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DEVELOPMENT AND IMPLEMENTATION OF A WATER MONITORING PLAN TO PREPARE FOR CRIMINAL AND TERRORISTIC CONTAMINATION OF A DRINKING WATER SYSTEM

Roger S. Fujioka
Audrey Y. Asahina
Dayna M. Sato
Bunnie S.Yoneyama

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PREPARED FOR
Honolulu Board of Water Supply
City and County of Honolulu
630 S. Beretania Street
Honolulu, Hawaii 96843

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Principal Investigator: Roger S. Fujioka, Ph.D.

WATER RESOURCES RESEARCH CENTER
University of Hawaii at Manoa
2540 Dole Street
Honolulu, Hawaii 96822

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EXECUTIVE SUMMARY

I. The Need for an Emergency Water Monitoring Plan

The deadliest acts of terrorism against the people and government of the United States occurred on September 11, 2001. Since the people responsible for these acts of terrorism lived in the United States and their organization was not identified, the US government concluded that they will strike again and that populated centers and critical infrastructures are likely targets. Today, the greatest identified threat to water utilities is acts of terrorism, especially intentional contamination of water supplies by extremely hazardous chemical and biological agents such as those selected for use in warfare. To address this new threat, President Bush alerted all public facilities, including water utilities, to develop plans to guard against acts of terrorism. One obvious problem for all water utilities is that their current water monitoring plan is not designed to detect intentional contamination of water supplies, especially by agents identified for use in biological and chemical warfare. Thus, the identified problem for the Honolulu Board of Water Supply (BWS) in 2001 was the need to develop an emergency water monitoring plan (EWMP) to address concerns related to intentional contamination of water supplies.

II. Project Development and Goals

Since there were no published guidelines in the development of an EWMP, BWS requested the services of Roger Fujioka of Water Resources Research Center (WRRC) at the University of Hawaii and funded a research study to develop an interim water monitoring plan. The primary goals of this funded study were to develop an EWMP for BWS and to evaluate its effectiveness to rapidly and reliably detect intentional contamination of Honolulu's municipal water system by hazardous chemicals and pathogens. The secondary goal was to train BWS laboratory personnel involved in the implementation of this EWMP. To address this secondary goal, BWS initially agreed to assign a microbiologist to this project but none was assigned. As a result, water samples were collected and transported to the University of Hawaii where all the assays were conducted. Training of BWS laboratory personnel was limited to periodic training sessions at the University of Hawaii.

III. Rationale and Description of the Three-Tiered EWMP

When a water utility is informed that its water system may be contaminated, it is faced with two immediate questions of concern. First, what is the nature (chemical, biological) of the contaminant? Second, what sections of the water systems are contaminated? To address these concerns, a three-tiered emergency water monitoring plan called EWMP was developed. Briefly, the first tier of testing (Tier One tests) is to determine the sites in a water system where contamination has occurred. The second tier of testing (Tier Two tests) is to identify the contaminating agent in water samples. The third tier of testing (Tier Three test) is to characterize the populations of THB isolates recovered from potable water samples so that

the range of bacteria that are naturally present in potable water sources can be established and bacteria that originated from an external source of contamination can be differentiated. The key to the success of this EWMP is selecting the tests to meet the objectives of this monitoring plan. Three guidelines were followed in the development of the EWMP. (1) Select commercially available methods that can be readily incorporated and used at the BWS laboratory. (2) Design a plan to be implemented after BWS has obtained creditable evidence of a water contamination event. (3) Evaluate the suitability of methods selected to implement EWMP because most of them have not been approved for analyzing potable water samples.

A diagram of the three-tiered EWMP is shown in Figure 1 (Chapter Two) and the seven steps to implement this EWMP are summarized below.

Step 1. The EWMP is triggered to start when BWS receives creditable information or evidence that its water system has been contaminated.

Step 2. Based on available evidence, BWS must determine the most likely type (chemical, biological) of contamination and must also determine the most likely sites of contamination in the water system so samples can be collected for evidence of contamination.

Step 3. The water system must be chlorinated to disinfect pathogens, which may be present in the water system.

Step 4. Suspected water samples, which were collected in Step 2, must be analyzed using the three Tier One tests: Microtox method to measure for presence of toxic chemicals, ATP method to measure for total concentrations of microorganisms, and InSpectra method to measure for levels of UV-absorbing organic chemicals or particles in water samples. For all positive water samples, initiate additional tests to determine whether practical methods such as filtration, adsorption, precipitation, and disinfection can be used to remove or neutralize the contaminant in the water sample.

Step 5. All positive Tier One tests must be analyzed by the following Tier Two tests: Ruggedized Advanced Pathogen Identification Device (R.A.P.I.D.) method to identify microbial pathogens and enzyme linked immunosorbent assay (ELISA) method to identify toxic chemicals in water samples.

Step 6. Isolates of THB and NTC recovered from potable water samples, should be analyzed by the RiboPrinter method as a means of determining those isolates of bacteria which originated from an external source such as a contamination event.

Step 7. Preparations should be made to use alternative sources of water if the primary sources of water are contaminated.

IV. Assessment and Recommendations of Tier One Tests

The objectives of Tier One tests are to analyze the many suspected potable water samples and to quickly determine which sites in the water system are free of contamination and which sites may be contaminated so that effective remedial action can be taken. Three Tier One methods were selected because no single method can be expected to detect both hazardous chemicals and biological agents. To be effective, Tier One methods must meet the following requirements: (1) the method must be commercially available and can rapidly (minutes) measure a water quality parameter that will change in response to a contamination event, (2) the variation in the measured concentrations for that water quality parameter must be modest and predictable, and (3) the concentration of the water quality parameter to signal a possible contamination event must be determined. These three requirements were used to evaluate the suitability of the Tier One methods.

The Microtox method was selected as a Tier One test to rapidly measure concentrations of toxic chemicals in water. The primary reasons for selecting the Microtox method were its long history of successful use to detect toxic chemicals in many types of water and acceptance of this method in *Standard Methods for the Examination of Water and Wastewater*. Moreover, this method has been reported to detect toxicity when tested against more than 1,300 known toxic chemicals. This test uses a marine luminescent bacterium (*Vibrio fischeri*) that produces light as a by-product of its normal metabolic process. When the light-producing population of *V. fischeri* is added to water samples without toxic chemicals, their metabolism will not be affected and the light produced will remain close to 100%. If the water samples contain toxic chemicals, they will inhibit the metabolism of the bacterial population and cause loss of light output. The percentage of light loss (e.g., 99%) can be correlated to the degree of toxicity in the water sample. The suitability of the Microtox method was evaluated based on meeting the three requirements of Tier One methods. The results showed that this method met all three requirements. However, due to normal variation in light output by the bacterial population in ambient potable water, the action level to suspect toxic levels of chemical agents in water was established at >40% loss of light after the standard 15 minute assay. The Microtox method was recommended as a Tier One test to screen potable water for presence of toxic chemicals and to signal a contamination event.

The Pallchek Luminometer System was selected as a Tier One method to rapidly measure concentrations of adenosine triphosphate (ATP) and indirectly measure concentrations of total microbial load in that water sample. Since every viable cell uses ATP for its metabolic reaction, the concentration of total ATP is an estimate of total viable cells per water sample Elevated levels of ATP in potable water can be used to signal contamination by microorganisms such as pathogens. The primary reasons for selecting the Pallchek method were its capacity to process large volumes (100 to 500 ml) of water and availability of reagents to increase the sensitivity of the assay. ATP in water samples is measured by its reaction with luciferase enzyme to produce light and the amount of light produced is proportional to ATP concentrations in all viable cells. The suitability of the Pallchek method

was evaluated based on meeting the three requirements of Tier One method. The results showed that this method partially met the first requirement but did not meet the other two requirements. The limitation of the Pallchek method was the wide variation of ATP concentrations measured in ambient potable water samples and the insensitivity of this method to detect health-related concentrations (100 to 2,000 CFU/100 ml) of *E. coli* added to potable water samples. The poor performance of the Pallchek ATP method raised the question of whether the limitation was the Pallchek method or the technology used in the measurement of ATP. To address this question, Profile-1 was selected as the alternative ATP assay and the same water samples were assayed by these two methods. Similar results were obtained by these two methods, indicating that the limitation is related to the technology of measuring ATP in water. It was concluded that the wide variation in ATP measurements in BWS potable water samples is most likely due to variable concentrations, physiological states and kinds of microorganisms (bacteria, yeast, protozoa) in potable water. The Pallchek ATP method was not recommended as a Tier One method to detect a contamination event in potable water.

The InSpectra method was selected as a Tier One method to rapidly measure presence of UV-absorbing components (organic matter, nitrates, suspended solids) in water and provide concentrations of six common water quality parameters: biological oxygen demand (BOD), chemical oxygen demand COD), total organic carbon (TOC) total suspended solids (TSS), nitrates (NO_3) and surfactants (SUR). The primary reason for selecting this method was the chance of detecting a water quality change based on the six different water quality parameters. The suitability of the InSpectra method was assessed based on meeting the three requirements of Tier One method. The results showed that InSpectra method failed to meet all three requirements. The limitation of this method was related to the wide variation and unrealistic concentrations of some of the six water quality parameters in potable water samples. The reason for the poor performance of the InSpectra method was determined to be due to the fact this method measures one set of water quality parameter (UV absorption spectra) and then calculates the concentrations of six other water quality parameters using algorithim-determined data stored in its software package. However, the data stored in the InSpectra software package were determined to be inappropriate for BWS potable groundwater sources. The InSpectra method was not recommended as a Tier One method to detect a contamination event in potable water.

V. Assessment and Recommendations of Tier Two Tests

The objective of Tier Two tests is to confirm the presence or absence of specific hazardous chemical agents or biological agents in water samples, which were determined to be presumptivly contaminated using Tier One tests. Today, PCR technology is considered to be the most feasible and reliable gene-probe test to identify biological agents (microorganisms). The Ruggedized Advanced Pathogen Identification Device (R.A.P.I.D.) was selected as a Tier Two method to identify biological agents, especially pathogens in water samples. R.A.P.I.D. is an automated system that uses the advanced quantitative PCR procedure specifically

designed to identify pathogens, which are most likely to be used by terrorists. The primary reason for selecting this method was the ease of operating this complex instrument by laboratory personnel with minimal training in molecular microbiology. For this study, only training in the use of the R.A.P.I.D. method was completed because no suspected pathogen was recovered from BWS potable water samples. Based on the capabilities of R.A.P.I.D. we recommend that this method be used as Tier Two method to identify biological agents, especially pathogens in water. However, since the R.A.P.I.D. has not been upgraded, we recommend that BWS consider adopting newer and more sophisticated systems such as the GeneXpert developed by Cepheid.

The enzyme linked immunosorbent assay (ELISA) method was selected as a Tier Two test to rapidly identify many toxic chemicals in water samples. The primary reason for selecting this method was the ease of completing this method by laboratory personnel whose laboratories are not equipped with sophisticated equipment needed to identify complex chemicals. The ELISA method uses antigen-antibody reaction, which is the basic reaction to identify many pathogens in clinical hospitals. The key to this method was the development of antibody reagents by commercial companies which will react specifically with the chemical reactive groups (antigen) that characterize the different toxic chemicals. In the ELISA method, the antigen-antibody reaction is associated with an enzymatic reaction resulting in a color reaction, which can be read either visually or using a photometer to determine the relative amount of the hazardous chemical in the water sample. Many of the toxic chemicals expected to be used by terrorists are available in ELISA test format with specific reagents for each toxic chemical. For this study, only training in the use of the ELISA method was completed because toxic chemicals were not recovered from BWS potable water samples. Based on the reliability and feasibility of the ELISA method, we recommend that this method be used as a Tier Two method to identify toxic chemicals in water.

In summary, the R.A.P.I.D. method and ELISA method were shown to be feasible and reliable tests. However, under actual contamination conditions, these two methods have some limitations because reagents for all pathogens and toxic chemicals are not available for these two methods. Moreover, to use these two methods, the analyst must pre-select the reagents to test for each specific pathogen or toxic chemical. If the wrong reagents are selected, the contaminant will not be identified. However, negative results are valuable because they show that certain hazardous chemical or biological agents are not the contaminant in water. Since reference laboratories are better equipped to identify and characterize toxic chemicals and pathogens, the water utility should forward samples to reference laboratories.

VI. Assessment and Recommendations of Tier Three Test

The objective of Tier Three test is to characterize colonies of total heterotrophic bacteria (THB) recovered from potable water samples and to determine if they can be used as markers for a contamination event. Concentrations of THB in potable water are routinely determined by water utilities. The use of THB in the EWMP is based on two hypotheses. The first

hypothesis is that during a contamination event, fast-growing colonies of total heterotrophic bacteria (THB) and some non-target colonies (NTC) recovered from potable water can be used as markers for an external source of contamination. This hypothesis is based on the expectation that the polluting solution prepared by terrorists will likely contain at least three classes of bacteria. The first class represents pathogenic bacteria deliberately added to cause disease and death in the population. The second class represents populations of bacteria deliberately added as a decoy to make it difficult to detect the actual pathogen. The third class includes those populations of bacteria that will be unavoidably associated with the polluting solution. This third class represents populations of bacteria that enter and multiply in the polluting solution as an external contaminant and will not be known to the terrorist group. It should be noted that this third class of bacteria can also be expected in polluting solution containing hazardous chemical agents. The three classes of bacteria can be expected to form fast-growing colonies of THB and NTC recovered from potable water samples and can be differentiated from THB naturally present in potable water, which form slow-growing THB colonies and usually do not form NTC. The second hypothesis is that the RiboPrinter method will characterize each THB isolated from potable water into their genetic ribogroup and the results can be used to identify THB isolates from potable water as bacteria normally present in water from those bacteria which originated from an external source such as a contamination event.

The RiboPrinter microbial characterization system (DuPont Qualicon, Wilmington, DE) or RiboPrinter method was selected as the Tier Three method because of its unique capability of identifying most human pathogenic bacteria, most environmental species of bacteria, and can characterize all unidentified bacteria into their distinct genetic ribogroups. This capability is especially useful for potable water samples because unlike other methods, this method can differentiate the populations of THB in potable water into distinct ribogroups. Moreover, the distribution of these ribogroups in potable water sources can be used to characterize the ambient populations of THB in that source of water. This method uses a complex and expensive equipment but its operation has been automated to identify up to 32 isolated bacteria per day. The RiboPrinter was leased for a two-year period and this lease included all maintenance costs, technical support, and reagents at discounted rate. The focus of the current study was to use the RiboPrinter method to characterize the ambient populations of THB isolates recovered from BWS potable water so that they can be easily differentiated from bacterial populations that originate from external contaminating sources. For this study, a total of 630 THB isolates and 111 NTC recovered from potable water samples were analyzed by the RiboPrinter method. The THB isolates included 140 from 45 well sites, 86 from 26 reservoir sites, 331 from 90 distribution sites, 27 from 4 tunnel sites, 33 from 4 shaft sites and 13 from 2 GAC sites.

Based on analyzing these water samples, the following conclusions were reached regarding the use of the RiboPrinter method. (1) Although the procedure to operate the RiboPrinter was easy, the procedure had to be modified to successfully process colonies of THB recovered from potable water. (2) The validity of this method is dependent on following standardized

procedures and using certified reagents. However, the frequency with which some of the reagents did not function properly was unacceptably high. Although the company replaced these reagents at no additional cost, many working hours were wasted. (3) The RiboPrinter method was able to identify a small fraction (13 to 29%) of the THB isolates based on matching the riboprint of the bacterial isolate with those in the DuPont data base. Several of the THB isolated from potable water sources were identified as *Legionella* spp. or *Vibrio* spp. and these identifications are not likely to be correct because these bacteria require special types of growth media and are not expected to grow as THB colonies. These results indicate that the DuPont data base used by the RiboPrinter may not be entirely reliable for THB isolates and casts some doubt on the reliability of the other identified THB isolates. (4) This method successfully characterized all THB isolates into their genetic ribogroups but failed to determine a predictable distribution of ribogroups in potable water sources because most of the ribogroups differed from each other and each ribogroup comprised a minor fraction of all THB isolates. The significance of this finding is that since the ambient populations of THB recovered from potable water are comprised of numerous different ribogroups, it would be difficult to recognize the ribogroup of a contaminating bacteria as being different.

Two explanations were given for the great variation in THB isolates after they were characterized into their ribogroups. One possible reason is that the populations of ambient THB in potable water sources are comprised of so many different species of bacteria that it is very likely that a different species of bacteria will be recovered when a different THB isolate is processed. Under this condition, it will not be possible to characterize a predictable distribution of ribogroups for THB isolates from potable water sources. The second and more likely reason is that this method uses a 92% similarity index, which is too stringent in placing unidentified THB isolates into the same ribogroup. As a result, many unidentified bacteria belonging to the same species will be placed into different ribogroups and they would be considered different species. In this regard, the DuPont data base identifies many bacteria to the same species but they are often within 85% similarity index and they belong to different ribogroups. Since the use of similarity index by the RiboPrinter method cannot be changed, DuPont Qualicon scientists recommended that we use the bionumeric software called GelCompar (Applied Maths, Austin, TX) to reanalyze the riboprints of the THB isolates generated by the RiboPrinter method. As a result, all of the riboprints of THB generated by the RiboPrinter method were re-analyzed using the GelCompar method to group together riboprints at 80% similarity index in an attempt to group together the closely related unidentified riboprints. The results obtained showed that the GelCompar method was successful in grouping more of the THB riboprints into clusters. However, even at 80% similarity index, the GelCompar method formed too many clusters and most clusters contained a minor fraction of the total THB isolates. These results provide evidence that the ambient populations of THB in potable water sources are so diverse that it was not possible to characterize the ambient populations of THB into some predictable distribution of ribogroups.

The RiboPrinter method may still be useful in identifying and or characterizing the fast-growing THB colonies as markers for a contamination event. However, due to the many limitations of this method, the high cost involved and the length of time to obtain data, we recommend that the RiboPrinter method not be used as Tier Three to characterize the THB isolates from potable water sources.

VII. Final Project Assessment and Recommendations

In the final assessment of this project, the most important question is whether the EWMP is feasible, reliable and effective. In this regard, the primary goals for this study were to develop an EWMP and to evaluate the effectiveness of this plan to rapidly and reliably detect intentional contamination of the BWS water system by hazardous chemicals and pathogens. To address these goals a three-tiered EWMP was devised and commercially available methods were used to analyze water samples. The proposed EWMP was only partially successful because many of the methods failed to provide reliable data needed to meet the objectives of this plan. For example, only the Microtox method was approved as a Tier One method to screen for toxic chemicals. Thus, the EWMP still needs a Tier One test method to rapidly detect changes related to contamination with biological agents. The R.A.P.I.D. method was approved as a Tier Two method to identify pathogens in potable water samples. The ELISA method was approved as a Tier Two method to identify toxic chemicals in potable water samples. The RiboPrinter method was not approved as the Tier Three method to characterize the THB isolates recovered from potable water and to identify those THB isolates which originated from an external source such as a contamination event.

Two problems were recognized in the implementation of the EWMP. The first problem is that we selected commercially available tests that had not been developed specifically to analyze potable groundwater. These methods did not perform as well as expected. The second problem was the apparent complexity of the biological composition of potable water. We assumed that because groundwater has low concentrations of THB, the composition of total microorganisms in potable water would also be relatively low in numbers and diversity. This apparently is not the case as the diverse populations of microorganisms in potable water was the cause for the failure of the Tier One test to measure for ATP and the Tier Three test to characterize the colonies of THB recovered from potable water. In retrospect, it may not be possible to develop a reliable EWMP based on using commercially available methods. Evidence for this conclusion is based on the observation that in December of 2001 a published plan describing an emergency water monitoring plan for water utilities was not available. As a result, we developed our EWMP without reference to other similar plans. Moreover, in December 2005, the publication of an effective EWMP is still not available.

Although the proposed EWMP was shown to be only partially effective, we believe the premise and experimental design for this plan is valid. Therefore, the EWMP should be accepted as an interim plan that needs to be improved and expanded to use other types of measurements. The experimental design of the EWMP was based on detecting a component

of the terrorist polluting solution in potable water samples and to use this measurement as a marker of the contaminating source. This kind of monitoring data can be used to identify the sites in water system which are contaminated and sites which are not contaminated. Although our testing methods were not successful, this experimental approach is still valid. In this regard, there are many other chemical, physical, and biological constituents in the terrorist polluting solution and detection of any of these components in potable water can be used as markers for that source of contamination. The challenge is to find a component in that polluting solution, and a method that can reliably detect its presence in potable water. This kind of challenge can only be met by a research project specifically designed to select a suitable monitoring method to detect a component of the external contaminating solution. In this regard, detecting bacterial populations in the polluting solution is still a valid approach and use of molecular methods may be the best technology. A promising example of this approach is to apply DNA microarray technology to rapidly detect contamination of pathogens and other microorganisms in potable water. The promise of this technology is that it can simultaneously detect hundreds of different kinds of pathogens, other microorganisms, as well as their metabolic products in one test. Thus, this kind of technology has the potential of characterizing potable water sources and then determining when that source of water is contaminated by external sources of microorganisms. Currently, the limitation of DNA microarray technology is that this molecular method can only detect high concentrations of microorganisms and cannot detect health-related concentrations of pathogens in potable water. However, sample concentration and amplification methods are being evaluated to overcome these limitations. Based on the need to rapidly test water for numerous types of microorganisms and pathogens, the future promise is in the application molecular methods.

Implementation of a EWMP must be recognized as a difficult task. The key to a successful EWMP is advanced planning, designating those with key responsibilities and then providing them with continuous training. As laboratory supervisors, the chief microbiologist and chief chemist must work together and be responsible for the water monitoring aspects of the EWMP. During an actual contamination event, when people are becoming ill and there is panic in the community, these supervisors will be asked many difficult questions relating to the results of the tests, other available tests and comments made by other scientists throughout the country. These laboratory supervisors must be adequately trained to answer these questions. In this regard, the training of these laboratory supervisors should not be limited to operating a specific instrument used to detect hazardous chemical or biological agent. Instead, a plan for continuous training for the laboratory supervisors and their staff on the theory and application of the methods used to monitor for hazardous chemical and biological agents should be implemented. Additional training should be focused on use of molecular methods because these methods can be expected to be used more extensively in the future and these methods can be expected to change rapidly. Other areas of training should include public health consequences of contamination at water utilities and problems related to public communication during these events. Finally, laboratory supervisors should be encouraged to establish professional relationships with other scientists and laboratory

supervisors throughout the country. These contacts can serve as resources to provide answers and recommendations during periods of crisis.

In conclusion, other water utilities are faced with the same problem as BWS in the development of a reliable EWMP. Agencies and water utilities that are actively involved in developing an EWMP are as follows: (1) EPA, (2) CDC, (3) AWWA, (4) Pittsburgh Water and Sewer Authority, (5) East Bay Municipal Utility District, (6) Metropolitan District of Southern California, and (7) San Francisco Public Utilities Commission. We recommend that BWS communicate with these agencies in the development of an effective EWMP. Currently, the most practical recommendation for BWS is to investigate the usefulness of the Hach Event Monitor Trigger System as an automated, on-line system to detect contamination of potable water systems. This method was developed by Hach Company for the specific purpose of developing a method to detect contamination of potable water. This test measures a combination of five water quality parameters (chlorine, turbidity, conductivity, pH, total organic carbon). Each of these water quality parameters by itself does not provide specific data for a contamination event but together the measurements are used in what is described as an "intelligent algorithim" to determine when a contamination event may have occurred as well as to identify the possible type of contaminant. Use of algorithm to predict a condition is now used as a means of obtaining water quality data quickly to signal a possible contamination event. However, there is danger in the use of algorithm-based data because they are collected under one set of conditions and may not be applicable when applied to water under a different set of conditions. To address the problem of site specificity, Hach Company recommends that their system be initially installed at the site where it will be used for several months to determine the background concentrations of the five water quality parameters. The background concentrations for the five water quality parameter will then be used to establish an action level for that source of water. That action level is the trigger point to signal a possible contamination event.

The development of this new method by Hach company points out the way in which commercial companies are developing tests specifically for an EWMP. This approach is superior to the application of commercially available methods that were designed to be applied to many situations and do not perform well enough to reliably analyze potable water. Since the Hach Event Monitor Trigger System is available and is being evaluated, we recommend that BWS contact a Hach representative such as Dan Kroll (Chief Scientist for Threat Agent Chemistry, 800-604-3493) to obtain the latest evaluative reports regarding their new method. We also recommend that BWS contact an EPA representative such as Matthew Magnuson (National Homeland Security Research Center, 513-569-7321) to get an update on EPA's plan to develop and evaluate an EWMP at one water utility in the United States sometime in 2006.

CHAPTER ONE

TERRORISM AND ITS IMPACT ON WATER UTILITIES

I. Terrorism: Today's Greatest Threat to Water Utilities

Terrorism is defined as the committing of violent and terrifying acts against a population or government for political purposes that cannot be achieved by peaceful means. Terrorist acts often involve mass killing of people and/or violent destruction of public structures such as populated buildings, or those that provide essential services, such as government administration, public health, transportation, food, security, energy, and water. Historically, acts of terrorism occurred in countries outside the United States, so Americans felt secure in our own country. However, on September 11, 2001, a terrorist group implemented a coordinated attack on the United States by hijacking four commercial airplanes, crashing two of them into the twin towers of the World Trade Center in New York City and one into the Pentagon Building in Washington, D.C. Targeting buildings which are symbols of business prosperity and military planning, these attacks represent the deadliest acts of terrorism against the people and government of the United States, and most significantly, it occurred on US soil. The day of these terrorist attacks has become such a defining point in time for all Americans that this event has since been called 9/11. It should be noted that airplane fuel, which represents a hazardous chemical, was responsible for much of the damage to buildings and the killing of people. Soon after the 9/11 event, a more insidious form of terrorism occurred in the United States when anthrax spores, previously categorized as a biological warfare agent, were mailed to several places, including the US Congress. This act of terrorism showed that any public document, air, food, or water could be contaminated with hazardous chemical or microbial agents to cause disease, death, and unrest in the US population. Since the groups responsible for these acts of terrorism were already established in the country, the US government concluded that it is likely that they will strike again and that populated centers and critical infrastructures are likely targets. Today, the greatest identified threat to water utilities is acts of terrorism, especially intentional contamination of water supplies by extremely hazardous chemical or biological agents such as those selected for use in warfare (Kelle et al., 2001).

II. Regulations and Guidelines to Address Terrorism

After the 9/11 attack, the US government determined the need for new regulations and guidelines to combat terrorism.

A. The Public Health Security and Bioterrorism Preparedness and Response Act of 2002. Called the Bioterrorism Act, this legislation, was established in June of 2002. It directs all critical agencies in the US to implement new security plans as the most effective preventive measure against future acts of terrorism. This Act identifies the Environmental Protection Agency (EPA) as the lead federal agency to ensure that all public water utilities complete a

vulnerability assessment, implement a water security plan, and develop an emergency response plan for possible acts of terrorism.

B. Homeland Security Act of 2002.

This Act was passed by Congress in November of 2002 to establish a new Department of Homeland Security (DHS) as the lead federal agency to establish plans and policies to prevent and counter acts of terrorism in the United States. Other federal agencies with security responsibilities were placed under DHS. One responsibility of the DHS is to secure the nation's critical infrastructure, such as the nation's water utilities.

C. Response Protocol Toolbox (RPTB) for Responding to and Planning for Contamination Threats and Incidents.

In December of 2003, EPA published a planning document called Response Protocol Toolbox (www.epa.gov/safewater/watersecurity). This document provides information and guidelines for all water utilities to use in devising their own plans to address intentional contamination events. It is a comprehensive planning document that is comprised of the following six separate planning guides, which are referred to as modules:

1. Water utility planning guide. This planning guide provides information on contamination threats so utilities can update their emergency response plan, develop information management strategy, and enhance physical security.

2. Contamination threat management guide. This planning guide provides information on how to use available data for the purpose of determining when a credible threat exists so action can be taken.

3. Site characterization and sampling guide. This planning document provides information on how to characterize the water utility sites where contamination may have occurred, how to determine options for rapid field testing of water, and how to collect water samples for transport to reference laboratories for further analysis.

4. Analytical guide. This planning document provides information on available methods to analyze water samples for possible contaminants.

5. Public health response guide. This planning guide provides information relevant to public health response by agencies such as water utilities, and recommendations to initiate plans to work with public health organizations for the purpose of communicating with the public on issues related to protecting the public from contaminated water.

6. Remediation and recovery guide. This planning document provides information on procedures to remediate contaminated water facilities so the system can be made safe and productive again.

It should be noted that Module 4 provides guidelines for water utilities to develop their own monitoring plan in response to intentional contamination of water. This module also describes some safety procedures for laboratory personnel to follow when handling and shipping contaminated water samples. However, the information in this module is not a manual of directions to be used during an actual contamination event. In other words, this module provides guidelines on the various methods to be used to assay for the various types of contaminants but does not provide details on how to assay samples using the various methods. More details on the various methods can be found at the website of EPA's Environmental Testing and Verification (ETV) Program (www.epa.gov/etv).

III. Issues Related to Intentional Contamination of Water

A. Vulnerability and Selection of Water Utilities for Intentional Contamination.

To comply with the Bioterrorism Act, all water utilities serving >3,300 people should have completed their vulnerability assessments and should have updated their water security plans by June of 2004. Increasing the security of water utility facilities in the United States represents the single most effective plan of action to prevent acts of terrorism such as intentional contamination of public water supplies. Despite the implementation of these preventive measures, water utilities are still considered vulnerable to attack by terrorists. The four identified vulnerable sites for water utilities are the sources of water, the water treatment facilities, the storage facilities, and the distribution systems. Water utilities are targets for several acknowledged reasons. First, everyone needs to drink water on a daily basis, and people are confident that potable water from any piped system in the nation is safe to drink. Second, because potable water is piped into nearly every private and public building, it is always accessible by the public. Third, since water is piped to every sector of the community, it can serve as an effective vehicle to deliver hazardous chemical agents or microbial pathogens to the public.

B. Agents Most Likely to be Used to Contaminate Drinking Water Sources.

The Centers for Disease Control and Prevention (CDC) completed a public health assessment of the biological terrorism agents most likely to be used in biological warfare (Rotz et al., 2002). CDC placed these agents into three categories based on their potential public health effects and risk to national security. Category A agents are characterized as easily disseminated and transmitted from person to person, can cause high mortality, and can be expected to cause public panic. The agents in Category A include pathogens that cause diseases such as anthrax, plague, tularemia, smallpox, viral hemorrhagic fever, and botulism. Category B agents are characterized as moderate in their ability to be transmitted from person to person and to cause morbidity and mortality. The agents in Category B include pathogens that cause brucellosis, glanders disease, and Q fever, as well as chemical forms of toxins such as ricin from a bean and toxins from some bacteria such as *Clostridium perfringens* and *Staphylococcus aureus*. Category C includes emerging infectious agents that are not likely to cause widespread diseases but are potentially dangerous. The agents in Category C include Hanta viruses, Nipah virus, multi-drug-resistant tuberculosis, tickborne encephalitis virus,

tickborne hemorrhagic fever viruses, and yellow fever virus. It should be noted that many of these identified hazardous pathogens are not transmitted by water but by aerosols or by insects. Table 1 lists the pathogens and toxins that are most likely to be used by terrorists to contaminate drinking water sources (Burrows and Renner, 1999; States et al., 2004; Meinhardt, 2005). Some of these pathogens include those categorized and identified by CDC as biological warfare agents. Since many of the biological warfare agents are difficult to produce and to handle, it must be recognized that terrorists groups may choose to contaminate water with more readily available hazardous chemicals such pesticides, herbicides, and heavy metals and still succeed in causing fear among the general public.

C. Assessment of On-Line Monitoring Methods.

Most water managers believe that on-line monitoring methods represent the best approach to detect intentional contamination of water because this technology is designed for continuous and automatic monitoring of water quality. Thus, this approach can detect an intentional contamination event when it occurs. However, there are many problems associated with on-line monitoring methods. First, these methods are limited to measuring only few water quality parameters such as total chlorine, pH, alkalinity, conductivity, turbidity, and total organic carbon. Second, these parameters do not provide specific information as to the kind of pollutant in the water. Third, this method must be placed at strategic sites which can detect contamination from many vulnerable sites. Fourth, the variation in data obtained can be expected to be large and the monitoring instruments must be periodically recalibrated. Fifth, since data are collected continuously, one can expect problems related to collection, storage, and interpretation of data. Due to these basic problems, effective on-line monitoring methods to reliably detect contamination by many possible hazardous chemicals and pathogens have not yet been developed for water utilities.

On-line monitoring methods are complex and developed by companies that service water utilities. It should be noted that very recently (mid-2005), Hach Company reported an on-line monitoring system for distribution water called the Hach Event Monitor Trigger System. This system simultaneously monitors for five water quality parameters (chlorine, turbidity, conductivity, pH, and total organic carbon) and uses what is described as "intelligent algorithm" to determine when a contamination event has occurred and what the probable type of the contaminant is (Kroll and King, 2005; King et al., 2005). Currently, the capital cost for this system is approximately $50,000. Its effectiveness is currently being evaluated.

IV. Historicial Review of Water Monitoring Plans for Intentional Contamination

A. Developments During 2001 to 2003.

After the 9/11 event in 2001 an effective water monitoring plan to address intentional contamination of water was not published and was not available to water utilities. To address this need, EPA published the RPTB in 2002. The RPTB includes methods to monitor water for intentional contamination. However, this document did not provide a plan to monitor water. In 2003, the Pittsburg Water and Sewer Authority (PWSA) recognized the need for

water utilities to initiate a monitoring plan to address intentional contamination of water. Since EPA had not yet provided specific guidance on analytical methods to be used by utilities, PWSA undertook a study to evaluate and to choose some commercially available analytical methods for use under emergency conditions. The study resulted in the first published work to address monitoring strategies for intentional contamination of a utility (States et al., 2003). In that study, PWSA concluded that Eclox and Microtox are two promising commercially available tests that can be used to detect toxic chemicals in water. Both tests use reactions that measure light production rapidly (minutes) and detect presence of toxic chemicals in water samples. In the Eclox test, light-activated enzyme systems are used, whereas in the Microtox system, a light-activated bacterial population is used. The PWSA study also evaluated on-line monitoring technologies which measure chlorine residual, pH, turbidity, conductivity, and total organic carbon. The on-line monitoring methods were concluded to be inadequate in reliably determining when an intentional contamination event had occurred.

B. Developments During 2004.

In 2004, PWSA published a follow-up study that focused on evaluating other rapid methods such as enzyme-linked immunosorbent test to detect biotoxins, enzymatic reaction tests to detect pesticides or nerve agents, polymerase chain reaction (PCR) tests to detect pathogens, and chemical tests to detect volatile organic compounds (States et al., 2004). One conclusion of this second study was that many of these rapid tests produce variable results and that baseline concentrations must be established for each test to determine incidences of false positive and false negative results (States et al., 2004). The authors concluded that results of rapid tests must be considered presumptive and should be confirmed before decisions are made. Hrudey and Rizak (2004) evaluated the results of States et al. and concluded that even if the rates of false positive and false negative for these rapid tests are low, the true positives may be even lower and that these test results can lead to many decisions by the water utilility managers based on false positive results. Hrudey and Rizak questioned the reliability of rapid screening methods used to routinely monitor water and stressed the need to confirm screening test results.

C. Developments During 2005.

In 2005, Meinhardt (2005) reviewed how water utilities and public health agencies were preparing for intentional contamination of drinking water supplies. She pointed out that recently implemented security plans have greatly increased the security of many water utilities, thus reducing opportunities for contaminating water supplies. However, she concluded that water utilities are still vulnerable to intentional acts of contamination, that current monitoring plans cannot be relied on to prevent contamination from reaching the public, and that recognition of disease symptoms in the community may be the first sign of water contamination. She also pointed out that doctors and public health agencies need more training in working together to rapidly link disease outbreaks to contamination of water supplies.

In a 2005 EPA report, Allgeier (2005) reviewed the water contamination warning systems and concluded that distribution systems of water utilities are the most vulnerable site for contamination. Moreover, screening methods to detect intentional contamination of water are slow, insensitive, and often non specific. Allegeier concluded that current monitoring plans cannot be relied on to respond to contamination threats and incidents in a timely and appropriate way.

In a 2005 EPA report, Magnuson (2005) announced that EPA will implement a new Water Sentinel Program. The original intent of this program was to evaluate methods that can detect intentional contamination at several water utilities in several different cities. However, due to lack of funds, EPA now plans to initiate this Water Sentinel Program in one US city in 2006. This program will benefit all water utilities, because EPA will finally tackle the problem of devising and evaluating a water monitoring plan that all utilities can consider using. It should be noted that the earliest expected data for this study will most likely be sometime in 2007.

CHAPTER TWO

IDENTIFICATION OF THE PROBLEM: THE PROPOSED STUDY

I. Identification and Assessment of the Problem

After the 9/11/2001 event, President Bush alerted all public facilities, including water utilities, to develop new plans to guard against acts of terrorism. One obvious problem for all water utilities is that their current water monitoring plan is not designed to detect intentional contamination of water supplies, especially by agents identified for use in biological and chemical warfare. Thus, the identified problem for the Honolulu Board of Water Supply (BWS) in 2001 was the need to develop an emergency water monitoring plan (EWMP) to address concerns related to intentional contamination of water supplies. To address this identified need, BWS requested the services of Roger Fujioka of Water Resources Research Center (WRRC) at the University of Hawaii to develop an interim water monitoring plan. In December of 2001, Fujioka reviewed all available information and confirmed that the primary supporting organizations such as EPA and Centers for Disease Control and Prevention (CDC) had not yet developed an EWMP for water utilities to use during intentional contamination events. In the absence of any approved EWMP, Fujioka devised an experimental three-tiered EWMP based on using commercially available methods.

II. The Proposed Study

A. Project Goals and Agreements.

The primary goals of this study were to develop an EWMP for BWS and to evaluate its effectiveness to rapidly and reliably detect intentional contamination of Honolulu's municipal water system by hazardous chemicals and pathogens. The secondary goal of this study was to train BWS laboratory personnel in the implementation of this EWMP by analyzing water samples at the BWS laboratory. To address this secondary goal, BWS initially agreed to assign a laboratory microbiologist to this study so that a laboratory analyst would be trained in the methods and would be analyzing samples at its laboratory. However, due to manpower problems, BWS was not able to assign a laboratory microbiologist to this project. As a result, BWS water samples were collected and transported to the University of Hawaii where all the assays were conducted. Training of BWS laboratory personnel was limited to periodic training sessions at the University of Hawaii.

III. Description of Materials and Methods

All requests to obtain water samples, to obtain bacterial isolates or to use equipment from BWS were coordinated with Owen Narikawa, Chief Microbiologist at BWS. WRRC provided sterile water containers, and BWS personnel collected water samples using their approved procedures and generally delivered the water samples to WRRC. On some occasions, water samples were picked up from BWS facilities by WRRC laboratory personnel. Most of the

samples were analyzed at the University of Hawaii. Several types of water samples obtained for analysis are described below (see items A through E). Methods used to assay the water samples are also described below (see items F and G).

A. Well Water Samples.
Water samples were obtained directly from BWS wells, often before chlorination or any other treatment. Thus, the water quality of well samples closely represents that of deep (e.g., 400 to 600 feet below surface) groundwater aquifers, which are naturally protected. For wells, the contribution of biofilm growth is considered minimal because the surface area of piping is small relative to the volume of water being pumped up under pressure.

B. Tunnel and Shaft Water Samples.
Tunnel and shaft water samples represent groundwater from sources that are much shallower than deep well water sources. These shallower water sources are generally located in protected areas and are of high quality.

C. Reservoir Water Samples.
Reservoir water samples represent groundwater that has been pumped from wells, tunnels, and shafts and stored in tanks. These tanks are placed at strategic and elevated locations throughout the island to supply water to the distribution lines for public consumption. Reservoir tanks are generally sealed, but contamination by external sources such as wind, dust, insects, and birds can occasionally occur through air vents. The tanks are generally disinfected with chlorine to maintain low concentrations of bacteria. Biofilm or growth of bacteria on the interior walls of the reservoir tanks can be expected, and some bacteria from biofilm growth can be expected to be released into the water.

D. Distribution Water Samples.
Water stored in reservoir tanks is released to flow through the network of BWS distribution pipelines, which transmit water to consumers. Distribution water samples were obtained from public faucets and represent the quality of water consumed by the public. The inner walls of distribution pipes represent large surface area for growth of biofilm and bacteria from this biofilm are released into the water.

E. Samples of Water Treated by Granulated Activated Carbon.
Granulated activated carbon (GAC) is used to remove residual pesticides in some water sources. Samples of water were obtained after treatment with GAC. These water samples were selected for analysis because the population of bacteria that grow as biofilm on GAC can be expected to differ from those that grow on walls of pipes and reservoir tanks.

F. Assay for Total Heterotrophic Bacteria (THB).
Total heterotrophic bacteria (THB) generally represent natural populations of bacteria in potable water sources. Concentrations of THB are routinely measured by water utilities for use in characterizing the microbial quality at the sampling site. Theoretically, when normal

levels of THB in water sources are greatly increased or greatly reduced, this change can signal a contamination event. However, current EPA guidelines state that definitive interpretation of THB counts in potable water cannot be made because the ambient species that comprise THB populations have not been determined. More recent interpretations of THB measurements in potable water indicate that their concentrations in water cannot be assumed to represent a health risk to consumers. However, the THB assay can provide useful information during a contamination event because the contaminating agent may contain bacteria that will grow as THB colonies and there is a good chance that these colonies will form larger and faster-growing colonies than that of ambient populations of THB in potable water. If one can isolate and characterize these colonies and determine which ones originated from an external source of contamination, these THB isolates can be used as markers for the contamination event. Thus, the THB assay can be used to detect an intentional contamination event.

In this study, the membrane filtration method as described in *Standard Methods for the Examination of Water and Wastewater* (APHA, AWWA, WEF, 1998) was used to enumerate THB. Briefly, 25 to 50 ml of water samples were filtered through a 47-mm (Gelman GN6) membrane with a 0.45-μm pore size and then the membrane was placed on mHPC agar. After incubation for 5 days at 25°C, all visible colonies were counted. It should be noted that although this assay counts total heterotrophic bacteria, many other bacteria as well as other microorganisms (protozoa, fungi, viruses) in water are not enumerated by this assay. In this regard, mHPC counts are generally estimated at 0.1% to 1% of all viable bacteria in any water sample.

G. Assay for Bacteria that Grow as Non-Target Colonies on mEndo Agar.

Potable water sources are routinely assayed for coliform bacteria using mEndo agar, as described in *Standard Methods for the Examination of Water and Wastewater* (APHA, AWWA, WEF, 1998). On mEndo agar, coliform bacteria grow and form typical target colonies, which can be easily identified based on their color. Some bacteria grow on mEndo agar and form colonies that differ in appearance from target colonies. These non-coliform colonies, called non-target colonies (NTC), are observed periodically on mEndo agar and represent populations of bacteria that grow naturally in water, that grow as biofilm on the lining of pipes, or that originate from an external source (broken pipe) of contamination. For example, NTC are often observed during repairs of pipes when opportunities for contamination from external sources are likely. Most of the time, NTC do not represent a health hazard and no action is required. However, it should be recognized that bacteria associated with an intentional contamination event will likely result in an increase in NTC on mEndo agar. Thus, under some conditions, NTC may represent a health hazard.

IV. Guidelines in the Development of Three-Tiered Emergency Water Monitoring Plan

Water utilities must be vigilant to contamination events. Evidence for contamination events may be in the form of telephone calls, physical evidence of contamination at some sites, detectable water quality parameter changes (taste, odor, color), or clinical symptoms among people. When a water utility is informed that its water system may be contaminated, it is faced with two immediate questions of concern. First, what is the nature (chemical, biological) of the contaminant? Second, what sections of the water systems are contaminated? To address these two concerns, a three-tiered emergency water monitoring plan called EWMP was developed. Briefly, the first tier of testing (Tier One tests) is to determine the sites in water system where the contamination has occurred. The second tier of testing (Tier Two tests) is to identify the contaminating agent in water samples. The third tier of testing (Tier Three test) is to characterize the populations of THB isolates recovered from potable water samples so that the range of bacteria that are naturally present in potable water sources can be established and bacteria that originated from an external source of contamination can be differentiated.

Guidelines used in the development of the EWMP are as follows. (1) Select commercially available methods that can be readily incorporated and used at the BWS laboratory. (2) Design a plan to be implemented after BWS has obtained creditable evidence of a water contamination event. (3) Evaluate the suitability of methods selected to implement EWMP because most of them have not been approved for analyzing potable water samples.

In addition to the three stated guidelines, the experimental design of Tier Three of the EWMP is based on two hypotheses. The first hypothesis is that as a result of an external contamination event, fast-growing colonies of THB and NTC on mEndo agar can be used as markers for the terrorist group's polluting solution. In this regard, the polluting solution refers to the solution that contains either a hazardous chemical agent or a hazardous biological agent and is used to pollute or contaminate the drinking water system. This hypothesis is based on the expectation that the polluting solution prepared by terrorists will likely contain at least three classes of bacteria. The first class represents pathogenic bacteria deliberately added to cause disease and death in the population drinking that water. The second class represents populations of bacteria deliberately added as a decoy to make it difficult to detect the actual pathogen. The third class includes those populations of bacteria that will be unavoidably associated with the polluting solution. This third class represents populations of bacteria that enter and multiply in the polluting solution as an external contaminant and will not be known to the terrorist group. (It should be noted that this third class of bacteria can also be expected in polluting solution containing hazardous chemical agents). The three classes of bacteria can be expected to form fast-growing colonies of THB and NTC on mEndo agar. In contrast ambient populations of THB from potable water sources form slow-growing and relatively smaller colonies. The second hypothesis used in the development of the EWMP is that the RiboPrinter method will be able to characterize all THB isolates recovered

from potable water samples into their genetic ribogroups. Moreover, there will be a distinct distribution of ribogroups, which will represent the kinds of bacteria naturally present in potable water. Finally, the ribogroups of bacteria that can grow as THB but originated from an external source, different from potable water, will be easily differentiated from the ribogroups of THB naturally found in potable water. To test this second hypothesis, the Tier Three method was selected to characterize the ambient populations of THB in the three major potable water sources (wells, reservoir tanks, distribution lines) so that they can be easily differentiated from bacterial populations intentionally added to the water system.

V. Objectives and Reasons for the Selection of Tier One Tests

The objectives of Tier One tests are to analyze the many suspected potable water samples and to quickly determine which sites in the water system are free of contamination and which sites may be contaminated so that effective remedial action can be taken. Three Tier One methods were selected because no single method can be expected to detect both hazardous chemicals and biological agents. To be effective, Tier One methods must meet the following requirements: (1) the method must be commercially available and can rapidly (minutes) measure a water quality parameter, which can be expected to change in response to a contamination event, (2) the variation in the measured concentrations for the water quality parameter must be modest and predictable, and (3) the concentration of the water quality parameter to signal a possible contamination event must be determined. These three requirements were used to evaluate the suitability of the Tier One methods. A recognized limitation of Tier One method is that it will not identify the contaminant and may not provide enough information to determine if the contaminant is a chemical agent or a biological agent. Since no single Tier One test can be expected to detect contamination by both hazardous chemicals and biological agents, three methods using different technologies were selected as Tier One tests for the EWMP.

A. The Microtox Assay for Toxic Chemicals in Water.

The Microtox method was selected as a Tier One test because it meets the criterion of a commercially available test that can quickly determine the concentrations of a class of pollutants (toxic chemicals) in water. The Microtox instrument is shown in Figure 1. The primary reagent for this test is a marine luminescent bacterium (*Vibrio fischeri*) that produces light as a by-product of its normal metabolic process. When the light-producing population of *V. fischeri* is added to water samples without toxic chemicals, their metabolism will not be affected and therefore the light produced will not be affected and should be measured as 100% light output using a luminometer. If the water samples contain toxic chemicals, the toxic property of the chemical will inhibit the metabolism of the bacterial population and cause loss of light output. The difference between the light output in the control water sample and that in the test water sample containing toxic chemicals is used to determine the percent loss of light by the bacterial population. The percentage of light loss (e.g., 99%) is the percent

effect of the toxic chemicals and can be correlated to the degree of toxicity in the water sample.

Reasons for selecting the Microtox method are as follows. (1) It is a test method with long history of successful use and is designed to rapidly (15 minutes for a single sample) quantitate the effects of acutely toxic chemicals in water. This method allows for the processing of many samples, and when this is done, the processing time is reduced to approximately 10 minutes per sample. Moreover, some continuous monitoring version of this is method is now available. (2) The results of this method have been standardized and the toxicity effect (EC_{50}) established for over 1,300 known toxic chemicals, including heavy metals, pesticides, fungicides, rodenticides, chlorinated solvents, industrial chemicals, and other toxic chemicals (Kaiser and Palabrica, 1991). (3) The results of this test have been shown to correlate with the results of approved bioassay tests using whole animals such as fish (Qureshi et al., 1982). (4) This method can detect the toxic effects resulting from the interaction of multiple chemicals. (5) This method uses *V. fischeri* as the test organism in a dehydrated form for ease of storage. When ready for use, it is activated by the addition of water. This greatly simplifies this bioassay method, as compared to methods that use multi-cellular animals such as fish or daphnia, which must be continuously cultured or maintained in the laboratory. (6) The Microtox method has been published as an approved method in *Standard Methods for the Examination of Water and Wastewater* (APHA, AWWA, WEF, 1998) for various uses including wastewater effluent monitoring, groundwater testing and hazardous waste testing. (7) Results of this test can be used to determine how water treatment methods (dilution, disinfection, filtration, adsorption, precipitation, heat) can be used to remove, dilute or inactivate the toxic chemicals in water. (8) The Microtox method has already been adopted to screen drinking water during periods of suspected water contamination, such as during the 1984 Olympics in Los Angeles, during the 1996 Olympics in Atlanta, and at the US Pentagon after 9/11. The current estimate is that as many as 75 drinking water utilities have already incorporated the use of Microtox to test their water for toxic chemical contamination.

B. The Pallchek Luminometer System to Measure Adenosine Triphosphate as a Test for Total Concentrations of Viable Cells.

The Pallchek Luminometer System (see Figure 2) was selected as a Tier One method to measure adenosine triphosphate (ATP) because it meets the criterion of a commercially available test that can rapidly (minutes) determine the concentrations of a class of pollutants (viable microorganisms) in water. Using this method, elevated levels of ATP in potable water may signal contamination of the water system by microorganisms and this in turn, can be used as a signal that an external contamination event has occurred. ATP in water samples is measured by its reaction with luciferase enzyme to produce light. The concentration of ATP is related to the light produced, which is measured by a luminometer and read as relative light units (RLU). Since every viable cell contains and uses ATP for its metabolic reaction, the concentration of ATP can be related to total viable cells per sample. One limitation of the ATP assay is that viruses will not be detected because they do not produce ATP. However,

most large volume preparations of viruses such as those used as the polluting solution by terrorists can also be expected to contain populations of bacteria that can be detected by the ATP assay.

The ATP Pallchek Luminometer System (Pall Life Sciences, Ann Arbor, MI) was selected for the following reasons. (1) This system is especially suitable for analysis of water because it is designed to filter large volumes (100 to 500 ml) through a membrane to concentrate microbial populations in water onto the surface of the membrane. High sensitivity reagents can be added directly onto the membrane to initiate the ATP reaction. (2) A luminometer is part of this instrument. It measures light produced within a minute after the reagents are added and measures total viable biomass or total concentrations of viable cells (bacteria) in the water sample. (3) Pall Life Sciences literature reported that the minimum level of detection was 10 to 300 organisms in water samples.

C. The Profile-1 ATP Assay.

The Profile-1 ATP method (New Horizons Diagnostics, Columbia, MD) uses the same technology as the Pallchek method to measure ATP and was used as a check on the variable results obtained by the Pallchek method. The Profile-1 equipment (see Figure 3) was selected as the alternative ATP assay for the following reasons. (1) Lee and Deininger (1999) used this method and reported a correlation between increasing concentrations of THB and ATP measurements in surface drinking water samples. (2) This method was reported to be much more sensitive than other ATP methods and therefore small volumes (1 to 25 ml) of water samples were used in the assays. (3) This method uses a somatic releasing agent, which eliminated ATP provided by non-bacterial cells. (4) Diposable, ATP-free membranes (fitravettes) are provided by the manufacturer, which eliminated the need to pre-wash filters as was required using the Pallchek method. (5) Sensitivity of this method can be increased by using a more sensitive luminometer. For our study, the Profile-1 Bioluminometer Model 3560 with 10 X sensitivity was used. (6) This method was reported to be able to detect 200 viable bacterial cells in water samples.

D. The InSpectra Test for Organic Chemicals and Particulates in Water.

The InSpectra method (Azur Environmental, Carlsbad, CA) was selected as a Tier One method because it meets the criterion of a commercially available method that can quickly (one minute) measure the presence of UV absorbing components (organic matter, nitrates, suspended solids) in water and provide concentrations of six common water quality parameters: biological oxygen demand (BOD), chemical oxygen demand COD), total organic carbon (TOC) total suspended solids (TSS), nitrates (NO_3) and surfactants (SUR.). The InSpectra instrument (see Figure 4) is a specially designed spectrophotometer that measures the absorption spectrum of water sample using UV wavelengths (205 to 330 nm). Components in water (organic matter, nitrates, particulates) are absorbed by different UV wavelengths. The characteristic way in which these compounds in wastewater, industrial-water and natural-water samples absorb UV wavelengths were measured in hundreds of samples and these reference UV spectra were compared to the concentrations of six water

quality parameters, which were independently measured by approved methods. In summary, the InSpectra method does not directly measure the concentrations of the six water quality parameters but determines their concentrations based on the measured UV spectrum for that water sample and comparison to many reference spectra stored in its software. In the application of this method, elevated concentrations of one or more of the six water quality parameters can be used as evidence for a change in some water quality parameter and this data can be used as signal for a contamination event.

VI. Objectives and Reasons for the Selection of Tier Two Methods

The primary objective of Tier Two methods is to confirm the positive signals obtained by Tier One methods by identifying the hazardous chemical or biological agent in the water samples. A secondary objective of Tier Two tests is to exclude some suspected contaminants. This secondary objective is of practical importance because Tier Two methods are charged with identifying the contaminating agent in water but the possible contaminating agents are many. Under this condition, the most logical approach is to make a prioritized list of chemical and biological agents which can be transmitted via water. If human disease symptoms are available, this additional information can be used in adjusting the prioritized list of possible hazardous agents. In the application of Tier Two methods, several of these hazardous agents in the prioritized table must be tested for with the expectations that most of the confirmation tests will be negative. However, negative confirmation tests are useful in eliminating those hazardous agents as possible contaminants. Guidelines for selection of Tier Two methods are commercial availability of methods and their capability of feasibly and reliably identifying the most likely hazardous chemicals or pathogens that can be transmitted by water. Since detection of chemical and biological agents requires methods using different technologies, two methods were selected for Tier Two methods.

A. Polymerase Chain Reaction Method: The Most Feasible Method to Identify Pathogens.
The polymerase chain reaction (PCR) method has been determined to be the most reliable and feasible genetic method to identify most microorganisms, especially pathogens. This method is based on the fact that every microorganism is comprised of different genes that code for the different functions required by that organism. Genes are specific sequences nucleic acid or nucleotides. The PCR technique has been shown to be the most feasible method to detect these specific sequences of nucleotides in bacteria, viruses and protozoa by using selective primers, which hybridize to nucleotides on both sides of that unique sequence of nucleotides. The PCR reaction then replicates that sequence of nucleotides between the two primers and the resulting product is called the amplicon. If the entire sequence of that amplicon is identical to the sequence found in the pathogen, it can be concluded that the sample contains that same pathogen. In standard PCR, the amplicon is detected as a single band on a gel and is characterized by a specific molecular weight that reflects the specific number and kinds of bases in that amplicon. For standard PCR, gel electrophoresis is used as a second step to identify the amplicon; therefore the method usually takes a whole day or two days. The quantitative PCR, or QPCR, procedure provides faster results (few hours) because it uses

fluorescence, which can be recorded directly by a computer screen as the specific amplicon is being replicated. In addition, a melting curve of the amplicon can be determined to confirm that the correct amplicon has been formed,

For this study the Ruggedized Advanced Pathogen Identification Device (R.A.P.I.D.) was selected as the Tier Two test to confirm the presence of a specific microbial pathogen in water samples because it meets the criterion of being a commercially available method that can specifically identify some of the most hazardous pathogens in a relatively short period of time (hours to less than a day). The R.A.P.I.D. system (see Figure 5) was selected for the following reasons. (1) This is one of a few commercially available instruments that has been automated (all reagents are available in kit form) and designed to detect pathogens that had been previously identified as likely to be used by terrorists. (2) This unit was previously purchased by BWS and their personnel were already trained in its use. (3) This system uses QPCR technology, which not only identifies the pathogens but can provide information as to approximate concentration of the pathogen within a few hours. (4) This system does not require the isolation or culture of the desired microorganism and can be detected in samples that contain many other microorganisms. (5) Communications with the technical staff of the manufacturer of this instrument (Idaho Technology, Salt Lake City, UT) indicated that more reagents for more pathogens would be developed in the future.

B. Enzyme-Linked Immunosorbent Assay Method for the Identification of Chemical Contaminants in Water Samples.

The enzyme linked lmmunosorbent assay (ELISA) method was selected as a Tier Two test to rapidly identify many toxic chemicals in water samples. The primary reason for selecting this method was the ease of completing this method by laboratory personnel whose laboratories are not equipped with sophisticated equipment needed to identify complex chemicals. The ELISA method uses antigen-antibody reaction, which is the basic reaction to identify many pathogens in clinical hospitals. The key to this method was the development of antibody reagents by commercial companies which will react specifically with the chemical reactive groups (antigen) that characterize the different toxic chemicals. In the ELISA method, the antigen-antibody reaction is associated with an enzymatic reaction resulting in a color reaction, which can be read either visually or using a photometer to determine the relative amount of the hazardous chemical in the water sample. Results of this kind of test can be obtained in 1 to 4 hours. Many of the toxic chemicals expected to be used by terrorists are available in ELISA test format with specific reagents for each toxic chemical.

The ELISA tests supplied by Strategic Diagnostic Inc. (SDI, Newark, DE) were selected for the following reasons. (1) SDI has consolidated most of the commercially available ELISA products used to detect the different toxic chemicals. This has simplified matters for consumers who now need only to purchase most of the reagents from one company. (2) Many of the toxic chemicals expected to be used by terrorists are available in ELISA test format. (3) Communications with the SDI technical staff indicated that the company would be producing more ELISA kits to expand the number of toxic chemicals that can be confirmed.

VII. Objective and Reasons for the Selection of Tier Three Test

The objective of Tier Three test is to characterize colonies of total heterotrophic bacteria (THB) recovered from potable water samples and to determine if they can be used as markers for a contamination event. Concentrations of THB in potable water are routinely determined by water utilities. The use of THB in the EWMP is based on two hypotheses. The first hypothesis is that during a contamination event, fast-growing colonies of total heterotrophic bacteria (THB) and some non-target colonies (NTC) recovered from potable water can be used as markers for an external source of contamination. This hypothesis is based on the expectation that the polluting solution prepared by terrorists will likely contain at least three classes of bacteria. The first class represents pathogenic bacteria deliberately added to cause disease and death in the population. The second class represents populations of bacteria deliberately added as a decoy to make it difficult to detect the actual pathogen. The third class includes those populations of bacteria that will be unavoidably associated with the polluting solution. This third class represents populations of bacteria that enter and multiply in the polluting solution as an external contaminant and will not be known to the terrorist group. It should be noted that this third class of bacteria can also be expected in polluting solution containing hazardous chemical agents. The three classes of bacteria can be expected to form fast-growing colonies of THB and NTC recovered from potable water samples and can be differentiated from THB naturally present in potable water, which form slow-growing THB colonies and usually do not form NTC. The second hypothesis is that the RiboPrinter method will characterize each THB isolate from potable water into a genetic ribogroup and the results can be used to identify the range of ribogroups for THB isolates whose source is potable water and THB bacteria from externals sources can be recognized based on differences in their ribogroups.

The RiboPrinter microbial characterization system (DuPont Qualicon, Wilmington, DE) or RiboPrinter method (see Figure 6) was selected as the Tier Three method because it has the unique capability of identifying most human pathogenic bacteria, most environmental species of bacteria relevant to man and can characterize all unidentified bacteria into their distinct genetic ribogroups. The application of this method was to characterize the populations of THB colonies from potable water samples as a means of differentiating THB colonies which are naturally present in potable water source from those that originated from an external source of contamination.

The theory of the RiboPrinter method is based on current knowledge that all bacteria can be identified to genus and species based on the sequence of nucleotides in their genes that code for their 16S ribosomal RNA. The RiboPrinter equipment is an automated system used to identify most of the bacteria relevant to man based on the specific sequence of nucleotides that code for 16S ribosomal RNA gene without actually determining the exact sequence of nucleotides. Instead, the different sequences of nucleotides that make up the ribosomal gene for different bacteria are determined by cutting that gene at specific sites using the restriction enzyme called *Eco*R1 into specific sized fragments. The resulting gene fragments are hybridized to chemiluminescent-labeled DNA probes specific for ribosomal genes. The visualized pattern of gene fragments is called a riboprint. Each riboprint pattern represents a

"fingerprint pattern" for that specific bacterium. Each different riboprint is similar to a distinct bar code given to each different item in a supermarket. The RiboPrinter automatically compares the riboprint of the newly tested bacterium with all the riboprints in its (DuPont) data base. If the test riboprint matches the riboprint of some identified bacteria, it is identified to that species. If the riboprint does not match up to any riboprint in its data base, this bacterium is characterized as a new ribogroup. If another unknown bacterium has the same riboprint, it will be placed into this same ribogroup. Thus, the same species of unidentified bacteria in a water sample can be grouped together into a common ribogroup, whereas another species of unidentified bacteria will be placed into a different ribogroup. In summary, the RiboPrinter method is capable of characterizing identified and unidentified populations of THB bacteria into different ribogroups and these ribogroups can be used to determine the sources of these bacteria.

The RiboPrinter method was selected for the following reasons. (1) At the time this study was initiated, this method was considered to be one of the most sophisticated automated methods to identify most of the bacteria (>4,000 riboprints) relevant to man, including most human pathogens. (2) The DuPont Qualicon Company provided a lease agreement to use the RiboPrinter equipment for the two-year period of this study. This eliminated the need to purchase this expensive equipment and reduced the cost of reagents during this period. (3) This automated system is capable of identifying an unknown bacterium within 8 hours and can process up to 32 samples within a day. (4) Since this system has the capability of identifying pathogenic bacteria and environmental bacteria and can characterize the unidentified THB isolates recovered from potable water samples into distinct ribogroups, it has the potential to identify the populations of bacteria in the polluting solution used for intentional contamination of potable water systems.

VIII. Diagram of the Proposed Three-Tiered EWMP

A summarized diagram of this three-tiered EWMP is shown in Figure 7. Explanations for the sequential steps involved in the implementation of this EWMP are outlined as follows.

Step 1. The EWMP is triggered to start when BWS receives creditable evidence (information, physical evidence, disease symptoms) that its water system has been contaminated.

Step 2. Based on available evidence, BWS must determine the most likely type (chemical, biological) of contamination and must also determine the most likely sites of contamination in the water system so samples can be collected for evidence of contamination. This often means having to test water samples from many suspected sites. Those designated to collect water samples must be informed that the water samples may be contaminated and they must have been trained in the use of proper attire and proper sampling procedures. Extra water samples should be collected for re-testing and to send to reference laboratories. Assume that external sources of contamination will result in fast-growing THB colonies or NTC. Therefore, examine all water samples previously analyzed for THB and total coliform for fast-growing THB or increased concentrations of NTC. Select all suspected THB colonies and NTC and purify so they can be tested by Tier Three method.

Step 3. As a precaution, highly chlorinate the water system to disinfect microbial pathogens, which may be associated with the contamination event.

Step 4. Suspected water samples, which were collected in Step 2, must be analyzed by the three Tier One tests: Microtox method to measure for presence of toxic chemicals, ATP method to measure for total concentrations of microorganisms, and InSpectra method to measure for levels of UV-absorbing organic chemicals or particles in water samples. For all Tier One positive tests: (1) initiate additional tests to determine whether practical methods such as filtration, adsorption, precipitation, and disinfection can be used to remove or neutralize the contaminant in the water sample, (2) analyze for concentrations and colony characterization of THB, (3) analyze for concentrations of total coliform and observe for NTC. Purify suspected colonies of THB and NTC so they can be tested by Tier Three method.

Step 5. All positive Tier One tests must be analyzed by the following Tier Two tests: Ruggedized Advanced Pathogen Identification Device (R.A.P.I.D.) method to identify microbial pathogens and enzyme linked immunosorbent assay (ELISA) method to identify toxic chemicals in water samples. All samples positive by Tier Two tests should be forwarded to Bioterrorism Laboratory of the Hawaii State Department of Health, where approved identification systems are available to identify most of the hazardous chemical and biological agents.

Step 6. Analyze purified isolates of THB and NTC recovered from water samples during Steps 2 and 4 by Tier Three method. The RiboPrinter method can be expected to identify these isolates as pathogens, as species of environmental bacteria or as unidentified bacteria characterized to specific ribogroups. Based on these results, determine if the THB isolated from the water samples originated from potable water as ambient THB or originated from an external source. All THB colonies characterized as originating from an external source should be suspected as originating from the contaminating source. Their presence can be used as markers for the polluting solution in the water system. Send these THB isolates to Bioterrorism Laboratory of the Hawaii State Department of Health, where approved identification systems are available to identify most of the hazardous biological agents.

Step 7. Preparations should be made to use alternative sources of water if the primary sources of water are contaminated. During a contamination event, all major sources of potable water should be considered targets for contamination. Under these conditions, the safety of these potable sources will be questioned. As a result, identify other alternative sources of water (e.g., springs, stream water) which are not likely to be contaminated and can be used as an alternative or emergency water supply during these emergency conditions. The ambient quality of these water sources should already have been determined.

CHAPTER THREE

EVALUATION OF TIER ONE MONITORING METHODS

I. Evaluation of Microtox Method to Measure Toxic Chemicals in Water

A. Application of Method.

There is a need for a commercially available Tier One method to rapidly (1 to 15 minutes) screen potable water samples for contamination by toxic chemicals or chemical agents with poisonous or toxic properties. Toxic chemicals comprise one of the major classes of contaminant and their presence in water should be suspected during an intentional contamination event. Under emergency conditions many water samples must be rapidly tested to determine the location of contamination in the water system. Under these conditions, the currently used standardized methods, which use whole animals to assay for toxicity in water, would not be suitable as a rapid Tier One test because they cannot be completed in minutes and the procedures are too complicated to analyze many samples. The Microtox method was selected as the Tier One test to detect chemicals with acute toxic properties in water. The reasons for selecting the Microtox test as a Tier One method were previously summarized in Chapter Two. The suitability of the Microtox method as a Tier One test, was evaluated based on meeting the following requirements of Tier One methods: (1) the method must be commercially available and can rapidly (minutes) and reliably measure a water quality parameter, which can be expected to change in response to a contamination event, (2) the ranges of measurement for that water quality parameter must be modest and predictable, and (3) the concentration of the water quality parameter to signal a possible contamination event must be determined.

B. Training to Use Method.

A Microtox instrument was purchased by WRRC several years ago and WRRC personnel were trained in its use. More recently, the Hawaii Department of Health (DOH) and the County of Maui each purchased a Microtox unit. On February 8, 2002, a water quality methods workshop organized by Roger Fujioka was held at the DOH laboratory auditorium. During this workshop, Gary Evereklian of Strategic Diagnostic Inc. discussed the theory of the Microtox test method and how this method has been used to monitor for toxic chemicals in wastewater and in drinking water. Immediately following that workshop, Evereklian held a hands-on training session on the use of the Microtox instrument at the DOH Environmental Laboratory, where laboratory personnel including those from DOH and BWS, were trained in the use of Microtox as well as the Delta Tox instruments. On July 30, 2004, BWS personnel (Owen Narikawa, Ronald Saito, Dean Tamura, and Karl Iwasaki) were provided additional training in the use of Microtox at the University of Hawaii by Audrey Asahina. Using a Power Point presentation, Asahina explained the theory and procedure in the use of Microtox method. After this formal presentation, the BWS personnel were taken to the WRRC laboratory where they were trained to operate the Microtox instrument using reagents that were already prepared.

C. Previous Findings.

The Microtox method was used by WRRC in several research projects to determine levels of acutely toxic chemicals in several environmental sources of water. For example, it was used to measure for toxic chemicals in effluent samples from the Wahiawa Wastewater Treatment Plant (Billingsley, 1990), in storm water samples from Oahu (McParland, 1991), and in samples from Waimanalo Stream (Paulino, 1994). Acutely toxic chemicals were not consistently detected in the samples from these three sources. In Wahiawa Wastewater Treatment Plant effluent, chlorine was determined to be a consistently toxic component of the treated effluent. In addition, the Microtox test was used yearly as a graduate class (CE 636/PH690) experiment to determine levels of toxicity in environmental samples from areas such as Ala Wai Canal. For all of these studies, BWS tap water was used as the negative control and phenol or some other toxic chemical was used as positive control. Based on the results at the time of these projects, we concluded that acutely toxic chemicals are not present in BWS potable water sources and in environmental waters of Hawaii. It should be noted that the Microtox method measures toxicity based on the reduction of light output by the population of bacteria (*V. fischeri*) and that all biological agents are susceptible to some variation. As a result, only readings showing >20% reduction in light were determined as significant in detecting toxicity in water samples.

D. Results and Conclusions.

As stated earlier (see Chapter Two), relative toxicity in a water sample is measured as the loss of light or percent effect of toxic chemicals after exposure of *V. fischeri* to water samples. For the present study, 10 ambient water samples each from well sites, reservoir tank sites, and distribution sites were assayed by the Microtox method. The results of the observed percent effect after reaction times of 5 and 15 minutes are presented in Table 2. The percent effect after 15 minutes have been reported to provide more reliable results. The percent effect after the shorter reaction time is used when the concentration of toxic chemicals in water sample is so high that a dramatic toxic effect can be clearly observed after 5 minutes. In this regard, during a probable contamination event, the 5-minute result should be read to determine whether the samples contain high levels of acutely toxic chemicals. As presented in Table 2, the results of the percent effect after 15 minutes for well water samples ranged from -10.49% to 29.26%, with a mean of 5.81%; for reservoir water samples, from 1.81% to 18.71% with a mean of 10.01%; and for distribution site water samples, from -14.55% to 35.48% with a mean of 1.14%.

The following conclusions can be drawn from these results. (1) Negative percent effect readings (e.g., -10.49%) are occasionally observed, due to water sample causing an increase in the level of light output by the population of *V. fischeri*. This kind of effect called "hormesis" has been reported in Microtox literature and is believed to be caused by components in water samples that stimulate the metabolism and light output of *V. fischeri*. (2) For individual ambient water samples, the percent effect ranged from -14.5% to 35.48%. However, the mean percent effect for the three sources of water (well, reservoir, distribution site) ranged from

1.14% to 10.01%. These results demonstrate the range of percent effect readings that can be expected when ambient potable water samples are analyzed using the Microtox method. These results are similar to results of our previous projects that used ambient BWS potable water samples as controls and are similar to those of States et al., (2004), who reported variations in percent effect when Microtox method was used to analyze potable water samples. As a result, States et al., (2004) concluded that 20% inhibition or percent effect is the minimum detection limit to reliably detect toxicity in water. We agree that 20% inhibition can be used as a minimum average effect but for individual ambient water samples, percent effect can range from 20 to 40%. In this regard, some variations in Microtox results occur because water quality factors (e.g., pH, salinity, oxygen level), which are not related to presence of toxic chemicals, can also have a measurable effect on bacterial metabolism of *V. fischeri*. (3) For an intentional contamination event, the toxicity of added chemicals can be expected to be very high. Under these conditions, we recommend that for the 5-minute percent effect, any reading >50% should be used as evidence that a contamination event may have occurred. For a 15-minute effect, >40% effect should be used as evidence that a contamination event may have occurred.

E. Final Assessment and Recommendations.

The suitability of the Microtox method was assessed based on meeting the three requirements of an effective Tier One method. The first requirement of reliably and feasibly measuring toxicity levels in water as a water quality parameter which can be expected to change during a contamination event was met. The second requirement of measuring moderate and predictable ranges of toxicity levels in potable water samples was met. In this regard, for individual potable water samples, the range of Microtox readings was established at -14.55% to 35.48% toxic effect, but for well water, reservoir water, and distribution water, the mean range was 1.14% to 10.01% (Table 2). The third requirement of setting a reasonable action level to signal a possible contamination event was met. In this regard, the recommended action level to conclude that a contamination may have occurred for a given sample is >50% effect for a 5-minute reading and greater than 40% effect for a 15-minute reading. Based on these data and other accumulated data on the use of the Microtox method, we recommend that BWS adopt this method as a Tier One test because when there is a need to screen many water samples for contamination by toxic chemicals, this is the most feasible method. Moreover, this method has been standardized and approved for detection of toxic chemicals in many types of water. It should be noted that a portable, single test version of the Microtox method called Delta Tox is now available. If BWS needs portability to assay for toxicity at sites away from the laboratory, this portable version should be considered.

II. Evaluation of ATP Pallchek Luminometer Method to Measure Total Microbial Load in Water

A. Application of Method.

There is a recognized need for a commercially available Tier One test method that can be used to rapidly (minutes) screen water samples for contamination by biological agents such as

pathogens. Hazardous biological agents comprise one of the major classes of contaminant which should be suspected in water during an intentional contamination event. Under emergency conditions many water samples must be rapidly tested to determine the location of contamination in the water system, so standard culture methods used to measure levels of biological agents in water would not be suitable as a rapid Tier One test because they are too slow. As a result, a rapid chemical assay for ATP in water as an indirect measurement for total viable concentrations of microorganisms was selected as the Tier One test. Increase in total microbial load in water samples is indicative that biological agents have contaminated water. Total ATP in a water sample is measured by an enzymatic reaction in which ATP reacts with luciferase enzymes to produce light. The amount of light produced is measured as relative light units and is proportional to concentrations of ATP. Since all viable cells contain and use ATP as energy to drive the biochemical reactions in cells, the concentrations of ATP in water indirectly measures total microbial concentration. The reasons for selecting the ATP Pallchek Luminometer System were previously summarized (see Chapter Two). The suitability of the Pallchek method as a Tier One test, was evaluated based on meeting the following requirements of Tier One methods: (1) the method must be commercially available and can rapidly and reliably measure a water quality parameter, which can be expected to change in response to a contamination event, (2) the ranges of measurement for that water quality parameter must be modest and predictable, and (3) the concentration of the water quality parameter to signal a possible contamination event must be determined.

B. Training to Use Method.

The Pallchek instrument was purchased by BWS. On December 10, 2002, Barry Schubel of Pall Life Sciences conducted a hands-on training session at the BWS laboratory to train BWS and WRRC personnel on the use of the Pallchek Luminometer System. During the training session, standard reagents were used and 100 ml water samples from BWS were analyzed. In our discussion with Schubel, we stated that BWS water samples are characterized by low (<10 CFU/ml) total heterotrophic bacteria and that ATP levels may be below the detection limit of the Pallchek method. Schubel made the following recommendations to increase the sensitivity of the ATP assay. (1) Use the high sensitivity reagents to detect lower concentrations of total bacteria in water samples. (2) Process larger volumes of water so that larger numbers of bacteria are trapped on the membrane for subsequent analysis. (3) Follow the guidelines as outlined in the manual to prevent contamination and to reduce nonspecific background levels of ATP. In this regard, Schubel cautioned that the high sensitivity reagents tend to give higher background levels of light (e.g., 100 to 150 relative light units), so this level must be subtracted from the final reading of the test to obtain a true measurement of ATP in the water sample. On February 11, 2005, the final training session was held for BWS personnel (Owen Narikawa, Dean Tamura, Karl Iwasaki) at WRRC by Audrey Asahina, who used a Power Point presentation to review all the methods used in this study. After this formal presentation, the BWS personnel were taken to the WRRC laboratory where Dayna Sato demonstrated the procedures to operate the Pallchek instrument and the Profile-1 instrument.

C. Processing Conditions.

To increase the sensitivity of the ATP assay and to prevent external contamination, the following guidelines were established for processing the water samples using the Pallchek Luminometer System. (1) Use high sensitivity reagents to increase the sensitivity of the assay. (2) Use larger volumes of water (450–500 ml) to increase the number of bacteria captured on the membrane. (3) Use the 47-mm (Gelman GN6) membrane with a 0.45-μm pore size and with the flat side up to enhance the spreading of the reagents on the membrane. (4) Prepare ATP-free water by filtering deionized water through a 0.22-μm pore size membrane, followed by autoclaving. Use this water to wash funnel and membrane to reduce contaminating levels of ATP. (5) Use gloves, a laminar flow hood and keep reagents away from light to prevent external contamination. Hydrate and then stabilized reagents by storing at room temperature for at least 30 minutes before use.

To process water samples, wash sterile filter funnel unit three times with 300 ml of ATP-free water. Place GN6 membrane on the filter holder and wash with 100 ml of ATP-free water. Filter 450 ml of water sample through this washed membrane followed by a second wash using 100 ml of ATP-free water. Transfer membrane to a petri dish and place the dish onto the aluminum plate of the Pallchek Luminometer System. Add 150 μl of extractant reagent onto the membrane and use an ATP-free plastic spreader to spread this reagent evenly onto the surface of the membrane for 15 seconds. This procedure releases ATP from the cells. Add 100 μl of the luciferase enzyme reagent to the membrane and spread evenly to initiate the ATP reaction. Close the cover, activate the vacuum to seal the unit and read the light output as relative light units. This measurement includes light produced from the test water as well as background light produced by the reagents and the apparatus. Subtract the background level for the reagents and apparatus from sample reading to obtain the actual RLU of the sample. To obtain background level, filter 100 ml of ATP-free water through a membrane and read RLU after all reagents were added to the filter. This procedure was repeated three times. The average of these three readings was taken as the background level of RLU for that day. According to Pall Life Science, the background level should range from 100 to 150 RLU. In these experiments, background levels ranged from10 to 360 RLU but most reagent background levels were within the 100 to150 RLU range.

For most potable water samples assayed for ATP, concentrations of total heterotrophic bacteria counts were also measured by filtering 25 ml of water sample and counting all colonies on the membrane placed onto mHPC agar after 5 days at 25 ± 2°C.

D. Confirmation in the Measurement of ATP.

To demonstrate that the Pallchek method is reliable, various concentrations of purified ATP were diluted into water and then the Pallchek method was used to assay for ATP. The results of two experiments are plotted in Figure 8, which shows the linear relationships (R^2 = 0.9947, R^2 = 0.9584) between increasing RLU readings and increasing concentrations (0.2 to

50 picogram) of ATP. These results verify that the Pallchek method can be relied on to measure purified concentrations of ATP in water.

E. Results of Analyzing Groundwater Sources.

A total of 70 groundwater samples (56 chlorinated, 14 non-chlorinated) from wells, tunnels, and shafts were analyzed for THB and for ATP. The results (Table 3) show that the concentrations of THB in these samples range from 0 to 891 CFU/100 ml with a geometric mean of 68 CFU/100 ml (<1 to 9.0 CFU/ml with geometric mean of <1 CFU/ml). Based on previously established concentrations of THB in other potable water systems, these results show that the THB levels in these groundwater samples were consistently low such that the microbial quality of groundwater sources can be characterized as being stable and excellent. When these same water samples were assayed for ATP, the measurements ranged from 0 to 4,822 RLU/100 ml for chlorinated samples with a geometric mean of 264 RLU/100 ml. For non-chlorinated samples, the ATP measurements ranged from 0 to 9,095 RLU/100 ml with a geometric mean of 439 RLU/100 ml (Table 3). To determine if the ATP assay can be used as a reliable surrogate test for THB, the log of the RLU readings of each sample was plotted against the measured levels of THB. The results (Figure 9) show a poor relationship (R^2 = 0.0097) between ATP and THB measurements, indicating no reliable correlation between these two measurements. The variation in the RLU readings for these 70 samples is displayed in Figure 10, which shows that 58/70, or 82.8%, of the water samples had readings of <1,000 RLU/100 ml and 17.2% of the samples had readings that range from >1,000 to 9,909 RLU/100 ml. Sometimes, the 95% threshold value is used to establish an action level. However, the 95% threshold value for all RLU measurements for groundwater samples was approximately 4,000 RLU/100 ml. This threshold is too high to be used to signal a reliable contamination event.

Based on the results of ATP measurements for groundwater samples the following conclusions were made. (1) Concentrations of THB cannot be correlated to ATP measurements in the same water samples. (2) Variations in RLU readings for ambient groundwater samples are not related to THB concentrations. (3) The most likely explanation for the wide variation in ATP measurements is the variable concentrations, physiological states, and kinds of microorganisms (bacteria, yeast, protozoa) in groundwater samples. These three factors are known to produce variable levels of ATP. (4) Due to the wide variation in measurements a reasonable level of ATP in groundwater samples to signal a contamination event cannot be recommended.

F. Results of Analyzing Reservoir Water Samples.

A total of 100 chlorinated water samples from reservoir storage tanks were analyzed for THB and for ATP. The results (Table 3) show that the concentrations of THB in these samples range from 0 to 2,938 CFU/100 ml with a geometric mean of 70 CFU/100 ml (<1 to 30 CFU/ml with a geometric mean of <1 CFU/ ml). Based on previously established concentrations of THB in other potable water systems, these results show that the THB levels in these reservoir water samples were consistently low such that and the microbial

quality of the reservoir water sources can be characterized as being stable and excellent. When these same water samples were assayed for ATP, the measurements ranged from 16 to 822,217 RLU/100 ml with a geometric mean of 665 RLU/100 ml (Table 3). To determine if the ATP assay can be used as a reliable surrogate test for THB, the log of the RLU readings of each sample was plotted against the measured levels of THB. The results (Figure 11) show a poor relationship ($R^2 = 0.0253$) between RLU readings and measurements of THB, indicating no reliable correlation between these two measurements. The variation in the RLU readings for these 100 samples is displayed in Figure 12, which shows that 70/100, or 70% of the water samples had readings of <1,000 RLU/100 ml and 30% had readings that range from >1,000 to 822,217 RLU/100 ml. Sometimes, the 95% threshold value is used to establish an action level. However, the 95% threshold value for all RLU measurements for reservoir water samples was approximately 30,000 RLU/100 ml. This threshold is too high to be used to signal a reliable contamination event.

Based on the results of ATP measurements for reservoir water samples, the following conclusions were made. (1) Concentrations of THB cannot be correlated to ATP measurements in the same water samples. (2) Variations in RLU readings for ambient reservoir water samples are not related to THB concentrations. (3) The most likely explanation for the wide variation in ATP measurements is the variable concentrations, physiological states and kinds of microorganisms (bacteria, yeast, protozoa) in reservoir water samples. These three factors are known to produce variable levels of ATP. (4) Due to the wide variation in measurements, a reasonable level of ATP in reservoir water samples to signal a contamination event cannot be recommended.

G. Results of Analyzing Distribution Water Samples.

A total of 184 chlorinated water samples from distribution pipes were analyzed for THB and for ATP. The results (Table 3) show that the concentrations of THB in these samples range from 4 to 3,720 CFU/100 ml (<1 to 37 CFU/ml) with a geometric mean of 360 CFU/100 ml (3.6 CFU/ml). Based on previously established concentrations of THB in other potable water systems, these results show that the THB levels in these distribution water samples were consistently low such that the microbial quality of the distribution water sources can be characterized as being good. When these same water samples were assayed for ATP, the measurements ranged from 21 to 62,179 RLU/100 ml with a geometric mean of 1,067 RLU/100 ml (Table 3). To determine if the ATP assay can be used as a reliable surrogate test for THB, the log of the RLU readings of each sample was plotted against the measured levels of THB. The results (Figure 13) show a poor relationship ($R^2 = 0.1125$), between measurements of ATP and THB, indicating no reliable correlation between these two measurements. The variation in the RLU readings for these 184 samples is displayed in Figure 14, which shows that 95/184, or 51.6%, of the water samples had readings of <1,000 RLU/100 ml and 48.4% of the samples had readings that ranged from >1,000 to 62,179 RLU/100 ml. Sometimes, the 95% threshold value is used to establish an action level. However, the 95% threshold value for all RLU measurements for distribution water samples

was approximately 9,000 RLU/100 ml. This threshold is too high to be used to signal a reliable contamination event.

Based on the results of ATP measurements for distribution water samples, the following conclusions were made. (1) Concentrations of THB cannot be correlated to ATP measurements in the same water samples. (2) Variations in RLU readings for ambient distribution water samples are not related to THB concentrations. (3) The most likely explanation for the wide variation in ATP measurements is the variable concentrations, physiological states, and kinds of microorganisms (bacteria, yeast, protozoa) in distribution water samples. These three factors are known to produce variable levels of ATP. (4) Greater variation in RLU readings were observed for ambient distribution water as compared to water samples from well or reservoir sources. These results most likely reflect the fact that biofilm growth is more predominant in distribution lines than at well or reservoir sites. (5) Due to the wide variation in measurements, a reasonable level of ATP in distribution water samples to signal a contamination event cannot be recommended.

H. Sensitivity of ATP Assay to Concentrations of *Escherichia coli.*

The critical question is whether the sensitivity of the ATP assay can reliably detect contamination of water samples when a bacterial pathogen is added. For these experiments, various concentrations of stationary phase culture *E. coli* were added to either buffer or distribution water samples and then ATP levels measured using the Pallchek method. We used stationary phase culture of *E. coli* because most bacterial preparations used for contamination would be in the stationary or even in the death phase. It should be noted that the ATP concentrations for bacteria in the stationary phase is much less than that for bacteria in the growth phase. The objective of the first experiment was to add 123,785, and 5,850 CFU of *E. coli* into 500 ml of BWS distribution water sample and to analyze the entire volume for concentrations of ATP. The results (Table 4) show that before the addition of *E. coli*, the water sample had an ATP concentration of 1,264 RLU/100 ml. After addition of 123 and 785 CFU of *E. coli* to this water sample, the RLU readings did not show an increase, indicating that the ATP assay was not sensitive enough to detect the addition of 123 and 785 CFU of *E. coli* to the 500 ml water sample tested. When 5,850 CFU of *E. coli* were added to 500 ml sample, the ATP reading increased by only 176 RLU/100 ml to a final reading of 1,440 RLU/100 ml. Based on previous analyses, a change of 176 RLU/100 ml may be related to normal variation rather than detection of the additional 5,850 CFU of *E. coli*. These preliminary results indicate that the ATP assay is not sensitive enough to detect the addition of 123,785 and 5,850 CFU of *E. coli* to 500 ml of BWS water sample. A serious implication of these results is that the ATP reaction would not be a reliable means of detecting a health-related concentration of bacteria associated with a contamination event.

The objective of the next experiment was to determine the minimum concentrations of stationary phase *E. coli* which could be detected by the Pallchek ATP method. For this experiment tenfold increments of *E. coli*, from <10 to 10^8 CFU/100 ml were added to sterile buffer samples or distribution water samples and then the entire 100 ml samples analyzed for

concentrations of ATP. Buffer solution represents the control sample because there are no microorganisms in the buffer sample and the added *E. coli* is the only source of ATP. In contrast, BWS potable water samples contain unknown concentrations of microorganisms and they are sources of ATP. The results of the experiment using buffer are summarized in Table 5 and show that for buffer alone and in buffer solutions containing 2 and 3 CFU/100 ml of *E. coli*, the measured level of ATP was 0 RLU/100 ml. When concentrations of *E. coli* in water samples were increased to 102 CFU/100 and to 2,000 CFU/100 ml the respective ATP measurements were 6 RLU/100 ml and 52 RLU/100 ml. However, these low measurements may not be significant because they are within the background level of 10 to 360 RLU/100 ml. When the concentration of *E. coli* was increased to 21,600 CFU/100 the concentrations of ATP reached a moderate level of 467 RLU/100 ml. Further tenfold increases in concentrations of *E. coli* resulted in corresponding tenfold increases in measurements of RLU/100 ml. These results indicate that in buffer solution, the reliable detectable limit for *E. coli* is approximately 21,000 CFU/100 ml.

For comparison, similar concentrations of *E. coli* were added to 100 ml samples of distribution water and then the 100 ml samples were assayed for ATP. The results (Table 6) show that this ambient potable water sample was characterized by 590 RLU/100 ml of ATP. After addition of 2, 27, 282, and 2,660 CFU/100 ml of *E. coli*, the measured levels of ATP in these samples did not change appreciably and the 2,660 CFU/100 ml of *E. coli* resulted in ATP reading of 560 RLU/100 ml These results indicate that the Pallchek ATP method is not sensitive enough to detect the addition of up to 2,660 CFU/100 ml of *E. coli* added to distribution water. The results (Table 6) show a tenfold increase in *E. coli* to 26,000 CFU resulted in only a two-fold increase in RLU to approximately 1,140 RLU/100 ml. It was not until 314,000 CFU/100 ml of *E. coli* were added to the potable water sample that a significant reading of 4,040 RLU/100 ml was observed. Additional tenfold increases in *E. coli* concentrations resulted in tenfold increases in ATP readings. Thus, in potable water characterized by 590 RLU of ATP, the reliable detectable limit for *E. coli* is between 26,000 to 314,000 CFU/100 ml. These results indicate that the detectable limit for *E. coli* is higher in ambient potable water than in sterile buffer. The most logical explanation is that the ambient populations of microorganisms in potable water interfere with the detection of moderate but health-related concentrations of 2,660 to 26,000 CFU/100 ml of *E. coli*. These results indicate that a serious limitation of the ATP assay is that it cannot be relied on to detect concentrations of bacteria which can have a health effect for the public.

I. General Conclusions.

For this study, the Pallchek ATP method was used to measure the concentrations of ATP in three major sources of potable water (groundwater, reservoir water, distribution water). Based on the data obtained using the Pallchek ATP method, the following conclusions were made. (1) Concentrations of THB in BWS potable water samples did not correlate with ATP measurements in the same water samples. (2) Variations in RLU readings of ambient potable water samples are not related to THB concentrations. The most likely explanation for the wide variation in ATP measurements in BWS potable water samples is the variable

concentrations, physiological states and kinds of microorganisms (bacteria, yeast, protozoa) in these sources of water. These variable concentrations, physiological states and kinds of microorganisms produce variable levels of ATP and account for the great variability of ATP readings in potable water samples. (3) Greater variation in RLU readings was observed for ambient distribution water as compared to water samples from well or reservoir sources. These results most likely reflect the fact that biofilm growth contribute to ATP levels and that biofilm in distribution pipes can be expected to be more extensive than in groundwater and reservoir sources of water. (5) The ambient populations of microorganisms in potable water interfere with the detection of *E. coli* added to potable water samples

J. Final Assessment and Recommendations.

The suitability of the Pallchek ATP method was assessed based on meeting the three requirements of an effective Tier One method. This ATP method partially met the first requirement of rapidly measuring a water quality parameter (total microbial load) that can be expected to change in response to a contamination event. However, since the ATP assay was not sensitive enough to detect health related concentrations (100 to 2,000 CFU/100 ml) of *E. coli*, this method may not be reliable enough to measure a contamination event. Another serious limitation of the ATP assay was related to the wide variations and unpredictable levels of ATP measured in ambient potable water samples. As a result, this ATP method could not meet the following two remaining requirements of a reliable Tier One method: (1) the ranges of measurement for that water quality parameter must be modest and predictable, and (2) the concentration of the water quality parameter to signal a possible contamination event must be determined. Based on these assessments, we recommend that BWS not adopt the Pallchek ATP method as a reliable Tier One test to screen potable water samples for the purpose of detecting a contamination event.

III. Evaluation of Profile-1, an Alternative ATP Assay Method

A. Application of Method.

The performance of the Pallchek ATP method failed to reach its objective of reliably detecting microbial contamination in BWS potable water samples, raising the question of whether an alternative ATP method could overcome all the problems related to the Pallchek method. To address this question, Profile-1 was selected as the alternative ATP assay. Reasons for selecting the Profile-1 method are summarized in Chapter Two.

B. Training to Use Method.

On February 11, 2005, the final training session was held for BWS personnel (Owen Narikawa, Dean Tamura, Karl Iwasaki) at WRRC. During this session, Audrey Asahina used a Power Point presentation to review all the methods used in this study. After this formal presentation, the BWS personnel were taken to WRRC laboratory where Dayna Sato demonstrated the procedures to operate the Pallchek ATP method and the Profile-1 ATP method.

C. Results of Comparative Study.

To demonstrate that the Profile-1 method can reliably measure ATP, various concentrations of purified ATP were dissolved in water and then these samples were assayed for ATP. The results of two experiments plotted in Figure 15 show reliable linear relationships (R^2 = 0.9622, R^2 = 0.9992) between increasing RLU readings and increasing concentrations from 0.2 to 10 picogram of ATP. These results verify that the Profile-1 system can be relied on to measure purified ATP concentrations in water. Moreover, the results indicate that the Profile-1 method is able to detect lower concentrations of ATP than the Pallchek method.

To compare the effectiveness of the Profile-1 ATP assay with that of the Pallchek method, approximately 10 water samples from each of the three general sources of water (groundwater, reservoir tank, distribution pipe) were assayed for ATP using both methods. For this study, 25-ml water samples were assayed by the Profile-1 method and 450-ml water samples by the Pallcheck method. The results, which are summarized in Table 7, show that when the same water samples were assayed using the two different methods, the measured concentrations were similar in some cases and different in other cases. For some samples the RLU readings were higher using Profile-1 method, but for other samples, the RLU readings were higher using the Pallchek method. The geometric means of the respective Profile-1 and Pallchek ATP assays for the 11 groundwater samples were 153 and186 RLU/100 ml, 275 and 282 RLU/100 for the 10 reservoir water samples and of 99 to 157 RLU/100 ml for the 10 distribution water samples (Table 7).

D. Summary Assessment of Methods.

Based on the comparative assessment of the Pallchek, and Profile-1 ATP methods, the following conclusions were made. (1) The Profile-1 method appears to be more sensitive at detecting ATP concentrations than the Pallchek method. This conclusion is based on the observation that only 25-ml samples were used in the Profile-1 method as compared to 450-ml samples used by Pallchek method. (2) Of the two methods, the Profile-1 is more feasible and can process more samples because it does not require washing of the equipment and filter with ATP-free water and because it requires lower volumes of water for testing. (3) Overall, both methods gave similar ATP measurements for the same set of BWS potable water samples. These results suggest that the limitations determined for the Pallchek ATP method cannot be totally overcome by using an alternative ATP method such as Profile 1.

IV. Evaluation of the InSpectra Method for UV Absorbing Components in Water

A. Application of Method.

In our experimental design for Tier One tests, there is a recognized need for a rapid test which can detect changes in some general water quality parameters, which would not be measured by the other two Tier One tests (ATP, Microtox). The results of this test can be used either to supplement the results of the other two Tier One tests or to provide independent data on water quality to signal a contamination event. A candidate is the commercially available InSpectra method, which uses a specially designed UV spectrophotometer to scan water

samples and within a minute provide data on the concentrations of six water quality parameters: BOD, COD, TOC, TSS, NO_3, and SUR. The experimental design is to use the InSpectra instrument to determine changes in these six water quality parameters in BWS water samples. Theoretically, when the ambient concentration of one or more of the six water quality parameters is exceeded in a potable water sample, this can be taken as evidence that the quality of water has changed and may represent a contamination event. However, the interpretation of the InSpectra data is complicated by the fact that this method does not analyze for the six parameters but determines their respective concentrations by comparing the characteristic UV absorption pattern measured in the water sample with an algorithim of UV absorption spectrum stored in its internal software. Organic matter, nitrates, and particulates are water sample components, which are absorbed by UV light. Thus, the InSpectra method actually measures changes in water quality based on concentrations of UV-absorbing components. Reasons for selecting the InSpectra method as a Tier One test are previously summarized in Chapter Two. The suitability of the Pallchek method as a Tier One test, was evaluated based on meeting the following requirements of Tier One methods: (1) the method must be commercially available and can rapidly (minutes) and reliably measure a water quality parameter, which can be expected to change in response to a contamination event, (2) the ranges of measurement for that water quality parameter must be modest and predictable, and (3) the concentration of the water quality parameter to signal a possible contamination event must be determined.

B. Training to Use Method.

BWS purchased a new InSpectra instrument from Strategic Diagnostic, Inc. Training in its use was part of a general workshop on the instruments available for rapid analysis of contaminants in water samples conducted by Gary Evereklian of Strategic Diagnostic Inc. This workshop was held at the State Department of Health Laboratory Auditorium on February 8, 2002. Following that workshop, a hands-on training session for BWS and WRRC personnel on the use of the InSpectra instrument was conducted by Evereklian at the BWS laboratory.

C. Experimental Results.

For this study, the InSpectra instrument was set for natural waters so that reference UV spectra from natural waters (rivers, lakes, wells) were used to be compared to the measurement for potable water samples. In preliminary studies the levels of BOD, COD, TOC, TSS, and SUR in the BWS potable water samples resulted in undetectable readings (<1 mg/l) and were below the detection limit of the InSpectra method. However, nitrate readings of 1.3–1.8 mg/l were observed in many water samples.

To better evaluate the InSpectra method, deionized water and 10 ambient water samples from well sites, reservoir tank sites, and distribution water sites were assayed. When deionized water was assayed, all six water quality parameters were below detectable levels of <1 mg/l (data not shown) illustrating that InSpectra method does not detect false positive signals. As for the tests on the 10 well water samples tested, all samples showed undetectable levels of

TSS and 9 of 10 samples showed undetectable levels for COD, BOD, TOC and SUR (surfactants) but 7 of 10 samples had nitrate readings ranging from 1.4 to 8.2 mg/l (Table 8). The well sample from Waialee Well I showed high levels of COD (40.5 mg/l), BOD (37.5 mg/l), TOC (33.5 mg/l), nitrates (8.2 mg/l) and surfactants (46.5 mg/l) (Table 8). The results of the test on the 10 reservoir samples showed that all 10 samples had undetectable levels of TSS and 7 of 10 samples had undetectable levels of COD, BOD, TOC and SUR (Table 9). On the other hand, most of the samples (7 of 10) had measurable levels (1.1 to 13.1 mg/l) of nitrates and 3 of 10 samples had elevated levels (24.6 to 50.5 mg/l) of COD, BOD, TOC and SUR (Table 9). The results on the 10 distribution water samples showed that all 10 samples had undetectable levels of TSS but 9 of 10 samples had elevated levels (9.1 to 47.5 mg/l) of COD, BOD, TOC, and SUR (Table 10).

The results of the InSpectra method showed that the measured levels of some of the water quality parameters (COD, BOD, TOC, SUR) were unrealistically high for potable water. To show that these measurements were incorrect, some of the samples with high TOC were measured for TOC using standardized methods and the results showed concentrations of <1 mg/l. Based on these results, we concluded that the measured concentrations of the six water quality parameters using the InSpectra method are not reliable. However, it should be noted that InSpectra method was measuring different concentrations of UV-absorbing materials in the different sources of potable water. In this regard, elevated concentrations of the water quality parameters were found with low frequency (1/10) in well water, moderate frequency (3/10) in reservoir water and, high frequency (9/10) in distribution water samples. These results indicate that as water is pumped from wells, stored in reservoirs, and then released into distribution lines, there is an increase in UV-absorbing components in these waters. Thus it is clear that distribution water contains more UV-absorbing compounds than well water and water stored in reservoir tanks.

D. Assessment of Data.

The results of analyzing BWS potable water samples with the InSpectra method were unsatisfactory because of the undetectable levels of most of the six water quality parameters in most samples and because of the unrealistically high concentrations of COD, BOD, TOC, and SUR in some samples. This problem is clearly related to the fact that the InSpectra method does not measure for the six water quality parameters but determines their concentrations based on comparing the UV spectra of the test water with reference UV spectra stored in its software. In this method, the assumption is made that reference UV spectra are relevant to the UV spectra for BWS potable water samples. Clearly, this assumption cannot be made and therefore the calculated concentrations of the six water quality parameters for BWS potable water samples are not valid. This conclusion was supported by scientists from SDI/Azur Company who were consulted to assist us in interpreting the InSpectra data. We were able to communicate with the scientists in Italy who developed this method. These scientists concurred that the algorithim they used to establish the concentrations for the specific measurements of the six water quality parameters cannot be reliably applied to Honolulu's potable groundwater samples. They pointed out that the

natural water setting of the InSpectra test was optimized for water samples with elevated and measurable levels of these six water quality parameters and that the reference spectra stored in the software are not appropriate for Honolulu's groundwater samples.

E. Final Assessment and Recommendation.

The InSpectra method is an example of a rapid test that measures one set of water quality parameter (UV absorption spectra) and calculates the concentrations of six other water quality parameters using algorithim determined data stored in its software package. However, the data stored in the InSpectra software package were determined to be inappropriate for BWS potable groundwater sources. As a result, when BWS potable water samples were analyzed by the InSpectra method, the specific measurements of the six water quality parameters were not accurate and these data could not be used to determine specific changes in the quality of water. As a result, the InSpectra method failed to meet the following three stated requirements of a Tier One method: (1) the method must be commercially available and can rapidly (minutes) and reliably measure a water quality parameter, which can be expected to change in response to a contamination event, (2) the ranges of measurement for that water quality parameter must be modest and predictable, and (3) the concentration of the water quality parameter to signal a possible contamination event must be determined.

The InSpectra method is not recommended for use by BWS as a Tier One method to detect a contamination event.

CHAPTER FOUR

EVALUATION OF TIERS TWO AND THREE MONITORING METHODS

I. Assessment of Ruggedized Advanced Pathogen Identification Device (R.A.P.I.D.) as Tier Two Test to Identify Pathogens

A. Application of Method.

There is a need for a commercially available Tier Two test that can be used to reliably confirm and identify pathogens as a class (bacteria, viruses, protozoa) of contaminants in water. Tier Two tests are generally used after a Tier One test provides evidence that a contamination event has occurred. If the contaminant is a biological agent, the PCR method is the most feasible Tier Two technology to confirm and identify pathogens in water samples. For this study, the commercially available R.A.P.I.D., that uses real-time PCR or QPCR technology was selected as the Tier Two method. Reasons for selecting this method were previously summarized in Chapter Two.

B. Training to Use Method.

Initially, BWS sent two of their laboratory personnel (Carl Braun, Dean Tamura) to Salt Lake City to be trained on the use of R.A.P.I.D. by instructors at the Idaho Technology, Inc. facility. This three-day training course took place on November 12–14, 2001. Another three-day training session took place in Honolulu on January 14–16, 2002 by Idaho Technology instructors (Matt Scullion, Halle Millford). Laboratory personnel from BWS (Owen Narikawa, Leslie Inouye, Karl Iwasaki) and from WRRC (Roger Fujioka, Bunnie Yoneyama, Audrey Asahina) took part in this training. On June 21, 2002, Dean Tamura took a refresher course in the procedure to operate the R.A.P.I.D. at the WRRC laboratory.

C. Assessment in the Use of R.A.P.I.D.

Only training in the use of the R.A.P.I.D. method was conducted because no suspected pathogen was recovered from BWS potable water samples. Some of the relevant comments on the use of R.A.P.I.D. are as follows: (1) R.A.P.I.D. is a complex instrument, but it has been designed for ease of use to analyze water samples for the most likely pathogens to be used by terrorists. However, reagents are available for only a limited number of pathogens (anthrax, brucellosis, tularemia, plague, botulism, smallpox, listeriosis, *E. coli* 0157, *Salmonella* species, *Campylobacter* species, *Cryptosporidium* species). (2) R.A.P.I.D. can be used to detect for the presence of most other pathogens. However, people trained in its use have not been trained to assay for other pathogens because reagents in kit form and procedures have not been optimized. (3) One limitation in the use of this PCR method is that it detects both dead (non-infectious) and live (infectious) cells or virus units. Since health risks are related to the presence and concentrations of infectious pathogens and not to that of non-infectious pathogens, there will always be some limitation in interpreting the health risk related to PCR data. For example, the PCR reaction will likely detect pathogens after they have been purposely chlorinated and made non-infectious. (4) Another limitation is deciding

which pathogens to test for and then selecting reagents specific to these pathogens. This creates a problem when one does not know which pathogens have contaminated water samples. In this regard, biological agents likely to be transmitted by water are listed in Table 1.

Currently, QPCR technology is the cutting edge PCR technique used to identity many different pathogens. R.A.P.I.D. is a commercially developed QPCR method designed for laboratories whose personnel have limited training in the use of molecular methods. We recommend that BWS adopt this method as the Tier Two test to identify pathogens in water samples. However, R.A.P.I.D. has not been upgraded and additional reagents to detect many other pathogens have not been produced. As a result, we recommend that BWS consider adopting newer and more sophisticated systems such as the GeneXpert system developed by Cepheid (www.cepheid.com). Since molecular methods will be used more frequently in the future, we recommend that BWS laboratory personnel be provided training in the use of other molecular methods as well. Finally, since reference laboratories are better equipped to assay for various pathogens, BWS should be prepared to request the assistance of the Hawaii State Department of Health Bioterrorism Laboratory in identifying pathogens in water samples

II. Assessment of Enzyme Linked Immunosorbent Assay (ELISA) Method As Tier Two Test to Identify Toxic Chemicals

A. Application of Method.

When Tier One test is positive, the commercially available ELISA method is a Tier Two test that can be used to identify toxic chemicals as a class of contaminant in water. The ELISA method uses antigen-antibody reaction to identify a specific toxic chemical resulting in a standard colorimetric test. Thus, this test is designed for use by laboratories which are not equipped with specialized equipment to assay for complex chemicals. Reagents in kit form are available for many known toxic chemicals. Reasons for selecting the ELISA method are summarized in Chapter Two.

B. Training to Use Method.

The spectrophotometer for the ELISA method was purchased by WRRC for use in earlier projects, so WRRC personnel were already trained in its use. The theory, description of reagents and procedure for the ELISA Method was initially presented by Gary Evereklian of Strategic Diagnostic Inc. during the February 8, 2002 workshop at the State Department of Health Laboratory Auditorium. Laboratory personnel from many laboratories in the state of Hawaii, including those from WRRC, DOH and BWS, attended this workshop. Immediately after this workshop, Evereklian held a hands-on training session on the use of ELISA method at the DOH Environmental Microbiology laboratory for laboratory personnel from WRRC, DOH and BWS.

C. Assessment in the Use of ELISA Method.

Only training in the use of the ELISA method was conducted because toxic chemicals were not recovered from BWS potable water samples. Some of the relevant comments on the use of ELISA technology are as follows: (1) ELISA kits and reagents have been specifically designed as feasible methods for basic water laboratories to analyze water samples for the most likely toxic chemicals to be used by terrorists. (2) A limitation of the ELISA technology is that kits and reagents have not been developed to detect all toxic chemicals. (3) In the use of ELISA technology, one must decide which toxic chemicals to test for and then select specific reagents to identify them. In this regard, toxic chemicals likely to be transmitted by water are listed in Table 1.

ELISA method is the most feasible, commercially developed method to identify many toxic chemicals. It uses an alternative technology to measure for toxic chemicals based on antigen-antibody reaction. The ELISA method is recommended for use by BWS as a Tier Two test and for use by laboratories without specialized equipment and trained personnel to analyze for complex toxic chemicals. However, since BWS has a chemistry laboratory with trained chemists and specialized instruments, we recommend that it take the lead in the identification of toxic chemicals in water samples. Finally, since reference laboratories are better equipped to assay for most of the toxic chemicals, BWS should be prepared to request the assistance of the Hawaii State Department of Health Bioterrorism and Chemistry Laboratories in identifying toxic chemicals in water samples.

III. Evaluation of RiboPrinter Method as a Tier Three Test

A. Application of Method.

The purpose of selecting the RiboPrinter method as a Tier Three test was to characterize the populations of THB colonies from potable water samples as a means of differentiating THB colonies which are naturally present in potable water source from those that originated from an external source of contamination. The reasons for selecting this method were summarized in Chapter Two. The application of this method was to address the hypothesis that during a contamination event, fast-growing colonies of THB and NTC recovered from potable water can be used as markers for the external source of contamination. This hypothesis is based on the expectation that the polluting solution used by terrorists to contaminate water systems will contain bacteria, which are not normally found in potable water and they will grow as fast-growing colonies in the standard THB assay.

A recognized limitation of this experimental approach is that ambient populations of THB in potable water sources have not been identified or characterized. As a result, they must first be characterized so they can be easily differentiated from bacterial populations that originate from external contaminating sources. Thus, the focus of the current study was to use the RiboPrinter method to characterize the ambient populations of THB isolates recovered from three major sources of potable water. The expectation was that most of the THB isolates from BWS potable water sources will not be identified by the RiboPrinter method but will be

characterized by their placement into specific ribogroups. In this regard, if two THB isolates cannot be identified but are placed into the same ribogroup, they will be assumed to belong to the same species. If 10% of the THB isolates are placed into the same ribogroup, they will represent a predominating species of bacteria for that source of water. Thus, based on the distribution of THB isolates into ribogroups, one can potentially characterize the populations of THB for that source of water. In practical terms, the usefulness of characterizing the THB populations is to determine what kinds of bacteria (ribogroups) are normally present in potable water sources and what kinds of bacteria (ribogroups) are not. During a potential contamination event, isolates of THB characterized into ribogroups that differ from those of ambient populations should be suspected as originating from the polluting solution used by terrorists.

B. Training to Use the RiboPrinter Method.

The RiboPrinter system was installed at Snyder 303 on the University of Hawaii Manoa campus and made operational on July 18, 2002. The initial four-day (August 5–8, 2002) training session to operate the instrument and to interpret the results was conducted at the University of Hawaii by Elizabeth Mangiaterra of DuPont Qualicon. This training session included lectures and hands-on operation of the RiboPrinter. Personnel from WRRC (Roger Fujioka, Bunnie Yoneyama, Zerong You, Audrey. Asahina, Adrian. Sentell, Gayatri Vithanage, Dayna. Sato) and from BWS (Owen Narikawa, Ron. Saito, Karl Iwasaki, Dean Tamura) were trained. The second training session, which included advanced data analysis, was held on October 27–28, 2003 and conducted by Elizabeth Mangiaterra. Personnel from WRRC (Audrey Asahina, Gayatri Vithanage, Dayna Sato, Roger Fujioka) and BWS (Owen Narikawa, Dean Tamura, Karl Iwasaki) were trained. During this advanced training session, Mangiaterra reviewed all the riboprint and ribogroup data generated by this project and concluded that most of the isolates from the BWS drinking water belonged to different ribogroups.

C. Results of Analyzing Well Water Samples.

One hundred forty THB isolates from 45 different well sites were analyzed by the RiboPrinter method. The results show that only 35 of 140, or 25%, of the THB isolates had riboprints that matched the DuPont data base and could be identified (Table 11). The results show that the identified THB isolates were comprised of the following 21 different bacterial species (Table 12): *Acinetobacter lwoffii*, *Bacillus megaterium*, *B. pumilus*, *B. thuringiensis*, *Delftia acidovorans*, *Escherichia coli*, *Flavobacterium* species, *Glaciecola pallidula*, *Lactobacillus pontis*, *Legionella pneumophila*, *L. pneumophila* ss *fraseri*, *Ochrobactrum anthropi*, *Pseudomonas aeruginosa*, *Sphingomonas paucimobilis*, *Staphylococcus hominis*, *S. pasteuri*, *S. warneri*, *S. xylosus*, *Stenotrophomonas maltophilia*, *Vibrio vulnificus*, and *Weisella halotolerans*. Of the 35 identified THB isolates, 21 were individual species that differed from all other THB isolates (Table 12). *D. acidovorans* and *P. aeruginosa* were the most commonly identified THB isolates in well water, with five each (Table 12). Since these 35 identified THB isolates represent only 25% of the total isolates and were comprised of 21 different species, most of them differed from each other. It should be noted that some of the

identified bacteria (*Legionella pneumophila*, *Legionella* spp., *Vibrio vulnificus*) are probably incorrect because they grow only on special types of media and will not grow on THB growth medium.These results indicate that the RiboPrinter method may not always be reliable in identifying bacteria recovered from potable well water sources.

The results show that 105 of 140 or 75%, of the THB isolates in well water samples could not be identified and that 78 of 140 or 55.7% of unidentified THB isolates had riboprints which did not match up with that of any other isolate (Table 11). Thus, these riboprints were not grouped with any other riboprint but formed distinct ribogroups containing only one riboprint each. The results indicate that 55.7% of the THB isolates recovered from well water differed from all the other THB isolates and most likely are different species of bacteria. The results also show that only 27 of 140 or 19%, of the unidentified THB isolates had riboprints that were similar to those of other THB isolates and therefore were grouped, i.e. they were placed into individual ribogroups comprised of members of the same species of bacteria (Table 11). The 27 unidentified but grouped THB isolates were placed into 11 different ribogroups, with most (8 of 11) ribogroups containing only two THB isolates (Table 11). Thus, most of these 27 unidentified but grouped THB isolates differed from each other. Taken together, the results show that the populations of THB in well water samples comprised numerous different ribogroups and each ribogroup comprised a population of bacteria which represented a minor fraction of all the THB isolates. As a result, the RiboPrinter method was not able to characterize the community of THB in well water samples into a predictable distribution of ribogroups.

D. Results of Analyzing Reservoir Water Samples.

Eighty six THB isolates from 26 different reservoir tanks were analyzed by the RiboPrinter method. The results show that only 25 of 86, or 29.1%, of the THB isolates had riboprints that matched the DuPont data base and could be identified (Table 11). The following 15 different bacterial species were identified: *Acinetobacter lwoffii*, *Bacillus cereus*, *B. sphaericus*, *B. thuringiensis*, *Lactococcus lactis*, *Legionella moravica*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *P. alcaligenes*, *P. putida*, *Staphylococcus epidermidis*, *S. pasteuri*, *S. warneri*, *Stenotrophomonas maltophilia*, *Vibrio cholerae*. Of the 25 identified THB isolates, 8 were individual species that differed from all other THB isolates (Table 12). *B. thuringiensis* was the most common THB isolate in reservoir water, with four identified (Table 12). Since these 25 identified THB isolates represent only 29.1% of the total isolates and were comprised of 15 different species, most of them differed from each other. It should be noted that some of the identified bacteria (*Legionella moravica*, *Vibrio cholerae*) are incorrectly identified because they grow only on special growth media and will not grow on THB growth medium. These results indicate that the RiboPrinter method may not always be reliable in identifying bacteria recovered from potable reservoir water sources.

The results show that 61 of 86, or 70.9%, of the THB isolates recovered from reservoir water samples could not be identified and that 45 of 86, or 52.3%, of these had riboprints which did not match up with that of any other isolate (Table 11). Thus, these riboprints were not

grouped with any other riboprint but formed distinct ribogroups containing only one riboprint each. The results indicate that that 52.3% of the THB isolates recovered from reservoir tanks differed from all the other THB isolates and most likely are different species of bacteria. The results also show that only 16 of 86 (18.6%) of the unidentified THB isolates had riboprints that were similar to those of other THB isolates and therefore were grouped, i.e., they were placed into individual ribogroups comprised of the same species of bacteria (Table 11). The 16 unidentified but grouped THB isolates were placed into 7 different ribogroups, with most (5 of 7) ribogroups containing only two THB isolates (Table 11). Thus, most of these 16 unidentified but grouped THB isolates differed from each other. Taken together, the results show that the populations of THB in reservoir water samples comprised numerous different ribogroups and each ribogroup comprised a population of bacteria which represented a minor fraction of all the THB isolates. As a result, the RiboPrinter method was not able to characterize the community of THB in reservoir water samples into a predictable distribution of ribogroups.

E. Results of Analyzing Distribution Pipe Water Samples.

A total of 331 THB isolates from 90 distribution sites were analyzed by the RiboPrinter method. The results show that only 45 of 331, or 13.6%, of the THB isolates had riboprints that matched the DuPont data base and could be identified (Table 11). The following 23 different bacterial species were identified: *Acinetobacter baumannii*, *Aerococcus viridans*, *Bacillus cereus*, *B. fusiformis*, *B. megaterium*, *B. thuringiensis*, *Delftia acidovorans*, *Enterobacter cloacae*, *Flavobacterium* sp., *Galciecola pallidula*, *Legionella pneumophila*, *Pseudomonas alcaligenes*, *P. putida*, *Ralstonia pickettii*, *Salinivibrio coasticola* ss *coasticola*, *Sphingomonas aromaticivorans*, *Staphylococcus haemoyticus*, *S. pasteuri*, *S. warneri*, *Stenotrophomonas maltophila*, *Terracoccus luteus*, *Vibrio cholerae*, and *Vibrio* species. Of the 45 identified THB isolates, 23 were individual species that differed from all other THB isolates (Table 12). *B. cereus* was the most common THB isolate in distribution water samples, with ten identified (Table 12). Since these 45 THB isolates represented only 13.6% of the total isolates and were identified as 23 different species, most of them differed from each other. It should be noted that some of the identified bacteria (*Legionella pneumophila*, *Vibrio* spp.) are incorrelty identified because they grow only on special growth media and will not grow on THB growth medium. These results indicate that the RiboPrinter identification method may not always be reliable in identifying bacteria recovered from potable reservoir water sources.

The results show that 286 of 331,or 86.4%, of the THB isolates recovered from distribution water samples could not be identified and that 188 of 331, or 56.8%, of these had riboprints which did not match up with that of any other isolate (Table 11). Thus, these riboprints were not grouped with any other riboprint but formed ribogroups containing only one riboprint each. The results indicate that 56.8% of the THB isolates recovered from distribution water pipes differed from all the other THB isolates and most likely are different species of bacteria. The results also show that only 98 of 331, or 29.6%, of the unidentified THB isolates had riboprints that were similar to those other THB isolates and therefore were

grouped, i.e., they were placed into individual ribogroups, comprised of members of the same species of bacteria (Table 11). The 98 unidentified but grouped THB isolates were placed into 27 different ribogroups, with most (15 of 27) ribogroups containing only two THB isolates. Thus, most of these 98 unidentified but grouped THB isolates differed from each other. Taken together, the results show that the populations of THB in distribution water samples comprised numerous different ribogroups and each ribogroup comprised a population of bacteria which represented a minor fraction of all the THB isolates. As a result, the RiboPrinter method was not able to characterize the community of THB in distribution water samples into a predictable distribution of ribogroups.

F. Results of Analyzing Water Samples From Tunnel, Shaft and Granular Activated Carbon Treatment Sites.

Tunnels and shafts represent sources of shallow groundwater withdrawn for potable use by BWS. Tanks containing granular activated carbon (GAC) are used to remove pesticides from some groundwater sources. Since environmental conditions at these three sites differ from well water sites, the populations of bacteria in water from these sites can be expected to differ. As a result, THB isolates were recovered from the three sources and then analyzed by the RiboPrinter method. A total of 27 samples from four tunnel sites were analyzed, and only 4 of 27 (14.8%) had riboprints that matched the DuPont data base. The bacterial species identified were *Bacillus cereus*, *B. thuringiensis*, *Chryseobacterium meningospeticum*, and *Idiomarina zobelii*. The remaining 23 of 27 (85.2%) of the THB isolates could not be identified. Of these, only 5 had riboprints which matched the riboprint of other THB isolates) and were placed into two common ribogroups (Table 11). These results indicate that most of the THB isolates from tunnel water were comprised of different species of bacteria.

A total of 33 water samples from four shaft sites were analyzed, and only 4 of 33 (12%) of the THB isolates had riboprints that matched the DuPont data base. The bacterial species identified were *Bacillus pulmilus*, *Lactococcus lactis*, *Pseudomonas aeruginosa*, and *Pseudomonas putida.* The remaining 29 of 33 (87.9%) of the THB isolates could not be identified. Of those, 10 had riboprints which matched the riboprint of other THB isolates and were placed into four ribogroups (Table 11). Most (3 of 4) of these ribogroups contained two isolates each. These results indicate that most of the THB isolates from shaft water were comprised of different species of bacteria.

A total of 13 water samples from two GAC sites, were analyzed, and 12 of 13, or 92.3%, of the isolates could not be identified and were placed in separate ribogroups (Table 11). Only 1 of 13, or 7.7%, of the THB isolates had riboprint that matched the DuPont data base. The bacterial species identified was *Glaciecola pallidula.* These results indicate that most of the THB isolates from GAC water samples were comprised of different species of bacteria.

In summary, based on the limited numbers of samples analyzed, high percentages (85.2 to 92.0%) of the THB isolates recovered from tunnel, shaft and GAC sites could not be identified. Moreover, most of the unidentified isolates were distributed into different

ribogroups, with each ribogroup containing a population of bacteria that represented a minor fraction of all the THB isolates. As a result, the RiboPrinter method was not able to characterize the community of THB in these sources of water into some predictable distribution of ribogroups.

G. Results of Analyzing Non-Target Colonies From mEndo Medium.

Non-target colonies (NTC) are occasionally observed on mEndo medium. They are formed by bacteria that can grow on mEndo medium, but they themselves do not grow into typical target colonies. The results show that when low numbers of NTC were assayed, none could be identified from wells (3/3), reservoir (2/2) and GAC (1/1) sources. When much higher numbers were assayed in distribution water samples, the results show that 72 of 105 (69%) of the THB isolates recovered could not be identified but that 33 of 105 (31%) of the NTC isolates could be identified (Table 13). The following11 bacterial species were identified (Table 14): *Acinetobacter baumannii*, *Aeromonas hydrophila*, *Bacillus megaterium*, *B. thuringiensis*, *Enterobacter cloacae*, *Escherichia coli*, *Flavobacterium* sp., *Pseudomonas aeruginosa*, *P. pseudoalcaligenes*, *Staphylococcus haemolyticus*, and *Stenotrophomonas maltophilia. Pseudomonas aeruginosa* was the most common species of bacteria that forms NTC on mEndo agar, with twelve identified.

H. Assessment and Recommendation of Riboprinter Method as Tier Three Test.

The RiboPrinter method was used to characterize THB isolates recovered from various BWS potable water sources, including 140 THB isolates from 45 well water sites, 86 from 26 reservoir water samples, 331 THB isolates from 90 distribution water sites, 27 from 4 tunnel water sites, 33 from 4 shaft water sites and 13 from 2 GAC sites. Thus, a total of 630 THB isolates were analyzed by the RiboPrinter method. In addition, 111 non-target bacteria recovered on mEndo agar medium were analyzed by the RiboPrinter method. Based on analyzing these water samples, the following assessments were made regarding the use of the RiboPrinter method.

The first assessment is that the processing procedure using the RiboPrinter method had to be modified to successfully analyze THB isolates from potable water. When the standard procedure to process THB isolates was followed, the quality of the riboprints for many isolates did not align properly or was too poor to be read. It was clear that the standard procedure recommended for the RiboPrinter method was optimized for fast growing bacteria and not for slow growing bacteria such as the THB from potable sources. Based on recommendations by DuPont Qualicon technical services, this problem was generally solved by increasing the incubation time for growth of THB isolates so that more cells could be analyzed.

The second assessment is that the successful and efficient use of the RiboPrinter method requires reliable services from DuPont Qualicon because the interpretation of the data is dependent on following their approved procedures and using their certified reagents. In this regard, the frequency with which some of the reagents did not function properly was much

higher than expected and was disruptive to our work schedule. Sometimes, this was due to poor shipping conditions but at other times it was related to poor quality control before the reagents were shipped. Although the company replaced these reagents at no additional cost, many working hours were wasted.

The third assessment is that this method was only able to identify a small fraction (13 to 29%) of the THB isolates recovered from potable water sources and there was some question on the reliability of this identification system. The identification of the THB isolates is based on matching the riboprint produced with those in the DuPont data base. Several of the THB isolated from potable water sources were identified as *Legionella* spp. or *Vibrio* spp. and these identifications are not likely to be correct because these bacteria require special types of growth media and are not expected to grow as THB colonies. Moreover, *Vibrio* species are marine bacteria and are not expected to be found in fresh groundwater sources. These results indicate that the DuPont data base used by the RiboPrinter may not be entirely reliable for THB isolates and casts some doubt on the reliability of the other identified THB isolates.

The fourth assessment is that this method successfully characterized each THB isolate into their genetic ribogroups but the expected distribution of ribogroups in potable water sources could not be determined because most of the ribogroups differed from those of other THB isolates. Since most THB isolates represented a minor fraction of all THB isolates, it was not possible to predict the range of ribogroups expected in BWS potable water sources. The significance of this finding is that since the range of ribogroups that characterizes ambient populations of THB could not be determined, it will be difficult to to determine if the THB originated from water or originated from an external source of contamination. It should be noted that this study did not characterize fast-growing colonies of THB, which we hypothesized would characterize bacteria from an external source of contamination.

In summary, the question to be considered is whether the RiboPrinter method should be used as a Tier Three test to detect external contamination of bacteria in potable water sources. The results of this study did not directly test our hypothesis that contamination of potable water could be detected by characterizing the fast-growing colonies of THB because these kinds of THB were not available to be tested. Based on previous reports (Allerberger and Fritschel, 1999, Fritschel, 2001) the RiboPrinter method can be expected to identify fast-growing bacteria from water and food sources as pathogens or as species of bacteria from environmental sources. However, to be successfully used as a Tier Three test, the RiboPrinter method should also be able to characterize the THB recovered from potable water under ambient or non-contaminating conditions. This specific objective was the focus of the present study. However, the results of the present study showed that ambient THB isolates were characterized into numerous different ribogroups, such that a predictable distribution of ribogroups in potable water sources could not be determined. Thus, based on ribogroup characterization of THB recovered from potable water, it would be difficult to determine if the THB originated from water or from an external source of contamination. In addition, the cost of operating the RiboPrinter is high and the cost of the reagents has increased. Finally,

some serious limitations in the use of the RiboPrinter method to analyze THB colonies from potable water sources were documented. As a result, we do not recommend that this method be adopted by BWS as a Tier Three method to characterize the THB isolates from potable water.

I. Assessment of the Limitation of the RiboPrinter Method.

Two possible explanations were given for the failure of the RiboPrinter method to achieve its goal of characterizing the THB isolates from potable water into some expected distribution of ribogroups. The first possible reason is that there are so many different species of unidentified THB in potable water sources that is unlikely that the same species of bacteria will be recovered as a THB isolate, even from the same source of water. The second and more likely reason is that the RiboPrinter method uses a 92% similarity index to determine whether unidentified THB isolates should be placed into a common ribogroup and presumably represent the same species of bacteria. However, using such a high similarity index, many of the same species of unidentified bacteria can be expected to be separated into different ribogroups such that they will be considered to belong to different species of bacteria. This conclusion is based on the observation that bacteria identified as the same species of bacteria in the DuPont data base are often within an 85% similarity index and belong to different ribogroups. This inherent problem of the RiboPrinter method can explain why the data obtained failed to meet our working hypothesis that the RiboPrinter method should characterize each ambient THB isolate into its genetic ribogroup and that this should lead to some predictable distribution ribogroups in potable water sources. An expectation of our working hypothesis was that some species of THB isolates would comprise a predominating population (>10%) of bacteria characteristic of that source of water. The data generated by the RiboPrinter method indicated that this working hypothesis could not be met because the THB isolates from the BWS potable water sources were placed into so many different ribogroups that essentially none of the ribogroups comprised 10% of the THB isolates, and thus none could be characterized as belonging to a predominating group or species of bacteria. In this regard, some of the unidentified bacteria placed into different ribogroups probably belong to the same species of bacteria, even though it was not possible to determine which ones. Due to this condition, the data could not be used to demonstrate that some species of THB isolates actually represent a predominating group. In the absence of predominating ribogroups, the predictable populations of THB in potable water sources could not be determined. As stated earlier, some predictable distributions of ambient THB isolates based on their ribogroups are needed to characterize THB naturally present in water. Without being able to characterize the ribogroups naturally present in water, it would be difficult to recognize the presence of THB isolates which are different and which may have originated from some external source, such as during a contamination event.

IV. Reassessment of the RiboPrinter Data Using GelCompar Method

A. Selection and Application of Method.

The use of a stringent 92% similarity index in the grouping of unidentified riboprints was cited as the most likely reason why the RiboPrinter method failed to characterize the populations of THB isolates from potable water sources into some predictable distribution of ribogroups. If the similarity index could be changed to 85%, many of the same species of unidentified bacteria could be placed into common ribogroups. However, DuPont Qualicon scientists informed us that the RiboPrinter software does not allow us to change the similarity index from 92% to a lower index such as 85%. It was recommended that we apply the bionumeric software called GelCompar (Applied Maths, Austin, TX) to re-analyze the riboprints of the THB isolates generated by the RiboPrinter method. The usefulness of the GelCompar method is that that it allows the user to select the similarity index, such that more similar riboprint patterns can be grouped into common clusters. In this regard, the clusters will represent the grouping of similar or presumably the same species of THB isolates. For this study, all of the riboprints generated by the RiboPrinter method were re-analyzed using the GelCompar method to group riboprints at an 80% similarity index.

B. Clustering the Riboprints from Well Water Samples.

The riboprints of the 140 THB isolates from well water samples were re-analyzed using the GelCompar method. The results show that 39 of 140, or 27.8%, of the THB isolates had riboprints that differed by more than 80% similarity with all other THB isolates (Table 15). These 39 THB isolates were categorized as unclustered because they could not be grouped with any other THB isolate. Thus, they represent species of bacteria that differ from all other THB isolates. The results also show that 101 of 140, or 72.1%, of the THB isolates were grouped into 27 clusters (Table 15). To determine if the 27 clusters were comprised of predominating groups of bacteria, the number of THB isolates distributed in each cluster was determined (Table 16). In this regard, a predominating group of bacteria should be comprised of at least 10% of the total population, or 14 of the140 THB isolates recovered in a cluster. The 11 clusters with 2 THB isolates were the most frequently observed, and each of these clusters represented a minor fraction (2/140) of all THB isolates. The maximum number of THB isolates per cluster was 10, and this occurred in only 1 cluster. The 10 isolates in this cluster comprised less than 10% of the total isolates, so they are not considered a predominating group. In conclusion, although the GelCompar method was able to place more of the THB isolates into clusters than the RiboPrinter method, there were still too many clusters and each cluster contained a minor fraction of the total THB isolates. As a result, re-analysis of the RiboPrinter data using the GelCompar method failed to characterize the community of THB in well water samples into a predictable distribution of clusters.

C. Clustering the Riboprints from Reservoir Water Samples.

The riboprints of the 86 THB isolates from reservoir tank samples were re-analyzed by using the GelCompar method. The results show that 34 of 86, or 39.5%, of the THB isolates had riboprints that differed by more than 80% similarity with all other THB isolates (Table 15).

These 34 THB isolates were categorized as unclustered because they could not be grouped with any other THB isolate. Thus, they represent species of bacteria that differ from all other THB isolates. The results also show that 52 of 86, or 60.5%, of the THB isolates were grouped into 12 clusters (Table 15). To determine if the 12 clusters were comprised of predominating groups of bacteria, the number of THB isolates distributed in each cluster was determined (Table 16). In this regard, a predominating group of bacteria should be comprised of at least 10% of the total population or 9 of the 86 THB isolates recovered in a cluster. The 5 clusters with 3 THB isolates were the most frequently observed, and each of these clusters represented a minor fraction (3/140) of all THB isolates. The maximum number of THB isolates per cluster was 12, and this occurred in only 1 cluster. The 12 isolates in this cluster comprised 14% of the total isolates, and so this cluster was considered a predominating population in the reservoir water samples. In conclusion, the GelCompar method was able to place more of the THB isolates into clusters than the RiboPrinter method and one cluster formed a predominating population. Despite this, 74 of 86, or 86%, of the THB isolates from this source of water represented minor populations. This indicates that the reliable characterization of a population of bacteria requires more than one predominating group. As a result, re-analysis of the RiboPrinter data using the GelCompar method failed to characterize the community of THB in reservoir water samples into a predictable distribution of clusters.

D. Clustering the Riboprints from Distribution Water Samples.

The riboprints of the 331 THB isolates from distribution water samples were re-analyzed using the GelCompar method. The results show that 85 of 331or 25.7%, of the THB isolates had riboprints that differed by more that 80% similarity with all other THB isolates (Table 15). These 85 THB isolates were categorized as unclustered because they could not be grouped with any other THB isolate. Thus, they represent species of bacteria that differ from all other THB isolates. The results also show that 246 of 331or 74.3.1%, of the THB isolates were grouped into 80 clusters (Table 15). To determine if the 80 clusters were comprised of predominating groups of bacteria, the number of THB isolates distributed in each cluster was determined (Table 16). In this regard, a predominating group of bacteria should be comprised of at least 10% of the total population, or 33 of the 331 THB isolates recovered in a cluster. The 42 clusters with 2 THB isolates were the most frequently observed cluster, and each of these clusters represented a minor fraction (2/331) of all THB isolates. The maximum number of THB isolates per cluster was 14, and this occurred in only 1 cluster. The 14 isolates in this cluster comprised less than 10% of the total isolates, so they are not considered a predominating group. In conclusion, although the GelCompar method was able to place more of the THB isolates into clusters than the RiboPrinter method, there were still too many clusters and each cluster contained a minor fraction of the total THB isolates. As a result, re-analysis of the RiboPrinter data using the GelCompar method failed to characterize the community of THB in distribution water samples into a predictable distribution of clusters.

E. Assessment of Data.

For this phase of the study, all of the riboprints generated of THB isolates using the RiboPrinter method were re-analyzed using the GelCompar method to cluster riboprints at an

80% similarity index in an attempt to group the closely related unidentified riboprints. The objective was to determine if the resulting clustering pattern would allow some of the THB isolates to form predominating groups. The working hypothesis for this objective was that the population of THB in a potable water source can be predictably characterized if some of the clusters contain at least 10% of the total number of THB tested. The GelCompar method was successful in grouping more of the THB riboprints into clusters than the RiboPrinter method. However, none of the clusters for well and distribution water samples formed predominating groups, and only one cluster for the reservoir water samples was characterized as containing more than 10% of the total THB isolates. Thus, even at an 80% similarity index, the GelCompar method formed too many clusters that with one exception contained a minor fraction of the total THB isolates.

In summary, the GelCompar Method was not able to characterize the populations of unidentified THB in the three major sources of potable water into some predictable distribution of clusters. These results support the alternative theory that the potable water sources are comprised of so many different species of bacteria that none of the ambient THB populations forms a predominating population.

CHAPTER FIVE

PROJECT ASSESSMENT AND RECOMMENDATIONS

I. Assessment of the EWMP

In the final assessment of this project, the most important question is whether the EWMP is feasible, reliable and effective. In this regard, the primary goals for this study were to develop an EWMP and to evaluate the effectiveness of this plan to rapidly and reliably detect intentional contamination of the BWS water system by hazardous chemicals and pathogens. To address these goals a three-tiered EWMP was devised and commercially available methods were used to analyze water samples. The proposed EWMP was only partially successful because some of the methods failed to provide reliable data needed to meet the objectives of this plan. For example, only the Microtox method was approved as a Tier One method to screen for toxic chemicals. Thus, the EWMP still needs a Tier One test method to rapidly detect changes related to contamination with biological agents. The R.A.P.I.D. method was approved as a Tier Two method to identify pathogens in potable water samples. The ELISA method was approved as a Tier Two method to identify toxic chemicals in potable water samples. The RiboPrinter method was not approved as the Tier Three method to characterize the THB isolates recovered from potable water and to identify those THB isolates which originated from an external source such as a contamination event.

Two problems were recognized in the implementation of the EWMP. The first problem is that we selected commercially available tests that had not been developed specifically to analyze potable groundwater. These methods did not perform as well as expected. The second problem was the apparent complexity of the biological composition of potable water. We assumed that because groundwater has low concentrations of THB, the composition of total microorganisms in potable water would also be relatively low in numbers and diversity. This apparently is not the case as the diverse populations of microorganisms in potable water was the cause for the failure of the Tier One test to measure for ATP and the Tier Three test to characterize the colonies of THB recovered from potable water. In retrospect, it may not be possible to develop a reliable EWMP based on using commercially available methods. Evidence for this conclusion is based on the observation that in December of 2001 a published plan describing an emergency water monitoring plan for water utilities was not available. As a result, we developed our EWMP without reference to other similar plans. Moreover, in December 2005, the publication of an effective EWMP is still not available.

II. Need to Improve the EWMP

Although the proposed EWMP was shown to be only partially effective, we believe the premise and experimental design for this plan is valid. Therefore, the EWMP should be accepted as an interim plan that needs to be improved and expanded to use other types of measurements. The experimental design of the EWMP was based on detecting a component

of the terrorist polluting solution in potable water samples and to use this measurement as a marker of the contaminating source. This kind of monitoring data can be used to identify the sites in water system which are contaminated and sites which are not contaminated. Although our testing methods were not successful, this experimental approach is still valid. In this regard, there are many other chemical, physical and biological constituents in the terrorist polluting solution and detection of any of these components in potable water can be used as markers for that source of contamination. The challenge is to find a component in that polluting solution and a method that can reliably detect its presence in potable water. This kind of challenge can only be met by a research project specifically designed to select a suitable monitoring method to detect a component of the external contaminating solution. In this regard, detecting bacterial populations in the polluting solution is still a valid approach and use of molecular methods may be the best technology. A promising example of this approach is to apply DNA microarray technology (Lemarchand et al., 2004) to rapidly detect contamination of pathogens and other microorganisms in potable water. The promise of this technology is that it can simultaneously detect hundreds of different kinds of pathogens, other microorganisms, as well as their metabolic products in one test. Thus, this kind of technology has the potential of characterizing potable water sources and then determining when that source of water is contaminated by external sources of microorganisms. Currently, the limitation of DNA microarray technology is that this molecular method can only detect high concentrations of microorganisms and cannot detect health-related concentrations of pathogens in potable water. However, sample concentration and amplification methods are being evaluated to overcome these limitations. Based on the need to rapidly test water for numerous types of microorganisms and pathogens, the future promise is in the application molecular methods.

III. Recommendations to Develop a Reliable EWMP

Other water utilities are faced with the same problem as BWS in the development of a reliable EWMP. Agencies and water utilities that are actively involved in developing an EWMP are as follows: (1) EPA, (2) CDC, (3) AWWA, (4) Pittsburgh Water and Sewer Authority, (5) East Bay Municipal Utility District, (6) Metropolitan District of Southern California, and (7) San Francisco Public Utilities Commission. We recommend that BWS communicate with these agencies in the development of an effective EWMP. Currently, the most practical recommendation for BWS is to investigate the usefulness of the Hach Event Monitor Trigger System as an automated, on-line system to detect contamination of potable water systems. This method was developed by Hach Company for the specific purpose of developing a method to detect contamination of potable water. This test measures a combination of five water quality parameters (chlorine, turbidity, conductivity, pH, total organic carbon). Each of these water quality parameters by itself does not provide specific data for a contamination event but together the measurements are used in what is described as an "intelligent algorithim" to determine when a contamination event may have occurred as well as to identify the possible type of contaminant. Use of algorithm to predict a condition is now used as a means of obtaining water quality data quickly to signal a possible contamination event.

However, there is danger in the use of algorithm-based data because they are collected under one set of conditions and may not be applicable when applied to water under a different set of conditions. This was clearly the reason for the failure of the InSpectra method, which we included for use in our EWMP. To address the problem of site specificity, Hach Company recommends that their system be initially installed at the site where it will be used for several months to determine the background concentrations of the five water quality parameters. The background concentrations for the five water quality parameter will then be used to establish an action level for that source of water. That action level is the trigger point to signal a possible contamination event.

The development of this new method by Hach company points out the way in which commercial companies are developing tests specifically for an EWMP. This approach is superior to the application of commercially available methods that were designed to be applied to many situations and do not perform well enough to reliably analyze potable water. Since the Hach Event Monitor Trigger System is available and is being evaluated, we recommend that BWS contact a Hach representative such as Dan Kroll (Chief Scientist for Threat Agent Chemistry, 800-604-3493) to obtain the latest evaluative reports regarding their new method. We also recommend that BWS contact an EPA representative such as Matthew Magnuson (National Homeland Security Research Center, 513-569-7321) to get an update on EPA's plan to develop and evaluate an EWMP at one water utility in the United States sometime in 2006.

IV. The Need for Continuous Training

Implementation of an EWMP must be recognized as a difficult task. The key to a successful EWMP is advanced planning, designating those with key responsibilities and then providing them with continuous training. As laboratory supervisors, the chief microbiologist and chief chemist must work together and be responsible for the water monitoring aspects of the EWMP. During an actual contamination event, when people are becoming ill and there is panic in the community, these supervisors will be asked many difficult questions relating to the results of the tests, other available tests and comments made by other scientists throughout the country. These laboratory supervisors must be adequately trained to answer these questions. In this regard, the training of these laboratory supervisors should not be limited to operating a specific instrument used to detect hazardous chemical or biological agent. Instead, a plan for continuous training for the laboratory supervisors and their staff on the theory and application of the methods used to monitor for hazardous chemical and biological agents should be implemented. Additional training should be focused on use of molecular methods because these methods can be expected to be used more extensively in the future and these methods can be expected to change rapidly. Other areas of training should include public health consequences of contamination at water utilities and problems related to public communication during these events. Finally, laboratory supervisors should be encouraged to establish professional relationships with other scientists and laboratory

supervisors throughout the country. These contacts can serve as resources to provide answers and recommendations during periods of crisis.

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FIGURES

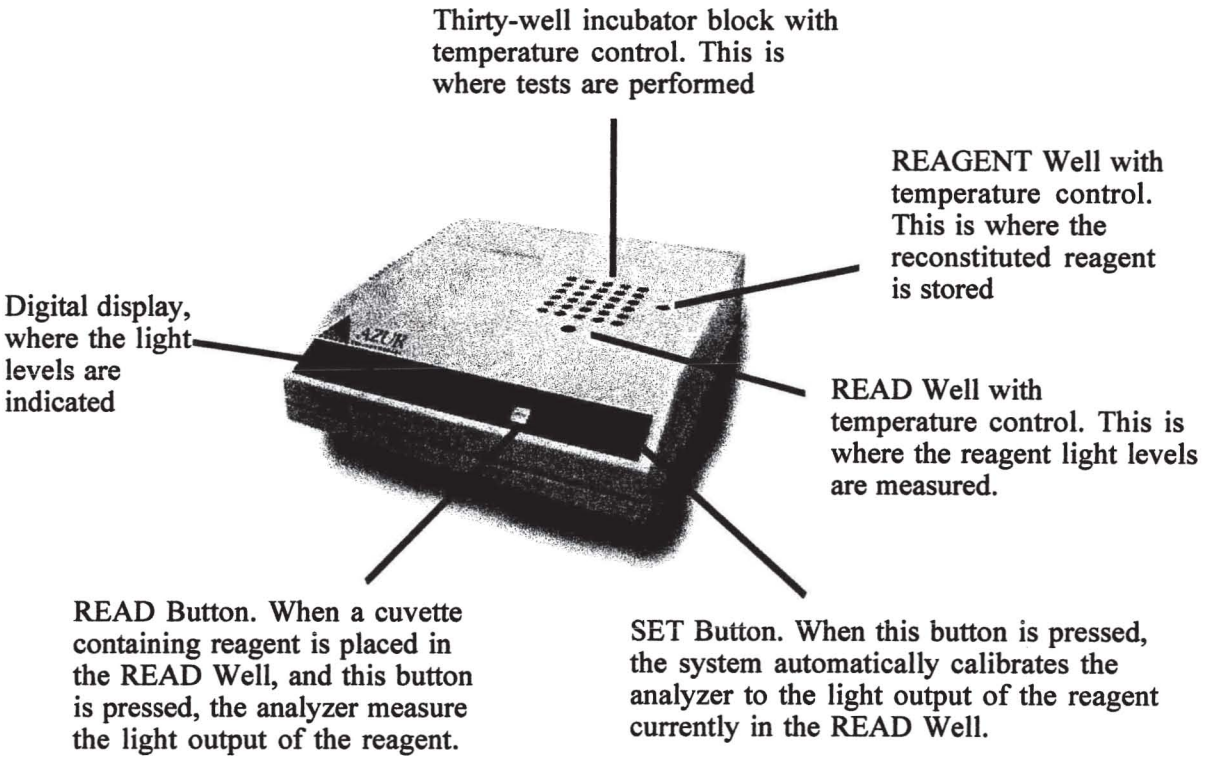


Figure 1. Microtox instrument

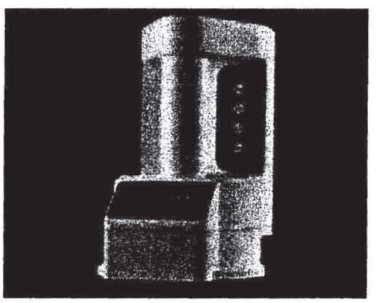


Figure 2. Pallchek instrument

Figure 3. New Horizon instrument – Profile 1

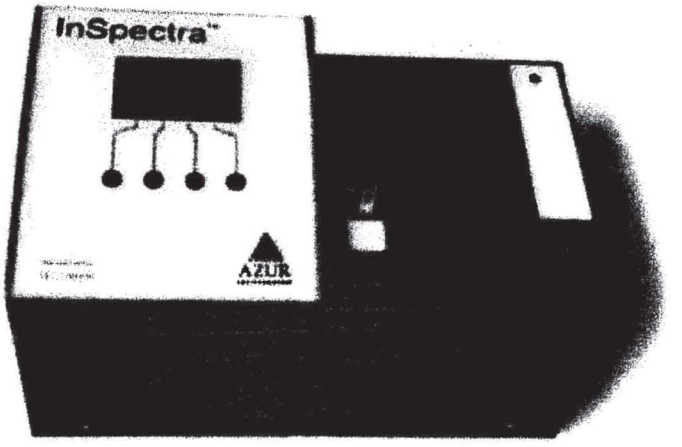


Figure 4. InSpectra instrument

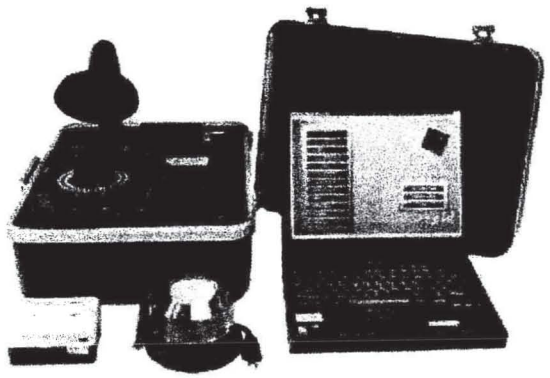


Figure 5. R.A.P.I.D. instrument

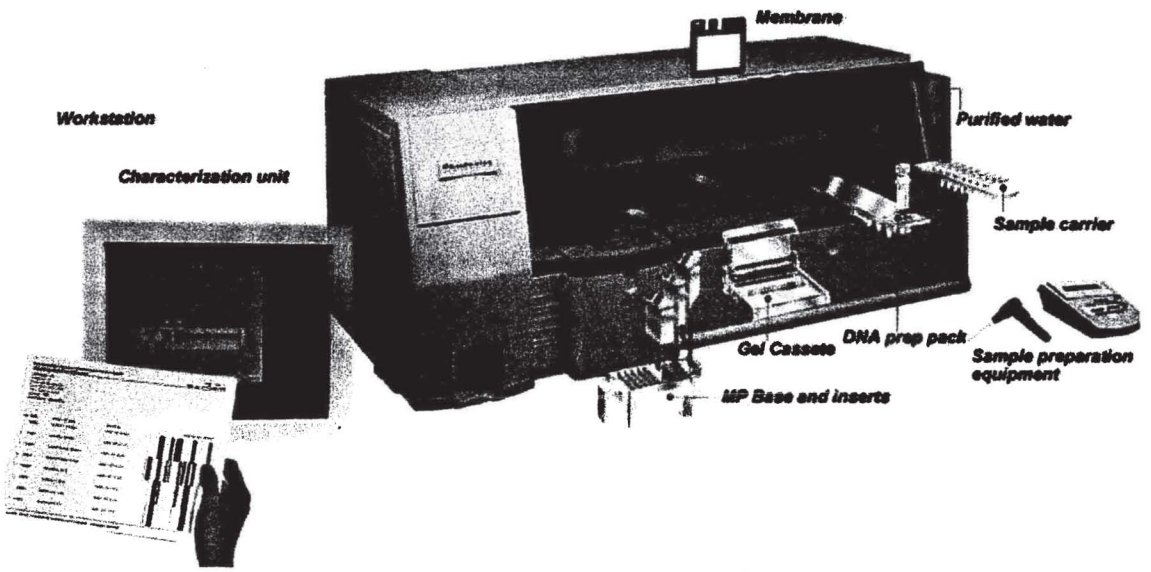


Figure 6. RiboPrinter instrument

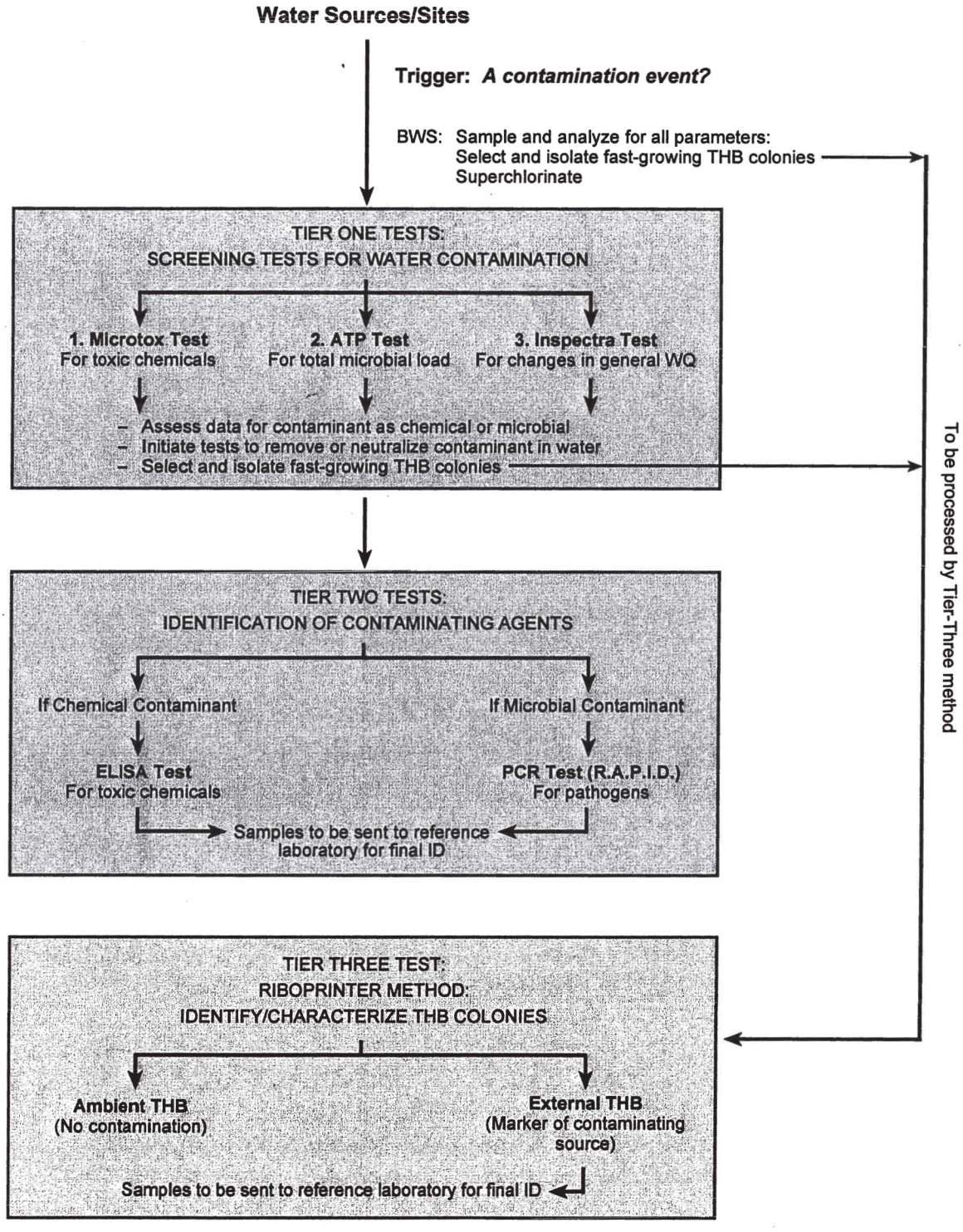


Figure 7. Diagram of three-tiered emergency water monitoring plan

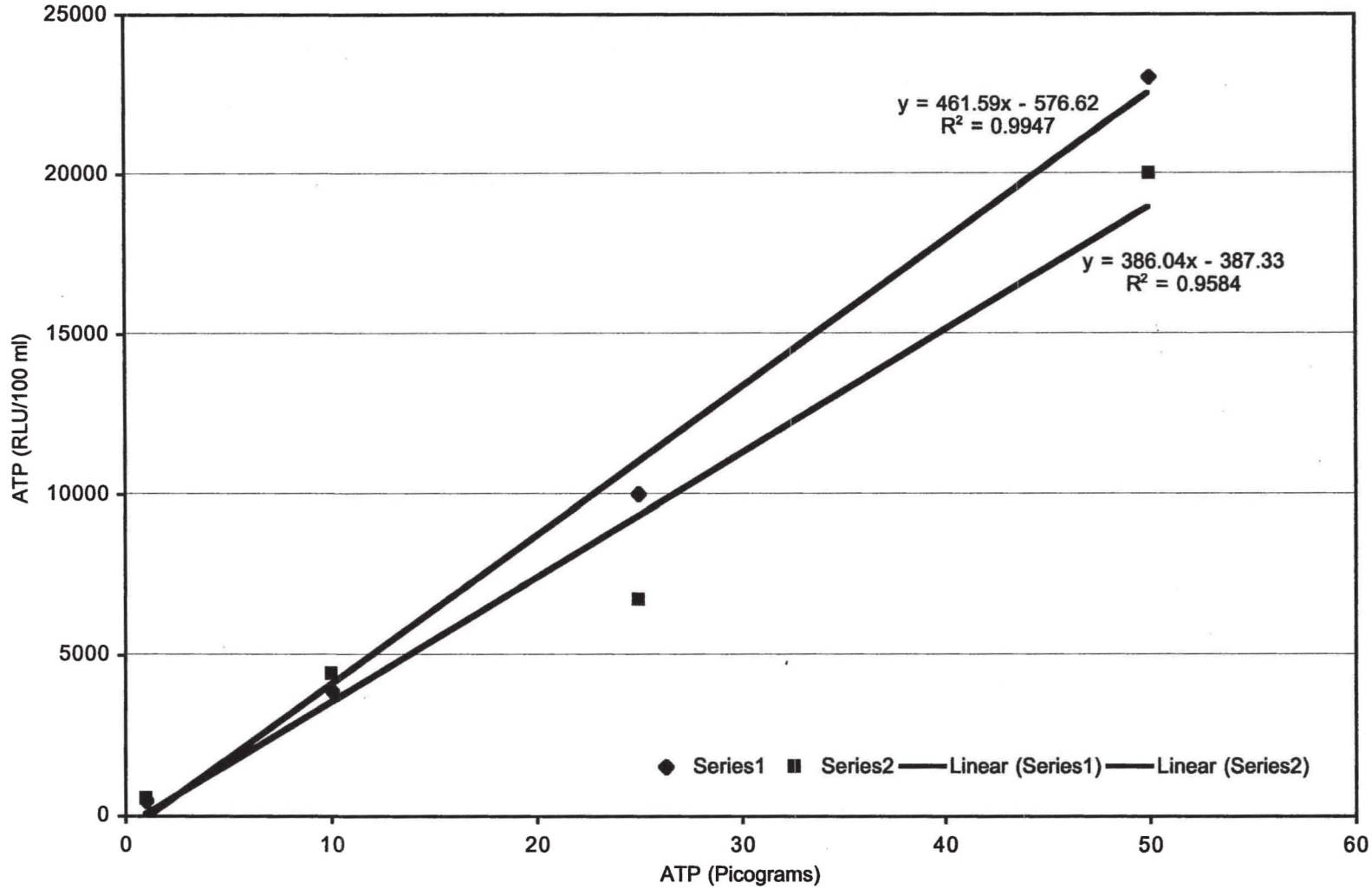


Figure 8. Correlating ATP (RLU/100 ml) measurements with increasing concentrations of purified ATP in ATP-free water by Pallchek method

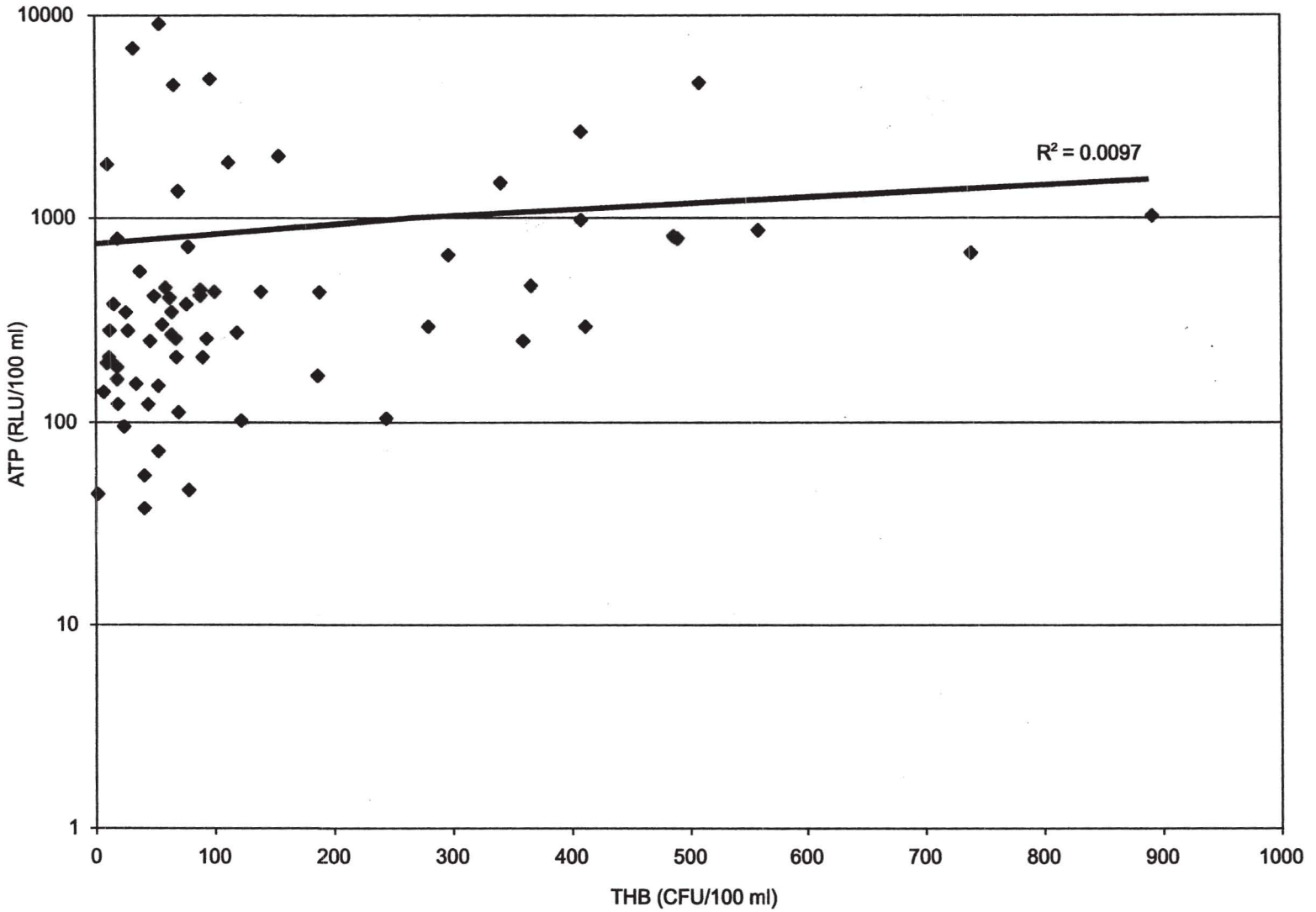


Figure 9. Correlating ATP (RLU/100 ml) measurements and THB or total heterotrophic bacteria (CFU/100 ml) in 70 groundwater (wells, tunnels, shafts) samples

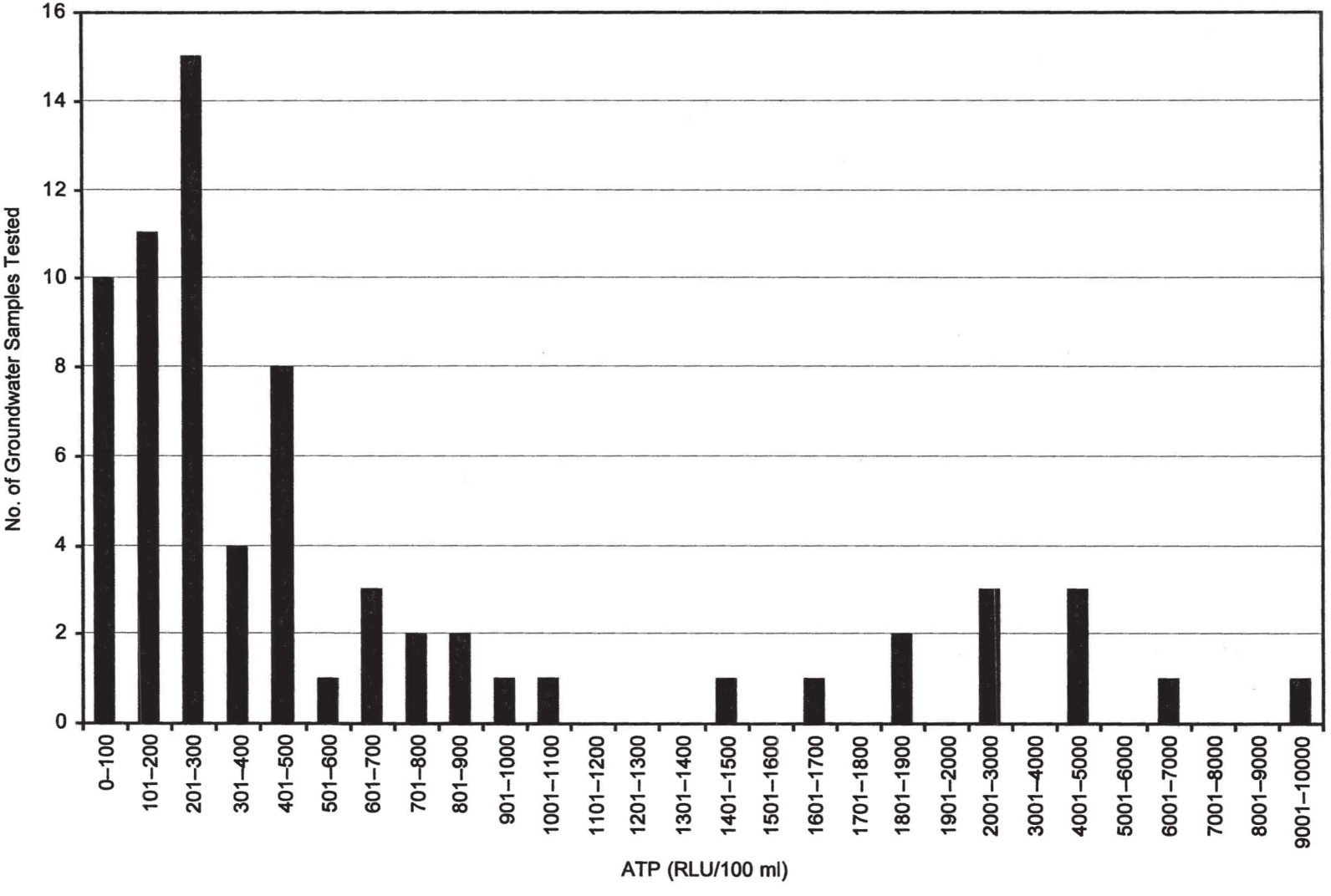


Figure 10. Variation in ATP (RLU/100 ml) measurements in each of 70 water samples obtained from groundwater sources (wells, tunnels, shafts)

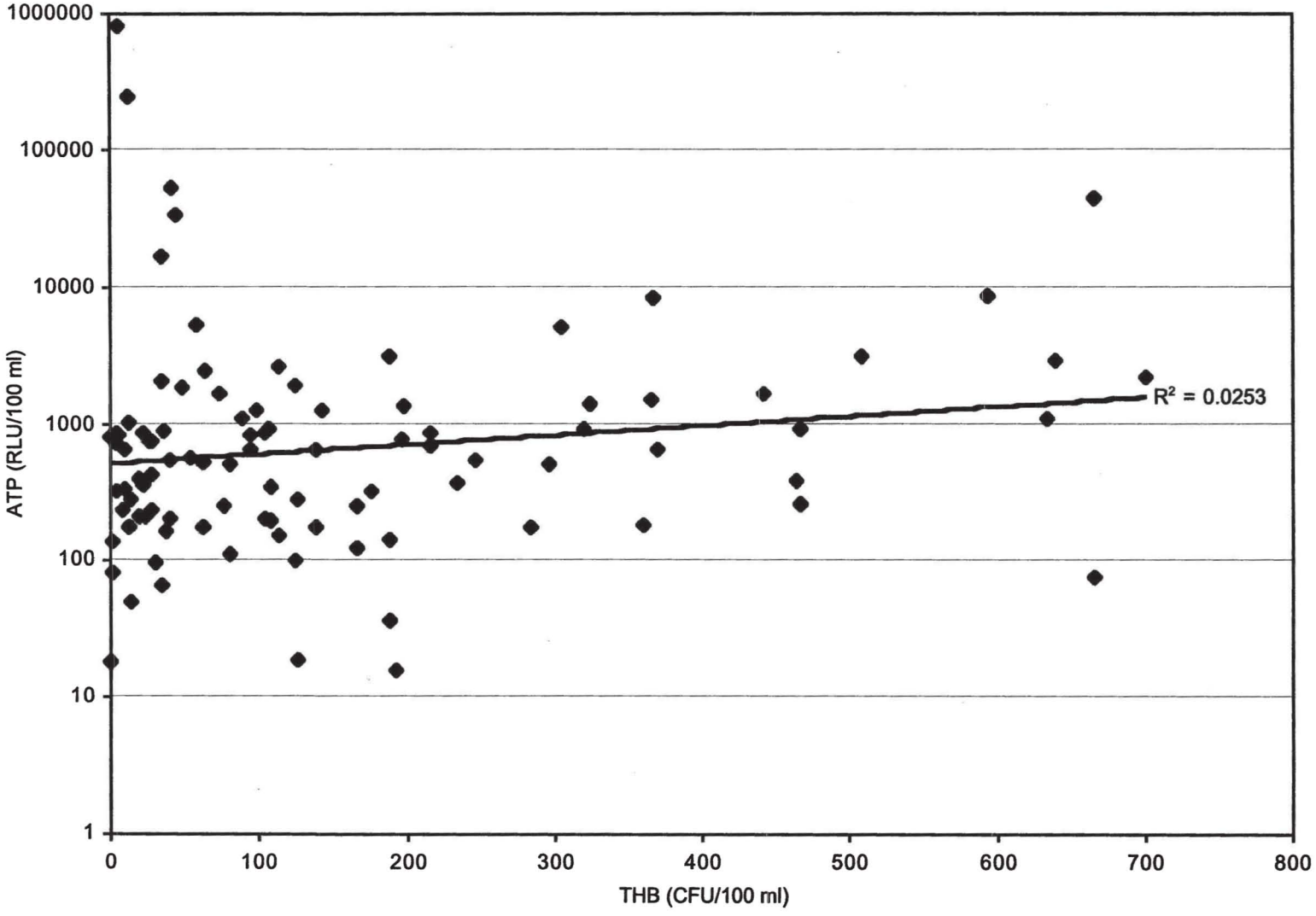


Figure 11. Correlating ATP (RLU/100 ml) measurements and THB or total heterotrophic bacteria (CFU/100 ml) in 100 reservoir water samples

Figure 12. Variation in ATP (RLU/100 ml) measurements in each of 100 water samples obtained from reservoir tanks

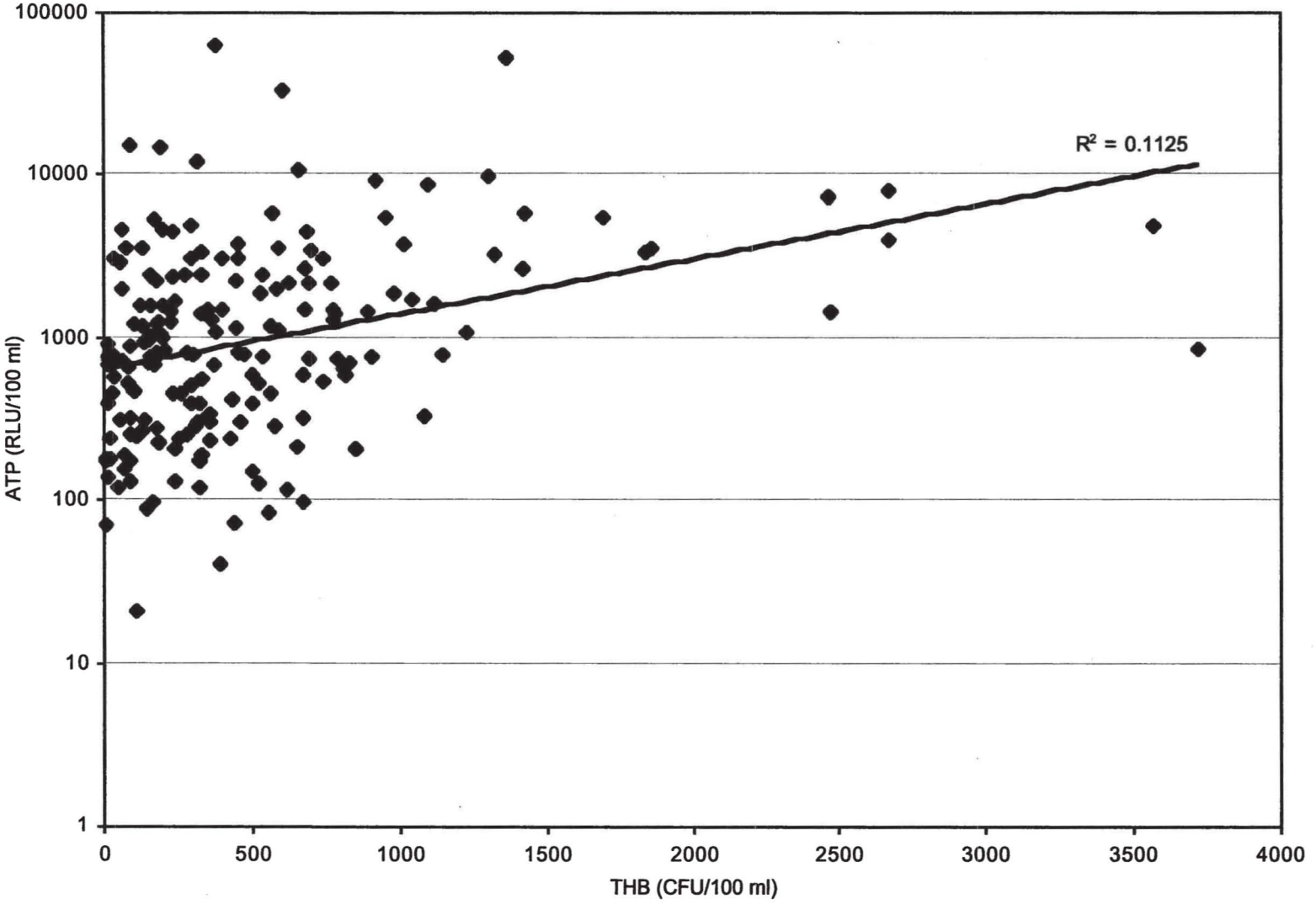


Figure 13. Correlating ATP (RLU/100 ml) measurements and THB or total heterotrophic bacteria (CFU/100 ml) in 184 distribution water samples

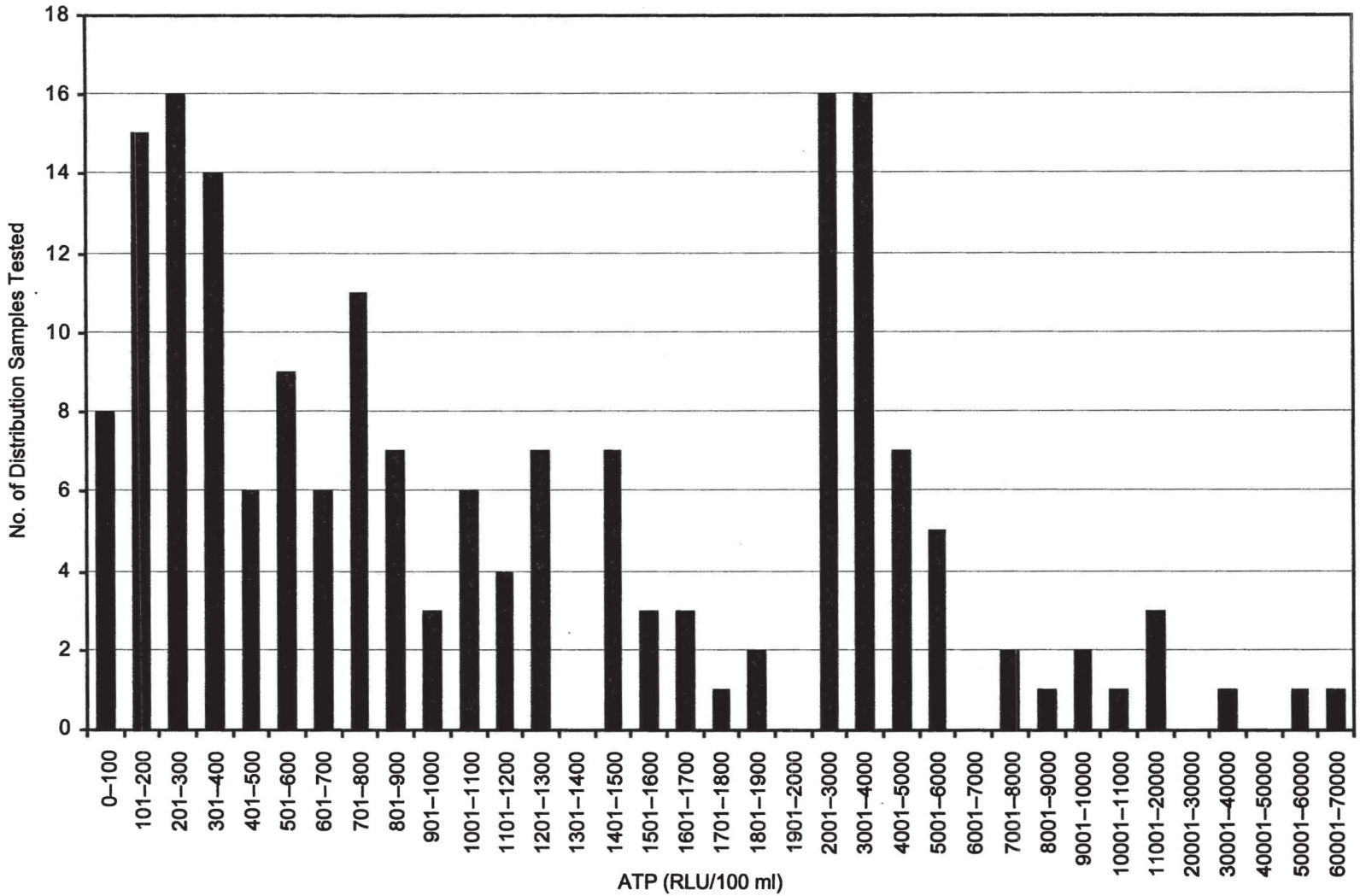


Figure 14. Variation in ATP (RLU/100 ml) measurements in each of 184 water samples obtained from distribution pipes

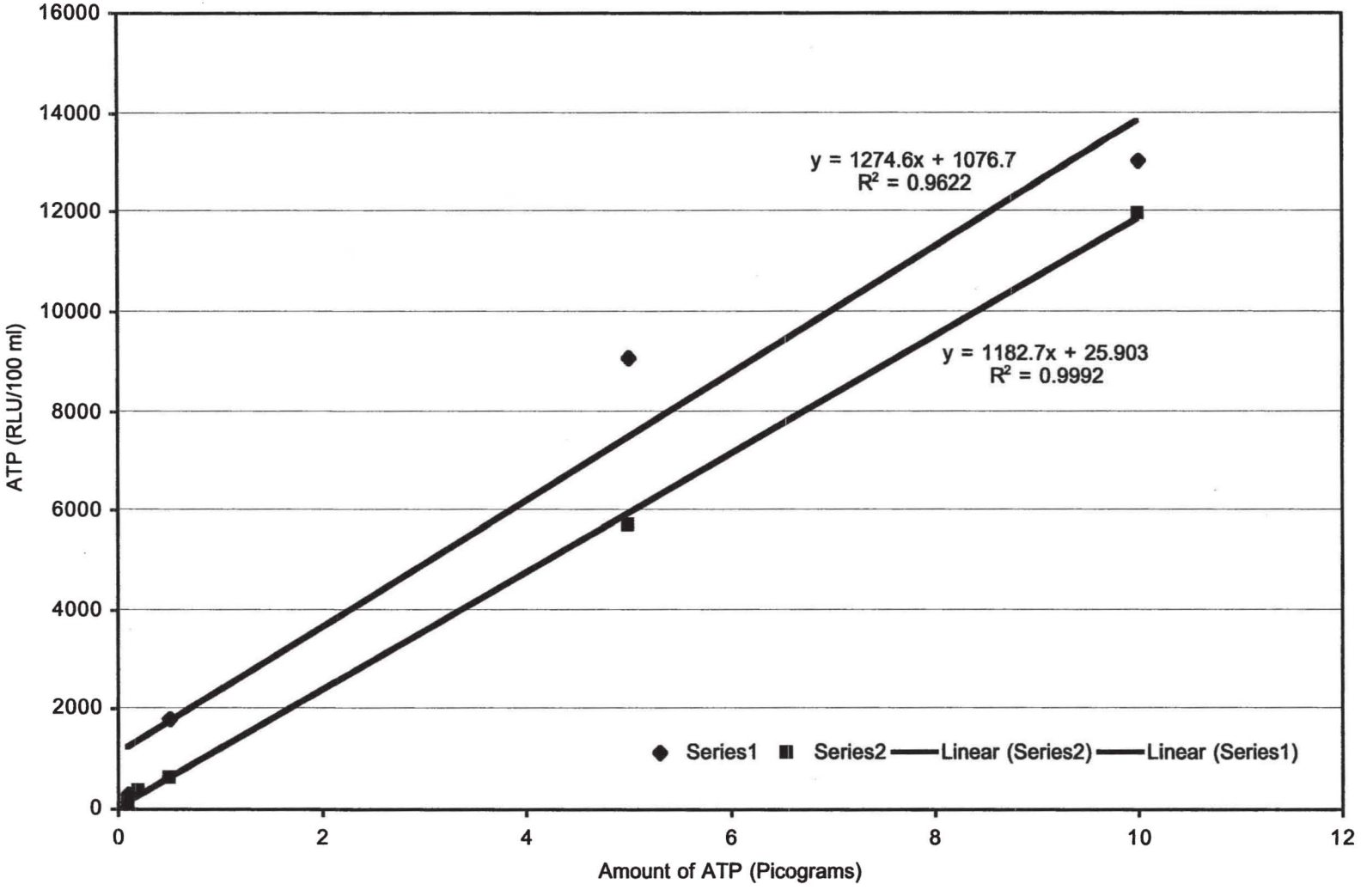


Figure 15. Correlating ATP (RLU/100 ml) measurements with increasing concentrations of purified ATP in ATP-free water by Profile-1 method

TABLES

Table 1. Agents or Toxins Identified as Likely to be Used For Intentional Contamination of Drinking Water Supplies

Agent or Toxin	Expected Disease or Human Response	CDC Category[a]
Biological agents or biotoxins categorized by Centers for Disease Control and Prevention		
Bacillus anthracis	Anthrax	Category A
Brucella melintensis	Brucellosis	Category B
Brucella suis	Brucellosis	Category B
Crytosporidium parvum	Gastroenteritis	Category B
Escherichia coli O157:H7	Bloody diarrhea	Category B
Salmonella spp.	Gastroenteritis	Category B
Shigella spp.	Gastroenteritis	Not categorized
Yersinia pestis	Plague	Category A
Vibrio cholerae	Cholera	Category B
Variola major	Smallpox virus	Category A
Hepatitis A virus	Hepatitis	Not categorized
Enteric viruses	Paralysis	Not categorized
Botulinum toxin	Toxic poisoning	Category A
Ricin	Toxic poisoning	Category B
Staphylococccal enterotoxin	Toxic poisoning	Category B
Toxic chemical agents		
Aflatoxin	Toxic poisoning	
Anatoxin	Toxic poisoning	
Microcystin	Toxic poisoning	
Saxitoxin	Toxic poisoning	
Tricothecene mycotoxin (T-2)	Toxic poisoning	
Tetrodotoxin	Toxic poisoning	
Industrial-grade hazardous chemicals		
Heavy metals	Toxic poisoning	
Herbicides	Toxic poisoning	
Insecticides	Toxic poisoning	
Rodenticides	Toxic poisoning	
Fungicides	Toxic poisoning	

[a]Category A = highest priority biological disease agent designated for biowarfare; Category B = second highest priority biological disease agent designated for biowarfare; not categorized = risk as disease agent not categorized for biowarfare.

Table 2. Microtox Analysis of Ambient Honolulu Board of Water Supply Potable Water Samples to Determine Range of Toxicity Effects

Sampling Site	%Effect After 5 minutes	%Effect After 15 minutes
Wells		
Moanalua Well	0	-1.096
Wilder Well	29.06	29.26
Beretania Station	-3.784	-0.0106
Waianae Well III Pump 1	9.317	12.19
Waianae Well III Pump 2	1.931	1.745
Aina Koa Well	-4.041	-10.49
Wailupe Well	12.77	11.73
Manoa Well	-3.377	-3.123
Waialee Well I	5.006	0.9251
Waianae Well II	9.333	16.93
Mean	NC[a]	5.81
Reservoir Tanks		
Pacific Heights Reservoir	11.47	14.42
Waahila 405 Reservoir	12.67	8.671
Moanalua 405 #2 Reservoir	21.79	18.71
Moanalua 405 Reservoir	7.370	7.298
Nuuanu 822 Reservoir	0	6.031
Nuuanu 640 Reservoir	12.12	12.67
Nuuanu 405 Reservoir	0	2.733
Bella Vista 180 Reservoir	4.521	9.972
Punchbowl 180 Reservoir	12.23	17.78
Diamond Head Reservoir	0	1.812
Mean	NC[a]	10.01
Distribution Pipes		
Waiau Fire Station	-10.80	-14.55
Nahele Neighborhood Park	5.736	8.458
Waiau Neighborhood Park	0	4.607
Haleiwa Seven-Eleven	-11.09	-9.459
Waialua Intermediate and High School	-12.43	-7.764
Kahuku Elderly Home	-6.696	-8.571
Kahuku District Park	-2.572	0.1913
Aiea Fire Station	0.0152	0.9029
Honolulu BWS	-0.3997	2.100
Crestview Community Park	13.38	35.48
Mean	NC[a]	1.14

[a]NC = not calculated.

Table 3. Measurements of Adenosine Triphosphate (ATP) and Total Heterotrophic Bacteria (THB) in Water Samples Obtained From Honolulu Board of Water Supply Groundwater Sources, Reservoir Tanks, and Distribution Pipes

Water Sources	No. of Samples Tested	ATP (RLU/100 ml)		THB (CFU/100 ml)	
		Range	Geomean	Range	Geomean
Groundwater[a]					
Chlorinated	56	0 – 4,822	264	0 – 891	68
Non-chlorinated	14	0 – 9,095	439	0 – 558	36
Reservoir Tanks					
Chlorinated	100	16 – 822,217	665	0 – 2,938	70
Distribution Pipes					
Chlorinated	184	21 – 62,179	1,067	4 – 3,720	360

[a]Wells, tunnels, and shafts.

Table 4. Detection of ATP Concentrations After Addition of *E. coli* to 500 ml of Distribution Water Characterized by 1,264 RLU/100 ml

Amount of *E. coli* (CFU/500 ml) Added to Tap Water	ATP Concentration (RLU/100 ml)
0	1,264
123	1,229
785	1,189
5,850	1,440

Table 5. Detection of ATP Concentrations After Addition of *E. coli* to 100 ml of ATP-Free Sterile Buffer Water

Amount of *E. coli* (CFU/100 ml) Added to Buffer Water	ATP Concentration (RLU/100 ml)
0	0
2	0
3	0
102	6
2,000[a]	52
21,600	467
168,000	4,480
1,720,000	34,400
15,800,000	560,000
204,000,000	5,250,000

[a]Calculated concentration.

Table 6. Detection of ATP Concentrations After Addition of *E. coli* to 100 ml of Distribution Water Characterized by 590 RLU/100 ml

Amount of *E. coli* (CFU/100 ml) Added to Tap Water	ATP Concentration (RLU/100 ml)
0	590
2	490
27	385
282	990
2,660	560
26,000	1,140
314,000	4,040
3,140,000	35,400
24,800,000	395,000
319,000,000	2,450,000

Table 7. Comparative ATP Measurements in Honolulu Board of Water Supply Potable Water Samples by Pallchek Versus Profile-1 Methods

Sampling Site	Profile-1 (RLU/100 ml)	Pallchek (RLU/100 ml)
Groundwater Sites		
HCS-1 Halawa Shaft	420	159
HCS-20 Moanalua Well #1	92	65
HPS-2 Halawa Wells #1	20	69
HS-4 Beretania Station L/S	56	93
HPS-6 Aiea 260 #1	148	851
HCS-29 Manoa Well	412	271
HS-14 Wilder Wells #4	52	37
HS-12 Palolo Tunnel	1,544	291
HCS-2 Kalihi Shaft	200	551
HS-4 Beretania Station H/S	70	491
HCS-11 Manoa Tunnel	352	211
Geomean	153	186
Reservoir Tanks		
HCR-60 Moanalua 405 #2	124	211
HCR-53 Nuuanu 822 Reservoir	852	811
HCR-46 Punchbowl 180 Reservoir	288	171
HCR-51 Nuuanu 640 Reservoir	240	171
HCR-30 Diamond Head 180	68	75
HCR-47 Bella Vista 180 Reservoir	552	163
HCR-59 Moanalua 405 #1	60	731
HCR-50 Nuuanu 405 Reservoir	96	391
Waahila 405 Res	1,940	191
Pacific Heights Reservoir	812	971
Geomean	275	282
Distribution Pipes		
WH-12 Helemano Elem. School	44	560
B102 Officers Beach	120	42
HP-15 Waiau Park	224	840
WE-4 Wailee Station	56	18
WA-5 Sunset Beach Fire Station	132	122
HP-16 Newtown Park	84	240
WH-2 Kemoo Farm	124	180
HP-17 Waiau Fire Station	124	54
MI-06 Makaulau Comm. Park	88	220
Waialua Fire Station	88	400
Geomean	99	157

Note: Profile-1 method analyzed 25-ml water samples; Pallcheck method analyzed 450-ml water samples.

Table 8. Detection of Total Suspended Solids (TSS), Chemical Oxidation Demand (COD), Biological Oxidation Demand (BOD), Total Organic Carbon (TOC), Nitrates (NO_3), and Surfactants (SUR) in Honolulu Board of Water Supply Groundwater Samples Using InSpectra Method

Sample Source	Sample No.	Pathway (mm)	TSS (mg/l)	COD (mg/l)	BOD (mg/l)	TOC (mg/l)	NO_3 (mg/l)	SUR (mg/l)
Aina Koa Well	HCS-17	10	<1	<1	<1	<1	2.7	<1
Palolo Tunnel	HCS-12	10	<1	<1	<1	<1	<1	<1
Waianae Tunnel	WAS-4	10	<1	<1	<1	<1	1.7	<1
Waianae I	WNS-12	10	<1	<1	<1	<1	3.3	<1
Wailupe Well	HCS-30	10	<1	<1	<1	<1	<1	<1
Waianae Well III Pump #2	WNS-08	10	<1	<1	<1	<1	8	<1
Waianae Plant Tunnel	WNS-05	10	<1	<1	<1	<1	1.4	<1
Waianae Well III Pump #1	WNS-08	10	<1	<1	<1	<1	7.8	<1
Manoa Well	HCS-29	10	<1	<1	<1	<1	<1	<1
Waialee Well I		5	<1	40.5	37.5	33.5	8.2	46.5

Table 9. Detection of Total Suspended Solids (TSS), Chemical Oxidation Demand (COD), Biological Oxidation Demand (BOD), Total Organic Carbon (TOC), Nitrates (NO_3), and Surfactants (SUR) in Honolulu Board of Water Supply Reservoir Water Samples Using InSpectra Method

Sample Source	Sample No.	Pathway (mm)	TSS (mg/l)	COD (mg/l)	BOD (mg/l)	TOC (mg/l)	NO_3 (mg/l)	SUR (mg/l)
Waialua 225 Res.	WAR-01	5	<1	24.8	29.8	24.6	11.5	37.0
Haleiwa 277 Res.	HCR-63	5	<1	31.5	29.6	26.4	13.1	36.5
Palolo Res. 2	HCR-36	10	<1	<1	<1	<1	<1	<1
Kahuku 228 Res.	KHR-01	10	<1	<1	<1	<1	1.1	<1
Punchbowl 180 Res.		10	<1	<1	<1	<1	1.5	<1
Kunia 440 Res. #1	WNR-4	10	<1	<1	<1	<1	1.2	<1
Bellavista Res. 180	HCR-47	10	<1	<1	<1	<1	1.4	<1
Kunia 228 Res. #1	WUR-03	10	<1	<1	<1	<1	<1	<1
Kunia 228 Res. #2	WUR-03	10	<1	<1	<1	<1	<1	<1
Kawela 228 Res.		5	<1	33.5	40.5	33.5	12	50.5

Table 10. Detection of Total Suspended Solids (TSS), Chemical Oxidation Demand (COD), Biological Oxidation Demand (BOD), Total Organic Carbon (TOC), Nitrates (NO_3), and Surfactants (SUR) in Honolulu Board of Water Supply Distribution Water Samples Using InSpectra Method

Sample Source	Sample No.	Pathway (mm)	TSS (mg/l)	COD (mg/l)	BOD (mg/l)	TOC (mg/l)	NO_3 (mg/l)	SUR (mg/l)
Haliewa 7-11	WA-07	5	<1	30.0	36.0	29.8	11.8	45.0
Aiea Fire Station	HP-02	5	<1	29.0	35.0	28.8	15.0	43.5
Waialua Intermediate and High School	WA-08	5	<1	36.0	38.5	32.5	9.1	47.5
Manana Park	HP-18	5	<1	30.0	36.0	29.8	11.6	45.0
Pearlridge Comm. Park	HP-19	5	<1	29.2	35.0	28.8	12.4	43.5
Halawa Heights	HP-1	5	<1	26.6	32.0	26.4	12.2	40.0
Crestview Comm. Park	WP-02	5	<1	26.4	32.0	26.2	23.0	39.5
Kahuku District Park	KH-04	5	<1	30.0	36.0	29.8	11.0	45.0
Honolulu BWS	HC-9	5	<1	28.8	34.5	28.6	11.9	43.0
Kahuku Elderly Home	KH-03	10	<1	<1	<1	<1	<1	<1

Table 11. RiboPrinter Analysis of Total Heterotrophic Bacteria (THB) Isolates Recovered From Honolulu Board of Water Supply Potable Water Sources

THB Isolates	Potable Water Sources					
	Wells	Reservoir Tanks	Distribution Pipes	Tunnels[a]	Shafts[a]	GAC[a]
No. Tested	140	86	331	27	33	13
% Identified	25.0% (35/140)	29.1% (25/86)	13.6% (45/331)	14.8% (4/27)	12.1% (4/33)	7.7% (1/13)
% Not Identified	75.0% (105/140)	70.9% (61/86)	86.4% (286/331)	85.2% (23/27)	87.9% (29/33)	92.0% (12/13)
% Grouped[b]	19.3% (27/140)	18.6% (16/86)	29.6% (98/331)	18.5% (5/27)	30.3% (10/33)	0% (0/13)
No. of Ribogroups[c]	11	7	27	2	4	0
% Not Grouped[d]	55.7% (78/140)	52.3% (45/86)	56.8% (188/331)	66.7% (18/27)	57.6% (19/33)	92.3% (12/13)

[a]Tunnel and shaft sources refer to shallow groundwater sites where water is withdrawn for potable use by the Honolulu Board of Water Supply; and GAC (= granular activated carbon) sources refer to tanks containing groundwater treated with GAC to remove pesticides.
[b]Grouped = more than one riboprint per ribogroup.
[c]Number of different ribogroups that are comprised of more than one riboprint.
[d]Not grouped = one riboprint per ribogroup.

Table 12. RiboPrinter Identification of Total Heterotrophic Bacteria (THB) Isolates Recovered From Honolulu Board of Water Supply Potable Water Sources

Wells	Reservoir Tanks	Distribution Pipes
Acinetobacter Iwoffii (1)	*Acinetobacter Iwoffii* (2)	*Acinetobacter baumannii* (1)
Bacillus megaterium (1)	*Bacillus cereus* (2)	*Aerococcus viridans* (1)
Bacillus pumilus (2)	*Bacillus sphaericus* (2)	*Bacillus cereus* (10)
Bacillus thuringiensis (3)	*Bacillus thuringiensis* (4)	*Bacillus fusiformis* (1)
Delftia acidovorans (5)	*Lactococcus lactis* (2)	*Bacillus megaterium* (1)
Escherichia coli (1)	*Legionella moravica* (1)	*Bacillus thuringiensis* (3)
Flavobacterium sp. (1)	*Micrococcus luteus* (1)	*Delftia acidovorans* (1)
Glaciecola pallidula (1)	*Pseudomonas aeruginosa* (2)	*Enterobacter cloacae* (2)
Lactobacillus pontis (1)	*Pseudomonas alcaligenes* (1)	*Flavobacterium* sp. (1)
Legionella pneumophila (1)	*Pseudomonas putida* (1)	*Glaciecola pallidula* (4)
Legionella pneumophila ss fraseri (1)	*Staphylococcus epidermidis* (1)	*Legionella pneumophila* (2)
Ochrobactrum anthropi (1)	*Staphylococcus pasteuri* (1)	*Pseudomonas alcaligenes* (2)
Pseudomonas aeruginosa (5)	*Staphylococcus warneri* (3)	*Pseudomonas putida* (2)
Sphingomonas paucimobilis (1)	*Stenotrophomonas maltophilia* (1)	*Ralstonia pickettii* (1)
Staphylococcus hominis (2)	*Vibrio cholerae* (1)	*Salinivibrio coasticola ss coasticola* (2)
Staphylococcus pasteuri (1)		*Sphingomonas aromaticivorans* (1)
Staphylococcus warneri (1)		*Staphylococcus haemolyticus* (1)
Staphylococcus xylosus (1)		*Staphylococcus pasteuri* (3)
Stenotrophomonas maltophilia (2)		*Staphylococcus warneri* (1)
Vibrio vulnificus (2)		*Stenotrophomonas maltophilia* (1)
Weissella halotolerans (1)		*Terracoccus luteus* (2)
		Vibrio cholerae (1)
		Vibrio sp. (1)

( ) = number of identified isolates.

Table 13. RiboPrinter Analysis of Non-Target Colonies (NTC) Recovered From Honolulu Board of Water Supply Potable Water Sources

Description	Wells	Reservoir Tanks	Distribution Pipes	GAC[a]
No. of Sites Samples	3	2	72	1
No. of iIolates	3	2	105	1
No. of Ribogroups	3	2	89	1
No. Identified	0	0	33	0
% Identified	0	0	31	0
% Not identified	100	100	69	100

[a]Refers to tanks containing groundwater treated with GAC (granular activated carbon) to remove pesticides.

Table 14. RiboPrinter Identification of Non-Target Colonies (NTC) Recovered From Honolulu Board of Water Supply Potable Water Samples

Wells	Reservoir Tanks	Distribution Pipes
—	—	*Acinetobacter baumannii* (3)
—	—	*Aeromonas hydrophila* (5)
—	—	*Bacillus megaterium* (1)
—	—	*Bacillus thuringiensis* (1)
—	—	*Enterobacter cloacae* (4)
—	—	*Escherichia coli* (1)
—	—	*Flavobacterium* sp. (1)
—	—	*Pseudomonas aeruginosa* (12)
—	—	*Pseudomonas pseudoalcaligenes* (1)
—	—	*Staphylococcus haemolyticus* (2)
—	—	*Stenotrophomonas maltophilia* (2)

Note: NTC were not recovered from any of the well or reservoir tank samples.

( ) = number of identified isolates.

Table 15. Cluster Analysis of Riboprints Generated by RiboPrinter Using GelCompar Method at 80% Similarity Index

THB Isolates	Potable Water Sources		
	Wells	Reservoir Tanks	Distribution Pipes
No. Tested	140	86	331
No. Clustered	101	52	246
% in Clusters	72.1% (101/140)	60.5% (52/86)	74.3% (246/331)
No. of Clusters	27	12	80
No. Not Clustered	39	34	85
% Not Clustered	27.8% (39/140)	39.5% (34/86)	25.7% (85/331)

Table 16. Riboprints of Total Heterotrophic Bacteria (THB) Isolates Placed into Clusters Using GelCompar Method at 80% Similarity Index

Cluster Data	Potable Water Sources		
	Wells	Reservoir Tanks	Distribution Pipes
Total No. of THB Isolates	140	86	331
Total No. of THB Clusters	27	12	80
Distribution of Isolates			
No. of Clusters with 2 isolates	11	2	42
No. of Clusters with 3 isolates	2	5	18
No. of Clusters with 4 isolates	5	2	12
No. of Clusters with 5 isolates	5	1	3
No. of Clusters with 6 isolates	3	0	2
No. of Clusters with 7 isolates	0	0	0
No. of Clusters with 8 isolates	0	1	1
No. of Clusters with 9 isolates	0	0	0
No. of Clusters with 10 isolates	1	0	0
No. of Clusters with 11 isolates	0	0	1
No. of Clusters with 12 isolates	0	1	0
No. of Clusters with 13 isolates	0	0	0
No. of Clusters with 14 isolates	0	0	1

APPENDIX A: TABLES

Appendix Table 1. Measurements of Adenosine Triphosphate (ATP) and Total Heterotrophic Bacteria (THB) in BWS Groundwater Sites (Wells, Tunnels and Shafts)

Date	BWS Sample No.	BWS Site Name	ATP RLU/450 ml	ATP RLU/100 ml	No. Colonies per Membrane	Sample Volume	THB CFU/100 ml
5/21/03	HPS-2	Pearl City Wells I, #1	0	0	4	25	16
5/21/03	HPS-2	Pearl City Wells I, #1	0	0	3	50	6
5/21/03	HS-5	Kaimuki Station- Low Service	0	0	0	25	0
5/21/03	HS-5	Kaimuki Station- Low Service	600	141	3	50	6
5/21/03	HS-11	Manoa Tunnel	1,200	267	16	25	64
5/21/03	HS-11	Manoa Tunnel	5,500	1,375	35	50	70
5/21/03	HS-12	Palolo Tunnel	21,700	4,822	24	25	96
5/21/03	HS-12	Palolo Tunnel	21,700	4,521	33	50	66
5/28/03	HS-17	Aina Koa Well	1,961	436	50	50	100
5/28/03	HS-22	Palolo Deep Well	2,061	458	30	50	60
5/28/03	HS-29	Manoa 405 Well	321	71	13	25	52
5/28/03	HS-30	Wailupe Well	551	123	22	50	44
5/28/03	HS-36	Kuliouou Well	3,561	791	9	50	18
5/28/03	WHS-2	Wahiawa Well II, #1	1,261	280	14	50	28
5/28/03	WNS-6	Makaha Well V	8,561	1,903	56	50	112
5/28/03	WNS-1	Makaha Well I	11,961	2,658	205	50	410
5/28/03	WNS-3	Makaha Shaft	1,861	414	44	50	88
5/28/03	MIS-1	Mililani Wells I, Pump #2	1,961	436	70	50	140
5/28/03	WNS-13	Makaha Well II	2,961	658	119	40	298
5/28/03	MIS-3	Mililani Wells III, Pump #1	1,561	347	32	50	64
5/28/03	WNS-7	Makaha Well VI	1,861	414	25	50	50
5/28/03	WHS-1	Wahiawa Wells I, Pump #1	6,661	1,480	171	50	342
5/28/03	MIS-2	Mililani Wells II, Pump #2	1,561	347	13	50	26
5/29/03	HS-20	Moanalua Wells	1,327	295	132	32	413
5/29/03	HS-14-1	Wilder Avenue Wells #1	1,727	384	38	50	76
5/29/03	WUS-3	Waipahu Wells I, #2	727	162	9	50	18

Appendix Table 1—*Continued*

Date	BWS Sample No.	BWS Site Name	ATP RLU/450 ml	ATP RLU/100 ml	No. Colonies per Membrane	Sample Volume	THB CFU/100 ml
5/29/03	WPS-2	Waipio Heights Wells I, Pump #2	1,327	295	140	50	280
5/29/03	MIS-4	Mililani Wells IV, Pump #2	4,427	984	205	50	410
5/29/03	WUS-8	Waipahu Wells II, Pump #2	2,127	473	183	50	366
5/29/03	WUS-6	Honouliui Wells I, #1	40,927	9,095	27	50	54
5/29/03	HS-37	Kapalama Wells #2	3,627	806	245	50	490
5/29/03	WUS-5	Makakilo Well	1,127	251	23	50	46
5/29/03	WNS-8	Waianae Well III, Pump #2	1,727	384	8	50	16
5/29/03	HS-2	Kalihi Shaft	897	199	7	50	14
5/29/03	WUS-1	Hoaeae Wells, Pump #1	1,227	273	59	50	118
5/29/03	WNS-4	Waianae Tunnel	2,027	451	44	50	88
5/29/03	WPS-1	Waipio Heights Wells, Pump #1	1,127	251	180	50	360
5/29/03	HS-1	Halawa Shaft	3,927	873	279	50	558
7/25/03	HS-15	Kalauao Wells Pump #4	3,049	677	369	50	738
7/28/03	HWS-15	Kahana Wells I, #2	8,356	1,857	5	50	10
7/28/03	HWS-7	Waihee Tunnel	3,256	724	39	50	78
7/28/03	HWS-3	Kuou Wells	496	110	35	50	70
7/28/03	HWS-4	Luluku Tunnel	1,256	279	6	50	12
7/28/03	HWS-23	Maakua Wells	936	208	45	50	90
7/28/03	HWS-20	Waimanalo Well II	166	37	20	50	40
7/28/03	HWS-14	Punaluu Wells III, #1	30,956	6,879	16	50	32
7/28/03	HWS-18	Luluku Well	686	152	17	50	34
7/28/03	HWS-2	Waimanalo Tunnels	2,456	546	19	50	38
7/28/03	HWS-10	Punaluu Wells II, #1	866	192	5	50	10
7/28/03	HWS-13	Haaula Well, #1	196	44	1	50	2
7/28/03	HWS-16	Kahaluu Well	246	55	20	50	40
7/28/03	HWS-6	Kahaluu Tunnel	20,956	4,657	255	50	510
7/28/03	HWS-19	Iolekaa Well	3,656	812	244	50	488

Appendix Table 1—*Continued*

Date	BWS Sample No.	BWS Site Name	ATP RLU/450 ml	ATP RLU/100 ml	No. Colonies per Membrane	Sample Volume	THB CFU/100 ml
7/28/03	HWS-21	Kuou Well II	206	46	39	50	78
7/30/03	WNS-1	Kamaile Wells Pump #1	4,641	1,031	405	45	891
7/30/03	WNS-5	Waianae Plantation Tunnel	751	167	93	50	186
7/30/03	WNS-12	Waianae Wells I	1,141	254	34	50	68
7/30/03	WNS-11	Waianae Wells II	1,141	254	47	50	94
7/30/03	HS-15-4	Kalauou Wells, #4	461	103	122	50	244
7/30/03	HPS-10	Newtown Wells #3	1,841	409	31	50	62
7/30/03	HPS-8	Halawa Wells, #2	421	94	12	50	24
7/30/03	HPS-9-3	Waiau Wells #3	551	123	9	50	18
7/30/03	HPS-3-1	PC Wells II, #1	841	187	9	50	18
7/30/03	HPS-1	PC Shaft, #1	9,041	2,009	77	50	154
7/30/03	HPS-11	PC Well III	1,941	431	94	50	188
7/30/03	WUS-4	Kunia Wells II, Pump #4	1,341	298	28	50	56
7/30/03	HPS-5	Kaonohi Wells I, #1	941	209	34	50	68
7/30/03	HPS-6	Aiea 260 Wells, #2	451	100	61	50	122
7/30/03	HPS-12	HECO- Waiau Wells, #1	671	149	26	50	52
7/30/03	HPS-7	Aiea Gulch Wells, #1	941	209	6	50	12

Appendix Table 2. Measurements of Adenosine Triphosphate (ATP) and Total Heterotrophic Bacteria (THB) in BWS Reservoir Sites

Date	BWS Sample No.	BWS Site Name	ATP RLU/450 ml	ATP RLU/100 ml	No. Colonies per Membrane	Sample Volume	THB CFU/100 ml
6/2/03	HCR-9	Hahaione 500	4,192	932	53	50	106
6/2/03	HCR-4	Kamehame 500	3,292	732	13	50	26
6/2/03	HPR-6	Kaonohi 277 res.	1,792	398	10	50	20
6/2/03	HPR-13	Waiau 550 res.	1,592	354	11	50	22
6/2/03	HCR-6	Kaluanui 170	4,892	1,087	29	33	88
6/2/03	MIR-1	Mililani 685 res.	4,592	1,020	6	50	12
6/2/03	HCR-47	Bella Vista 180	23,892	5,309	29	50	58
6/2/03	HCR-30	Diamond Head 180	3,892	865	52	50	104
6/2/03	HCR-2	Koko Head 170	10,892	2,420	32	50	64
6/2/03	WHR-2	Wahiawa 1075 res.	302	67	17	50	34
6/2/03	HPR-11	Newtown 550 res.	622	138	1	50	2
6/2/03	HPR-5	Kaamilo 497 res.	5,592	1,243	71	50	142
6/2/03	WHR-5	Wahiawa 1361 (2)	37,892	8,420	99	27	367
6/2/03	HCR-46	Punchbowl 180	2,492	554	27	50	54
6/2/03	HCR-1	Kalama 170 Tr.	2,492	554	N/A	15	N/A
6/4/03	HCR-18	Waialae Iki 180	695	154	57	50	114
6/4/03	HCR-11	Niu 170	3,155	701	108	50	216
6/4/03	HCR-65	Halawa 550	955	212	10	50	20
6/4/03	WUR-3	Kunia 228 res.	4,155	923	28	6	467
6/4/03	WUR-19	Waikele 395 (1)	2,255	501	>148	50	296
6/4/03	HCR-13	Hawaii Loa 475	325	76	233	35	666
6/4/03	HPR-3	Aiea 782	635	141	94	50	188
6/4/03	HCR-10	Kuliouou 350	1,455	323	2	50	4
6/4/03	WHR-6	Melemanu 808 res.	5,655	1,257	49	50	98
6/4/03	HPR-	Pearl City 285 (2)	365	81	1	50	2
6/4/03	WUR-10	Makakilo 920 res.	1,055	234	4	50	8
6/4/03	HCR-16	Aina Haina 170	4,955	1,101	317	50	634

Appendix Table 2—*Continued*

Date	BWS Sample No.	BWS Site Name	ATP RLU/450 ml	ATP RLU/100 ml	No. Colonies per Membrane	Sample Volume	THB CFU/100 ml
6/4/03	MIR-4	Mililani 1150 res.	9,355	2,079	17	50	34
6/4/03	MIR-2	Mililani 865 res.	3,855	857	2	50	4
7/11/03	HCR-19	Waialae Iki 405	790	176	69	50	138
7/11/03	HCR-26	Aina Koa 640 Res.	1,640	364	117	50	234
7/11/03	HCR-28	Aina Koa 1100 Res.	2,240	498	40	50	80
7/11/03	HCR-25	Aina Koa 405 Res.	12,940	2,876	320	50	640
7/11/03	HCR-15	Hawaii Loa 1125	1,040	231	14	50	28
7/11/03	HCR-12	Niu Valley 297	1,140	253	38	50	76
7/11/03	HCR-48	Pacific Heights 578	13,940	3,098	94	50	188
7/11/03	HCR-33	Wilhelmina 811 Res.	2,940	653	185	50	370
7/11/03	HCR-24	Aina Koa 180 Res.	1,140	253	83	50	166
7/11/03	HCR-49	Pacific Heights 915	2,940	653	47	50	94
7/11/03	HCR-27	Aina Koa 865 Res.	1,240	276	63	50	126
7/11/03	HCR-14	Hawaii Loa 800	890	198	54	50	108
7/11/03	HCR-53	Nuuanu 822 Res.	22,940	5,098	152	50	304
7/11/03	HCR-7	Kaluanui 500	1,940	431	14	50	28
7/18/03	HCR-18	Waialae Iki 180 Res.	7,577	1,684	37	50	74
7/18/03	HCR-55	Alewa 850	239,977	53,328	21	50	42
7/18/03	HCR-34	Wilhemina 1100	3,377	750	14	50	28
7/18/03	HCR-23	Waialae 180 Res.	3,277	728	2	50	4
7/18/03	HCR-29	Aina Koa 1370 Res.	3,699,977	822,217	3	50	6
7/18/03	HCR-65	Halawa 550 Res.	73,977	16,439	17	50	34
7/18/03	HCR-33	Wilhemina 811	4,177	928	160	50	320
7/18/03	HCR-31	Wilhemina 405	2,477	550	10	25	40
7/18/03	HCR-60	Moanalua 405 Res (2)	227	50	7	50	14
7/18/03	HCR-50	Nuuanu 405	6,377	1,417	162	50	324
7/18/03	HCR-63	Halawa 277	1,177	262	233	50	466

Appendix Table 2—*Continued*

Date	BWS Sample No.	BWS Site Name	ATP RLU/450 ml	ATP RLU/100 ml	No. Colonies per Membrane	Sample Volume	THB CFU/100 ml
7/18/03	HCR-54	Alewa 597	6,077	1,350	99	50	198
7/18/03	HCR-21	Waialae Iki 865 Res.	1,477	328	5	50	10
7/18/03	HCR-59	Moanalua 405 Res (1)	507	113	40	50	80
7/25/03	HCR-38	St. Louis 640 Res.	2,949	655	5	50	10
7/25/03	HCR-40	St. Louis 1100 Res.	8,549	1,900	62	50	124
7/25/03	HCR-43	Roundtop 705	119,949	26,655	1,469	50	
7/25/03	HCR-39	St. Louis 865 Res.	3,849	855	108	50	216
7/25/03	HCR-57	Kalihi 405 Res.	2,449	544	111	45	247
7/25/03	HCR-63	Halawa 277 Res.	1,749	389	232	50	464
7/25/03	HCR-62	Aliamanu 385 Res.	919	204	20	50	40
7/25/03	HCR-7	Kaluanui 500	3,849	855	11	50	22
7/25/03	HCR-29	Aina Koa 1370 Res.	1,099,949	244,433	6	45	13
7/25/03	HCR-61	Aliamanu 180 Res.	789	175	31	50	62
7/25/03	HCR-68	Kalihi 614 Res.	11,949	2,655	57	50	114
8/1/03	HWR-19	Hauula 180	3,761	836	47	50	94
8/1/03	KHR-1	Kahuku Res. 228	81	18	0	50	0
8/1/03	HWR-4	Ahuimanu 272	921	205	52	50	104
8/1/03	HWR-12	Pohakupu 272 (2)	1,061	236	7	25	28
8/1/03	HWR-11	Pohakupu 272 (1)	9,961	2,214	350	50	700
8/1/03	HWR-5	Ahuimanu 500	1,461	325	88	50	176
8/1/03	HWR-15	Waimanalo 230	3,461	769	98	50	196
8/1/03	HWR-2	Kahana 315	441	98	15	50	30
8/1/03	HWR-3	Waihee 265	821	182	180	50	360
8/1/03	HPR-12	Waiau 285 Res.	2,861	636	69	50	138
8/1/03	HWR-1	Punaluu 180 Res.	7,461	1,658	221	50	442
8/1/03	HPR-11	Kaonohi 550 Res.	451	100	62	50	124
8/1/03	HWR-17	Waimanalo 364 (2)	199,961	44,436	333	50	666

Appendix Table 2—*Continued*

Date	BWS Sample No.	BWS Site Name	ATP RLU/450 ml	ATP RLU/100 ml	No. Colonies per Membrane	Sample Volume	THB CFU/100 ml
8/1/03	HPR-14	Waiau 850 Res.	791	176	6	50	12
8/1/03	HPR-20	Pearl City 1050 Res.	8,361	1,858	24	50	48
8/1/03	HWR-9	Kapaa 272 Res.	3,661	814	3	50	6
8/1/03	HPR-8	Kaonohi 850 Res.	961	214	12	50	24
9/26/03	WUR-8	Makakilo 440	1,244	276	7	50	14
9/26/03	WUR-6	Honouliuli 228	3,644	810	0	50	0
9/26/03	WUR-4	Kunia 440 (1)	1,544	343	54	50	108
9/26/03	WUR-1	Waipahu 224 (1)	6,744	1,499	183	50	366
9/26/03	WUR-7	Honouliuli 440	744	165	19	50	38
9/26/03	WAR-2	Haleiwa 225	4,044	899	18	50	36
9/26/03	WUR-9	Makakilo 675 (1)	2,344	521	31	50	62
9/26/03	WUR-2	Waipahu 228 (1)	13,944	3,099	254	50	508
9/26/03	WUR-5	Kunia 440 (2)	38,944	8,654	297	50	594
9/29/03	WAR-1	Waialua 225	149,944	33,321	22	50	44
9/29/03	WNR-9	Makaha 242 (2)	786	175	142	50	284
9/29/03	WNR-1	Nanakuli 350 Res.	166	37	94	50	188
9/29/03	WNR-4	Waianae 390 (1)	72	16	96	50	192
9/29/03	WNR-3	Waianae 242	566	126	83	50	166
9/29/03	WNR-5	Waianae 390 (2)	86	19	63	50	126

Appendix Table 3. Measurements of Adenosine Triphosphate (ATP) and Total Heterotrophic Bacteria in BWS Distribution Sites

Date	BWS Sample No.	BWS Site Name	ATP RLU/450 ml	ATP RLU/100 ml	No. Colonies per Membrane	Sample Volume	THB CFU/100 ml
5/23/03	HC-5	1955 Young St.	4,611	1,025	150	50	300
5/23/03	HC-6	Waikiki Fire Station	1,611	358	68	50	136
5/23/03	HC-11	Jack-in-the-Box Waikiki	18,611	4,136	120	30	396
5/23/03	HC-14	Kanewai Playground	3,811	847	40	50	80
5/23/03	HC-20	Diamond Head Line Booster	1,611	358	26	50	52
6/10/03	WP-2	Crestview Community Park	1,773	394	6	50	12
6/10/03	WN-6	Pililaau Plgd.	1,073	238	127	50	254
6/10/03	WP-1	BWS Waipio Heights Wells Control Station	15,973	3,550	37	50	74
6/10/03	WH-8	Harry and Jeannette Weinberg Silvercrest	973	216	327	50	654
6/10/03	WN-8	Maili Elementary School	16,973	3,772	508	50	1,016
6/10/03	WN-9	Leihoku Elementary School	4,273	950	68	50	136
6/10/03	WN-4	BWS Waianae Corp.Yard	6,373	1,416	163	50	326
6/10/03	WH-11	95-023 Waihau Street	5,273	1,172	280	50	560
6/10/03	MI-1	Kipapa Park	6,373	1,416	391	50	782
6/10/03	WN-7	Nanakuli Fire Station	2,673	594	408	50	816
6/10/03	WP-5	Waipio Neighborhood Park	1,473	327	542	50	1,084
6/10/03	WP-3	Gentry Waipio Shopping Center	21,973	4,883	1,785	50	3,570
6/10/03	WP-6	Better Brands	9,973	2,216	90	50	180
6/10/03	WH-6	Wahiawa Elementary School	3,273	727	22	35	63
6/10/03	MI-3	Noholoa Neighborhood Park	2,273	505	149	50	298
6/13/03	HP-18	Manana Park	1,750	389	131	44	298
6/13/03	HP-8	Momilani Elementary School	3,350	744	394	50	788
6/13/03	HC-22	Manoa Fire Station	7,050	1,567	80	50	160
6/13/03	HP-15	Waiau Neighborhood Park	149,950	33,322	301	50	602
6/13/03	HC-33	Ala Wai Clubhouse	3,550	789	573	50	1,146

Appendix Table 3—*Continued*

Date	BWS Sample No.	BWS Site Name	ATP RLU/450 ml	ATP RLU/100 ml	No. Colonies per Membrane	Sample Volume	THB CFU/100 ml
6/13/03	HC-9	630 South Beratania Street	4,350	967	75	50	150
6/13/03	HP-17	Waiau Fire Station	3,050	678	184	50	368
6/13/03	HP-25	Kaahele Park	850	189	34	50	68
6/13/03	HC-16	Kaimuki Fire Station	1,350	300	221	48	460
6/13/03	HC-31	Wailae Iki Playground	1,450	322	337	50	674
6/13/03	HC-13	Wailae Beach Park	700	155	35	50	70
6/13/03	HP-21	Waiau Elementary School	320	71	2	50	4
6/13/03	HP-16	Nahele NP	590	131	45	50	90
6/13/03	HC-11	Jack in the Box Waikiki	580	129	120	50	240
6/13/03	HC-6	Waikiki Fire Station	1,550	344	177	50	354
6/18/03	HW-8	Keolu Elementary School	43,933	9,763	444	34	1,306
6/18/03	HM-1	Kalihi Uka Neighborhood Park	6,533	1,452	1,235	50	2,470
6/18/03	HW-10	Kaneohe 7-11	7,733	1,719	520	50	1,040
6/18/03	HC-1	Nimitz Fire Station	4,933	1,096	615	50	1,230
6/18/03	HM-4	2765 Pacific Heights Road	3,033	674	8	50	16
6/18/03	HC-36	Moanalua Golf Course	5,233	1,163	224	50	448
6/18/03	HW-6	Aikahi Fire Station	9,033	2,007	291	50	582
6/18/03	HW-19	Enchanted Lake Plgd.	13,933	3,096	225	50	450
6/18/03	HW-5	Kailua Fire Station	10,933	2,430	136	50	272
6/18/03	HC-8	Fern Playground	4,533	1,007	98	50	196
6/18/03	HW-4	Mid Pac Country Club	1,433	319	45	50	90
6/18/03	HW-20	Pali Golf Course	3,133	696	77	50	154
6/18/03	HW-9	Castle High School	25,933	5,763	284	50	568
6/18/03	HC-18	Lagoon Drive Chevron Service Station	2,433	541	371	50	742
6/18/03	HC-4	Building Industry Association Hawaii	3,033	674	87	50	174

Appendix Table 3—*Continued*

Date	BWS Sample No.	BWS Site Name	ATP RLU/450 ml	ATP RLU/100 ml	No. Colonies per Membrane	Sample Volume	THB CFU/100 ml
6/20/03	HW-7	Olomana Fire Station	2,044	454	132	50	264
6/20/03	HW-1	Waimanalo Shopping Center	54,944	12,210	114	36	317
6/20/03	HW-11	Kaneohe Fire Station	14,944	3,321	918	50	1,836
6/20/03	HC-26	Kalama Valley Park	1,744	388	160	50	320
6/20/03	HW-18	Waimanalo District Park	10,944	2,432	268	50	536
6/20/03	HW-2	Waimanalo Beach Park	3,544	788	85	46	185
6/20/03	HC-25	Hawaii Kai Fire Station	1,244	276	89	50	178
6/20/03	HC-27	Kamiloiki Neighborhood Park	3,744	832	94	46	204
6/20/03	HW-3	Jehovah Witness Hall	39,944	8,876	549	50	1,098
6/20/03	HC-30	869 Alamuku Street	3,444	765	7	50	14
6/20/03	HW-21	Heeia NP	3,144	699	415	50	830
6/20/03	HC-29	Niu Valley Intermediate School	2,344	521	42	50	84
6/20/03	HW-12	46-445-D Kahuhipa Street	16,944	3,765	228	50	456
6/20/03	HC-28	Hahaione Neighborhood Park	10,944	2,432	166	50	332
6/20/03	HC-19	Kawaikui Beach Park	13,944	3,099	148	50	296
6/23/03	WU-17	Ewa Elementary School	5,681	1,262	92	50	184
6/23/03	WU-1	Silva Store	10,681	2,373	115	50	230
6/23/03	HC-15	Alewa Booster #1	7,581	1,685	121	50	242
6/23/03	WU-13	Kaleiopuu Park	1,081	240	12	50	24
6/23/03	WU-2	Makakilo Booster #2	6,581	1,462	112	50	224
6/23/03	HC-21	Booth District Park	3,681	818	141	50	282
6/23/03	WU-21	Palailai Mall	6,681	1,485	175	50	350
6/23/03	HC-37	C.P.B. 960 Mapunapuna Street	13,681	3,040	17	50	34
6/23/03	HC-7	Honolulu BWS	3,481	773	267	50	534
6/23/03	WU-16	Kunia NP	5,381	1,196	65	50	130
6/23/03	WU-3	Makakilo Booster #4	15,681	3,485	350	50	700
6/23/03	HC-34	Nuuanu Fire Station	9,681	2,151	384	50	768

Appendix Table 3—*Continued*

Date	BWS Sample No.	BWS Site Name	ATP RLU/450 ml	ATP RLU/100 ml	No. Colonies per Membrane	Sample Volume	THB CFU/100 ml
6/23/03	HC-38	Moanalua Fire Station	8,381	1,862	263	50	526
6/23/03	WU-20	Kapolei Elementary School	5,581	1,240	91	50	182
6/23/03	HC-12	Central Fire Station	1,781	396	251	50	502
6/25/03	WU-18	Holomua Elementary School	1,147	255	43	50	86
6/25/03	WU-5	Honowai Park	9,047	2,010	30	50	60
6/25/03	HC-3	Kewalo Basin Park	23,947	5,321	86	50	172
6/25/03	WU-10	Waipahu Elementary School	777	173	5	50	10
6/25/03	HM-8	2553 D Booth Road	5,547	1,233	89	50	178
6/25/03	WU-11	Waipahu Intermediate School	617	137	6	50	12
6/25/03	WU-4	St. Joseph School	2,047	455	115	50	230
6/25/03	HM-3	3609 Nuuanu Pali Drive	3,447	766	452	50	904
6/25/03	HC-39	Salt Lake Chevron	10,947	2,433	79	50	158
6/25/03	WU-14	Puuloa Plgd.	20,947	4,655	98	50	196
6/25/03	HC-2	Kalihi Kai Fire Station	11,947	2,655	339	50	678
6/25/03	HC-40	Alianamu Park	3,647	810	88	50	176
6/25/03	WU-6	Hans L'Orange Park	5,547	1,233	52	50	104
6/25/03	WU-9	Waipahu Fire Station	21,947	4,877	148	50	296
6/25/03	HC-35	1972 Ala Mahamoe Street	1,147	255	140	50	280
7/7/03	WE-2	SSB Chevron	1,862	414	215	50	430
7/7/03	WE-6	Sunset Beach Support Park	8,462	1,881	492	50	984
7/7/03	WE-1	Sunset Beach Church of Christ	25,962	5,769	712	50	1,424
7/7/03	WA-6	Waimea Bay BP	1,362	303	158	50	316
7/7/03	WA-9	Haleiwa Chevron	68,962	15,325	46	50	92
7/7/03	WE-4	UH Waialee Exp. Station	9,962	2,214	111	25	444
7/7/03	WA-4	Waialua Recreation Center	15,962	3,547	65	50	130
7/7/03	WE-5	Turtle Bay Golf Course	7,062	1,569	61	50	122
7/7/03	WA-2	Waialua Fire Station	6,662	1,481	387	50	774

Appendix Table 3—*Continued*

Date	BWS Sample No.	BWS Site Name	ATP RLU/450 ml	ATP RLU/100 ml	No. Colonies per Membrane	Sample Volume	THB CFU/100 ml
7/7/03	WA-8	Waialua Inter/High School	4,662	1,036	39	25	156
7/7/03	WA-5	Sunset Beach Fire Station	11,962	2,658	711	50	1,422
7/7/03	MI-3	Noholoa Neighborhood Park	5,862	1,303	181	50	362
7/7/03	MI-11	Kuahelani Park	4,062	903	8	50	16
7/7/03	WA-3	Haleiwa Community Center	12,962	2,881	26	50	52
7/14/03	WE-3	Ehukai Beach Park	1,062	236	89	25	356
7/14/03	WU-7	Waipahu Recreation Center	279,804	62,179	190	50	380
7/14/03	WN-11	Makaha Valley Playground	5,704	1,268	114	50	228
7/14/03	WN-13	Koa Iki Head Start	14,804	3,290	661	50	1,322
7/14/03	WH-11	95-023 Waihau Street	9,704	2,156	311	50	622
7/14/03	WH-2	Kemoo Farms Snack Bar	2,604	579	16	50	32
7/14/03	WU-15	Waikele Fire Station	2,504	556	164	50	328
7/14/03	WH-14	142 Lake View Circle	3,504	779	237	50	474
7/14/03	MI-1	Kipapa Park	4,704	1,045	95	50	190
7/14/03	WN-1	Makaha Valley Plantation	94	21	27	25	108
7/14/03	MI-15	Mililani Mauka Fire Station	6,604	1,468	447	50	894
7/14/03	WN-2	Keau Beach Park	5,804	1,290	193	25	772
7/14/03	MI-2	Mililani Fire Station	1,104	245	55	50	110
7/14/03	WH-13	Melemanu Neighborhood Park	15,804	3,512	294	50	588
7/14/03	MI-14	Mililani Middle School	10,804	2,401	83	50	166
7/16/03	MI-9	Hokuahiahi Neighborhood Park	2,630	584	336	50	672
7/16/03	MI-3	Noholoa Neighborhood Park	670	149	251	50	502
7/16/03	WH-7	Wahiawa Recreation Center	4,830	1,073	187	50	374
7/16/03	WH-5	Wahiawa Fire Station	239,930	53,318	683	50	1,366
7/16/03	HM-1	Kalihi Uka Neighborhood Park	24,930	5,540	478	50	956
7/16/03	MI-11	Kuahelani Park	810	180	3	50	6
7/16/03	MI-13	Kaloapau Neighborhood Park	7,030	1,562	100	50	200

Appendix Table 3—*Continued*

Date	BWS Sample No.	BWS Site Name	ATP RLU/450 ml	ATP RLU/100 ml	No. Colonies per Membrane	Sample Volume	THB CFU/100 ml
7/16/03	WP-5	Waipio Neighborhood Park	41,930	9,318	459	50	918
7/16/03	WP-3	Gentry-Waipio Shopping Center	1,230	273	64	50	128
7/16/03	WH-6	Wahiawa Elementary School	2,030	451	14	50	28
7/16/03	WP-5	Waipio Neighborhood Park	2,030	451	282	50	564
7/16/03	WH-10	Leileihua High School	330	73	221	50	442
7/16/03	WH-8	Harry and Jeannette Weinberg Silvercrest	1,030	229	94	50	188
7/16/03	MI-10	Mililani Golf Club	3,530	784	14	50	28
7/16/03	HW-1	Waimanalo SC	2,130	473	50	50	100
7/21/03	HP-19	Pearlridge Community Park	406	90	36	25	144
7/21/03	HP-15	Waiau Neighborhood Park	2,876	639	405	50	810
7/21/03	HP-14	Halawa Xeriscape Garden	3,476	772	39	25	156
7/21/03	HP-23	Webling Elementary School	35,976	7,995	667	25	2,668
7/21/03	HM-1	Kalihi Uka Neighborhood Park	936	208	212	25	848
7/21/03	HP-13	Kaonohi Booster #2	5,076	1,128	147	25	588
7/21/03	HP-12	98-337 Pono Street	436	97	41	25	164
7/21/03	HP-11	Napuanani Park	19,976	4,439	172	25	688
7/21/03	HP-10	Aiea Booster #3	13,976	3,106	99	25	396
7/21/03	HP-9	Pearl City Rec Center	566	126	131	25	524
7/21/03	HP-1	99-739 Halawa Heights Road	776	172	80	25	320
7/21/03	HP-2	Aiea Fire Station	9,776	2,172	173	25	692
7/21/03	HP-7	Pearl City Booster #1	1,076	239	106	25	424
7/21/03	HP-5	869 Hoomalu Street	786	175	23	25	92
7/23/03	KH-1	Kahuku Hospital	3,688	820	113	25	452
7/23/03	HW-22	Kapunahala Playground	32,888	7,308	615	25	2,460
7/23/03	KH-3	Kahuku Elderly Home	24,888	5,531	424	25	1,696
7/23/03	HW-17	Hauula Fire Station	3,988	886	22	25	88

Appendix Table 3—*Continued*

Date	BWS Sample No.	BWS Site Name	ATP RLU/450 ml	ATP RLU/100 ml	No. Colonies per Membrane	Sample Volume	THB CFU/100 ml
7/23/03	KH-4	Kahuku District Park	15,888	3,531	464	25	1,856
7/23/03	HW-21	Heeia NP	3,388	753	174	25	696
7/23/03	WA-1	Pupukea Booster #2	1,488	331	87	25	348
7/23/03	WE-1	Sunset Beach Church of Christ	3,888	864	930	25	3,720
7/23/03	HW-13	Ahuimanu Elementary School Plgd.	2,388	531	131	25	524
7/23/03	HW-14	Waihee Line Booster	538	120	12	25	48
7/23/03	WA-7	Haleiwa 7-11	6,688	1,486	170	25	680
7/23/03	HW-15	Kaaawa Fire Station	20,888	4,642	15	25	60
7/23/03	KH-2	Kahuku High School	798	177	6	25	24
7/23/03	HW-16	Punaluu Beach Park	13,888	3,086	186	25	744
7/23/03	HW-23	Kainalu Elementary School	1,288	286	77	25	308
7/23/03	WA-5	Sunset Beach Fire Station	17,888	3,975	668	25	2,672
7/25/03	HM-1	Kalihi Uka Neighborhood Park	2,649	589	126	25	504
7/25/03	HP-15	Waiau NP	65,949	14,655	48	25	192
9/26/03	WA-2	Waialua Fire	47,944	10,654	329	50	658
9/26/03	WE-6	Sunset Beach Support Park	934	207	121	50	242
9/26/03	WA-6	Waimea Beach Park	14,944	3,321	165	50	330
9/26/03	WA-3	Haleiwa Community Surf Center	19,944	4,432	115	50	230
9/29/03	WE-3	Ehukai Beach Park	7,344	1,632	558	50	1,116
9/29/03	WN-6	Pililaau Playground	186	41	194	50	388
9/29/03	WN-7	Nanakuli Fire Station	376	83	277	50	554
9/29/03	WN-12	Kaupuni Neighborhood Park	536	119	161	50	322
9/29/03	WN-4	BWS Waianae Corp. Yard	1,276	283	289	50	578
9/29/03	WN-14	Kamehameha School Koaliku Drake	866	192	166	50	332
9/29/03	WN-3	Ulehawa Beach Park #2	526	117	309	50	618
9/29/03	WN-10	Kamaile Elementary School	1,376	306	177	50	354
9/29/03	WN-5	Pokai Bay Beach Park	446	99	337	50	674

Appendix Table 4. THB Well Isolates Analyzed by the RiboPrinter

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
1	Hoaeae Wells	Well	WUS-01	WUS-01B	277-41-S-1	Delftia acidovorans	DUP-18546	0.9
2	Hoaeae Wells	Well	WUS-01	WUS-01C	277-41-S-2	None		
3	Hoaeae Wells	Well	WUS-01	WUS-01D	277-41-S-3	None		
4	Hoaeae Wells	Well	WUS-01	WUS-01G	277-41-S-4	None		
5	Hoaeae Wells	Well	WUS-01	WUS-01E1	277-42-S-1	None		
6	Hoaeae Wells	Well	WUS-01	WUS-01E2	277-42-S-2	None		
7	Moanalua Wells	Well	HS-20	HS-20A	277-42-S-3	None		
8	Moanalua Wells	Well	HS-20	HS-20(3A)	277-46-S-6	None		
9	Moanalua Wells	Well	HS-20	HS-20(3B)	277-46-S-7	None		
10	Moanalua Wells	Well	HS-20	HS-20(3C)	277-46-S-8	Delftia acidovorans	DUP-18546	0.86
11	Wilder Avenue Wells	Well	HS-14(4)	HS-14(4)A	277-47-S-4	None		
12	Wilder Avenue Wells	Well	HS-14(4)	HS-14(4)B	277-47-S-4	None		
13	Wilder Avenue Wells	Well	HS-14(4)	HS-14(4)C	277-47-S-4	None		
14	Wilder Avenue Wells	Well	HS-14(4)	HS-14(4)D	277-47-S-7	None		
15	Wilder Avenue Wells	Well	HS-14(4)	HS-14(4)E	277-47-S-7	None		
16	Makaha Well I	Well	WNS-03	WNS-03A	277-48-S-4	None		
17	Makaha Well I	Well	WNS-03	WNS-03B	277-48-S-5	None		
18	Makaha Well I	Well	WNS-03	WNS-03C	277-48-S-5	None		
19	Makaha Well I	Well	WNS-03	WNS-03D	277-48-S-7	None		
20	Makaha Well II	Well	WNS-13	WNS-13A	277-50-S-1	Delftia acidovorans	DUP-18546	0.87
21	Makaha Well II	Well	WNS-13	WNS-13B	277-50-S-2	None		
22	Palolo Deep Well	Well	HS-22	HS-22A	277-52-S-1	Glaciecola pallidula	DUP-18328	0.91
23	Mililani Wells II	Well	MIS-02	MIS-02C	277-52-S-2	None		
24	Manoa 405 Wells	Well	HS-29	HS-29A	277-52-S-3	None		
25	Wailupe Well	Well	HS-30	HS-30C	277-52-S-4	None		
26	Kuliouou Well	Well	HS-36	HS-36A	277-52-S-5	Stenotrophomonas maltophilia	DUP-18768	0.86
27	Wahiawa Wells I	Well	WHS-01	WHS-01A	277-52-S-6	None		
28	Wahiawa Wells I	Well	WHS-01	WHS-01B	277-52-S-7	Ochrobactrum anthropi	DUP-18219	0.93
29	Wahiawa Wells II	Well	WHS-02	WHS-02A	277-52-S-8	None		
30	Aina Koa Well	Well	HS-17	HS-17A	277-55-S-1	None		
31	Wailupe Well	Well	HS-30	HS-30B	277-55-S-3	None		
32	Mililani Wells I	Well	MIS-01	MIS-01A	277-50-S-8	None		
33	Mililani Wells I	Well	MIS-01	MIS-01B	277-55-S-5	None		
34	Makaha Well V	Well	WNS-06	WNS-06B	277-55-S-6	None		
35	Makaha Well VI	Well	WNS-07	WNS-07A	277-55-S-7	None		
36	Makaha Well VI	Well	WNS-07	WNS-07B	277-55-S-8	None		
37	Waipahu Wells II	Well	WUS-08	WUS-08A	277-61-S-1	None		
38	Waipahu Wells II	Well	WUS-08	WUS-08B	277-61-S-2	None		

Appendix Table 4—*Continued*

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
39	Waianae Well III	Well	WNS-08	WNS-08A	277-61-S-3	None		
40	Waianae Well III	Well	WNS-08	WNS-08B	277-61-S-4	Bacillus thuringiensis	DUP-6040	0.9
41	Waianae Well III	Well	WNS-08	WNS-08C	277-61-S-5	None		
42	Hoaeae Wells	Well	WUS-01	WUS-01A	277-62-S-1	None		
43	Moanalua Well	Well	HS-20	HS-20A	277-62-S-2	None		
44	Makakilo Well	Well	WUS-05	WUS-05A	277-62-S-3	None		
45	Makakilo Well	Well	WUS-05	WUS-05B	277-62-S-4	None		
46	Honouliuli Wells I	Well	WUS-06	WUS-06A	277-62-S-5	Pseudomonas aeruginosa	DUP-18083	0.93
47	Mililani Wells IV	Well	MIS-04	MIS-04B	277-62-S-6	None		
48	Wilder Avenue Wells	Well	HS-14-1	HS-14-1A	277-62-S-8	Flavobacterium species	DUP-18211	0.9
49	Punaluu Wells III	Well	HWS-14	HWS-14A	277-63-S-4	None		
50	Waipio Height Well	Well	WPS-01	WPS-01A	277-63-S-5	None		
51	Newtown Wells	Well	HPS-10	HPS-10A	277-63-S-6	None		
52	Newtown Wells	Well	HPS-10	HPS-10B	277-63-S-7	None		
53	Kunia Wells II	Well	WUS-04	WUS-04A	277-63-S-8	Lactobacilllus pontis	DIP-13160	0.87
54	Hoaeae Wells	Well	WUS-01	WUS-01A	277-61-S-6	None		
55	Hoaeae Wells	Well	WUS-01	WUS-01B	277-42-S-1	None		
56	Waipio Heights Wells II	Well	WPS-03	WPS-03B	277-80-S-4	None		
57	Waiau Wells	Well	HPS-09	HPS-09A	277-47-S-3	None		
58	Waiau Wells	Well	HPS-09	HPS-09B	277-50-S-1	Delftia acidovorans	DUP-18546	
59	Kalihi Station	Well	HS-3-LS	HS-3-LSA	277-57-S-6	Acinetobacter lwoffii	DUP-16763	0.92
60	Kalihi Station	Well	HS-3-LS	HS-3-LSB	277-47-S-3	None		
61	Honouliuli Wells I	Well	WUS-06	WUS-06A	277-82-S-1	None		
62	Honouliuli Wells I	Well	WUS-06	WUS-06B	277-82-S-2	None		
63	Honouliuli Wells I	Well	WUS-06	WUS-06C	277-82-S-3	Legionella pneumophila	DUP-18315	0.96
64	Pearl City Well I	Well	HPS-02	HPS-02A	277-82-S-4	Weissella halotolerans	DUP-135-3	0.91
65	Pearl City Well I	Well	HPS-02	HPS-02B	277-82-S-5	Sphingomonas paucimobilis	DUP-10112	0.91
66	Pearl City Well III	Well	HPS-11	HPS-11A	277-82-S-6	Stenotrophomonas maltophilia	DUP-11077	0.91
67	Pearl City Well III	Well	HPS-11	HPS-11B	277-82-S-7	Legionella pneumophila ss. Fraseri	DUP-18287	0.93
68	Pearl City Well III	Well	HPS-11	HPS-11C	277-82-S-8	None		
69	Wilder Avenue Wells	Well	HS-14-4	HS-14-4F	277-83-S-1	None		
70	Wilder Avenue Wells	Well	HS-14-4	HS-14-4G	277-83-S-2	Bacillus thuringiensis	DUP-16815	0.96
71	Wilder Avenue Wells	Well	HS-14-4	HS-14-4	277-83-S-3	None		
72	Waipahu Wells	Well	WUS-03	WUS-03A	277-83-S-6	None		
73	Waipahu Wells	Well	WUS-03	WUS-03B	277-83-S-7	Staphylococcus hominis	DUP-16658	0.96
74	Waipahu Wells II	Well	WUS-08	WUS-08C	277-83-S-8	None		
75	HECO Waiau Wells	Well	HPS-12	HPS-12A	277-84-S-1	None		
76	Kapalama Well	Well	HS-37-1	HS-37-1B	277-84-S-6	None		

Appendix Table 4—*Continued*

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
77	Waianae Well III	Well	WNS-08	WNS-08D	277-84-S-8	None		
78	Honouliuli Wells II	Well	WUS-07	WUS-07A	277-85-S-1	None		
79	Honouliuli Wells II	Well	WUS-07	WUS-07B	277-85-S-2	None		
80	Honouliuli Wells II	Well	WUS-07	WUS-07C	277-85-S-3	None		
81	Beretania Station Low Service	Well	HS-4,LS	HS-4, LSA	277-50-S-8	None		
82	Beretania Station High Service	Well	HS4, HS	HS-4, HAS	277-85-S-5	None		
83	Beretania Station High Service	Well	HS-4, HS	HS-4, HSB	277-85-S-6	None		
84	Kalauao Wells	Well	HS-15	HS-15A	277-85-S-3	None		
85	Kalihi Station	Well	HS-03, HS	HS-03, HAS	277-85-S-8	Bacillus megaterium	DUP-14711	0.9
86	Kaonohi Wells I	Well	HPS-05	HPS-05A	277-86-S-5	None		
87	Kaonohi Wells I	Well	HPS-05	HPS-05B	277-86-S-5	None		
88	Kaonohi Wells I	Well	HPS-05	HPS-05C	277-86-S-7	None		
89	Kaonohi Wells I	Well	HPS-05	HPS-05D	277-54-S-5	Bacillus thuringiensis	DUP-6040	0.91
90	Luluku Well	Well	HWS-18	HWS-18A	277-88-S-1	Staphylococcus warneri	DUP-18755	0.96
91	Luluku Well	Well	HWS-18	HWS-18B	277-88-S-2	Bacillus pumilus	DUP-18516	0.94
92	Luluku Well	Well	HWS-18	HWS-18C	277-88-S-3	Staphylococcus pasteuri	DUP-4235	0.93
93	Luluku Well	Well	HWS-18	HWS-18D	277-88-S-2	Bacillus pumilus	DUP-18516	0.94
94	Pearl City Well III	Well	HPS-11	HPS-11B	277-93-S-1	Vibrio vulnificus	DUP-16918	0.88
95	Pearl City Well III	Well	HPS-11	HPS-11D	277-93-S-1	Vibrio vulnificus	DUP-16918	0.87
96	Palolo Deep Well	Well	HS-22, 2	HS-22, 2A	277-93-S-4	None		
97	Beretania Station	Well	HS-04, HS	HS-04, HSC	277-93-S-5	Staphylococcus xylosus	DUP-4249	0.92
98	Mililani Wells II	Well	MIS-02	MIS-02, #6	277-102-S-2	None		
99	Kaahumanu Wells	Well	HCS-26	HCS-26A	277-41-S-1	Delftia acidovorans	DUP-18546	0.87
100	Kaahumanu Wells	Well	HCS-26	HCS-26C	277-41-S-1	None		
101	Kaahumanu Wells	Well	HCS-26	HCS-26D	277-102-S-5	None		
102	Kaahumanu Wells	Well	HCS-26	HCS-26E	277-41-S-1	None		
103	Wahiawa Wells I	Well	WHS-01, #1	WHS-01, #1A	277-102-S-7	None		
104	Wahiawa Wells I	Well	WHS-01, #1	WHS-01, #1C	277-102-S-8	None		
105	Kaamilo Wells	Well	HCS-19	HCS-19A	277-50-S-8	None		
106	Aiea 260 Wells	Well	HPS-06	HPS-06D	277-104-S-4	None		
107	Punaluu Wells II	Well	HWS-10	HWS-10B	277-128-S-1	None		
108	Punaluu Wells II	Well	HWS-10	HWS-10C	277-121-S-2	None		
109	Punaluu Wells III	Well	HWS-14	HWS-14B	277-128-S-3	None		
110	Punaluu Wells III	Well	HWS-14	HWS-14C	277-128-S-4	None		
111	Iolekaa Wells	Well	HWS-19	HWS-19A	277-128-S-5	None		
112	Iolekaa Wells	Well	HWS-19	HWS-19B	277-128-S-6	None		
113	Manoa 405 Wells	Well	HS-29	HS-29B	277-128-S-7	None		
114	Manoa 405 Wells	Well	HS-29	HS-29C	277-128-S-8	None		

Appendix Table 4—*Continued*

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
115	Manoa 405 Wells	Well	HS-29	HS-29D	277-130-S-2	None		
116	Hoaeae Well Pump 1	Well	WUS-01 (P1)	WUS-01 (P1) H	277-47-S-7	None		
117	Hoaeae Well Pump 1	Well	WUS-01 (P1)	WUS-01 (P1) I	277-140-S-2	None		
118	Hoaeae Well Pump 4	Well	WUS-01 (P4)	WUS-01 (P4) J	277-140-S-3	None		
119	Hoaeae Well Pump 4	Well	WUS-01 (P4)	WUS-01 (P4) K	277-140-S-3	None		
120	Hoaeae Well Pump 4	Well	WUS-01 (P4)	WUS-01 (P4) L	277-140-S-5	None		
121	Hoaeae Well Pump 4	Well	WUS-01 (P4)	WUS-01 (P4) M	277-140-S-6	None		
122	Kaonohi Wells I	Well	HPS-05	HPS-05E (P1)	277-146-S-1	None		
123	Kaonohi Wells I	Well	HPS-05	HPS-05F (P1)	277-146-S-2	None		
124	Kaonohi Wells I	Well	HPS-05	HPS-05G (P1)	277-146-S-2	None		
125	Kaonohi Wells I	Well	HPS-05	HPS-05H (P1)	277-146-S-4	Escherichia coli	DUP-14194	0.93
126	Kaonohi Wells I	Well	HPS-05	HPS-05I (P1)	277-146-S-5	None		
127	Kaonohi Wells I	Well	HPS-05	HPS-05J (P1)	277-146-S-2	None		
128	Kaonohi Wells I	Well	HPS-05	HPS-05K (P1)	277-146-S-2	None		
129	Kaonohi Wells I	Well	HPS-05	HPS-05 (P1)	277-146-S-8	Staphylococcus hominis	DUP-15246	0.87
130	Waipahu Wells II	Well	WUS-08	WUS-08D	277-148-S-3	None		
131	Kalauao Wells	Well	HCS-15	HCS-15A	277-148-S-5	None		
132	Kalauao Wells	Well	HCS-15	HCS-15B	277-148-S-6	None		
133	Kalauao Wells	Well	HCS-15	HCS-15C	277-148-S-7	None		
134	Makakilo Well	Well	WUS-05	WUS-05C	277-167-S-1	Pseudomonas aeruginosa	DUP-18083	0.96
135	Makakilo Well	Well	WUS-05	WUS-05D	277-167-S-2	None		
136	Makakilo Well	Well	WUS-05	WUS-05E	277-167-S-1	Pseudomonas aeruginosa	DUP-18083	0.94
137	Makakilo Well	Well	WUS-05	WUS-05F	277-146-S-5	None		
138	Makakilo Well	Well	WUS-05	WUS-05G	277-167-S-5	None		
139	Makakilo Well	Well	WUS-05	WUS-05H	277-167-S-1	Pseudomonas aeruginosa	DUP-18083	0.95
140	Makakilo Well	Well	WUS-05	WUS-05I	277-167-S-1	Pseudomonas aeruginosa	DUP-18083	0.89

Appendix Table 5. THB Tunnel Isolates Analyzed by the RiboPrinter

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
1	Palolo Tunnel	Tunnel	HS-12	HS-12A	277-49-S-1	None		
2	Palolo Tunnel	Tunnel	HS-12	HS-12B	277-49-S-2	None		
3	Palolo Tunnel	Tunnel	HS-12	HS-12C	277-49-S-3	None		
4	Palolo Tunnel	Tunnel	HS-12	HS-12E	277-49-S-5	None		
5	Palolo Tunnel	Tunnel	HS-12	HS-12F	277-49-S-6	None		
6	Palolo Tunnel	Tunnel	HS-12	HS-12G	277-49-S-7	None		
7	Palolo Tunnel	Tunnel	HS-12	HS-12H	277-49-S-8	None		
8	Manoa Tunnel	Tunnel	HS-11	HS-11A	277-50-S-8	None		
9	Waianae Tunnel	Tunnel	WNS-04	WNS-04B	277-61-S-6	None		
10	Waianae Tunnel	Tunnel	WNS-04	WNS-04C	277-83-S-4	Bacillus cereus	DUP-6082	0.9
11	Waianae Tunnel	Tunnel	WNS-04	WNS-04D	277-83-S-5	Bacillus thuringiensis	DUP-6032	0.91
12	Waianae Plant Tunnels	Tunnel	WNS-05	WNS-05A	277-84-S-7	None		
13	Palolo Tunnel	Tunnel	HWS-12	HWS-12I	277-88-S-5	None		
14	Palolo Tunnel	Tunnel	HWS-12	HWS-12J	277-88-S-5	None		
15	Palolo Tunnel	Tunnel	HWS-12	HWS-12K	277-88-S-7	Chryseobacterium meningosepticum	DUP-16699	0.86
16	Palolo Tunnel	Tunnel	HWS-12	HWS-12L	277-88-S-8	Idiomarina zobellii	DUP-18431	0.87
17	Palolo Tunnel	Tunnel	HS-12	HS-12M	277-93-S-6	None		
18	Palolo Tunnel	Tunnel	HS-12	HS-12N	277-93-S-7	None		
19	Palolo Tunnel	Tunnel	HS-12	HS-12O	277-93-S-8	None		
20	Palolo Tunnel	Tunnel	HS-12	HS-12P	277-172-S-1	None		
21	Palolo Tunnel	Tunnel	HS-12	HS-12Q	277-172-S-2	None		
22	Palolo Tunnel	Tunnel	HS-12	HS-12R	277-172-S-3	None		
23	Palolo Tunnel	Tunnel	HS-12	HS-12S	277-172-S-4	None		
24	Palolo Tunnel	Tunnel	HS-12	HS-12T	277-172-S-5	None		
25	Palolo Tunnel	Tunnel	HS-12	HS-12U	277-172-S-4	None		
26	Palolo Tunnel	Tunnel	HS-12	HS-12V	277-172-S-4	None		
27	Palolo Tunnel	Tunnel	HS-12	HS-12W	277-172-S-8	None		

Appendix Table 6. THB Shaft Isolates Analyzed by the RiboPrinter

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
1	Pearl City Shaft	Shaft	HPS-01(1)	HPS-01(1)A	277-45-S-1	None		
2	Pearl City Shaft	Shaft	HPS-01(1)	HPS-01(1)B	277-45-S-1	None		
3	Pearl City Shaft	Shaft	HPS-01(1)	HPS-01(1)C	277-45-S-3	None		
4	Pearl City Shaft	Shaft	HPS-01(1)	HPS-01(1)D	277-45-S-4	None		
5	Pearl City Shaft	Shaft	HPS-01(1)	HPS-01(1)E	277-45-S-5	None		
6	Pearl City Shaft	Shaft	HPS-01(1)	HPS-01(1)F	277-45-S-6	Pseudomonas putida	DUP-12014	0.93
7	Pearl City Shaft	Shaft	HPS-01(1)	HPS-01(1)G	277-45-S-4	None		
8	Pearl City Shaft	Shaft	HPS-01(1)	HPS-01(1)H	277-45-S-8	None		
9	Pearl City Shaft	Shaft	HPS-01(1)	HPS-01(1)I	277-46-S-1	None		
10	Pearl City Shaft	Shaft	HPS-01(1)	HPS-01(1)J	277-46-S-2	None		
11	Pearl City Shaft	Shaft	HPS-01(1)	HPS-01(1)K	277-47-S-1	None		
12	Pearl City Shaft	Shaft	HPS-01(1)	HPS-01(1)N	277-47-S-2	None		
13	Pearl City Shaft	Shaft	HPS-01(1)	HPS-01(1)O	277-47-S-3	None		
14	Makaha Shaft	Shaft	WNS-02	WNS-02A	277-50-S-3	None		
15	Makaha Shaft	Shaft	WNS-02	WNS-02B	277-50-S-3	None		
16	Makaha Shaft	Shaft	WNS-02	WNS-02C	277-50-S-3	None		
17	Makaha Shaft	Shaft	WNS-02	WNS-02D	277-50-S-3	None		
18	Makaha Shaft	Shaft	WNS-02	WNS-02E	277-50-S-7	Bacillus pumilus		
19	Halawa Shaft	Shaft	HS-01	HS-01B	277-61-S-7	None		
20	Halawa Shaft	Shaft	HS-01	HS-01C	277-61-S-8	None		
21	Kalihi Shaft	Shaft	HS-02	HS-02A	277-62-S-7	Pseudomonas aeruginosa	DUP-6406	0.89
22	Kalihi Shaft	Shaft	HS-02	HS-02C	277-47-S-7	None		
23	Kalihi Shaft	Shaft	HS-02	HS-02D	277-50-S-8	None		
24	Kalihi Shaft	Shaft	HS-02	HS-02E	277-84-S-4	Lactococcus lactis	DUP-12763	0.85
25	Kalihi Shaft	Shaft	HS-02	HS-02F	277-84-S-5	None		
26	Pearl City Shaft	Shaft	HPS-01	HPS-01P	277-86-S-1	None		
27	Pearl City Shaft	Shaft	HPS-01	HPS-01Q	277-86-S-2	None		
28	Pearl City Shaft	Shaft	HPS-01	HPS-01R	277-86-S-3	None		
29	Pearl City Shaft	Shaft	HPS-01	HPS-01S	277-86-S-4	None		
30	Kalihi Shaft	Shaft	HS-02	HS-02B	277-47-S-7	None		
31	Pearl City Shaft	Shaft	HPS-01	HPS-01Q	277-101-S-8	None (Rerun)		
32	Pearl City Shaft	Shaft	HPS-01	HPS-01Q	277-103-S-7	None (Rerun)		
33	Pearl City Shaft	Shaft	HPS-01	HPS-01Q	277-86-S-2	None (Rerun)		

Appendix Table 7. THB Reservoir Isolates Analyzed by the RiboPrinter

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
1	Niu 170	Reservoir	HCR-11	HCR-11A	277-56-S-1	None		
2	Niu 170	Reservoir	HCR-11	HCR-11C	277-56-S-2	Pseudomonas aeruginosa	DUP-11042	0.94
3	Hawaii Loa 475	Reservoir	HCR-13	HCR-13A	277-47-S-4	None		
4	Hawaii Loa 475	Reservoir	HCR-13	HCR-13B	277-56-S-4	Pseudomonas putida	DUP-12014	0.9
5	Hawaii Loa 475	Reservoir	HCR-13	HCR-13C	277-56-S-5	None		
6	Waialae Iki 180	Reservoir	HCR-18	HCR-18A	277-56-S-6	None		
7	Waialae Iki 180	Reservoir	HCR-18	HCR-18B	277-56-S-7	None		
8	Waialae Iki 180	Reservoir	HCR-18	HCR-18C	277-56-S-8	None		
9	Halawa 550	Reservoir	HCR-65	HCR-65B	277-57-S-1	None		
10	Kunia 228	Reservoir	WUR-03	WUR-03E	277-57-S-2	Bacillus thuringiensis	DUP-6033	0.93
11	Makakilo 920	Reservoir	WUR-10	WUR-10A	277-57-S-3	None		
12	Makakilo 920	Reservoir	WUR-10	WUR-10B	277-57-S-2	Bacillus thuringiensis	DUP-6033	0.94
13	Aiea 782	Reservoir	HPR-03	HPR-03A	277-57-S-5	Stenotrophomonas maltophilia	DUP-10113	0.91
14	Aiea 782	Reservoir	HPR-03	HPR-03B	277-57-S-6	Acinetobacter lwoffii	DUP-16763	0.89
15	Aiea 782	Reservoir	HPR-03	HPR-03C	277-57-S-6	Acinetobacter lwoffii	DUP-16763	0.88
16	Aiea 782	Reservoir	HPR-03	HPR-03D	277-47-S-3	None		
17	Aina Haina 170	Reservoir	HCR-16	HCR-16B	277-54-S-5	Bacillus thuringiensis	DUP-6040	0.94
18	Melemanu 808 Reservoir	Reservoir	WHR-06	WHR-06A	277-58-S-2	None		
19	Melemanu 808 Reservoir	Reservoir	WHR-06	WHR-06B	277-58-S-3	Bacillus thuringiensis	DUP-6031	0.9
20	Melemanu 808 Reservoir	Reservoir	WHR-06	WHR-06C	277-58-S-4	Pseudomonas aeruginosa	DUP-18040	0.95
21	Melemanu 808 Reservoir	Reservoir	WHR-06	WHR-06D	277-58-S-5	None		
22	Melemanu 808 Reservoir	Reservoir	WHR-06	WHR-06E	277-58-S-6	None		
23	Melemanu 808 Reservoir	Reservoir	WHR-06	WHR-06F	277-58-S-7	None		
24	Bella Vista 180	Reservoir	HCR-47	HCR-47A	277-64-S-1	Staphylococcus warneri	DUP-4227	0.92
25	Bella Vista 180	Reservoir	HCR-47	HCR-47B	277-64-S-3	None		
26	Hahaione 500	Reservoir	HCR-09	HCR-09A	277-64-S-3	None		
27	Hahaione 500	Reservoir	HCR-09	HCR-09B	277-64-S-4	None		
28	Hahaione 500	Reservoir	HCR-09	HCR-09C	277-64-S-5	None		
29	Hahaione 500	Reservoir	HCR-09	HCR-09D	277-64-S-5	None		
30	Hahaione 500	Reservoir	HCR-09	HCR-09E	277-64-S-7	None		
31	Hahaione 500	Reservoir	HCR-09	HCR-09F	277-64-S-5	None		
32	Kaamilo 497	Reservoir	HPR-05	HPR-05A	277-65-S-1	Bacillus cereus	DUP-13207	0.92
33	Kaamilo 497	Reservoir	HPR-05	HPR-05B	277-65-S-2	Bacillus cereus	DUP-6050	0.91
34	Diamond Head 180	Reservoir	HCR-30	HCR-30A	277-65-S-3	None		
35	Diamond Head 180	Reservoir	HCR-30	HCR-30B	277-65-S-4	None		
36	Diamond Head 180	Reservoir	HCR-30	HCR-30C	277-47-S-3	None		
37	Diamond Head 180	Reservoir	HCR-30	HCR-30D	277-65-S-6	None		
38	Kunia 440 (2)	Reservoir	WUR-05	WUR-05A	277-65-S-7	None		

Appendix Table 7—*Continued*

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
39	Kunia 440 (2)	Reservoir	WUR-05	WUR-05B	277-65-S-8	None		
40	Waipahu 228 (1)	Reservoir	WUR-01	WUR-01A	277-66-S-1	None		
41	Waipahu 228 (1)	Reservoir	WUR-01	WUR-01B	277-66-S-2	None		
42	Waipahu 228 (2)	Reservoir	WUR-02	WUR-02A	277-66-S-3	None		
43	Haleiwa 225	Reservoir	WAR-02	WAR-02A	277-66-S-5	None		
44	Haleiwa 225	Reservoir	WAR-02	WAR-02B	277-66-S-6	Bacillus sphaericus	DUP-16999	0.95
45	Haleiwa 225	Reservoir	WAR-02	WAR-02C	277-66-S-7	None		
46	Haleiwa 225	Reservoir	WAR-02	WAR-02D	277-66-S-8	None		
47	Makakilo 675	Reservoir	WUR-09	WUR-09A	277-67-S-1	Bacillus sphaericus	DUP-14766	0.89
48	Makakilo 675	Reservoir	WUR-09	WUR-09B	277-67-S-2	Staphylococcus pasteuri	DUP-4221	0.96
49	Makakilo 675	Reservoir	WUR-09	WUR-09C	277-67-S-3	None		
50	Makakilo 675	Reservoir	WUR-09	WUR-09D	277-67-S-4	None		
51	Makakilo 675	Reservoir	WUR-09	WUR-09F	277-67-S-6	Staphylococcus epidermidis	DUP-4124	0.92
52	Makakilo 675	Reservoir	WUR-09	WUR-09G	277-67-S-7	None		
53	Honouliuli 440	Reservoir	WUR-07	WUR-07A	277-68-S-5	None		
54	Honouliuli 440	Reservoir	WUR-07	WUR-07B	277-68-S-6	None		
55	Kunia 440 (1)	Reservoir	WUR-04	WUR-04A	277-68-S-7	None		
56	Kunia 440 (1)	Reservoir	WUR-04	WUR-04B	277-68-S-8	None		
57	Nanakuli 350	Reservoir	WNR-01	WNR-01A	277-69-S-1	Staphylococcus warneri	DUP-18769	0.97
58	Waianae 242	Reservoir	WNR-03	WNR-03A	277-65-S-4	None		
59	Waianae 242	Reservoir	WNR-03	WNR-03B	277-69-S-3	Micrococcus luteus	DUP-14702	0.87
60	Waianae 242	Reservoir	WNR-03	WNR-03C	277-69-S-4	None		
61	Waianae 242	Reservoir	WNR-03	WNR-03E	277-69-S-4	None		
62	Waianae 242	Reservoir	WNR-03	WNR-03F	277-69-S-6	Lactococcus lactis	DUP-5210	0.9
63	Waianae 390 (2)	Reservoir	WNR-05	WNR-05-1A	277-69-S-6	Lactococcus lactis	DUP-5210	0.91
64	Waianae 390 (2)	Reservoir	WNR-05-1	WNR-05-1C	277-70-S-3	None		
65	Waianae 390 (2)	Reservoir	WNR-05-1	WNR-05-1D	277-69-S-1	Staphylococcus warneri	DUP-18769	0.96
66	Waianae 390 (2)	Reservoir	WNR-05-2	WNR-05-2A	277-70-S-5	None		
67	Waianae 390 (2)	Reservoir	WNR-05-2	WNR-05-2B	277-70-S-6	None		
68	Waianae 390 (2)	Reservoir	WNR-05-2	WNR-05-2C	277-70-S-6	None		
69	Waianae 390 (2)	Reservoir	WNR-05-2	WNR-05-2D	277-70-S-6	None		
70	Kapunahala 272	Reservoir	HWR-08	HWR-08A	277-129-S-1	Legionella moravica	DUP-18348	0.94
71	Kapunahala 272	Reservoir	HWR-08	HWR-08B	277-129-S-2	None		
72	Kapunahala 272	Reservoir	HWR-08	HWR-08C	277-129-S-3	None		
73	Kapunahala 272	Reservoir	HWR-08	HWR-08D	277-129-S-3	None		
74	Kapunahala 272	Reservoir	HWR-08	HWR-08E	277-129-S-5	None		
75	Kapunahala 272	Reservoir	HWR-08	HWR-08F	277-129-S-6	Vibrio cholerae	DUP-6605	0.85
76	Kapunahala 272	Reservoir	HWR-08	HWR-08G	277-129-S-7	None		

Appendix Table 7—*Continued*

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
77	Kapunahala 272	Reservoir	HWR-08	HWR-08H	277-129-S-8	None		
78	Kapunahala 272	Reservoir	HWR-08	HWR-08I	277-130-S-1	None		
79	Koko Head 405	Reservoir	HCR-03	HCR-03A	277-132-S-1	None		
80	Koko Head 405	Reservoir	HCR-03	HCR-03B	277-132-S-2	None		
81	Koko Head 405	Reservoir	HCR-03	HCR-03C	277-131-S-1	None		
82	Koko Head 405	Reservoir	HCR-03	HCR-03E	277-131-S-1	Pseudomonas alcaligenes	DUP-10127	0.86
83	Koko Head 405	Reservoir	HCR-03	HCR-03F	277-132-S-5	None		
84	Koko Head 405	Reservoir	HCR-03	HCR-03G	277-132-S-6	None		
85	Kalama 170	Reservoir	HCR-01	HCR-01A	277-132-S-7	None		
86	Kalama 170	Reservoir	HCR-01	HCR-01B	277-132-S-8	None		

Appendix Table 8. THB Distribution Isolates Analyzed by the RiboPrinter

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
1	Diamond Head Line Booster	Distribution	HC-20	HC-20A	277-48-S-2	Legionella pneumophila	DUP-18287	0.89
2	Diamond Head Line Booster	Distribution	HC-20	HC-20B	277-48-S-2	Legionella pneumophila	DUP-18287	0.89
3	1955 Young Street	Distribution	HC-05	HC-05A	277-51-S-2	None		
4	1955 Young Street	Distribution	HC-05	HC-05B	277-51-S-3	None		
5	Kanewai Playground	Distribution	HC-14	HC-14C	277-51-S-4	None		
6	Kanewai Playground	Distribution	HC-14	HC-14D	277-51-S-4	None		
7	Diamond Head Line Booster	Distribution	HC-20	HC-20A	277-48-S-2	Pseudomonas putida	DUP-12010	0.89
8	Diamond Head Line Booster	Distribution	HC-20	HC-20B	277-48-S-2	Pseudomonas putida	DUP-12010	0.89
9	Jack-In-The-Box	Distribution	HC-11	HC-11A	277-50-S-8	None		
10	BWS Waianae Corp. Yard	Distribution	WN-04	WN-04A	277-53-S-1	None		
11	BWS Waianae Corp. Yard	Distribution	WN-04	WN-04B	277-53-S-2	None		
12	Nanakuli Fire Station	Distribution	WN-07	WN-07A	277-53-S-4	Sphingomonas aromaticivorans	DUP-10031	0.88
13	Nanakuli Fire Station	Distribution	WN-07	WN-07B	277-53-S-5	None		
14	Nanakuli Fire Station	Distribution	WN-07	WN-07C	277-47-S-3	None		
15	Maile Elementary School	Distribution	WN-08	WN-08A	277-53-S-7	None		
16	Maile Elementary School	Distribution	WN-08	WN-08B	277-53-S-8	Flavobacterium species	DUP-18217	0.88
17	Noholoa Neighborhood Park	Distribution	MI-03	MI-03A	277-54-S-1	None		
18	Noholoa Neighborhood Park	Distribution	MI-03	MI-03B	277-50-S-1	Delftia acidovorans	DUP-18546	0.87
19	Gentry Waipio Shopping Center	Distribution	WP-03	WP-03A	277-54-S-3	None		
20	Gentry Waipio Shopping Center	Distribution	WP-03	WP-03B	277-54-S-3	None		
21	Waipio Neighborhood Park	Distribution	WP-05	WP-05A	277-54-S-5	Bacillus thuringiensis	DUP-6040	0.93
22	Better Brands	Distribution	WP-06	WP-06A	277-54-S-6	None		
23	Better Brands	Distribution	WP-06	WP-06B	277-54-S-7	None		
24	Harry and Jeannette Weinberg Silvercrest	Distribution	WH-08	WH-08A	277-54-S-8	None		
25	Kamaile Elementary School	Distribution	WN-10	WN-10A	277-69-S-7	Bacillus fusiformis	DUP-14809	0.88
26	Kamehameha Schools Hoaliku Drake	Distribution	WN-14	WN-14A	277-69-S-8	None		
27	Waimanalo Shopping Center	Distribution	HW-01	HW-01A	277-101-S-1	None		
28	Waimanalo Shopping Center	Distribution	HW-01	HW-01B	277-101-S-2	None		
29	Waimanalo Beach Park	Distribution	HW-02	HW-02A	277-101-S-3	None		
30	Waimanalo Beach Park	Distribution	HW-02	HW-02B	277-101-S-4	None		
31	Waimanalo District Park	Distribution	HW-18	HW-18A	277-101-S-5	None		
32	Enchanted Lake Playground	Distribution	HW-19	HW-19B	277-101-S-6	None		
33	Wahiawa Recreation Center	Distribution	WH-07	WH-07D	277-101-S-7	Bacillus thuringeinsis	DUP-16815	0.89
34	Leihoku Elementary School	Distribution	WN-09	WN-09A	277-103-S-1	Bacillus cereus	DUP-13209	0.86
35	Leihoku Elementary School	Distribution	WN-09	WN-09B	277-57-S-2	Bacillus cereus	DUP-6048	0.88
36	Waipio Neighborhood Park	Distribution	WP-05	WP-05A	277-103-S-3	None		

Appendix Table 8—*Continued*

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
37	Waipio Neighborhood Park	Distribution	WP-05	WP-05B	277-103-S-4	None		
38	3609 Nuuanu Pali Drive	Distribution	HM-03	HM-03A	277-103-S-5	None		
39	3609 Nuuanu Pali Drive	Distribution	HM-03	HM-03B	277-103-S-6	None		
40	Waipio Neighborhood Park	Distribution	WP-05	WP-05A	277-103-S-3	None		
41	Waipio Neighborhood Park	Distribution	WP-05	WP-05B	277-103-S-4	None		
42	3609 Nuuanu Pali Drive	Distribution	HM-03	HM-03C	277-104-S-5	None		
43	Koa Iki Headstart	Distribution	WN-13	WN-13B	277-104-S-6	None		
44	BWS Makakilo Booster #2	Distribution	WU-02	WU-02A	277-57-S-2	Bacillus thuringiensis	DUP-6028	0.91
45	Kaleiopuu Playground	Distribution	WU-13	WU-13A1	277-111-S-2	None		
46	Kunia Neighborhood Park	Distribution	WU-16	WU-16D	277-111-S-3	None		
47	Kunia Neighborhood Park	Distribution	WU-16	WU-16F	277-111-S-3	None		
48	Ewa Town Center	Distribution	WU-19	WU-19C	277-111-S-5	None		
49	Watanabe Floral	Distribution	MI-05	MI-05A	277-111-S-6	None		
50	Watanabe Floral	Distribution	MI-05	MI-05B	277-111-S-7	None		
51	Ewa Elementary School	Distribution	WU-17	WU-17A	277-112-S-1	None		
52	Ewa Elementary School	Distribution	WU-17	WU-17D	277-112-S-2	None		
53	Kunia Neighborhood Park	Distribution	WU-16	WU-16C	277-61-S-6	None		
54	Kaleiopuu Playground	Distribution	WU-13	WU-13D	277-112-S-4	None		
55	Kaleiopuu Playground	Distribution	WU-13	WU-13E	277-85-S-3	None		
56	Kaleiopuu Playground	Distribution	WU-13	WU-13F	277-112-S-6	None		
57	Mililani Golf Course	Distribution	MI-10	MI-10C	277-57-S-2	Bacillus cereus	DUP-6048	0.91
58	Ewa Town Center	Distribution	WU-19	WU-19A	277-113-S-1	None		
59	Kuahelani Park	Distribution	MI-11	MI-11B	277-113-S-2	None		
60	Kuahelani Park	Distribution	MI-11	MI-11C	277-47-S-3	None		
61	Ahuimanu Elementary School Playground	Distribution	HW-13	HW-13A	277-113-S-5	None		
62	BWS Waihee Line Booster	Distribution	HW-14	HW-14A	277-113-S-6	None		
63	BWS Waihee Line Booster	Distribution	HW-14	HW-14B	277-111-S-7	None		
64	BWS Waihee Line Booster	Distribution	HW-14	HW-14C	277-113-S-8	None		
65	Kaaawa Fire Station	Distribution	HW-15	HW-15A	277-65-S-	Salinivibrio costicola ss costicola	DUP-18262	0.87
66	Kaaawa Fire Station	Distribution	HW-15	HW-15B	277-114-S-2	None		
67	Kaaawa Fire Station	Distribution	HW-15	HW-15C	277-114-S-3	None		
68	Kaaawa Fire Station	Distribution	HW-15	HW-15E	277-47-S-3	None		
69	Puunaluu Beach Park	Distribution	HW-16	HW-16A1	277-114-S-5	Vibrio species	DUP-16535	0.88
70	Puunaluu Beach Park	Distribution	HW-16	HW-16B	277-57-S-5	Stenotrophomonas maltophilia	DUP-10113	0.85
71	Puunaluu Beach Park	Distribution	HW-16	HW-16C	277-114-S-7	None		
72	Puunaluu Beach Park	Distribution	HW-16	HW-16D	277-57-S-5	None		
73	Hauula Fire Station	Distribution	HW-17	HW-17C	277-115-S-1	None		

Appendix Table 8—*Continued*

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
74	Hauula Fire Station	Distribution	HW-17	HW-17D	277-115-S-2	None		
75	Hauula Fire Station	Distribution	HW-17	HW-17E	277-115-S-3	None		
76	Heeia Neighborhood Park	Distribution	HW-21	HW-21A	277-115-S-4	None		
77	Heeia Neighborhood Park	Distribution	HW-21	HW-21B	277-115-S-5	None		
78	Heeia Neighborhood Park	Distribution	HW-21	HW-21C	277-115-S-6	None		
79	Honolulu BWS	Distribution	HC-09	HC-09A	277-47-S-3	None		
80	2765 Pacific Heights Road	Distribution	HM-04	HM-04A	277-118-S-1	None		
81	1972 Ala Mahamoe Street	Distribution	HC-35	HC-35D	277-47-S-3	None		
82	2765 Pacific Heights Road	Distribution	HM-04	HM-04C	277-118-S-3	None		
83	2765 Pacific Heights Road	Distribution	HM-04	HM-04D	277-118-S-4	None		
84	2765 Pacific Heights Road	Distribution	HM-04	HM-04E	277-118-S-5	Bacillus megaterium	DUP-16768	0.92
85	2765 Pacific Heights Road	Distribution	HM-04	HM-04F	277-118-S-6	None		
86	2765 Pacific Heights Road	Distribution	HM-04	HM-04G	277-118-S-7	None		
87	2765 Pacific Heights Road	Distribution	HM-04	HM-04H	277-118-S-3	None		
88	98337 Pono Street	Distribution	HP-12	HP-12A	277-54-S-7	None		
89	98337 Pono Street	Distribution	HP-12	HP-12B	277-54-S-7	Glaciecola pallidula	DUP-18328	0.89
90	98337 Pono Street	Distribution	HP-12	HP-12C	277-119-S-3	None		
91	Nuuanu Fire Station	Distribution	HC-34	HC-34C	277-119-S-4	None		
92	BWS Alewa Booster #1	Distribution	HC-15	HC-15A	277-47-S-3	None		
93	BWS Alewa Booster #1	Distribution	HC-15	HC-15B	277-65-S-4	None		
94	Puunaluu Beach Park	Distribution	HW-16	HW-16E	277-119-S-7	None		
95	Nahele Neighborhood Park	Distribution	HP-16	HP-16A	277-120-S-1	None		
96	Nahele Neighborhood Park	Distribution	HP-16	HP-16B	277-85-S-3	None		
97	Nahele Neighborhood Park	Distribution	HP-16	HP-16C	277-120-S-3	None		
98	Waiau Fire Station	Distribution	HP-17	HP-17A	277-120-S-4	None		
99	Waiau Fire Station	Distribution	HP-17	HP-17B	277-120-S-5	None		
100	Waiau Fire Station	Distribution	HP-17	HP-17C	277-120-S-6	None		
101	1972 Ala Mahamoe Street	Distribution	HC-35	HC-35A	277-120-S-7	None		
102	1972 Ala Mahamoe Street	Distribution	HC-35	HC-35B	277-120-S-8	None		
103	BWS Pearl City Booster	Distribution	HP-06	HP-06A	277-121-S-1	None		
104	BWS Pearl City Booster	Distribution	HP-06	HP-06B	277-121-S-2	None		
105	Kailua Fire Station	Distribution	HW-05	HW-05B	277-121-S-4	None		
106	Mid Pac Country Club	Distribution	HW-04	HW-04A	277-121-S-5	None		
107	95-023 Waihau Street	Distribution	WH-11	WH-11A	277-121-S-7	None		
108	95-023 Waihau Street	Distribution	WH-11	WH-11B	277-121-S-8	None		
109	Momilani Elementary School	Distribution	HP-08	HP-08A	277-122-S-1	None		
110	Castle High School	Distribution	HW-09	HW-09A	277-122-S-2	None		
111	Castle High School	Distribution	HW-09	HW-09B	277-122-S-3	None		

Appendix Table 8—*Continued*

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
112	Aikahi Fire Station	Distribution	HW-06	HW-06A	277-54-S-7	Glaciecola pallidula	DUP-18328	0.87
113	Aikahi Fire Station	Distribution	HW-06	HW-06B	277-122-S-5	None		
114	1972 Ala Mahamoe Street	Distribution	HC-35	HC-35C	277-122-S-7	Ralstonia pickettii	DUP-16381	0.88
115	Manana Park	Distribution	HP-18	HP-18A	277-123-S-1	None		
116	Manana Park	Distribution	HP-18	HP-18B	277-54-S-7	Glaciecola pallidula	DUP-18328	0.88
117	Melemanu Neighborhood Park	Distribution	WH-13	WH-13B	277-123-S-4	None		
118	BWS Aiea Booster #3	Distribution	HP-10	HP-10A	277-123-S-8	None		
119	46-445 D Kahuhipa Street	Distribution	HW-12	HW-12A1	277-54-S-5	Bacillus cereus	DUP-6082	0.93
120	46-445 D Kahuhipa Street	Distribution	HW-12	HW-12B	277-120-S-1	None		
121	46-445 D Kahuhipa Street	Distribution	HW-12	HW-12C	277-123-S-8	None		
122	46-445 D Kahuhipa Street	Distribution	HW-12	HW-12D	277-85-S-3	None		
123	46-445 D Kahuhipa Street	Distribution	HW-12	HW-12E	277-120-S-1	None		
124	98-337 Pono Street	Distribution	HP-12	HP-12D	277-124-S-6	None		
125	98-337 Pono Street	Distribution	HP-12	HP-12E	277-124-S-7	None		
126	Aina Koa Playground	Distribution	HC-32	HC-32A	277-124-S-8	None		
127	Pearl City Rec. Center	Distribution	HP-09	HP-09A	277-54-S-7	Glaciecola pallidula	DUP-18328	0.86
128	Pearl City Rec. Center	Distribution	HP-09	HP-09B	277-61-S-6	None		
129	Pearl City Rec. Center	Distribution	HP-09	HP-09C	277-125-S-3	None		
130	Pearl City Rec. Center	Distribution	HP-09	HP-09E	277-125-S-4	None		
131	Pearl City Rec. Center	Distribution	HP-09	HP-09F	277-121-S-2	None		
132	Kaneohe Fire Station	Distribution	HW-11	HW-11B	277-125-S-7	None		
133	Ahuimanu Elementary School Playground	Distribution	HW-13	HW-13A	277-125-S-7	None		
134	Enchanted Lake Playground	Distribution	HW-19	HW-19C	277-127-S-1	None		
135	Enchanted Lake Playground	Distribution	HW-19	HW-19D	277-127-S-1	None		
136	Kainalu Elementary School	Distribution	HW-23	HW-23C	277-123-S-8	None		
137	Kainalu Elementary School	Distribution	HW-23	HW-23D	277-127-S-1	None		
138	Heeia Neighborhood Park	Distribution	HW-21	HW-21E	277-127-S-5	None		
139	Heeia Neighborhood Park	Distribution	HW-21	HW-21F	277-127-S-6	Enterobacter cloacae	DUP-15327	0.91
140	Heeia Neighborhood Park	Distribution	HW-21	HW-21H	277-47-S-3	None		
141	Heeia Neighborhood Park	Distribution	HW-21	HW-21I	277-127-S-6	Enterobacter cloacae	DUP-15327	0.9
142	Kaneohe 7-Eleven	Distribution	HW-10	HW-10A	277-47-S-3	None		
143	Kaneohe 7-Eleven	Distribution	HW-10	HW-10C	277-130-S-4	None		
144	Kaneohe 7-Eleven	Distribution	HW-10	HW-10E	277-130S-5	None		
145	Waialae Iki Playground	Distribution	HC-31	HC-31B	277-131-S-1	None		
146	Waialae Iki Playground	Distribution	HC-31	HC-31C	277-131-S-1	Pseudomonas alcaligenes	DUP-10127	0.86
147	Waialae Iki Playground	Distribution	HC-31	HC-31D	277-131-S-3	Bacillus cereus	DUP-6050	0.89
148	Waialae Iki Playground	Distribution	HC-31	HC-31E	277-131-S-4	None		

Appendix Table 8—*Continued*

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
149	Waialae Iki Playground	Distribution	HC-31	HC-31F	277-131-S-1	Pseudomonas alcaligenes	DUP-14776	0.85
150	Waialae Iki Playground	Distribution	HC-31	HC-31G	277-85-S-3	None		
151	Booth District Park	Distribution	HC-21	HC-21B	277-131-S-8	None		
152	Waimanalo District Park	Distribution	HW-18	HW-18B	277-121-S-2	None		
153	Waimanalo District Park	Distribution	HW-18	HW-18D	277-133-S-2	None		
154	Olomana Fire Station	Distribution	HW-07	HW-07B	277-121-S-2	None		
155	Kaneohe 7-Eleven	Distribution	HW-10	HW-10F	277-133-S-4	Staphylococcus haemolyticus	DUP-14741	0.86
156	Booth District Park	Distribution	HC-21	HC-21C	277-133-S-5	None		
157	Jehova Witness Hall	Distribution	HW-03	HW-03B	277-133-S-6	None		
158	Kainalu Elementary School	Distribution	HW-23	HW-23A	277-133-S-7	None		
159	Waialae Iki Playground	Distribution	HC-31	HC-31B (Redo)	277-131-S-1	None		
160	August Ahrens School	Distribution	WU-08	WU-08B	277-47-S-3	None		
161	August Ahrens School	Distribution	WU-08	WU-08C	277-142-S-2	None		
162	August Ahrens School	Distribution	WU-08	WU-08D	277-142-S-3	None		
163	August Ahrens School	Distribution	WU-08	WU-08E	277-142-S-4	Bacillus cereus	DUP-12561	0.96
164	Aiea Fire Station	Distribution	HP-02	HP-02E	277-140-S-3	None		
165	Aiea Fire Station	Distribution	HP-02	HP-02F	277-140-S-3	None		
166	Waipahu Recreation Center	Distribution	WU-07	WU-07A	277-121-S-2	None		
167	Waipahu Recreation Center	Distribution	WU-07	WU-07B	277-142-S-8	None		
168	Jack in the Box	Distribution	HC-11	HC-11A	277-143-S-1	None		
169	Jack in the Box	Distribution	HC-11	HC-11D	277-47-S-3	None		
170	Jack in the Box	Distribution	HC-11	HC-11F	277-143-S-3	None		
171	99-739 Halawa Heights Road	Distribution	HP-01	HP-01C	277-140-S-3	None		
172	99-739 Halawa Heights Road	Distribution	HP-01	HP-01D	277-143-S-5	None		
173	99-739 Halawa Heights Road	Distribution	HP-01	HP-01A	277-140-S-5	None		
174	99-739 Halawa Heights Road	Distribution	HP-01	HP-01B	277-140-S-5	None		
175	Aiea Fire Station	Distribution	HP-02	HP-02E (Redo)	277-140-S-5	None		
176	Honowai Park	Distribution	WU-05	WU-05B	277-123-S-8	None		
177	Honowai Park	Distribution	WU-05	WU-05C	277-145-S-3	Vibrio cholerae	DUP-6607	0.88
178	Honowai Park	Distribution	WU-05	WU-05E	277-145-S-4	None		
179	Kalama Valley Park	Distribution	HC-26	HC-26A	277-145-S-5	Acinetobacter baumannii	DUP-16939	0.91
180	Waikiki Fire Station	Distribution	HC-06	HC-06D	277-145-S-6	None		
181	L'Orange Park	Distribution	WU-06	WU-06A	277-123-S-8	None		
182	Napuanani Park	Distribution	HP-11	HP-11A	277-149-S-1	None		
183	Aiea Fire Station	Distribution	HP-02	HP-02F	277-149-S-4	None		
184	Aiea Fire Station	Distribution	HP-02	HP-02G	277-46-S-7	Terracoccus luteus	DUP-18429	0.94

Appendix Table 8—*Continued*

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
185	Aiea Fire Station	Distribution	HP-02	HP-02H	277-149-S-6	Bacillus cereus	DUP-13210	0.9
186	Honolulu BWS	Distribution	HC-09	HC-09B	277-149-S-7	None		
187	Honolulu BWS	Distribution	HC-09	HC-09C	277-149-S-8	None		
188	Halawa Xeriscape Garden	Distribution	HP-14	HP-14C	277-150-S-1	None		
189	BWS Aiea Booster #3	Distribution	HP-10	HP-10C	277-150-S-4	None		
190	Waikiki Fire Station	Distribution	HC-06	HC-06B	277-150-S-5	None		
191	Halawa Xeriscape Garden	Distribution	HP-14	HP-14E	277-150-S-6	None		
192	Honowai Park	Distribution	WU-05	WU-05F	277-123-S-8	None		
193	Aiea Fire Station	Distribution	HP-02	HP-02E	277-150-S-8	None		
194	Ewa Town Center	Distribution	WU-19	WU-19B	277-57-S-2	Bacillus cereus	DUP-6048	0.93
195	Holomua Elementary School	Distribution	WU-18	WU-18A	277-168-S-1	None		
196	Holomua Elementary School	Distribution	WU-18	WU-18B	277-168-S-2	None		
197	Holomua Elementary School	Distribution	WU-18	WU-18C	277-168-S-3	None		
198	Holomua Elementary School	Distribution	WU-18	WU-18D	277-168-S-4	None		
199	Holomua Elementary School	Distribution	WU-18	WU-18E	277-168-S-5	None		
200	Holomua Elementary School	Distribution	WU-18	WU-18F	277-168-S-6	None		
201	Holomua Elementary School	Distribution	WU-18	WU-18H	277-168-S-7	None		
202	Waipahu Fire Station	Distribution	WU-09	WU-09A	277-168-S-8	None		
203	Honowai Park	Distribution	WU-05	WU-05G	277-169-S-1	None		
204	Honowai Park	Distribution	WU-05	WU-05H	277-120-S-1	None		
205	Honowai Park	Distribution	WU-05	WU-05I	277-120-S-1	None		
206	Honowai Park	Distribution	WU-05	WU-05J	277-169-S-4	None		
207	Honowai Park	Distribution	WU-05	WU-05K	277-169-S-5	None		
208	Honowai Park	Distribution	WU-05	WU-05L	277-169-S-5	None		
209	Honowai Park	Distribution	WU-05	WU-05M	277-148-S-3	None		
210	Honowai Park	Distribution	WU-05	WU-05N	277-123-S-8	None		
211	Ewa Elementary School	Distribution	WU-17	WU-17B	277-171-S-1	None		
212	Ewa Elementary School	Distribution	WU-17	WU-17C	277-171-S-2	None		
213	Ewa Elementary School	Distribution	WU-17	WU-17F	277-171-S-3	None		
214	Ewa Elementary School	Distribution	WU-17	WU-17G	277-123-S-8	None		
215	Ewa Elementary School	Distribution	WU-17	WU-17H	277-123-S-8	None		
216	Ewa Elementary School	Distribution	WU-17	WU-17J	277-171-S-6	None		
217	Aiea Fire Station	Distribution	HP-02	HP-02E (Redo)	277-140-S-5	None		
218	Jehova Witness Hall	Distribution	HW-03	HW-03A	277-169-S-5	None		
219	Jehova Witness Hall	Distribution	HW-03	HW-03C	277-174-S-2	None		
220	Jehova Witness Hall	Distribution	HW-03	HW-03E	277-169-S-4	None		
221	Jehova Witness Hall	Distribution	HW-03	HW-03F	277-169-S-4	None		

Appendix Table 8—*Continued*

Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
222	Jehova Witness Hall	Distribution	HW-03	HW-03G	277-174-S-5	Bacillus cereus	DUP-13207	0.89
223	Jehova Witness Hall	Distribution	HW-03	HW-03H	277-174-S-6	None		
224	Jehova Witness Hall	Distribution	HW-03	HW-03I	277-174-S-7	None		
225	Jehova Witness Hall	Distribution	HW-03	HW-03J	277-174-S-8	None		
226	Waimanalo Beach Park	Distribution	HW-02	HW-02C	277-174-S-5	Bacillus cereus	DUP-13212	0.87
227	Waimanalo Beach Park	Distribution	HW-02	HW-02E	277-175-S-2	None		
228	Waimanalo Beach Park	Distribution	HW-02	HW-02F	277-175-S-3	None		
229	Waimanalo Beach Park	Distribution	HW-02	HW-02G	277-61-S-6	None		
230	Waimanalo Beach Park	Distribution	HW-02	HW-02H	277-175-S-5	None		
231	Waimanalo Beach Park	Distribution	HW-02	HW-02I	277-175-S-6	None		
232	Waimanalo Beach Park	Distribution	HW-02	HW-02J	277-175-S-7	None		
233	Waimanalo Beach Park	Distribution	HW-02	HW-02K	277-175-S-8	None		
234	Waimanalo Shopping Center	Distribution	HW-01	HW-01C	277-176-S-1	None		
235	Waimanalo Shopping Center	Distribution	HW-01	HW-01G	277-176-S-2	None		
236	Waimanalo Shopping Center	Distribution	HW-01	HW-01H	277-176-S-3	None		
237	Waimanalo Shopping Center	Distribution	HW-01	HW-01I	277-176-S-4	None		
238	Waimanalo Shopping Center	Distribution	HW-01	HW-01J	277-176-S-5	None		
239	Waimanalo Shopping Center	Distribution	HW-01	HW-01K	277-176-S-6	None		
240	Waimanalo Shopping Center	Distribution	HW-01	HW-01L	277-176-S-7	None		
241	Waimanalo Shopping Center	Distribution	HW-01	HW-01M	277-176-S-8	None		
242	Olomana Fire Station	Distribution	HW-07	HW-07C	277-177-S-1	None		
243	Olomana Fire Station	Distribution	HW-07	HW-07D	277-177-S-2	None		
244	Olomana Fire Station	Distribution	HW-07	HW-07E	277-177-S-3	None		
245	Olomana Fire Station	Distribution	HW-07	HW-07G	277-177-S-4	None		
246	Olomana Fire Station	Distribution	HW-07	HW-07I	277-177-S-5	None		
247	Olomana Fire Station	Distribution	HW-07	HW-07J	277-177-S-6	None		
248	Waimanalo Shopping Center	Distribution	HW-01	HW-01E	277-177-S-7	None		
249	Manoa Fire Station	Distribution	HC-22	HC-22C	277-177-S-8	None		
250	Mid Pac Country Club	Distribution	HW-04	HW-04C	277-175-S-3	None		
251	Mid Pac Country Club	Distribution	HW-04	HW-04D	277-52-S-8	None		
252	Mid Pac Country Club	Distribution	HW-04	HW-04E	277-178-S-3	None		
253	Mid Pac Country Club	Distribution	HW-04	HW-04F	277-178-S-4	None		
254	Mid Pac Country Club	Distribution	HW-04	HW-04G	277-66-S-1	None		
255	Mid Pac Country Club	Distribution	HW-04	HW-04H	277-66-S-1	None		
256	Kaneohe 7-Eleven	Distribution	HW-10	HW-10G	277-178-S-7	None		
257	Kaneohe 7-Eleven	Distribution	HW-10	HW-10H	277-178-S-8	None		
258	Waimanalo District Park	Distribution	HW-18	HW-18E	277-175-S-3	None		
259	Waimanalo District Park	Distribution	HW-18	HW-18G	277-179-S-2	None		

Appendix Table 8—*Continued*

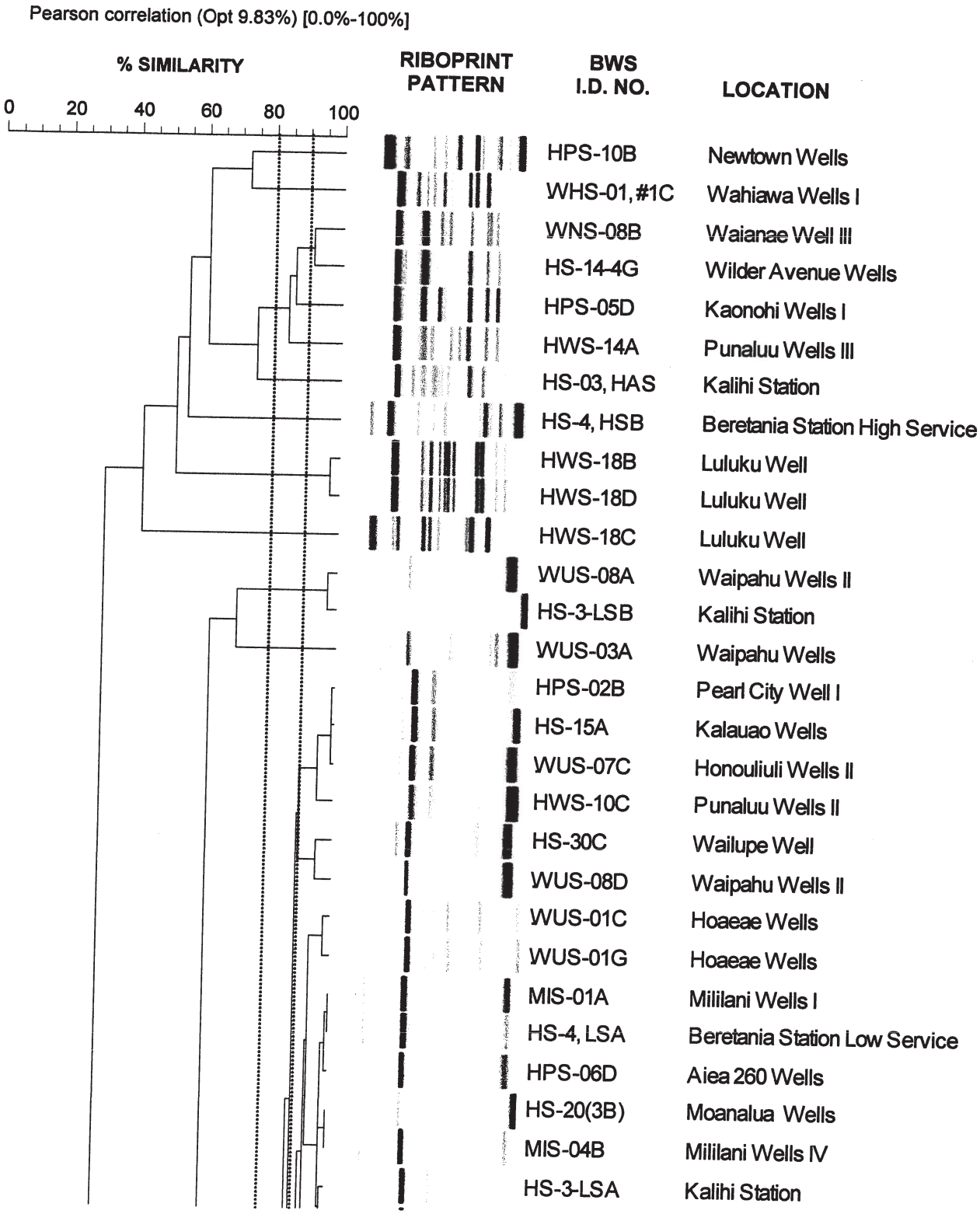
Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
260	Waimanalo District Park	Distribution	HW-18	HW-18H	277-61-S-6	None		
261	Keolu Elementary School	Distribution	HW-08	HW-08A	277-179-S-4	None		
262	Keolu Elementary School	Distribution	HW-08	HW-08B	277-179-S-5	None		
263	Keolu Elementary School	Distribution	HW-08	HW-08C	277-179-S-6	None		
264	Palolo Fire Station	Distribution	HC-23	HC-23A	277-179-S-7	None		
265	Palolo Fire Station	Distribution	HC-23	HC-23B	277-179-S-8	None		
266	Enchanted Lake Playground	Distribution	HW-19	HW-19A	277-127-S-1	None		
267	Enchanted Lake Playground	Distribution	HW-19	HW-19E	277-120-S-1	None		
268	Enchanted Lake Playground	Distribution	HW-19	HW-19F	277-180-S-5	None		
269	Enchanted Lake Playground	Distribution	HW-19	HW-19G	277-180-S-6	None		
270	Enchanted Lake Playground	Distribution	HW-19	HW-19J	277-180-S-7	Aerococcus viridans	DUP-11116	0.94
271	Enchanted Lake Playground	Distribution	HW-19	HW-19K	277-180-S-8	None		
272	Holomua Elementary School	Distribution	WU-18	WU-18A*	277-46-S-7	None		
273	Holomua Elementary School	Distribution	WU-18	WU-18B*	277-181-S-2	None		
274	Holomua Elementary School	Distribution	WU-18	WU-18C*	277-181-S-3	None		
275	Holomua Elementary School	Distribution	WU-18	WU-18D*	277-181-S-4	None		
276	Holomua Elementary School	Distribution	WU-18	WU-18E*	277-181-S-5	None		
277	Holomua Elementary School	Distribution	WU-18	WU-18F*	277-181-S-6	None		
278	Holomua Elementary School	Distribution	WU-18	WU-18H*	277-53-S-8	Salinivibrio costicola ss costicola	DUP-18262	0.86
279	Puuloa Playground	Distribution	WU-14	WU-14A	277-127-S-1	None		
280	Puuloa Playground	Distribution	WU-14	WU-14D	277-123-S-8	None		
281	Puuloa Playground	Distribution	WU-14	WU-14E	277-127-S-1	None		
282	Kunia Neighborhood Park	Distribution	WU-16	WU-16G	277-182-S-4	None		
283	Kunia Neighborhood Park	Distribution	WU-16	WU-16H	277-182-S-5	None		
284	Ewa Elementary School	Distribution	WU-17	WU-17E	277-169-S-4	None		
285	Aiea Fire Station	Distribution	HP-02	HP-02E (Redo)	277-140-S-5	None		
286	Better Brands	Distribution	WP-06	WP-06A	277-112-S-1	None		
287	Better Brands	Distribution	WP-06	WP-06B	277-183-S-2	None		
288	Better Brands	Distribution	WP-06	WP-06C	277-183-S-3	None		
289	Better Brands	Distribution	WP-06	WP-06D	277-183-S-4	Staphylococcus warneri	DUP-18618	0.94
290	Better Brands	Distribution	WP-06	WP-06F	277-183-S-6	None		
291	Better Brands	Distribution	WP-06	WP-06G	277-183-S-7	None		
292	Better Brands	Distribution	WP-06	WP-06H	277-183-S-8	None		
293	Momilani Elementary School	Distribution	HP-08	HP-08A1	277-184-S-1	None		
294	Momilani Elementary School	Distribution	HP-08	HP-08B	277-184-S-2	None		
295	Momilani Elementary School	Distribution	HP-08	HP-08C	277-184-S-3	None		
296	Momilani Elementary School	Distribution	HP-08	HP-08D	277-184-S-4	None		

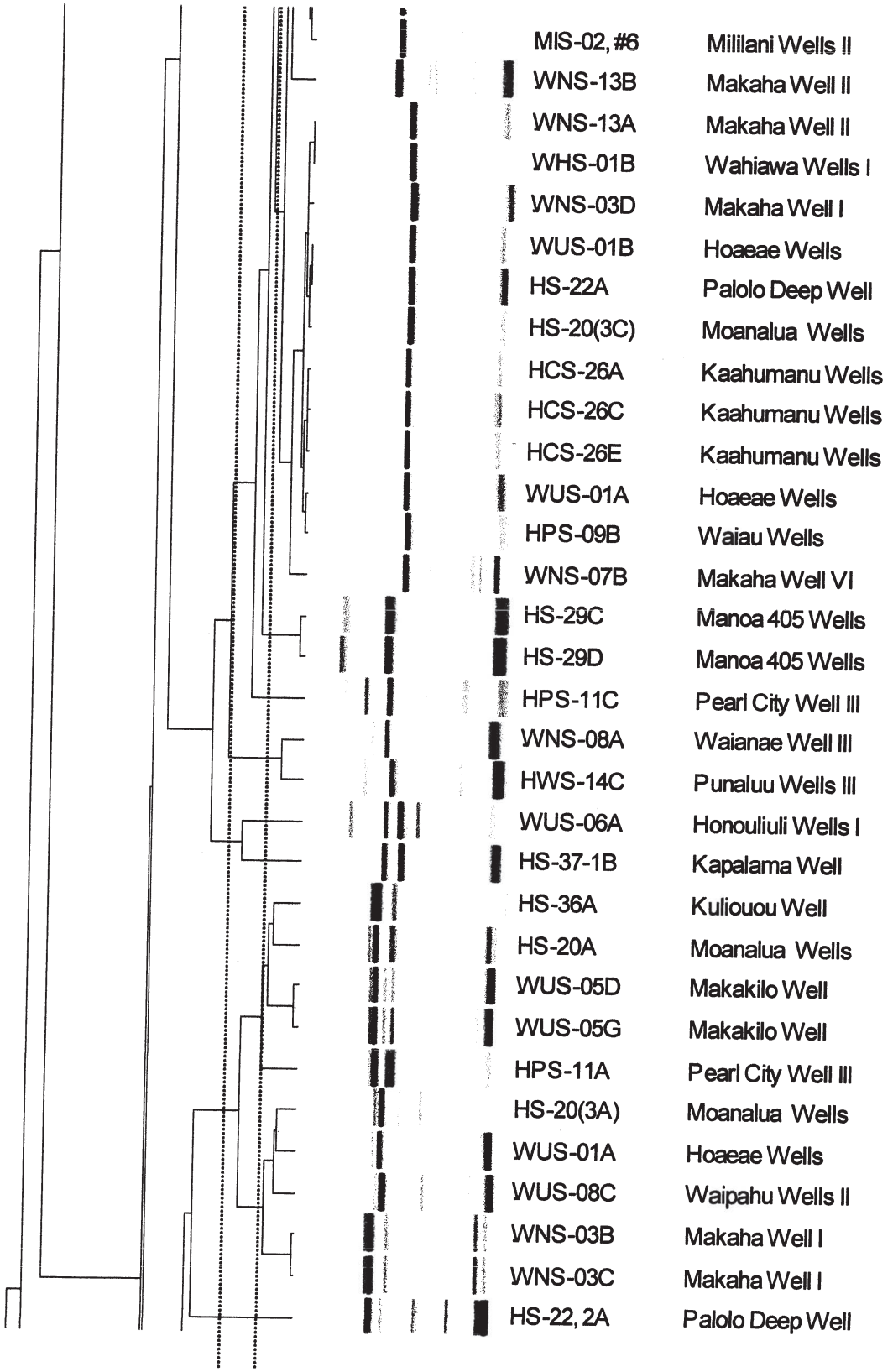
Appendix Table 8—*Continued*

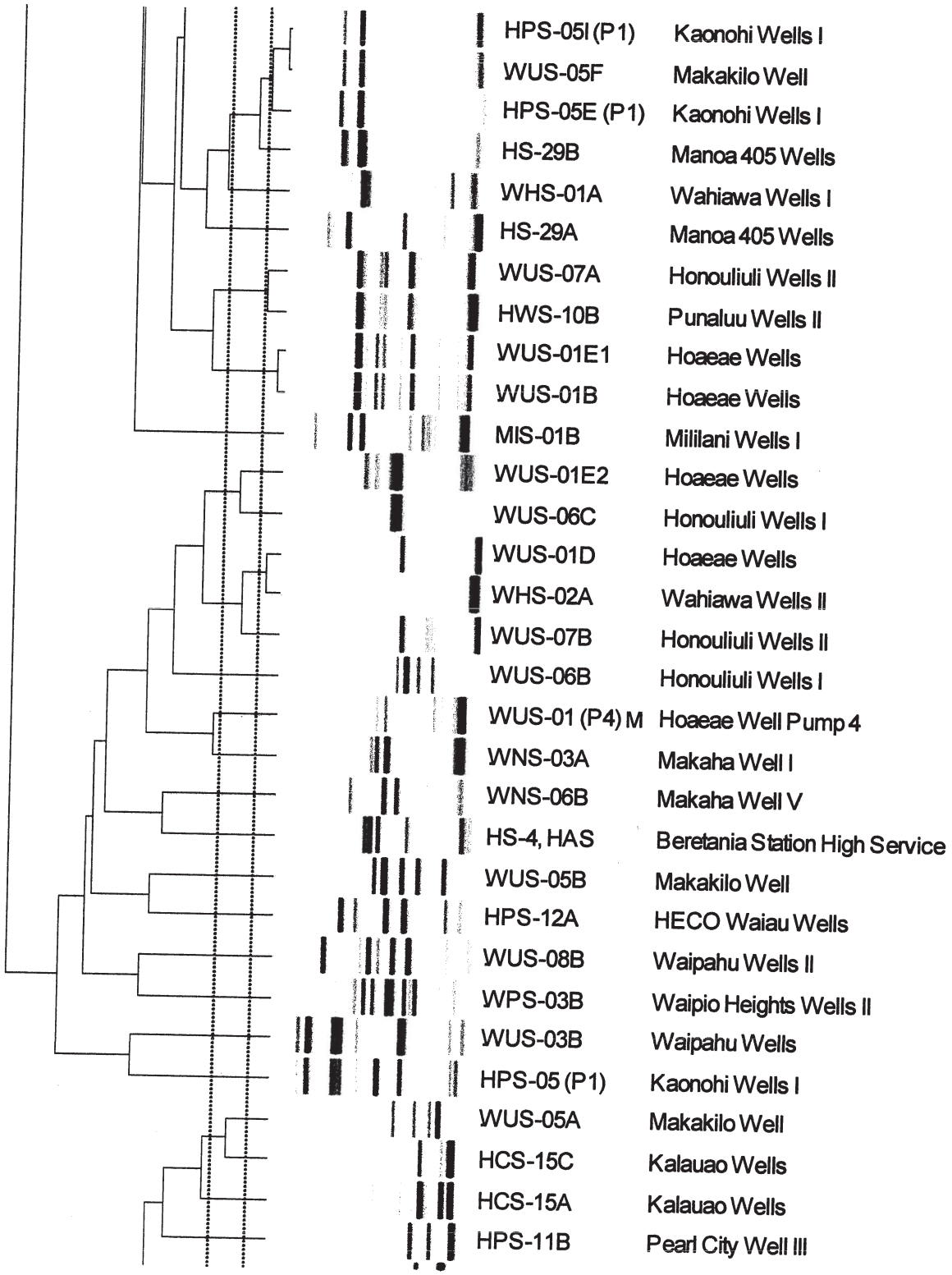
Sample No.	Location	Source	BWS No.	ID No.	Ribogroup	DuPont ID	DuPont ID Label	DID Sim
297	Momilani Elementary School	Distribution	HP-08	HP-08E	277-53-S-8	None		
298	Momilani Elementary School	Distribution	HP-08	HP-08F	277-67-S-2	Staphylococcus pasteuri	DUP-4221	0.9
299	Momilani Elementary School	Distribution	HP-08	HP-08G	277-184-S-7	None		
300	Momilani Elementary School	Distribution	HP-08	HP-08I	277-184-S-8	None		
301	Crestview Community Park	Distribution	WP-02	WP-02A	277-181-S-4	None		
302	Crestview Community Park	Distribution	WP-02	WP-02B	277-183-S-4	Staphyloccocus warneri	DUP-18618	0.91
303	Crestview Community Park	Distribution	WP-02	WP-02C	277-185-S-3	None		
304	Crestview Community Park	Distribution	WP-02	WP-02D	277-67-S-2	Staphylococcus pasteuri	DUP-4221	0.88
305	Kawaikui Beach Park	Distribution	HC-19	HC-19A	277-47-S-3	None		
306	Kawaikui Beach Park	Distribution	HC-19	HC-19B	277-185-S-6	None		
307	942 Spencer Street	Distribution	HC-17	HC-17A	277-185-S-7	None		
308	942 Spencer Street	Distribution	HC-17	HC-17C	277-148-S-3	None		
309	Waiau Elementary School	Distribution	HP-21	HP-21A	277-47-S-3	None		
310	Waiau Elementary School	Distribution	HP-21	HP-21B	277-186-S-2	None		
311	Waiau Elementary School	Distribution	HP-21	HP-21E	277-47-S-3	None		
312	Waiau Elementary School	Distribution	HP-21	HP-21F	277-47-S-3	None		
313	Waiau Elementary School	Distribution	HP-21	HP-21G	277-186-S-5	None		
314	Nahele Neighborhood Park	Distribution	HP-16	HP-16D	277-120-S-1	None		
315	Nahele Neighborhood Park	Distribution	HP-16	HP-16E	277-85-S-3	None		
316	Nahele Neighborhood Park	Distribution	HP-16	HP-16F	277-186-S-8	None		
317	Waialae Beach Park	Distribution	HC-13	HC-13E	277-61-S-6	None		
318	Waialae Beach Park	Distribution	HC-13	HC-13B	277-187-S-2	None		
319	Waialae Beach Park	Distribution	HC-13	HC-13D	277-187-S-2	None		
320	Waialae Iki Playground	Distribution	HC-31	HC-31H	277-187-S-4	None		
321	Waialae Iki Playground	Distribution	HC-31	HC-31I	277-187-S-5	None		
322	Waialae Iki Playground	Distribution	HC-31	HC-31J	277-187-S-6	None		
323	BWS Waipio Heights Wells Control Station	Distribution	WP-01	WP-01B	277-187-S-7	None		
324	BWS Waipio Heights Wells Control Station	Distribution	WP-01	WP-01C	277-187-S-8	None		
325	B102A	Distribution			277-188-S-1	None		
326	B106A	Distribution			277-188-S-2	None		
327	B109C	Distribution			277-188-S-3	None		
328	B109D	Distribution			277-188-S-3	None		
329	Waikiki Fire Station	Distribution	HC-06	HC-06A	277-188-S-5	None		
330	Waikiki Fire Station	Distribution	HC-06	HC-06B	277-188-S-6	None		
331	Crestview Community Park	Distribution	WP-02	WP-02E	277-46-S-7	Terracoccus luteus	DUP-18429	0.97

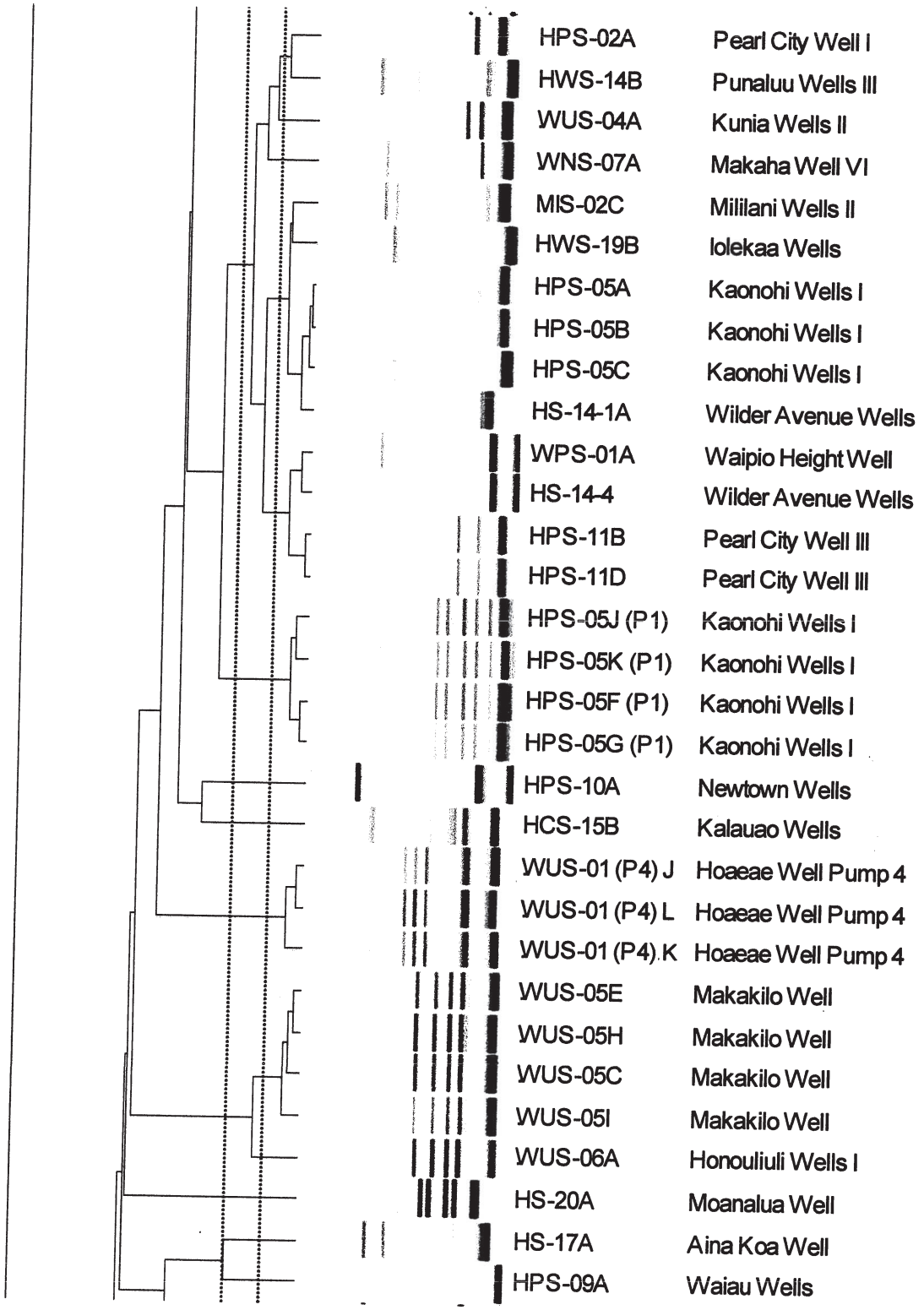
APPENDIX B: FIGURES

Figure B.1. Clustering the dendrograms of THB riboprints from well sites using GelCompar method at 80% similarity index




MIS-02, #6
Mililani Wells II
WNS-13B
Makaha Well II
WNS-13A
Makaha Well II
WHS-01B
Wahiawa Wells I
WNS-03D
Makaha Well I
WUS-01B
Hoaeae Wells
HS-22A
Palolo Deep Well
HS-20(3C)
Moanalua Wells
HCS-26A
Kaahumanu Wells
HCS-26C
Kaahumanu Wells
HCS-26E
Kaahumanu Wells
WUS-01A
Hoaeae Wells
HPS-09B
Waiau Wells
WNS-07B
Makaha Well VI
HS-29C
Manoa 405 Wells
HS-29D
Manoa 405 Wells
HPS-11C
Pearl City Well III
WNS-08A
Waianae Well III
HWS-14C
Punaluu Wells III
WUS-06A
Honouliuli Wells I
HS-37-1B
Kapalama Well
HS-36A
Kuliouou Well
HS-20A
Moanalua Wells
WUS-05D
Makakilo Well
WUS-05G
Makakilo Well
HPS-11A
Pearl City Well III
HS-20(3A)
Moanalua Wells
WUS-01A
Hoaeae Wells
WUS-08C
Waipahu Wells II
WNS-03B
Makaha Well I
WNS-03C
Makaha Well I
HS-22, 2A
Palolo Deep Well


HPS-05I (P1) Kaonohi Wells I
WUS-05F Makakilo Well
HPS-05E (P1) Kaonohi Wells I
HS-29B Manoa 405 Wells
WHS-01A Wahiawa Wells I
HS-29A Manoa 405 Wells
WUS-07A Honouliuli Wells II
HWS-10B Punaluu Wells II
WUS-01E1 Hoaeae Wells
WUS-01B Hoaeae Wells
MIS-01B Mililani Wells I
WUS-01E2 Hoaeae Wells
WUS-06C Honouliuli Wells I
WUS-01D Hoaeae Wells
WHS-02A Wahiawa Wells II
WUS-07B Honouliuli Wells II
WUS-06B Honouliuli Wells I
WUS-01 (P4) M Hoaeae Well Pump 4
WNS-03A Makaha Well I
WNS-06B Makaha Well V
HS-4, HAS Beretania Station High Service
WUS-05B Makakilo Well
HPS-12A HECO Waiau Wells
WUS-08B Waipahu Wells II
WPS-03B Waipio Heights Wells II
WUS-03B Waipahu Wells
HPS-05 (P1) Kaonohi Wells I
WUS-05A Makakilo Well
HCS-15C Kalauao Wells
HCS-15A Kalauao Wells
HPS-11B Pearl City Well III


HPS-02A Pearl City Well I
HWS-14B Punaluu Wells III
WUS-04A Kunia Wells II
WNS-07A Makaha Well VI
MIS-02C Mililani Wells II
HWS-19B Iolekaa Wells
HPS-05A Kaonohi Wells I
HPS-05B Kaonohi Wells I
HPS-05C Kaonohi Wells I
HS-14-1A Wilder Avenue Wells
WPS-01A Waipio Height Well
HS-14-4 Wilder Avenue Wells
HPS-11B Pearl City Well III
HPS-11D Pearl City Well III
HPS-05J (P1) Kaonohi Wells I
HPS-05K (P1) Kaonohi Wells I
HPS-05F (P1) Kaonohi Wells I
HPS-05G (P1) Kaonohi Wells I
HPS-10A Newtown Wells
HCS-15B Kalauao Wells
WUS-01 (P4) J Hoaeae Well Pump 4
WUS-01 (P4) L Hoaeae Well Pump 4
WUS-01 (P4) K Hoaeae Well Pump 4
WUS-05E Makakilo Well
WUS-05H Makakilo Well
WUS-05C Makakilo Well
WUS-05I Makakilo Well
WUS-06A Honouliuli Wells I
HS-20A Moanalua Well
HS-17A Aina Koa Well
HPS-09A Waiau Wells

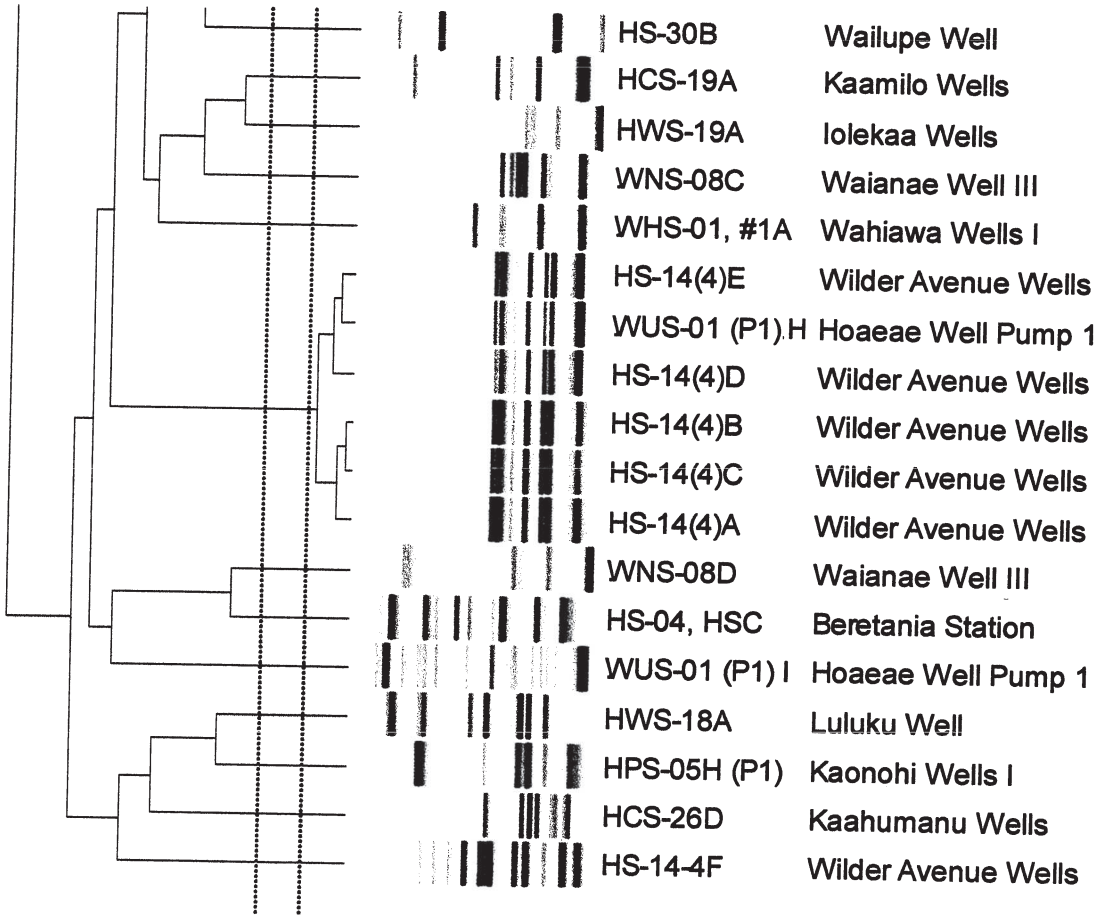

HS-30B
Wailupe Well
HCS-19A
Kaamilo Wells
HWS-19A
Iolekaa Wells
WNS-08C
Waianae Well III
WHS-01, #1A
Wahiawa Wells I
HS-14(4)E
Wilder Avenue Wells
WUS-01 (P1).H
Hoaeae Well Pump 1
HS-14(4)D
Wilder Avenue Wells
HS-14(4)B
Wilder Avenue Wells
HS-14(4)C
Wilder Avenue Wells
HS-14(4)A
Wilder Avenue Wells
WNS-08D
Waianae Well III
HS-04, HSC
Beretania Station
WUS-01 (P1) I
Hoaeae Well Pump 1
HWS-18A
Luluku Well
HPS-05H (P1)
Kaonohi Wells I
HCS-26D
Kaahumanu Wells
HS-14-4F
Wilder Avenue Wells

Figure B.2. Clustering the dendrograms of THB riboprints from tunnel sites using GelCompar method at 80% similarity index

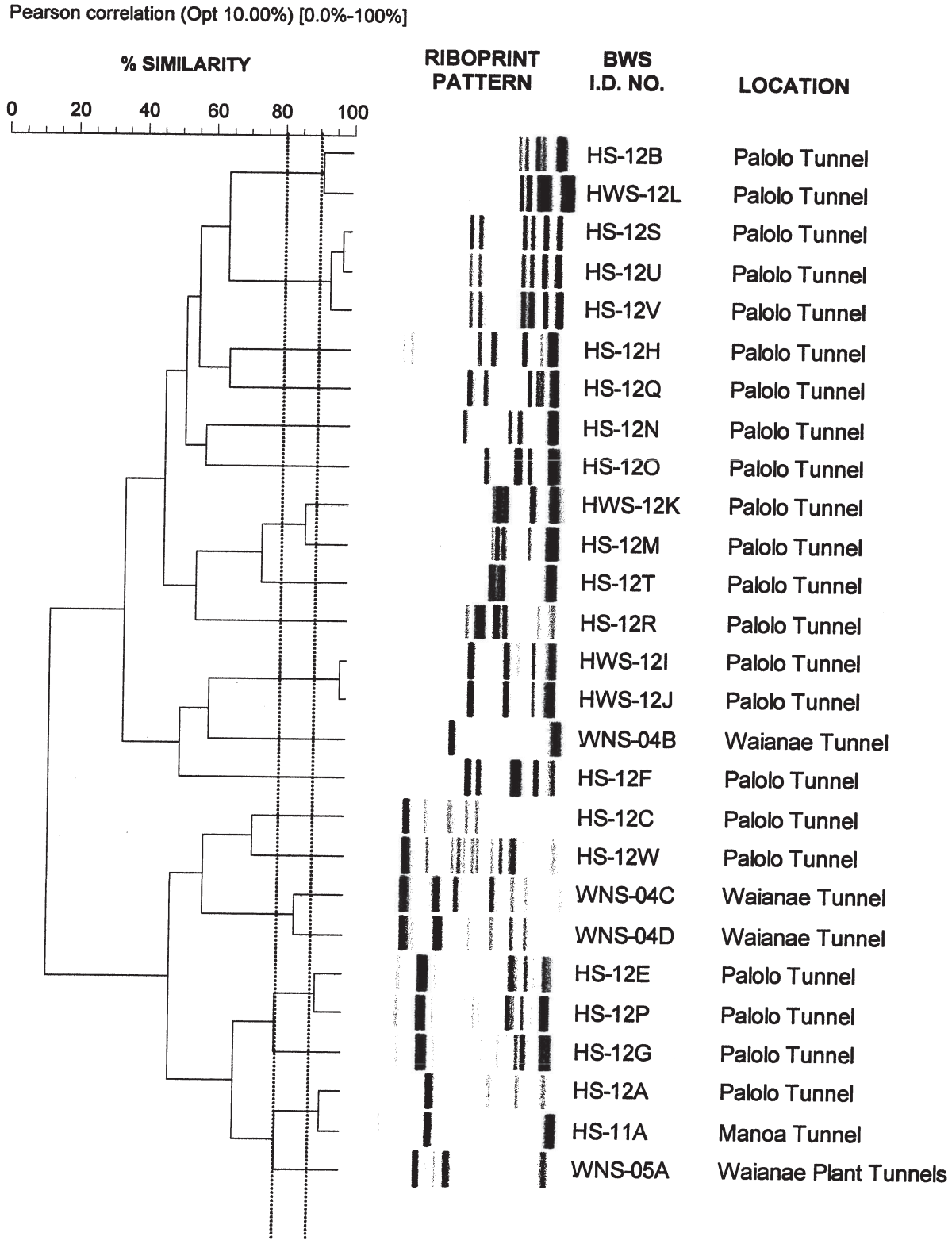
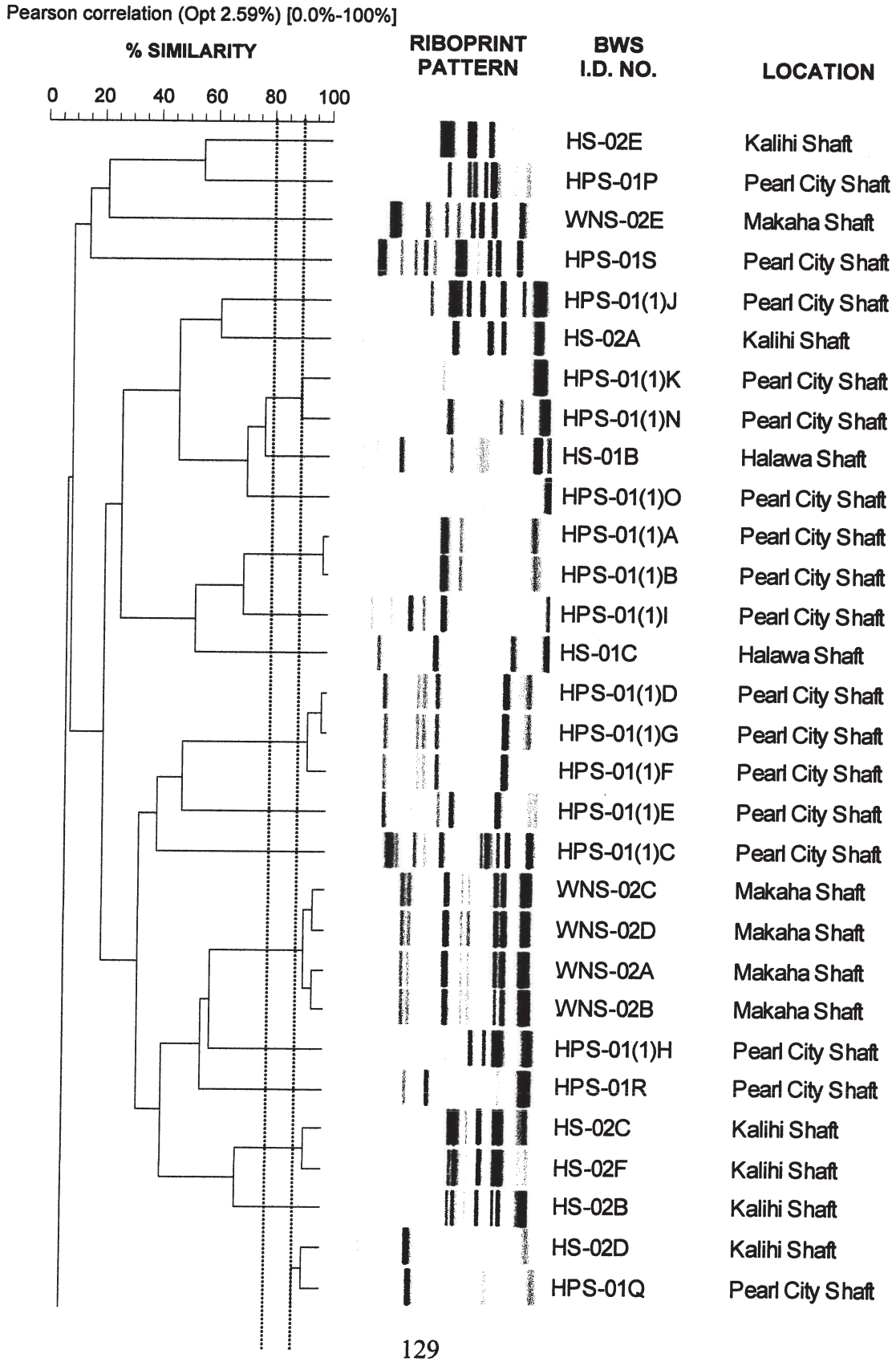
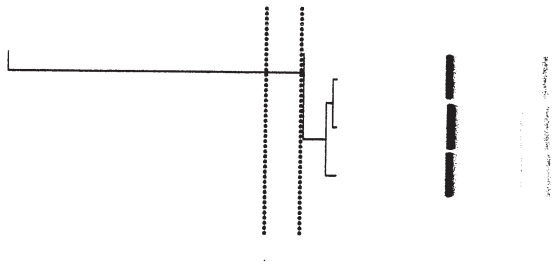


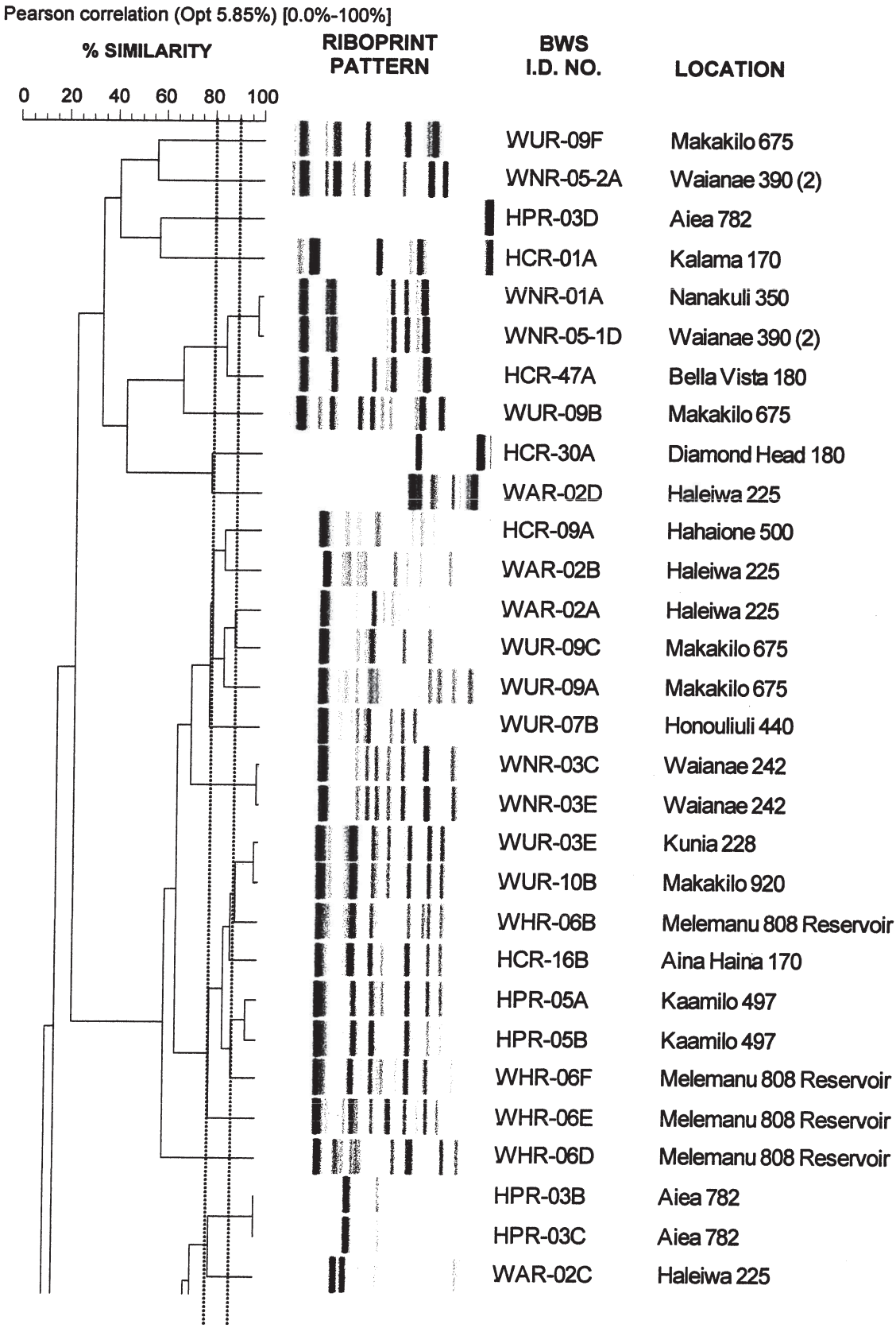
Figure B.3. Clustering the dendrograms of THB riboprints from shaft sites using GelCompar method at 80% similarity index

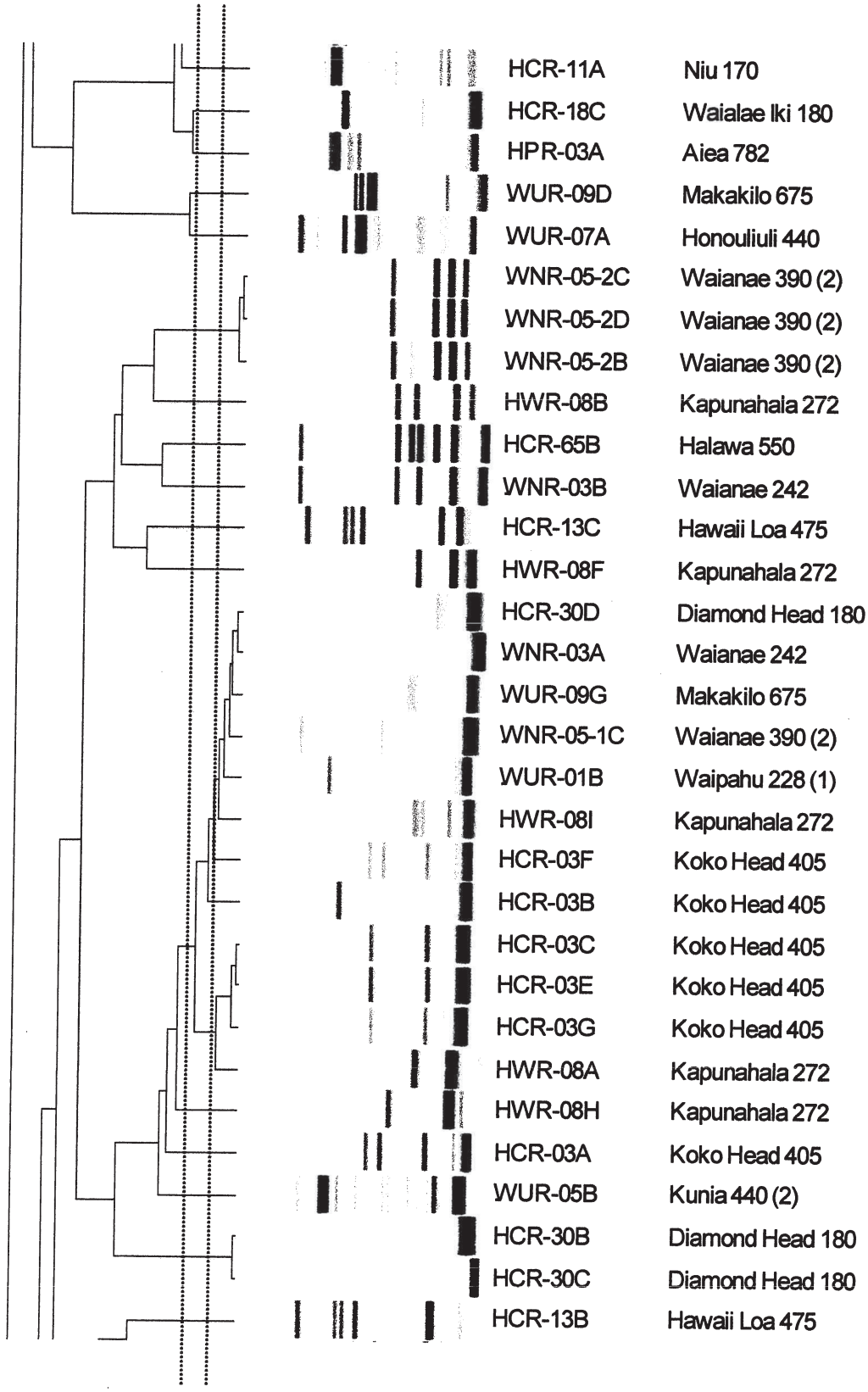




HPS-01Q	Pearl City Shaft
HPS-01Q	Pearl City Shaft
HPS-01Q	Pearl City Shaft

Figure B.4. Clustering the dendrograms of THB riboprints from reservoir sites using GelCompar method at 80% similarity index




HCR-11A Niu 170
HCR-18C Waialae Iki 180
HPR-03A Aiea 782
WUR-09D Makakilo 675
WUR-07A Honouliuli 440
WNR-05-2C Waianae 390 (2)
WNR-05-2D Waianae 390 (2)
WNR-05-2B Waianae 390 (2)
HWR-08B Kapunahala 272
HCR-65B Halawa 550
WNR-03B Waianae 242
HCR-13C Hawaii Loa 475
HWR-08F Kapunahala 272
HCR-30D Diamond Head 180
WNR-03A Waianae 242
WUR-09G Makakilo 675
WNR-05-1C Waianae 390 (2)
WUR-01B Waipahu 228 (1)
HWR-08I Kapunahala 272
HCR-03F Koko Head 405
HCR-03B Koko Head 405
HCR-03C Koko Head 405
HCR-03E Koko Head 405
HCR-03G Koko Head 405
HWR-08A Kapunahala 272
HWR-08H Kapunahala 272
HCR-03A Koko Head 405
WUR-05B Kunia 440 (2)
HCR-30B Diamond Head 180
HCR-30C Diamond Head 180
HCR-13B Hawaii Loa 475

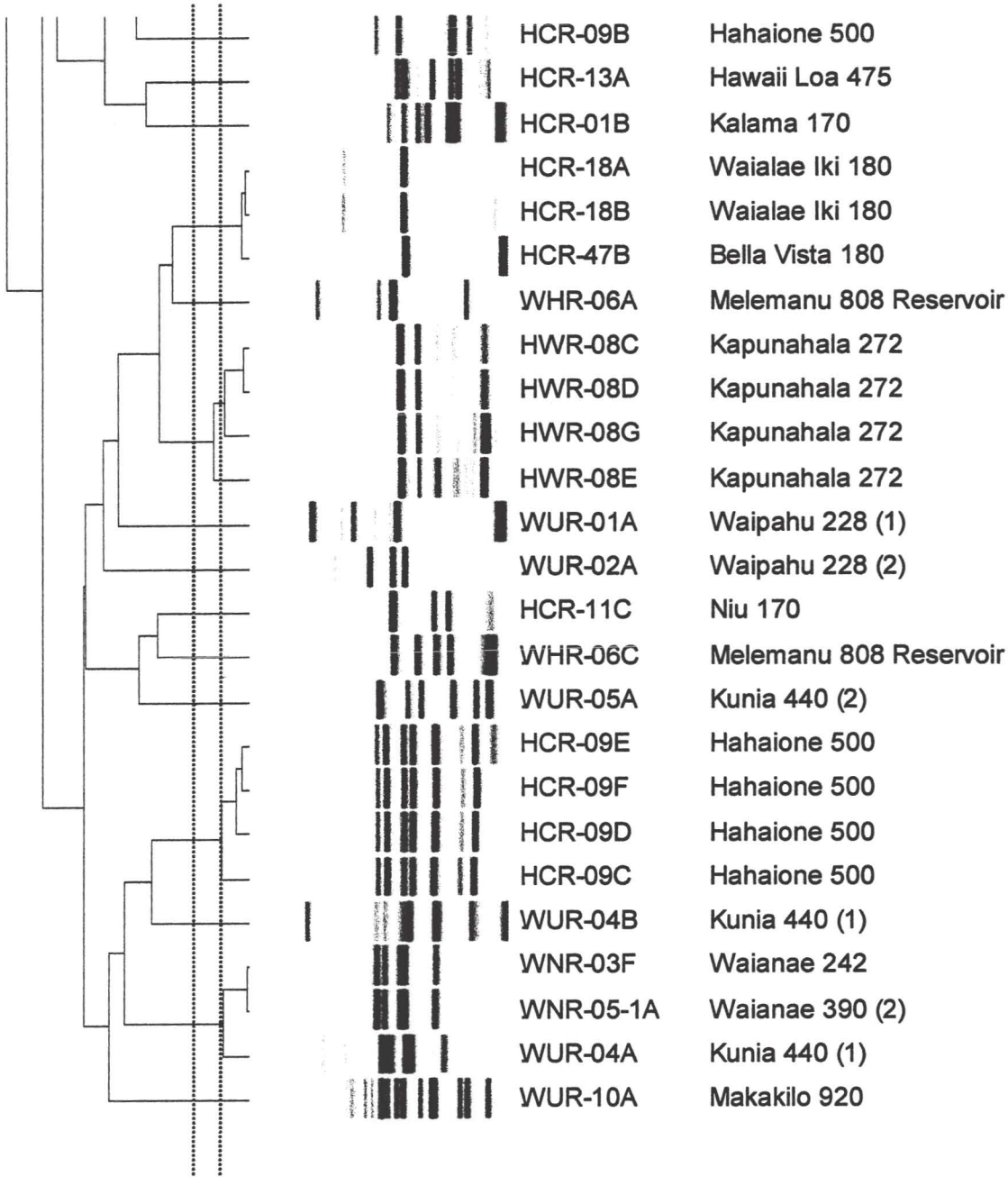
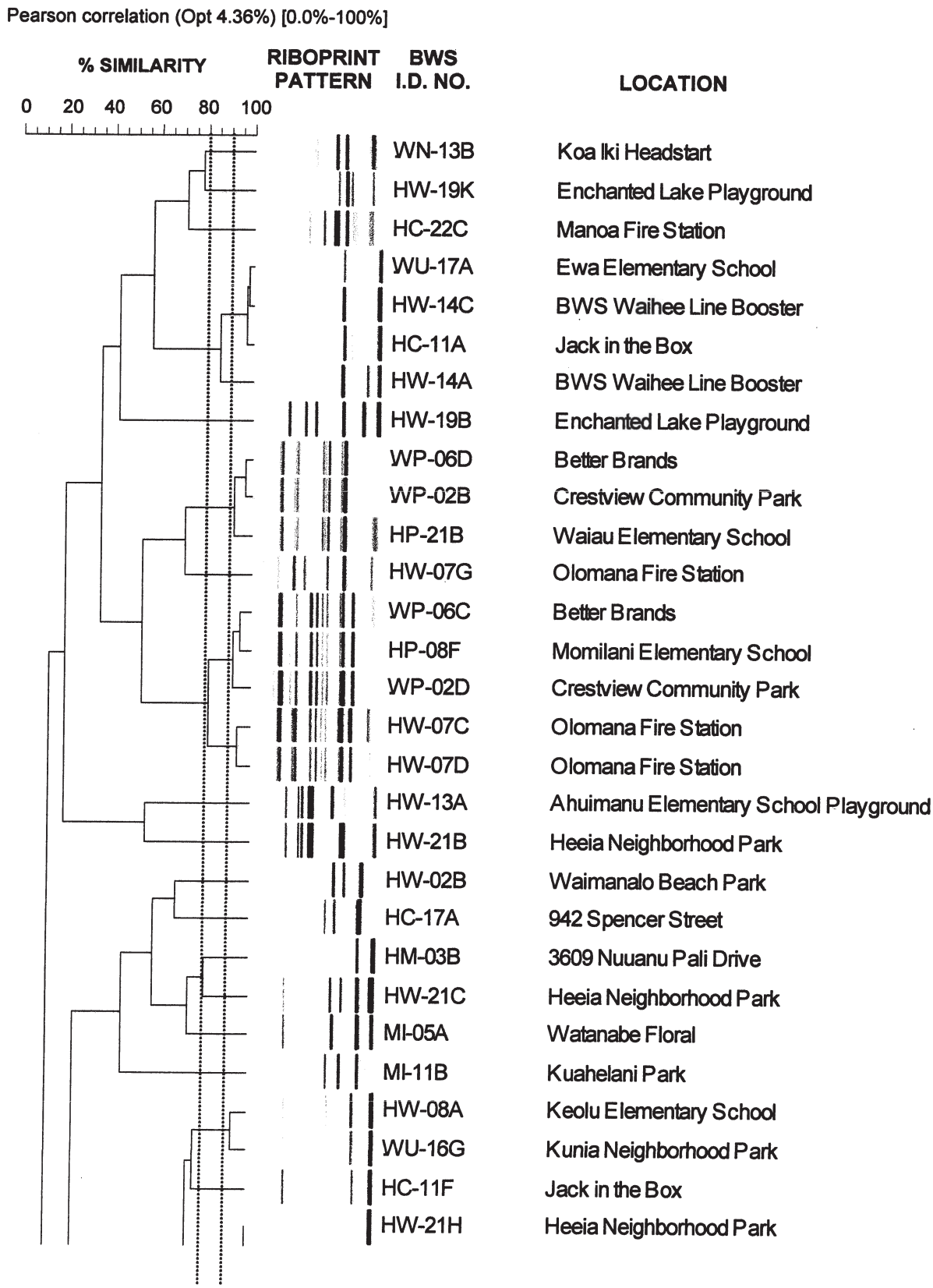
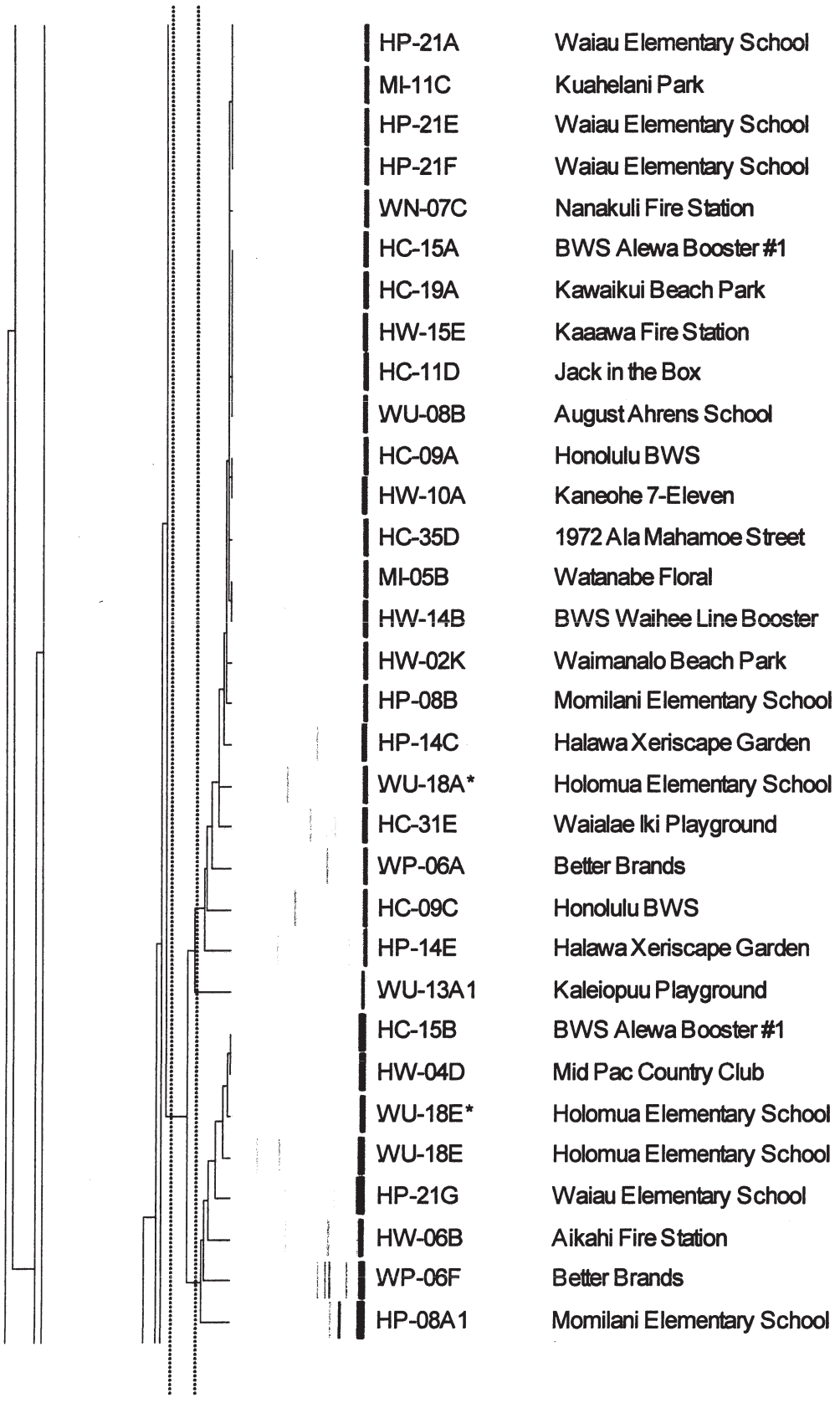
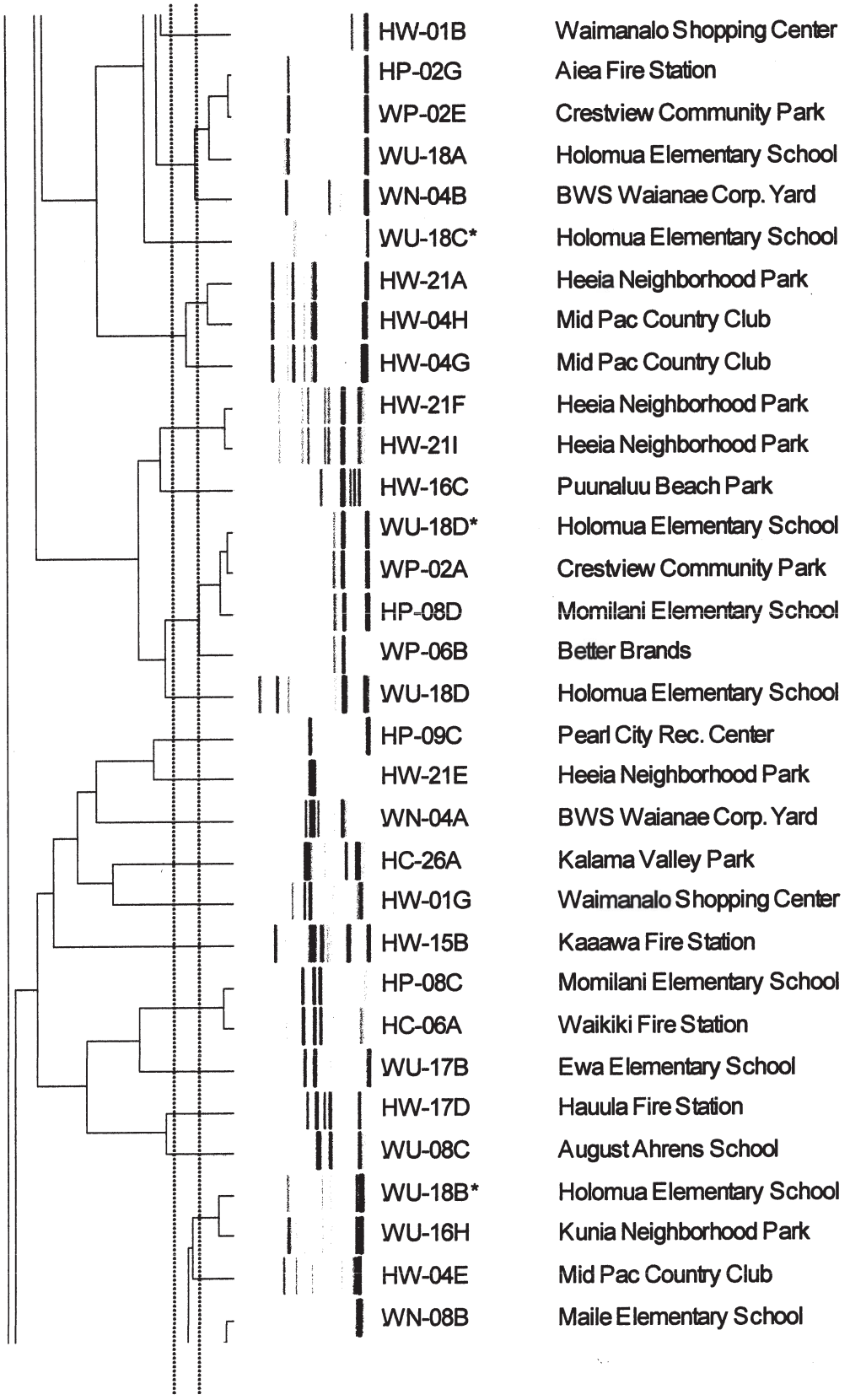
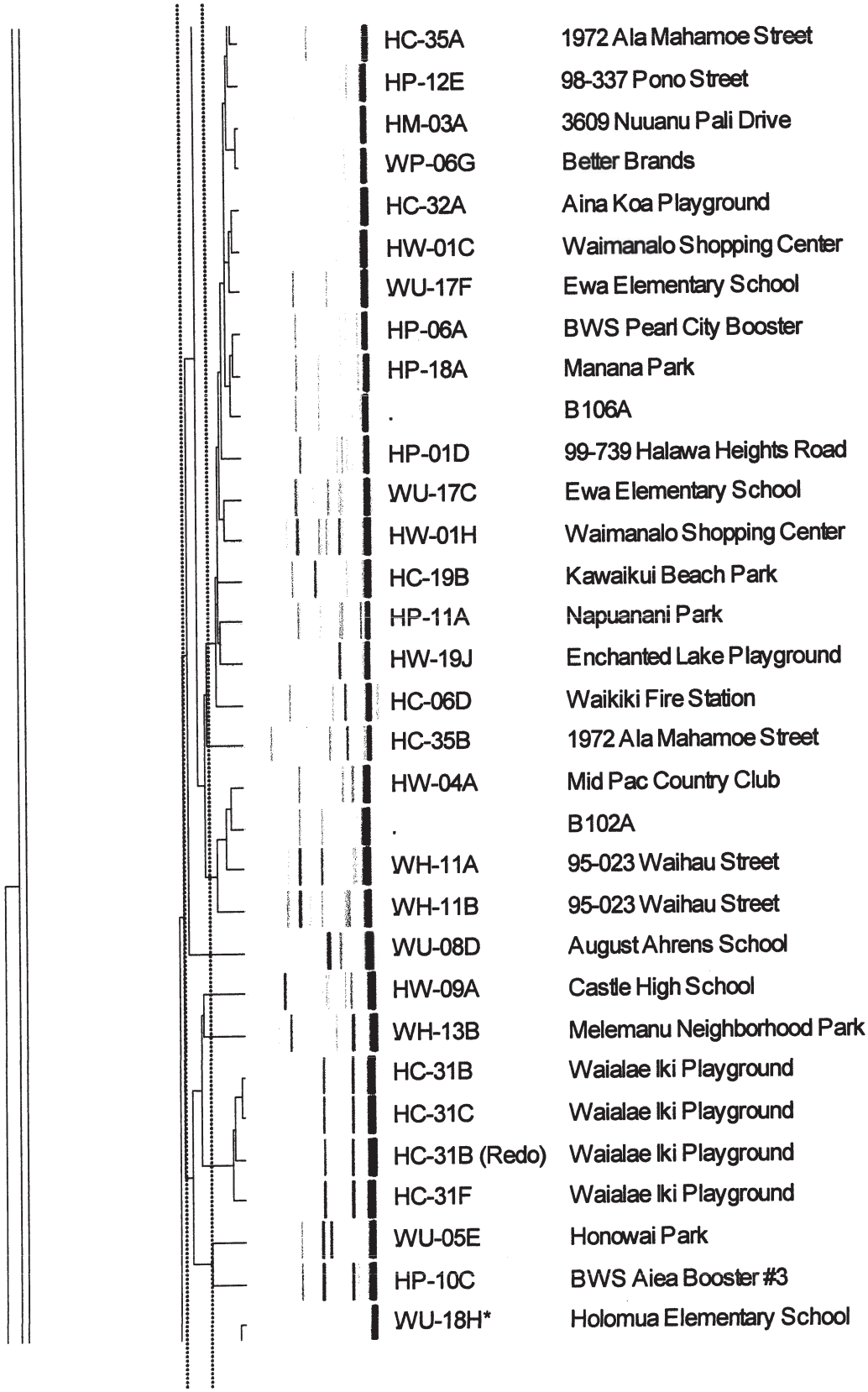

HCR-09B Hahaione 500
HCR-13A Hawaii Loa 475
HCR-01B Kalama 170
HCR-18A Waialae Iki 180
HCR-18B Waialae Iki 180
HCR-47B Bella Vista 180
WHR-06A Melemanu 808 Reservoir
HWR-08C Kapunahala 272
HWR-08D Kapunahala 272
HWR-08G Kapunahala 272
HWR-08E Kapunahala 272
WUR-01A Waipahu 228 (1)
WUR-02A Waipahu 228 (2)
HCR-11C Niu 170
WHR-06C Melemanu 808 Reservoir
WUR-05A Kunia 440 (2)
HCR-09E Hahaione 500
HCR-09F Hahaione 500
HCR-09D Hahaione 500
HCR-09C Hahaione 500
WUR-04B Kunia 440 (1)
WNR-03F Waianae 242
WNR-05-1A Waianae 390 (2)
WUR-04A Kunia 440 (1)
WUR-10A Makakilo 920

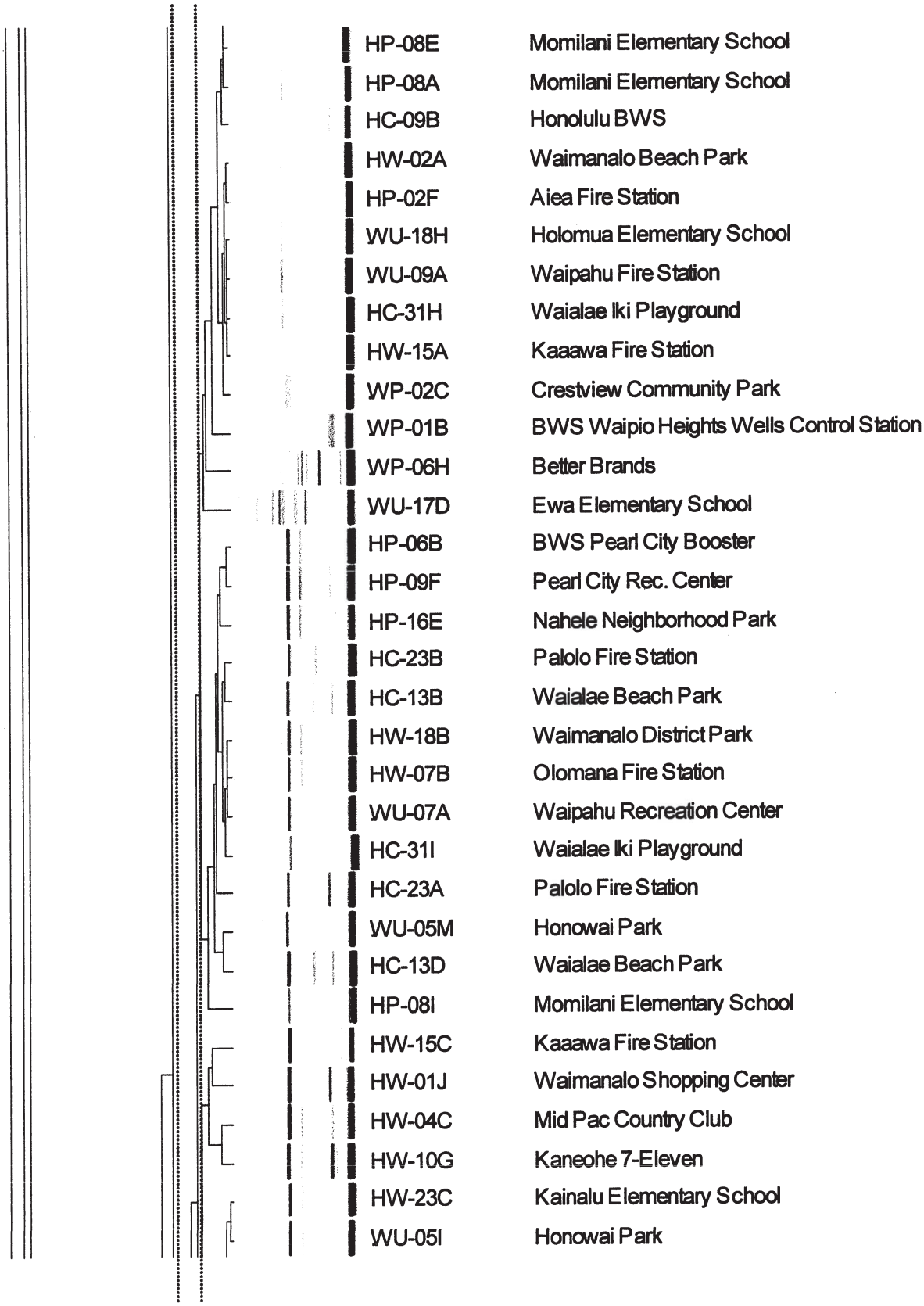
Figure B.5. Clustering the dendrograms of the THB riboprints from distribution sites using GelCompar method at 80% similarity index

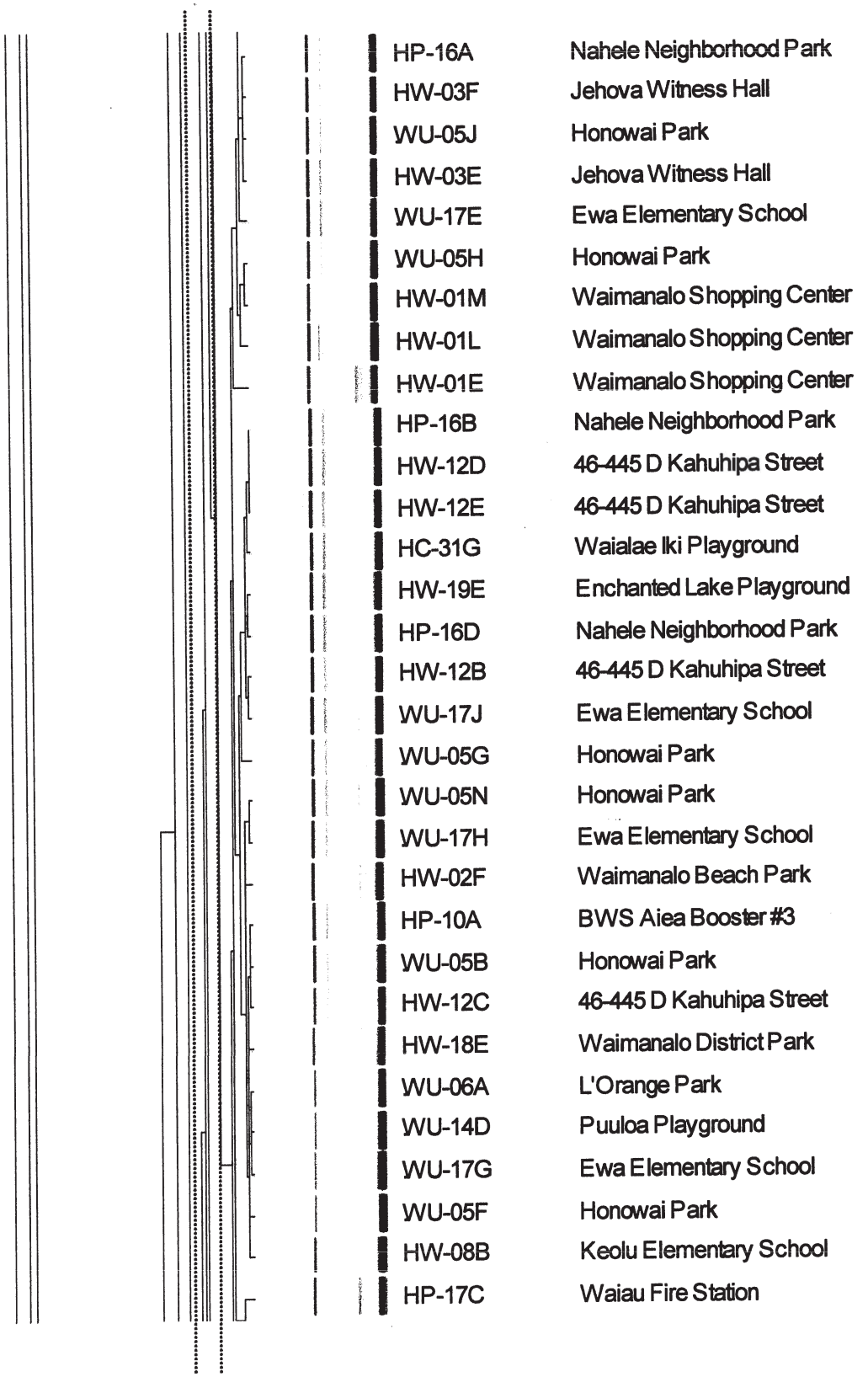


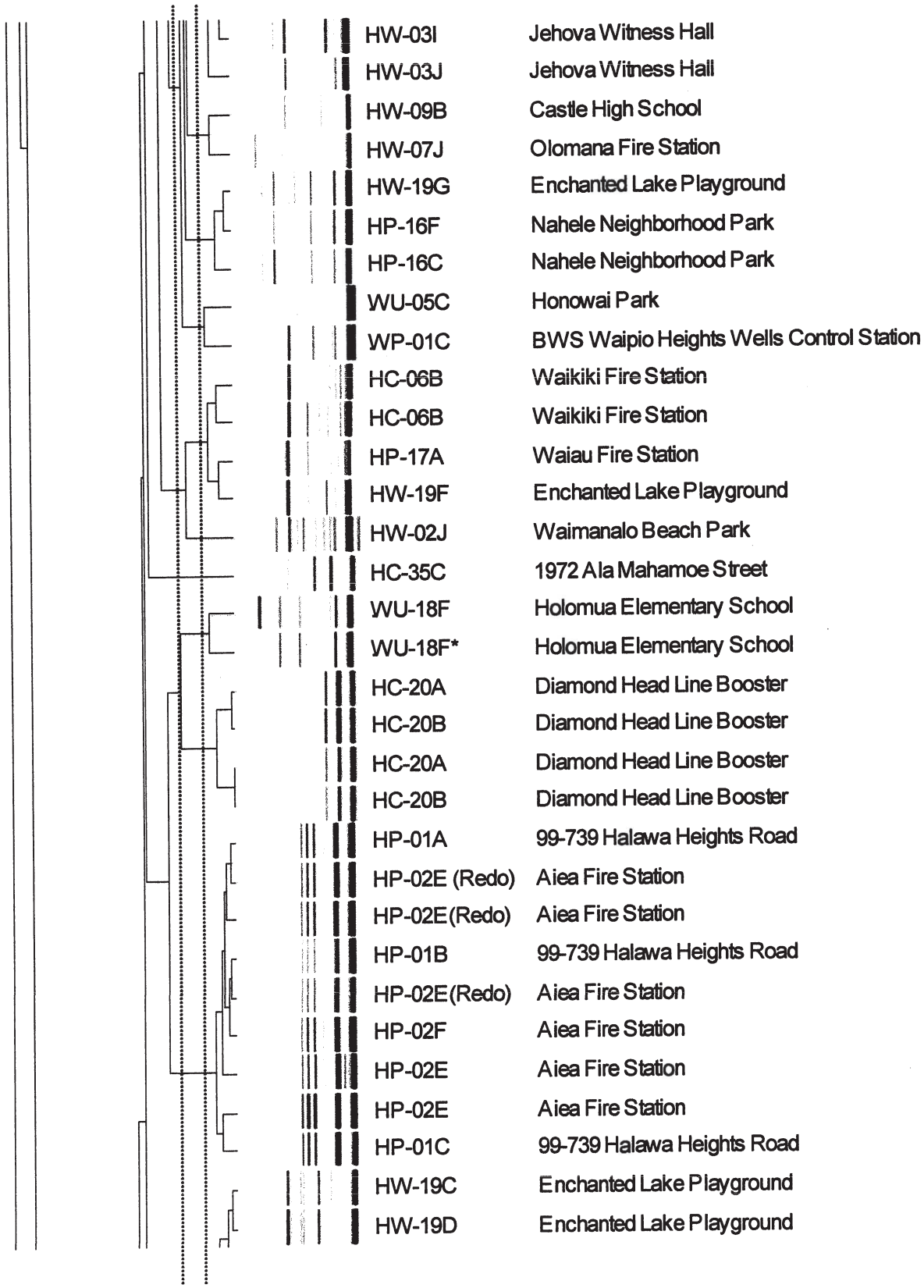

HP-21A Waiau Elementary School
MI-11C Kuahelani Park
HP-21E Waiau Elementary School
HP-21F Waiau Elementary School
WN-07C Nanakuli Fire Station
HC-15A BWS Alewa Booster #1
HC-19A Kawaikui Beach Park
HW-15E Kaaawa Fire Station
HC-11D Jack in the Box
WU-08B August Ahrens School
HC-09A Honolulu BWS
HW-10A Kaneohe 7-Eleven
HC-35D 1972 Ala Mahamoe Street
MI-05B Watanabe Floral
HW-14B BWS Waihee Line Booster
HW-02K Waimanalo Beach Park
HP-08B Momilani Elementary School
HP-14C Halawa Xeriscape Garden
WU-18A* Holomua Elementary School
HC-31E Waialae Iki Playground
WP-06A Better Brands
HC-09C Honolulu BWS
HP-14E Halawa Xeriscape Garden
WU-13A1 Kaleiopuu Playground
HC-15B BWS Alewa Booster #1
HW-04D Mid Pac Country Club
WU-18E* Holomua Elementary School
WU-18E Holomua Elementary School
HP-21G Waiau Elementary School
HW-06B Aikahi Fire Station
WP-06F Better Brands
HP-08A1 Momilani Elementary School

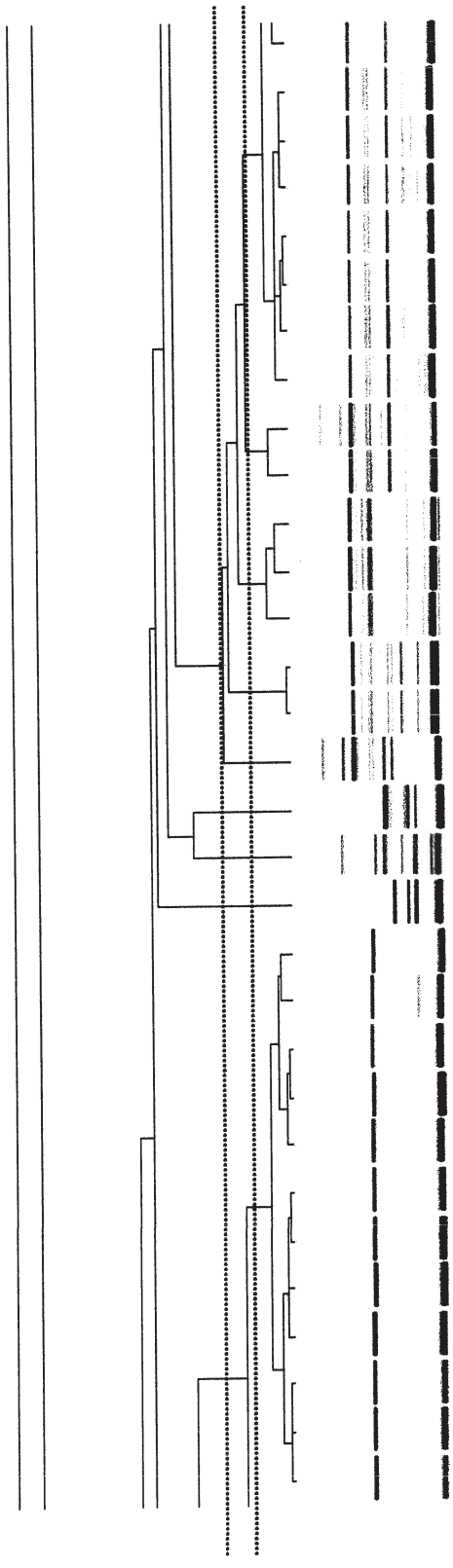

HW-01B Waimanalo Shopping Center
HP-02G Aiea Fire Station
WP-02E Crestview Community Park
WU-18A Holomua Elementary School
WN-04B BWS Waianae Corp. Yard
WU-18C* Holomua Elementary School
HW-21A Heeia Neighborhood Park
HW-04H Mid Pac Country Club
HW-04G Mid Pac Country Club
HW-21F Heeia Neighborhood Park
HW-21I Heeia Neighborhood Park
HW-16C Puunaluu Beach Park
WU-18D* Holomua Elementary School
WP-02A Crestview Community Park
HP-08D Momilani Elementary School
WP-06B Better Brands
WU-18D Holomua Elementary School
HP-09C Pearl City Rec. Center
HW-21E Heeia Neighborhood Park
WN-04A BWS Waianae Corp. Yard
HC-26A Kalama Valley Park
HW-01G Waimanalo Shopping Center
HW-15B Kaaawa Fire Station
HP-08C Momilani Elementary School
HC-06A Waikiki Fire Station
WU-17B Ewa Elementary School
HW-17D Hauula Fire Station
WU-08C August Ahrens School
WU-18B* Holomua Elementary School
WU-16H Kunia Neighborhood Park
HW-04E Mid Pac Country Club
WN-08B Maile Elementary School


HC-35A 1972 Ala Mahamoe Street
HP-12E 98-337 Pono Street
HM-03A 3609 Nuuanu Pali Drive
WP-06G Better Brands
HC-32A Aina Koa Playground
HW-01C Waimanalo Shopping Center
WU-17F Ewa Elementary School
HP-06A BWS Pearl City Booster
HP-18A Manana Park
. B106A
HP-01D 99-739 Halawa Heights Road
WU-17C Ewa Elementary School
HW-01H Waimanalo Shopping Center
HC-19B Kawaikui Beach Park
HP-11A Napuanani Park
HW-19J Enchanted Lake Playground
HC-06D Waikiki Fire Station
HC-35B 1972 Ala Mahamoe Street
HW-04A Mid Pac Country Club
. B102A
WH-11A 95-023 Waihau Street
WH-11B 95-023 Waihau Street
WU-08D August Ahrens School
HW-09A Castle High School
WH-13B Melemanu Neighborhood Park
HC-31B Waialae Iki Playground
HC-31C Waialae Iki Playground
HC-31B (Redo) Waialae Iki Playground
HC-31F Waialae Iki Playground
WU-05E Honowai Park
HP-10C BWS Aiea Booster #3
WU-18H* Holomua Elementary School

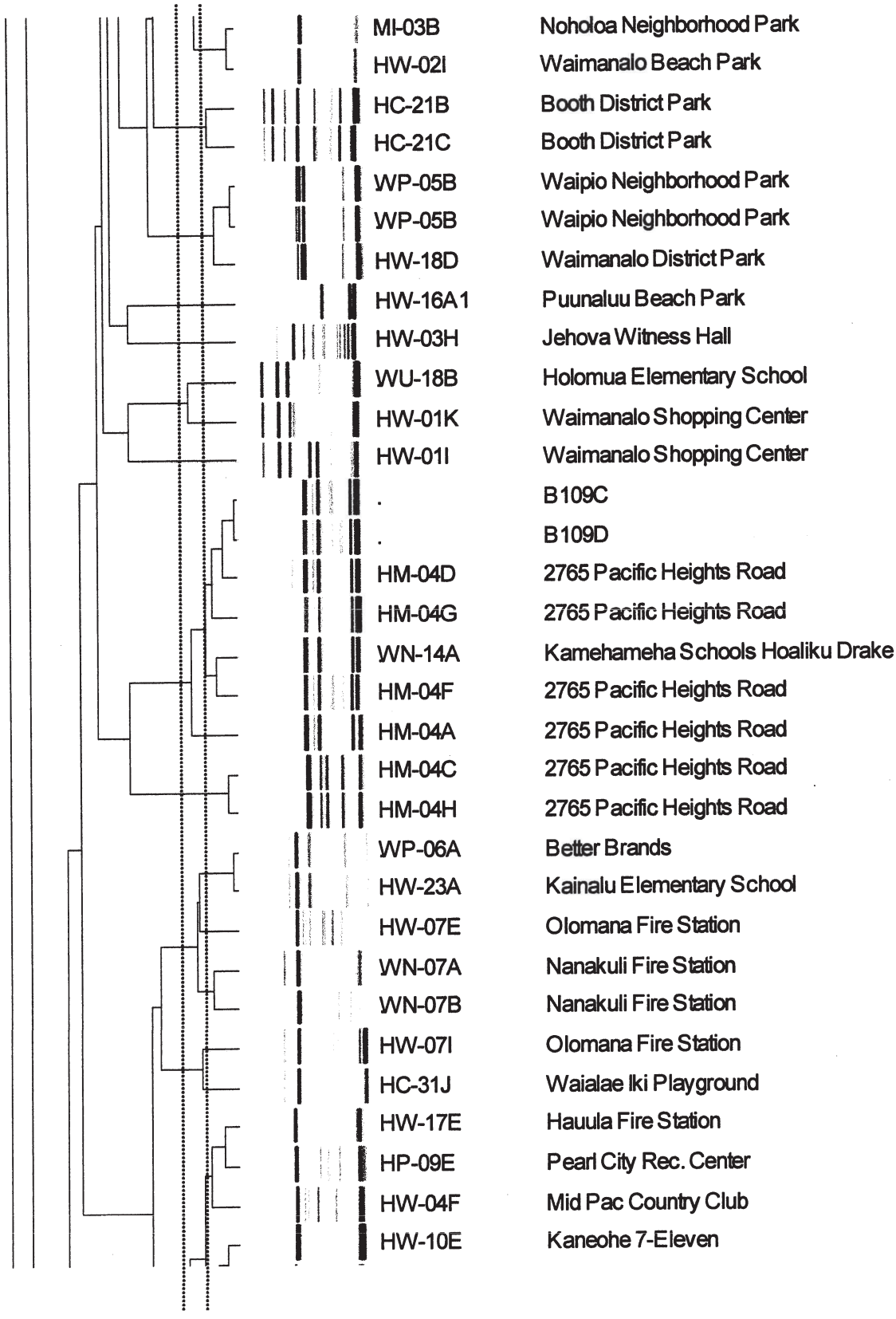

HP-08E Momilani Elementary School
HP-08A Momilani Elementary School
HC-09B Honolulu BWS
HW-02A Waimanalo Beach Park
HP-02F Aiea Fire Station
WU-18H Holomua Elementary School
WU-09A Waipahu Fire Station
HC-31H Waialae Iki Playground
HW-15A Kaaawa Fire Station
WP-02C Crestview Community Park
WP-01B BWS Waipio Heights Wells Control Station
WP-06H Better Brands
WU-17D Ewa Elementary School
HP-06B BWS Pearl City Booster
HP-09F Pearl City Rec. Center
HP-16E Nahele Neighborhood Park
HC-23B Palolo Fire Station
HC-13B Waialae Beach Park
HW-18B Waimanalo District Park
HW-07B Olomana Fire Station
WU-07A Waipahu Recreation Center
HC-31I Waialae Iki Playground
HC-23A Palolo Fire Station
WU-05M Honowai Park
HC-13D Waialae Beach Park
HP-08I Momilani Elementary School
HW-15C Kaaawa Fire Station
HW-01J Waimanalo Shopping Center
HW-04C Mid Pac Country Club
HW-10G Kaneohe 7-Eleven
HW-23C Kainalu Elementary School
WU-05I Honowai Park

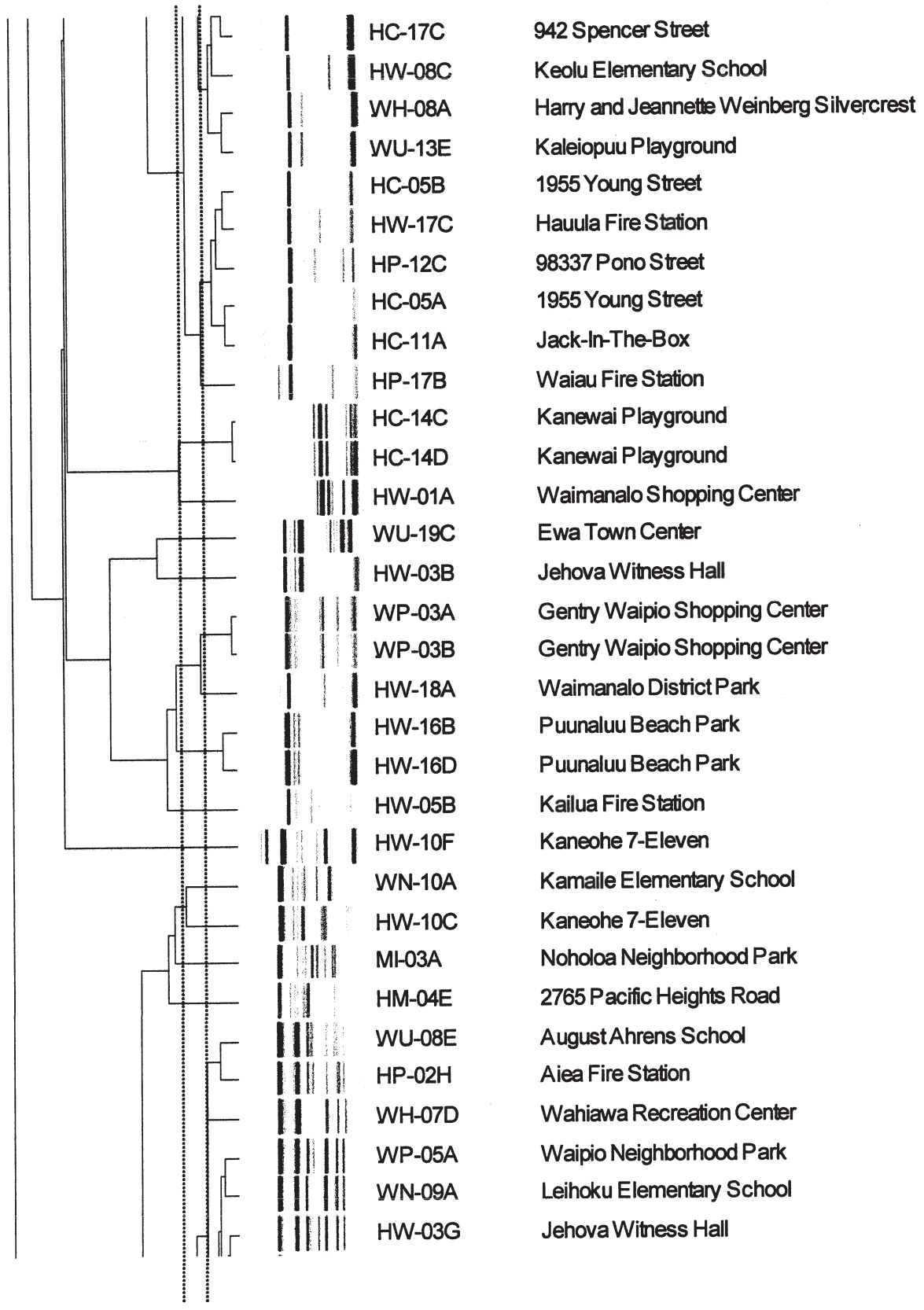

HP-16A Nahele Neighborhood Park
HW-03F Jehova Witness Hall
WU-05J Honowai Park
HW-03E Jehova Witness Hall
WU-17E Ewa Elementary School
WU-05H Honowai Park
HW-01M Waimanalo Shopping Center
HW-01L Waimanalo Shopping Center
HW-01E Waimanalo Shopping Center
HP-16B Nahele Neighborhood Park
HW-12D 46-445 D Kahuhipa Street
HW-12E 46-445 D Kahuhipa Street
HC-31G Waialae Iki Playground
HW-19E Enchanted Lake Playground
HP-16D Nahele Neighborhood Park
HW-12B 46-445 D Kahuhipa Street
WU-17J Ewa Elementary School
WU-05G Honowai Park
WU-05N Honowai Park
WU-17H Ewa Elementary School
HW-02F Waimanalo Beach Park
HP-10A BWS Aiea Booster #3
WU-05B Honowai Park
HW-12C 46-445 D Kahuhipa Street
HW-18E Waimanalo District Park
WU-06A L'Orange Park
WU-14D Puuloa Playground
WU-17G Ewa Elementary School
WU-05F Honowai Park
HW-08B Keolu Elementary School
HP-17C Waiau Fire Station

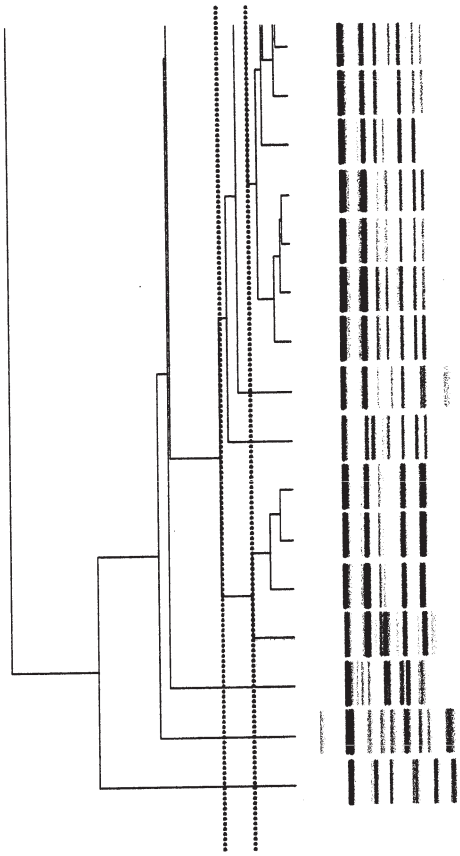

HW-03I Jehova Witness Hall
HW-03J Jehova Witness Hall
HW-09B Castle High School
HW-07J Olomana Fire Station
HW-19G Enchanted Lake Playground
HP-16F Nahele Neighborhood Park
HP-16C Nahele Neighborhood Park
WU-05C Honowai Park
WP-01C BWS Waipio Heights Wells Control Station
HC-06B Waikiki Fire Station
HC-06B Waikiki Fire Station
HP-17A Waiau Fire Station
HW-19F Enchanted Lake Playground
HW-02J Waimanalo Beach Park
HC-35C 1972 Ala Mahamoe Street
WU-18F Holomua Elementary School
WU-18F* Holomua Elementary School
HC-20A Diamond Head Line Booster
HC-20B Diamond Head Line Booster
HC-20A Diamond Head Line Booster
HC-20B Diamond Head Line Booster
HP-01A 99-739 Halawa Heights Road
HP-02E (Redo) Aiea Fire Station
HP-02E(Redo) Aiea Fire Station
HP-01B 99-739 Halawa Heights Road
HP-02E(Redo) Aiea Fire Station
HP-02F Aiea Fire Station
HP-02E Aiea Fire Station
HP-02E Aiea Fire Station
HP-01C 99-739 Halawa Heights Road
HW-19C Enchanted Lake Playground
HW-19D Enchanted Lake Playground



WU-07B	Waipahu Recreation Center
WU-05L	Honowai Park
HW-03A	Jehova Witness Hall
WU-05K	Honowai Park
HW-19A	Enchanted Lake Playground
WU-14A	Puuloa Playground
WU-14E	Puuloa Playground
HW-03C	Jehova Witness Hall
WU-13F	Kaleiopuu Playground
HW-23D	Kainalu Elementary School
HW-11B	Kaneohe Fire Station
HW-13A	Ahuimanu Elementary School Playground
WU-19A	Ewa Town Center
WU-16D	Kunia Neighborhood Park
WU-16F	Kunia Neighborhood Park
WU-13D	Kaleiopuu Playground
WN-08A	Maile Elementary School
HW-02E	Waimanalo Beach Park
HC-34C	Nuuanu Fire Station
HW-06A	Aikahi Fire Station
HP-12D	98-337 Pono Street
HP-12A	98337 Pono Street
HP-09A	Pearl City Rec. Center
HP-12B	98337 Pono Street
HP-18B	Manana Park
HP-09B	Pearl City Rec. Center
WP-06B	Better Brands
HC-13E	Waialae Beach Park
WU-16C	Kunia Neighborhood Park
HW-18H	Waimanalo District Park
HW-02G	Waimanalo Beach Park


MI-03B Noholoa Neighborhood Park
HW-02I Waimanalo Beach Park
HC-21B Booth District Park
HC-21C Booth District Park
WP-05B Waipio Neighborhood Park
WP-05B Waipio Neighborhood Park
HW-18D Waimanalo District Park
HW-16A1 Puunaluu Beach Park
HW-03H Jehova Witness Hall
WU-18B Holomua Elementary School
HW-01K Waimanalo Shopping Center
HW-01I Waimanalo Shopping Center
. B109C
. B109D
HM-04D 2765 Pacific Heights Road
HM-04G 2765 Pacific Heights Road
WN-14A Kamehameha Schools Hoaliku Drake
HM-04F 2765 Pacific Heights Road
HM-04A 2765 Pacific Heights Road
HM-04C 2765 Pacific Heights Road
HM-04H 2765 Pacific Heights Road
WP-06A Better Brands
HW-23A Kainalu Elementary School
HW-07E Olomana Fire Station
WN-07A Nanakuli Fire Station
WN-07B Nanakuli Fire Station
HW-07I Olomana Fire Station
HC-31J Waialae Iki Playground
HW-17E Hauula Fire Station
HP-09E Pearl City Rec. Center
HW-04F Mid Pac Country Club
HW-10E Kaneohe 7-Eleven


HC-17C 942 Spencer Street
HW-08C Keolu Elementary School
WH-08A Harry and Jeannette Weinberg Silvercrest
WU-13E Kaleiopuu Playground
HC-05B 1955 Young Street
HW-17C Hauula Fire Station
HP-12C 98337 Pono Street
HC-05A 1955 Young Street
HC-11A Jack-In-The-Box
HP-17B Waiau Fire Station
HC-14C Kanewai Playground
HC-14D Kanewai Playground
HW-01A Waimanalo Shopping Center
WU-19C Ewa Town Center
HW-03B Jehova Witness Hall
WP-03A Gentry Waipio Shopping Center
WP-03B Gentry Waipio Shopping Center
HW-18A Waimanalo District Park
HW-16B Puunaluu Beach Park
HW-16D Puunaluu Beach Park
HW-05B Kailua Fire Station
HW-10F Kaneohe 7-Eleven
WN-10A Kamaile Elementary School
HW-10C Kaneohe 7-Eleven
MI-03A Noholoa Neighborhood Park
HM-04E 2765 Pacific Heights Road
WU-08E August Ahrens School
HP-02H Aiea Fire Station
WH-07D Wahiawa Recreation Center
WP-05A Waipio Neighborhood Park
WN-09A Leihoku Elementary School
HW-03G Jehova Witness Hall



HW-02C	Waimanalo Beach Park
HW-12A1	46-445 D Kahuhipa Street
HC-31D	Waialae Iki Playground
MI-10C	Mililani Golf Course
WU-19B	Ewa Town Center
WN-09B	Leihoku Elementary School
WU-02A	BWS Makakilo Booster #2
HW-16E	Puunaluu Beach Park
HM-03C	3609 Nuuanu Pali Drive
WP-05A	Waipio Neighborhood Park
WP-05A	Waipio Neighborhood Park
HP-08G	Momilani Elementary School
HW-18G	Waimanalo District Park
HW-02H	Waimanalo Beach Park
WU-18C	Holomua Elementary School
HW-10H	Kaneohe 7-Eleven