WRRC-98-01

VULNERABILITY TO PATHOGENS: WATER QUALITY MONITORING AND ASSESSMENT STUDY

VULNERABILITY TO PATHOGENS: WATER QUALITY MONITORING AND ASSESSMENT STUDY

Roger S. Fujioka
and
Bunnie S. Yoneyama

WRRC Project Completion Report: WRRC 98-01

October 1997

Prepared for:

Honolulu Board of Water Supply
City and County of Honolulu
630 South Beretania Street
Honolulu, Hawaii 96843

Agreement Number: Contract No. C33814
Project Period: August 15, 1994 to August 31, 1997
Principal Investigator: Roger S. Fujioka

Water Resources Research Center
University of Hawaii at Manoa
2540 Dole Street
Honolulu, Hawaii 96822

CHAPTER ONE
INTRODUCTION TO STUDY

I. Groundwater Disinfection Rule (GWDR)

The Groundwater Disinfection Rule (GWDR) is one of the few regulations mandated by the amended Safe Drinking Water Act (SDWA) of 1986 which is still being formulated. The basis of the GWDR is to implement a general goal of USEPA to eliminate water borne diseases by requiring all potable water supplies to be disinfected. The need to disinfect groundwater sources was fueled by nation wide statistics cited by USEPA which indicated that groundwater systems a) have been implicated in nearly half of all water borne disease outbreaks, b) are responsible for 85% of coliform MCL violations, c) provide water for approximately 50% of nation's population and d) are often untreated and only 55% of these systems undergo some sort of disinfection (Macler and Pontius, 1997). The 1986 SDWA also set an MCLG of zero for pathogens such as human enteric viruses, giardia and even *Legionella* bacteria but did not require monitoring for these pathogens. Instead, reliance was placed on good management practices and disinfection to meet this goal. To implement this goal, the first proposed draft groundwater disinfection rule was circulated as a "strawman" document by USEPA in 1992 (Grubbs and Pontius, 1992). This proposed rule was highly criticized by some members of the utilities, state and county regulators, as well as those representing the private and academic sectors. A major criticism was the top-down approach and the assumptions that were used to implement a restrictive plan which essentially indicated that routine disinfection of all groundwater sources would be the effective solution. As a result of these criticisms, this initial USEPA plan for the GWDR was withdrawn by USEPA.

To develop a satisfactory GWDR, USEPA appointed Dr. Bruce Macler of USEPA Region IX to be the manager for the formation of the new GWDR. Dr. Macler's approach has been to provide as much information as possible on the needs for a GWDR and to use a bottom-up approach to reach a consensus for a GWDR plan. As a result, he has worked diligently and tirelessly to obtain input from stakeholders and all sectors of the community through numerous teleconferences, seminars, workshops, and conferences in order to reach a consensus for an implementable GWDR. In 1996 the SDWA was reauthorized and a timetable of August 1999 was established to implement the new GWDR (Macler and Pontius, 1997). Dr. Paul Berger, USEPA microbiologist, has been given the authority to assist Dr. Macler in establishing the microbiological monitoring requirements for the GWDR.

As summarized by Macler (1997), the philosophical approach of the current GWDR is now focused on best management practices and a variety of barriers rather than only treatment barriers. The barriers identified are: a) protection from groundwater contamination, b) well and system integrity, c) distribution system protection and d) monitoring.

II. Impact of GWDR on Honolulu Board of Water Supply

The Honolulu Board of Water Supply (HBWS) is the public water supplier for the island of Oahu where nearly 80% of the population in the state of Hawaii resides. Currently, nearly 100% of the approximately 150 mgd of water provided by the HBWS is categorized as groundwater. Historically, and up to 1990, the HBWS distributed this source of groundwater to the public without routine disinfection and was still able to meet the coliform drinking water standard. Public confidence in the overall quality of drinking water provided by HBWS has historically been excellent and the most numerous complaints by the public occur when chlorine is added to the drinking water following spot chlorination due to pipe repairs or when the reservoir tanks occasionally become positive for coliform. These reservoir tanks are strategically placed throughout the island as a means to store water and to supply neighborhoods with water using gravity flow. However, each reservoir tank must have vents to enable water levels in these tanks to rise and fall. These same vents are potential sources of contamination with coliform bacteria because they may allow dust and insects to enter the tank.

New regulations under the 1986 amendments to the Safe Drinking Water Act have altered the management of water by the HBWS. One of these new regulations is the Surface Water Treatment Rule (Pontius, 1990a) which states that groundwater sources which are under the influence of surface waters must be categorized and treated as surface waters. Although most of the groundwater sources used by the HBWS are deep aquifers, a small percentage (<1%) are shallow groundwater sources called tunnels or springs. Recently, these waters were evaluated and the few sources which were determined to be under the influence of surface waters were deleted as drinking water sources. Two other new rules have had a significant impact. The new total coliform rule (Pontius, 1990b) records a violation when any coliform is present in 100 ml of water sample and requires an additional test to confirm whether that coliform is a member of total coliform, or fecal coliform or *E. coli*. Another rule called the public notification rule (Pontius, 1990b) requires the water utility to go to immediate public notification of possible health hazard if the coliform bacteria initially detected is confirmed as a fecal coliform or *E. coli*. Public notification results in public distrust of the water utility which is a serious management problem. Together, these two rules place great pressure on water utilities to provide coliform free water. To address these new regulations, the HBWS has begun a program of selectively chlorinating those reservoir tanks and wells which have had a history of coliform contamination. However, since the coliform contamination from the wells and tanks is minimal and sporadic, the dosage of chlorine used is low (0.1 mg/l residual) and at this low level of chlorination, the water which reaches the distribution system contains only trace levels of chlorine which are generally undetectable by taste. Under these conditions, public complaint of bad tasting (chlorinated) water has not increased and HBWS is meeting the more recent and more stringent coliform rule.

The impending Groundwater Disinfection Rule (GWDR) may greatly impact the operation of the HBWS because the basic intent of this rule is to protect the public from groundwater sources which may become contaminated with water-borne pathogens by

routinely disinfecting all groundwater used for potable purposes. This new rule was initially described in the first Draft Groundwater Disinfection Rule (Grubbs and Pontius, 1992) which essentially indicated that the best national policy was to routinely disinfect all groundwater sources used for drinking and to maintain a disinfectant residual of 0.2 mg/l at the start of the distribution system. A variance to this rule could be obtained if the water utility was able to show that its groundwater sources were not vulnerable to contamination with feces or sewage. It was the publication of the first draft of the GWDR which prompted the HBWS to initiate the present study to obtain information to demonstrate that its groundwater sources are not vulnerable to contamination with sewage.

Although the first draft of the GWDR has been withdrawn by USEPA and a new GWDR is currently being formulated, the water utility which chooses to apply for a variance will still be required to demonstrate that its groundwater aquifers are not vulnerable to contamination with sewage. In this regard, the HBWS has traditionally not disinfected its groundwater on a routine basis and has publicly stated its position that since its groundwater sources are not vulnerable to contamination with sewage, it will seek a variance to the planned GWDR (Honolulu Advertiser, 1993).

III. Overall Goal and Experimental Design of Study

The overall goal of this study was to establish a microbial water quality monitoring program to determine whether HBWS deep groundwater sources and water in the potable distribution system may be vulnerable to contamination by fecal matter. The objective of this study was to obtain monitoring data to assess the microbial quality of groundwater as a prerequisite for HBWS to seek a variance to the upcoming GWDR.

It should be noted that at the start of this study, the guiding document was the first draft of the GWDR proposed by USEPA (Grubbs and Pontius, 1992). The initial design of this study was made after consultation with the USEPA officials who authored the first draft of the GWDR. After this initial GWDR plan was withdrawn by USEPA, and Dr. Bruce Macler of USEPA was appointed as manager to formulate a new GWDR, the intent of this study was to be an active contributor in the process of formulating the specifics for the new GWDR. Despite a change in the implementation of the GWDR, the basic criteria to determine whether a groundwater should or should not be routinely disinfected will still depend on establishing whether the groundwater sources are or are not vulnerable to contamination by feces or sewage. Monitoring the groundwater for fecal microbial indicators will be a requirement to determine whether the groundwater is vulnerable to contamination with sewage.

The experimental design of this study followed three approaches. The first approach was to obtain as many water samples as could be handled from groundwater well sites and from distribution sites which are being used by HBWS. This approach would ensure that samples representing all sources of drinking water would be analyzed. The second approach was to analyze larger volumes of sample than the minimal 100 ml which is the current

monitoring protocol. This approach would increase the sensitivity of each assay. The third approach was to monitor water samples for several potential microbial fecal indicators. This approach would better characterize the quality of the water with respect to vulnerability to contamination. Since selection of the fecal indicator to be monitored under the GWDR has yet to be determined, this study had to anticipate which fecal indicators would be used in the final GWDR. Since additional information is often required to better interpret the quality of groundwater; the proposed monitoring program also included determination of other water quality parameters such as total heterotrophic bacteria, turbidity, total organic carbon and residual chlorine.

IV. Sampling Sites

A. Identification of Sample Sites. The HBWS has divided the island of Oahu into the following seven water districts: 1) Honolulu. 2) Pearl Harbor. 3) Windward. 4) Waialua/Kahuku. 5) Wahiawa. 6) Waianae. 7) Ewa (see Figure 1). The identification and description of each well from which water samples were obtained are summarized in Table 1 whereas Table 2 identifies the limited number of tunnel and spring sites. Table 3 identifies the sites where water samples were obtained from the distribution system. The source of all groundwater is rainfall and like most islands, the windward is the wet side of the island while the leeward is the dry side of the island. This is accentuated on the island of Oahu due to the predominant northeastern tradewinds which transport warm, moisture laden air to the steep, lofty Koolau Mountains which string across the northeastern end of the island. The ocean air is cooled by the high rising mountains resulting in daily rainfall on the Koolau Mountains which then act as a natural means to collect and to allow water to seep into its groundwater basin. As a result, the largest groundwater aquifers and those containing the highest number of wells are in the Pearl Harbor, Windward and Honolulu water districts.

B. Collection of Water Samples. Wells and distribution systems where water samples were collected are under the security of the HBWS. All water samples were collected by HBWS personnel using their standardized and approved method of collecting water. The University of Hawaii team provided the HBWS with the sterile sample containers, retrieved the water samples from the HBWS and analyzed these samples for indicator bacteria within eight hours of collection.

CHAPTER TWO
PHASE 1: MONITORING FOR FECAL INDICATOR BACTERIA

I. Identification of a Need

The coliform group of bacteria is commonly referred to as fecal indicator bacteria because historically the presence of this group of bacteria in water was used to indicate that the water was contaminated with fecal matter or sewage. Although coliform bacteria have been very useful for monitoring the pollution of surface water sources, they have been less useful in the monitoring of groundwater sources because of reported incidences of transmission of water borne diseases by groundwater even when these waters met coliform standards. These results are consistent with the known characteristics of fecal bacteria and sewage borne pathogens such as human enteric viruses. For example, it has been well established that fecal bacteria are effectively filtered out by soil and die off within a few weeks. On the other hand, human enteric viruses are much smaller, much more stable than bacteria and therefore can more easily be transported for long distances through the soil profile to contaminate groundwaters. Moreover, because the infectious dose of these viruses is much lower than pathogenic fecal bacteria, groundwater contamination by human enteric viruses present an increased risk of water borne diseases. Recently Craun et al (1997) reviewed the groundwater monitoring data and concluded that coliform bacteria are not reliable indicators of groundwater quality because many groundwater samples which were negative for coliform bacteria were positive for human enteric viruses. However, it should be noted that the volume of sample used to assay for fecal bacteria is usually 100 ml while the volume of sample assayed for human enteric viruses is in the order of 200 to 400 liters. Based on volume of sampled assayed, the monitoring data for fecal indicator bacteria and for human enteric viruses are not be comparable. These results indicate that larger volumes of water should be analyzed for coliform bacteria.

In summary, the available evidence indicate that the traditional method of analyzing 100 ml of water for coliform bacteria is not a reliable means to determine whether groundwater may be contaminated with sewage borne pathogens such as human enteric viruses. Despite this recognition, there is still a desire by many in the water industry (regulators, educators, managers, laboratory analysts) to maintain the system of monitoring groundwater for bacteria of fecal origin because the methodology, the theory and the interpretation of recovering fecal bacteria in water samples are familiar to the water industry.

II. Objective and Experimental Design

The objective of Phase 1 of this study was to develop a reliable fecal bacteria monitoring program to determine the hygienic quality of potable sources of water from well sites and from distribution sites used by the HBWS and to determine whether these sources of water are vulnerable to contamination with sewage.

Three experimental approaches were taken in establishing the experimental design for this phase of the study. First, water samples were analyzed for all of the fecal bacteria (total coliform/fecal coliform/*E. coli*, fecal streptococci, *C. perfringens*) which are currently being discussed as probable candidates to monitor groundwater under the upcoming GWDR. Second, the minimum 100 ml and a larger but still reasonable 1000 ml volume of sample water was analyzed for the most commonly used fecal indicator bacteria (total coliform, fecal coliform, *E. coli*, fecal streptococci) as a means to increase the sensitivity of the test method. Third, other relevant water quality parameters (total heterotrophic bacteria, hydrogen sulfide bacteria, turbidity, total organic carbon, chlorine residual) were determined to better characterize the quality of the water.

In the selection of fecal indicator bacteria, a conscious effort was made to include bacteria representing different sizes, different shapes, as well as different physiological and genetic groups. Since these different classes of bacteria have differing survival and movement characteristics within a soil profile, data obtained from several classes of bacteria will be superior to data collected from one bacteria alone. The different classes of bacteria and the representative groups selected are as follows:

a) Gram negative, fecal bacteria: total coliform, fecal coliform, *E. coli.*
b) Gram positive, fecal bacteria: fecal streptococci.
c) Gram positive, spore-forming, anaerobic fecal bacteria: *Clostridium pefringens.*
d) Hydrogen sulfide producing bacteria: an experimental group of water quality bacteria.
e) Total heterotrophic bacteria: total viable count of all aerobic bacteria.

III. Materials and Methods

A. Sampling Design and Methods Used. The University of Hawaii team devised the sampling plan and analyzed all the samples. To increase the sensitivity of this study, a total of 1,100 ml of water (one 100 ml sample, two 500 ml samples) from each of the sites were added to presence/absence broth and incubated at 37°C for 48 hours (Standard Methods APHA, 1995) as a screening test for the presence of coliform group of bacteria as well as fecal streptococci group of bacteria. No change in turbidity, color or gas production in the presence/absence broth sample indicated a negative test for total coliform and fecal streptococci bacteria. A change to yellow color and/or gas production were considered presumptively positive for total coliform and the broth was subcultured to brilliant green lactose bile broth (BGLBB) and incubated for 48 hours at 37°C to confirm for the presence of total coliform. Only confirmed total coliform results were reported as positive for total coliform. Confirmed total coliform positive samples were further subcultured into EC plus MUG broth and incubated at 45°C for 24 hours. Growth in EC plus MUG media and presence of gram negative rod-shaped cells were considered positive confirmation for fecal coliform. An additional MUG positive reaction, fluorescence, in this same medium was confirmation for the presence of *E. coli*. Some cultures were also streaked onto EMB agar to

look for typical green sheened colonies. Standard Methods (APHA, 1989) indicated that the presence/absence broth can be used to screen for the growth of fecal streptococci as well as total coliform. In preliminary studies, we determined that water samples added to presence/absence broth and directly to azide dextrose broth gave similar results indicating that fecal streptococci will grow in presence/absence broth. Thus, after 48 hours of incubation, all presence/absence broth samples which showed evidence of bacterial growth (turbidity) were subcultured into azide dextrose broth and incubated at 37°C for 48 hours. Any growth (turbidity) in azide dextrose broth was considered presumptively positive for fecal streptococci bacteria and positive samples were then subcultured on bile esculin azide agar or m-enterococcus agar. Growth of typical target colonies such as brownish-black colonies on bile esculin agar and presence of gram positive cocci cells were taken as confirmation of fecal streptococci. Separate 100 ml samples were analyzed for *C. perfringens* on mCP medium utilizing the method as described by Bisson and Cabelli (1979), for total heterotrophic bacteria on mHPC medium as described in Standard Methods (APHA, 1989) and for hydrogen sulfide producing bacteria utilizing the method as described by Kromoredjo and Fujioka (1991). This microbial sampling design is summarized in Figure 2. Water samples were also analyzed for turbidity using a turbidimeter (Hach Model 2100A) and for total organic carbon using a TOC analyzer (Shimadzu Model 5000).

IV. Results and Discussion

A. Well Water. Under current regulations, water samples which do not contain total coliform bacteria in 100 ml samples are considered uncontaminated. For this study, 1,100 ml of untreated groundwater obtained directly from 39 wells located in the seven water districts were assayed for total coliform and fecal streptococci. In addition, 100 ml water samples were assayed for hydrogen sulfide bacteria, *C. perfringens* and total heterotrophic bacteria while smaller volumes of samples were needed to test for turbidity, and total organic carbon. The results of each of these assays are listed in Appendix A and show that multiple samples (2-4) were obtained from the majority of the monitoring wells with the exception of seven wells where single samples were obtained.

Of a total of 80 well water samples assayed for the various fecal bacteria, one well water sample (HS4-LS) collected during the early phase of this study was considered unsatisfactory because this well was not operating for some time and was not sufficiently flushed before a sample was obtained. This was the only water sample which was positive for total coliform, fecal coliform, *E. coli* and *C. perfringens*. The sample was also characterized by elevated total heterotrophic bacteria. This sample was not included in the final assessment and therefore the well water analysis was based on the 79 well water samples which were properly collected.

All well water samples which were negative for total coliform (72/79) are summarized in Table 4 and show that the percentages of these samples from the different water use districts ranged from 71 to 100%. Since the number of samples analyzed from

some districts were few, the percentages obtained could not be used to compare the quality of well water from one district to another. It was more reliable to assess the quality of groundwater by using all the data. Thus, 72/79 or 91.1% of the groundwater samples were negative for coliform bacteria and collectively these waters had an average turbidity of 0.65 NTU and an average total organic carbon of 3.4 mg/l. The average turbidity (1.27 NTU) and average TOC (3.87 mg/l) of the water samples which were positive for total coliform were similar suggesting that these water quality parameters did not drastically change in water samples from which coliform bacteria were recovered. Table 5 summarizes the percentage of the 79 well water samples which were positive for the various fecal bacteria and show that 7/79 or 8.9% were positive for total coliform, 2/79 or 2.5% were positive for fecal coliform and fecal streptococci, 1/79 or 1.3% was positive for *E. coli,* 4/79 or 5.1% were positive for hydrogen sulfide bacteria and 0/79 or 0% was positive for *C. perfringens*.

The correlation between the recovery of the various fecal indicators in the seven positive well water samples is summarized in Table 6 and shows that total coliform was the most frequently isolated of the fecal indicator bacteria. *C. perfringens* was never recovered from any of the well water samples whereas fecal streptococci was recovered from only two samples and hydrogen sulfide bacteria from four samples. It should be noted that water samples which were negative for total coliform were generally also negative for other fecal indicator bacteria, although there was one coliform negative sample which was positive for hydrogen sulfide bacteria. The total heterotrophic bacterial count of all the well water samples which were positive for coliform bacteria ranged from 19->400 CFU/100 ml (Table 6) with an average count of 87 CFU/100 ml and this was similar to well water samples which were negative for coliform bacteria which had an average heterotrophic bacteria count of 47 CFU/100 ml. These results again indicate that the other water quality parameters did not drastically change in water samples from which coliform bacteria were recovered.

Another objective of this study was to determine the effect of sample volume on the efficiency of recovering coliform bacteria. Table 7A shows that only 3/7 coliform positive were detected when the minimum volume of 100 ml was assayed. Thus, if only 100 ml of water sample were tested the percentage of coliform positive samples would have been 3/79 or 3.8%. By increasing the sample volume to 1,000 ml 4 additional water samples were determined to be positive for total coliform which increased the percent of positive coliform to 7/79 or 8.9%. These results show that by increasing the volume of sample to be tested, the sensitivity for recovery of total coliform and fecal coliform bacteria more than doubled.

B. Springs and Tunnels. These sources of water are considered shallow groundwater as compared to the typical groundwater wells. They comprise only a small fraction of the potable water supply. Water samples from only one spring and one tunnel site were obtained for analysis (see Appendix B). The results summarized in Table 5 show that the single spring and single tunnel water sample were both positive for presence of total coliform, fecal coliform, *E. coli,* fecal streptococci, hydrogen sulfide bacteria but not for *C. perfringens*. Although limited in number, these results suggest that spring and tunnel sources of water are vulnerable to contamination most likely from soil, since these indicators

are present in Hawaii's soil environment. However, the actual source for these fecal bacteria were not determined.

C. Distribution Water. Although distribution sites located in the seven water use districts predominantly obtain water from wells and reservoir in that district, it should be noted that the entire distribution system to all water districts is inter-connected. Moreover, HBWS has been selectively chlorinating those wells and reservoir tanks from which coliform bacteria have previously been detected. As a result, approximately 50% of the reservoir tanks are chlorinated but this chlorinated water is mixed in the distribution system with water from non-chlorinated reservoir tanks. The same sampling design used to assay groundwater samples was used to analyze distribution water samples. In addition, since selective low level chlorination was now a routine practice, residual chlorine measurements were made for water samples obtained from distribution sites. The measurable levels of chlorine were divided into three categories (0-weak, trace, >0.05 mg/l). Most of the water samples obtained from distribution sites (142/168) contained either 0-weak or trace levels of chlorine while 26/168 water samples contained at least 0.05 mg/l of chlorine (Table 8). The concentrations of turbidity and total organic carbon in these three categories of water were very similar indicating a similarity in the overall quality of all water in the distribution system. However, water samples with the highest measurable level of chlorine did result in the lowest average concentration of total heterotrophic bacteria. This observation most likely reflects the disinfecting effect of the added chlorine.

The results of the analyses of the water samples from each of the 85 distribution sites are summarized in Appendix C and show that each of the sites was analyzed 1-4 times resulting in a total of 152 water analyses. The results in Table 9 show that 149/152 or 98.02% of distribution water samples were negative for any of the fecal indicator bacteria and collectively these waters had an average turbidity of 0.75 NTU and an average TOC of 4.15 mg/l. Table 5 summarizes the percentages of the 152 distribution water samples which were positive for the various fecal indicator bacteria and show that 3/152 or 1.97% of the samples were positive for total coliform. None of the 152 samples was positive for fecal coliform, *E. coli*, fecal streptococci, *C. perfringens* or hydrogen sulfide bacteria.

The three water samples which were positive for coliform bacteria are listed in Table 7B. Of these three positive samples, only one was positive when 100 ml of water was analyzed. Using standard procedures, 151 of 152 or 99.3% of the samples would have been negative for coliform bacteria. By increasing the assay volume from 100 ml to 1,000 ml, two more positive samples were obtained increasing the percentage of coliform positive samples from 0.7% to 2%. All three coliform positive samples from the distribution system could not be confirmed for fecal coliform and did not contain any other fecal bacteria. Thus, although these three water samples were positive for total coliform, they do not appear to be contaminated with sewage. The source of this total coliform contamination was not determined.

CHAPTER THREE
PHASE 2: MONITORING FOR FECAL BACTERIAL VIRUSES

I. Identification of a Need

Human enteric viruses have been listed in the 1986 SDWA as one of the pathogens with a MCLG of zero and therefore these viruses should not be present in drinking water. Due to their small size, highly infectious nature and their pathogenicity, human enteric viruses are the most likely group of pathogens to contaminate groundwater sources. However, the assay method for human enteric viruses is too complex, too time consuming and too costly for routine monitoring of water. There is a need to develop a monitoring method for groundwater which can provide reliable data to show that groundwater sources are or are not vulnerable to fecal contamination and in particular contamination with human enteric viruses.

A review of the scientific literature reveals a consensus conclusion that the male-specific RNA bacterial viruses (FRNA phage) are the most reliable and feasible surrogates for the presence or absence of human enteric viruses in groundwater because this group of bacterial viruses has the same size, shape and genetic composition as human enteric viruses (IAWPRC, 1991). Moreover, like human enteric viruses, FRNA phages require living cells for multiplication and will not be able to multiply in the ambient environment because the host bacteria for FRNA phages requires temperatures exceeding 30^{0}C to support the growth cycle of this group of phages. Finally, FRNA phages are consistently found in measurable concentrations at least 100 times greater than human enteric viruses.

II. Objective and Experimental Design of Phase 2

The objective of Phase 2 of this study was to monitor groundwater and distribution water samples for FRNA phages as the best available means of determining whether the groundwater sources used by the HBWS are vulnerable to contamination with sewage and in particular with human enteric viruses.

The experimental approach of this study was to analyze 1 liter water samples obtained from wells, springs/tunnels and distribution system sites for coliphage and in particular FRNA phage.

III. Materials and Methods

The standard double agar method as described by Debartolomeis and Cabelli (1991) was used to visualize and quantitate the number of infectious FRNA phages in the sample as visible plaque forming units (PFU). FRNA phages infect only piliated *E. coli* strains. For this monitoring study, two piliated *E. coli* strains were used to assay for FRNA phages

because they have been reported to be sensitive to different groups of phages. The primary *E. coli* strain was the HS(pFamp)R developed by Cabelli and known to be specific for FRNA phage while being resistant to most DNA somatic coliphages. The second strain of *E. coli* selected was ATCC 15597 which detects both FRNA phages as well as DNA somatic coliphages. For confirmation studies, *E. coli* strain B was also used because this strain of *E. coli* allows only somatic phages to multiply.

The standard method for phage assay (APHA, 1995) is limited to an analysis of 100 ml of water sample. This is the same volume used to assay for indicator bacteria. To process 1,000 ml of water sample, a membrane adsorption and elution method (Borrega et. al. 1991) similar to that used for concentrating human enteric viruses from water was used. In this procedure, 1,000 ml of water was filtered through a 47 mm, 1MDS electropositive filter (Cuno, Meriden, CT) to allow the electronegatively charged phages to adsorb onto the filter while allowing the water to filter through. The virus adsorbed to the filter was then eluted off the filter by passing 4 ml of 3% beef extract at pH 8 through the filter. This was done twice. The two eluates were pooled and assayed for plaques on *E. coli* strain HS(pFamp)R and *E. coli* 15597. Using a known FRNA phage (MS2) added to 1,000 ml of water, the efficiency of recovering the FRNA phage by this method averaged about 50% but ranged from 23-66%. This was approximately the same efficiency as reported by Borrego et al (1991) and Sobsey (personal communication). Although this assay produces quantitative results, it has been well established that this method is not very efficient in recovering low concentrations of phages from large volumes of water because in good quality water the phages adsorb on so strongly to the membrane that elution is not effective while in poor quality water, the phages do not adsorb on efficiently to the membrane.

To increase the sensitivity of the method to recover phage, a pre-enrichment method commonly used for food samples and also used to analyze water samples for viruses by Yanko (personal communication) was employed. In this method, live *E. coli* strain HS (pFamp)R cells which are selective for the growth of only FRNA phages and some nutrients were added to 1,000 ml of water sample and incubated for 24-48 hours at 37°C. Under these conditions, the *E. coli* cells will multiply and even one phage in the 1,000 ml water sample will have the opportunity to infect one *E. coli* cell to produce thousands of new progeny FRNA phages which will in turn infect other *E. coli* cells. Thus, after the 48 hour incubation period, there should be thousands of phages or none at all in the enriched water sample. From this flask, 30 ml of the enriched sample was then filtered through a 0.45 um pore size membrane to remove bacterial cells while allowing the phages to pass through this filter. The filtrate was then assayed for the presence of phage. Although this method is not quantitative, it is a very sensitive assay which under laboratory testing conditions was able to detect a calculated concentration of 1 PFU of FRNA phage added to 1,000 ml of water.

IV. Results and Discussion

Initially 53 water samples (1,000 ml/sample) from 32 well sites, 11 distribution sites and 7 Springs or Tunnel sites were assayed for coliphages using the membrane

adsorption/elution method and the plaque assay on *E. coli* strain HS(pFamp)R and *E. coli* strain 15597. The results summarized in Table 10 show that all 53 samples were negative for the recovery of FRNA and somatic phages. These results provide monitoring data that when the currently recommended membrane adsorption/elution method was used, the groundwater and distribution sources of water used by HBWS contain undetectable levels of FRNA phage and somatic phage.

Rather than continuing to analyze more water samples using the membrane adsorption/elution method, we initiated a second round of analysis for FRNA virus using an experimental method utilizing pre-enrichment. This method greatly enhanced the ability to recover low concentrations of FRNA phage from 1,000 ml of water. Using this method, all 24 well water and 3 Spring or Tunnel water samples were negative for FRNA phage (Table 11). However, 3 of 52 (5.8%) water samples obtained from 49 distribution sites were positive for FRNA phage using this method. The phage recovered from the three distribution water samples (WA-5, WU-1, WU-6) were confirmed as FRNA phage since they produced plaques on *E. coli* strain HS(pFamp)R which is highly specific for FRNA phage but not on *E. coli* strain B which is selective for somatic phage. Within three months, water samples from sites WA-5 and WU-6 were retested and determined to be negative for FRNA phage.

In summary, the pre-enrichment method provided strong confirmatory evidence that the groundwater sources used by HBWS are not contaminated with FRNA phage and that the groundwater sources are not vulnerable to contamination with sewage. However, when the pre-enrichment method was used, FRNA phage was recovered from 3/52 water samples from the distribution system indicating a sporadic and low frequency contamination of distribution sources of water by FRNA phage. There is insufficient data is to determine the source of these bacterial viruses in the distribution system.

CHAPTER FOUR
PHASE 3: MONITORING FOR LEGIONELLA BACTERIA

I. Identification of a Need

Legionella bacteria was listed in the 1986 SDWA with a MCLG of zero because some of the species of *Legionella* are pathogenic to man and drinking water sources have been implicated in the transmission of Legionnaires disease to man. However, unlike other water-borne diseases, Legionnaires disease is caused by the inhalation of aerosolized water containing pathogenic *Legionella* rather than by drinking the water. Aerosolization of tap water used for cooling water, for showering, for humidifiers, for whirlpool baths, decorative fountains, grocery store mist machine and for respiratory care equipment have been reported as sources for the transmission of Legionnaires disease and other disease symptoms caused by pathogenic species of *Legionella* (Freije, 1996). However, an MCL has never been established for *Legionella* bacteria n drinking water sources because of the following findings: (1) *Legionella* spp. (approximately 40) are known to be commonly present in most environmental waters and have been detected in water from drinking water distribution systems. (2) Only a few species of *Legionella* are pathogenic to man. (3) Only a limited segment of the human population (elderly, those with chronic lung diseases, immunocompromised) are highly susceptible to legionellosis and most of the diseases occur in health care facilities (hospital). (4) The culture method for *Legionell*a bacteria is slow, costly and often unreliable. The Center for Disease Control does not recommend that all waters be routinely monitored for *Legionella* bacteria. (5) The presence and concentrations of *Legionella* in water sources have often not been associated with diseases. (6) The ideal growth range for *Legionella* spp. is between 35-46^{0}C. Therefore, these bacteria are normally found in low concentrations in most water sources and multiply only in heated water systems such as hot water tanks. (7) Recent evidence indicates that most of the multiplication of *Legionella* spp. occur within living amoeba cells rather than freely in water.

Although there have been numerous studies documenting the presence of *Legionella* bacteria in sources of surface water, there have been relatively fewer studies on the presence of *Legionella* bacteria from groundwater sources where normal temperatures are well below the optimum multiplication temperature (35-46°C) of this group of bacteria. In this regard, the approved method to detect the presence of *Legionella* bacteria in water samples is by culture. This is a slow (1-2 weeks), tedious, and relatively expensive method which limits the number of water samples that can be tested. Also the culture method often results in false negatives. There is a need for a rapid test which can be used to monitor many groundwater samples for presence of *Legionella* spp. and in particular the pathogenic *L. pneumophila.*

The polymerase chain reaction (PCR) method is the only rapid and feasible method which can simultaneously analyze many water samples for the presence of all species of *Legionella* (*Legionella* spp.) as well as for *L. pneumophila*, the pathogenic species responsible for 85-90% of legionellosis. The basis of this method is the detection of a gene

sequence specific to all species of *Legionella* and another gene sequence specific only to *L. pneumophila* by selecting primers which will amplify these specific gene sequences. This test has become especially feasible because Perkin Elmer Corporation has produced a commercial test kit which uses PCR technology and which contains all of the necessary reagents to complete the testing of water samples for *Legionella* bacteria within a few hours to a day. The limitations of this PCR method is that it is a qualitative test which detects both dead and live cells of *Legionella*. As a result, it is a research method rather than an approved monitoring method.

II. Objectives and Experimental Design of Phase 3

The objective of phase 3 of this study was to use a PCR-based gene probe method called EnvironAmp Legionella Kit produced by Perkin-Elmer Corporation as a conservative screening test to determine whether the cells of *Legionella* spp. and *L. pneumophila* can be detected in potable water samples obtained from well and distribution sources used by the HBWS.

The experimental design was to follow the procedure as outlined in the EnvironAmp Legionella kit but to filter 1,000 ml of water rather than a 10 ml sample through the membrane filter as a means to increase the sensitivity of the assay. Representative water samples from well sites, from distribution system sites and from a few Tunnel and Spring sites were selected for this monitoring study.

III. Materials and Methods. The EnvironAmp kits with all the necessary reagents were obtained from Perkin Elmer Corporation. The procedure provided by the kit manufacturer was followed except that 1,000 ml of water rather than only 10 ml of water sample were filtered through the 25 mm (0.45 um porosity) membrane filters (HVLP, Millipore Corp.) All bacteria in the water including *Legionella* were trapped onto the surface of the filter. It should be noted that in contrast to water samples tested in other reported studies, the water samples obtained in this study were so clear that 1,000 ml of water could be readily filtered through this filter. By increasing the volume of water analyzed from 10 to 1,000 ml, the probability of recovering *Legionella* spp. from water samples was increased. The filter was then placed into a sterile 1.5 ml microcentrifuge tube containing lysing agent to extract the DNA from all bacterial cells. Portions of this DNA extract sample was then added to PCR reagents and the samples were placed into the thermocycler with the specific primers to amplify or replicate the genes common to all *Legionella* species as well as primers to amplify the gene specific to only *Legionella pneumophila*. The presence of *Legionella* is determined by a visual colored reaction on a filter paper strip which represents the presence of the specific genes of *Legionella* spp. Thus, the results of this test are qualitative (positive or negative) rather than quantitative. However, based on the observed colored endpoint reaction, Perkin Elmer has calculated that the test kit can detect not only a level of 10^3 cells per ml but even reactions as low as 50-100 cells/ml.

IV. Results and Discussion

A. Well Water. The results of analyzing well water samples are summarized in Table 12 and show that a total of 16 of 28 (57.1 %) well water samples representing 15 of 26 (57.6%) well sources were positive for *Legionella* spp. bacteria using the PCR gene probe method. Of these, 2 of 28 (7.1%) of water samples representing 1 of 26 (3.8%) well sources were positive for the pathogenic *L.pneumophila.* The positive water samples were obtained from Hoaeae well (WUS-1). Water samples from this source were positive for *L. pneumophila* in two out of three samples suggesting that this source of water differs from other well sources and that there may be sporadic contamination with a surface water source. Taken together, these results indicate that some species of *Legionella* bacteria can be commonly detected in nearly 60% of groundwater sources using a gene probe assay. In contrast, the pathogenic *L. pneumophila* species was detected in groundwater samples from only one well.

B. Springs and Tunnel Water. The results of analyzing 2 Springs and 3 Tunnel water samples *for Legionella* bacteria are summarized in Table 12 and show that one Spring sample (Makiki) was negative for any *Legionella* bacteria. Satisfactory data could not be obtained from the second Spring (Alewa) because water from this spring contained some component which interfered with the PCR method. The three Tunnel water samples were positive for *Legionella* spp. but negative for *L. pneumophila.* Although insufficient samples were taken, the evidence indicates that *Legionella* spp. can be expected to be recovered from Spring and Tunnel water.

C. Distribution Water. The results of analyzing water samples from distribution sites are summarized in Table 13 and show a total of 42 of 44 (95.5%) distribution sites were positive for some species of *Legionella.* Of these, 5 of 44 (11.4%) were positive for *L. pneumophila.* The five water samples which were positive for the pathogenic *L. pneumophila* were obtained from five different distribution sites. These results indicate that some species of *Legionella* bacteria can be readily detected in most (>90%) of the water samples from distribution systems using a gene probe assay. These results indicate that *Legionella* spp. of bacteria as well as *L. pneumophila* are more likely to be present in distribution water samples than in groundwater samples. These results support previous findings that distribution sources contain more ecosystems (piping, tanks, biofilm, sediments, amoeba, elevated temperature in water heaters) where *Legionella* bacteria can multiply than can be found in groundwater environments.

CHAPTER FIVE
PROJECT SUMMARY AND CONCLUSIONS

I. Motivation For This Study

The mission of USEPA is to ensure that all public potable water supplies are free of infectious sewage borne pathogens. To accomplish this, USEPA has passed legislation that requires all surface sources of potable water to undergo two treatment processes (filtration and disinfection). Currently USEPA is finalizing the Groundwater Disinfection Rule (GWDR) which proposes to require that all groundwater sources of potable water undergo disinfection with a maintenance of chlorine residual in the distribution pipes. However, the GWDR must consider conditions which will allow a variance to this rule if the utility can demonstrate that their groundwater is not vulnerable to contamination with sewage. In this regard, the HBWS is one of those public utilities which has had a long history of providing untreated, non-disinfected groundwater as the source of potable water. Moreover, the consumers have publically voiced their approval and pride of being served safe, pure and good tasting water characterized by no chlorine taste. However, HBWS has recently initiated limited and selective chlorination of drinking water sources to meet the new and much more stringent regulation called the "total coliform rule". This limited and selective chlorination does not result in drinking water with a discernible chlorine taste. However, under the current guidelines of the upcoming GWDR, all groundwater sources must be chlorinated to a level which will add a distinct chlorine taste to the water in the distribution pipe. In this regard, the HBWS has publically stated that since its groundwater sources are not vulnerable to contamination with sewage, there is no need to chlorinate all groundwater sources. As a result, the HBWS has indicated its intent to seek a variance to the stringent disinfection requirements of the upcoming GWDR.

II. Goal and Strategies of this Study

The overall goal of this study was to extensively monitor samples of water from production wells and from the distribution system for several kinds of microorganisms as a reliable means to determine whether these potable sources of water managed by the HBWS are or are not vulnerable to sewage contamination.

The first strategy of this study was to monitor larger than required volumes of water to increase the sensitivity of the monitoring test. The second strategy was to monitor the water for all of the fecal indicators which are currently being considered for monitoring under the proposed GWDR.

III. STATUS OF GWDR

Dr. Bruce Macler, manager of the current GWDR has for several years accepted the input of stakeholders, regulators and scientists through various forums such as

teleconferences, seminars, workshops, publications and national conferences to formulate an acceptable GWDR. The following are some of the key meetings where important decisions were made in the formulating the details of the GWDR.

1. USEPA Workshop on Regulating the Revised GWDR. July, 1996. Newport Beach, CA.

This was the first major and formal USEPA workshop involving stakeholders and scientists to discuss all issues of the GWDR and the expected regulatory language. One recommendation of this conference was the selection of candidate microbial indicators (*E. coli*, enterococci, *Clostridium perfringens*, somatic phage, FRNA phage, enteric virus by PCR) to meet the monitoring requirement of the GWDR.

2. Groundwater Foundation Conference. Sept. 5-6, 1996. Boston, MA. Under the Microscope: Examining Microbes in Groundwater. Groundwater Disinfection Rule.

This was a national conference which highlighted the GWDR and led to an open discussion of this upcoming rule to a national audience. At this conference, Dr. Paul Berger of USEPA was assigned to head the microbiological team of the GWDR. R. Fujioka attended and participated in this conference.

3. NWRI Conference. March 16-17, 1997. Irving, CA. Groundwater Disinfection Regulations Benefits Conference.

This conference sponsored by National Water Research Institute (NWRI) was comprised of invited individuals representing a diverse group of professionals who are knowledgeable about water resource issues. The objective of this conference was to discuss the benefits which should be considered in the development of the GWDR. R. Fujioka attended and participated in this conference.

4. USEPA Workshop. April 1-2, 1997. San Antonio, TX.

This was the second major workshop sponsored by USEPA for stakeholders, regulators and scientists to begin to fine tune all issues related to the GWDR. The consensus agreement reached with regard to monitoring groundwaters for microorganism was as follows: 1) Volume of sample not to exceed 1,000 ml. 2) Cost for microbial assay not to exceed $50. 3) Number of samples to be limited to 2-4 times per year.

5. USEPA Specialty Workshop. Sept. 18-19, 1997. Analytical Methods for Monitoring Microbes in Groundwater.

Selected microbiologists from throughout the nation were invited to this workshop to discuss the advantages and disadvantages of each of the proposed microbial monitoring methods to be used in the GWDR. The draft conclusions and recommendations of this workshop are currently being circulated to the attendees. R. Fujioka attended and participated in this workshop.

It should be noted that R. Fujioka participated in the GWDR teleconference on August 14, 1997 which was led by Dr. Paul Berger. A major focus of that teleconference was to determine the microbial monitoring requirements of the GWDR. Due to concerns on the limitations being considered for the microbial monitoring program of the GWDR, R. Fujioka wrote an evaluation of each of the microbial monitoring methods and his own assessment for a monitoring program for the GWDR. This evaluative plan was e-mailed to Paul Berger and to Bruce Macler in preparation for the GWDR workshop scheduled for September 18-19, 1997 by USEPA. The full context of this e-mail response dated August 16, 1997 is included as Appendix D.

IV. Assessment of Potable Water Quality Based on Monitoring for Fecal Bacteria

A. Well Water. As documented in Chapter Two of this report, a total of 79 water samples obtained from 39 wells were monitored for various fecal bacteria by analyzing a standard volume (100 ml) and an increased volume (1,000 ml) of water. When the standard 100 ml volumes of water were analyzed, 3/79 or 3.8% of the well water samples were positive for total coliform. However, when the sample volume was increased tenfold to 1,000 ml, 7/79 or 8.9% of the well samples were positive for total coliform. These results support our original prediction that increasing the volume of water sample to be assayed for fecal indicator bacteria will increase the sensitivity of the tests. Total coliform was the most frequently recovered of the fecal bacteria tested. In comparison, fecal coliform and fecal streptococci were positive in 2/79 or 2.5% of the well water samples and *E. coli* was recovered in 1/79 or only 1.3% of the samples. None of the well samples were positive for *C. perfringens*. It should be noted that hydrogen sulfide bacteria was recovered in 4/79 or 5.1% of the well water samples and this frequency was more closely related to the frequency of recovering total coliform bacteria. Although hydrogen sulfide bacteria is not considered a fecal bacteria, under comparable conditions, the recovery of hydrogen sulfide bacteria from potable water correlates favorably with recovery of total coliform bacteria.

A major question is whether the low levels of coliform recovered from the groundwater samples are evidence that the groundwater is vulnerable to contamination with sewage or whether the level of contamination is due to non-sewage source. To address this question, several important factors were considered. First, the surface of all soil in Hawaii is known to contain high concentrations of total coliform, fecal coliform, *E. coli* but not *C. perfringens* (Hardina and Fujioka, 1991, Byappanahali, Roll and Fujioka, 1996). The absence of most of these bacteria in groundwater samples indicates that the soil profile is able to filter out these bacteria which are present on the surface of the soil. Second, based on the monitoring assessment plan (see Appendix D), the absence specifically of *C. perfringens* in groundwater samples indicates that the groundwater is not contaminated with sewage and the integrity of the soil profile is good. Third, the recovery of predominantly total coliform but not fecal coliform from the few positive groundwater samples indicates that the source of contamination is not sewage but some other source. Although the source of the total coliform was not determined, it has been reported in other studies that non-fecal sources of

total coliform include contamination of samples with soil, dust, insects, plants and even biofilm within pipes.

The best assessment of the data is that the low percentages of total coliform in groundwater samples is due to a non-sewage source and the groundwater is not vulnerable to sewage contamination. One recommendation for future monitoring is to continue to monitor groundwater for fecal bacteria using larger than stipulated volumes of water such as 1,000 ml. Another recommendation is for the HBWS to save the total coliform isolates from groundwater samples for further characterization to provide additional data to determine the source of these coliform bacteria.

B. Spring and Tunnel Waters. There were too few samples from these sources to make definitive conclusions about the quality of spring and tunnel waters. Since, *C. perfringens* was not recovered in any of these water samples, the monitoring data indicate that the source of contamination is more likely soil rather than sewage. This conclusion is strengthened by the fact that these sources of water are in remote areas away from human habitation. The limited data indicated that Alewa Spring water and a tunnel source of water are vulnerable to contamination with surface soil. Thus, spring and tunnel sources of water are much more vulnerable to contamination with fecal indicator bacteria than well water.

C. Distribution Water. Total coliform was recovered in 3/152 or 1.97% of the distribution water samples tested. None of the other fecal indicators (fecal coliform, *E. coli*, enterococci, *C. perfringens)* was recovered from these 152 distribution water samples. Although these results indicate that the quality of water from distribution sources is better than water obtained directly from wells, two conditions favor the lower incidences of recovering fecal bacteria from distribution sites. First, those wells and reservoirs which supply the water to the distribution system and from which coliform bacteria have been previously detected are routinely chlorinated. The data suggests that chlorine will reduce total bacterial load in potable water within the distribution system. Second, the distribution sites are generally indoors and better protected from wind, soil, and insects than well sites. Thus, opportunities for contamination of water samples obtained from distribution sites are less than when water samples are obtained from well sites. The recovery of only total coliform bacteria and the absence of all other fecal bacteria such as fecal coliform, *E. coli* and *C. perfringens* indicate that the low level contamination of water samples obtained from distribution sites is not due to fecal contamination. Thus, the quality of the water in the distribution system is good and this most likely reflects the selective chlorination program instituted by the HBWS.

D. Overall Conclusions. The analysis of the HBWS distribution water samples indicates that the microbial quality of water reaching the consumers is generally excellent and that most of the water samples met the total coliform rule. The current low level chlorination program used by the HBWS appears to be very successful in ensuring that the microbial quality of water in the distribution system is consistently good. Taken together, these results indicate that the HBWS is knowledgeable and capable of managing the quality of its drinking water source. However, there were low incidences in which total coliform

bacteria were recovered from this potable water source. Since most of the coliform detected were not fecal coliform and these same water samples were negative for the presence of more specific fecal bacteria such as *E. coli*, fecal streptococci or *C. perfringens*, the detection of total coliform most probably reflects a non-sewage source. Thus, the fecal bacterial monitoring evidence indicates that the potable water sources of HBWS are not vulnerable to sewage contamination.

E. Recommendations. It was not the intent of this study to determine the source of the total coliform detected in the few potable water samples. This is not an easy task and many utilities have tried and failed. However, we recommend that an attempt be made to determine the source of total coliform recovered in potable water sources because this information can lead to two desirable outcomes. First, the biochemical, antigenic or genetic characterization of total coliform bacteria recovered from potable water sources may help determine their source (sewage or non-sewage) and their public health significance. In this regard, there are now many molecular methods which can address this need. Second, knowing the source of these total coliform bacteria, an effective preventive procedure to reduce or totally eliminate the probability of recovering total coliform in any potable water supply can be devised.

V. Assessment of Potable Water Quality Based on Monitoring for Coliphages

A. Data Based on Standard Phage Assay. As documented in Chapter Three of this study the standard membrane adsorption/elution method using 1,000 ml volumes of water sample was initially employed to monitor all potable sources of water. Using this assay method, all 53 water samples obtained from wells, springs, tunnels and distribution sources were negative for coliphages and in particular FRNA phage. Since FRNA phage is the best surrogate for human enteric viruses, these data indicate that these sources of potable water are not contaminated with human enteric viruses.

B. Data Based on Experimental Phage Assay. Recognizing that the standard phage assay is not very sensitive in recovering low concentrations of phage in large volumes of water, an experimental and non-quantitative method which is very sensitive and designed to detect low concentrations of FRNA phage was used in the second round of analysis. Using this sensitive method, FRNA phage was not recovered from any of the samples of water obtained from wells and spring/tunnel. These results present additional and supportive evidence that the groundwater sources used by HBWS are not vulnerable to contamination with sewage and with human enteric viruses. However, this experimental phage assay detected FRNA phage in 3 of 52 or 5.7% of the water samples obtained from distribution systems. Repeat analysis of water samples obtained from 2 of the 3 positive distribution sites, resulted in no recovery of phage. These results suggest that the contamination with phage in the distribution system is sporadic.

Since FRNA phage was recovered from three water samples obtained from the distribution system, additional studies should be conducted to determine the source of these

phage and whether it constitutes a health risk. In this follow up study, all possible sources should be considered. The first possibility is that the phage may have resulted from the *E. coli* strain used to assay the water and not from the water sample itself. Although, we have no evidence for this and all of our control reactions do not indicate that this is happening, Dr. William Yanko of County Sanitation District of Los Angeles (personal communication) reported that somatic phage had lysogenized within one of his *E. coli* cell line. During the test, the lysogenized somatic phage would be expressed at a low frequency resulting in the release of a fully formed somatic phage which would then multiply in the assay procedure. Since FRNA phage are less likely to lysogenize than somatic phage and since we determined that FRNA phage was recovered from water samples, the possibility that the source of the FRNA was the *E. coli* host cells is not likely. The second possible source of FRNA phage in some distribution water samples is the reservoir tanks because these tanks are susceptible to coliform contamination. Moreover, bird feces is a likely source of these coliform bacteria and FRNA phage. As stated earlier, the HBWS uses minimal doses of chlorine to control the concentrations of coliform bacteria. However, since FRNA phages are much more resistant to chlorine inactivation than coliform bacteria, infectious FRNA phages can be expected to resist low level chlorination and may occasionally be present in the distribution system. It should be noted that the feces of birds are not sources of human enteric viruses and therefore the presence of FRNA phage due to bird feces has a lower health risk than FRNA phage from human sewage.

C. Overall Conclusions. The results of this study provide data that using the most sensitive method to assay for coliphages, groundwater sources used by HBWS are not contaminated with the FRNA phage which is the best surrogate for human enteric viruses. Based on these results, we conclude that HBWS groundwater sources are not vulnerable to contamination with sewage. However, the monitoring data showed that sporadic and low frequency recovery of FRNA phage from distribution water sites occurred only when the experimental and very sensitive phage enrichment detection procedure was used. Although this method has not been properly evaluated by different laboratories and is not an approved monitoring method, the data obtained indicate a source of contamination in some point in the distribution system. In our evaluation of possible contaminating sources, we believe that the most vulnerable sites are the reservoir tanks and the most likely source is bird feces. If it can be shown that the source of FRNA phage is from the reservoir tank, one can reasonably conclude that the source of FRNA phage does not represent human feces (sewage) and therefore the health risk associated with this source of FRNA phage in drinking water cannot be correlated with presence of human enteric viruses.

D. Recommendations. Further studies should be done to fully assess the applicability of the experimental, enrichment method to recover FRNA phage and to determine the source of FRNA phage recovered in the three water samples obtained from the distribution system. For future studies, emphasis should be placed on conducting water quality studies at the various reservoir tanks for several reasons. First, this source of potable water was not monitored as part of this study. Second, this source of water is vulnerable to contamination with coliform bacteria. Third, some of the reservoir tanks are being chlorinated by HBWS and it is well known that chlorination is much more effective in disinfecting coliform

bacteria than FRNA phage. Fourth, determining the source of FRNA phage can result in a better risk assessment determination of the health risk associated with detecting FRNA phage in few water samples obtained from the distribution system. Fifth, if the source of FRNA phage is known, applicable strategy and procedures can be implemented to prevent the contamination by FRNA phage in the future.

VI. Assessment of Potable Water Quality Based on Monitoring for Legionella Bacteria

A. Expectations. At the start of this study, *Legionella* bacteria was associated with surface sources of water and not with groundwater. There was an expectation that groundwaters were not suitable habitats for *Legionella* bacteria. Based on this expectation, our study design was to assay larger volumes of water samples and to use the PCR method to detect for the presence or absence of *Legionella* spp. and *L. pneumophila* in groundwater samples from the various wells. The major usefulness of the PCR method is to determine whether a water sample is negative for *Legionella* spp. or positive for *L. pneumophila.* A negative test for *Legionella* spp. indicates that *Legionella* is not present in that water sample and therefore that source of water pose no health risk for transmission of legionellosis. A positive *L. pneumophila* indicates that cells of the most common pathogenic *Legionella* sp. are present in that source of water and therefore that source of water may be a potential source for the transmission of legionellosis disease. Water samples which are positive for *Legionella* spp. by PCR are difficult to interpret because this test detects for the presence of the many species of *Legionella* which are not pathogenic as well as those which are pathogenic.

B. Detectable levels of *Legionella* in Potable Water Using a Gene Probe Test. As documented in Chapter four of this report, a PCR based gene probe method was used to detect for the presence or absence of *Legionella spp.* and *L. pneumophila* bacteria in various drinking water samples. Although, the PCR method is not an USEPA approved monitoring method it was the method of choice because it is the only feasible method capable of analyzing many water samples for the presence or absence of this group of bacteria. Using this method, the rate of detecting *Legionella* bacteria in various potable water sources tested can be summarized as follows:

Source of Water	Percent (# pos/# tested) *Legionella* spp.	Percent (# pos/# tested) *Legionella pneumophila*
1. Wells	15/26 (57.6%)	1/26 (3.8%)
2. Tunnel	3/3 (100%)	0/3 (0%)
3. Spring	0/1 (0%)	0/1 (0%)
4. Distribution	42/44 (95.5%)	5/44 (11.4%)

These results clearly show that by using the gene probe assay, bacterial cells with genes specific to *Legionella* spp. were readily detected in most of the water samples obtained from well and distribution sites used by the HBWS. The data show that the

detection rates of *Legionella* spp. and *L. pneumophila* were much lower in water samples obtained from wells than from water samples obtained from the distribution system. These results are expected since it is well known that there are many more opportunities for *Legionella* bacteria to multiply in distribution ecosystems than in groundwater ecosystems.

C. Interpretation of Data. Reliable interpretation of gene probe method data alone is difficult because the data is not quantitative and detects both dead and live cells. In this regard, all microbial water quality standards, including MCLG are based on viable concentrations of the microorganism. Thus, the MCLG of zero for *Legionella* means that no viable cells of any species of *Legionella.* should be present in potable waters. Under this guideline, dead or non-viable concentrations of *Legionella* spp., including *L. pneumophila* cells are permissible in potable water. Thus, the data obtained from this study must be related to other studies in which culturable levels of *Legionella* spp. were measured. In this regard, it should be noted that there have been numerous studies documenting that culturable concentrations of *Legionella* spp. are prevalent in water samples obtained from the distribution systems and that even the pathogenic *L. pneumophila* can often be recovered from potable and chlorinated drinking water systems. However, only limited studies have been done to determine the prevalence of *Legionella* in groundwater sources.

The first comprehensive study to analyze groundwater samples for *Legionella* bacteria was reported by USEPA in 1994 (Lieberman et al, 1994). In that study, the groundwater sources were determined not to be under the influence of surface water but these groundwater sources were determined to be vulnerable to sewage contamination. This was confirmed by recovering some fecal indicator bacteria in nearly 90% of these groundwater samples. Using the USEPA approved culture method, nearly 50% these groundwater samples was positive for *Legionella spp.* and of these, approximately 20% was positive for *L. pneumophila*. Although this study did not use the PCR method to measure for *Legionella,* the results of this study show that groundwaters which are obviously contaminated with fecal bacteria can be expected to have a high prevalence rate of culturable *Legionella spp.* as well as an appreciable prevalence rate of culturable pathogenic *L. pneumophila.*.

A more recent and more relevant study was reported by Lye et al (1997). In that study, they compared culturable method to the gene probe method using the EnvironAmp method and analyzed non vulnerable groundwater samples from 16 states. Using the EnvironAmp method they detected Legionella spp. of bacteria in 84% of the 58 groundwater samples but only 7% of these water samples was positive by culture method. Significantly, *L. pneumophila* was never detected in these groundwater samples by either PCR or by culture. In that same study, they reported that 68% of 47 water samples from distribution systems was positive for *Legionella* spp. by PCR but only 15% of these same water samples was positive for *Legionella* spp. by culture method. In that study, *L. pneumophila* was not detected by either the PCR method or by the culture method. The results of that study are especially relevant because it demonstrates that the incidence rate of detecting *Legionella* spp. is much higher when the PCR method is used compared to culturable methods. These results verify the existing knowledge that only a fraction of the total number of *Legionella*

bacteria cells in natural environmental waters are viable and are of public health significance. Since, PCR method detects the presence of dead and live cells, the data generated from PCR methods are always very conservative and much higher than the actual incidence of viable *Legionella* in water samples.

D. Overall Conclusion. The available evidence now indicates that viable concentrations of *Legionella spp.* and even *L. pneumophila* can be readily recovered from potable water distribution systems, even in highly chlorinated systems. The reason for this is the many opportunities and ecosystems in distribution systems which will allow the *Legionella* bacteria to multiply. Thus, although the prevalence and concentrations of *Legionella* spp. and *L. pneumophila* are low in groundwater sources, the concentrations of these bacteria can be expected to increase in the distribution system. Moreover, since transmission of legionellosis is not by drinking these bacteria in the water but by the inhalation of aerosolized droplets of water containing the pathogenic species of *Legionella,* the health risk of contracting legionellosis is very low under most conditions. The high risk locations such as health care facilities where the susceptible population is concentrated, have been identified. As of this date, USEPA and CDC have yet to propose a water quality standard for concentrations of *Legionella* in drinking water sources. Based on the available evidence the acceptable level of *Legionella* spp. should be relatively high and approximately 1,000 cells/ml (Dennis Lye, personal communication).

E. Final Assessment and Recommendations. 1. Based on information available today, it is not scientifically sound for USEPA to maintain that the MCLG for *Legionella* in potable water be established at zero because this is an unachievable goal. Thus, MCLG of zero for *Legionella* should be deleted from the SDWA and replaced with proper guidance language. In this regard, we recommend that the HBWS consult with sister agencies such as Hawaii State Department of Health and American Water Works Association to request that USEPA clarify the issue of *Legionella* in potable water systems.

2. USEPA should provide more up to date guidance on the expected concentrations of *Legionella* spp. and *L. pneumophila* in potable water systems and recommend procedures to reduce the concentrations of these bacteria in water systems, especially water systems used by populations who are at high risk to *Legionella* infections. Guidance should include monitoring programs for highly susceptible sites such as at health care facilities.

3. Detectable rates of *Legionella* and *L. pneumophila* in the various sources of potable water used by HBWS and detected by the PCR method were higher than expected. These results may be due to the fact that higher volumes of water were assayed to increase the sensitivity of the test. Another possibility is that groundwater in tropical areas may be more conducive to the growth of more environmental types of *Legionella* spp. Thus, the higher incidence of detecting *Legionella* spp. but not *L. pneumophila* may not necessarily be related to an increase in health risk. Since the PCR method detects both dead as well as viable concentrations of *Legionella* bacteria, measurements using PCR will always result in much higher detection incidences than measurements based on culture methods. Based on previous studies which showed that less than 10% of the gene probe positive well water

samples were positive when culture methods were used, and based on the fact that we analyzed ten times the normal volume of water sample, we conclude that the levels of viable *Legionella* species in the groundwater sources used by HBWS may be closer to 1.0% of the PCR detectable levels. Based on all the information available, the data obtained cannot be used to associate a health risk with legionellosis to consumers of the waters distributed by HBWS. As a result, we do not recommend any changes to HBWS in the treatment and distribution of potable groundwater to consumers.

4. Based on the data, two recommendations are made. First, some *Legionella* spp. detected by the gene probe test may represent endogenous, non-pathogenic spp. which are unique to groundwaters in tropical island aquifers. Thus, it is recommended that well waters be assayed by culturable techniques to show that viable *Legionella* bacteria can be recovered from groundwater samples. The viable *Legionella* spp. recovered from this groundwater source should be identified to its species and compared with those already known to determine if *Legionella* spp. recovered from groundwater represent a unique species. The second recommendation is to follow up on sites where water samples were positive for *L. pneumophila* on at least two separate samples suggesting a potential problem related to contamination or ecology at these sites. In this regard, *L. pneumophila* was detected on two occasions at a well site (Hoaeae Well/WUS-1) and at a distribution site (Crestview Community Playground/WP-2). The data indicates that it is prudent to examine and evaluate these two sites for possible contamination from some external sources or conditions which may be favorable for the growth of *L. pneumophila*.

REFERENCES CITED

American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1995. *Standard Methods for the Examination of Water and Wastewater*, 18th edition. American Public Health Association, Washington, D.C.

Byappanahalli, M., and R. Fujioka. 1996. Evidence for prevalence, persistence and multiplication of fecal coliform and *E. coli* in the soil environment of Hawaii. Abstract of the 96th General Meeting of the American Society for Microbiology. Pg. 467.

Bisson, J.W., and V.J. Cabelli. 1979. Membrane filtration enumeration methods for *Clostridium perfringens*. *Appl. Environ. Microbiol.* 37:55-66.

Borrego, J.J., R. Cornax, D. Preston, S. Farrah, B. McElhaney, and G. Bitton. 1991. Development and application of new positively charged filters for recovery of bacteriophages from water. *Appl. Environ. Microbiol.* 57:1218-1222.

Charriere, G., D.A.A. Mossel, P. Beaudeau, and H. Leclerc. 1994. Assessment of the marker value of various components of the *coli-aerogenes* group of Enterobacteriaceae and of a selection of *Enterococcus* spp. for the official monitoring of drinking water supplies. *J. of Appl. Bacteriol.* 76:336-344.

Craun, G.F. 1984. Health aspects of groundwater pollution. In G. Bitton and C.P. Gerba (ed.), *Groundwater pollution microbiology*. John Wiley & Sons, Inc., New York. pp. 135-179.

Craun, G.F., P.S. Berger, and R.L. Calderon. 1997. Coliform bacteria and waterborne disease outbreaks. *J. AWWA*. 89:96-104.

Debartolomeis, J., and V.J. Cabelli. 1991. Evaluation of an *Escherichia coli* host strain for enumeration of F male-specific bacteriophages. *Appl. Environ. Microbiol.* 57:1301-1305.

Frahm, E. and U. Obst. 1995. Comparison of two rapid tests for the detection of legionellaceae in water: A microbiological-immunological method and a commercial gene-probe test kit. *Wat. Sci. Tech.* Vol. 31, No. 5-6, pp. 403-406.

Freije, M.R. 1996. Legionellae Control in Health Care Facilities. 131 pp. HC Information Resources, Inc.

Fujioka, R., P. Borthakur, and B. Yoneyama. 1995. Assessing the need to disinfect Hawaii's groundwater sources under the groundwater disinfection rule. In *Am. Wat. Works Assn. Proceedings of the 1995 Water Quality Technology Conference*, New Orleans, LA. Part II—Session 4A through ST7, pp. 1625-1635.

Fujioka, R.S., and L.K. Shizumura. 1985. *Clostridium perfringens*, a reliable indicator of stream water quality. *J. Water Pollut. Control Fed.* 57:986-992.

Grubbs, T.R., and F.W. Pontius. 1992. USEPA releases draft ground water disinfection rule. *J. AWWA* 84(9):25-31.

Hardina, C.M., and R.S. Fujioka. 1991. Soil: The environmental source of *E. coli* and enterococci in Hawaii's streams. *Environ. Toxicol. Water Qual.* 6:185-195.

Havelaar, A.H., K. Furuse, and W.H. Hogeboom. 1986. Bacteriophages and indicator bacteria in human and animal feces. *J. Appl. Bacteriol.* 60:255-262.

Havelaar, A.H., M. van Olphen, and Y.C. Drost. 1993. F-specific RNA-bacteriophages are adequate model organisms for enteric viruses in fresh water. *Appl. Environ. Microbiol.* 59:2956-2962.

Honolulu Advertiser. 1993. Pending EPA rules may affect isle water. Thursday June 24, A8.

IAWPRC. 1991. Review Paper: bacteriophages as model viruses in water quality control. *Wat. Res.* 25:529-545.

Kromoredjo, P. and R. Fujioka. 1991. Evaluating three simple methods to assess the microbial quality of drinking water in Indonesia. Environ. Toxicol. and Water Quality 6:259-270.

Lieberman, R.J., L.C. Shadix, B.S. Newport, S.R. Cout, S. E. Buescher, R.S. Safferman, R.E. Stetler, D. Lye, G.S. Fout, and D.R. Dahling. 1994. Source water microbial quality of some vulnerable public ground water supplies. Water Quality Technology Conference, Proceedings. Pg. 1425-1436. AWWA, Denver, Co.

Lye, D., G. Shay Fout, S.R. Crout, R. Danielson, C.L. Thio, and C.M. Paszko-Kolva. 1997. Survey of ground, surface, and potable waters for the presence of *Legionella* species by EnviroAmp PCR *Legionella* kit, culture, and immunofluorescent staining. *Wat. Res.* Vol. 31, No. 2, pp. 287-293.

Macler, B.A. and F.W. Pontius. 1997. Update on the ground water disinfection rule. *J. AWWA.* 89:17-115.

Miller, L.A., J.L. Beebe, J.C. Butler, W. Martin, R. Benson, R.E. Hoffman, and B.S. Fields. 1993. Use of polymerase chain reaction in an epidemiologic investigation of Pontiac fever. *J. Infect. Dis.* 168:769-772.

Pontius, F.W. 1990. Complying with the new drinking water quality regulations. *J. AWWA* 82(2):32.

Pontius, F.W. 1990. New regulations for total coliforms. *J. AWWA* 82(8):16-22.

Pontius, F.W. 1993. Strengthening the surface water treatment rule. *J. AWWA* 85(12):20-22, 119.

Roll, B. and R.S. Fujioka. 1995. Detection of *Legionella* bacteria in sewage by polymerase chain reaction and standard culture method. *Wat. Sci. Tech.*, Vol. 31, No. 5-6, pp. 409-416.

Stout, J.E., V.L. Yu, P. Muraca, J. Joly, N. Troup, and L.S. Tompkins. 1992. Potable water as a cause of sporadic cases of community-acquired legionnaires' disease. *N. Engl. J. Med.* 326:151-155.

ACKNOWLEDGMENTS

This study was funded by a grant from the Honolulu Board of Water Supply. The authors acknowledge the skillful assistance of Pritty Borthakur, Bo Lin, Amy Cao and Audrey Asahina in analyzing the water samples. The authors acknowledge the excellent technical and administrative support provided by the Honolulu Board of Water Supply in the completion of this study. In particular the administrative guidance and timely information provided by Mr. Erwin Kawata, project officer and the cooperative efforts of Mr. Owen Narikawa, supervisor of microbiology laboratory, who obtained the water samples for this project.

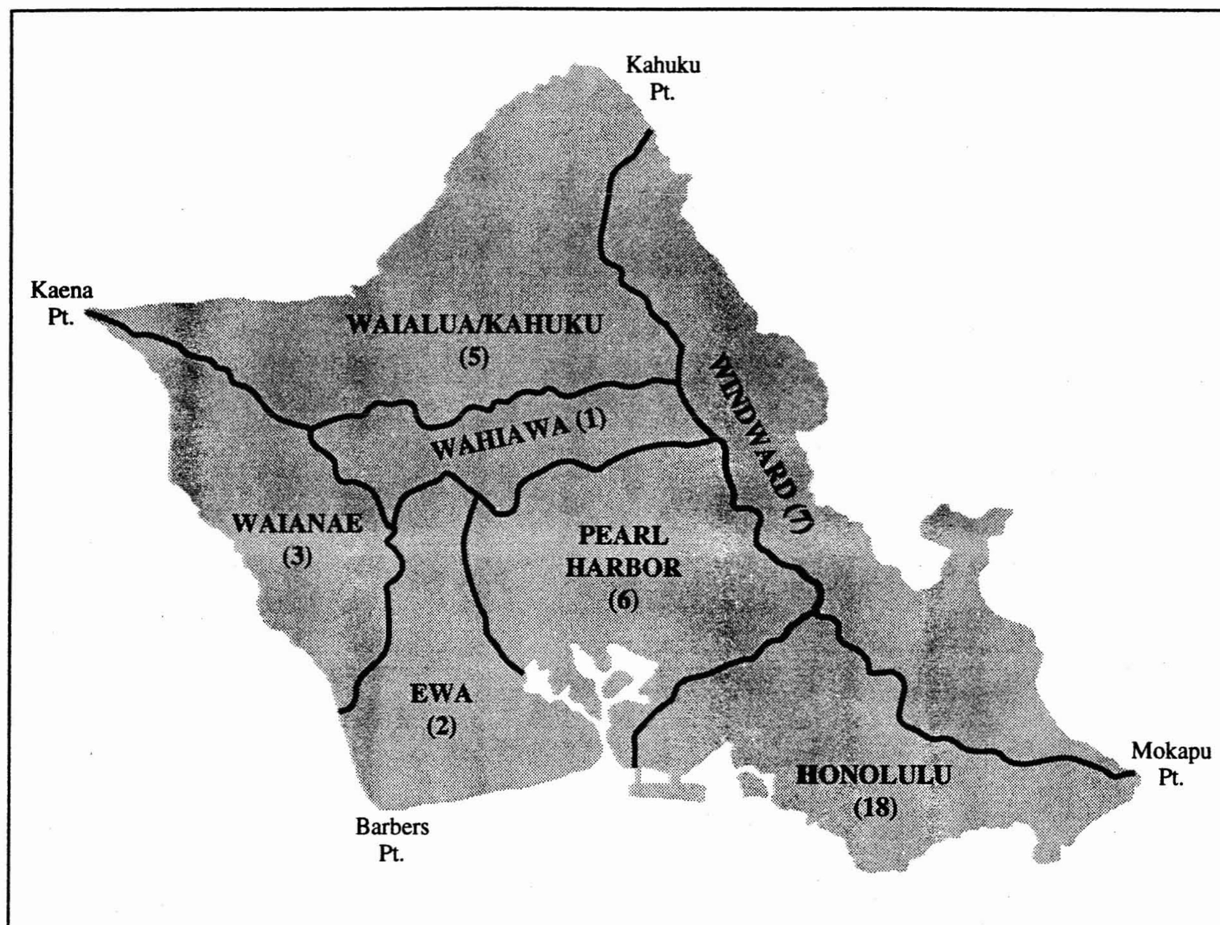

Figure 1. Map of Oahu showing the seven water districts (Honolulu, Pearl Harbor, Windward, Waialua/Kahuku, Wahiawa, Waianae, Ewa) used by the Honolulu Board of Water Supply. Numbers in parenthesis indicate the number of wells sampled from each of the water districts.

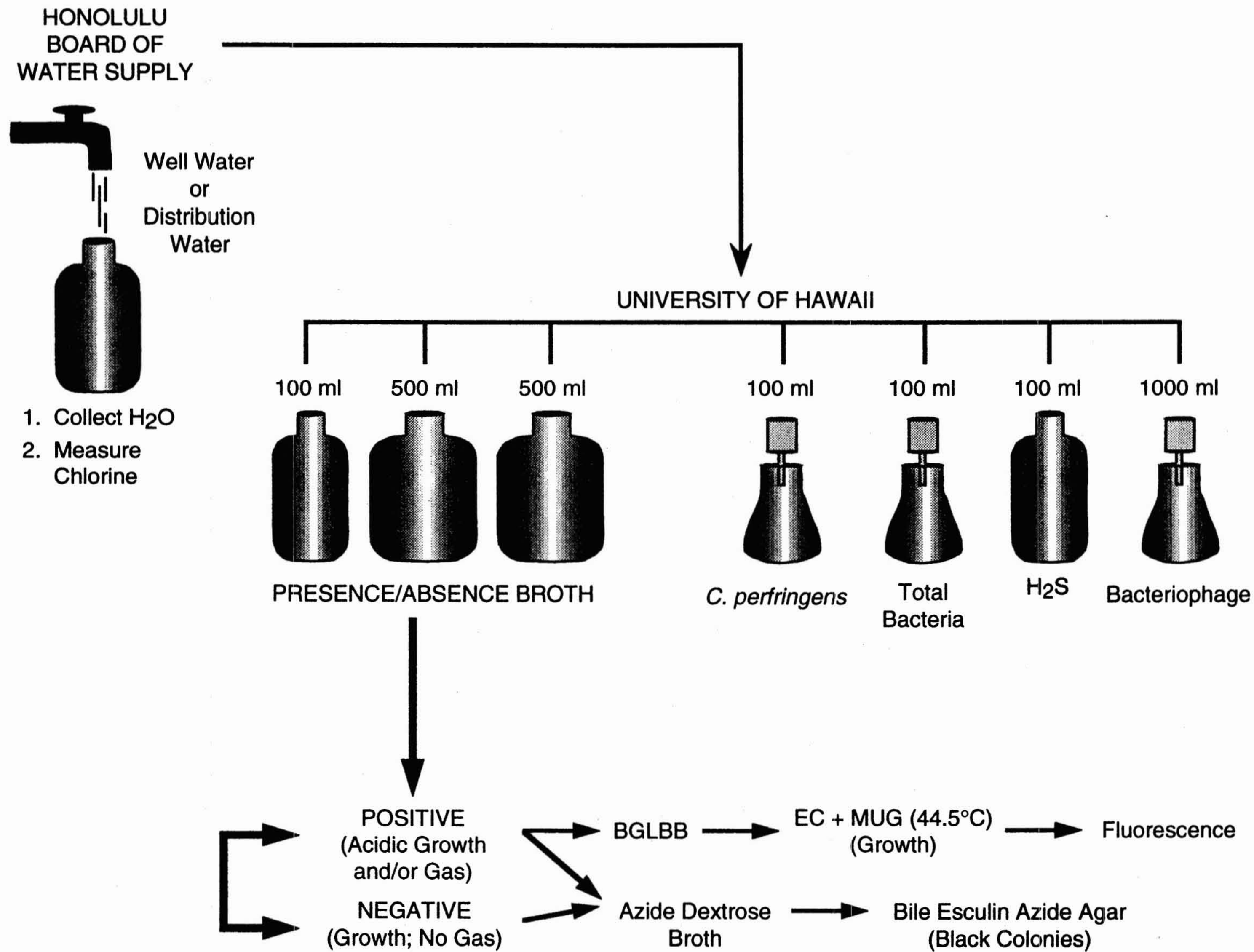

Figure 2. Water sampling and microbial analyses plan used by the University of Hawaii

Table 1. Identification of each HBWS well site sampled in the seven water use districts of Oahu.

Water-Use District	Site No.	Well Name	Well Number
Honolulu	HS-36	Kuliouou	1843-01
	HS-30	Wailupe I	1745-01
	HS-23	Waialae Iki	1746-02
	HS-17	Aina Koa	1746-01
	HS-22	Palolo	1847-01
	HS-5	Kaimuki	Hi-Service
	HS-4	Beretania	Low Service
	HS-14	Wilder	1849-15
	HS-29	Manoa II	1948-01
	HS-28	Jonathan Springs	2052-12
	HS-3	Kalihi Station	Low Service
	HS-16	Kalihi Uka	2250-01
	HS-2	Kalihi Shaft	2052-08
	HS-20	Moanalua	2152-12
	HS-1	Halawa Shaft	2354-01
	HS-15	Kalauao	2355-13
	HS-19	Kaamilo	2356-58
	HS-18	Punanani	2457-05
Pearl Harbor	HPS-2	Pearl City I	2458-03
	WPS-2	Waipio Heights I	2459-23
	WUS-3	Waipahu I	2400-03
	WUS-1	Hoaeae	2301-38
	WUS-4	Kunia II	2402-01
	MIS-1	Mililani I	2800-01
Windward	HWS-20	Waimanalo II	1943-01
	HWS-3	Kuou I	2348-05
	HWS-16	Kahaluu	2651-03
	HWS-15	Kahana	3353-03
	HWS-10	Punaluu II	3553-06
	HWS-22	Kaluanui	3554-04
	HWS-13	Hauula	3655-01

Table 1—*Continued*

Water-Use District	Site No.	Well Name	Well Number
Wahiawa	WHS-1	Wahiawa I	2901-11
Waialua/Kahuku	WAS-1	Waialua	3405-01
	WAS-2	Haleiwa	3405-03
	WES-1	Waialee I	4101-07
	WES-2	Waialee II	4101-08
	KHS-1	Kahuku	4057-15
Waianae	WNS-1	Kamaile	2712-30
	WNS-2	Makaha Shaft	2812-01
	WNS-5	Waianae Plantation Tunnels	2908
Ewa	WUS-6	Honouliuli I	2303-02
	WUS-7	Honouliuli II	2303-04

Table 2. Identification of each HBWS spring and tunnel site sampled in the water use districts of Oahu.

District	Site No.	Site Name
Honolulu	AS	Alewa Springs
	PS	Palolo Springs
	NT	Nuuanu Tunnel
	HS-12	Palolo Tunnel
Windward	HWS-2	Waimanalo Tunnel
	HWS-6	Kahaluu Tunnel
Waianae	WNS-4	Waianae Tunnel

Table 3. Identification of each HBWS distribution site sampled in the seven water use districts of Oahu.

Water-Use District	Site No.	Site Name
Honolulu	HC-3	BWS Ala Moana Garage
	HC-6	Waikiki Fire Station
	HC-9	630 South Bertania Street
	HC-13	Waialae Beach Park
	HC-14	UH Auxiliary Services Building
	HC-19	Wailupe Beach Park
	HC-22	Manoa Fire Station
	HC-26	Kalama Valley Park
	HC-27	Kamiloiki Neighborhood Park
	HC-31	Waialae Iki Playground
	HC-33	Ala Wai Clubhouse
	HC-35	Moanalua Gardens
	HC-38	Moanalua Fire Station
Pearl Harbor	HP-4	Pearl City Fire Station
	HP-14	Halawa Xeriscape Garden
	MI-1	Kipapa Park
	MI-2	Mililani Fire Station
	MI-3	Noholoa Neighborhood Park
	MI-4	Mililani District Park #1
	MI-5	Watanabe Floral
	MI-6	Mililani Recreation Center #1
	MI-7	Mililani Town Center
	MI-8	Mililani 685 Residence Station
	MI-9	Hokuahiahi Neighborhood Park
	MI-10	Mililani Golf Course
	MI-11	Kuahelani Park
	WP-1	Waipio Heights Wells
	WP-2	Crestview Community Park
	WP-4	Waipio Heights Wells II
	WP-5	Waipio Neighborhood Park
	WU-4	St. Joseph's School

Table 3—*Continued*

Water-Use District	Site No.	Site Name
Pearl Harbor (continued)		
	WU-5	Honowai Park
	WU-6	L'Orange Park
	WU-7	Waipahu Recreation Center
	WU-8	August Ahrens School
	WU-9	Waipahu Fire Station
	WU-10	Waipahu Elementary School
	WU-11	Waipahu Intermediate School
	WU-12	Waipahu High School
	WU-13	Kaleiopuu Playground
Windward	HW-1	Waimanalo Cafe
	HW-2	Waimanalo Beach Park
	HW-4	Mid-Pac Country Club
	HW-8	Keolu Elementary School
	HW-10	45-620 Kam Highway
	HW-13	Ahuimanu Elementary Playground
	HW-14	Waihee Line Booster
	HW-15	Kaaawa Fire Station
	HW-16	Punaluu Beach Park
	HW-17	Hauula Fire Station
	HW-18	Waimanalo District Park
	HW-20	Pali Golf Course
Wahiawa	WH-2	Kemoo Farm Snack Bar
	WH-3	Iliahi Elementary School
	WH-4	BWS Wahiawa Corporation Yard
	WH-5	Wahiawa Fire Station
	WH-6	Wahiawa Elementary School
	WH-8	Wahiawa Freshwater Park
	WH-9	Whitmore Playground
	WH-10	Wahiawa Intermediate School

Table 3—*Continued*

Water-Use District	Site No.	Site Name
Waialua/Kahuku	KH-1	Kahuku Hospital
	KH-2	Kahuku Fire Station
	KH-3	Kahuku Elderly Homes
	WA-1	Pupukea Booster Station #2
	WA-2	Waialua Fire Station
	WA-3	Haleiwa Community Center
	WA-5	Sunset Fire Station
	WA-6	Waimea Bay Beach Park
	WA-7	Haleiwa McDonald's
	WE-1	Sunset Beach Church of Christ
	WE-2	Sunset Beach Chevron
	WE-3	Ehukai Beach Park
	WE-4	Waialee Experimental Station
	WE-5	Turtle Bay Pro Shop
Waianae	WN-1	Sheraton Makaha
	WN-2	Keaau Beach Park
	WN-3	Ulehawa Beach Park #2
	WN-4	BWS Waianae Yard
	WN-5	Pokai Bay Beach Park
	WN-6	Pililaau Playground
	WN-7	Nanakuli Fire Station
	WN-8	Maili Elementary School
	WN-9	Leihoku Elementary School
	WN-10	Kamaile Elementary School
	WN-11	Makaha Valley Playground
Ewa	WU-1	Silva's Store
	WU-2	Makakilo Booster Station #2
	WU-3	Makakilo Booster Station #4
	WU-14	Puuloa Playground

Table 4. The turbidity and total organic carbon (TOC) of well water samples collected from seven water use districts of Oahu which were negative for coliform bacteria.

Water Use District	No. Neg. for Coliform/ No. Samples Tested	Turbidity Avg. (NTU)	TOC Avg. (mg/l)
Honolulu	36/38 (94.7%)	0.75 (37)*	3.52 (37)*
Pearl Harbor	14/14 (100%)	0.44 (8)*	1.175 (8)*
Windward	10/12 (83.3%)	0.71 (6)*	5.98 (6)*
Waialua/Kahuku	5/7 (71.4%)	0.22 (4)*	2.52 (5)*
Wahiawa	2/2 (100%)	1.2 (1)*	3.97 (1)*
Waianae	3/4 (75%)	0.2 (2)*	5.02 (2)*
Ewa	2/2 (100%)	0.45 (2)*	2.66 (2)*
Total Average	72/79 (91.1%)	0.65 (60)*	3.4 (61)*

*(No.) = number of samples tested.

Table 5. Percent recovery of various fecal indicators from all water samples obtained from well, spring or tunnel, and distribution sources.

Bacterial Indicators	No. Positive/No. Samples		
	Wells	Springs/Tunnels	Distribution
TotalColiform	7/79 (8.9%)	2/2 (100%)	3/152 (1.97%)
Fecal Coliform	2/79 (2.5%)	2/2 (100%)	0/152 (0%)
E. coli	1/79 (1.3%)	2/2 (100%)	0/152 (0%)
Fecal Streptococci	2/79 (2.5%)	2/2 (100%)	0/152 (0%)
H_2S Bacteria	4/79 (5.1%)	2/2 (100%)	0/152 (0%)
Clostridium perfringens	0/79 (0%)	0/2 (0%)	0/152/ (0%)

Table 6. Correlation of recovering coliform bacteria with other indicator bacteria from well (A) and distribution (B) water samples.

Sample ID	Coliform	FS	H_2S	TB (CFU/100 ml)
A. WELL WATER				
HS-15	+	–	+	10
HS-30	+	–	–	35
HWS-15	+	–	+	45
HWS-16	+	–	–	76
KHS-1	+	–	–	37
KHS-1	+	+	+	6
WNS-1	+	+	+	>400
B. DISTRIBUTION WATER				
WU-8	+	–	–	ND
HW-2	+	–	ND	ND
WE-4	+	–	–	58

FS = fecal streptococci
H_2S = hydrogen sulfide producing bacteria
TB = total heterotrophic bacteria
ND = not done

Table 7. The effect of sample volume on recovering coliform bacteria from well (A) and distribution (B) sources of water and the confirmation of coliform isolates as fecal coliform and *E. coli*.

Sample Source	Total Coliform Presence/Absence Broth Confirmed in BGLBB			Fecal Coliform (growth at 44.5°C) in EC-MUG	*E. coli* (fluorescence) in EC-MUG
	100 ml	500 ml	500 ml		
A. WELL WATER					
HS-30	+	+	+	+	+
KHS-1	+	+	+	–	–
HS-15	+	+	–	–	–
KHS-1	–	+	+	–	–
WNS-1	–	+	+	–	–
HWS-16	–	–	+	+	–
HWS-15	–	–	+	–	–
B. DISTRIBUTION WATER					
WE-4	+	+	+	–	–
WU-8	–	–	+	–	–
HW-2	–	–	+	–	–

Table 8. Correlation of chlorine residual concentrations in 168 distribution water samples and concentrations of total heterotrophic bacteria, turbidity and total organic carbon (TOC).

Chlorine Residual	Number of Samples	Total Bacteria		Turbidity Avg. (NTU)	TOC Avg. (mg/l)
		CFU/100 ml	Times>400		
0-weak	67 (39.9%)	82	6	0.67	4.14
Trace	75 (44.6%)	32	2	0.75	4.12
>0.05 mg/l	26 (15.5%)	6	0	1.09	4.18

Table 9. The turbidity and total organic carbon (TOC) of distribution water samples collected from seven water use districts of Oahu which were negative for coliform bacteria.

Water Use District	No. Neg. for Coliform/ No. Samples Tested	Turbidity Avg. (NTU)	TOC Avg. (mg/l)
Honolulu	20/20 (100%)	0.35 (7)*	3.15 (7)*
Pearl Harbor	39/40 (97.5%)	0.26 (5)*	4.36 (6)*
Windward	29/30 (96.7%)	1.52 (2)*	4.86 (2)*
Waialua/Kahuku	26/27 (96.3%)		
Wahiawa	16/16 (100%)	0.25 (1)*	4.12 (1)*
Waianae	11/11 (100%)	1.41 (9)*	4.03 (9)*
Ewa	8/8 (100%)	0.35 (4)*	5.49 (4)*
Total Average	149/152 (98.0%)	0.75 (28)*	4.15 (29)*

*(No.) = number of samples tested.

Table 10. Recovery of FRNA coliphage from Oahu well sites, distribution sites, springs or tunnels using (membrane adsorption/elution) concentration method.

H_2O Sample Source (No.)	Total No. of Samples Tested	No. of Samples Positive for Coliphage
Wells (32)	35	0
Distribution sites (11)	11	0
Springs or tunnels (7)	7	0

Table 11. Recovery of FRNA coliphage from Oahu well sites, distribution sites, springs or tunnels using the enrichment method.

H_2O Sample Source (No.)	Total No. of Samples Tested	No. of Samples Positive for Coliphage	% Samples Positive for Coliphage
Wells (24)	24	0	0
Distribution sites (49)	52	3	5.8
Springs or tunnels (3)	3	0	0

Table 12. Water sample obtained from spring and tunnel sources (A) and from well sources (B).

Date	Sample	Station/ Station Identification	*L. pneumophila*	L. species
A. Spring and Tunnel Sources				
12/28/95	AS	Alewa Springs	no result	no result
12/28/95	HS-10	Makiki Springs	–	–
11/30/95	HWS-2	Waimanalo Tunnel	–	+
11/30/95	HWS-6	Kahaluu Tunnel	–	+
11/20/95	WNS-4	Waianae Tunnel	–	+
B. Well Sources				
04/08/96	HPS-2	Pearl City I	–	+
12/28/95	HS-2	Kalihi Shaft	–	+
12/28/95	HS-3-LS	Kalihi Station	–	–
12/28/95	HS-4-LS	Beretania Station	–	–
12/28/95	HS-5-HS	Kaimuki Station	–	–
12/28/95	HS-14-4	Wilder Wells	–	–
12/28/95	HS-17	Aina Koa Wells	–	–
12/28/95	HS-36	Kuliouou Wells	–	+
12/19/95	HWS-3	Kahaluu	–	–
02/01/96	HWS-10	Punaluu II	–	–
04/10/96	HWS-13	Hauula	–	+
12/19/95	HWS-15	Kahana	–	–
01/11/95	HWS-16	Waimanalo II	–	+
12/19/95	HWS-18	Luluku Well	–	+
03/11/96	MIS-1	Mililani Well I	–	+
05/30/96	MIS-3	Mililani Well III	–	+
02/12/96	WAS-1	Waialua	–	+
03/04/96	WAS-2	Haleiwa	–	–
03/11/96	WHS-1	Wahiawa I	–	+
01/09/96	WNS-1	Kamaile	–	–
11/15/95	WNS-5	Waianae Plantation Tunnel	–	+
03/11/96	WPS-2	Waipio Heights I	–	+
01/09/96	WUS-1*	Hoaeae	–	–
05/30/96	WUS-1*	Hoaeae	+	+
06/05/96	WUS-1*	Hoaeae	+	+
05/30/96	WUS-2	Kunia-1	–	+
01/09/96	WUS-3	Waipahu I	–	–
02/29/96	WUS-6	Honouliuli I	–	+

*WUS-1: Ran in triplicate.

Table 13. Detection of Legionella from various water distribution sites utilizing the enviroamp Legionella detection kit.

Date	Sample	Station/ Station Identification	*L. pneumophila*	L. species
12/12/95	HC-6	Waikiki Fire Station	–	+
12/12/95	HC-9	630 S. Beretania St.	–	+
12/12/95	HC-13	Waialae Beach Park	–	+
12/12/95	HC-22	Manoa Fire Station	–	+
12/12/95	HC-26	Kalama Valley Park	–	+
11/07/95	HP-4	P.C. Fire Station	–	+
11/07/95	HP-14	Halawa Xeriscape Gdn.	–	+
12/19/95	HW-10	45-620 Kam Hwy.	–	+
12/19/95	HW-14	Waihee Ln. Bstr.	–	+
12/19/95	HW-16	Punaluu Beach Park	–	+
12/19/95	HW-17	Hauula Fire Station	–	+
03/04/96	KH-1	Kahuku Hospital	–	+
03/04/96	KH-2	Kahuku Fire Station	+	+
12/11/95	MI-1	Kipapa Park	–	+
01/29/96	MI-2	Mililani Fire Station	–	+
01/29/96	MI-4*	Mililani District Park #1	–	+
05/30/96	MI-4*	Mililani District Park #1	–	+
12/11/95	MI-8	Mililani 685 Res. Sta.	–	+
01/29/96	MI-11*	Kuahelani Park	–	+
05/30/96	MI-11*	Kuahelani Park	+	+
02/12/96	WA-2	Waialua Fire Station	–	+
11/07/95	WA-3	Haleiwa Community Ctr.	–	–
02/12/96	WA-5	Sunset Fire Station	–	+
12/11/95	WH-2	Kemoo Farm Snack Bar	–	+
01/29/96	WH-4	BWS Wahiawa Corp. Yd.	–	+
01/29/96	WH-6	Wahiawa Elem. School	–	+
02/12/96	WH-8	Wahiawa Freshwater Pk.	–	+
02/12/96	WH-10	Wahiawa Int. School	–	+
01/09/96	WN-2	Keaau Beack Park	–	+
11/15/95	WN-4*	BWS Waianae Yard	+	+
06/05/96	WN-4*	BWS Waianae Yard	–	+
11/15/95	WN-5	Pokai Bay Beach Park	–	+

Table 13—*Continued*

Date	Sample	Station/ Station Identification	*L. pneumophila*	L. species
01/09/96	WN-7	Nanakuli Fire Station	–	+
05/30/96	WN-8*	Maili Elementary School	+	+
06/05/96	WN-8*	Maili Elementary School	–	+
01/09/96	WN-9	Leihoku Elem. School	–	+
06/05/96	WN-10	Kamaile Elem. School	–	–
01/09/96	WN-11	Makaha Val. Plgd.	–	+
12/11/95	WP-1	Waipio Heights Wells	–	+
05/30/96	WP-2*	Crestview Comm. Pk.	+	+
06/17/96	WP-2*	Crestview Comm. Pk.	+	+
12/11/95	WP-4	Waipio Heights Wells II	–	+
06/17/96	WP-5	Waipio Nbrhd. Park	–	+
11/15/95	WU-1	Silva's Store	–	+
02/20/95	WU-2	Makakilo Bstr. #2	–	+
01/09/96	WU-5*	Honowai Park	–	+
06/05/96	WU-5*	Honowai Park	–	+
11/15/95	WU-6	L'Orange Park	–	+
02/20/96	WU-9	Waipahu Fire Station	–	+
01/09/96	WU-11	Waipahu Int. School	–	+

*Ran duplicate samples.

Appendix A: Results of Analyzing All Water Samples from HBWS Well Sites

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
11/7/94	HPS02	100	+/-			-		-	12	0	
11/7/94	HPS02	1000	+/-			-					
2/16/95	HPS02	100	-/-					+	10	0	
2/16/95	HPS02	1000	+/-			-					
2/16/95	HPS02	1000									ND
10/6/94	HS01	100	-/-					-	55	0	
10/6/94	HS01	1000	+/-			+	-				
1/26/95	HS01	100	-/-					-	18	0	
1/26/95	HS01	1000	-/-								
2/28/95	HS01	100	-/-					-	13	0	
2/28/95	HS01	1000	+/-			+ (G+c)	-				
2/28/95	HS01	1000									ND
10/6/94	HS02	100	+/-			-		-	26	0	
10/6/94	HS02	1000	+/-			-					
1/26/95	HS02	100	-/-					-	21	0	
1/26/95	HS02	1000	+/-			+	-				
2/28/95	HS02	100	+/-			-		-	8	0	
2/28/95	HS02	1000	+/-			-					
2/28/95	HS02	1000									ND
2/15/96	HS02	1000									-
10/6/94	HS03(LS)	100	+/-			+	-	-	32	0	
10/6/94	HS03(LS)	1000	+/-			+	-				
1/26/95	HS03(LS)	100	-/-					-	19	0	
1/26/95	HS03(LS)	1000	-/-								
2/28/95	HS03(LS)	100	+/-			-		-	3	0	
2/28/95	HS03(LS)	1000	+/-			-					
2/28/95	HS03(LS)	1000									ND
2/15/96	HS03(LS)	1000									-
* 11/28/94	HS04(LS)	100	-/+	+	+			-	TNTC	2	
* 11/28/94	HS04(LS)	1000	-/+	+	+						
2/15/96	HS04(LS)	1000									-
10/6/94	HS05(HS)	100	+/-			-		-	TNTC	0	
10/6/94	HS05(HS)	1000	+/-			-					
1/26/95	HS05(HS)	100	-/-					-	27	0	

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	C.P.	Phage
1/26/95	HS05(HS)	1000	+/-			-					
2/28/95	HS05(HS)	100	+/-			+ (G+c)	-	-	12	0	
2/28/95	HS05(HS)	1000	+/-			+ (G+c)	-				
2/28/95	HS05(HS)	1000									ND
2/15/96	HS05(HS)	1000									-
10/6/94	HS14	100	-/-					-	TNTC	0	
10/6/94	HS14	1000	+/-			-					
1/26/95	HS14	100	-/-						15		
1/26/95	HS14	1000	+/-			+	-				
3/16/95	HS14	100	-/-					-	9	0	
3/16/95	HS14	1000	+/-			-					
3/16/95	HS14	1000									ND
2/15/96	HS14	1000									-
10/6/94	HS15	100	+/-			-		-	0	0	
10/6/94	HS15	1000	+/-			-					
3/16/95	HS15	100	+/+	+	- 45	-		3	10	0	
3/16/95	HS15	1000	+/+	+	- 45	-					
3/16/95	HS15	1000									ND
10/20/94	HS16	100	+/-			-		-	35	0	
10/20/94	HS16	1000	+/-			-					
3/30/95	HS16	100	+/-			+ (G+c)	-	-	3	0	
3/30/95	HS16	1000	+/-			-					
3/30/95	HS16	1000									ND
3/23/95	HS17	100	+/-			-		-	29	0	
3/23/95	HS17	1000	+/-			-					
3/23/95	HS17	1000									ND
2/15/96	HS17	1000									-
10/6/94	HS18	100	-/-					-	14	0	
10/6/94	HS18	1000	-/-								
3/16/95	HS18	100	-/-					-	3	0	
3/16/95	HS18	1000	-/-								
3/16/95	HS18	1000									ND
10/10/94	HS19	100	-/-					-	6	0	
10/10/94	HS19	1000	-/-								

Appendix A: Results of Analyzing All Water Samples from HBWS Well Sites

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
1/9/95	HS19	100	+/-			-		-	5	0	
1/9/95	HS19	1000	+/-			-					
2/16/95	HS19	100	-/-					-	160	0	
2/16/95	HS19	1000	-/-								
2/16/95	HS19	1000									ND
10/6/94	HS20	100	+/-			-		-	46	0	
10/6/94	HS20	1000	+/-			-					
1/26/95	HS20	100	-/-					-	171	0	
1/26/95	HS20	1000	+/-			-					
3/16/95	HS20	100	+/-			-		-	60	0	
3/16/95	HS20	1000	+/-			+ (G+c)	-				
3/16/95	HS20	1000									ND
7/2/95	HS20	1000									ND
10/20/94	HS22	100	-/-					-	35	0	
10/20/94	HS22	1000	-/-								
3/23/95	HS22	100	+/-			+ (G+c)	-	-	7	0	
3/23/95	HS22	1000	+/-			-					
3/23/95	HS22	1000									ND
10/20/94	HS23	100	-/-					-	27	0	
10/20/94	HS23	1000	-/-								
3/23/95	HS23	100	-/-					-	18	0	
3/23/95	HS23	1000	+/-			+ (G+c)	-				
3/23/95	HS23	1000									ND
10/20/94	HS28	100	+/-			-		-	47	0	
10/20/94	HS28	1000	+/-			-					
3/30/95	HS28	100	-/-					-	11	0	
3/30/95	HS28	1000	+/-			+ (G+c)	-				
3/30/95	HS28	1000									ND
2/15/96	HS28	1000									-
10/20/94	HS29	100	+/-			-		-	17	0	
10/20/94	HS29	1000	+/-			-					
3/30/95	HS29	100	+/-			+ (G+c)	-	-	148	0	
3/30/95	HS29	1000	+/-			-					
3/30/95	HS29	1000									ND

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
10/20/94	HS30	100	+/+	+	+(GS)	-		-	35	0	
10/20/94	HS30	1000	+/+	+	+(GS)	-					
5/18/95	HS36	100	-/-					-	12	0	
5/18/95	HS36	1000	-/-								ND
2/15/96	HS36	1000									-
12/19/94	HWS03	100	+/-			+	-	-	3	0	
12/19/94	HWS03	1000	+/-			+	-				
1/10/95	HWS03	100	+/-			+	-	-	153	0	
1/10/95	HWS03	1000	+/-			+	-				
12/19/94	HWS10	100	-/-					-	4	0	
12/19/94	HWS10	1000	-/-								
1/10/95	HWS10	100	-/-					-	3	0	
1/10/95	HWS10	1000	-/-								
2/1/96	HWS10	1000									-
12/19/94	HWS13	100	+/-			-		-	1	0	
12/19/94	HWS13	1000	+/-			-					
1/10/95	HWS13	100	+/-			-		-	2	0	
1/10/95	HWS13	1000	+/-			-					
2/1/96	HWS13	1000									-
2/28/95	HWS15	100	+/-			-		3	45	0	
2/28/95	HWS15	1000	+/-	+	+37(GS)	-					
2/28/95	HWS15				-45						
2/28/95	HWS15	1000									ND
11/28/94	HWS16	100	+/-			-		-	12	0	
11/28/94	HWS16	1000	+/-			-					
4/24/95	HWS16	100	+/-			-		-	76	0	
4/24/95	HWS16	1000	+/-	+	+	-					
4/24/95	HWS16	1000									ND
2/1/96	HWS16	1000									-
11/21/94	HWS20	100	+/-			+	-	-	9	0	
11/21/94	HWS20	1000	+/-			+	-				
4/24/95	HWS20	100	-/-					-	73	0	
4/24/95	HWS20	1000	+/-			-					
4/24/95	HWS20	1000									ND

Appendix A: Results of Analyzing All Water Samples from HBWS Well Sites

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
11/28/94	HWS22	100	+/-			+	-	-	4	0	
11/28/94	HWS22	1000	+/-			+	-				
11/1/94	KHS01	100	+/-	+	- (pink)	-		-	37	0	
11/1/94	KHS01	1000	+/-	+	- (pink)	-					
5/18/95	KHS01	100	+/-	+	-	-		2	6	0	
5/18/95	KHS01	1000	+/-	+	-	+ (G+c)	+/+				
5/18/95	KHS01	1000									ND
2/5/96	KHS01	1000									-
10/6/94	MIS01	100	-/-					-	137	0	
10/6/94	MIS01	1000	-/-								
3/16/95	MIS01	100	+/-			-		-	107	0	
3/16/95	MIS01	1000	+/-			-					
3/16/95	MIS01	1000									ND
2/5/96	MIS01	1000									-
2/6/95	WAS01	100	-/-					-	50	0	
2/6/95	WAS01	1000	-/-								
2/6/95	WAS01	1000									ND
3/1/96	WAS01	1000									-
2/6/95	WAS02	100	-/-					-	25	0	
2/6/95	WAS02	1000	+/-			+	-				
2/6/95	WAS02	1000									ND
3/1/96	WAS02	1000									-
2/6/95	WES01	100	-/-					-	10	0	
2/6/95	WES01	1000	-/-								
11/1/94	WES02	100	+/-			+	-	-	3	0	
11/1/94	WES02	1000	+/-			+	-				
5/1/95	WES02	100	-/-					-	1	0	
5/1/95	WES02	1000	-/-								
5/1/95	WES02	1000									ND
2/5/96	WES02	1000									-
12/19/94	WHS01	100	-/-					-	74	0	
12/19/94	WHS01	1000	-/-								
4/17/95	WHS01	100	+/-			-		-	59	0	
4/17/95	WHS01	1000	+/-			-					

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
4/17/95	WHS01	1000									ND
2/5/96	WHS01	1000									-
12/6/94	WNS01	100	+/-			+	-	-	0	0	
12/6/94	WNS01	1000	+/-			+	-				
4/17/95	WNS01	100	+/+	-		-		2	400+	0	
4/17/95	WNS01	1000	+/+	+	-	+ (G+c)	+/+				
4/17/95	WNS01	1000									ND
2/29/96	WNS01	1000									-
12/6/94	WNS02	100	+/-			-		-	4	0	
12/6/94	WNS02	1000	+/-			-					
4/17/95	WNS02	100	-/-					-	47	0	
4/17/95	WNS02	1000	+/-			-					
4/17/95	WNS02	1000									ND
11/17/95	WNS05	1000									-
10/20/94	WPS02	100	+/-			-		-	89	0	
10/20/94	WPS02	1000	+/-			-					
1/9/95	WPS02	100	+/-			-		-	13	0	
1/9/95	WPS02	1000	+/-			-					
5/18/95	WPS02	100	-/-					-	5	0	
5/18/95	WPS02	1000	+/-			-					
5/18/95	WPS02	1000									ND
2/5/96	WPS02	1000									-
10/6/94	WUS01	100	+/-			-		-	27	0	
10/6/94	WUS01	1000	+/-			-					
3/23/95	WUS01	100	-/-					-	6	0	
3/23/95	WUS01	1000	+/-			-					
3/23/95	WUS01	1000									ND
2/29/96	WUS01	1000									-
10/6/94	WUS03	100	+/-			-		-	2	0	
10/6/94	WUS03	1000	+/-			-					
3/30/95	WUS03	100	+/-			-		-	3	0	
3/30/95	WUS03	1000	+/-			+ (G+c)	-				
3/30/95	WUS03	1000									ND
4/17/95	WUS03	100	+/-			+ (G+c)	-	-	19	0	

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
4/17/95	WUS03	1000	+/-			+ (G+c)	-				
4/17/95	WUS03	1000									ND
2/29/96	WUS03	1000									-
2/14/95	WUS04	100	-/-					-	36	0	
2/14/95	WUS04	1000	+/-			-					
2/14/95	WUS04	1000									ND
4/17/95	WUS04	100	-/-			-		-	25	0	
4/17/95	WUS04	1000	+/-			-					
4/17/95	WUS04	1000									ND
2/29/96	WUS04	1000									-
2/29/96	WUS06	1000									-
10/6/94	WUS07	100	+/-			+	-	-	2	0	
10/6/94	WUS07	1000	+/-			+	-				
2/14/95	WUS07	100	-/-					-	77	0	
2/14/95	WUS07	1000	+/-			+	-				

P/A = Presence-Absence Test (growth/gas)
BGLBB = Brilliant Green Lactose Bile Broth
EC(EMB) = EC Agar(EMB Agar)
AZD = Azide Dextrose Broth
ENT/PSE = Enterococcus Agar/Pfizer Selective Enterocccus Agar
H2S = Hydrogen Sulfide Broth
mHPC = membrane Heterotrophic Plate Count Agar
CP = Clostridium perfringens Agar
Phage = Assay for phage by Adsorption/Elution or Enrichment (+ or -)
+ = positive for growth
- = negative for growth
G+c = gram positive cocci cells
GS = green sheen
ND = not detectable

Appendix B: Results of Analyzing All Water Samples from HBWS Spring and Tunnel Sites

Springs											
Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H2S	mHPC	CP	Phage
5/18/95	AS	100	+/+	+	+	-		+		0	
5/18/95	AS	1000	+/+	+	+	+(G+c)	+/+				ND
7/2/95	AS	1000									ND
8/31/95	AS	1000									ND
7/12/95	PS	1000									ND
Tunnels											
Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H2S	mHPC	CP	Phage
8/31/95	HS12	1000									ND
11/29/95	HWS02	1000									-
11/29/95	HWS06	1000									-
5/18/95	NT	100	+/-	+	+	+(G+c)	+/+	+		0	
5/18/95	NT	1000	+/-	+	+	+(G+c)	+/+				ND
11/17/95	WNS04	1000									-
	P/A = Presence-Absence Test (growth/ gas)										
	BGLBB = Brilliant Green Lactose Bile Broth										
	EC(EMB) = EC Broth (EMB Agar)										
	AZD = Azide Dextrose Broth										
	ENT/PSE = Enterococcus Agar/ Pfizer Selective Enterococcus Agar										
	H2S = Hydrogen Sulfide Broth										
	mHPC = membrane Heterotrophic Plate Count Agar										
	CP = Clostridium perfringens Agar										
	Phage = Assay for phage by Adsorption/Elu on (0) or Enrichment (+ or -)										
	+ = positive for growth										
	- = negative for growth										
	G+c = gram positive cocci cells										
	GS = green sheen										
	ND = not detectable										

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
4/11/95	HC03	100	+/-			-		-	86	0	
4/11/95	HC03	1000	+/-			-					
4/11/95	HC03	1000									ND
11/29/95	HC03	1000									-
9/20/94	HC06	100	-/-					-	TNTC	0	
9/20/94	HC06	1000	-/-								
11/7/94	HC06	100	+/-			+	-	-	4	0	
11/7/94	HC06	1000	+/-			+	-				
5/31/95	HC06	1000									ND
11/29/95	HC06	1000									-
7/27/94	HC09	100	-/-			-					
7/27/94	HC09	1000	-/-			-					
11/7/94	HC09	100	-/-					-	11	0	
11/7/94	HC09	1000	-/-								
5/31/95	HC09	1000									ND
9/20/94	HC13	100	+/-			-			200	0	
9/20/94	HC13	1000	+/-			-					
11/7/94	HC13	100	+/-			+	-	-	1	0	
11/7/94	HC13	1000	+/-			+	-				
5/31/95	HC13	1000									ND
5/31/95	HC14	1000									ND
9/20/94	HC19	100	-/-						55	0	
9/20/94	HC19	1000	+/-			-					
11/7/94	HC19	100	+/-			+	-	-	2	0	
11/7/94	HC19	1000	+/-			+	-				
11/29/95	HC19	1000									-
9/20/94	HC22	100	-/-						57	0	
9/20/94	HC22	1000	-/-								
11/7/94	HC22	100	+/-			+	-	-	1	0	
11/7/94	HC22	1000	+/-			+	-				
11/29/95	HC22	1000									-
9/20/94	HC26	100	-/-						11	0	
9/20/94	HC26	1000	+/-			-					
11/7/94	HC26	100	+/-			+	-	-	3	0	

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
11/7/94	HC26	1000	+/-			+	-				
9/20/94	HC27	100	-/-						9	0	
9/20/94	HC27	1000	-/-								
11/7/94	HC27	100	+/-			-		-	1	0	
11/7/94	HC27	1000	+/-			-					
9/20/94	HC31	100	-/-						65	0	
9/20/94	HC31	1000	-/-								
12/6/94	HC31	100	+/-			+	-	-	109	0	
12/6/94	HC31	1000	+/-			+	-				
11/29/95	HC31	1000									-
9/20/94	HC33	100	+/-			-			4	0	
9/20/94	HC33	1000	+/-			-					
12/6/94	HC33	100	+/-			+	-	-	14	0	
12/6/94	HC33	1000	+/-			+	-				
1/4/95	HC35	100	-/-					-	TNTC	0	
1/4/95	HC35	1000	-/-								
11/29/95	HC38	1000									-
9/20/94	HP04	100	-/-					-	4	0	
9/20/94	HP04	1000	-/-								
1/9/95	HP04	100	-/-					-	11	0	
1/9/95	HP04	1000	-/-								
5/31/95	HP04	1000									ND
9/20/94	HP14	100	+/-			-			2	0	
9/20/94	HP14	1000	+/-			-					
1/9/95	HP14	100	+/-			-		-	603	0	
1/9/95	HP14	1000	+/-			-					
11/6/95	HP14	500									-
4/11/95	HW01	1000									ND
7/20/94	HW02	100	-/-			+	-				
7/20/94	HW02	1000	+/+	+	-	-					
12/19/94	HW02	100	+/-			-		-	1	0	
12/19/94	HW02	1000	+/-			-					
1/10/95	HW02	100	+/-			-		-	3	0	
1/10/95	HW02	1000	+/-			-					

Appendix C: Results of Analyzing All Water Samples from HBWS Distribution Sites

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
11/29/95	HW02	1000									-
7/20/94	HW04	100	+/-			-					
7/20/94	HW04	1000	+/-			+	-				
12/19/94	HW04	100	+/-			+	-	-	1	0	
12/19/94	HW04	1000	+/-			+	-				
1/10/95	HW04	100	+/-			+	-	-	5	0	
1/10/95	HW04	1000	+/-			+	-				
7/20/94	HW08	100	+/-			-					
7/20/94	HW08	1000	-/-			+	-				
12/19/94	HW08	100	+/-			+	-	-	2	0	
12/19/94	HW08	1000	+/-			+	-				
1/10/95	HW08	100	+/-			+	-	-	1	0	
1/10/95	HW08	1000	+/-			+	-				
7/20/94	HW10	100	-/-			+	-				
7/20/94	HW10	1000	+/-			+	-				
12/19/94	HW10	100	+/-			-		-	3	0	
12/19/94	HW10	1000	+/-			-					
1/10/95	HW10	100	+/-			-		-	2	0	
1/10/95	HW10	1000	+/-			-					
11/29/95	HW10	1000									-
7/20/94	HW13	100	-/-			+	-				
7/20/94	HW13	1000	+/-			-					
12/19/94	HW13	100	+/-			+	-	-	3	0	
12/19/94	HW13	1000	+/-			+	-				
1/10/95	HW13	100	+/-			+	-	-	2	0	
1/10/95	HW13	1000	+/-			+	-				
7/20/94	HW14	100	+/-			-					
7/20/94	HW14	1000	+/-			+	-				
12/19/94	HW14	100	+/-			-		-	7	0	
12/19/94	HW14	1000	+/-			-					
1/10/95	HW14	100	+/-			-		-	3	0	
1/10/95	HW14	1000	+/-			-					
11/29/95	HW14	1000									-
4/11/95	HW15	1000									ND

Appendix C: Results of Analyzing All Water Samples from HBWS Distribution Sites

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/RSE	H_2S	mHPC	CP	Phage
7/20/94	HW16	100	+/-			-					
7/20/94	HW16	1000	+/-			-					
12/19/94	HW16	100	+/-			+	-	-	2	0	
12/19/94	HW16	1000	+/-			+	-				
1/10/95	HW16	100	+/-			+	-	-	3	0	
1/10/95	HW16	1000	+/-			+	-				
4/11/95	HW16	1000									ND
11/29/95	HW16	1000									-
7/20/94	HW17	100	-/-			-					
7/20/94	HW17	1000	+/-			+	-				
12/19/94	HW17	100	+/-			-		-	2	0	
12/19/94	HW17	1000	+/-			-					
1/10/95	HW17	100	+/-			-		-	25	0	
1/10/95	HW17	1000	+/-			-					
11/29/95	HW17	1000									-
7/27/94	HW18	100	-/-			-					
7/27/94	HW18	1000	-/-			-					
12/19/94	HW18	100	+/-			-		-	5	0	
12/19/94	HW18	1000	+/-			-					
1/10/95	HW18	100	+/-			-		-	5	0	
1/10/95	HW18	1000	+/-			-					
7/27/94	HW20	100	-/-			-					
7/27/94	HW20	1000	-/-			-					
12/19/94	HW20	100	+/-			+	-	-	7	0	
12/19/94	HW20	1000	+/-			+	-				
1/10/95	HW20	100	+/-			+	-	-	3	0	
1/10/95	HW20	1000	+/-			+	-				
11/29/95	HW20	1000									-
9/6/94	KH01	100	-/-					-	0	0	
9/6/94	KH01	1000	-/-								
11/1/94	KH01	100	-/-					-	3	0	
11/1/94	KH01	1000	-/-								
12/4/95	KH01	1000									-
9/6/94	KH02	100	-/-					-	0	0	

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
9/6/94	KH02	1000	-/-								
11/1/94	KH02	100	+/-			-		-	TNTC	0	
11/1/94	KH02	1000	+/-			-					
12/4/95	KH02	1000									-
9/6/94	KH03	100	-/-					-	0	0	
9/6/94	KH03	1000	-/-								
11/1/94	KH03	100	-/-					-	3	0	
11/1/94	KH03	1000	-/-								
12/4/95	KH03	1000									-
11/21/94	MI01	100	+/-			+	-	-	58	0	
11/21/94	MI01	1000	+/-			+	-				
11/17/95	MI01	1000									-
11/21/94	MI02	100	+/-			-		-	2	0	
11/21/94	MI02	1000	+/-			-					
11/21/94	MI03	100	+/-			+	-	-	3	0	
11/21/94	MI03	1000	+/-			+	-				
9/26/94	MI04	100	+/-			-		-	19	0	
9/26/94	MI04	1000	-/-								
11/21/94	MI04	100	-/-					-	21	0	
11/21/94	MI04	1000	-/-								
11/17/95	MI04	1000									-
1/17/95	MI05	100	-/-					-	21	0	
1/17/95	MI05	1000	+/-			+	-				
9/26/94	MI06	100	+/-			+	-	-	4	0	
9/26/94	MI06	1000	+/-			+	-				
1/17/95	MI06	100	-/-						4	0	
1/17/95	MI06	1000	+/-			+	-				
9/26/94	MI07	100	-/-					-	4	0	
9/26/94	MI07	1000	-/-								
1/17/95	MI07	100	-/-						7	0	
1/17/95	MI07	1000	+/-			-					
9/26/94	MI08	100	-/-					-	66	0	
9/26/94	MI08	1000	-/-								
1/17/95	MI08	100	-/-						9	0	

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
1/17/95	MI08	1000	+/-			-					
11/17/95	MI08	1000									-
1/17/95	MI09	100	-/-						9	0	
1/17/95	MI09	1000	+/-			-					
1/17/95	MI10	100	-/-						15	0	
1/17/95	MI10	1000	+/-			+	-				
1/17/95	MI11	100	-/-						10	0	
1/17/95	MI11	1000	+/-			+	-				
11/17/95	MI11	1000									-
10/10/94	WA01	100	-/-					-	5	0	
10/10/94	WA01	1000	-/-								
11/1/94	WA01	100	-/-					-	1	0	
11/1/94	WA01	1000	-/-								
12/4/95	WA01	1000									-
10/10/94	WA02	100	-/-					-	3	0	
10/10/94	WA02	1000	-/-								
11/1/94	WA02	100	+/-			+	-	-	6	0	
11/1/94	WA02	1000	+/-			+	-				
12/4/95	WA02	1000									-
9/12/94	WA03	100	-/-					-	0	0	
9/12/94	WA03	1000	+/-			+	-				
11/1/94	WA03	100	-/-					-	3	0	
11/1/94	WA03	1000	-/-								
11/6/95	WA03	500									-
12/4/95	WA03	1000									-
9/12/94	WA05	100	-/-					-	0	0	
9/12/94	WA05	1000	-/-								
11/1/94	WA05	100	+/-			-		-	1	0	
11/1/94	WA05	1000	+/-			-					
12/4/95	WA05	1000									+
3/1/96	WA05	1000									-
9/12/94	WA06	100	-/-					-	0	0	
9/12/94	WA06	1000	-/-								
11/1/94	WA06	100	-/-					-	2	0	

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
11/1/94	WA06	1000	+/-			-					
12/4/95	WA06	1000									-
10/10/94	WA07	100	-/-					-	4	0	
10/10/94	WA07	1000	-/-								
11/1/94	WA07	100	+/-			-		-	1	0	
11/1/94	WA07	1000	+/-			-					
9/6/94	WE01	100	-/-					-	0	0	
9/6/94	WE01	1000	-/-								
11/1/94	WE01	100	+/-			+	-	-	3	0	
11/1/94	WE01	1000	+/-			+	-				
12/4/95	WE01	1000									-
9/6/94	WE02	100	-/-					-	0	0	
9/6/94	WE02	1000	-/-								
9/6/94	WE03	100	-/-					-	0	0	
9/6/94	WE03	1000	-/-								
11/1/94	WE03	100	+/-			-		-	1	0	
11/1/94	WE03	1000	+/-			-					
12/4/95	WE03	1000									-
9/6/94	WE04	100	-/-					-	5	0	
9/6/94	WE04	1000	+/-			+	-				
11/1/94	WE04	100	+/-	+	-	+	-	-	58	0	
11/1/94	WE04	1000	+/-	+	-	+	-				
12/4/95	WE04	1000									-
9/6/94	WE05	100	-/-					-	0	0	
9/6/94	WE05	1000	-/-								
11/1/94	WE05	100	+/-			-		-	2	0	
11/1/94	WE05	1000	+/-			-					
12/4/95	WE05	1000									-
9/12/94	WH02	100	-/-					-	1	0	
9/12/94	WH02	1000	+/-			-					
10/10/94	WH02	100	-/-					-	59	0	
10/10/94	WH02	1000	-/-								
1/29/96	WH02	1000									-
9/26/94	WH03	100	-/-					-	6	0	

Date	Site	Volume	P/A	BGLBB	FC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
9/26/94	WH03	1000	+/-			-					
10/10/94	WH03	100	+/-			-		-	90	0	
10/10/94	WH03	1000	+/-			-					
9/12/94	WH04	100	+/-			+	-	-	2	0	
9/12/94	WH04	1000	+/-			+	-				
10/10/94	WH04	100	+/-			-		-	53	0	
10/10/94	WH04	1000	+/-			-					
1/29/96	WH04	1000									-
9/12/94	WH05	100	-/-					-	2	0	
9/12/94	WH05	1000	-/-								
10/10/94	WH05	100	+/-			-		-	TNTC	0	
10/10/94	WH05	1000	+/-			-					
9/12/94	WH06	100	-/-					-	0	0	
9/12/94	WH06	1000	-/-								
10/10/94	WH06	100	+/-			+	-	-	257	0	
10/10/94	WH06	1000	+/-			+	-				
1/29/96	WH06	1000									-
9/12/94	WH08	100	-/-					-	4	0	
9/12/94	WH08	1000	+/-			-					
11/21/94	WH08	100	+/-			-		-	16	0	
11/21/94	WH08	1000	+/-			-					
9/12/94	WH09	100	-/-					-	1	0	
9/12/94	WH09	1000	-/-								
11/21/94	WH09	100	-/-					-	6	0	
11/21/94	WH09	1000	-/-								
1/29/96	WH09	1000									-
9/26/94	WH10	100	-/-					-	30	0	
9/26/94	WH10	1000	-/-								
11/21/94	WH10	100	-/-					-	6	0	
11/21/94	WH10	1000	-/-								
1/4/95	WN01	100	+/-			+	-	-	52	0	
1/4/95	WN01	1000	+/-			+	-				
6/7/95	WN01	1000									ND
1/4/95	WN02	100	-/-					-	TNTC	0	

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	Cl_2	Phage
1/4/95	WN02	1000	-/-								
3/12/96	WN02	1000									-
1/4/95	WN03	100	-/-					-	TNTC	0	
1/4/95	WN03	1000	-/-								
1/4/95	WN04	100	-/-					-	2	0	
1/4/95	WN04	1000	-/-								
11/17/95	WN04	1000									-
1/4/95	WN05	100	-/-					-	145	0	
1/4/95	WN05	1000	-/-								
11/17/95	WN05	1000									-
1/4/95	WN06	100	-/-					-	9	0	
1/4/95	WN06	1000	-/-								
3/12/96	WN06	1000									-
1/4/95	WN07	100	-/-					-	59	0	
1/4/95	WN07	1000	-/-								
3/12/96	WN07	1000									-
1/4/95	WN08	100	+/-			+	-	-	TNTC	0	
1/4/95	WN08	1000	+/-			+	-				
3/12/96	WN08	1000									-
1/4/95	WN09	100	-/-					-	TNTC	0	
1/4/95	WN09	1000	-/-								
3/12/95	WN09	1000									-
1/4/95	WN10	100	-/-					-	15	0	
1/4/95	WN10	1000	-/-								
3/12/96	WN10	1000									-
1/4/95	WN11	100	+/-			+	-	-	4	0	
1/4/95	WN11	1000	+/-			+	-				
10/10/94	WP01	100	-/-					-	73	0	
10/10/94	WP01	1000	+/-			-					
3/1/96	WP01	1000									-
10/10/94	WP02	100	+/-			-		-	4	0	
10/10/94	WP02	1000	+/-			-					
10/10/94	WP04	100	+/-			-		-	370	0	
10/10/94	WP04	1000	+/-			-					

[illegible]	[illegible]	[illegible]	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	[illegible]	m-FC	[illegible]	Phage
3/1/96	WP04	1000									-
10/10/94	WP05	100	+/-			-		-	5	0	
10/10/94	WP05	1000	+/-			-					
3/1/96	WP05	1000									-
9/28/94	WU01	100	+/-			-		-	60	0	
9/28/94	WU01	1000	+/-			+	-				
12/6/94	WU01	100	+/-			-		-	0	0	
12/6/94	WU01	1000	+/-			-					
6/7/95	WU01	1000									ND
11/17/95	WU01	1000									+
9/28/94	WU02	100	-/-					-	1	0	
9/28/94	WU02	1000	-/-								
12/6/94	WU02	100	+/-			+	-	-	0	0	
12/6/94	WU02	1000	+/-			+	-				
3/12/96	WU02	1000									-
9/28/94	WU03	100	-/-					-	4	0	
9/28/94	WU03	1000	+/-			-					
12/6/94	WU03	100	-/-					-	1	0	
12/6/94	WU03	1000	-/-								
8/3/94	WU04	100	-/-			-					
8/3/94	WU04	1000	-/-			-					
12/6/94	WU04	100	-/-					-	0	0	
12/6/94	WU04	1000	-/-								
8/3/94	WU05	100	-/-			-					
8/3/94	WU05	1000	-/-			-					
12/6/94	WU05	100	+/-			+	-	-	148	0	
12/6/94	WU05	1000	+/-			+	-				
8/3/94	WU06	100	-/-			-					
8/3/94	WU06	1000	-/-			-					
12/6/94	WU06	100	+/-			+	-	-	23	0	
12/6/94	WU06	1000	+/-			+	-				
11/17/95	WU06	1000									+
3/12/96	WU06	1000									-
8/3/94	WU07	100	-/-			-					

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
8/3/94	WU07	1000	+/-			+	-				
12/6/94	WU07	100	+/-			+	-	-	0	0	
12/6/94	WU07	1000	+/-			+	-				
8/3/94	WU08	100	-/-			-					
8/3/94	WU08	1000	+/+	+	-	-					
12/6/94	WU08	100	+/-			-		-	0	0	
12/6/94	WU08	1000	+/-			-					
8/3/94	WU09	100	-/-			-					
8/3/94	WU09	1000	-/-			+	-				
3/12/96	WU09	1000									-
8/3/94	WU10	100	-/-			-					
8/3/94	WU10	1000	-/-			-					
6/7/95	WU10	1000									ND
8/3/94	WU11	100	-/-			-					
8/3/94	WU11	1000	-/-			-					
8/3/94	WU12	100	-/-			-					
8/3/94	WU12	1000	-/-			-					
1/4/95	WU12	100	+/-			-		-	6	0	
1/4/95	WU12	1000	+/-			-					
8/3/94	WU13	100	-/-			-					
8/3/94	WU13	1000	-/-			-					
1/4/95	WU13	100	+/-					-	136	0	
1/4/95	WU13	1000	+/-								
3/12/96	WU13	1000									-
9/28/94	WU14	100	-/-					-	2	0	
9/28/94	WU14	1000	-/-								
1/4/95	WU14	100	+/-			+	-	-	102	0	
1/4/95	WU14	1000	+/-			+	-				ND

P/A = Presence-Absence Test (growth/gas)
BGLBB = Brilliant Green Lactose Bile Broth
EC(EMB) = EC broth (EMB Agar)
AZD = Azide Dextroas Broth

Date	Site	Volume	P/A	BGLBB	EC(EMB)	AZD	ENT/PSE	H_2S	mHPC	CP	Phage
	ENT/PSE= Enterococcus Agar/Pfizer Enterococcus Agar										
	H2S = Hydrogen Sulfide Broth										
	mHPC = membrane Heterotrophic Plate Count										
	CP = Clostridium perfringens Agar										
	Phage = Assay for phage by Adsorption/Elution or Enrichment (+ or -)										
	+ = positive for growth										
	- = negative for growth										
	G+c = gram positive cocci cells										
	GS = green sheen										
	ND = not detectable										

Date: Sat, 16 Aug 1997 15:26:11 -1000 (HST)
From: Roger Fujioka <roger@hawaii.edu>
To: Paul@epamail.epa.gov
Cc: Bruce@epamail.epa.gov
Subject: GWDR

Paul and Bruce: I am e-mailing you my most current assessment on monitoring requirements for GWDR given the parameters as recently outlined by both of you. The opportunity to discuss the basis for my assessment do not fit in well with a teleconference and is best stated in writing for both of you to review.

I. Latest Macler and Berger premise for monitoring of groundwater for microorganisms as part of GWDR.

A. To determine whether goundwater is contaminated with sewage and is therefore vulnerable to sewage borne pathogens. Measurement for pathogens is not a requirement.

B. Of the sewage-borne pathogens, human enteric viruses is the group of pathogens most likely to contaminate groundwater because its small size and ability to maintain infectivity far longer than bacteria enables this group of pathogens the best chance to be transported through the soil profile to reach the groundwater in an infectious state. Moreover,the infectious dose of human enteric viruses is very low.

C. List of likely candidate indicators to monitor for:

1) Coliform group:total coliform, fecal coliform, E. coli
2) Enterococci
3) C. perfrinens
4) Coliphage (somatic or FRNA)
5) Human enteric virus by PCR

D. Cost for monitoring test a major factor: $50-100?

E. Frequency of monitoring a major factor: Quarterly or monthly?

F. Volume to be sampled a major factor in shipping: 1 liter?

G. Method of assay should be feasible, reliable and inexpensive.

II. My assessment of proposed indicators:

A. Coliform group.

ADVANTAGES

1. They are found in highest concentrations in sewage.
2. Monitoring methods are feasible, reliable and inexpensive.
3. Applicable when sewage contamination of groundwater occurs quickly and not sieved through intact soil profile.

DISADVANTAGES

1. Their survivability under environmental conditons is poor and they can be expected to die off much sooner than viruses.
2. They are larger than viruses and will be filtered out by soil profile before human viruses.

B. Enterococci

ADVANTAGES

1. Their concentrations in sewage is high and consistently enumerable.
2. Monitoring methods are feasible, reliable and inexpensive.
3. Applicable when sewage contamination of groundwater occurs quickly and not sieved through intact soil.

DISADVANTAGES

1. Their survivability in the environment is better than coliform but this group of bacteria will still die off before human viruses.
2. They are larger than viruses and will be filtered out by soil profile before human viruses.

C. Clostridium perfringens.

ADVANTAGES

1. C. perfringens is consistently found in moderate and enumerable levels in sewage and at concentrations higher than pathogens.
2. Monitoring method is similar to membrane filtration method used for E. coli and enterococci currently used by most water laboratory. As a result, this method can easily process larger volumes of clean water. Although the method is not described in Standard Methods, most water laboratories will be able to quickly perform this method. Thus, this method can be classified as feasible, reliable and slightly more expensive in terms of reagents but not in terms of laboratory skill.
3. This method is applicable when sewage contamination of groundwater occurs without effective seiving action of intact soil profile.
4. C. perfringens spores are very stable to environmental conditons and these spores may survive longer than human viruses once they are exposed to environmental conditions.

DISADVANTAGES

1. The spores of C. perfringens are larger than viruses and therefore they will be filtered out by soil much more effectively than human enteric viruses.
2. Their are some disadvantages to the method. The growth medium is not yet commercially available. Incubation is under anaerobic conditions and one key reagent (indoxy B-D-glucuronide) is still expensive.

D. Coliphage (FRNA phage).

FRNA phages was selected over somatic phage because the FRNA structure,their RNA genome, their inability to multiply in host under environmental conditons, and their stability to environmental conditions are similar to human enteric viruses. In contrast, somatic phages are dissimilar to human enteric viruses in these important properties.

ADVANTAGES

1. They are found in moderate and enumerable concentrations in sewage and at concentrations higher than human enteric viruses.

2. Monitoring methods can still be classified as feasible, reliable and inexpensive because typical water laboratories can be taught to conduct this test with existing equipment.
3. The structure and survivability of FRNA phages are similar to human enteric viruses and therefore their movement through all soil profiles should be similar to those of human enteric viruses.

DISADVANTAGES

1. Laboratory personnel will have to be trained to do assays for FRNA phages and will need to be able to maintain the efficiency of their host cell line. These requirements are easy for some water laboratories (eg reference, regional laboratories) but may not be easy for some basic laboratories.
2. The monitoring method to recover FRNA phage has been standardized for only the direct assay method using sample volumes of approximately 4 ml of sample. To process sample volume of 100 ml, the direct method becomes more expensive and and will require more set up time.
3. For monitoring of larger volumes such as 500 ml or 1,000 ml, the sample will have to be concentrated on membranes and eluted in a method similar to human enteric viruses. Although this method has been reported by several laboratories, this method has yet to be standardized and the efficiency of this method has not been reliably determined. Some work will have to be done to standardized this large volume assay for FRNA phage.
4. Methods to confirm that the recovered plaque is due to FRNA phages are also not as easy or standardized as bacterial methods.

E. Human enteric viruses by direct PCR.

The ICR assay method for culture of human enteric viruses requires sampling large volumes of water and involves extensive methodology of eluting viruses from expensive membrane cartridge, of inefficient method to concentrate the viruse as well as long and very expensive method to culture for viruses using cell culture. Thus, this method has been concluded to be ideal but not feasible for monitoring groundwater under the GWDR. However, direct PCR for human enteric viruses of water concentrates using small volumes of water is considered a possible feasible method.

ADVANTAGES

1. Assay method measures for the group of pathogens (human enteric viruses) of most concern for contamination of groundwater.Thus, the assay measures the movement of human enteric viruses through any soil profile system.
2. Monitoring method has the following characteristics with regard to virus assays. It is rapid: 1-2 days instead of weeks. It is effective and specific:can be made to concurrently detect genes of most of the species and serotypes of water borne human enteric viruses. It is sensitive: It can detect as few as 10 virus particles reliably.
3. The method can be easily scaled up to assay larger volumes of water.

DISADVANTAGES

1. The method is not a standard method and currently can be completed in relatively few laboratories with specially trained personnel in specially equipped laboratories.

2. Reagents for test are expensive and there are inherent problems of false positive, false negative and confirmation of results.
3. Assay method detects dead as well as live virus particles and this has created problems in true health risk assessments based on PCR data alone.

III. My Proposal for Selection of Indicators for Monitoring

Establishing of monitoring regimem must consider the desirability based on scientific data and be modified by the reality of being able to implement the established rule. Some important considerations are as follows.

1. Sample frequency. For practical reasons, the traditional approach of requiring higher frequency of samples (once a month) for monitoring wells serving many people (>10,000?) and minimum sampling frequency (quarterly) for wells serving smaller population of people (<,3000?) is reasonable. It is also reasonable to limit well monitoring to two samples for wells serving small population for only portion of year. A more serious requirement is whether every well drilled will require sampling or only representative wells from a defined aquifer.

2. Sample volume. In previous comparison of coliform positives versus human virus positive, small volumes (100 ml) were used for coliform assay and larger volume (50 gallons) were used for human viruses. Thus, based on volumes of assay, this comparison is not valid. Larger volumes of water samples should be assayed for fecal indicators which had been limited to 100 ml volumes. This will not substantially increase the cost of the assay but will increase the sensitivity of the assay. As a result, I propose that a sample volume of 500 ml or 1,000 ml of groundwater should be assayed for.

3. Groundwater contamination by sewage may occur under three condtions. First,rather quickly because the soil profile is not intact and this allows sewage to reach groundwater without being effectively sieved or filtered out by intact soil profile. Second, there is enough intact soil profile so the seiving or filtering capacity of the soil profile can effectively seive or filter out the microorganisms in the sewage. Third, only under severe conditions (flooding or yearly heavy rains). Some methods are applicable for one condition while other methods are more applicable for both conditons.

4. A proposed monitoring Strategy: C. perfringens and Human virus by PCR.

There will be a requirement for at least two assay procedures to address the three conditions by which sewage percolates through soil to contaminate groundwater (see above 3).

Assay method one: C. perfringens. This is the most conservative method to assay for groundwater contamination when insufficient seiving of the soil profile occurs since this indicator will remain viable for a long period while it percolates through the soil and while it remains in the groundwater. The method is feasible and can be easily scaled up to process 1,000 ml. Results from this assay should address questions related to gross contamination with a reliable and feasible test most laboratories can easily master.

Assay method two: Human enteric virus by direct PCR. This is the only method whose data will provide direct evidence for presence or absence of many types of human enteric viruses. Thus, use of this method will directly address the expected question of whether the test used is actually measuring for the possible contamination of groundwater with

human enteric viruses. The data from this assay will be relevant to all three of the groundwater contamination conditions as described earlier. The limitation that this method will recover both dead and live viruses is less of a problem for groundwater monitoring because groundwater should be protected from the contamination of all intact human enteric virus particle, even dead viruses. I recommend that at minimum of 1,000 ml of water be processed to detect for human viruses by the PCR method. Larger volumes of water can be easily handled by this method. Standardization of this method is still a requirement.

Use of these two methods will provide more information than any other two methods.

Alternative method two: FRNA phage. If the PCR method is determined not to be feasible,the second method of choice should be to assay for FRNA phage because data from this method will also address the three groundwater contaminating conditions. The advantage of this assay is that all sewage consistently contains moderate concentrations of this group of phage and therefore the absence of FRNA phage in groundwater is a reliable index that the soil profile has effectively filtered out the sewage borne contaminants, if present. Moreover, many more laboratories can be expected to be able to complete this method as compared to the PCR method. If this method is used, a 500 ml volume to be assayed for C. perfringens and a 500 ml volume to be assayed for FRNA virus would be a feasible minimum strategy.

I would be interested to hear your response to my assessment of the monitoring issues for the GWDR. As you know, the Honolulu Board of Water Supply is very interested in the GWDR. In this regard, I have completed the monitoring of Honolulu's groundwater for the various bacterial and FRNA phage indicators. Moreover, I have just obtained approval to monitor groundwater for human enteric viruses. I would be interested in attending the workshop in Cincinnati on methods for monitoring groundwater that is scheduled for September.
Roger Fujioka.