to enumerate coliforms for the assessment of drinking water quality. The MF method is developed based on the metabolic definition of coliforms i.e. to detect and enumerate the presence of coliforms from their production of acid during the fermentation of lactose. Newer detection methods based on the enzymatic behavior of coliforms have also been developed to detect the presence of coliforms and *E.coli.*

6.2 How Membrane Filtration Works

Colonies are the individual "dots" that grow on surfaces of membranes. A colony is formed with the accumulation of the same type of bacteria that have grown dense enough to be seen with the eye. A single colony is not a single bacterium. Instead, it can contain millions and millions of individual and identical bacteria (Lisle, 1993). It is presumed that every colony begins with a single bacterium or so-called "colony forming unit" (CFU). The bacterium will start to grow and divide, making a clone of itself. The incubation period (e.g. 22 to 24 hours for TC) is required to allow for enough bacteria to grow and become dense enough to see. Also, since every bacterium in the colony is a clone of the original bacterium, it can be assumed that all bacteria in that colony are identical, assuming no other colony is touching it.

The MF membrane has uniformly sized holes or pores of diameter 0.45 μm. This pore size is slightly smaller than the diameter of a typical TC or other bacteria of interest. As the water sample is drawn through the filter by a vacuum pump, the water passes through the pores, but the TC and anything larger in size than 0.45 μm are caught on the surface or trapped in the pores of the membrane. The membrane filter is then removed, saturated with a specific culture medium and these bacteria are supplied with the necessary nutrients and moisture for growth.

6.3 Advantages of Membrane Filtration over Multiple Tube Fermentation Method

The advantages of the membrane filtration (MF) method over the traditional multiple-tube fermentation (MTF) method whose results are interpreted using Most Probably Number (MPN) method are summarized below (Grabow and Du Preez, 1979; Rompré et al., 2001):

- Gives more accurate results within 16 to 24 hours instead of 48 to 96 hours for MPN;
- Gives a direct count, whereas MPN evaluations are based on statistical estimates;
- Colonies can easily be picked from membranes for further identification;
- Larger volumes of water can be tested, thus improving sensitivity and reliability;
- *Clostridium perfringens* and coliphages may interfere with MPN evaluations;
- MF petri dishes take up less incubator space than MPN tubes;
- MF technique is relatively simple to carry out;
- MF may be conveniently applied in field conditions.

6.4 Methodology of MF Test

The MF test is significantly more complex than the P/A test discussed in previous chapters. There are more steps and many precautions are needed to ensure that external contamination of samples is avoided. Therefore, in the following description of the MF methodology, 10 steps are identified and then elaborated upon, as required.

Instruments used: Millipore portable MF setup, culture medium (e.g. m-Coliblue24®), Oxford pipette, candle, lighter, tweezers, incubator (See Figure 6-1 and Figure 6-2 for the test setup.).

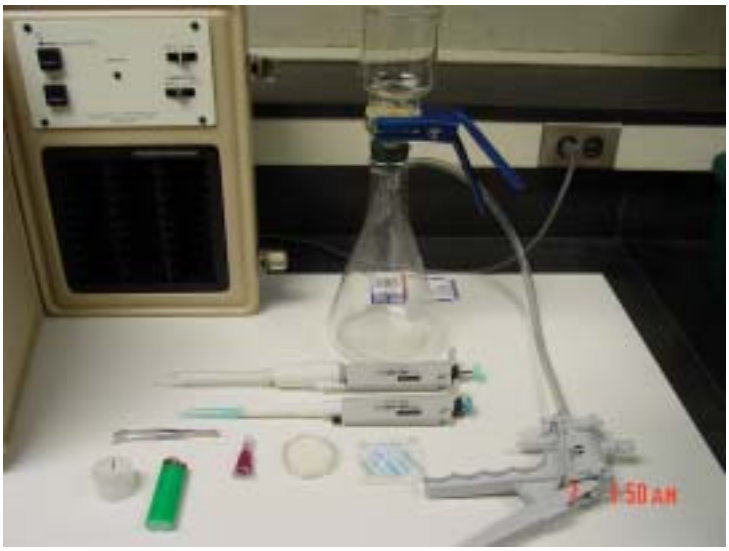

Figure 6-1: Millipore glass MF setup with Millipore incubator on the left.

Figure 6-2: Portable Millipore stainless filter holder.

Procedures:

1. Sterilize the portable Millipore MF stainless steel filter holder for 15 minutes.

Ideally, the portable MF stainless steel filter holder (shown in Figure 6-2) should be sterilized in between every water sample. However, this can become very time-consuming if a large number of samples are to be tested. Sterilization of the portable MF filter holder takes 15 minutes, but the sterilization of the glass kit (shown in Figure 6-1) can take up to an hour in the air oven. Therefore, to save time, when the author tested different water samples at various dilutions, the portable MF stainless steel filter holder was only sterilized in between water samples and not between dilutions. To minimize cross-contamination among dilutions of the same sample, the more dilute (in terms of coliform concentration) sample was filtered followed by the less dilute sample. This was especially important with non-potable water samples with high number of indicator bacteria present. Sufficient sterile rinse water is also used to rinse the funnel in between filtrations to flush away residue in the funnel. Standard Methods (1998) also suggested that a sterile blank be inserted after filtration of 10 samples to check for possible cross-contamination. This suggestion was also followed by the author.

2. Label petri dish and pour m-ColiBlue24® medium onto absorbent pad. Decant extra medium.

 When using culture medium pre-packaged in 2 ml plastic ampules (from Millipore), the medium is simply poured into the petri dish and the excess decanted. When pouring the medium, it is important to ensure every surface of the absorbent pad is uniformly soaked. The medium is decanted by tilting the petri dish and pouring away the excess, leaving behind about one drop at the bottom. The petri dish should not be shaken when decanting.

If the culture medium is self-prepared, there is a need to verify the new batch against a previously acceptable lot for satisfactory performance before use. Blank tests should always be carried out first. For the first few tests, parallel tests using the previous and new batch should be conducted to cross check their recoveries.

3. Flush about 30 ml of distilled water through filter once.
4. Place 0.45 µm filter paper on the filter support base using sterile tweezers.

 Millipore carried out a study on the effect of membrane filter pore size on microbial recovery and colony morphology (Millipore, 2002). While Millipore recommended the use of 0.7 µm pore size for the recovery of FC colonies, Millipore's study confirmed that both the 0.7 µm and the standard 0.45 µm pore size filters gave the most consistent recoveries for TC colonies (> 90%) during filtration. These recovery results were compared to controls using spread plates. The larger pore size filters can also be used for difficult-to-filter samples e.g. high turbidity, or where larger sample volumes are needed. However, for most practical purposes when testing water samples in Nepal, the 0.45 µm pore size can be used for the recovery of TC, FC and *E.coli.*

5. Pipette specified volume of sample into funnel. Move the whole apparatus in a swirling motion to stir the sample.

 For drinking water samples, standard 100 ml volumes are used. For contaminated water supplies, smaller volumes may be used in order to yield 20 to 80 TC colonies (20 to 60 FC colonies) for easy counting and to prevent overcrowding on the filter paper. When less than 10

ml of sample (diluted or undiluted) is to be filtered, approximately 10 ml of sterile dilution water is added to the funnel before sample addition and the entire dilution is filtered (Standard Methods, 1998) (See next section on dilutions).

6. Run filtration.
7. Rinse funnel with about 30ml of distilled water twice.

 With filter still in place, the interior surface of the funnel is rinsed by filtering twice 30 ml portions of sterile dilution water. Rinsing between samples prevents carry-over contamination.

8. Remove filter carefully with sterilized tweezers and place filter into petri dish in a rolling motion.

 The filter paper is placed onto the absorbent pad in a rolling motion to prevent the trapping of air bubbles. The air bubbles may prevent the absorbing of media to the top of the filter paper, therefore resulting in the uneven growth of colonies.

9. Invert petri dish and place into incubator set at 35°C for 24 hours.

 The petri dish is inverted to prevent condensation from dripping down onto the membrane filters and disturbing the growth of the colonies.

10. Count number of coliform forming units (CFU) under magnifying glass and express as CFU/100ml.

6.5 Sampling Volumes for TC/FC/E.coli Tests

Sample volume is generally governed by bacterial density. An ideal sample volume for TC testing yields approximately 20 to 80 coliform colonies, and not more than 200 colonies of all types per filter (Standard Methods, 1998). For FC testing, a sample volume producing 20 to 60 coliform colonies is ideal (Standard Methods, 1998). When filtering samples where the coliform number is uncertain, three different volumes should be used. There is, however, no specified rule on the volumes that should be tested. Instead, the researcher should select a range of

volumes that he or she thinks would yield the ideal range of coliform colonies for easy enumeration. For example, when the author was sampling drinking water sources in Kathmandu, he chose 5, 10, 20 ml for enumerating TC and 10, 20, 50 ml volumes for enumerating FC and *E.coli.* When the sample volume is less than 10 ml, 10 ml of sterile dilution water is added to the filter funnel before filtration. This increase in water volume aids in the uniform dispersion of the bacterial suspension over the entire effective filtering surface. Table 6-1 and Table 6-2 show the suggested sample volumes for MF tests of TC and FC for various water source types.

Table 6-1: Suggested sample volumes for MF-TC test (Standard Methods, 1998).

	Volume (☼) To Be Filtered (ml)							
Water Source	**100**	**50**	**10**	**1**	**0.1**	**0.01**	**0.001**	**0.0001**
Drinking water	☼							
Swimming pools	☼							
Wells, springs	☼	☼	☼					
Lakes, reservoirs	☼	☼	☼					
Water supply intake			☼	☼	☼			
Bathing beaches			☼	☼	☼			
River water				☼	☼	☼	☼	
Chlorinated sewage				☼	☼	☼		
Raw sewage					☼	☼	☼	☼

Table 6-2: Suggested sample volumes for MF-FC test (HACH, 2001).

	Volume (◙) To Be Filtered (ml)						
Water Source	**100**	**50**	**10**	**1**	**0.1**	**0.01**	**0.001**
Lakes, reservoirs	◙	◙					
Wells, springs	◙	◙					
Water supply intake		◙	◙	◙			
Natural bathing waters		◙	◙	◙			
Sewage treatment plant, secondary effluent		◙	◙	◙			
Farm ponds, rivers				◙	◙	◙	
Storm water run-off				◙	◙	◙	
Raw municipal sewage					◙	◙	◙
Feedlot run-off					◙	◙	◙

For very small sample volumes (<1 ml), a series of dilutions should be carried out for better accuracy. For a 1:10 (0.1 ml) dilution, 1 ml sample is added to 9 ml sterile buffer and thoroughly mixed. 1 ml of the mixture is then filtered. Similarly, for a 1:100 (0.01 ml) dilution, 1 ml of

sample is mixed with 99 ml sterile buffer and 1 ml of the mixture is filtered. If further dilutions are required, the mixture can be further diluted by repeating the same procedures.

6.6 Classical Metabolic Methods of Coliform Detection

Classical culture methods using MF are generally based on metabolic reactions of the coliform bacteria. These methods are developed from the metabolic rather than from taxonomic definitions of the coliform bacteria. Bacteria that produce a red colony with metallic (gold or green) sheen within 24 hours incubation at 35±0.5ºC on an Endo-type medium are considered members of the coliform group according to this definition (Standard Methods, 1998). The sheen area may vary in size from partial to complete coverage of the colony surface. Atypical colonies which are dark red, mucoid or nucleated and without a metallic sheen may occasionally appear (Rompré et al., 2001). These colonies should also be counted as coliforms (Standard Methods, 1998). Generally pink, blue, white, or colorless colonies lacking sheen are considered non-coliforms and should not be counted. It is also shown that the total colony count on the m-Endo medium has no consistent relationship to the total number of bacteria present in the sample (Standard Methods, 1998).

It should be noted that most literature that describes the inadequacy of the MF test is associated with the use of the m-Endo medium to enumerate TC. The enumeration of TC by MF with m-Endo medium is not totally specific; for example, typical colonies with a metallic sheen may also be produced occasionally by non-coliform organisms (Grabow and Du Preez, 1979). Conversely, atypical colonies which may be missed during counting may be coliforms. *Aeromonas* shares many characteristics with the coliform species and can inflate TC densities. This will give a false indication of water quality or produce false positive results. Of all positive confirmed TC tests, as many as 9 to 58% can be attributed to *Aeromonas* (Clark et al., 1982; Grabow and Du Preez, 1979; Katamay, 1990). When the m-FC medium is used to isolate thermotolerant coliforms, it is important to adhere to the narrow range (±0.2ºC) of incubation temperature of 44.5ºC. This is because as little as 44 – 0.2ºC will yield a much higher percentage of certain non-fecal *Klebsiella* sp., thus giving rise to false positive results (Katamay, 1990). As little as 45 + 0.2ºC will inhibit the growth of many strains of *E.coli*, giving rise to possible false negative results (Katamay, 1990). Therefore, it is assumed that some "safety

factor" is taken into account in the narrower specified range of ±0.2ºC. At the same time, 10% of *E.coli* are anaerogenic, and gas variability has been reported (Meadows et al., 1980). About 15% of *Klebsiella pneumoniae* can be found in pristine sites and not of fecal origin, are thermotolerant and will produce a positive fecal-coliform test (Meadows et al., 1980).

Presence of high numbers of background heterotrophic bacteria can decrease coliform recovery by MF. The presence of excessive numbers of non-coliform bacteria can influence the growth rate of TC on the culture media and inhibit the development of the diagnostic green-to-golden metallic sheen with the m-Endo broth (Lisle, 1993). This could lead to an underestimation of the TC count in a water sample. That is why Standard Methods states that if the background count exceeds 200 colonies per 100 ml, the results should be reported as "too numerous to count" (TNTC). Geidreich et al. (1978) and Clark (1980) inferred that when the Heterotrophic Plate Count (HPC) exceeds 500 per ml of sample, interference with coliform detection on the membrane will take place. Burlingame et al. (1983) further investigated the effect of non-coliform bacteria presence on the detection of coliforms using the m-Endo medium. Four types of non-coliform bacteria, *Pseudomonas aeruginosa, Aeromonas hydrophila, Bacillus* sp., and *Flavobacterium* sp. were introduced at different concentrations to a water sample to study their interference effect on coliform enumeration. They showed that *P. aeruginosa* can reduce coliform counts at levels as low as 30 per ml and eliminated coliform detection at levels of about 400 per ml. *A. hydrophila* reduced coliform counts at only 2 per ml and eliminated coliform detection at about 10 per ml. Many of these HPC organisms do not produce colonies on the membrane filter with m-Endo medium (Burlingame et al., 1983). *Bacillus* sp. and *Flavobacterium* sp. did not have any effect on coliform counts even when they were added at densities greater than 1000 per ml. These results show that some HPC organisms can interfere with coliform colony sheen production at densities considerably lower than what was previously determined (Burlingame et al., 1983). Therefore, these studies demonstrate that the presence of a high HPC count may signify the presence of antagonistic non-coliform bacteria which inhibit the growth of coliform bacteria on the membrane filter, thus underestimating the coliform density.

Water samples of high turbidity can interfere with the development of distinct, isolated colonies on the membrane (Lisle, 1993). Bacteria prefer to grow on solid surfaces, thus turbidity particles

lying close to each other on the membrane can provide a surface for them to spread on. If the turbidity is high enough, the growth of both desired coliform and undesired background organisms will cover the entire membrane instead of forming distinct isolated colonies. This causes the formation of colonies that are 'joined' together, which makes counting difficult. Together with high HPC count, turbid samples can lead to possible false negative outcomes.

In general, only a small portion (0.1 to 15%) of the total bacterial population can be enumerated by cultivation-based methods (Amann et al., 1990). In addition, MF test is unable to recover stressed or injured coliforms (Rompré et al., 2001). Drinking water treatment, disinfection, and stress induced to the coliforms during their collection and transfer to the petri dish, can injure the coliforms. Not all coliforms are able to survive the change in their living environment. When these injured coliforms are filtered from the water sample, they often die off or are unable to grow and multiply to form a positive coliform colony. This can result in an underestimate of the TC count.

As a consequence of these shortcomings of the classical MF method, a large number of modified media is currently in use e.g. m-Endo in North America (Standard Methods, 1998) and Singapore (Teper, 2002), Tergitol-TTC (Triphenyltetrazoliumchloride) medium in Europe (AFNOR, 1990), MacConkey agar in South Africa, and Teepol in Britain (Rompré et al., 2001). No universal medium currently exists which allows optimal enumeration of various coliform species originating from different environments and present in a wide variety of physiological states.

6.7 Enzymatic Methods of Coliform Detection

The previous discussion demonstrates many limitations of the classical methods of coliform detection based on their metabolic behavior. The two most important limitations are its lack of specificity and interference by background heterotrophic bacteria. An alternative method of coliform detection called the "Defined Substrate Technology (DST)" was patented by IDEXX with Colilert® in 1993 to detect the presence of TC and *E.coli.*

DST uses a defined substrate as a vital nutrient source for the target microbe. In this case, the two targeted microorganisms are TC and *E.coli*. Only the target microbes are fed and no other substrates are provided for other bacteria. During the process of substrate utilization, a chromogen[9] or a fluorochrome[10] is released from the defined substrate thus indicating the presence of target microbes (Edberg and Edberg, 1988; Rompré et al., 2001). Specifically, two substrate nutrient-indicators, o-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG) are used in DST. ONPG is metabolized by the coliform enzyme β-galactosidase changing from colorless to yellow. MUG is metabolized by the *E.coli* enzyme β-glucuronidase to create fluorescence. Since most non-coliforms do not have these enzymes, they are unable to grow and interfere. This greatly improves the specificity of the DST compared to the past methods based on coliform metabolism.

These enzymatic reactions are rapid and sensitive. They can yield results in 4 to 24 hours and have a detection limit of 1 CFU per 100 ml for TC and *E.coli* (Edberg and Edberg, 1988). DST was first introduced as a P/A test, and developed into a Most Probable Number test. For example, the Colilert® test comes in both P/A and MPN format. The standard Colilert® test yields results in 24 hours and the Colilert®-18 gives results in 18 hours, both at an incubation temperature of 35±0.5ºC. These tests detect both TC and *E.coli* simultaneously in a single medium. Because of the specificity of this test, no confirmation test is required, thus bypassing the need for a time-consuming presumptive step followed by an isolation confirmed step. In a performance evaluation, β-D-glucuronidase-positive reactions were observed in 94 to 96% of the *E.coli* isolates tested (Kilian and Bulow, 1976; Feng and Hartman, 1982; Edberg and Kontnick, 1986). The reagents can also be stored for up to 12 months without the need for refrigeration.

Katamay et al. (1990) performed a study comparing the DST with MF and found them to be in agreement (r^2 of 0.93 by linear regression). There was no effect of non-coliform heterotrophs on the ability of the DST test to enumerate TC and *E.coli.* Several other authors have also developed agar media based on DST to enumerate TC and *E.coli.* Brenner et al. (1993)

[9] Chromogen is a substance that is released during enzymatic action and indicate a color change.
[10] Flurochrome is a substance that is released during enzymatic action and cause fluorescent under long-wave UV light.

developed the MI agar medium, containing the fluorogenic MUG and the choromogenic indoxyl-β-D-glucuronide (IBDG) to simultaneously detect TC and *E.coli* in waters. The method was shown to be sensitive, selective, specific and rapid (available in 24 hours) (Brenner et al., 1996). Gaudet et al. (1996) and Ciebin et al. (1995) associated MUG with classical m-TEC, and 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Glu) developed by Watkins et al. (1988) with classical lauryl tryptose agar and found similar or higher recovery of TC and *E.coli.*

There are a few limitations of DST. First, DST is a direct test for TC and *E.coli* but it should not be used as a confirmed test (Katamay, 1990). A direct test means that the target organism is detected directly in a single medium, thus by-passing the need for an isolation procedure prior to confirmation. DST, however, cannot be used to confirm coliforms and *E.coli* that are isolated from a positive presumptive test. Second, if *Aeromonas hydrophila* are present in concentrations greater than 20,000 per ml, a false positive may result (Edberg, 1989). Third, DST should not be incubated longer than 28 hours. If the test shows positive results after 28 hours, it should be voided (Katamay, 1990).

6.8 Modified Membrane Filtration Culture Media for Total Coliform

Commercial agar media currently available includes classical agar media modified with specific chromogenic and/or fluorogenic substrates for the detection of β-D-glucuronidase and/or β-D-galactosidase. They include the Chromocult® Coliform Agar (Merck, Germany) and m-ColiBlue24® broth (HACH, USA). The Chromocult® Coliform Agar requires incubation at 35 to 37ºC and the m-ColiBlue24® broth requires incubation at 35ºC.

Chromocult® contains the chromogenic Salmon-GAL substrate which is cleaved by β-D-galactosidase produced by coliforms to form a salmon to red color of the coliform colonies. For *E.coli* detection, *E.coli* cleaves both Salmon-GAL and chromogenic X-glucuronide to produce a dark-blue to violet colonies which are easily distinguishable from other coliform colonies (Merck, 2000). Chromocult® also contains Tergitol® 7 which inhibits the growth of Gram-positive bacteria as well as some selected Gram-negative bacteria.

m-ColiBlue24® is a lactose-based medium, containing inhibitors to selectively eliminate growth of non-coliforms. The TC colonies are highlighted by a non-selective dye, 2,3,5-Triphenyltetrazoliumchloride (TTC) which produces red-colored colonies. The *E.coli* colonies on the other hand, will show up as blue colonies, resulting from the action of a β-glucuronidase enzyme on 5-Bromo-4-Chloro-3-Indolyl-β-D-glucuronide (BCIG, also has the commercial name "X-Glu"). According to studies (Lupo et al., 1977; Covert et al., 1989; Edberg et al., 1988; Jacobs et al., 1986;; Sartory and Howard, 1992; Brenner et al., 1993; Cenci et al., 1993;), the performance of the m-ColiBlue24® is better than m-Endo for the detection of TC and it is especially good for the detection of *E.coli.* See Table 6-3 for a performance summary of the m-ColiBlue24® medium.

Table 6-3: Performance summary of tests carried out with m-ColiBlue24® medium on TC and *E.coli* recovery (HACH, 1999).

		Reference Positive	**Reference Negative**	**Total**		
m-ColiBlue24	Positive	234	6	240	Sensitivity[1]	234/234 = 100.0%
(*E.coli)*	Negative	0	250	250	Specificity[2]	250/256 = 97.7%
	Total	234	256	490	False Positive Error[3]	6/240 = 2.5%
					Undetected Target Error[4]	0/234 = 0%
					Overall Agreement[5]	(234+250)/490= 98.8%
		Reference Positive	**Reference Negative**	**Total**		
m-ColiBlue24	Positive	183	67	250	Sensitivity[1]	183/185 = 98.9%
(TC)	Negative	2	248	250	Specificity[2]	248/315 = 78.7%
	Total	185	315	300	False Positive Error[3]	67/250 = 26.8%
					Undetected Target Error[4]	2/185 = 1.1%
					Overall Agreement[5]	(183+248)/500= 86.2%
		Reference Positive	**Reference Negative**	**Total**		
m-Endo	Positive	149	61	210	Sensitivity[1]	149/154 = 96.8%
(TC)	Negative	5	245	250	Specificity[2]	245/306 = 80.1%
	Total	154	306	460	False Positive Error[3]	61/210 = 29.0%
					Undetected Target Error[4]	5/154 = 3.2%
					Overall Agreement[5]	(149+245)/460= 85.7%

[1]Sensitivity = Ref (+) ∩[11] Test (+) / Reference Total
[2]Specificity = Ref (-) ∩ Test (-) / Reference Total
[3]False Positive Error = Ref (-) ∩ Test (+)/ Reference Total
[4]Undetected Target Error = Ref (+) ∩ Test (-) / Reference Total
[5] Overall Agreement = (Sensitivity samples + Specificity samples) / Total samples

[11] ∩ refers to the cell intersected with e.g. Look down the Reference Positive column and across the Test Positive Row.

There is no mention of the reference test that was used.

From Table 6-3, for the detection of *E.coli,* m-ColiBlue24® has a very low false positive error of 2.5% and a zero undetected target error when compared to the reference methods. Overall agreement between the m-ColiBlue24® and *E.coli* reference methods was 98.8% for *E.coli* recovery. For TC, while m-ColiBlue24® has a relatively high false positive error of 26.8% and undetected target error of 1.1%, both percentages are still lower than the m-Endo percentages of 29.0% and 3.2% respectively. Overall agreement for TC recovery was 86.2% for m-ColiBlue24® and 85.7% with m-Endo. These results show that the m-ColiBlue24® is a good medium to detect *E.coli* and it also shows an improvement over the traditional m-Endo broth when used to detect total coliforms.

6.9 Selecting Culture Media for Different Indicator Organisms

During the past nine months of this project, the author primarily used two different types of culture media to enumerate TC: m-Endo medium, and m-ColiBlue24® medium. The m-Endo broth is a standard culture medium stated in the Standard Methods for TC whereas the m-ColiBlue24® broth is a relatively new broth used to detect both TC and *E.coli* simultaneously. The author also had the opportunity to use Merck's Chromocult® when working in the ENPHO lab in Kathmandu. For FC enumeration, m-FC medium and EC medium were used. Finally, for *E.coli,* m-ColiBlue24® medium and EC with MUG medium were used. For TC enumeration, the samples were incubated at 35±0.5ºC for 22 to 24 hours. For FC and *E.coli* enumeration, the samples were incubated at 44.5±0.2ºC for 24 hours. These media (except the Chromocult®) are used with the disposable plastic 47 mm petri dish costing US$0.26 each (Millipore, 2002), and 0.45 μm or 0.7 μm white gridded S-Pak filters costing US$0.42 each (Millipore, 2002).

The objective of this next section is to compare the various media and recommend the best medium to use in the field for each indicator organism. The three criteria that are used for assessment are:

1) Ease of result interpretation;
2) Cost;
3) Ease of media preparation.

6.10 Total Coliform Media – m-Endo, m-ColiBlue24®, Chromocult®

The color and appearance of the colonies that show up with the m-Endo, m-ColiBlue24®, Chromocult® broth after incubation at 35±0.5ºC for 22 to 24 hours can be summarized in Table 6-4 below:

Table 6-4: Different coliform colony colors with different culture media.

	Colony Color	**Target Organism**
m-Endo	Dark Red with/without metallic sheen	TC
	Pink, blue, white or colorless	Non-coliform
m-ColiBlue24®	Red and blue	TC
	Blue	*E.coli*
Chromocult®	Salmon to red	TC
	Dark blue to violet	*E.coli*
	Light green	Other strains of *Shigella*, *Yersinia*, *Salmonella*
	Colorless	Other gram negative bacteria

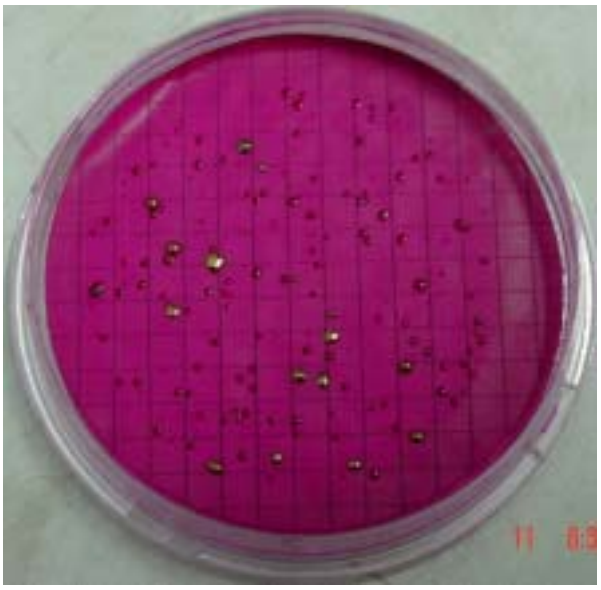

Figure 6-3: m-Endo medium showing dark red coliform colonies with metallic sheen.

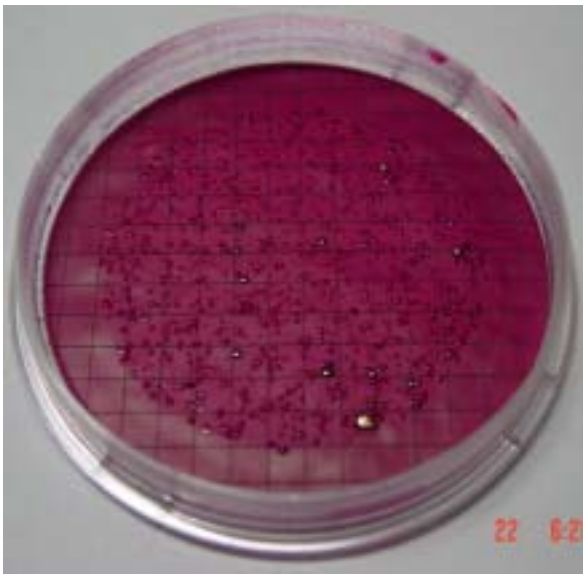

Figure 6-4: m-Endo medium showing a few coliform colonies with metallic sheen, but also with many background colonies which makes counting difficult.

Figure 6-5: Plastic ampules are pre-packed with 2 ml of (from left to right) m-Endo, m-ColiBlue24®, m-FC media from Millipore.

Figure 6-3 and Figure 6-4 show how a typical TC colony would show up on the m-Endo medium. Apparently, the colonies that are either dark red or have a metallic sheen do not show up very clearly. Some colonies may present a whole range of the color red. Very often, the analyst has to judge carefully before deciding if a particular colony is TC or not. The presence of a high number of background colonies also makes the counting and differentiation of coliform colonies difficult and tedious with m-Endo medium. Figure 6-5 shows the pre-packed plastic ampules containing 2 ml of culture media for use with MF. Each m-Endo ampule or test costs US$0.74 (HACH, 2002) to US$1.02 (Millipore, 2002) and requires no media preparation.

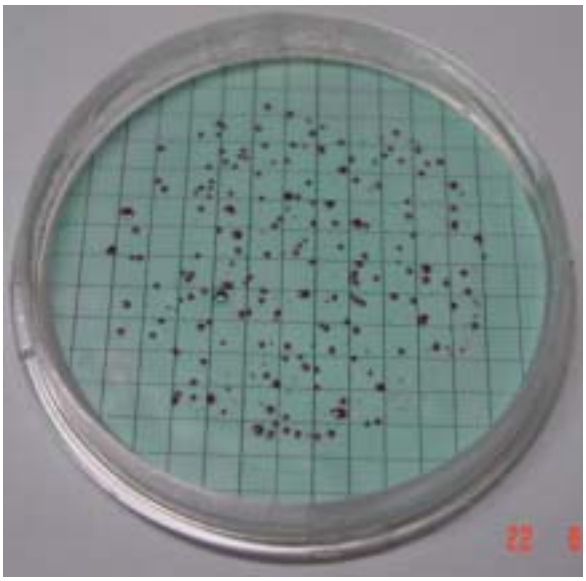

Figure 6-6: m-ColiBlue24® medium showing coliform colonies as red colonies and *E.coli* (only one *E.coli* colony) as blue colonies.

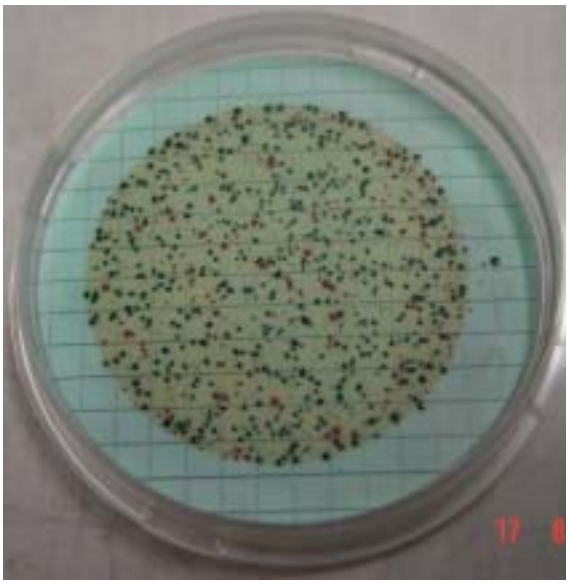

Figure 6-7: m-ColiBlue24® medium showing a sample crowded with blue colonies (*E.coli*) and red colonies (TC). Despite the overcrowding, the colonies still show up distinctly which makes counting possible. Brown background is a result of a high iron content in the water sample.

Figure 6-6 and Figure 6-7 show how a typical TC and *E.coli* colony would show up on the m-ColiBlue24® media. Most of the TC and *E.coli* colonies show up as distinct red and blue colonies, even when the filter paper is overcrowded with colonies. There is little interference growth of other non-identifiable colonies. The author encountered no any other color colonies other than the specified blue and red colonies. This makes differentiation and counting of the colonies easier and more certain. The m-ColiBlue24® medium comes pre-packed in plastic

ampules as shown in Figure 6-5. Each ampule or test cost US$1.50 (HACH, 2002) to US$1.70 (Millipore, 2002) and requires no media preparation.

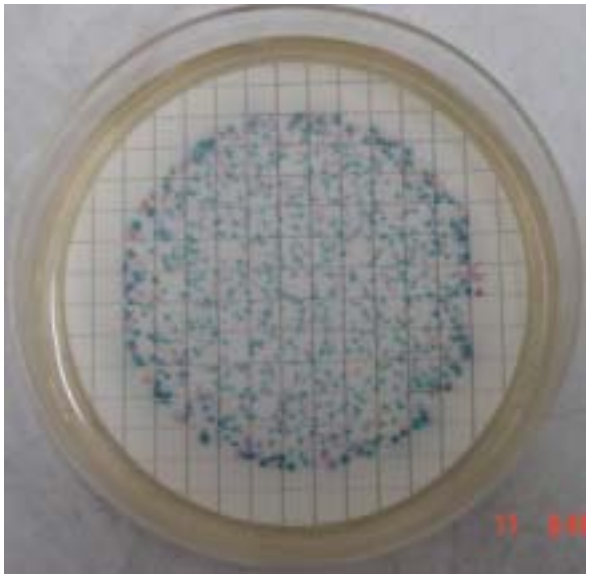

Figure 6-8: Chromocult® agar medium showing coliform as salmon pink colonies and *E.coli* as blue colonies (overcrowding).

Figure 6-8 shows how a typical TC and *E.coli* colony would show up on the Chromocult® agar medium. Most of the *E.coli* colonies show up as distinct blue colonies but the TC colonies are not as distinct as with the m-ColiBlue24® media. Frequently, there are some very light pink (near colorless) colonies which may or may not be considered as coliforms. Chromocult® comes in 100g or 500g of agar from Merck which costs US$345 per 500g bottle (VWR, 2002) or US$108.50 per 100g bottle. According to Merck's preparation instructions, 26.5g is suspended in every liter of demineralized water by heating in a boiling water bath. Assuming about 55 ml[12] of prepared solution is used per plate; this would translate to a cost of about US$1.01 to US$1.58 per sample (Merck, 2002) for culture medium cost alone. Although the 500g bottle is about one-third cheaper than m-ColiBlue24® on a unit cost basis, it is only practical if a large number of samples are tested in a relatively short time. This is because the powdered medium typically only has a 12-month shelf-life after the bottle is opened. The Chromocult® also uses a different type of petri dish that is 100 mm in diameter. It costs US$0.25 each (Hach, 2002).

[12] Assume using 10cm petri dish, pour to 0.7 cm thick, will give a volume of π*5^2*0.7 = 55.0 cm^3.

Table 6-5: Summary of TC culture media in terms of cost, ease of result interpretation, and medium preparation.

TC Culture Medium	Medium Cost Per Sample	Total Cost Per Sample	Ease of Interpretation	Ease of Preparation
m-Endo (pre-packed)	US$0.74 (HACH, 2002)	US$1.42	Difficult	Easy (None)
m-ColiBlue24® (pre-packed)	US$1.50 (HACH, 2002)	US$2.18	Easy	Easy (None)
Chromocult (self-prepared)	US$1.58 using 100g (VWR, 2002) US$1.01 using 500g (VWR, 2002)	US$2.25 US$1.68	Medium	Medium

Considering all three criteria in Table 6-5, it is recommended that m-ColiBlue24® is the best medium to use out of the three described because TC (and *E.coli*) colonies show up the most distinctly. It only costs a little more than Chromocult®, and require no media preparation.

6.11 Fecal Coliform Media – m-FC with rosalic acid, EC

For the enumeration of FC using MF, the m-FC with rosalic acid medium and EC medium are compared (See Figure 6-9 and Figure 6-10 below).

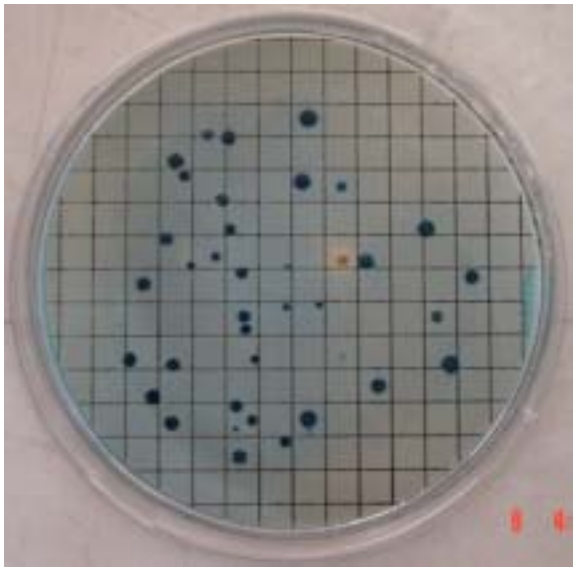

Figure 6-9: m-FC with rosalic acid medium showing FC as distinctive blue colonies with little interference.

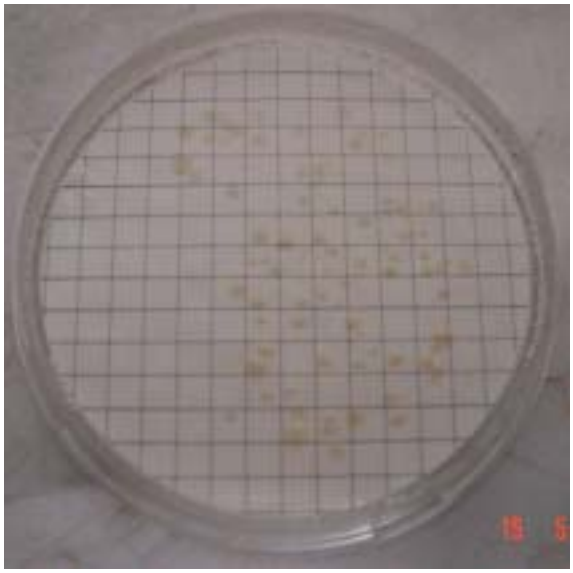

Figure 6-10: EC medium showing FC as cream colored colonies that are less distinctive compared to the m-FC medium.

Figure 6-9 shows the distinctive dark blue FC colonies on the m-FC with rosalic acid medium after incubation at 44.5±0.2ºC for 24 hours. The addition of rosalic acid helps to reduce

background microorganisms (Millipore, 2002). Sometimes, non-FC may show up as cream or grey colonies. Throughout the author's laboratory work, only one sample produced this kind of cream or grey colored colonies out of 34 total samples tested. The m-FC with rosalic acid medium comes pre-packed in 2 ml plastic ampules (as shown in Figure 6-5) and costs US$0.83 (HACH, 2002) to US$1.02 (Millipore, 2002). These colonies were not counted as FC. The m-FC medium can also be self-prepared by mixing 3.7 g of the medium in a 100 ml of purified water containing 1 ml of 1% rosalic acid in 0.2N NaOH. The medium is mixed with repeated stirring and heated to boiling. The m-FC medium comes either in a 500 g bottle for US$88.25 (VWR, 2002) or 100 g bottle for US$37.75. This translates to US$0.013 per 2 ml for the 500 g bottle and US$0.028 for the 100 g bottle. Note these prices do not include the costs of rosalic acid and NaOH. Nonetheless, if the medium can be self-prepared, it can cost up to 20 times less than purchasing pre-packed ampules. This is significantly more economical, if the media can be readily prepared in the lab.

The EC medium is prepared by the same procedure as the m-FC medium. The EC medium was prepared by the author in the ENPHO lab in Kathmandu (See Figure 6-11). The medium was kept refrigerated in between uses and allowed to be kept for a week before a new batch must be prepared again.

Figure 6-11: EC with MUG medium (looks exactly the same as the EC medium) prepared from BD/Difco powdered medium.

The cost of the EC medium is US$22.79 for the 100 g bottle (VWR, 2002) which translates to only US$0.017 per 2 ml of medium. While the self-prepared EC medium costs about half the self-prepared m-FC medium, its cream-colored FC colonies (See Figure 6-10) do not show up as distinctly as blue-colored colonies with the m-FC medium.

Table 6-6: Summary of FC culture media in terms of cost, ease of result interpretation, and medium preparation.

FC Culture Medium	Medium Cost Per Sample	Total Cost Per Sample	Ease of Interpretation	Ease of Preparation
m-FC with rosalic acid (pre-packed)	US$0.83 (HACH, 2002)	US$1.51	Easy	Easy (None)
m-FC with rosalic acid (self-prepared)	US$0.028 using 100g (HACH, 2002) US$0.013 using 500g (VWR, 2002)	US$0.71 US$0.69	Easy	Medium
EC (self-prepared)	US$0.017 using 100g (VWR, 2002)	US$0.70	Medium	Medium

Therefore, after again considering all three criteria (See Table 6-6) as applied to m-FC versus EC media, it is recommended that m-FC with rosalic acid is the best medium to use since the FC colonies show up as distinct blue colonies. If possible, the medium can be self-prepared in the lab to significantly cut costs (by at least 20 times).

6.12 E.coli Media – m-ColiBlue24®, EC with MUG

The appearance of the colonies when using m-ColiBlue24®, EC with MUG medium after incubation at 44.5±0.2ºC for 24 hours are shown in Figure 6-7, Figure 6-12, and Figure 6-13 below.

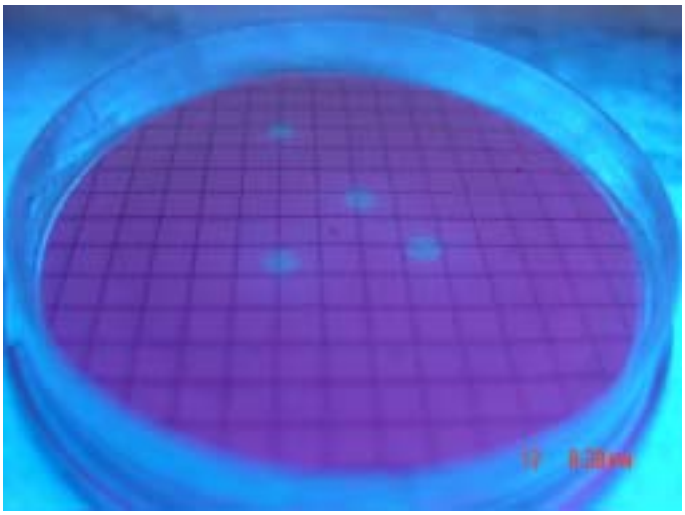

Figure 6-12: EC with MUG medium showing *E.coli* colonies fluorescing under a long-wavelength (366nm) ultraviolet lamp.

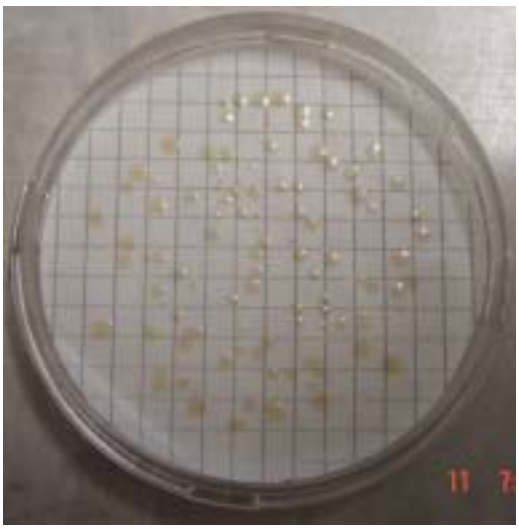

Figure 6-13: *E.coli* colonies on a EC with MUG medium not under a ultraviolet lamp.

The fluorescence in Figure 6-12 is the result of the metabolism MUG by the β-glucuronidase enzyme produced by *E.coli* when placed under a long-wavelength (366nm) ultraviolet (UV) lamp. The *E.coli* colonies show up very distinctly and there was little interference from background growth. The only disadvantage of this approach is that it requires the use of a UV lamp. During the testing of this medium, the *E.coli* colonies show up fairly distinctly even without the use of a UV lamp as shown in Figure 6-13. It was also found that all the cream colored colonies fluoresced under UV lamp. Therefore, the researcher can count all colonies without the use of a UV lamp after he or she has verified that every colony fluoresces with a UV lamp.

The EC with MUG medium is prepared in the exact same way as the EC medium. It is, however, more expensive than the EC medium and costs US$79.95 for the 100 g bottle (VWR, 2002) which translates to US$0.060 per 2 ml of medium. Compared to the m-ColiBlue24® medium which can be used to detect the presence of TC and *E.coli* simultaneously, the self-prepared EC with MUG medium is still significantly cheaper.

Table 6-7: Summary of *E.coli* culture media in terms of cost, ease of result interpretation, and medium preparation.

***E.coli* Culture Medium**	**Medium Cost Per Sample**	**Total Cost Per Sample**	**Ease of Interpretation**	**Ease of Preparation**
m-ColiBlue24® (pre-packed)	US$1.50 (HACH, 2002)	US$2.18	Easy	Easy (None)
EC with MUG (self-prepared)	US$0.060 using 100g (VWR, 2002)	US$0.74	Easy	Medium

Therefore, there are two separate recommendations for the best medium to use for the detection of *E.coli* (See Figure 6-7). If only *E.coli* is to be detected independently from TC, EC with MUG will be a more economical option without sacrificing ease of result interpretation. But if TC is also to be detected, the m-ColiBlue24® medium should be used since it can simultaneously detect both TC and *E.coli*.

6.13 Summary of Culture Media Recommendations for Membrane Filtration

The following culture media are proposed for use in MF for the various indicator organisms as shown in Table 6-8.

Table 6-8: Summary of selected MF culture medium to use for each indicator organism.

Indicator Organism to be Detected by MF	**Culture Medium**	**Medium Cost Per Sample**	**Total Cost Per Sample**
Total Coliform	m-ColiBlue24® (pre-packed)	US$1.50 (HACH, 2002)	US$2.18
Fecal Coliform	m-FC (self-prepared)	US$0.028 using 100g (VWR, 2002)	US$0.71
E.coli	EC with MUG (self-prepared) m-ColiBlue24® (pre-packed)	US$0.060 using 100g (VWR, 2002) US$1.50 (HACH, 2002)	US$0.74 US$2.18

Note: m-ColiBlue24® can be used to enumerate TC and *E.coli* simultaneously.

- The enumeration capability of MF enables determination of the level of contamination in drinking water samples.
- MF is especially valuable when used to assess the treatment efficiencies of a water filter. The ability to quantify the level of contamination in the water sample before and after filtration allows the removal efficiency of the filter to be calculated (See Chapter 8).

- MF-FC is the proposed indicator test in the work of the MIT Nepal Water Project when enumeration is required for evaluating the rate of microbial removal in filters. There are several reasons:

  1) FC shows up as distinct blue colonies on the membrane filter which are easy to identify and count.

  2) TC concentration in raw water is usually too high and fluctuates more when compared to FC. This can become difficult for the researcher who is trying to carry out filter test runs with a relatively constant raw coliform count since more dilutions are required. Instead, FC usually exists in a smaller concentration, thus this makes sample preparation with a constant FC concentration easier and more predictable.

Chapter 7 : MANUFACTURING CERAMIC WATER FILTERS IN NEPAL

7.1 Selection of Ceramic Filters in Nepal

Although point-of-use treatment is relatively uncommon in Nepal, especially outside of the major urban areas, ceramic filters are probably the most commonly used point-of-use drinking water treatment options in Nepal. The Indian and Nepali white ceramic clay candle filters are currently the most widely available ceramic filter in use. However, these candle filters suffer from very low flow rates between 0.2 to 0.3 liters per hour and unsatisfactory microbial removal when no disinfection is used (Sagara, 2000). The basic water requirement for drinking is about 4 liters per person per day (Davis, 2002). If there are 5 people in an average household, at least 20 liters of water is required per day. Therefore, the candle filters will not be able to provide sufficient quantity of drinking water to use for the household. An alternative point-of-use household water filter with a higher flow rate and better microbial removal is needed.

The terracotta ceramic disk filter was selected by the author as a possible solution for study. Ceramic filters are preferred over other filter media because Nepal has a long and established tradition in ceramic pottery making. The raw materials for ceramic making are easily available and many people are trained in this trade. Ceramic filters are also relatively cheap and easy to manufacture without requiring any sophisticated machinery. The author studied another Indian terracotta ceramic filter called the TERAFIL in detail prior to going to Nepal (See Chapter 8). The author also studied another reference ceramic filter from Nicaragua called the "Potters for Peace" filter. By learning the raw materials used and their manufacturing procedures, the author hoped to replicate a similar type of terracotta ceramic filter disk under the local conditions in Nepal.

7.2 Local Ceramics Cooperative in Thimi

When the author was in Kathmandu, Nepal, he visited a local ceramics cooperative called "Madhyapur Clay Crafts" in Thimi in order to initiate a collaboration. Thimi is a small town about 30 minutes outside of Kathmandu and the town's two main occupations are ceramic manufacture and agriculture. Ceramics in Thimi is the traditional occupation and industry of the Thimi people, who have been making pottery in traditional ways for hundreds of years. The

concentration of ceramic pottery making can be found in "Potters Square". See Figure 7-1, Figure 7-2, Figure 7-3, and Figure 7-4 for some photos taken in Thimi's Potters Square.

Figure 7-1: Traditional "Potters Wheel" using an old tire and spinning it by hand with a stick.

Figure 7-2: Pottery making in open courtyards where finished pots are left to dry.

Figure 7-3: Pots ready to be fired in the traditional way are covered with hay and ash.

Figure 7-4: Pots are fired between 3-5 days in covered ash mound with small side vents emitting smoke.

The owner of the ceramic workshop is Mr. Hari Govinda Prajapati, an experienced ceramic handicrafts maker. Hari specializes in the manufacture of terracotta, earthenware, stoneware ceramics, and water filter candles. He is also a designer and constructor of various types of kilns. In the area of household water treatment, Hari manufactures a ceramic candle filter using white clay after having studied this technology in India and imported the proper type of white kaolin clay from India, which is unavailable in Nepal (See Figure 7-5).

The Thimi candle filter manufactured by Hari and the Indian manufactured filters were studied and tested by Junko Sagara in her thesis in 2000, but she found the flow rates and the microbial removal inadequate. During the author's visits to Thimi, Hari showed himself to be very knowledgeable about ceramics manufacture. He was also very helpful and enthusiastic in designing a terracotta ceramic filter for household water treatment according to design specifications decided by the author.

Figure 7-5: White clay candle filter

7.3 Making A Ceramic Filter in Thimi

During the three weeks in Nepal, the author was able to produce several prototypes of a terracotta filter in the shape of a ceramic disk. The filter was then cemented to the bottom of a ceramic container which was also made by Hari. Figure 8-4 and Figure 8-5 in Chapter 8.3 shows photos of the completed Thimi ceramic filters. The objective of this part of the project was to learn, to collaborate in the design, and to document the manufacturing process of ceramic filters using locally available material and equipment in Nepal. Since it was the first time Hari made a terracotta ceramic filter in the shape of a disk, several trial and error attempts were required using different raw materials and firing temperature. Basically, three materials were used: local red pottery clay, saw dust, and rice husk ash. The author chose to design a filter disk smaller than the 9-inch TERAFIL. The smaller filter disks have a 6-inch diameter to facilitate transportation back to MIT. The upper container that housed the filter and a lower collecting container were also made from clay. The total height of the assembly is about 25 centimeters (cm). See Figure 8-4 and Figure 8-5 in Chapter 8.3 for pictures of the Thimi ceramic filter assembly.

Presently, Hari is manufacturing a similar terracotta ceramic filter for ENPHO for arsenic removal. The ENPHO arsenic ceramic filter is shaped like the Indian white clay candle filter but is shorter, wider, and made of terracotta clay. The component proportions are 1 part clay to 2.5 parts sawdust. The mixture is fired at a temperature of 1100°C. According to tests carried out by S.K. Hwang (2002), this filter has a flow rate between 2 and 4 liters per hour. Figure 7-6 below shows a close-up of the ENPHO filter.

Figure 7-6: ENPHO arsenic ceramic filter

After several discussions with Hari, mixtures of the following ingredient proportions were proposed as shown in Table 7-1:

Table 7-1: Proportions of red clay, sawdust, and rice husk ash used in the first set of prototypes fired at 1000°C.

	A	B	C	D (3")	D(1")	E
Red clay (parts)	4	4	4	4	Same as D(3")	1
Sawdust (parts)	6	5	4	3		2.5
Rice husk ash (parts)	0	1	2	3		0
Water for mixing (parts)	1 2/3	1 2/3	1 ½	1 1/3		>2
Measured porosity[1] (by Hari)	51%	54%	51%	49%		83%
Adjusted Flow rate[2]	1.1 L/hr	0.7 L/hr	0.6 L/hr	0.3 L/hr	1.0 L/hr	3.2 L/hr

[1]Method of porosity measurement is unclear.
[2]Refer to Chapter 8.5 on how the adjusted flow rate is calculated.

Hari recommended that the proportion of red clay remain constant while varying the sawdust and rice husk ash proportions. The sawdust is burnt off during the high temperatures of firing thus leaving behind more pores in the filter. Therefore, a greater porosity of the filter can be achieved with more sawdust which also means higher flow rates. However, too much sawdust will also weaken the ceramic structure thus causing cracks to form. Ash will reduce pore size and shrinkage of the ceramic filter during firing thus reducing the possibility of cracking. All these materials are sieved through an approximate 40 mesh (0.425 μm diameter) sieve. Water is added to aid mixing of the different materials. Since sawdust absorbs water, more water will be needed to increase the workability of the mixture if there is more sawdust present.

The filters were made into 6-inch diameter disks of 3-inch thickness. 1-inch thickness versions of designs D and E were also made. These filters were then allowed to dry for 5 to 7 days before they were fired in the kiln at 1000°C. The result was all filters fired well except the 1-inch filter E cracked after firing. The reason given by Hari was that when water in the mixture vaporizes, it expands during firing, thus causing cracks to form. The higher water content is a result of the higher sawdust proportion in the mixture. If this filter is allowed to dry for a longer time, it should be less likely to crack (Prajapati, 2002).

7.3.1 Manufacturing Costs and Time

According to Hari, the production cost of one 9-inch terracotta filter disk is about NRs 75 (US$1). The production cost of both the upper and lower ceramic container is NRs 190 (US$2.50).

Including the time required for clay preparation and assuming the use of a hand mold for finishing, two persons can make 50 filter disks per day. More disks can be made if a press machine is used (Prajapati, 2002).

7.3.2 Preliminary Flow Rate Testing

A method to determine the approximate flow rates of the first set of fired filters was required. By knowing the flow rate, changes to the subsequent sets of filters could be proposed and unnecessary time would not be wasted carrying out tests on those filters whose flow rates were too slow. Therefore, the author thought of fitting those filters into makeshift plastic containers that could be conveniently purchased in the market place. Some time was spent searching for the correct container size. The bottoms of these containers were cut with a mini-saw and the filter disks were fitted into the containers (See Figure 7-7 and Figure 7-8). Silicone was applied to waterproof and seal any gaps between the plastic container and the filter disk. The silicone took more than 3 days to completely dry. Water was fed to the inverted container and the amount of water collected in a certain time was noted and their approximate flow rates were measured and normalized (See Chapter 8.5).

Figure 7-7: Cutting the bottom part of the plastic containers purchased from marketplace.

Figure 7-8: Filter disk placed in the plastic containers and silicone applied all around for water sealing.

As it turned out, the silicone did not bond strongly enough and some water was leaking in some of the tests. In any case, an approximate flow rate was obtained for all the filters and it is recorded in Table 7-1. As expected, the greater the proportion of sawdust, the faster the flow rate (although the measured porosity was not proportional to the sawdust proportions). The 1-inch filter also recorded a flow rate more than 3 times that of the 3-inch filter.

Based on these preliminary results, Hari made another set of filters (all of which are 3-inch thick) using similar proportions, but fired at a higher temperature of 1070°C with the hope of increasing porosity and hence flow rates.

7.4 Filter Manufacturing Procedure

This section describes the 7 steps that comprise the manufacturing process of the Thimi ceramic filters.

1. **Prepare** the raw materials.

The red pottery clay is widely available in the vicinity of Thimi and it is usually purchased by the cart loads. The type of clay Hari and the author used to make the Thimi ceramic filter disks is the same type of normal red clay used by the local potters to make ceramic pots and containers. It is sandy and has enough plasticity to bind sawdust and ash (Prajapati, 2002). The chemical formula of the clay is given in Table 7-2.

Table 7-2: Chemical composition of pottery clay used in Thimi (Prajapati, 2002).

Chemical	Percent Composition
SiO_2	65.80
Al_2O_3	15.82
TiO_2	0.86
Fe_2O_3	5.78
MnO	1.78
MgO	1.78
CaO	0.71
Na_2O	1.12
K_2O	2.72
P_2O_5	0.09
Unaccounted chemical	3.54

The sawdust is collected from the furniture or wood industry. These are usually the discarded wood filings from sawing. Finally, the ash is obtained from burnt rice husk. All these ingredients are sieved through a size 40 mesh sieve before they are used. See Figure 7-9.

Figure 7-9: Three basic raw materials (from left to right) – Red pottery clay, rice husk ash, and sawdust.

2. **Mix** by hand.

The different ingredients are mixed together according to the specified proportions in Table 7-1. In Thimi, a small green bowl of unknown exact volume (See Figure 7-10 and Figure 7-11) was used as a simple standard measuring device to measure out the specified "parts" of each ingredient. After adding the ingredients into a larger basin, a suitable amount of water (half a bowl) was added to increase workability when the mixture is mixed by hand. The remaining volume of water was added until the mixture was thoroughly mixed.

Figure 7-10: Hari measuring the various proportions using a green bowl.

Figure 7-11: Proportions mixed in a red plastic basin.

3. **Press** in mold.

A plaster mold was fabricated by Hari specially to make these filter disks. The mold was lined with paper along its sides and the bottom to prevent the mixture from sticking. The mold was filled with the mixture to the top and compressed by hand during the process (See Figure 7-12). The excess was scrapped away from the top (See Figure 7-13). The mold was then carefully inverted to prevent the mixture from falling apart. The paper that stuck to the mixture was peeled away carefully. The mixture was labeled for easy identification (See Figure 7-14).

Figure 7-12: Mixture placed in a plaster mold made by Hari. The mold has an inner diameter of 6" and depth of 3".

Figure 7-13: Excess is scrapped off to form a smooth surface after pressing and filling the mixture to the top.

Figure 7-14: The mold is carefully inverted to remove the mixture and is labeled for easy identification.

4. **Dry** (5-7 days).

The finished mixtures were laid out to dry in the sun for 5 to 7 days (See Figure 7-15). The higher the sawdust content, the more water is absorbed thus requiring longer drying period. According to Hari, the dryer the mixture, the less likely they are to crack during firing.

Figure 7-15: Mixtures allowed to dry for 5-7 days before firing.

5. **Fire** (1000-1070°C).

After 5 to 7 days of drying under January climatic conditions, the dried mixtures were ready to be fired in the kiln (See Figure 7-16). The kilns were heated to 1000 and 1070°C (for two separate firings) and the mixtures were fired for 12 hours to form finished ceramic disks. The kiln has a maximum firing temperature of 1150°C. According to Hari, the firing temperature and firing period are the most important parameters of the manufacturing process. A longer firing time was preferred because the mixture had a lot of carbon materials (from the sawdust) which had to be oxidized slowly. If insufficient firing time were provided, these carbon materials would remain inside the filter disc even if a higher firing temperature was used (Prajapati, 2002). Notice the fired ceramic disks had a lighter color and became slightly smaller due to shrinkage (See Figure 7-17).

Figure 7-16: Dried mixtures are placed in the kiln and fired at a temperature of 1000-1070°C for 12 hours.

Figure 7-17: Filters after firing and ready to be affixed. Lighter color in filters after firing.

6. **Cement** into ceramic/metal containers.

The fired ceramic disks were then fitted into the prepared ceramic containers (also fabricated by Hari) and cemented with white cement (See Figure 7-18).

Figure 7-18: 6-inch diameter ceramic containers also fabricated by Hari.

7. **Dry** (2 days).

During the drying process, the white cement applied should not be too dry as cracks may form. Therefore, it was important to continuously wet the cement with a damp cloth when the cement was left to dry for 24 to 48 hours.

At the end of the three weeks, two 3-inch filter disks (A and D) were cemented into separate top containers and those filters with their matching bottom containers with attached metal spigots were brought back to MIT for further flow rate and microbial testing (See Chapter 8).

Chapter 8 : ASSESSMENT OF CERAMIC WATER FILTERS

8.1 Two Filters Studied: TERAFIL and Thimi Ceramic Filters

The first goal of the author's ceramic filter study was to try to produce a cheap household point-of-use ceramic filter using locally manufactured materials that improved upon the ceramic candle filters already in use mainly in urban areas of Nepal (See Chapter 7). The second goal of the author's filter study was to evaluate the manufactured filters based on their ability to producing filtered water that meets WHO Drinking Water Guidelines. Other than the ceramic filter disks made in Thimi, another ceramic filter, the TERAFIL, was also studied in this thesis.

In Fall 2001, a Indian terracotta ceramic filter called TERAFIL, was donated and sent to the MIT laboratory for evaluation by Surendra Khuntia, Scientist and Divisional Director of the Regional Research Laboratory in Bhubaneswar, India. Over 1000 of these filter units were distributed to affected villages of Orissa, India during the devastating cyclone in late 1999. The TERAFIL at MIT was evaluated based on its flow rate, turbidity, and microbial removal performance using both P/A and MF enumeration methods (See Chapter 4, Chapter 5, and Chapter 6 for more details on these methods). A similar TERAFIL unit was also evaluated in ENPHO laboratory in Kathmandu, Nepal in January 2002. As has already been discussed in Chapter 7, the author also visited a local candle filter manufacturer in Thimi, Nepal and made prototypes of a terracotta ceramic filter similar to the TERAFIL. Two of these Thimi ceramic filters were brought back to MIT for testing in February 2002.

8.2 Indian TERAFIL Terracotta Ceramic Filter

The TERAFIL terracotta filter consists of two cylindrical metal buckets with a TERAFIL ceramic disk filter fitted in the middle by means of ordinary grey cement. See Figure 8-1 and Figure 8-2 for photos of the entire TERAFIL filter assembly. Figure 8-3 shows the TERAFIL filter disk itself. Raw water is poured in the upper container, passes through the filter, and then into the lower collection container with an attached spigot. The TERAFIL filter ceramic disk is manufactured from a mixture of red clay (ordinary pottery clay), river sand, wood sawdust and burnt at a high temperature in a low cost kiln. In this respect, it differs from the filter disks made in Thimi which do not contain additional river sand but contain rice husk ash. The red terracotta

clay, which is used to prepare domestic earthenwares, is abundantly available in many parts of India and elsewhere in the world. The wood sawdust is burnt and the clay particles are sintered around the sand particles, leaving pores in between. According to Khuntia (2001), the pores in a well-sintered TERAFIL are within 1 to 5 microns, and the pores are not interconnected. Thin clay membrane of 50 to 100 micron thickness separates the pores and is responsible for the separation of most larger-sized bacteria. The removal of most suspended particles occurs at the top surface of the TERAFIL, forming a layer of sediments, which over time, may cause clogging to the filter and reduce flow rates. Therefore, it is recommended by Khuntia (2001) that the top of the TERAFIL clay disk be scrubbed once a day with a soft nylon brush or similar material to remove the sediments and open new pores. Since the pores of the filter are not continuous and interconnected, the core of the TERAFIL should not get clogged. With proper maintenance, the TERAFIL is expected to last more than 5 years (Khuntia, 2001).

Figure 8-1: TERAFIL filter tested in MIT.

Figure 8-2: TERAFIL filter tested in ENPHO.

Figure 8-3: TERAFIL ceramic filter disk.

Currently, the TERAFIL is being marketed and disseminated in Orissa by M/S Orissa Renewable Energy Development Agency, Government of Orissa, Bhubaneswar and a few private micro industries. Production cost is Indian Rs 15 to 20 (US$1 = Rs 43) for the TERAFIL and Rs 130 for the complete set with the filter disk plus two ceramic containers instead of the metal

containers shown in Figure 8-1 and Figure 8-2. Retail cost is Rs 25 and Rs 180 for the full set including ceramic containers. At this low cost, this filter is afforded to the general population to those in India for whom it is currently available.

8.3 Thimi Terracotta Ceramic Filter

The Thimi ceramic filter was fabricated using locally available materials in Thimi, as already described in Chapter 7. The photos in Figure 8-4 show the two Thimi ceramic filters that were brought back to MIT in January 2002. Similar to the TERAFIL, the ceramic filter disk in the Thimi ceramic filters is cemented into the base of the upper container. These ceramic filter disks are made from local pottery clay, saw dust, and rice husk ash. The detailed manufacturing procedures are described in Chapter 7.

Figure 8-4: Two Thimi ceramic filters with ceramic filter disks of different compositions that are brought back to MIT.

Figure 8-5: Top view of the upper container showing the ceramic filter disk A.

8.4 Other Studies on the TERAFIL

There have been 5 prior studies carried out on the TERAFIL:

1) **CSIR (RRL),** *Laboratory Tests on TERAFIL between August and September 1999* – Council of Scientific and Industrial Research (CSIR), Bhubaneswar, India.

2) **CSIR (Orissa),** *Report on Performance of Terracotta Water Filters (fitted with TERAFIL) Distributed in Super Cyclone Affected Areas of Orissa During October 1999 to March 2000* – Council of Scientific and Industrial Research (CSIR), Bhubaneswar, India;
3) **EAWAG (Switzerland),** Wolfgang Köster, Beat H. Birkhofer, Martin Wegelin. *Report on Removal of Bacteria and Bacteriophages with the Help of the 'TERAFIL' Filter Device* – Swiss Federal Institute for Environmental Science and Technology, Switzerland (EAWAG), between October and November 2000;
4) **SIIR (New Delhi),** *Final Report on Study of the Effectiveness of TERRACOTTA FILTER – A Household Water Treatment Device* – SIIR, New Delhi, India, (undated).
5) **ENPHO (Kathmandu),** *Five Months Performance Trial of Home Based Filters – two sand filters and one TERAFIL filter* – Environment and Public Health Organization (ENHPO), Kathmandu, Nepal, between January and June 2001.

The results from these studies are summarized in Table 8-1:

Table 8-1: Summary of TERAFIL performance as tested by five different laboratories.

	CSIR (RRL) Bhubaneswar	**CSIR (Orissa) Bhubaneswar**	**EAWAG Switzerland**	**SIIR New Delhi**	**ENPHO Kathmandu**
Flow rate [L/hr]	**2**	**2.5 – 3**	**1.8 – 2.5**	**2.7 Avg**	**1 – 11 (5 Avg)**
Turbidity, Raw [NTU]	184	660 – 133	N.A.	100	27
Turbidity, Filtered [NTU]	1	1	N.A.	<1 – 6.5	0.2
% Turbidity Removal	**>99%**	**>99%**	N.A.	**>93%**	**>99%**
TC, Raw [CFU/100ml]	>1100	N.A.	N.A.	426 – 1300	241 (FC[2])
Total Coliform, Filtered [CFU/100ml]	7	N.A.	N.A.	4 – 58	9 (FC[2])
% TC Removal	**>99%**	N.A.	**93 – 99% (*E.coli*[1])**	**95 – 99%**	**93 – 96%**
Iron, Raw [mg/L]	3.6	0 – 20.5	N.A.	9.7 – 19.7	2.9
Iron, Filtered [mg/L]	0.3	0 – 1.6	N.A.	0.5 – 1.0	0.015
% Iron Removal	**92%**	**>90%**	N.A.	**>90%**	**>99%**
Cleaning (if any)	Not indicated	Once in 1 to 7 days	Once a week	Not indicated	Once a week

N.A. – No Available results.
[1] *E.coli* was spiked in the raw water sample and their removal was measured instead of TC.
[2] FC removal was measured instead of TC.

Three reports, CSIR (RRL), CSIR (Orissa), and ENPHO showed the TERAFIL to be capable of excellent turbidity removal and good microbial and iron removal, if cleaning is regularly and

properly carried out. However, the overall recommendations varied between studies. For example, both the CSIR (Orissa) and ENPHO reports found good results with the TERAFIL. ENPHO compared the TERAFIL with two other biosand filters and determined that the TERAFIL worked better, was easier to clean, and provided more consistent results than the biosand filters. In this favor, the TERAFIL also had a very low manufacturing cost, could be locally made, and provided a generally consistent although not perfect performance. It is "worthy of serious consideration of wider scale application in Nepal" (ENPHO, 2001).

On the other hand, EAWAG strongly stated that the TERAFIL cannot be recommended for filtration of raw water to produce potable water. "Microbial removal is only satisfactory with a new filter unit, or alternatively with a thoroughly cleaned and disinfected one. The terracotta disk will likely allow the growth of microbial biofilms on its surface and inside the porous structure." (EAWAG, 2000) The SIIR report also found that microorganisms were not effectively removed and break-point was found even after the 2nd cycle. SIIR recommended that water should be further disinfected after filtration to make the treated water fit for human consumption.

In this chapter, the results of tests carried out by the author on the two TERAFIL units are discussed and compared with those summarized in Table 8-1 by previous researchers.

8.5 Methodology of Filter Testing

The performances of the filters were assessed based on 3 main criteria:

1) Flow rate;
2) Turbidity Removal;
3) Microbial Removal.

1. Flow rate Testing

The flow rate of the filters were approximately measured. The TERAFIL filter was filled with water to a certain measured height representing two-thirds full in the upper cylindrical container. For the Thimi ceramic filters, water was filled to almost the top of the upper container. The decreases in water level after a fixed period of time in both filters were measured. The volume

of water that passed through the filter was calculated by multiplying the surface area of the container by the drop in water level. Refer to Figure 8-6 and Figure 8-7 for dimensions of the top container of both TERAFIL and Thimi ceramic filters.

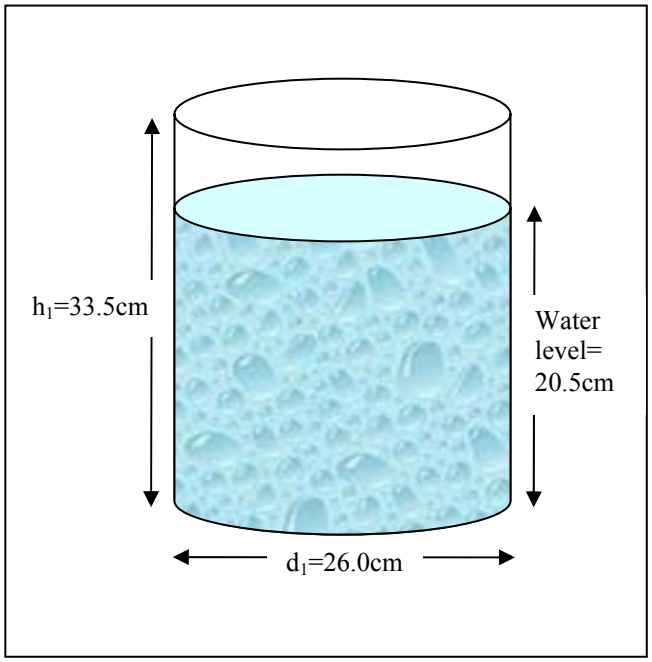

Figure 8-6: Simplified diagram showing the top container of the TERAFIL filter and water level.

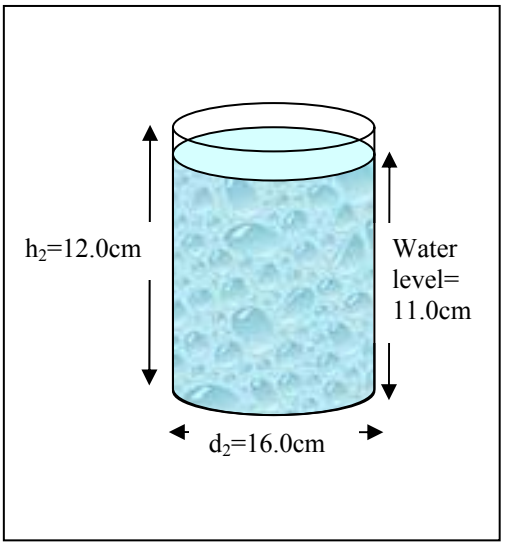

Figure 8-7: Simple diagram showing the top container of the Thimi ceramic filter and water level.

Both filters were allowed to be saturated with water before starting the timing. For the TERAFIL, the drop in water level was measured after 2 hours. The amount of water that filtered through was divided by 2 hours to obtain the flow rate in liters per hour. For the Thimi filter, the drop in water level was measured after 24 hours or more because the container is significantly smaller and the permeability of the ceramic filter is lower, thus resulting in a much lower flow rate. Of course, the author is aware that a higher starting water level will result in a greater flow rate because of the greater hydraulic head. The larger surface area of the TERAFIL also contributed to a greater flow rate compared to the smaller Thimi ceramic filters. Therefore, in order to compare the flow rates between the two types of filters, the measured flow rates of the Thimi ceramic filters had to be normalized for these two factors. The normalization is as follows:

$$\text{Normalized flow rate} = \text{measured flow rate} \times h_1/h_2 \times (d_1/d_2)^2$$

h_1 is the hydraulic head in TERAFIL (20.5cm).

h_2 is the hydraulic head in Thimi filter (11.0cm).
d_1 is the TERAFIL diameter (26.0cm).
d_2 is the Thimi filter diameter (16.0cm).

The author is also aware that it is an over-simplification to assume that the flow rate measured using the above methods represents the true average flow rate of the filter. Instead, a more accurate method would be to monitor the flow rate at equal time intervals e.g. ½ hour. One should expect a declining flow rate after each time interval because of a continuously falling hydraulic head. Therefore, the reported flow rates should be understood as approximate averages.

2. Turbidity Testing

The turbidity of the water sample was tested with the HACH 2100P turbidimeter. A small volume of 20 ml of the sample was placed in the sample cell bottle. The exterior surface of the bottle was wiped clean of fingerprints with the provided cleaning cloth which has been dabbed with oil before placing in the meter. The WHO Drinking Water Guidelines require a turbidity less than or equal to 5 NTU (WHO, 1996).

3. Microbial Testing

The microbial tests of the raw water samples were generally carried out within 2 to 3 hours of collection at the source, except for the Dhobi Khola river samples which were refrigerated. Extra care was taken when collecting the filtered samples from the spout in the bottom container to avoid contamination. The filtered water was collected directly into sterile sampling bottles, after allowing it to run for half a minute to flush out any deposits in the spout. The bottom container was also thoroughly washed and rinsed with sterile rinse water between filter runs. The microbial tests included P/A tests and MF tests of TC, FC, and *E.coli* as described in Chapter 4, Chapter 5, and Chapter 6. In Fall 2001 at MIT, only the MF-TC test was used. WHO Drinking Water Guidelines require zero TC or *E.coli* to be found in every 100 ml of sample.

8.6 Variations in Test Conditions

Due to changing environments and laboratory setups, the 4 different filters: TERAFIL (MIT), TERAFIL (ENPHO), and two Thimi ceramic filters were tested under different conditions.

Efforts were made to keep as many of the test parameters constant as possible. The tests were carried out at 2 different sites: MIT laboratory Room 1-047, Massachusetts, U.S.A. and the ENPHO laboratory, Kathmandu, Nepal. The TERAFIL (MIT) was tested between November and December 2001. The TERAFIL (ENPHO) was tested in January 2002 in ENPHO, Kathmandu. The two Thimi ceramic filters were tested in March 2002 at MIT.

Four sets of filter runs (Preliminary Test, Test MA, MB, and MC) were carried out on the TERAFIL (MIT), including the first set which was called the "Preliminary Test". The first set was so called because the author was learning the laboratory techniques for the first time. Each set consisted of three filter runs for a total of 12 runs. After each run, the filter was "cleaned" by scrubbing the top surface of the ceramic filter with a plastic scrubber provided by the filter manufacturer to remove any sediments that would accumulate and clog the filter surface. For the latter 6 of the 12 runs, the filter would also be "flushed" with sterile rinse water once to ensure that the filter pores were free of any remaining raw water. In the first "real" test set (Test MA), the filter was "flushed" but not "cleaned" between runs. In the second test set (Test MB), the filter was "cleaned" and "flushed" between runs. In the third test set (Test MC), silicone sealant was applied to the top of the white cement that was used to bond the ceramic filter to the container (See Figure 8-8 and Figure 8-9). This was to test the hypothesis that bacteria would pass through some of the cracks visible in the cement. The filter was also "cleaned" and "flushed" between runs.

Figure 8-8: TERAFIL (MIT) showing the original white cement used to bond the ceramic filter to the metal container.

Figure 8-9: TERAFIL (MIT) showing the silicone added on top of the white cement after drying.

For the TERAFIL (ENHPO), two sets of filter runs (Test EA and EB) were carried out. In the first test set (Test EA), the filter was "cleaned" and "flushed" with chlorine-free tap water between runs. In the second test set (Test EB), the filter was coated with colloidal silver to test the disinfection properties of colloidal silver.

Colloidal silver is known for its germicidal effect on microorganisms and has been used in a similar household ceramic filter appropriate for developing countries called the "Potters for Peace" filter from Nicaragua (Rivera, 2001). The colloidal silver solution used for the Potters for Peace filter comes in small 20 ml bottles in a concentration of 0.34% and is packaged under the brand name of "Microdyn", a product commonly available in shops in Mexico. The author's method for coating the ceramic filter with the colloidal silver based on previous instructions from Ron Rivera (2001) Potters for Peace filter was as follows:

1. 2 ml of Microdyn colloidal silver was diluted in 250 ml of distilled water.
2. About 50 ml of the dilution was brushed onto the top surface of the filter.
3. Remaining 200 ml of the dilution was poured onto the filter and allowed to pass through the filter.
4. The filter was allowed to dry for 24 hours.
5. The filter was flushed through once with clean, unchlorinated tap water before carrying out filter Run EB.

For Thimi ceramic filter A and filter D, one set of filter runs (Test AH and DH respectively) was carried out for each filter. Filter A has a composition of 4 parts clay and 6 parts sawdust. Filter D has a composition of 4 parts clay, 3 parts sawdust, and 3 parts ash. The filters were "cleaned" and "flushed" in between each run.

8.6.1 Raw Water Sample

Different water sources were used for the raw water samples at MIT and ENPHO. At MIT, the raw water was collected from the Charles River (CRW) in the afternoons. The water was always collected at the same location, about 100 feet east of the Harvard Bridge, on the north end of the river (See Figure 8-10). The turbidity of CRW remained fairly constant in the range of 2 to 4 NTU. However, the microbial quality of the CRW varied significantly during the period of

testing (Fall 2001 and Spring 2002). The CRW contained between 500 to 210,000 TC per 100 ml. Therefore, the collected sample had to be diluted to obtain a reasonable colony count on the membrane filter.

At ENPHO, the raw water was collected once from a nearby river called the Dhobi Khola (See Figure 8-11 and Figure 8-12). The river was so contaminated with municipal waste, animal feces, and all other wastes of unknown origin, that the collected water had to be diluted significantly. This original sample was kept refrigerated during the two-week period of testing. The Dhobi Khola water sample had a very high FC concentration of about 16,000 CFU per ml. Therefore, a very small volume, 3.5 ml of the Dhobi Khola river water was diluted in 6 liters of unchlorinated tap water and 6 liters of well water. The well water was collected from a well in a nearby household from the ENPHO office (See Figure 8-13). The well water had a very yellowish appearance and a very high turbidity of about 100 NTU. It was found to contain very high iron content but no microbial contamination. The well sample tested negative for TC, FC and *E.coli.* The purpose of mixing this well water to the Dhobi Khola sample was to introduce turbidity to the highly diluted raw water sample. The final diluted sample had a water quality of about 50 NTU and 500 FC per 100ml. In the end, however, the coliform counts vary considerably due to uncertainty associated with natural variability despite the author's best efforts to obtain a consistent raw water source.

Figure 8-10: Location near Harvard bridge where water samples are collected from the Charles River.

Figure 8-11: Collecting river samples from a "very" polluted Dhobi Khola River in Kathmandu, Nepal.

Figure 8-12: Comparison of the Dhobi Khola River sample with distilled water.

Figure 8-13: Collecting high turbidity water from a well near the ENPHO lab.

8.6.2 MF Setup

Both laboratories at MIT and ENPHO were equally well-equipped. At MIT during Fall 2001, the Millipore glass MF setup was used. Between samples, the glassware was sterilized in an air oven at 170ºC for an hour. At both ENPHO and MIT during Spring 2002, the Millipore portable MF setup was used instead. The portable setup can be quickly sterilized in 15 minutes by flaming with methanol in between samples. (See Chapter 6 for a more detailed discussion on MF). The portable MF setup had the advantages of a faster sterilization than the traditional glass MF setup.

At MIT, Milli-Q water was used as rinse water in between filtrations. At ENPHO, pre-bottled sterile, non-pyrogenic water was used. While these waters should be sterilized to ensure that they were bacteria-free, such procedures were considered too elaborate and time-consuming. Instead, blanks with the rinse water which tested negative were carried out for both P/A and MF tests at the beginning of each week to ensure that no prior contamination had occurred.

8.7 Test Results and Discussion

The results of the filter tests are summarized in Table 8-2 and Table 8-3 and discussed below.

Table 8-2: TERAFIL filter test performance under lab conditions.

	MIT TERAFIL Preliminary Test			**Lab Test MA (No clean between runs)**			**Lab Test MB (clean between runs)**			**Lab Test MC (sealant and clean)**		
	P1	**P2**	**P3**	**MA1**	**MA2**	**MA3**	**MB1**	**MB2**	**MB3**	**MC1**	**MC2**	**MC3**
Flow rate (L/hr)	0.9	1.7	-	1.5	1.3	1.1	1.5	1.3	1.3	1.8	1.8	1.9
Turbidity, R(NTU)	2.38	7.54	3.72	4.15	4.66	3.36	2.99	2.44	3.98	3.17	3.3	3.17
Turbidity, F(NTU)	0.28	0.49	0.89	0.79	0.41	0.36	0.47	0.63	0.43	1.09	2.2	1.87
% reduction	88.2%	93.5%	76.1%	81.0%	91.2%	89.3%	84.3%	83.2%	89.2%	65.6%	33.3%	41.0%
H2S,R(P/A)	P	P	P	P	P	P	P	P	P	P	P	P
H2S,F(P/A)24h	-	-	-	P	0.5P	A	A	A	A	P	P	P
H2S,F(P/A)48h	A	P	P	P	0.5P	P	P	P	P	P	P	P
TC/*E.coli*,R(P/A)	P/A	P/A	P/A	P/A	P/A	P/A	P/A	P/A	P/A	P/A	P/A	P/A
TC/*E.coli*,F(P/A) 24h	-	-	-	P/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A
TC/*E.coli*,F(P/A) 48h	A/A	P/A	P/A	P/A	P/A	P/A	P/A	P/A	P/A	P/A	P/A	P/A
TC,R(CFU/100ml)	30400	210000	44000	8750	12333	1889	1417	1000	1000	1375	962	500
TC,F(CFU/100ml)	60	240	20	1	4	4	5	34	47	19	20	18
% TC Removal	99.80%	99.90%	99.95%	99.99%	99.97%	99.66%	99.36%	96.60%	95.30%	98.62%	97.92%	96.40%

Table 8-3: TERAFIL and Thimi ceramic filter test performance under lab conditions.

	ENPHO TERAFIL Lab Test EA			**Lab Test EB (colloidal silver)**			**Thimi Filter A Lab Test AH**			**Thimi Filter D Lab Test DH**		
	EA1	**EA2**	**EA3**	**EB1**	**EB2**	**EB3**	**AH1**	**AH2**	**AH3**	**DH1**	**DH2**	**DH3**
Flow rate (L/hr)	5.9	6.9	6.1	4.9	6.6	6.9	0.29	0.26	0.23	0.24	0.23	0.23
Turbidity,R(NTU)	50.1	45.9	70.3	58.6	40.4	38.1	3.4	3.8	3	3.4	3.8	3
Turbidity,F(NTU)	0.64	1.25	1.24	0.56	0.59	0.82	0.56	0.6	0.96	1.47	1	1.21
% reduction	98.7%	97.3%	98.2%	99.0%	98.5%	97.8%	83.5%	84.2%	68.0%	56.8%	73.7%	59.7%
H2S,R(P/A)	P	P	P	P	P	P	P	P	P	P	P	P
H2S,F(P/A)) 24hrs	0.5P	P	P	A	A	A	A	A	A	A	A	A
H2S,F(P/A) 48hrs	P	P	P	A	A	A	A	A	A	A	A	A
TC/*E.coli*,R(P/A)	P/P	P/P	P/P	P/A	P/A	P/A	P/A	P/P	P/P	P/A	P/P	P/P
TC/*E.coli*,F(P/A) 24hrs	P/A	P/P	P/P	P/A	P/A	P/A	-	-	-	-	-	-
TC/*E.coli*,F(P/A) 48hrs	P/P	P/P	P/P	P/A	P/A	P/A	A/A	P/P	P/A	P/A	P/A	P/A
TC,R(CFU/100ml)	222	1200	2200	680	14500	7450	648	625	1295	Same as AH		
TC,F(CFU/100ml)	1	29	42	7	342	460	9	28	4	20	69	46
% TC Removal	99.55%	97.58%	98.09%	98.97%	97.64%	93.83%	98.61%	95.52%	99.69%	96.91%	88.96%	96.45%
FC,R(CFU/100ml)	56	900	2300	125	6850	1740	N.A.	43	15	Same as AH		
FC,F(CFU/100ml)	0	2	2	0	260	350	N.A.	0	0	N.A.	0	0
% FC Removal	100%	99.78%	99.91%	100%	96.20%	79.89%	N.A.	100%	100%	N.A.	100%	100%
*E.coli,*R (CFU/100ml)	30	880	2800	190	7000	1425	18	28	48	Same as AH		
*E.coli,*F (CFU/100ml)	1	1	4	2	260	290	0	1	1	0	1	1
% *E.coli* Removal	96.67%	99.89%	99.86%	98.95%	96.29%	79.58%	100%	96.43%	97.92%	100%	96.43%	97.92%

1. Flow rate Results

The TERAFIL (MIT) had a flow rate ranging from 1.1 to 1.9 L/hr. In Test MA when the filter was not cleaned in between runs, the flow rate decreased from 1.5 to 1.3 to finally 1.1 L/hr. A layer of sediment could be observed on the top surface of the filter. In fact, there was about 1 cm of water remaining on top of the filter after 24 hours between Run MA2 and MA3. This meant that some of the pores in the upper part of the filter were clogged, thus causing the flow rate to decrease. When the filters were cleaned between Tests MB and MC, the flow rate between runs was more consistent at 1.3 and 1.8 L/hr respectively and standing water did not remain in the upper container. However, no explanation could be provided for the consistently higher flow rates for Test MC over Test MB.

The TERAFIL (ENPHO) had a significantly higher flow rate than the TERAFIL (MIT). The TERAFIL (ENPHO) had a flow rate ranging from 5.9 to 6.9 L/hr (Run EB1 has a flow rate of only 4.9 L/hr because the filter was not pre-saturated before the test). Both TERAFIL filters came from the same Indian manufacturer and so, theoretically, they should have the same performance. However, this significant difference in flow rate could not be explained. A likely reason is the lack of quality control during manufacturing. As the microbial results later show, the higher flow rates was achieved without sacrifice of the microbial removal rates, and this is of considerable interest.

Both Thimi ceramic filters had a very low normalized flow rate between 0.2 to 0.3 L/hr, comparable to the white clay candle filters studied by Sagara (2000). Filter A was slightly faster with a normalized flow rate of 0.26 L/hr, while Filter D had a normalized flow rate of 0.23 L/hr. The higher flow rate of Filter A can possibly be explained by its higher proportion of sawdust than Filter D. Either way, these prototypes had relatively similar flow rates that were too low to be practical. But since it was the first time this type of ceramic filter was being made in Nepal and only the second time the author attempted to make such ceramic filter disks himself, many improvements to the design are possible.

2. Turbidity Results

The TERAFIL (MIT) had good turbidity removal rate ranging from 76% to 94%. Most removal rates exceeded 80% except Test MC which achieved only 33% to 66% removal. The turbidity of the raw CRW samples ranged from 2 to 4 NTU and the turbidity of the filtered water was 0.8 NTU or less. Except in Test MC when additional sealant was applied, turbidity removal rates fell to 33% and 66% from raw water turbidity of about 3 NTU. Again, this drop in turbidity removal cannot be explained since the filter in Test MC was subjected to the same conditions as previous tests. On the other hand, an inverse relationship between the turbidity removal rate and filter flow rate can be identified (See section on Correlation of Results).

The TERAFIL (ENPHO) performed exceptionally well at removing turbidity with removal rates ranging between 97% and 99%. For example, in Run EB1, the filter was able to reduce the raw sample turbidity of 58.6 NTU to 0.56 NTU in the filtered sample. Again, this high rate of turbidity removal was not significantly affected by the higher flow rate.

Both Thimi ceramic filters had reasonable turbidity removal rates ranging from 57% to 84%. Filter A removed turbidity better than Thimi filter D with an average removal rate of 79% as opposed to an average removal rate of 63%. Only Filter A was able to produce a filtered turbidity of less than 1 NTU when the raw turbidity was between 3 and 4 NTU. The filtered turbidity in Filter D were all between 1 and 1.5 NTU.

3. Microbial Results

During the microbial testing of the TERAFIL (MIT), both P/A-TC and P/A-H_2S tests and the MF-TC tests were carried out. In every run, the raw CRW showed a "Presence" in all P/A-TC and P/A-H_2S tests. Since, no *E.coli* was present as indicated by the P/A-*E.coli* test in all CRW samples, its removal by the filter could not be assessed. In filtered samples, all of them showed a "Presence" in the P/A-TC test after 48 hours (except run P1), thus these results were not useful in showing any filter performance benefit. Similarly, all filtered samples produced a "Presence" P/A-H_2S result (except run P1-Absence and run MA2-0.5 Presence) after 48 hours. In a shorter 24 hours, however, all three P/A-H_2S tests in Test MB showed "Absence" results. As previously established in Chapter 5, the rate at which a "Presence" result is produced is related to the

concentration of H_2S-producing bacteria. Therefore, it was possible that there were less H_2S bacteria in the filtered samples of Test MB compared to Test MA and MC, both of which showed a "Presence" result in all the runs in 24 hours (except Run MA2-0.5Presence). Unfortunately, the correlation of this result was not evident from the enumerated TC results.

As mentioned above, the microbial removal rates for the TERAFIL (MIT) have been established based on the enumeration capability of the MF-TC test only. Removal rates are calculated by comparing the TC counts in the water before and after filtration. The Preliminary Test MF results were discarded because the raw TC counts were too high and very approximate because of the high colony densities on the membrane filters. Looking at Test MA, MB, and MC, it can be seen that the TERAFIL (MIT) was able to achieve a TC removal rate between 95% (from 1,000 to 47 CFU/100ml) to 99.99% (8,750 to 1 CFU/100ml).

The TERAFIL (ENPHO) was able to achieve similarly high microbial removal rates and with a significant improvement in flow rates. When P/A tests were used to assess its microbial removal efficiencies, no useful results were obtained. All P/A-TC tests showed "Presence" in both raw and filtered samples after 48 hours. On the other hand, for the P/A-H_2S test in Test EB, all 3 filtered samples showed "Absence" results. These results however, contradict the MF results which showed significant (between 0 to 460 CFU/100 ml) TC, FC and *E.coli* counts. Although this discrepancy may be explained by the lower sensitivity (about 5 CFU/100ml) of the 20 ml sample volume used in the P/A-H_2S test, the author ruled out the possibility that the "Absence" outcomes was a result of missed detection. Therefore, it was possible that these three P/A-H_2S results were all false-negative.

For the TERAFIL (ENPHO), all three indicator organisms: TC, FC, and *E.coli* were enumerated using MF. TC removal rates ranged from 94% to 99.55% when the original counts ranged from 222 to 14,500 CFU per 100 ml. There was no identifiable relationship between the raw sample counts and removal rates. FC removal rates ranged from 80% to 100% when the original counts ranged from 56 to 6,850 CFU per 100 ml. The 100% removal rates were obtained during two runs when original counts were very low at 56 and 125 CFU per 100 ml. *E.coli* removal rates were also similar to those of FC, ranging from 80% to 99.89%. The raw samples contain

between 30 to 7000 CFU per 100 ml and filtered samples contain between 1 and 290 CFU per 100 ml. In Runs EB2 and EB3, some breakthrough appeared to have taken place, with the unexpectedly high concentrations of TC, FC, and *E.coli* in the filtered samples. It was suspected to be caused by the much higher concentrations of TC, FC, and *E.coli* in the raw water sample. This led to a significantly greater number of TC, FC, and *E.coli* passing through the filter into the filtered samples. While this significantly increased the coliform counts, the overall microbial removal rates were still reasonable above 80%.

The application of colloidal silver also appeared to have no noticeable effect on the microbial removal rates. One possible explanation is because the actual pore size of the TERAFIL is noticeably larger than the Potters for Peace filter, despite the similar pore size specifications provided by the manufacturers. Therefore, the applied colloidal silver might have been unable to adhere to the ceramic structure in the author's application to the TERAFIL. Therefore, the colloidal silver was suspected to be flushed away during the filtration.

The 2 Thimi ceramic filters, A and D, showed similar microbial removal performance. In the P/A tests, the filtered samples of both filters again showed "Absence" in all the P/A-H_2S tests. Contrary to results from Test EB of the TERAFIL (ENPHO), these results showed good correlation with the MF-FC and MF-*E.coli* results. Both MF indicator results showed either 1 or 0 CFU per 100 ml. For the P/A-TC and P/A-*E.coli* tests, most of the filtered samples showed "Absence". Therefore again, these P/A test results were inconclusive in assessing the filter performance.

In MF tests, both Thimi ceramic filters achieved similar and very good TC removal rates ranging from 89% to 99.69%, with a starting TC count between 625 to 1,295 CFU per 100 ml. The filtered TC counts were between 4 and 69 CFU per 100 ml. The FC and *E.coli* counts in the raw sample were also low, both were 15 and 43 CFU per 100 ml. This also explained the complete removal of FC and the very high *E.coli* removal rates between 96% and 100%. If a higher concentration of FC and *E.coli* was present in the raw sample, one could expect their removal rates to decrease as well.

8.8 Correlation of Results

Correlation of the parameters flow rate, percent turbidity removal, percent TC removal, percent FC removal, percent *E.coli* removal, for both TERAFIL filters was done using the correlation coefficient function (CORREL) in Microsoft Excel. In the CORREL function, the performance data are compared with each other assuming a simplistic linear relationship. The correlation coefficient ranges from -1 to 1. The closer it is to 1 or -1, the stronger is the relationship between the two parameters. When it is positive, the parameters will vary in the same direction. When it is negative, the parameters will vary in opposite direction. These correlation coefficients are only computed for the two TERAFILs and not the Thimi ceramic filters because the measured parameters of the Thimi ceramic filters did not vary significantly to yield useful correlation coefficients. For example, all measured Thimi ceramic filter flow rates fall within ±0.1L/hr and the measured percent TC, FC, and *E.coli* removal are within ±5%. Table 8-4 and Table 8-5 show the correlation results for the TERAFIL (MIT) and TERAFIL (ENPHO).

Table 8-4: Correlation coefficients of various performance parameters for TERAFIL (MIT).

	Flow rate	**% Turbidity Removal**	**% TC Removal**	**% FC Removal**	**% *E.coli* Removal**
Flow rate		-0.70	-0.23	N.A.	N.A.
% Turbidity Removal			0.37	N.A.	N.A.
% TC Removal				N.A.	N.A.
% FC Removal					N.A.
% *E.coli* Removal					

These correlation coefficients are calculated from 12 filter runs.
"N.A." indicates that no tests on the parameter were carried out.

Table 8-5: Correlation coefficients of various performance parameters for TERAFIL (ENPHO).

	Flow rate	**% Turbidity Removal**	**% TC Removal**	**% FC Removal**	**% *E.coli* Removal**
Flow rate		-0.83	-0.66	-0.50	-0.43
% Turbidity Removal			0.58	0.33	0.25
% TC Removal				0.94	0.99
% FC Removal					0.99
% *E.coli* Removal					

These correlation coefficients are calculated from 6 filter runs.

- Flow rate is *negatively correlated* with microbial removal.

When flow rate is compared to the rate of microbial removal, the correlation coefficients are all negative. This means that an increase in flow rate will reduce microbial removal efficiencies. This seems intuitively obvious because the faster the water passes through the filter, the more likely it seems that microorganisms will be forced through the filter pores. This correlation is strongest between flow rate and TC removal. The correlation decreases with FC and *E.coli*. However, since these coefficients are not close to -1, their correlations with flow rates are not significant. On the other hand, the negative sign is useful in showing the inverse relationship between flow rate and microbial removal efficiencies.

The curious and as yet unexplained difference in flow rates between the two TERAFIL yet comparable microbial removals is not explained by this statistical correlation.

- Turbidity removal is *positively correlated* with microbial removal.

When turbidity removal rate is compared to microbial removal rate, a positive relationship is identified. This shows the possibility of microorganisms living among the suspended particles causing turbidity in water. When turbidity is reduced, the coliform counts also decrease accordingly. Again, these coefficients ranged between 0.25 and 0.58 and are not close to 1, so the correlation is not significant.

- Flow rate is *negatively correlated* with turbidity removal.

Flow rate is found to have a closer inverse relationship with the rate of turbidity removal. The correlation coefficients are -0.70 and -0.83 for the two TERAFIL filters. Similar to the discussion on microbial removal, the faster the flow rate, the more likely suspended particles in the water will pass through the filter pores. Therefore, in a filter design, it is important to achieve high flow rates without compromising the rate of turbidity and microbial removal.

The following two graphs in Figure 8-14 analyze the relationship of flow rate and turbidity removal further by plotting the measured values. Both graphs, especially the first graph, show a

similar trend of the turbidity removal approaching some peak value when flow rate decreases. This indicates that the turbidity cannot be totally removed even if the flow rate reaches zero. There is some maximum rate of turbidity removal, about 90%, that can be achieved with the TERAFIL (MIT). In the second graph, the TERAFIL (ENPHO) appears to maintain a high turbidity removal greater than 97% at a higher flow rate of 7 L/hr.

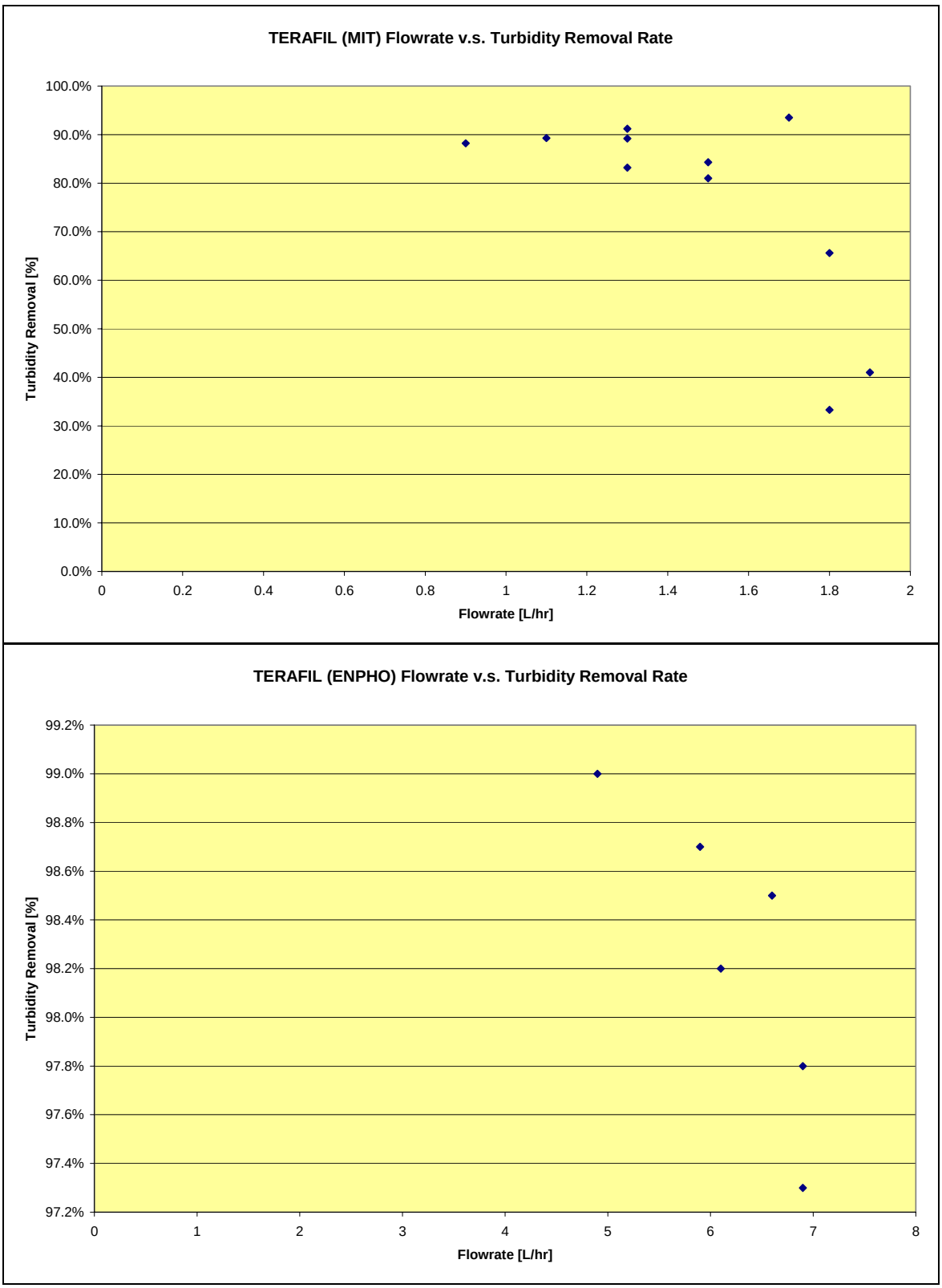

Figure 8-14: Two graphs plotting the flow rates vs. turbidity removal rates of TERAFIL (MIT) and TERAFIL (ENPHO).

8.9 Filter Tests Summary

Table 8-6: Performance summary of TERAFIL and Thimi ceramic filters.

	TERAFIL (MIT)	**TERAFIL (ENPHO)**	**Two Thimi Filters**
Flow rate [L/hr]	**1.1 – 1.9**	**5.9 – 6.9**	**0.2 – 0.3**
Turbidity, Raw [NTU]	2 – 4	38 – 70	3 – 4
Turbidity, Filtered [NTU]	<0.8 generally	0.5 – 1.2	0.5 – 1.5
% Turbidity Removal	**33 – 94%**	**97 – 99%**	**57 – 84%**
TC, Raw [CFU/100ml]	500 – 12,333	222 – 14,500	625 – 1,295
Total Coliform, Filtered [CFU/100ml]	1 – 43	1 - 460	4 – 69
% TC Removal	**95 – 99.99%**	**94 – 99%**	**89 – 99%**
FC, Raw [CFU/100ml]	N.A.	56 – 6,850	15 - 43
Fecal Coliform, Filtered [CFU/100ml]	N.A.	0 - 350	0
% FC Removal	N.A.	**80 – 100%**	**100%**
E.coli, Raw [CFU/100ml]	N.A.	30 – 7,000	18 – 48
E.coli, Filtered [CFU/100ml]	N.A.	1 – 290	0 – 1
% *E.coli* Removal	N.A.	**80 – 99%**	**96 – 100%**
Cleaning (if any)	Every 3 runs/ Every run	Every run	Every run

- TERAFIL showed excellent performance in terms of turbidity removal with rates exceeding 85% most of the time; and microbial removal rates exceeding 95% in 17 out of 18 runs, without disinfection.
- Turbidity of the filtered water only exceeded 1.0 NTU in 5 out of 18 runs, which is well below the WHO guideline value of ≤5 NTU. The TERAFIL (ENPHO) showed particularly impressive turbidity removal since it was able to reduce the raw water turbidity of 70 NTU to less than 1.3 NTU in the filtered water.
- Microbial removal rates always exceeded 95% (except in Run EB3). The most significant improvement of the TERAFIL (ENPHO) over the TERAFIL (MIT) was its faster flow rate, which was possible without sacrificing its microbial removal performance.
- These tests were carried out under controlled conditions in the laboratory. Factors such as the regular cleaning of the bottom container and scrubbing of filter disk can significantly affect the performance of the filter in the households.

- At Rs. 180 (US$4.20), the TERAFIL filter can be a cost-effective point-of-use treatment option if some form of post-disinfection is carried out.
- Both Thimi ceramic filters have similar turbidity and microbial removal performances but their flow rates were too slow for practical use.

8.10 Recommendations for Future Work

The variation in performance of the two TERAFILs demonstrated the possible inconsistency that can occur during its manufacturing process. It would be ideal if all TERAFIL manufactured have at least the same level of performance as the TERAFIL (ENPHO). More importantly, high microbial removal rates do not necessarily imply that the filtered water is drinkable. This is because the filtered water quality can still be unacceptable if highly contaminated raw water is used. Instead, it is recommended that a low chlorine dose be added to the filtered water if 100% microbial indicator removal is to be achieved. Chlorine is chosen to be added to the filtered water and not the raw water so that only a low dosage will be needed. This way, the TERAFIL filter should be able to achieve the WHO Drinking Water Guidelines of zero FC and *E.coli* per 100 ml sample. If only a low concentration of chlorine is required for complete disinfection, there will not be any problems with unpleasant taste associated with high residual chlorine.

The Thimi ceramic filters are manufactured for the first time and can be considered as prototype designs. They were found to be cheap and easy to manufacture. The raw materials used such as red clay, saw dust, and ash are commonly locally available in Nepal and elsewhere. However, the flow rates of these filters are too slow and changes are needed in order to improve the flow rates. These include adjustments in the proportions of clay versus sawdust. Compositions containing a high sawdust proportion may be less likely to crack during firing if a longer drying period is allowed. The firing temperature can also be varied although the effect should be discussed with Hari. Then again, when the flow rates are improved, the removal performance may decrease. Therefore, more prototypes need to be made and tested in Thimi before locally made filters of this filter disk design are ready for widespread implementation in Nepal and potentially elsewhere.

Chapter 9 : CONCLUSIONS AND RECOMMENDATIONS

The motivation for the first part of this thesis was to evaluate and recommend the most appropriate indicator organism or organisms for drinking water quality testing and monitoring for the MIT Nepal Water Project and similar MIT water and sanitation projects. This thesis began with a discussion of the various waterborne pathogens and the indicator organisms that were used to indicate their presence. The traditional coliform indicator was found to have shortcomings not least of which is that it is inappropriate for use in tropical developing countries. Instead, *E.coli* is being proposed here as the most suitable indicator of recent fecal contamination thus it is proposed as the indicator organism of choice for routine water quality monitoring. However, in circumstances where there may be a very small concentration of *E.coli,* fecal coliform is the next most appropriate indicator to use.

Future work is recommended in the following few areas. Less stringent drinking water guidelines or standards should be drafted for the developing countries which do not have the resources to maintain the same stringent standards of zero total coliforms per 100 ml of drinking water sample. The newer guidelines and standards should be based on the "acceptable risk" which people can be exposed to without falling sick. A cost-benefit approach could be used to determine this "acceptable risk". At the same time, focus should also be placed on the incremental improvement in water quality at the most affordable cost to the local community. The zero coliform guideline should serve as the medium-term goal and not an immediate requirement during water quality monitoring for these countries. Such a gradual transition would be more achievable by the developing countries where financial and technical resources are limited.

Two test methods commonly used to assess the microbial quality of drinking water were also studied extensively. The Presence/Absence test for total coliform is a useful and simple test that can be carried out both in the field and laboratory to indicate their presence. While it was previously established that total coliform is not an appropriate indicator of drinking water quality in tropical countries, it is still valuable when used as an indicator of treatment efficiencies in developed countries and of drinking water quality when disinfection is applied. Instead, the P/A-

H_2S test which detects hydrogen-sulfide producing bacteria, is found to be a good indicator test for *E.coli* presence and hence fecal contamination.

The second method, membrane filtration, allows indicator organisms to be counted in a water sample. The enumeration capability is particularly valuable in evaluating microbial removal efficiencies of point-of-use water filters. When this method is employed, the fecal coliform indicator is preferred because fecal coliforms are often present in the raw water and they are easily recovered and counted by this technique.

This thesis also explored several culture media used in membrane filtration to recover the three indicator organisms: total coliform, fecal coliform, and *E.coli*. These media were compared based on their relative costs, east of colony interpretation, and medium preparation. Conclusions are as follows: m-ColiBlue24® medium (pre-packed) is proposed for the recovery of total coliform primarily because of its ease of colony identification; m-FC with rosalic acid medium (self-prepared) is selected for the recovery of fecal coliform, because of its low cost and ease of colony interpretation. Either m-ColiBlue24® or EC with MUG medium (self-prepared) can be used to recover *E.coli* because the *E.coli* colonies show up most distinctly with both of these media.

A ceramic disk filter for point-of-use water treatment was fabricated by the author in collaboration with Hari Govinda Prajapati, a local pottery maker in Thimi, Nepal. The complete manufacturing process was described and documented with pictures. Recommendations on future work that needs to be carried out in this area are discussed in Chapter 8.10. These include improvements on the material composition of the filter and fine-tuning of the manufacturing process.

The technical performance of two ceramic water filters was evaluated in this thesis. Between the two, the TERAFIL showed good, although not flawless technical performance both in terms of turbidity and microbial removal. Turbidity removal rate exceeds 85% most of the time and the turbidity of the filtered water only exceeded 1.0 NTU in one third of the runs with a starting turbidity as high as 70 NTU. Microbial removal rates exceed 95% without disinfection in almost

all of the runs with a starting fecal coliform count as high as 14,500 CFU per 100 ml when the filter is cleaned after each use. The social acceptability of daily cleaning of the TERAFIL filter in order for it to sustain its level of technical performance has yet to be determined. The Thimi filters showed similar microbial removal rates but with slightly lower turbidity removal rates. In terms of flow rate, of the 2 TERAFIL units tested, 1 unit performed significantly better with a flow rate of up to 7 L/hr without a decrease in its microbial removal performance. The flow rate of the other TERAFIL reached a maximum of only 2 L/hr. The Thimi filters have flow rates about 0.3 L/hr which are too low to be of any practical use. Therefore, if all manufactured TERAFILs can have the same high flow rate without sacrificing the microbial performance, it would be a valid technology for the MIT Nepal Water Project to test on a pilot scale in rural areas of Nepal to determine both technical performance under field conditions and just as importantly, social acceptability and good practice. However, it is also recommended that a small application of chlorine and/or solar disinfection is used to completely reduce the indicator counts to zero per 100 ml, as required in the WHO Drinking Water Guidelines.

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APPENDIX A – ADDITIONAL TABLES AND DRINKING WATER GUIDELINES AND STANDARDS

Table A1: Potential waterborne pathogens and their associated diseases. (AWWA, 1999)

Organism	Major disease	Primary source
Bacteria		
Salmonella typhi	Typhoid fever	Human feces
Salmonella paratyphi	Paratyphoid fever	Human feces
Other salmonella sp.	Gastroenteritis (salmonellosis)	Human/animal feces
Shigella	Bacillary dysentery	Human feces
Vibrio cholerae	Cholera	Human feces, coastal water
Pathogenic Escherichia coli	Gastroenteritis	Human/animal feces
Yersinia enterocolitica	Gastroenteritis	Human/animal feces
Campylobacter jejuni	Gastroenteritis	Human/animal feces
Legionella pneumophila	Legionnaires' disease, Pontiac fever	Warm water
Mycobacterium avium intravellulare	Pulmonary disease	Human/animal feces, soil, water
Pseudomonas aeruginosa	Dermatitis	Natural waters
Aeromonas hydrophila	Gastroenteritis	Natural waters
Helicobacter pylori	Peptic ulcers	Saliva, human feces?
Enteric viruses		
Poliovirus	Poliomyelitis	Human feces
Coxsackievirus	Upper respiratory disease	Human feces
Echovirus	Upper respiratory disease	Human feces
Rotavirus	Gastroenteritis	Human feces
Norwalk virus and other caliciviruses	Gastroenteritis	Human feces
Hepatitis A virus	Infectious hepatitis	Human feces
Hepatitis E virus	Hepatitis	Human feces
Astrovirus	Gastroenteritis	Human feces
Enteric adenoviruses	Gastroenteritis	Human feces
Protozoa and other organisms		
Giardia lamblia	Giardiasis (gastroenteritis)	Human/animal feces
Cryptosporidium parvum	Cryptosporidiosis (gastroenteritis)	Human/animal feces
Entamoeba histolytica	Amoebic dysentery	Human feces
Cyclospora cayatanensis	Gastroenteritis	Human feces
Microspora	Gastroenteritis	Human feces
Acanthamoeba	Eye infection	Soil and water
Toxoplasma gondii	Flu-like symptoms	Cats
Naegleria fowleri	Primary amoebic meningoencephalitis	Soil and water
Blue-green algae	Gastroenteritis, liver damage, nervous system damage	Natural waters
Fungi	Respiratory allergies	Air, water?

Table A2: Waterborne pathogens and their significance in water supplies. (WHO, 1993, 2000 and AWWA, 1999)

Organism	Disease	Symptoms	Primary Source	Health Significance	Persistence in water supplies	Resistance to chlorine	Relative infective dose	Important animal reservoir
Bacteria								
Escherichia coli	Gastroenteritis	**Diarrhea**	**Human/animal feces**	High	Moderate	Low	High	Yes
Legionella pneumophila	Legionellosis	Acute respiratory illness	Warm water					
Leptospira	Leptospriosis	**Jaundice**, **fever**						
Salmonella typhi	Typhoid fever	**Fever**, **diarrhea**	**Human feces**	High	Long	Low	High	No
Salmonella	Salmonellosis	**Food poisoning**	**Human feces**	High	Moderate	Low	High	Yes
Shigella	Shigelloisis	**Bacillary dysentery**	**Human feces**	High	Short	Low	Moderate	No
Vibrio cholerae	Cholera	**Heavy diarrhea**, dehydration	**Human feces, coastal water**	High	Short	Low	High	No
Yersinia enterolitica	Yersinosis	**Diarrhea**	**Human/animal feces**	High	Long	Low	High (?)	No
Viruses								
Adenovirus	Respiratory disease			High		Moderate	Low	No
Enteroviruses (67 types, including **polio**, echo, etc.)	Gastroenteritis, heart anomalies, meningitis		Human feces	High	Long	Moderate	Low	No
Hepatitis A	Infectious hepatitis	**Jaundice**, **fever**	**Human feces**	High		Moderate	Low	No
Norwalk agent	Gastroenteritis	Vomiting	Human feces	High			Low	No
Reovirus	Gastroenteritis						Low	No
Rotavirus	Gastroenteritis		Human feces	High			Moderate	No (?)
Protozoa								
Balantidium coli	Balantidiasis	**Diarrhea**, **dysentery**						
Cryptosporidium	Cryptosporidiosis	**Diarrhea**	**Human/animal feces**	High	Long	High	Low	Yes
Entamoeba histolytica	Amebiasis	**Diarrhea**, bleeding	**Human feces**	High	Moderate	High	Low	No
Giardia lamblia	Giardiasis	**Diarrhea**, nausea, indigestion	**Human/animal feces**	High	Moderate	High	Low	Yes
Helminths								
Ascaris lumbricoides	Ascariasis	Roundworm infestation						
Enterobius vericularis	Enterobiasis	Pinworm						
Fasciola hepatica	Fascioliasis	Sheep liver fluke						
Hymenolepis nana	Hymenolepiasis	Dwarf tapeworm						
Taenia saginata	Taeniasis	Beef tapeworm						
T. solium	Taeniasis	Pork tapeworm						
Trichuris trichiura	Trichuriasis	Whipworm						

Organisms associated with the common diseases in Nepal are highlighted in **Bold.**

Table A3: WHO bacteriological quality of drinking water[a] (WHO, 1996)

Organisms	Guideline value
All water intended for drinking	
E.coli or thermotolerant coliform bacteria[b,c]	Must not be detectable in any 100ml sample
Treated water entering the distribution system	
E.coli or thermotolerant coliform bacteria[b]	Must not be detectable in any 100ml sample
Total coliform bacteria	Must not be detectable in any 100ml sample
Treated water in the distribution system	
E.coli or thermotolerant coliform bacteria[b]	Must not be detectable in any 100ml sample
Total coliform bacteria	Must not be of samples detectable in any 100ml sample. In the case of large supplies, where sufficient samples are examined, must not be present in 95% of samples taken throughout any 12-month period.

[a] Immediate investigative action must be taken if either *E.coli* or total coliform bacteria are detected. The minimum action in the case of total coliform bacteria is repeat sampling; If these bacteria are detected in the repeat sample, the cause must be determined by immediate further investigation.
[b] Although *E.coli* is the more precise indicator of fecal pollution, the count of thermotolerant coliform bacteria is an acceptable alternative. If necessary, proper confirmatory tests must be carried out. Total coliform bacteria are not acceptable indicators of the sanitary quality of rural water supplies, particularly in tropical areas where many bacteria of no sanitary significance occur in almost all untreated supplies.
[c] It is recognized that, in the great majority of rural water supplies in developing countries, fecal contamination is widespread. Under these conditions, the national surveillance agency should set medium-term targets for the progressive improvement of water supplies, as recommended in Volume 3 Guidelines for drinking-water quality.

Table A4: EPA National Primary Drinking Water Standards (USEPA, 2002)

Microorganisms	MCLG[1] (mg/L)[2]	MCL or TT[1] (mg/L)[2]	Potential Health Effects from Ingestion of Water	Sources of Contaminant in Drinking Water
Cryptosporidium	as of 01/01/02: zero	as of 01/01/02: TT [3]	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human and animal fecal waste
Giardia lamblia	zero	TT[3]	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human and animal fecal waste
Heterotrophic plate count	n/a	TT[3]	HPC has no health effects, but can indicate how effective treatment is at controlling microorganisms.	HPC measures a range of bacteria that are naturally present in the environment
Legionella	zero	TT[3]	Legionnaire's Disease, commonly known as pneumonia	Found naturally in water; multiplies in heating systems
Total Coliforms (including fecal coliform and *E. Coli*)	zero	5.0%[4]	Used as an indicator that other potentially harmful bacteria may be present[5]	Coliforms are naturally present in the environment; fecal coliforms and E. coli come from human and animal fecal waste.
Turbidity	n/a	TT[3]	Turbidity is a measure of the cloudiness of water. It is used to indicate water quality and filtration effectiveness (e.g., whether disease-causing organisms are present). Higher turbidity levels are often associated with higher levels of disease-causing microorganisms such as viruses, parasites and some bacteria. These organisms can cause symptoms such as nausea, cramps, diarrhea, and associated headaches.	Soil runoff
Viruses (enteric)	zero	TT[3]	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human and animal fecal waste

[1] Definitions:
Maximum Contaminant Level (MCL) - The highest level of a contaminant that is allowed in drinking water. MCLs are set as close to MCLGs as feasible using the best available treatment technology and taking cost into consideration. MCLs are enforceable standards.
Maximum Contaminant Level Goal (MCLG) - The level of a contaminant in drinking water below which there is no known or expected risk to health. MCLGs allow for a margin of safety and are non-enforceable public health goals.

[2] Units are in milligrams per liter (mg/L) unless otherwise noted. Milligrams per liter are equivalent to parts per million.

[3] EPA's surface water treatment rules require systems using surface water or ground water under the direct influence of surface water to (1) disinfect their water, and (2) filter their water or meet criteria for avoiding filtration so that the following contaminants are controlled at the following levels:

- *Cryptosporidium:* (as of January 1, 2002) 99% removal/inactivation
- *Giardia lamblia:* 99.9% removal/inactivation
- Viruses: 99.99% removal/inactivation
- *Legionella:* No limit, but EPA believes that if *Giardia* and viruses are removed/inactivated, *Legionella* will also be controlled.
- Turbidity: At no time can turbidity (cloudiness of water) go above 5 nephelolometric turbidity units (NTU); systems that filter must ensure that the turbidity go no higher than 1 NTU (0.5 NTU for conventional or direct filtration) in at least 95% of the daily samples in any month. As of January 1, 2002, turbidity may never exceed 1 NTU, and must not exceed 0.3 NTU in 95% of daily samples in any month.
- HPC: No more than 500 bacterial colonies per milliliter.

[4] No more than 5.0% samples total coliform-positive in a month. (For water systems that collect fewer than 40 routine samples per month, no more than one sample can be total coliform-positive). Every sample that has total coliforms must be analyzed for fecal coliforms. There may not be any fecal coliforms or *E. coli*.

[5] Fecal coliform and *E. coli* are bacteria whose presence indicates that the water may be contaminated with human or animal wastes. Disease-causing microbes (pathogens) in these wastes can cause diarrhea, cramps, nausea, headaches, or other symptoms. These pathogens may pose a special health risk for infants, young children, and people with severely compromised immune systems.

APPENDIX B – MANUFACTURING PROCEDURES OF SOME OTHER CERAMIC FILTERS

New Improved Earthenware Water Purifiers, a Sneak Preview of Candle Production

Dhaka, Bangladesh March 8, 2002

Earthenware water purification candles are set to be introduced within the coming weeks, to Bangladesh based organizations that are dedicated to safe water options for the poor. Shown above is our press operator, Mr. G. Mortuja Milon. He is one of our hard working crew, determined to get these low cost, US$0.25 (25 cent) candles to the poor, for 100% removal of the fecal coliforms that cause gastro intestinal diseases.

In his left hand he holds a newly produced earthenware candle, in his right an injection molded, plastic cover cap, ready for sealing onto the open end of a fired candle. Then the candle unit will be ready for insertion into the bottom of a clean bucket, to be suspended above a second container which will catch the purified water.

Following is a line tour of the production of these low cost, earthenware water purifiers. We believe that over time these candles will prove sustainable with respect to effectiveness, ease of use and low cost. And since the resources necessary in fabricating the candles are available almost anywhere, this production line is widely replicable.

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Photo 2: Parts of the Candle Die

The production system to be used for the earthenware candles consists of a die and press. Shown here are the parts of the die into which the candle's clay composition is pressed. All exterior parts of the die are made of iron, while those parts which make contact with the clay are made of polished stainless steel.

The right hand of Mr. Milon is shown on the spindle, which occupies the space that will become the inside of the candle. Clockwise from the spindle, the other parts include, beneath the left hand, the outer cladding of the die, then the two, half cylinder inserts for this. At bottom right is the cylindrical form used in the first pressing, for compacting the clay composition around the spindle. At bottom left is the form used for the second of the two pressings, to push clay over the top of the spindle, forming the closed end of the candle.

Photo: 3: Preparing to Charge the Die

In this photo Mr. Milon is getting ready to put together the half cylinder, stainless steel inserts.

Photo 4: Placing the Stainless Steel Inserts

Together these are pushed into the outer cladding

Photo 5: Pouring the Clay Composition Into the Die

Once the inserts are in place Mr. Milon begins to pour the damp clay composition into the die, around the spindle. He then tamps the material down with a stick, in effect a first pressing.

Photo 6: The New Improved Screw Press

After placing the cylindrical form (shown at bottom right in photo 2) into the die, pressure is applied to the clay material, thus compacting it. This is accomplished using this newly designed press, which offers a clear advantage over the hydraulic truck jacks previously used in candle pressing. (Truck jacks are not designed to withstand the repeated use necessary in candle pressing, and the hydraulics tend to fail after a few hundred pressings, or less.)

Photo 7: Preparing for the Second Pressing

Following the first pressing additional clay composition is poured into the die, on top of the spindle, as shown here. Then Mr. Milon places into the die the form shown at bottom left of photo 2. Following this is the second pressing during which the clay material will be compacted, covering the closed end of the candle. Note that the second pressing is not shown here, in appearance resembling the first, as shown in photo 6.

Photo 8: Removing the Candle from the Stainless Steel Insert

After the second pressing the candle is removed, the equipment ready for another candle. The entire process of forming a candle by the method shown takes about 5 minutes for a single operator, but with several helpers this time is reduced substantially.

Following this forming process the candles are dried then fired to about 800 degrees centigrade. This step gives them the strength necessary in ensuring a long lifetime. Prior to sale of the candles the last step is to saturate these with a very tiny amount of silver, the anti microbial which acts with the filtration process in ensuring the 100% removal of harmful bacteria.

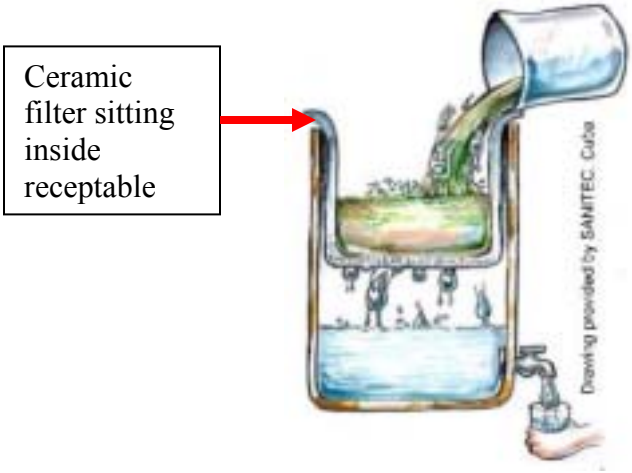
Photo 9: The Injection Mold, for Forming the Plastic Cover Caps of the Candle

The plastic cover cap is made using the injection mold shown here. This mold, the die and press shown in this photo essay cost $450.00. Along with the simple kiln and other processing equipment necessary in fabricating the candles these allow for a startup cost of less than $1000, or in some cases no more than about $400.00.

The candle price will be kept low by getting village potters producing them around the country. Thus the poor will be purchasing water purifiers made near their own community, by small entrepreneurs of similar means. Because of appropriate technology with respect to use of the candle, and it's manufacture, this offers an attractive alternative to the arsenic tainted water from tube wells. This alternative makes possible the collection of surface water.

Potter's For Peace (PFP) Filter

The Potter's For Peace filter unit consists of three separate parts: 1) a porous clay filter medium, 2) a larger clay recipient canister (a plastic bucket can be substituted) and lid, and 3) a spigot attached to the bottom. The filter medium itself is 31 cm in diameter, 24 cm high, holds 7.1 liters of water, and is shaped like a coned flowerpot. The filter medium sits inside the receptacle like a vegetable steamer sits inside the steaming pot. The filter is coated with colloidal silver as a microbicide/disinfectant. This filter should offer a flow rate of 1 ¾ L/hr. (PFP website http://www.potpaz.org/ , 2002).



Raw Materials Required

1. **Dry powder clay** (which can be obtained from the following sources)
    - Any clay available at the factory? "Depending on the clay's natural porosity, proportions of sawdust to clay will vary." (PFP website, 2001)
    - Pulverized? Grain size?
    - Brick clay (To better simulate local conditions and raw material. As in video, unwanted bricks are picked up from dump sites and manually mashed into fine grains.)
2. **Dry sawdust**
3. **Water**
4. **Filtered water** for dilution
5. **3.2% Colloidal Silver** (Microdyne) – 2ml per filter

Equipment Required

1. Screens
    - 25-35 mesh (larger)
    - 60 or 80 mesh (smaller)
2. (Mechanical mixer) – Not required if we just do the mixing manually.
3. (Potter's wheel/mold press) – Not required, if we just form the flower pot (or any shape) by hand.
4. **Buckets** for mixing (Prepare at least **2**)
5. **Temporary mold** e.g. **plastic pail** slightly smaller than lower receptacle – to simulate the actual dimensions of the PFP filter. (Or, as in video, we form the filter shape by hand in the

bucket, which is used as the temporary mold → filter shape is always smaller than the bucket)
6. Firing kiln
7. **Brush** for colloidal silver application
8. **250ml beaker** to dilute colloidal silver
9. **Stirrer**

MANUFACTURING PROCESS
(Own comments are in *italics.*)

1. Dry pulverized clay e.g. bricks is mashed to fine grains (size not specified).
2. Dry sawdust is screened between a 35 mesh and a 60 mesh, keeping only what stays between the two screens. (Size of sawdust important.)
3. 1 bucket of dry pulverized clay (60%) mixed with 0.8 bucket of dry sawdust (40%), either manually or in a mixer.
   (PFP suggests 50-50% ratio as a starting point for the above components.)
4. Slowly add water while trying to wedge until you get to a consistency that you find workable.
   Actual amount of water to be added is not specified. I guess that is not important, because "consistency" here should just mean "workability". As long as the mixture is not too dry or too wet to mould into shape, it should be fine.
5. "Throw them if you can." The filters are then formed by hand, turned on a potter's wheel, or press-molded. (See later for types of press mold)
   I have no idea what "throwing" means.
6. Make about 5 of them (in case they break).
7. Let them dry slowly and fire them when they are ready.
8. Filters are fired at 887°C degrees in a brick kiln. All battery of tests should be fired at the same cone. (Fuel source can come from wood scraps from industry.)
   "Try a few at cone 014 and then some at 010, etc. Keep in contact."
   I have no idea what this means, maybe the potter will know. I also need to know how long the filter has to be fired at this temperature.
9. Filters are allowed to cool.
   Any point from here onwards can be carried out back in MIT lab.
10. Filters are soaked for 24 hours to saturate the filter before flow testing.
11. The flow rate of each filter is tested. Measure the amount of water that seeped from the filter in one hour. Batteries of flow rate tests are run to determine adequate clay/burn material ratios. Ratios will differ for every clay deposit used. PFP design flow rate to be achieved is at least 1 ¾ L/hr.
    This sounds like a trial and error method. Since nature of clay (cetaris paribus) is highly variable depending on where the clay is obtained, different filter prototypes with varying clay:sawdust ratio are made to test for flow rates. Once the PFP design flow rate of 1 ¾ L/hr is achieved, the proportion of clay to sawdust is recorded and kept constant.
    → sawdust quality, size assumed to be constant. That is why the sieving process must be consistent.
    → mold press pressure, water added also assumed to be kept constant.
12. Filters are allowed to dry again.

13. 2 ml of 3.2 percent colloidal silver (Microdyne) in 250 ml of filtered water are applied with a brush to each filter.
    According to Daniele's PFP, 2/3 is applied to inner surface and the remaining 1/3 is applied to outside.
14. Filters are dried and prepared for sale or use.

"The PFP filter is currently sold for US$4.00 per filter to primarily NGOs interested in establishing their own water filtration program. Filters are sold without packaging or a finished water receptacle." (PFP website, 2001)

Further comments from Ron Riveria

"I spoke to Rod Bagley, a retired Corning Inc ceramist, about 2 years ago concerning the PFP approach. He said it is inherently difficult to control porosity in fired clay because of the complex chemistry involved in the sintering process. Rod suggested using the maximum amount of previously fired clay-ground to the desired particle size-in the initial process. Reid Harvey (http://www.geocities.com/ceramafrique/) replied that his concern was using enough unfired clay in his pressed clay to make sure the candle held together in the firing step but that he would look into increasing the level of 'grog' (sized fired clay) in his studies."

3 Ways to Make Mold Press (For Information ONLY)
"One method PFP encourages to accentuate consistency is the use of a press mold for forming the filter unit." (PFP website, 2001)
This section is perhaps more useful when in Thimi, Nepal.

Source: Email from Ron Rivera, Nov 22, 2001
"Attached are three ways that these presses have been made (there are other designs also):

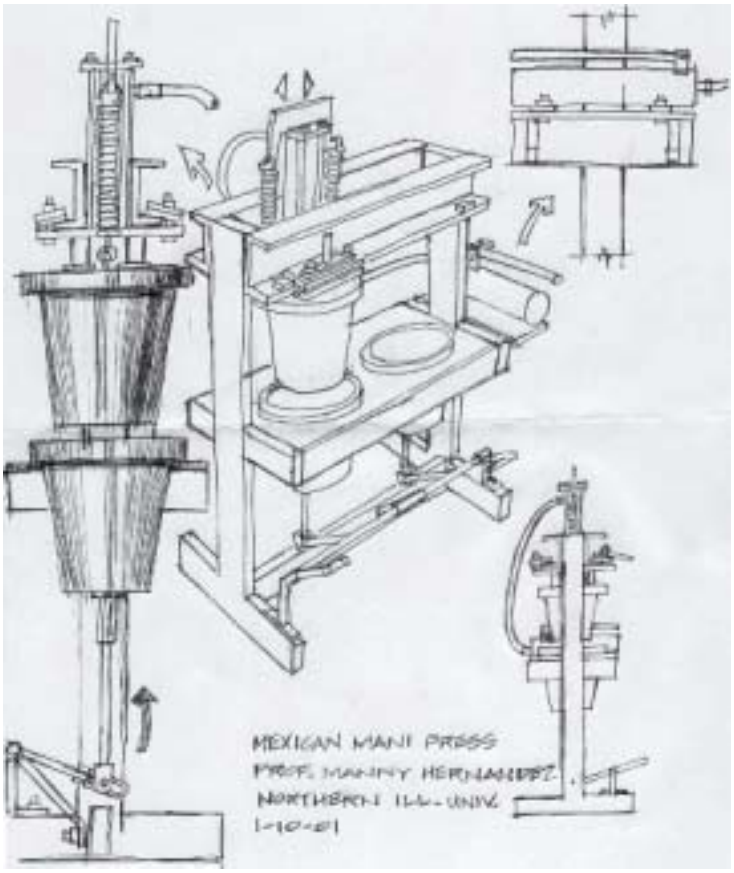
1) In Bangladesh (the red press) they actually made the aluminum molds at a local foundry, rented a large mechanics hydraulic press and started making prototypes, through trail and error based on a starting point of 50/50 clay and sawdust mix (in volume) and fired to about 860 degrees Centigrade they reached the correct filtration rate of 1 to 2 liters an hour. Danielle's research is going to tell us if that is the most appropriate rate but
that's what we have been doing for 20 years.

Bangladesh Press

2) The screw press was developed in Cambodia and they two had an aluminum foundry make the 2 molds. It's a killer to use but it works. They report very positive microbiological test results. Hopefully they will soon sell enough filters to actually adapt he screw press to a hydraulic system.

Your potter will get a good laugh at this technology and probably have allot of questions, please tell him or her to contact me, Potters have a different language about technology.

3) In Chihuahua Mexico the Tahumara Indians we work with use this press, the biggest problem is that the hydraulics breaks down allot and it a takes a long time to fix it. I highly recommend the use of a 12 to 15 ton hydraulic truck jack found easily in every country in the world. It's pretty inexpensive, there are many mechanics that fix them, and they are very portable and designed to be reliable."



Mexican Mani Press