

MANAGEMENT OF PATHOGENS ASSOCIATED WITH STORM DRAIN DISCHARGE

**RESULTS OF INVESTIGATIONS OF THE PRESENCE OF
HUMAN PATHOGENS IN URBAN STORM DRAINS**



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CONTENTS

| | |
|--|-------|
| Tables..... | iii |
| Acknowledgements | v |
| Executive Summary | vi |
| Research Approach | vivii |
| Results | viii |
| Significance of Microorganisms in Urban Drainage | ix |
| Suggested Directions | xii |
| Conclusions | xiii |
| Introduction..... | 1 |
| Biological Quality of Water..... | 2 |
| Waterborne Diseases | 3 |
| Indicator Organisms..... | 6 |
| Total Coliforms, Fecal Coliforms, and Enterococci..... | 7 |
| Standards for Biological Quality of Water..... | 9 |
| Indicator Organisms and the Environment | 13 |
| Problem Statement and Research Approach | 15 |
| Pathogen Selection..... | 17 |
| Research Approach | 19 |
| Baseline Studies..... | 21 |
| Field Investigations..... | 22 |
| Methods..... | 24 |
| Sample Collection and Preservation | 25 |
| Development of an Elution Protocol for the FALP Filter..... | 26 |
| Elution of FALP and 1-MDS Filters..... | 26 |
| Nucleic Acid Extraction and Purification..... | 27 |
| Nucleic Acid Amplification and Detection..... | 27 |
| Confirmation of The PCR Product..... | 28 |
| Baseline Sensitivity of PCR Assays..... | 31 |
| Inhibition of PCR..... | 31 |
| Baseline Recovery from FALP and 1-MDS Filters..... | 32 |
| Recovery from FALP Filters Processed at Each Sample Location..... | 32 |
| Recovery from 1-MDS Filters Processed at Each Sample Location..... | 33 |
| Results | 33 |
| Experimental Methods Development..... | 33 |
| Elution Protocol for the FALP Filter..... | 34 |
| Baseline Recovery and Sensitivity..... | 34 |
| Inhibition of PCR..... | 36 |
| Recovery from Processed FALP Filters..... | 38 |
| Recovery from Processed 1-MDS Filters..... | 40 |
| Bulk Measures of Water Quality..... | 40 |
| Overall Detection Limit of the PCR-Based Assays | 43 |
| Baseline Studies | 45 |
| Field Investigations | 50 |
| Discussion | 55 |
| Detection Limits | 56 |
| Pathogen Sources | 57 |
| Significance of Microorganisms in Urban Drainage..... | 58 |
| Suggested Directions..... | 60 |
| Conclusions..... | 63 |
| References..... | 65 |

TABLES

| | | |
|-----------|--|----|
| Table 1. | Principal waterborne diseases | 4 |
| Table 2. | Incidence of waterborne salmonellosis, typhoid fever and shigellosis in Israel from 1976 to 1997 | 5 |
| Table 3. | Ambient water quality criteria for marine and fresh waters used for full contact recreation. | 11 |
| Table 4. | Beach postings and closures in Southern California counties between 1999 and 2001. | 17 |
| Table 5. | Description of sample locations used in methods development | 20 |
| Table 6. | Baseline study sites..... | 21 |
| Table 7. | Sample sites used for field investigations | 23 |
| Table 8. | Molecular techniques used in the identification and confirmation of the selected bacteria, viruses, and protozoa in water samples collected from storm drains and surfaces in close proximity to the drains. | 29 |
| Table 9. | Composition of PCR and RT-PCR reaction mixtures. | 30 |
| Table 10. | Evaluation of three methods of eluting an FHLP filter processed with a water sample spiked with known concentrations of <i>E. coli</i> and <i>C. parvum</i> | 35 |
| Table 11. | Baseline recovery and sensitivity of the protocols used in the isolation and identification of pathogens spiked into RNase free water..... | 35 |
| Table 12. | Recovery of bovine enterovirus/fecal coliform and sensitivity of PCR assays used in the detection of pathogens in water | 37 |
| Table 13. | Recovery of bovine enterovirus/fecal coliform and sensitivity of PCR assays used in the detection of pathogens in water | 39 |
| Table 14. | Bulk measures of the physical, chemical, and biological quality of water samples collected from seven storm drains or surfaces in close proximity to the drains..... | 42 |
| Table 15. | Concentrations of selected pathogens in water samples collected from seven storm drains and surfaces located in close proximity to the drains..... | 44 |
| Table 16. | Summary of baseline experiment results. Values for PCR data for viruses, bacteria and protozoa can be read as: blank = non detect, 0 = detected in undiluted sample only, 1, 2, ...n = detected at the nth fold dilution | 47 |
| Table 17. | Summary of field investigation results. Values for PCR data for viruses, bacteria and protozoa can be read as: blank = non detect, 0 = detected in undiluted sample only, 1, 2, ...n = detected at the nth fold dilution. | 51 |

| | | |
|-----------|--|----|
| Table A-1 | Highway Drain Workbook - Gross Characteristics | 68 |
| Table A-2 | Highway Drain Workbook - Viruses | 75 |
| Table A-3 | Highway Drain Workbook - Bacteria and Protozoa..... | 91 |
| Table A-4 | UC Riverside Sample Details..... | 84 |
| Table A-5 | Sample Site Locations | 90 |
| Table A-6 | Baseline Study - Gross Characteristics | 91 |
| Table A-7 | Baseline Study - Indicator Organisms..... | 93 |
| Table A-8 | Baseline Study - Viruses | 96 |
| Table A-9 | Baseline Study - Bacteria and Protozoa | 98 |

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EXECUTIVE SUMMARY

Substantial evidence has accumulated that the indicator organisms used to evaluate the biological quality of water provide erroneous information under a number of circumstances. The organisms have been shown to reproduce and compete in warm soils, to be normal members of the microbial community in some instances, and to have survival rates lower than some important waterborne pathogens. Additionally, there have been important cases, such as the cryptosporidiosis outbreaks in Carrollton, Georgia and Milwaukee, Wisconsin, where indicator organisms were absent altogether. In considering urban drainage, such as that flowing onto beaches in California, the impact of this information is significant because of the need to address high indicator bacteria concentrations in the surf zone. California limits concentrations of total coliform bacteria, fecal coliform bacteria, and *Enterococcus* spp. in recreational waters. In 2001 there were 795 beach postings and 115 beach closures in the San Diego, Orange and Los Angeles counties. Nearly all of the postings were due to high bacterial counts of unknown origin while nearly all of the closures were due to known sewage spills.

Posting of beaches has a major economic impact on beach communities as well as the loss of recreational opportunities to beach users. If the high bacterial counts are not associated with high risks of pathogen contact, public health is not being protected by the postings and the economic and recreational losses incurred have no benefit. Moreover, other possible mitigating actions, such as diversion of dry weather urban drainage to storm sewers and storage and disinfection of wet weather flows would drain public health budgets without protecting public health. If biological water quality questions must be addressed, managing urban drainage presents major engineering problems, particularly following storms. For these reasons

determination of relationship between the indicator organism concentrations and presence of pathogens in urban drainage is extremely desirable.

The World Health Organization has succinctly stated the current situation with respect to indicator organisms and recreational waters in a 1999 document termed the *Annapolis Protocol*.

Present regulatory schemes for the microbiological quality of recreational water are primarily or exclusively based on percentage compliance with faecal indicator counts. A number of constraints are evident in the current standards and guidelines:

- *management actions are retrospective and can only be deployed after human exposure to the hazard;*
- *the risk to health is primarily from human excreta, the traditional indicators of which may also derive from other sources;*
- *there is poor inter-laboratory and international comparability of microbiological analytical data; and*
- *while beaches are classified as safe or unsafe, there is a gradient of increasing severity, variety and frequency of health effects with increasing sewage pollution and it is desirable to promote incremental improvements prioritising 'worst failures'*

Research Approach

The project was organized in three phases; methods development, baseline studies and field studies. In all three phases samples were analyzed for both indicator organisms (coliforms, fecal coliforms, *E. coli*, and enterococci) and for four viruses (adenovirus, enterovirus, hepatitis A virus, and rotavirus), five bacteria (enterohemorrhagic *Escherichia coli*¹, enterotoxigenic *Escherichia coli*, *Shigella*, *Salmonella*, and *Staphylococcus aureus*), and two protozoa (*Giardia lamblia* and *Cryptosporidium parvum*). Indicator organisms were monitored using traditional

methods and a majority of the analyses were done by commercial laboratories. Pathogen analysis was conducted using polymerase chain reaction (PCR) technology. This method requires extraction of deoxyribonucleic or ribonucleic acid (DNA or RNA) sequences, amplification of specific nucleic acid sequences using *primers* that define the sequence end points, and analyzing for the presence of the selected sequences using gel electrophoresis. PCR amplification allows detection of extremely small quantities of nucleic acid. The sequences defined by each primer set are specific to each organism. Methods development was required because of the complex nature of the samples. Environmental samples often contain materials that interfere with the molecular techniques used to detect pathogens. Additionally, methods of technique validation must be developed.

The purpose of the baseline studies was to develop background data from common areas that produce urban drainage. Samples for the baseline studies were taken from paved and grass areas of parks, roofs, residential lawns, ponds, storm drains and similar surfaces that would provide a broad picture of the microbial quality of urban water.

Ninety-seven field investigation samples were taken under both wet and dry conditions at 20 sites between Los Angeles and San Diego. The sites selected included urban drains and best management practice treatment installations (bmps).

Results

Methods development proved to be a difficult issue because of materials in environmental samples that inhibited and/or interfered with the molecular techniques used to determine the presence of pathogens. Dissolved constituents may cause direct inhibition while particulate material, such as tar and other hydrocarbons, foul filters and reduce recoveries.

¹ Enterohemorrhagic *Escherichia coli* (EHEC) is also listed as *Escherichia coli* O157:H7 and is the cause of potentially deadly hemolytic uremic syndrome.

Based on an analysis of recoveries and sensitivities, a detection limit analysis was formulated. Such an approach provides more meaningful interpretation of data, rather than merely reporting the presence or absence of a pathogen, a practice that is commonly used in PCR-based assays. In many cases the detection limits for environmental samples appear to be unsatisfactorily high and work needs to be directed toward improving the analytical methods.

The baseline studies were composed of 49 samples taken at 35 sites. Pathogens were detected in 10 of the samples, with two pathogens being detected in one sample for a total of 11 positives. Pathogens detected in the baseline studies were adenovirus (five samples), enterovirus (one sample), *Salmonella* (one sample), *Staphylococcus* (2 samples), *Giardia* (one sample), and *Cryptosporidium* (one sample). The virus positives must be taken as evidence of human contamination while the bacterial and protozoan positives are quite likely from animals or soil populations. Significant indicator organism concentrations were found in most of the baseline samples. In the samples having highest total coliform counts (MPN/100 mL), 5,000,000 from a park lawn washed down in Davis, 280,000 from a park lawn washed down in Laguna Niguel, and 200,000 from a paved surface at a park in Davis, there were no pathogens detected. The results for samples having high concentrations of fecal coliforms and *Enterococcus* were similar. No discernible correlation was observed between indicator organisms and the presence of pathogens, in part because of the ubiquitous presence of indicators.

Results of the field investigations were similar to the results of the baseline studies. Pathogens were detected in twelve of the ninety-seven samples. One of the samples had two positives, making a total of 13 pathogen detections. There was one detection of adenovirus. No other viruses were detected and there were no positives for *Giardia* or *Cryptosporidium*. All of the bacterial detections were enterotoxigenic *Escherichia coli* (4) or *Salmonella* (8). Two of the

samples that were positive for *Salmonella* had *Enterococcus* concentrations of 200,000 MPN/100 mL. However, there were no pathogens detected in a sample having total coliform, fecal coliform, and *Enterococcus* values of 1,600,000, 500, and 7,000 MPN/100 mL, respectively.

Significance of Microorganisms in Urban Drainage

In summary, indicator organisms are ubiquitous in urban drainage while human pathogens are sometimes found in urban drainage. Based on the MPN data available from the baseline studies and the field investigations there does not appear to be a correlation of any kind between the number of indicator organisms and the presence of pathogens. The fact that indicator organisms are present in soil and pavement from parks, drainage from parking lots, roofs, and residential lawns presents interesting issues in terms of regulation of water quality. Based on these investigations there is good evidence that public health is not protected by using the common indicators (total coliforms, fecal coliforms, fecal streptococci, *E. coli* and *Enterobacter*) in this context. However, pathogens are found in urban drainage. Whether there is greater danger from swimming in surf receiving urban drainage, from rolling around on park grass, or from chewing blades of park grass, is unknown. The detection limit problems associated with PCR analysis make defining the problem more difficult. However, regulators must begin to recognize that new approaches to protecting public health and recreational waters must be investigated. Decisions based on detection of indicator organisms often result in significant costs and may very often have no public health benefit.

Of the organisms selected for the study, the viruses and infective *Shigella* exclusively affect humans, and implicitly must have human sources. All the other bacteria and protozoa may have multiple non-human sources. No samples were found to contain infective *Shigella*, so the viruses appear to be the best indicators of human contamination.

Human waste may not necessarily contain these viruses, and could contain any of the non-human source pathogens. Therefore, only those samples that were positive for viruses were conclusive for presence of human contamination and no sample is conclusive for the absence. The field study locations were sampled repeatedly, and viruses are common enough in human waste that at least some of those samples would be expected to contain viruses if a site is subject to chronic human contamination. Incidental contamination is virtually impossible to detect.

Of the samples collected from drains in both study phases, the sites where collected can be categorized as having drainage predominantly from highway uses, predominantly from city uses, and a mixture of the two. Of 18 samples tested, none of those from highway sites were positive for viruses. One of 53 samples from mixed-use sites was found to contain the viruses, but 5 of 25 samples from city sites were positive for viruses. Additionally, one of the city sites, three of the mixed-use sites, and two of the highway sites produced samples in which a pathogenic bacteria or protozoa were found.

The data may also be categorized by hydrologic regime, as coming from more than 72 hrs since the last rain (dry), 1 to 72 hrs since the last rain (recent rain), and raining at the time of collection (wet). Fifty-four samples were collected during dry conditions. Four of these were found to contain viruses. Two of the 18 samples taken from sites having received recent rain contained viruses. In the 24 samples tested that were collected wet, no viruses were found.

There were six exclusively highway drainage sites sampled, with 22 total samples. Two samples, each from a different site, tested positive for bacteria, none were positive for viruses. The city sites, relatively remote from highways, were sampled almost exclusively during the dry season. Those samples were collected during the summer and fall before the rainy season collection on the highways. Five of the 28 samples were collected after recent rains, one of

which was positive for virus. Four of the city sites sampled under dry conditions tested positive for viruses, and one each was positive for bacteria and protozoa.

The mixed drainage sites were sampled mostly during dry and recent rain conditions. Only six samples, each from a different site, were taken from these sites during rains and neither viruses nor bacterial/protozoan pathogens were detected. Thirteen sites with mixed drainage sampled during dry conditions with a total of 46 samples. Six of the 46 samples tested positive for bacteria, representing 3 sites; none of the samples tested positive for viruses. Ten of the mixed drainage sites were sampled after recent rains. One of the 23 samples was found to contain viruses.

These results support the belief that highway facilities are not a significant source of human contamination of either urban drainage or storm drainage. Consideration should be given to setting a low priority on monitoring these facilities for microbial contamination.

Suggested Directions

Four specific research directions should be undertaken for the purposes of protecting the biological quality of and developing appropriate standards for recreational waters.

- (1) Improving the detection limits of molecular methods for analysis of environmental samples and the ability to provide quantitative results using these methods,
- (2) Further study of field sites where highway runoff and drainage from surrounding areas are mixed to establish whether highway runoff has been a contributor to pathogen detections,
- (3) Development of improved understanding of the contributions of urban drains to the microbial quality of the surf zone, and
- (4) Development of improved indicators of human contamination.

Conclusions

- Significant concentrations of indicator organisms are nearly ubiquitous in urban drainage.
- Pathogens can be found in urban drainage but there does not appear to be a relationship between the presence of pathogens and the concentration or presence of indicator organisms.
- Based on the number of pathogen detections in this study, 12 of 97 samples in the field investigations and 10 of 49 samples in the baseline studies, contact with pathogenic organisms in the urban environment is not a rare event.
- The most commonly detected pathogens in stormwater are those for which the principal reservoirs are domestic and/or wild animals.
- Molecular methods of detection of environmental pathogens, such as PCR, are very promising but inhibition and interferences associated with environmental samples can result in unacceptably high detection limits.
- The presence of human viruses may be a more suitable indicator of recent contamination with human wastes than coliforms, fecal coliforms, *E. coli*, or *Enterococcus* counts.
- Consideration should be given to both the probable origins of indicators and pathogens in setting policy and developing strategies for protecting recreational waters.
- With respect to control of waterborne disease, money used for improving sanitary sewers and wastewater treatment is probably more wisely invested than money used for treatment of urban drainage.
- Highway facilities, including park and rides and maintenance stations, do not appear to be a significant source of pathogens in urban drainage.

INTRODUCTION

Biological quality of discharges from storm drains has become an issue of concern to water quality and public health agencies, and to groups and individuals who are regularly in contact with receiving waters. The majority of the concern is with dry weather flow because of greater beach use during good weather. In California, dry weather flow in urban areas results from lawn and shrub irrigation, car washing, recreational use of water, washing down driveways, and similar activities. Sources of coliform and fecal coliform bacteria in dry weather drainage include animal droppings, naturally occurring soil bacteria, human wastes resulting from inappropriate and generally illegal defecation in parks and other areas, and possible contributions of bacteria from leaking municipal sewers. Pathogenic bacteria in the drainage are from the same sources as coliforms and fecal coliforms, although the numbers of specific pathogens may vary considerably.

Health risks associated with human pathogens in storm drain discharges are not well understood. Conventional methods of risk assessment involving the enumeration of coliform and fecal coliform organisms are currently widely being questioned. Protection of public health requires a much improved understanding of the applicability of current measures of biological water quality and the evaluation of new methods based on recent advances in molecular biology for the evaluation of health risks. The primary objective of this project was to assess the density and significance of human pathogens in storm drain discharge from an urban watersheds in Southern California. Specific project goals included:

- Selection and/or development of methods to detect and quantify specific human protozoan, bacterial and viral pathogens,

- Demonstration of the reproducibility and reliability of the methods in analysis of water from storm drains,
- Comparison of the results of pathogen detection and enumeration with results of standard total and fecal coliform tests,
- Establishment of *baseline* coliform and pathogen discharge rates from selected land uses

Biological Quality of Water

Recognition that disease can be carried with water is undoubtedly quite ancient. However, understanding that waterborne diseases were caused by microorganisms and viruses began only in the mid to late 19th century with the work of Pasteur, Koch and other early microbiologists. The classic example is the 1854 case in which John Snow, a London physician, was able to stop a cholera epidemic by removing the handle from Broad Street pump. During the same late 19th century period the association of human and animal wastes with disease transmission began to be understood, as well as the linkage between municipal wastewater disposal and contamination of municipal water supplies. Since that time a number of diseases have been identified that are commonly, or characteristically, transmitted through water. A list of the principal waterborne diseases, the causative organisms, and, if known, infectious doses is given in Table 1.

The biological quality of water is most commonly defined in terms of the potential presence of pathogenic organisms. The concept of the potential presence of pathogenic organisms is based on known contamination by human or animal wastes, conditions that are known to be conducive to contamination, and the presence of certain *indicator organisms* that are associated with human and animal wastes. An example of a known case of contamination was the failure of the ocean outfall discharge pipe of the Point Loma Wastewater Treatment

Plant in San Diego in 1997. Conditions known to be conducive to contamination include combined sewers and sanitary sewers that overflow into storm sewers at very high hydraulic loading rates. Such conditions are recognized in Southern California by the automatic posting of beaches following storms. The presence of indicator organisms, including the coliform organisms referred to above, is used as both a qualitative and quantitative measure of contamination. It is the use of indicator organisms for judging biological quality of recreational waters that poses the greatest difficulty and provides the basis for this project.

Waterborne Diseases

Most waterborne infections are contracted by ingestion and primarily impact the gastrointestinal tract. Some, such as hemolytic uremic syndrome, are systemic. Only a few of the diseases conventionally thought of as waterborne are infections of the skin, eyes, or ears. Records of incidence of waterborne diseases are limited because (a) most infections are relatively minor and the infected persons are not seen by a physician, (b) not all diseases are *notifiable*², and (c) many infections are not diagnosed because symptoms are relatively minor and treatment by a physician is not required.

In California, health care providers are specifically required to report incidences of all of the diseases listed in Table 1 except peptic ulcer to the local health officer. Additionally, all diseases that appear to be water associated must be reported. A summary of statistics on notifiable diseases are collected nationally by the Centers for Disease Control and Prevention, a division of the United States Public Health Service.

² Data on the incidence of 60 diseases are collected by the National Centers for Disease Control and Prevention for state health departments. In California all water associated infections must be reported (Title 17, California Code of Regulations, §2500)

Table 1.
Principal waterborne diseases

| Disease | Causative agent | Infectious dose | Characteristics |
|---|---|----------------------|--|
| Bacterial Infections | | | |
| Cholera | <i>Vibrio cholerae</i> | 10 ⁶ | Severe diarrhea, dehydration watery diarrhea often with abdominal cramping, nausea, vomiting fever and chills |
| Dysentery or Shigellosis | <i>Shigella spp.</i> | ≈ 10 | Bloody diarrhea, abdominal cramps, rectal pain. Most virulent species, <i>S. dysenteriae</i> , produces toxin causing hemolytic uremic syndrome. |
| Gastroenteritis or Campylobacteriosis | <i>Campylobacter jejuni</i> . | <500 | Watery diarrhea often with abdominal cramping, nausea, vomiting fever and chills |
| Hemorrhagic colitis & Hemolytic uremic syndrome | <i>Escherichia coli</i> O157:H7 | unknown ^a | Severe, systemic condition that occurs principally in children under 10 years of age. |
| Legionellosis | <i>Legionella pneumophila</i> | unknown | acute pneumonia, high fever, headache, cough, little sputum. |
| Leptospirosis | <i>Leptospira spp.</i> | unknown | Fever, kidney infection, may result in kidney failure. In some cases there is internal bleeding, including pulmonary hemorrhage. |
| Peptic ulcer | <i>Helicobacter pylori</i> | unknown | Sore or hole in the lining of the stomach or duodenum. |
| Salmonellosis | <i>Salmonella spp.</i> | | Headache, vomiting, diarrhea |
| Typhoid fever | <i>Salmonella typhi</i> | <1000 | Fatigue, headache, abdominal pain, elevated temperature. Approximately 4% death rate. |
| Protozoal Infections | | | |
| Amebiasis | <i>Entamoeba histolytica</i> | 1 | Abdominal discomfort, fatigue, diarrhea, flatulence, weight loss |
| Cryptosporidiosis | <i>Cryptosporidium parvum</i> | 1 | Diarrhea, abdominal discomfort |
| Giardiasis | <i>Giardia lamblia</i> | 1 | Diarrhea, abdominal discomfort |
| Viral Infections | | | |
| Hepatitis A | Hepatitis A | 1 | Fever, chills, abdominal discomfort, jaundice, dark urine |
| Viral Gastroenteritis | Norwalk agents, rotavirus and other viruses | 1 | Fever, headache, gastrointestinal discomfort, vomiting, diarrhea |

^aprobably similar to *Shigella*

Transmission of the diseases listed in Table 1 is quite commonly through a mode other than water, as is indicated by the data in Table 2. Contaminated food is a common mode of transmission for most waterborne diseases. Direct person to person, and in some cases animal to person, transmission occurs also.

Table 2.
Incidence of waterborne salmonellosis, typhoid fever and shigellosis in Israel from 1976 to 1997 (Source: Tulchisky *et al.*, 2000)

| Years | Disease | | | | | |
|-------|---------------|--------|-------------|--------|---------------|-------|
| | Salmonellosis | | Shigellosis | | Typhoid fever | |
| | Waterborne | Total | Waterborne | Total | Waterborne | Total |
| 76-80 | 979 | 10,101 | 6,557 | 32,839 | 112 | 596 |
| 81-85 | 157 | 12,386 | 10,180 | 44,152 | 76 | 629 |
| 86-90 | 244 | 17,127 | 1,524 | 29,070 | 0 | 216 |
| 91-95 | 260 | 28,986 | 260 | 25,874 | 0 | 0 |
| 96-97 | 0 | 11,481 | 0 | 7,274 | 0 | 0 |

Surface waters in even the most remote areas can be contaminated with pathogens through contact with infected animals and animal wastes. For example, birds and reptiles are common carriers of bacteria of the genus *Salmonella*. All species of *Salmonella* are considered to be human pathogens. Feces or carcasses of carriers washed into streams will thus result in contamination. Determining that water is contaminated is difficult. In even highly contaminated waters pathogen concentrations are generally quite low. Additionally, each pathogen requires a specific test be conducted to determine its presence. A negative test for *Salmonella* means that the organisms were not detected rather than that they were absent and provides no information about the presence of other pathogens. Screening for an entire list of pathogens, such as given in Table 1, requires a great deal of time as well as money. There is also a temporal factor to be considered. Waters that are subject to contamination may not contain pathogens at all times. For example, pathogens may be present only when storms wash animal feces from shore lines or

when municipal wastewater collection systems become overloaded and discharge to storm drains. Finally, the risk of epidemics of waterborne disease is very real when contamination occurs. Thus a method of detecting the probable presence of pathogens is desirable. Such a method would, when positive, indicate that human and/or animal wastes had contaminated water. When negative, the water could be considered uncontaminated. Note that a positive test would not mean that pathogens were present, only that there was a strong possibility of their presence.

Indicator Organisms

For over 100 years public health officials have sought suitable indicators of contamination [Stein, 1926]. Both chemical and microbial indicators have been investigated but at present the microbial indicators are strongly favored. Criteria for selection of microbial indicators include:

- The indicator organism must be a reliable measure of the probable presence of pathogens,
- The indicator organism concentrations must be significantly greater than pathogen concentrations,
- Methods of identification must be relatively simple,
- Estimation of the concentration present must be relatively simple.

To be a reliable measure of the probable presence of pathogens an indicator must be present whenever pathogens are present and be absent when water is uncontaminated by human or animal wastes. The two measures are not completely synchronous. A requirement for a co-presence of indicators and pathogens is clearly desirable. However, a more conservative approach is to select an indicator that is present whenever contamination occurs from sources that may contain pathogens. Thus municipal wastewater may be pathogen free but is an obvious

potential source of pathogens. Water that is contaminated with municipal wastewater is therefore a probable source of pathogens. However, if the indicator organisms selected were also present in runoff from ordinary soil nearly all natural drainage would be suspect. Thus the requirement that the indicator organism be absent when human or animal wastes have not contaminated water is necessary. Otherwise the spectrum of waters requiring some action would be virtually all encompassing.

The requirement that the indicator organism be present in larger numbers than pathogens is set to make detection easier. If the indicator organism was present in numbers similar to pathogens there would be little benefit to the concept. Methods of detection and enumeration of the indicator organisms should be simple. In the case of the most commonly used indicator organisms, total coliforms, fecal coliforms, *Escherichia coli*, and *Enterococcus* spp., growth media and methods have been developed that make both detection and enumeration possible in most laboratories. The growth media can be purchased inexpensively from a number of suppliers and glassware and incubators required are also inexpensive. Little training is required for laboratory technicians to run the tests.

Total Coliforms, Fecal Coliforms, and Enterococci

Development of tests for the microbial quality of water began early in the 20th century. The first tests used were heterotrophic plate counts and coliform enumeration [Stein, 1926]. Heterotrophic plate counts used were conducted at 20°C and 37°C using gelatin and agar, respectively. Colonies developing on gelatin plates were considered to be representative of flora naturally present in the aquatic environment. However, it was recognized that only a small fraction of the various species present would grow under the test conditions. The 37°C counts were thought to be more representative of the organisms associated with the gut of warm-

blooded animals. Coliform enumeration was conducted using lactose broth fermentation. Only a limited number of bacterial species ferment lactose and many of these are characteristic of the lower intestinal tract. Further development of the lactose fermentation technique resulted in the most probable number (MPN) estimate of the concentration of bacteria in the water [*Standard Methods*, 1998].

The presence of lactose fermenting bacteria was (and is) considered to be probable evidence of recent fecal contamination of a water. Further tests to detect the presence of organisms more characteristic of the gut of warm-blooded animals are focused on two groups of organisms, *Escherichia coli* (*E. coli*) and *Enterococcus* spp.. *Escherichia coli* is a lactose fermenting facultative anaerobe normally present in the human intestine. Techniques for detection and enumeration of *E. coli* have been developed (*Standard Methods*, 1998) that are straightforward and inexpensive. Today the conventional coliform test is often bypassed in potable water treatment plants and *E. coli* enumeration is conducted as a first step. *Enterococcus* spp. include *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus avium*, and *Enterococcus gallinarum* that like *E. coli* are characteristic of the colon. The prominence of particular *Enterococcus* species varies with the host and at one time there was hope that this information could be used to identify the source of pollution (human, cattle, bird....). However, a number of factors such as the relative die-off rates and impacts of methods of culturing have made the approach unfeasible. The *Enterococcus* group were formerly classified as members of the genus *Streptococcus* and are differentiated from that group by their ability to grow in 6.5% sodium chloride, at pH 9.6, and at 10°C and 45°C.

Standards for Biological Quality of Water

Two categories of standards for biological quality of water are in use, those for drinking water and those for recreational water. Drinking water standards are divided into source water standards and potable water standards. All current standards are based on the presence and estimated number concentration of coliform, fecal coliform, *E. coli*, and/or *Enterococcus* spp. The number concentration is estimated through a process of serial dilution and culturing stipulated in *Standard Methods* [1998] and reported as the most probable number (MPN) per 100 mL of sample. Two approaches are used, plate counts and fermentation tubes. The MPN is a statistically based method of estimating the number of viable organisms in the sample from the distribution of positive results in the diluted samples [Greenwood and Yule, 1917, Thomas, 1942]. Prior to 1986 both Federal and state standards were based only on coliforms and fecal coliforms. In 1986 the U.S. Environmental Protection Agency recommended the use of *E. coli* and *Enterococcus* spp. as the primary standards [USEPA, 1986]. At present California continues to use coliforms and fecal coliforms as standards for regulation, although *Enterococcus* is used for marine water standards. Current numerical standards for recreational waters are based on statistical analysis of data developed in several epidemiological studies. The World Health Organization analyzed data from 22 epidemiological studies and concluded that [Pruss, 1998, WHO, 1998]:

- "A causal relationship does exist between gastrointestinal symptoms and recreational water quality as measured by indicator-bacteria concentration. A strong and consistent association was reported with temporality and dose response relationships, as well as biological plausibility and analogy to clinical cases in drinking water pollution"

- "In 19 out of 22 studies, the rate of certain symptoms or symptom groups was significantly related to the count of faecal indicator bacteria in recreational water. Gastrointestinal symptoms were the most frequent health outcome for which significant dose-related associations were reported. Symptom rates were usually higher in the lower age groups."
- "Several indicators were used for describing water quality. Most probably, the indicators showing correlation with health outcome varied according to faecal contamination of the water or contamination by other bathers. Consequently, despite different indicators, the trend in reported associations was similar."
- "Associations between ear infections and microbiological indicators of faecal pollution and bather load have been reported. A significant dose-response relationship (with faecal coliforms) has been reported in one study [Fleisher *et al.*, 1996]. When compared to gastro-enteritis, the statistical probabilities are generally lower and are associated with higher faecal coliform concentrations than those for gastrointestinal symptoms and for acute febrile respiratory illness. A cause effect relationship between pollution or bather-derived pollution and ear infection is biologically plausible."
- " Increased rates of eye symptoms have been reported amongst bathers [e.g. Fleisher *et al*, 1996] and evidence suggests that bathing, regardless of water quality, compromises the eye's immune defenses leading to increased symptom reporting in marine waters. Despite biological plausibility, no credible evidence for increased rates of eye ailments associated with water pollution is available."
- " No credible evidence for an association of skin disease with either water exposure

or microbiological water quality is available."

A summary of the current U.S. and California standards for biological quality of water used for full contact recreation is given in Table 3.

Table 3.
Ambient water quality criteria for marine and fresh waters used for full contact recreation.

| Organism | Indicator organisms (MPN/100 mL) | |
|-----------------------------|--|----------------------------------|
| | US EPA guidance level ^a | California Standard ^b |
| Total Coliform | | |
| Single sample | NR ^c | 10,000 |
| Geometric mean ^d | NR | 1,000 |
| Fecal Coliform | | |
| Single sample | 400 ^e | 1,000 |
| Geometric mean ^d | 200 | 100 |
| <i>E. coli</i> | | |
| Single sample | 235-576 ^{f,g} | |
| Geometric mean ^d | 126 ^g | |
| <i>Enterococcus</i> spp. | | |
| Single sample | 61-151 ^{f,g} , 104-500 ^{f,h} | 104 |
| Geometric mean ^d | 33 ^g , 35 ^h | 35 |

^a Guidelines for fecal coliform are outlined in USEPA (1976) and *E. coli* and enterococci in USEPA (1986).

^b Standards established as part of Assembly Bill AB 411.

^c NR = not regulated.

^d Geometric mean based on a minimum of 5 samples over a 30-day period.

^e Not more than 10% of the samples collected over a 30-day period can exceed the specified value.

^f Dependent on the level and frequency of body contact with a particular water.

^g Recommended for fresh waters

^h Recommended for marine waters

At first glance the WHO [1998] conclusions would appear to be strong support for universal application of water quality standards based on indicator organisms. However, there is clear evidence that universal application is not appropriate. The World Health Organization provides several qualifications in a document termed the *Annapolis Protocol* [WHO, 1999].

Present regulatory schemes for the microbiological quality of recreational water are primarily or exclusively based on percentage compliance with faecal indicator counts. A number of constraints are evident in the current standards and guidelines:

- *management actions are retrospective and can only be deployed after human exposure to the hazard;*
- *the risk to health is primarily from human excreta, the traditional indicators of which may also derive from other sources;*

- *there is poor inter-laboratory and international comparability of microbiological analytical data; and*
- *while beaches are classified as safe or unsafe, there is a gradient of increasing severity, variety and frequency of health effects with increasing sewage pollution and it is desirable to promote incremental improvements prioritising 'worst failures'.*

Particular stress should be placed on the second constraint listed above: "the risk to health is primarily from human excreta." The epidemiological studies on which the 1998 conclusions were developed were nearly all for situations where direct contamination of recreational waters by sewage existed. A notable exception was the Santa Monica Bay Study [Haile, 1996] in which relative risks of swimming near storm drains were assessed. However, storm drains often receive sanitary sewer overflows and thus direct contamination may have been a factor in the Santa Monica Bay Study results. This is not the same situation as high indicator organism concentrations associated with undetermined sources as will be explained in the following section. Moreover, there were indications in some of the studies that organisms or viruses shed by bathers might be a factor in resulting infections.

Relative risks of swimming in contaminated water compared to less contaminated water reported in the WHO analysis varied from less than 1.0 to 3.5 and the Santa Monica Bay Study relative risk was 2. The indicator organisms used varied from study to study and the organism concentrations of the contaminated waters varied considerably.

Some consideration should be given to cases in which indicator organisms were absent and waterborne diseases were transmitted. The most interesting cases involve cryptosporidiosis resulting from drinking treated and disinfected municipal water. Although the causative organism, *Cryptosporidium parvum* was first identified in 1907, the connection with the disease was not recognized until 1976 [Nime *et al.*, 1976]. Outbreaks of cryptosporidiosis associated with water were not reported until 1984. The first surface water connected outbreak occurred in 1987 when the municipal water supply of Carrollton, Georgia became contaminated and 13,000 people became ill. In 1993, the water

supply of Milwaukee, Wisconsin became contaminated with *Cryptosporidium* and an estimated 400,000 people became ill. In both the Carrollton and Milwaukee cases the drinking water supplies were completely acceptable based on indicator organism standards for potable water [MacKenzie *et al.*, 1994].

Indicator Organisms and The Environment

Use of total coliforms, fecal coliforms, *E. coli*, and *Enterococcus* spp. as indicator organisms is based on the assumption that these organisms do not compete well in the natural environment. The assumption is based in part on the fact that these organisms reproduce at temperatures typical of body temperatures of warm-blooded animals (36°C - 42°C) and in nutrient rich environments. Soils and natural waters are typically less than 30°C and are relatively nutrient poor in comparison to the gut of warm-blooded animals. Experience has supported the assumption, to a degree, because the organisms are normally present in relatively low numbers in natural waters. However, there is a substantial amount of information in the literature documenting that indicator organisms are normal members of the microbial community in soil, that these bacteria can grow and reproduce in plants, including food crops [Solomen *et al.*, 2002], and that use of coliforms, fecal coliforms, *E. coli*, and *Enterococcus* spp. as probable evidence of human fecal contamination is very unreliable [Carillo *et al.* 1985; Chao and Feng, 1990; Rassoulzadegan and Sheldon, 1986; Solo-Gabriele *et al.* 2000]. Recent studies that have evaluated the occurrence [Bernhard *et al.*, 2000; Byamukama *et al.*, 2000; Francy *et al.*, 2000; Hirotani *et al.*, 1999; Obiri-Danso and Jones, 2000], survival [Monfort *et al.*, 2000; Nasser and Oman, 1999; Obiri-Danso and Jones, 2000], and regrowth [Hardina and Fujioka, 1991; Marino and Gannon, 1991; Solo-Gabrielle *et al.*, 2000] of indicator organisms in environmental habitats provide a sufficient body of data to collectively question the suitability of using conventional

indicator organisms to assess the biological quality of non-point source runoff and recreational waters (Berg, 1978; Cabelli, 1977; Olivieri, 1982).

Solo-Gabriele *et al.* [2000] reported that riverbank soil was the principal dry weather source of *E. coli* in a tidal stream in Fort Lauderdale, Florida. High concentrations of *E. coli* in the stream were associated with storm run-off and high tides. Possible sources of *E. coli* investigated included storm sewers, which had lower concentrations of the organism than did the river under dry weather conditions, and riverbank soil. In laboratory studies *E. coli* numbers increased as soil moisture decreased. The authors postulated that *E. coli* was able to out compete predators under dryer conditions. Predation by zooplankton has been observed to be a key factor in controlling bacterial populations in fresh and marine waters [Rassoulzadegan and Sheldon, 1986].

Carillo *et al.* [1985] determined that *E. coli* survive and grow in river water in Puerto Rico. The authors concluded that coliforms could become part of the normal flora in tropical freshwater environments and suggest that coliforms are poor indicators of recent human fecal contamination. Chao and Feng [1990] studied survival of *Escherichia coli* in river water and found that prefiltering and sterilization of the water greatly decreased the rate of die off. They concluded that predation was a key feature of die off in natural waters. Like Carillo *et al.*, Chao and Feng concluded that coliforms were unsatisfactory as indicators of fecal contamination in tropical waters.

McFeters *et al.* [1972, 1974] conducted studies using membrane filter chambers on the survival of coliforms and selected pathogens in stream and well water. They concluded that die off rates of coliforms and *Salmonella* were similar. The comparative survival of bacteria based on die off rates was: *Aeromonas* sp > *Shigella* > fecal *Streptococcus* > coliforms and *Salmonella*

> *Streptococcus equinus* > *Vibrio cholera* > *Salmonella typhi*. Kurhonen and Martikainen (1991) conducted studies on the survival of *Escherichia coli* and *Campylobacter jejuni* in untreated and filtered lake water and concluded that survival rates of *E. coli* were greater than those of *Campylobacter* and that both species survived better in filtered water. Thus there is evidence that die off rates in natural waters are due to predation and that some important pathogens may have lower die off rates than those for indicator organisms.

PROBLEM STATEMENT AND RESEARCH APPROACH

Substantial evidence has accumulated that the indicator organisms used to evaluate the biological quality of water provide erroneous information under a number of circumstances. The organisms have been shown to reproduce and compete in warm soils, to be normal members of the microbial community in some instances, and to have survival rates lower than some important waterborne pathogens. Additionally, there have been important cases when the indicator organisms were not present in which pathogens were present. In considering urban drainage, such as that flowing onto beaches in California, the impact of this information is significant because of the need to address high indicator bacteria concentrations in these waters. For example, in 2001 there were 795 beach postings and 115 beach closures in the San Diego, Orange and Los Angeles counties. A summary of posting and closure actions for these three counties in 1999, 2000, and 2001 is given in Table 4. Nearly all of the postings were due to high bacterial counts of unknown origin while nearly all of the closures were due to known sewage spills.

Storm drains that discharge to beaches and to near shore waters have been suspected sources of coliform bacteria and the cause of beach closures. Storm drains can be divided into two general classes, those that discharge directly into the surf zone and those that flow across the

beach and into the surf. The direct discharge drains are nearly all creeks or rivers that flow continually but have large increases in flow following storms. Examples include Bayona Creek and Malibu Creek on Santa Monica Bay. Drains that flow across the beach and into the surf zone are seen on almost every beach. They usually drain small areas and may not flow all of the time. Often the dry weather flow does not reach the surf but disappears into the sand. Because of the high coliform concentrations typically found in these drains, the City of San Diego has diverted flows of up to 200 gpm from these small drains to the sanitary sewer system. However, considering the low dry weather flows in the small drains flowing onto beaches, it would not appear that enough coliforms would enter the surf zone to result in elevated concentrations. Unfortunately, the field studies necessary to evaluate such a hypothesis have not been conducted.

Posting of beaches has a major economic impact on beach communities as well as the loss of recreational opportunities to beach users. If the high bacterial counts are not associated with high pathogen contact, public health is not being protected by the postings and the economic and recreational losses incurred have no benefit. Moreover, other possible mitigating actions, such as diversion of dry weather urban drainage to storm sewers and storage and disinfection of wet weather flows would drain public health budgets without protecting public health. If biological quality questions must be addressed, managing urban drainage presents major engineering problems, particularly following storms. For these reasons determination of relationship between the indicator organism concentrations and presence of pathogens in urban drainage is extremely desirable.

Table 4.
Beach postings and closures in Southern California counties between 1999 and 2001.

| | County | | | | | | | | |
|-----------------|-----------|-------|-------|-------------|------|------|--------|------|-------|
| | San Diego | | | Los Angeles | | | Orange | | |
| | 1999 | 2000 | 2001 | 1999 | 2000 | 2001 | 1999 | 2000 | 2001 |
| Postings | 97 | 274 | 187 | 109 | 325 | 263 | 136 | 283 | 345 |
| Sum of days | 617 | 2450 | 855 | 406 | 1150 | 1204 | 887 | 2376 | 10515 |
| Beach mile days | 33.7 | 168.9 | 51.5 | 39.8 | 126 | 93 | 175.1 | 609 | 904 |
| Closures | 32 | 47 | 58 | 6 | 6 | 6 | 21 | 40 | 51 |
| Sum of days | 116 | 310 | 359 | 12 | 12 | 12 | 208 | 152 | 182 |
| Beach mile days | 33.9 | 187 | 359.6 | 36.1 | 34.1 | 34.1 | 155.7 | 53.4 | 53.1 |

source: California State Water Resources Control Board

Pathogen Selection

A limited number of waterborne diseases are associated with recreational waters in the United States. Although eye, ear and skin infections are of some concern, the principal problems are with gastrointestinal and upper respiratory disorders. Causes of these diseases include viruses, bacteria, and protozoa, as noted in Table 1. A comprehensive survey was conducted of waterborne-disease outbreaks in drinking and recreational waters between 1986 and 1998 using the databases maintained by the Centers for Disease Control. The resulting pathogens were selected as a result of this survey.

Viruses

Enterovirus A group of RNA viruses that includes polioviruses, coxsackieviruses, and echoviruses. Sixty-one non-polio, human enteroviruses have been identified (23 Coxsackie A viruses, 6 Coxsackie B viruses, 28 echoviruses, and 4 other enteroviruses). Symptoms are usually mild and flu like. An estimated 10 to 15 million symptomatic infections occur in the United States each year.

Hepatitis A A RNA virus that causes liver disease. A vaccine is available for hepatitis A. Approximately 100,000 cases occur in the United States each year with 1500 of those cases diagnosed in California.

- Rotavirus A RNA virus that infects the lining of the intestine and causes diarrhea, especially in children. Worldwide, rotavirus is estimated to cause 800,000 infant deaths per year throughout the world.
- Adenovirus A DNA virus that is a major cause of respiratory illness and conjunctivitis. Adenovirus has also been identified as a cause of acute hemorrhagic cystitis (inflammation of urinary tract) and infant diarrhea. Serotypes 40 and 41 are frequently implicated in cases of gastroenteritis.
- Bacteria
- E. coli* ETEC Enterotoxigenic *Escherichia coli* adhere to the intestinal wall and secrete a heat labile toxin that causes severe diarrhea similar but generally less severe than cholera.
- E. coli* O157:H7 An enterohemorrhagic strain of *E. coli* that produces a *Shigella* like exotoxin that causes hemolytic uremic syndrome. The principal source of *E. coli* O157:H7 infections is inadequately cooked ground beef but waterborne outbreaks have occurred.
- Shigella* spp. A genera of bacteria that causes a severe form of diarrhea. One species, *Shigella sonnei*, is responsible for approximately two-thirds of the 18,000 cases diagnosed in the United States each year. Actual cases are believed to number considerably higher because mild cases are not treated.
- Salmonella* spp. All members of the genus *Salmonella* are considered to be human pathogens. Three members of the genus are of particular importance, *Salmonella typhi*, *Salmonella typhimurium*, and *Salmonella enteritidis*. *Salmonella typhi* causes typhoid fever. *Salmonella typhimurium* and *Salmonella enteritidis* are

principally associated with food poisoning but are also transmitted through water. *Salmonella* spp. are commonly found in wild animals and birds.

Staphylococcus aureus An opportunistic pathogen commonly associated skin infections but also with pneumonia and meningitis.

Protozoa

Cryptosporidium parvum As noted above, *Cryptosporidium parvum* was the responsible agent in the largest waterborne disease outbreak in the history of the United States.

Giardia lamblia This organism is the source of about 25 percent of the drinking water related outbreaks of infectious disease in the United States. The disease, giardiasis, is characterized by watery, foul smelling diarrhea, cramps, flatulence, nausea, and malaise.

The pathogens selected are responsible for the vast bulk of waterborne diseases in the United States. While it is possible that other pathogens are present in urban drainage, consistent presence of other pathogens without the presence of one or more of the selected pathogens in water contaminated by humans would be very unlikely.

Research Approach

The project was organized in three phases; methods development, baseline studies and field studies. In all three phases samples were analyzed for both indicator organisms (coliforms, fecal coliforms, *E. coli*, and enterococci) and for the pathogens listed above. Methods development was required because of the nature of the samples. Environmental samples often contain materials that interfere with the molecular techniques used to detect pathogens. Additionally, methods of technique validation must be developed. As part of the methods development field samples were obtained from sites described in Table 5.

Table 5.
Description of sample locations used in methods development

| Sample location | Paved and unpaved surfaces | | | Storm drains | | |
|------------------|----------------------------------|---|---------------------------------|-----------------------------|---|-------------------|
| | Type of surface | Dominant use | Surface area, (m ²) | Source of flow ^b | Dimensions of drain | Flow rate (L/min) |
| Encinitas | | | | | | |
| Unpaved | Dirt with patches of dried grass | Foot traffic | 37 | | | |
| Paved | Concrete | Driveway with frequent foot and vehicle traffic | 37 | | | |
| Storm Drain | | | | R | 1.2 m diameter concrete pipe | 1 |
| San Diego | | | | | | |
| Unpaved | Hard-packed soil and weeds | Parking lot with foot and vehicle traffic | 23 | | | |
| Paved | Asphalt | Parking lot with foot and vehicle traffic | 37 | | | |
| Storm Drain | | | | R, C | 0.61 m diameter concrete pipe | 4 |
| Huntington Beach | | | | | | |
| Unpaved | Iceplant | Landscape | 9 | | | |
| Paved | Concrete | Sidewalk with heavy foot traffic | 28 | | | |
| Storm Drain | | | | R | Unlined channel 6.1 m wide with approximately 0.61 m of water | |
| Irvine | | | | | | |
| Paved | Asphalt | Patio with heavy foot traffic | 37 | | | |
| Storm Drain | | | | R, C | Unlined creek 4.6 m wide with approximate 10 cm of water | |
| Malibu | | | | R, C | Unlined creek 3.1 m wide with approximately 15 cm of water | |
| Santa Monica | | | | R, C | 0.91 m diameter concrete pipe | 3 |
| Redondo Beach | | | | R, C | 1.2 m diameter concrete pipe | 5 |

^a All cities located within Southern California.

^b R = residential; C = commercial.

Baseline Studies

The purpose of the baseline studies was to develop background data from common areas that produce urban drainage. Samples for the baseline studies were taken from paved and grass areas of parks, roofs, residential lawns, ponds, storm drains and similar surfaces that would provide a broad picture of the microbial quality of urban water. A list of baseline study site locations, the type of surface, and the weather at the time of sampling is given in Table 6. When surfaces were dry tap water was used to wash the surface and obtain a runoff sample.

Table 6.

Baseline study sites

| Location | County | Weather | Surface |
|-------------------------------|--------|---------|---------|
| Davis (park) 1 | Yolo | Dry | Paved |
| Davis (park) 2 | Yolo | Dry | Paved |
| Davis (residence) | Yolo | Dry | Soil |
| Davis (park) | Yolo | Dry | Soil |
| Davis (roof) | Yolo | Dry | Roof |
| Elk Grove (pond) | Sac | Dry | Drain |
| Sacramento (Riverbend) | Sac | Dry | Drain |
| Sacramento Airport | Sac | Dry | Drain |
| San Diego (Ravina) | SD | Dry | Drain |
| San Diego (residence) | SD | Dry | Paved |
| San Diego (residence) | SD | Dry | Roof |
| San Diego (residence) | SD | Dry | Soil |
| San Diego(Solano Beach) | SD | Dry | Drain |
| San Diego (Del Mar) | SD | Dry | Drain |
| San Diego (La Jolla) | SD | Dry | Drain |
| Encinitas (Moonlight) | SD | Dry | Drain |
| Encinitas (firehouse) | SD | Dry | Paved |
| Encinitas (firehouse) | SD | Dry | Soil |
| Kearney Mesa (truck wash) | SD | Dry | Paved |
| Kearney Mesa (truck wash) | SD | Dry | Soil |
| Aliso Creek | Or | Dry | Drain |
| Laguna Niguel (park) | Or | Dry | Paved |
| Laguna Niguel (park) | Or | Dry | Soil |
| Laguna Niguel (senior center) | Or | Dry | Roof |
| Irvine (drain) | Or | Dry | Drain |
| Irvine (wash station) | Or | Dry | Paved |
| Huntington Beach | Or | Dry | Drain |
| Huntington Beach | Or | Dry | Paved |
| Huntington Beach | Or | Dry | Soil |
| Malibu Creek 1 | LA | Dry | Drain |
| Malibu Creek 2 | LA | Dry | Drain |
| Malibu Creek 3 | LA | Dry | Drain |

Table 6 continued

| Location | County | Weather | Surface |
|--------------------------|--------|---------|---------|
| Santa Monica (Ashland) 1 | LA | Dry | Drain |
| Santa Monica (Ashland) 2 | LA | Dry | Drain |
| Santa Monica (Ashland) 3 | LA | Dry | Drain |
| Redondo Beach 1 | LA | Dry | Drain |
| Redondo Beach 2 | LA | Dry | Drain |
| Redondo Beach 3 | LA | Dry | Drain |
| Simi Valley (park) | Ven | Wet | Drain |
| Simi Valley (residence) | Ven | Wet | Drain |
| Port Hueneme | Ven | Dry | Drain |
| Port Hueneme | Ven | Dry | Paved |
| Port Hueneme | Ven | Dry | Roof |
| Port hueneme | Ven | Dry | Soil |
| Arrundale Barranca | SB | Wet | Drain |
| Atascadero Creek | SB | Wet | Drain |
| Camino Park Barranca | SB | Wet | Drain |

Note that many of the baseline study sites are unlikely to be regulated. For example, discharge requirements are unlikely to be placed on stormwater runoff from residential roofs, residential lawns, and parks. Additionally, restrictions on park or residential lawn utilization would be unlikely to occur as the result of high indicator organism counts in stormwater runoff.

Field Investigations

Field investigation samples were taken under both wet and dry conditions at selected sites in Southern California. The sites selected included urban drains and best management practice treatment installations (bmps). Samples collected at bmps were taken only in wet weather because the units were dry at other periods. Seven drain samples were taken during wet weather. Thus the bmp samples provide a comparison between runoff from Caltrans facilities and runoff from the general urban environment. Descriptions of the sample sites are given in Table 7.

Table 7.
Sample sites used for field investigations
A. Dry weather

| Code | Name location | Direction | Description |
|----------------|---------------|--|---|
| A | Calabasas | 101 W, exit Calabasas Parkway, right shoulder off-ramp | Two underground drains combine and come to surface in the off-ramp's shoulder. |
| B | Malibu Creek | On the 101, exit the N1. | Small creek with flowing water. Nature area, with the Calabasas town upstream. |
| D | Lakewood | 405 S, exit Lakewood (Long Beach), right-hand shoulder on off-ramp. | Underground drain from under the fwy and off-ramp comes to surface. |
| F | Culver | 405 S in Irvine, Orange County, exit Culver, right shoulder off-ramp. | Two large drains come to surface and combine into one, continuing as an open channel along the off-ramp to the north. |
| G | Las Posas | 101 W in Ventura close to Oxnard, exit Las Posas south, continue a few miles to intersection with hwy 34 and the rail-road | Agricultural area, irrigation, fields are surrounded by wide, unpaved ditches. |
| I | Riverside | On Blaine St. close to UCR at the rail-road crossing | Underground drain surfaces and continues as an open channel along the rail-road. |
| K | Spruce | 60 E at intersection with the 91 fwy, exit at Spruce St, turn right, and look for the drain at the right after about 500 ft. | This is a large and deep concrete channel coming from a business area. |
| L | Cesar Chavez | The 101/10 intersection close to downtown LA. | This is a 'hill'/elevated area with a residential buildings and few small businesses. |
| SD1 | Montiel | Ditch parallel to fwy 15 in Escondido. | Concrete ditch, parallel to 15 fwy. |
| SD2 | El Camino | 78 fwy close to Carlsbad/Oceanside. | This is the Vista Marron Canyon Creek, South and parallel to fwy 78. |
| SD3 | Leucadia | 5 fwy north of Encinitas. | Long concrete ditch, west and parallel to the 5 fwy. |
| SD4 | Encinitas | Drain outlet in Encinitas. | Open drain outlet discharging onto the beach. |
| B. Wet weather | | | |
| E6 | Turnbull | 60 W, exit at Hacienda Boulevard North. | This bmp is very similar to the one at Rincon (E23). It collects water running down from the 60 fwy. |
| E8 | June Way | 60 W between the 605 and 710, exit at Wilcox Ave.,. | |
| E23 | Rincon | 210 W, just after intersection with the 2 fwy, exit Ocean View. | This site collects water from a few acres area with freeway only. Caltrans. |
| H1 | Fillmore | 210 W, exit at Paxton. | Granular medium filter BMP |
| H2 | Orcas | 210 W, exit at Wheatland. | This bmp collects water running down from the slope of the 210 fwy. |
| H3 | Altadena | 210 W, exit at Arroyo Blvd. | This bmp probably treats run-off from the premises of the maintenance station. |

METHODS

Methods of sampling, sample preservation, and analysis for indicator organisms followed standard procedures as will be noted in the descriptions below. Indicator organism analysis was contracted to commercial laboratories for all of the work in Southern California. During the field investigation portion of the project, sampling was carried out by Professor Marc Deshusses and Dr. Huub Cox of the University of California, Riverside. Collected samples were concentrated and preserved at UC Riverside before being transferred to UC Davis for pathogen analysis. Samples taken in Northern California were analyzed for indicator organisms in the departmental laboratories at UC Davis.

Pathogen analysis was conducted using polymerase chain reaction (PCR) technology. This method requires extraction of deoxyribonucleic or ribonucleic acid (DNA or RNA) sequences, amplification of specific nucleic acid sequences using *primers* that define the sequence end points, and analyzing for the presence of the selected sequences using gel electrophoresis. PCR amplification allows detection of extremely small quantities of nucleic acid. The sequences defined by each primer set are specific to each organism organism. . As will be explained in detail below, PCR analysis requires that sample material be separated from the aqueous sample in a number of steps that are relatively arduous. Additionally, the analysis can be inhibited by materials commonly found in surface and storm waters.

The methods description to follow refers specifically to the sample locations in Table 5. All subsequent samples (baseline and field studies) were analyzed according to these optimized protocols (pp. 25-35).

Sample Collection and Preservation

Runoff was generated from the paved and unpaved surfaces (Table 5) by washing a 9 m² area of the specified surface with 100 L of tap water, supplied to the local area as drinking water. Grab samples were taken from drains with flowing water.

At each sample location, water samples were collected in three 1-L Nalgene plastic containers for analyses of hardness, suspended solids, and indicator organisms as per Standard Methods, 20th edition (*Standard Methods*, 1998), methods 2340C, 2540D, and 9221E, respectively. In addition, water samples, ranging in volume from 20 to 100 L, were collected for the analysis of selected pathogens in sterile 20-L Nalgene plastic containers. The specific volume processed at each location reflected the concentration of suspended solids. The samples collected for pathogen analyses were concentrated on site by pumping the water (stainless steel progressive cavity pump, Ryan-Herco Products Corp, Sacramento, CA) through a 1.0 µm pore size 293mm FALP filter (Millipore, Bedford, MA), pre-wetted with methanol, and mounted on a sterilized stainless steel filter holder (Millipore). The filtrate was collected in sterile 20-L Nalgene plastic containers. The pH of the filtrate was adjusted to 5 with 1N HCl, and the liquid was pumped (flexible impeller pump, Ryan-Herco Products Corp, Sacramento, CA) through a 1-MDS cartridge filter (CUNO, Inc., Meridian, CT). The cartridge and FALP filters were placed in separate 2-L Nalgene plastic containers and shipped on ice, along with the 1-L grab samples collected for selected water quality analyses, back to the laboratories at the University of California at Davis (UCD). The filters were eluted and concentrated within 24 hours of arrival and the resulting solutions were stored at -20 °C until further analyses. The 1-L grab samples were processed immediately upon arrival for the specified water quality characteristics.

Development of an Elution Protocol for the FALP Filter.

Three methods of eluting the FALP filter were evaluated to maximize the recovery of bacteria and protozoa. A 10-fold dilution of an overnight culture of *E. coli* (ATCC 15597), grown in nutrient broth (Difco), was prepared in autoclaved water obtained from the storm drain at Sample Location 1. The initial titer of viable *E. coli* was enumerated on nutrient agar plates incubated overnight at 37 °C. The autoclaved solution was filtered through three separate 1.0 µm pore size 47 mm FALP filters (Millipore, Bedford, MA) pre-wetted with methanol. The filters were shaken gently for 15 minutes in sterile plastic containers containing three different solutions: 1) 0.2% Tween 20 (Bio-Rad Laboratories, Richmond, CA) 2) 1.5% (w/v) Beef Extract (Becton Dickinson and Company, Sparks, MD) in 1X PBS (137mM NaCl, 2.7mM KCl, 4.3mM Na₂HPO₄ 7H₂O, 1.4mM KH₂PO₄, pH 7.2) 3) 1% Tween 80 (Bio-Rad Laboratories) containing 1% (v/v) sodium dodecylsulfate, and 0.001% (v/v) Antifoam (Difco). The recovery of *E. coli* off the filter was evaluated by enumerating the concentration of viable organisms in each wash solution. The recovery of protozoa from the FALP filter was evaluated using a similar procedure as outlined above with *C. parvum*. The initial titer of oocysts and the concentration in the eluate were enumerated using a hemocytometer. The above procedures utilizing *E. coli* and *C. parvum* were repeated in triplicate. Solution 2 produced the largest percent recovery of both bacteria and protozoa and was consequently used in all subsequent analyses.

Elution of FALP and 1-MDS Filters.

The 293 mm FALP filters obtained from each sample location were cut in half and laid flat in a sterile plastic tray containing 100 mL of 1.5% (w/v) Beef Extract in 1X PBS. The filter was eluted by scrubbing the surface with a sterile nylon bristle brush (Fisher Scientific, Pittsburgh, PA) for 10 minutes. The extracts were collected in 50 mL centrifuge tubes. The

brush was rinsed with elution buffer and the liquid was pooled with the extracts. The tubes were spun in a centrifuge at 3000 x g for 12 minutes. After removing the supernatant, the pellet was weighed and distributed to a 2mL screw- capped microcentrifuge tube. The maximum mass allowed per tube was 300 mg. The 1-MDS cartridge filter was eluted and organically flocculated [Fout *et al.*, 1996]. The pellet was dissolved in 8 mL of buffer (0.15M Na₂HP0₄·7H₂0, pH 7.5) and concentrated using a Biomax-100K microconcentrator (Millipore) to a final volume of approximately 400 µL.

Nucleic Acid Extraction and Purification.

The nucleic acid was extracted from the FALP pellet using mechanical glass bead lysis as per Cullen and Hirsh [1998] and Miller *et al.* [1999] using a Mini-Bead Beater 8 (BioSpec Products, Bartlesville, OK) operated at 2,510 rpm for 2 minutes. The DNA pellets were redissolved in 100 µL of sterile double-distilled water and the solution was sequentially passed through PVPP and Sephadex G200 spin columns as per Tsai and Olson [1992] to remove substances that inhibit PCR. Viral nucleic acid was extracted from 10-fold serial dilutions prepared in double-distilled water of the concentrated sample using QIAmp Viral RNA Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's directions. The nucleic acid extracts were passed through Sephadex G200 spin columns to remove substances that inhibit PCR.

Nucleic Acid Amplification and Detection.

The molecular techniques used in the identification and confirmation of the selected bacteria, viruses, and protozoa are summarized in Table 8. Primers were synthesized by Life Technologies (Gibco BRL, Grand Island, NY), and stored in TE buffer at -20⁰C until use. The reaction mixtures for both PCR and RT-PCR are summarized in Table 9. All PCR reactions

were performed using a GeneAmp PCR System 9700 thermocycler (PE Biosystems, Foster City, CA). For each PCR assay, a positive and negative control was included. A miniaturized microcapillary electrophoresis chip (Agilent Technologies Inc., Germany) with a 2100 Bioanalyzer chip reader (Agilent Technologies Inc., Germany) was used to analyze the PCR products.

Confirmation of The PCR Product.

The PCR product from a positive sample (determined from Bioanalyzer results) was separated on a 1.5% agarose gel, stained with ethidium bromide, and transferred to a positively charged Nylon membrane (Boehringer Mannheim). The gel was depurinated in 0.25N HCl for 15 minutes, rinsed twice in ddH₂O, and then denatured in 0.5N NaOH for 30 min. Amplicons were transferred to the Nylon membrane in 10X SSC for 90 min. at 5 in. Hg using a vacuum blotter (Bio-Rad Laboratories). Nucleic acid was crosslinked to the membrane for 2 min. using a UVC 500 UV Crosslinker (Hoefer Scientific Instruments, San Francisco, CA).

Oligonucleotide probes (Life Technologies) were labeled using DIG Oligonucleotide Tailing Kit (Boehringer Mannheim Corp., Indianapolis, IN) according to manufacturer's instructions.

Hybridization and colorimetric detection of bound probe was performed using DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

Table 8.
Molecular techniques used in the identification and confirmation of the selected bacteria, viruses, and protozoa in water samples collected from storm drains and surfaces in close proximity to the drains.

| Organism | Stock culture ^a | Identification | | | Confirmation | | Southern blot ^c |
|------------------------------|----------------------------|-----------------------------------|--------------|--|--------------|--|----------------------------|
| | | Primers | Product size | PCR cycle sequence ^b | Product size | Nested PCR PCR cycle sequence | |
| Bacteria^d | | | | | | | |
| Fecal coliform | 15597 | Bej, <i>et al.</i> , 1991 | 153 | D10 ^e , D20s, A30s(60°C), E45s, E7 ^f , 30 cycles | | | |
| E. coli ETEC | 35401 | Wang <i>et al.</i> , 1997 | 117 | D10 ^e , D20s, A30s(56°C), E45s, E7 ^f , 35 cycles | | | Yes |
| E. coli O157:H7 ^g | 43890 | Wang <i>et al.</i> , 1997 | 361 | D10 ^e , D20s, A30s(56°C), E45s, E7 ^f , 35 cycles | | | No |
| Shigella spp. | 29903 | Achi <i>et al.</i> , 1996 | 320 | D10 ^e , D20s, A30s(53°C), E45s, E7 ^f , 35 cycles | | | Yes |
| S. aureus ⁱ | 25923 | Wang <i>et al.</i> , 1997 | 276 | D10 ^e , D20s, A30s(56°C), E45s, E7 ^f , 35 cycles | | | |
| Salmonella spp. | 13311 | Chiu <i>et al.</i> , 1996 | 275 | D10 ^e , D20s, A30s(58°C), E45s, E7 ^f , 30 cycles | | | Yes |
| Viruses^d | | | | | | | |
| Enterovirus | 1007 | Abbaszadegan <i>et al.</i> , 1993 | 149 | RT: 10min. 25°C, 30min. 42°C, 4°C hold PCR: D10 ^e , D20s, A30s(55°C), E45s, 30cycles | | | Yes |
| Bovine Enterovirus | VR-248 | Egger <i>et al.</i> , 1995 | 260 | RT: 10min. 25°C, 12min. 42°C, 4°C hold PCR: D10 ^e , D20s, A30s(51°C), E45s, 35cycles | | | Yes |
| Rotavirus | VR-2018 | Abbaszadegan <i>et al.</i> , 1999 | 211 | RT: 15min. 25°C, 45min. 42°C, 4°C hold PCR: D10 ^e , D20s, A30s(55°C), E45s, 35cycles | | | Yes |
| Hepatitis A | 2281 | Jothikumar <i>et al.</i> , 1998 | 247 | RT: 15min. 42°C, 4°C hold PCR: D10 ^e , D20s, A30s(55°C), E45s, 35cycles | | | Yes |
| Adenovirus | VR-1083 | Puig <i>et al.</i> , 1994 | 300 | D10 ^e , D20s, A30s(55°C), E45s, 30 cycles | 142 | D10 ^e , D20s, A30s(55°C), E45s, E7 ^f 30 cycles | No |
| Protozoa^d | | | | | | | |
| C. parvum | PRL ^h | Deng <i>et al.</i> , 1997 | 452 | D10 ^e , D20s, A30s(60°C), E45s, E7 ^f , 39 cycles | 210 | D10 ^e , D20s, A30s(66°C), E45s, E7 ^f , 39 cycles | Yes |
| G. lamblia | PRL ^h | Rochelle <i>et al.</i> , 1997 | 218 | D10 ^e , D20s, A30s(56°C), E45s, E7 ^f , 35 cycles | | | Yes |

^a All numbers are in reference to the American Type Culture Collection unless otherwise noted. The stock cultures were used as positive controls in the PCR reactions.

^b D = Denaturation; A = Annealing; E = Elongation. All times in minutes unless otherwise noted. All denaturation and elongation temperatures were 95 and 72 °C, respectively.

^c Hybridization temperature and probe sequence for southern blotting obtained from the same references as the primer sequences.

^d PCR performed on a Perkin-Elmer Thermocycler GeneAmp PCR System Model 9700.

^e Initial denaturation step that is not part of the cycle sequence.

^f Final elongation step that is not part of the cycle sequence.

^g Confirmation of PCR product obtained with multiple primer sets applied to template DNA extracted from the water sample as per Fratamico and Strobaugh (1998).

^h Parasitology Research Labs, Phoenix, AZ

ⁱ This organism was used as a spike to test for PCR inhibition.

Table 9.
Composition of PCR and RT-PCR reaction mixtures.

| Organism | PCR Reaction Mixtures ^a | | | | | RT-PCR Reaction Mixtures ^b | | | | | | |
|------------------------|------------------------------------|----------------|-----------------------|----------------|------------------|---------------------------------------|----------------|--|------------------|-------------|--|------------------|
| | MgCl ₂ (mM) | dNTP's (mM) | Primers (μM) | TAQ (Units) | Template (μL) | MgCl ₂ (mM) | dNTP's (mM) | Primers or random hexamers (μM) | RNase (Units) | DTT (mM) | Reverse Transcrip- tase (Units) | Template (μL) |
| | <hr/> | | | | | | | | | | | |
| Bacteria | | | | | | | | | | | | |
| <i>E. coli</i> (15597) | 2.5 | 0.20 | 0.20 | 1 | 5 | | | | | | | |
| <i>E. coli</i> ETEC | 3 | 0.25 | 0.25 | 1 | 5 | | | | | | | |
| <i>E. coli</i> O157:H7 | 3 | 0.25 | 0.25 | 1 | 5 | | | | | | | |
| <i>Shigella</i> spp. | 2 | 0.25 | 0.25 | 1 | 5 | | | | | | | |
| <i>S. aureus</i> | 3 | 0.25 | 0.25 | 1 | 5 | | | | | | | |
| <i>Salmonella</i> spp. | 1.5 | 0.25 | 0.25 | 1 | 5 | | | | | | | |
| Virus | | | | | | | | | | | | |
| Enterovirus | 2 | 0.20 | 0.15 | 1 | 20 | 7.5 | 0.20 | 1.25 ^c | 10 | | 15 | 6 |
| Bovine Enterovirus | 2 | 0.25 | 0.15 | 2.5 | 20 | 2.5 | 0.25 | 1.25 ^c | 10 | 5 | 15 | 5 |
| Rotavirus | 3.5 | 0.25 | 0.50 | 2.5 | 49 | 3 | 0.5 | 1.25 ^c | 32 | 15 | 50 | 10 |
| Hepatitis A | 1.5 | 0.25 | 1.5 | 2.5 | 20 | 1.5 | .20 | 1.5 ^d | 10 | 5 | 15 | 6 |
| Adenovirus | 1.5 | 0.20 | 0.08 (0.16 nested) | 2.5 | 7 (1 nested) | | | | | | | |
| Protozoa | | | | | | | | | | | | |
| <i>C. parvum</i> | 2 | 0.20 | 1.0 | 1.25 | 5 | | | | | | | |
| <i>G. lamblia</i> | 1.5 | 0.20 | 0.5 | 1 | 5 | | | | | | | |

^a Chemicals utilized in PCR were obtained from GeneAmp Gold PCR Reagent Kit (PE Biosystems, Foster City CA). Final volumes of reaction mixtures, adjusted with sterile dH₂O, were 50 μL.

^b Chemicals utilized in RT-PCR were obtained from GeneAmp Gold RNA PCR Kit (PE Biosystems, Foster City CA). Final volumes of reaction mixtures, adjusted with sterile dH₂O, were 20 μL except for rotavirus that had a final volume of 50 μL.

^c Random hexamers. An additional pre-incubation was performed at room temperature for 10 minutes prior to reverse transcription.

^d Primers.

Baseline Sensitivity of PCR Assays.

Stock cultures of the bacteria, virus, and protozoa used to obtain nucleic acid for positive controls in the PCR assays are summarized in Table 8. The bacterial nucleic acid was extracted from a 200 μ L aliquot of a 24-hour culture (grown in nutrient broth at 37°C) using a series of four freeze-thaw cycles. The organisms were frozen in an ethanol dry ice bath and thawed in boiling water. The stock cultures of protozoa were supplemented with Amphotericin B to retard fungal growth, and stored at 4 °C. Protozoal nucleic acid was also extracted using the above freeze-thaw procedure. Stock cultures of the viruses were divided into small aliquots and stored at -20 °C. Viral positive control nucleic acid was obtained using the QIAmp Viral RNA Kit (Qiagen) according to manufacturer's directions. All of the nucleic acid positive controls were stored at -20 °C.

A baseline detection limit for the PCR assay was established in RNase free reagent grade water for each of the pathogens of interest. Serial ten-fold dilutions were made with the nucleic acid extracted from the stock cultures. The target regions of the nucleic acid were amplified using appropriate volumes of each dilution. The concentration of organisms in each stock culture was coupled with the volumes of each culture placed in the PCR reaction tube to estimate the minimum number of organisms necessary to produce a detectable band on the Bioanalyzer. The values obtained with the above procedure are accurate within an order of magnitude and represent a conservative estimate of the minimum detection limit.

Inhibition of PCR.

The relative impact of inhibitory substance on PCR was evaluated in the water samples. An aliquot of the nucleic acid extract obtained from either the FALP filter or the 1-MDS filter was spiked with ca. 40 cells of *Staphylococcus aureus* prior to purification on the

PVPP/Sephadex G200 spin column. The detection limit was then determined using 10-fold serial dilutions of the purified, spiked samples. In addition, inhibition of the RT-PCR process was evaluated using the GeneAmp RNA PCR Control Kit (Perkin-Elmer). An aliquot of each nucleic acid extract, obtained from the 1-MDS filter, was spiked with control RNA, purified on a Sephadex G200 spin column, amplified using the appropriate RT-PCR protocol, and analyzed on the Bioanalyzer. The concentration of cells in the spiked sample (estimated to within an order of magnitude) was compared to the baseline sensitivity of the PCR assay to assess the relative impact of inhibitory substances on the minimum detection limit of the PCR-assays for each of the bacteria, viruses, and protozoa.

Baseline Recovery from FALP and 1-MDS Filters.

A baseline recovery from each filter type for each of the organisms of interest was established using 10 L of dechlorinated tap water spiked with each of the appropriate stock cultures. The initial titer of each organism was estimated using the appropriate PCR-based assay. The liquid was then filtered through the FALP and 1-MDS filters sequentially. The filters were eluted and the concentrations of organisms in the original sample were estimated using the procedures outlined above. The percent recovery of each organism off the appropriate filter was established by comparing the concentrations of organisms in the original sample estimated in the filter eluate to the initial titers.

Recovery from FALP Filters Processed at Each Sample Location.

To further evaluate the recovery of bacteria and protozoa at each sample location, the concentration of fecal coliform enumerated in the original sample using the multiple tube fermentation (MPN-MTF) test [*Standard Methods*, 1998, 9221E] was directly compared with the concentration enumerated in the filter eluate using an MPN-PCR assay. Ten-fold serial dilutions

were prepared with the filter eluate. Five tubes per dilution were processed using the primers and PCR protocol outlined in Bej *et al.* [1991]. The number of positive tubes per dilution were used in the Poisson Equation to statistically estimate the most probable number of fecal coliform bacteria in the original sample.

Recovery from 1-MDS Filters Processed at Each Sample Location.

The recovery of the viruses from the 1-MDS filter processed at each sample location was estimated using the following procedure. At each sample location, bovine enterovirus was spiked into the filtrate of the FALP filter. The 1-MDS filter was then processed as outlined above. The recovery of bovine enterovirus in each sample was established by comparing the concentration in the original sample estimated from the filter eluate with the initial titer. The recoveries of all the viruses were then corrected from the initial baseline values established in dechlorinated tap water to reflect the percent difference in the recovery of the bovine enterovirus at each sample location.

RESULTS

The results presented in this section include analysis of the experimental procedures for PCR, the results of methods development studies, the baseline and field studies, and the results of detection limit analysis for the methods development studies.

Experimental Methods Development

Results of the analysis of the elution protocol for The FALP filter, baseline recoveries and sensitivity, inhibition of PCR, and recoveries from FALP and 1-MDS filters are presented below.

Elution Protocol for the FALP Filter.

The recoveries of *E. coli* and *C. parvum* from FALP filters processed with an autoclaved storm drain sample spiked with known titers of the respective organisms are summarized in Table 10 for three different elution buffers. The solution containing 1.5% beef extract in 1X PBS produced the highest recovery and was consequently used in all subsequent analyses. Although the elution buffers may have produced a germicidal effect on the *E. coli*, thereby reducing the percent recovery, a significant effect is unlikely given that the recoveries of *E. coli* and *C. parvum* were comparable and the concentration of *C. parvum* was enumerated using a hemocytometer.

Baseline Recovery and Sensitivity.

The baseline recoveries and sensitivities of the isolation and identification protocols are summarized in Table 11 for stock cultures spiked into RNase free water. The sensitivities of the PCR assays used to detect the bacteria and protozoa are reported as colony forming units (cfu) and oocysts, respectively. The numbers of adenovirus and rotavirus are expressed as the tissue culture infective dose that affects 50% of the test units in an endpoint dilution series (TCID₅₀). The numbers of hepatitis A and enterovirus are expressed as radio-immuno focus units (RIFU) and plaque forming units (pfu), respectively. The sensitivity of the PCR assays summarized in Table 11 are consistent with previously reported values. The recovery of bacteria and protozoa from the FALP is greater than existing techniques utilizing glass- and yarn-wound cartridge filters [Kaucner *et al.*, 1998; LeChavallier *et al.*, 1991; Payment *et al.*, 1989; Nieminski *et al.*, 1995] and various size exclusion filters [Aldom *et al.*, 1995; Boulanger and Edelstein, 1995; Hansen and Ongerth, 1991; Shepherd and Wyn-Jones, 1996]. The baseline values established in

RNase free water constitute the maximum recovery and sensitivity possible with the reported protocols.

Table 10.

Evaluation of three methods of eluting an FALP filter processed with a water sample spiked with known concentrations of *E. coli* and *C. parvum*.

| Elution Method | Percent Recovery | |
|---|-----------------------------|-------------------------------|
| | <i>E. coli</i> ^a | <i>C. parvum</i> ^b |
| 0.2% Tween 20 | 54 | 60 |
| 1.5% beef extract in 1X PBS | 80 | 85 |
| 1% Tween 80 containing 1% sodium dodecylsulfate and 0.001% antifoam | 22 | 31 |

^a Recovery based on a viability assay using nutrient agar plates incubated overnight at 37 °C.

^b Concentration of oocysts measured with a hemocytometer.

Table 11.

Baseline recovery and sensitivity of the protocols used in the isolation and identification of pathogens spiked into RNase free water.

| Organism | Recovery ^a (%) | Sensitivity ^b |
|------------------------|---------------------------|--------------------------|
| Bacteria | | |
| <i>E. coli</i> O157:H7 | 80 | 2 |
| <i>E. coli</i> ETEC | 80 | 20 |
| <i>S. aureus</i> | 79 | 4 |
| <i>Shigella</i> | 82 | 40 |
| <i>Salmonella</i> | 81 | 7 |
| Protozoa | | |
| <i>G. lamblia</i> | 85 | 5 |
| <i>C. parvum</i> | 85 | 5 |
| Viruses | | |
| Enterovirus | 50 | 0.006 |
| Bovine Enterovirus | 56 | 0.05 |
| Rotavirus | 40 | 0.002 |
| Hepatitis A | 57 | 0.004 |
| Poliovirus | 50 | 0.9 |
| Adenovirus | 25 | 0.1 |

^a Recovery of bacteria and protozoa in reference to the FHLP filter. Recovery of viruses in reference to the 1-MDS filter.

^b Sensitivity of bacteria and protozoa expressed as the number of cfu and oocysts, respectively. Adenovirus, poliovirus, and rotavirus are expressed as TCID₅₀; hepatitis A is expressed as RIFU; and enterovirus and bovine enterovirus are expressed in pfu.

Inhibition of PCR.

The sensitivity of the PCR assays used to detect pathogens in the water samples collected from the storm drains or surfaces in close proximity to the drains was assessed by evaluating the minimum dilution of the purified filter extract necessary to prevent inhibition. The minimum dilutions of the filter extracts, spiked with stock cultures and processed with the Sephadex G-200 spin columns, necessary to produce a positive band in the respective PCR assay are summarized in Table 12 for the methods development sample locations. The water samples obtained from paved and unpaved surfaces contained a higher concentration of inhibitory substances in the purified extracts, thereby requiring a greater dilution to obtain a positive PCR result, than the water samples collected from the storm drains. Additionally, the inhibition of PCR in the nucleic acid extracts obtained from the 1-MDS filter was consistently less than in the extracts obtained from the FALP filter.

The overall sensitivity of the PCR assays at each of these sample location was estimated by coupling the minimum dilution necessary to eliminate inhibition (Table 12) with the baseline detection limit established in RNase free water (Table 11). For example, the minimum number of *Cryptosporidium* obtained from the concentrated filter extract at Sample Location 3 (unpaved surface) necessary to produce a positive PCR result is 5000 oocysts, 1000 times greater than the baseline sensitivity. The above procedure provides a conservative estimate of the sensitivity of each PCR assay and represents an upper bound of a value accurate within an order of magnitude.

Table 12.
Recovery of bovine enterovirus/fecal coliform and sensitivity of PCR assays used in the detection of pathogens in water

| Sample Location | Lowest dilution producing a positive result in spiked sample ^a | | Recovery of Bovine Enterovirus ^b (%) | Concentration of Fecal Coliform, MPN/100 mL | | | | | |
|------------------|---|---------|---|---|--------------------|--------------------|----------------------|--------------------|--------------------|
| | Bacteria and Protozoa | Viruses | | MTF Test ^c | | | MPN-PCR ^d | | |
| | | | | Mean | Upper ^e | Lower ^e | Mean | Upper ^e | Lower ^e |
| Encinitas | | | | | | | | | |
| Unpaved | 1000 | 10 | 5.0 | | | | | | |
| Paved | 100 | 100 | 4.0 | <2 | | | 120 | 470 | 50 |
| Storm Drain | 100 | 0 | 35.3 | 9,000 | 29,000 | 3,000 | 3,000 | 9,000 | 1,500 |
| San Diego | | | | | | | | | |
| Unpaved | 1000 | 100 | 4.0 | | | | | | |
| Paved | 1000 | 10 | 0.5 | | | | | | |
| Storm Drain | 100 | 0 | 42.4 | | | | | | |
| Huntington Beach | | | | | | | | | |
| Unpaved | 1000 | 10 | 49.5 | | | | | | |
| Paved | 1000 | 0 | 44.4 | | | | | | |
| Storm Drain | 1000 | 0 | 0.3 | 80 | 250 | 30 | 85 | 240 | 35 |
| Irvine | | | | | | | | | |
| Paved | 1000 | 10 | 55.5 | | | | | | |
| Storm Drain | 100 | 10 | 5.6 | | | | | | |
| Malibu | | | | | | | | | |
| 8 AM | 0 | 0 | 31.3 | | | | | | |
| 12 Noon | 10 | 0 | 31.3 | | | | | | |
| 4 PM | 10 | 0 | 31.3 | | | | | | |
| Santa Monica | | | | | | | | | |
| 8 AM | 0 | 0 | 0.2 | 500 | 2,000 | 200 | 25 | 75 | 10 |
| 12 Noon | 0 | 0 | 0.2 | 1,300 | 3,800 | 500 | 120 | 325 | 46 |
| 4 PM | 10 | 10 | 0.1 | | | | | | |
| Redondo Beach | | | | | | | | | |
| 8 AM | 10 | 0 | 0.3 | 8 | 24 | 3 | 0.35 | 1 | 0.1 |
| 12 Noon | 10 | 0 | 14 | | | | | | |
| 4 PM | 100 | 0 | 14 | | | | | | |

^a The lowest dilution producing a detectable band following PCR. *Staphylococcus* DNA (ca. 40 cells) spiked to an appropriate volume of each nucleic acid extract, from either the FALP or 1-MDS filters, prior to purification. Dilutions were made with the purified nucleic acid extracts. A 0-fold dilution corresponds to the undiluted purified extract.

^b The filtrate from the FALP filter was spiked with the bovine enterovirus. The recovery is from the 1-MDS cartridge filter.

^c Multiple tube fermentation test (Standard Methods, 20th edition, 9221E) performed on a grab sample collected from the specified location.

^d PCR-based assay performed with the purified filter extract utilizing a 10-fold end-point dilution sequence with 5 tubes per dilution and the primer set outlined in Bej *et al.* (1991). The Poisson Equation was used to estimate the most probable number of coliform per 100 mL of sample (MPN/100 mL).

^e Values based on a 95% confidence interval.

Recovery from Processed FALP Filters

The concentrations of fecal coliform enumerated with the multiple tube fermentation technique (MTF) and the MPN-PCR assay are summarized in Table 13 for the seven methods development sample locations. The MTF technique was employed with grab samples collected at the specified sample locations, whereas the MPN-PCR assay was performed on the purified extract obtained from the FALP filter. Comparison of the concentration of fecal coliform enumerated with the two techniques provides a basis for assessing the recovery off the FALP filter at each sample location. The concentration of fecal coliform enumerated with the MPN-PCR assay was less than the value obtained from the MTF technique at Sample Locations 6 and 7, suggesting the recovery was less than 100%. However, the concentrations enumerated with the two techniques in the storm drains at Sample Locations 1 and 3 were comparable, and the concentrations enumerated with MPN-PCR assay were greater than the MTF technique in the water sample collected from the paved surface at Sample Location 1. The specific reasons for the higher concentrations of fecal coliform enumerated with the MPN-PCR assay at Location 1 are unclear, but are likely due to the presence of viable but non-culturable organisms. Overall, the above procedure provided mixed results with no clear trend in the percent of organisms recovered from the FALP filter. Consequently, the recovery of bacteria and protozoa from water samples collected from the storm drains or surfaces in close proximity to the drains was assumed to be comparable to the baseline values.

Table 13.

Recovery of bovine enterovirus/fecal coliform and sensitivity of PCR assays used in the detection of pathogens in water

| Sample Location | Lowest dilution producing a positive result in spiked sample ^a | | Recovery of Bovine Enterovirus ^b (%) | Concentration of Fecal Coliform, MPN/100 mL | | | | | |
|------------------|---|---------|---|---|--------------------|--------------------|----------------------|--------------------|--------------------|
| | Bacteria and Protozoa | Viruses | | MTF Test ^c | | | MPN-PCR ^d | | |
| | | | | Mean | Upper ^e | Lower ^e | Mean | Upper ^e | Lower ^e |
| Encinitas | | | | | | | | | |
| Unpaved | 1000 | 10 | 5.0 | | | | | | |
| Paved | 100 | 100 | 4.0 | <2 | | | 120 | 470 | 50 |
| Storm Drain | 100 | 0 | 35.3 | 9,000 | 29,000 | 3,000 | 3,000 | 9,000 | 1,500 |
| San Diego | | | | | | | | | |
| Unpaved | 1000 | 100 | 4.0 | | | | | | |
| Paved | 1000 | 10 | 0.5 | | | | | | |
| Storm Drain | 100 | 0 | 42.4 | | | | | | |
| Huntington Beach | | | | | | | | | |
| Unpaved | 1000 | 10 | 49.5 | | | | | | |
| Paved | 1000 | 0 | 44.4 | | | | | | |
| Storm Drain | 1000 | 0 | 0.3 | 80 | 250 | 30 | 85 | 240 | 35 |
| Irvine | | | | | | | | | |
| Paved | 1000 | 10 | 55.5 | | | | | | |
| Storm Drain | 100 | 10 | 5.6 | | | | | | |
| Malibu | | | | | | | | | |
| 8 AM | 0 | 0 | 31.3 | | | | | | |
| 12 Noon | 10 | 0 | 31.3 | | | | | | |
| 4 PM | 10 | 0 | 31.3 | | | | | | |
| Santa Monica | | | | | | | | | |
| 8 AM | 0 | 0 | 0.2 | 500 | 2,000 | 200 | 25 | 75 | 10 |
| 12 Noon | 0 | 0 | 0.2 | 1,300 | 3,800 | 500 | 120 | 325 | 46 |
| 4 PM | 10 | 10 | 0.1 | | | | | | |
| Redondo Beach | | | | | | | | | |
| 8 AM | 10 | 0 | 0.3 | 8 | 24 | 3 | 0.35 | 1 | 0.1 |
| 12 Noon | 10 | 0 | 14 | | | | | | |
| 4 PM | 100 | 0 | 14 | | | | | | |

^a The lowest dilution producing a detectable band following PCR. *Staphylococcus* DNA (ca. 40 cells) spiked to an appropriate volume of each nucleic acid extract, from either the FALP or 1-MDS filters, prior to purification. Dilutions were made with the purified nucleic acid extracts. A 0-fold dilution corresponds to the undiluted purified extract.

^b The filtrate from the FALP filter was spiked with the bovine enterovirus. The recovery is from the 1-MDS cartridge filter.

^c Multiple tube fermentation test (Standard Methods, 20th edition, 9221E) performed on a grab sample collected from the specified location.

^d PCR-based assay performed with the purified filter extract utilizing a 10-fold end-point dilution sequence with 5 tubes per dilution and the primer set outlined in Bej *et al.* (1991). The number of positive tubes per dilution were used in conjunction with the Poisson Equation to estimate the most probable number of coliform per 100 mL of sample (MPN/100 mL).

^e Values based on a 95% confidence interval.

Recovery from Processed 1-MDS Filters

The recoveries of the viruses from the water samples collected at each of the methods development sample locations were estimated by correcting the baseline values summarized in Table 11 with the percent difference in the recovery of bovine enterovirus at each sample site. The percent difference in the recovery of bovine enterovirus was calculated by dividing the percent recovery at each sample location, summarized in Table 13, by the fractional baseline value of 0.56 from Table 11. The percent difference in the recovery of bovine enterovirus was then multiplied by the baseline values to obtain the recovery of a particular virus at a given sample location. For example, the calculated recovery of rotavirus from the unpaved surface at the San Diego site was 2.9%, calculated by multiplying the percent recovery of bovine enterovirus ($4\%/0.56 = 7.14\%$) by the fractional baseline value for rotavirus of 0.40 from Table 11. The above procedure provides a method of accounting for the variable influence of water quality characteristics on the concentration of viruses in a 1-MDS filter without spiking the actual pathogen into the water sample, an infeasible task with processing large volumes of an environmental sample.

Bulk Measures of Water Quality.

Bulk measures of the water quality at each of the seven methods development sample locations are summarized in Table 14. The suspended solids concentrations were measured to assess a potential correlation with inhibitory compounds co-extracted in the bead-beating protocol. Higher concentrations of solids may result in a higher concentration of inhibitory compounds removed during the extraction of nucleic acid. The suspended solids concentrations were pooled into four groups corresponding to the samples that required a 0-, 10-, 100-, and 1000-fold dilution to detect the bacteria and protozoa with the PCR assays. The median

concentration of suspended solids in the pooled groups corresponding to the 0-, 10-, 100-, and 1000-fold dilutions were 9.3, 3.3, 4, and 252 mg/L, respectively. The Kruskal-Wallis H-Test was used to evaluate the null hypothesis that the suspended solids concentrations within each of the pooled-groups fall within the same population. There was no statistical significance at the 95% confidence level between the concentration of suspended solids in the pooled groups corresponding to the 0-, 10-, and 100-fold dilutions. There was a statistical significance between the suspended solids concentrations in the pooled-group corresponding to the 1000-fold dilution and all other groups. The statistical significance of the suspended solids concentration in the pooled-group requiring a 1000-fold dilution suggests that the concentration of solids play a role in the production of inhibitory compounds. However, the statistically identical concentrations of suspended solids in the other pooled-groups would suggest the type of solids plays a much more dominant role than the concentration in the production of inhibitory compounds in the nucleic acid extraction protocol.

The hardness within the water samples was measured to assess a potential correlation with the recovery of bovine enterovirus off the 1-MDS filter. Water hardness has been documented by other researchers to potentially have a significant impact on the ability of viruses to adsorb to the 1-MDS filter [Lukasik *et al.*, 2000]. The recovery of viruses off the 1-MDS filter was correlated to the hardness at a 95% confidence level using a one-sided t-test applied to a Spearman rank correlation coefficient. However, there is no discernable relationship between the two variables, suggesting the presence of other compounds in the water that interfere with either the adsorption or elution of viruses from the 1-MDS filter.

Table 14.

Bulk measures of the physical, chemical, and biological quality of water samples collected from seven storm drains or surfaces in close proximity to the drains.

| Sample Location | Physical and Chemical Characteristics | | Indicator Organisms (MPN/100 mL) ^a | | |
|------------------|--|---|---|----------------|-------------|
| | Hardness (mg/L as CaCO ₃) | Suspended Solids ^a (mg/L) | Total Coliform | Fecal Coliform | Enterococci |
| Encinitas | | | | | |
| Unpaved | 12 | 128 | 3,000 | <2 | 30,000 |
| Paved | 46 | 230 | 500 | <2 | 20 |
| Storm Drain | 128 | 2.5 | 30,000 | 9,000 | 2,400 |
| Huntington Beach | | | | | |
| Unpaved | 200 | 303 | 1,600 | <2 | 17,000 |
| Paved | 14 | 68 | 500 | <2 | 13 |
| Storm Drain | 1,200 | NM | 140 | 80 | 14 |
| Irvine | | | | | |
| Paved | 14 | 555 | 30 | <2 | NM |
| Storm Drain | 46 | 4 | 80,000 | 1,700 | NM |
| Malibu | | | | | |
| 8 AM | 1,040 | 3.4 | <2 | <1.1 | <2 |
| 12 Noon | 680 | 3.2 | <2 | <1.1 | <2 |
| 4 PM | 960 | 3.2 | 30 | <1.1 | <2 |
| Redondo Beach | | | | | |
| 8 AM | 460 | 3.3 | 230 | 8 | 300 |
| 12 Noon | 380 | 3.6 | 110 | 8 | 50 |
| 4 PM | 720 | 7.5 | 140 | 13 | 50 |
| San Diego | | | | | |
| Unpaved | 360 | 3,390 | NM | NM | >2,000 |
| Paved | 50 | 201 | NM | NM | 42 |
| Storm Drain | 172 | 4 | >1,600 | 9,000 | 1,044 |
| Santa Monica | | | | | |
| 8 AM | 1,940 | 9.4 | 24,000 | 500 | <1.1 |
| 12 Noon | 1,740 | 9.3 | 24,000 | 1300 | <1.1 |
| 4 PM | 1,800 | 12.2 | 24,000 | 230 | <1.1 |

^a NM = not measured.

Overall Detection Limit of the PCR-Based Assays

Most of the water samples collected as part of this study contained compounds that either impacted the recovery of viruses off the 1-MDS filters or inhibited the PCR-based assays. The overall detection limit of each organism in a particular water sample was calculated by coupling the recovery off the appropriate filter with the sensitivity of the PCR-based assay established for a particular water sample to estimate the minimum number of organisms in the original sample necessary to produce a positive result.

The concentrations of selected bacteria, viruses, and protozoa in water samples collected from the seven storm methods development sites are summarized in Table 15. Detectable concentrations of adenovirus were found in the storm drains at Sample Locations 1 and 2. A detectable concentration of *Salmonella* spp. was found in the runoff from the drain at Sample Location 5. The concentrations of the remaining pathogens at each of the sample locations were below the detection limit of the isolation and identification procedures outlined above.

Every analytical method is characterized by a detection limit determined by the sampling conditions and the specific methodology employed. What can be said here is that a positive result that is verified by Southern blotting can be considered a definite fact. Thus positives recorded are valid measures of the presence of pathogens in the samples. The fact that some positives could be detected at one or two dilutions (e.g. *E. coli* ETEC at Culver/405 on January 25, 2001) is not a quantitative measure. As stated above, the methodology is only qualitative at this time. However, detection limits can be estimated and used as a measure of reliability of the PCR analysis for protecting public health.

Table 15.

Concentrations of selected pathogens in water samples collected from seven storm drains and surfaces located in close proximity to the drains.^a

| Sample Location | Bacteria ^a | | | | Protozoa ^b | | Virus ^c | | | | |
|------------------|---------------------------|------------------------|-----------------|-------------------|-----------------------------|----------------------------|--------------------|-------------|-------------|------------|-----------|
| | <i>E. coli</i> O157:H7 | <i>E. coli</i> ETEC | <i>Shigella</i> | <i>Salmonella</i> | <i>G.</i> <i>lamblia</i> | <i>C.</i> <i>parvum</i> | <i>Adenovirus</i> | Hepatitis A | Enterovirus | Poliovirus | Rotovirus |
| Encinitas | | | | | | | | | | | |
| Unpaved | <130 | <1300 | <2500 | <440 | <290 | <290 | <52 | <28 | <24 | <430 | <1.2 |
| Paved | <13 | <130 | <2,50 | <44 | <29 | <29 | <52 | <28 | <24 | <430 | <1.2 |
| Storm Drain | <13 | <130 | <250 | <44 | <29 | <29 | >0.5 | <0.03 | <0.02 | <0.4 | <0.001 |
| Huntington Beach | | | | | | | | | | | |
| Unpaved | <250 | <2,500 | <5,000 | <880 | <580 | <580 | <100 | <55 | <47 | <850 | <2.3 |
| Paved | <130 | <1,300 | <2,500 | <440 | <290 | <290 | <0.5 | <0.3 | <0.2 | <4.2 | <0.001 |
| Storm Drain | <130 | <1,300 | <2,500 | <440 | <290 | <290 | <5.6 | <2.9 | <2.5 | <46 | <0. |
| Irvine | | | | | | | | | | | |
| Paved | <130 | <1,300 | <2,500 | <440 | <290 | <290 | <0.5 | <0.3 | <0.2 | <4.2 | <0.001 |
| Storm Drain | <13 | <130 | <250 | <44 | <29 | <29 | <5.1 | <2.7 | <2.3 | <42 | <0.1 |
| Malibu | | | | | | | | | | | |
| 8 AM | <0.13 | <1.3 | <2.5 | <0.44 | <0.29 | <0.29 | <0.02 | <0.01 | <0.01 | <0.2 | <0.0006 |
| 12 Noon | <1.3 | <13 | <25 | >4.4 | <2.9 | <2.9 | <0.02 | <0.01 | <0.01 | <0.2 | <0.0006 |
| 4 PM | <1.3 | <13 | <25 | <4.4 | <2.9 | <2.9 | <0.03 | <0.01 | <0.01 | <0.2 | <0.0006 |
| Redondo Beach | | | | | | | | | | | |
| 8 AM | <1.3 | <13 | <25 | <4.4 | <2.9 | <2.9 | <87 | <46 | <40 | <710 | <2 |
| 12 Noon | <1.3 | <13 | <25 | <4.4 | <2.9 | <2.9 | <130 | <69 | <60 | <1,100 | <2.9 |
| 4 PM | <13 | <130 | <250 | <44 | <29 | <29 | <9 | <4.6 | <3.9 | <71 | <0.2 |
| San Diego | | | | | | | | | | | |
| Unpaved | <160 | <1600 | <3300 | <580 | <390 | <390 | <70 | <37 | <32 | <570 | <1.6 |
| Paved | <130 | <1300 | <250 | <440 | <290 | <290 | <52 | <28 | <24 | <430 | <1.2 |
| Storm Drain | <13 | <130 | <250 | <44 | <29 | <29 | >5.2 | <0.03 | <0.02 | <0.4 | <0.001 |
| Santa Monica | | | | | | | | | | | |
| 8 AM | <0.13 | <1.3 | <2.5 | <0.44 | <0.29 | <0.29 | <130 | <69 | <60 | <1,100 | <29.5 |
| 12 Noon | <0.13 | <1.3 | <2.5 | <0.44 | <0.29 | <0.29 | <130 | <69 | <60 | <1,100 | <29.5 |
| 4 PM | <1.3 | <13 | <25 | <4.4 | <2.9 | <2.9 | <1,300 | <690 | <600 | <11,000 | <29.5 |

^a Concentrations expressed in cfu/100 mL.

^b Concentrations expressed in oocysts/100 mL.

^c Adenovirus and rotavirus are expressed in TCID₅₀/100 mL; hepatitis A is expressed in RIFU/L; and poliovirus and enterovirus are expressed in pfu/L.

Baseline Studies

The baseline studies were composed of 49 samples and the results are summarized in Table 16. Note that the numbers in the pathogen columns indicate the maximum 10-fold dilution of detection. A 0 means that the pathogen was detected only in the undiluted sample while a 2 means that the pathogen was detected in a sample diluted by a factor of 100. Pathogens detected in the baseline studies were adenovirus (five samples), enterovirus (one sample), *Salmonella* (one sample), *Staphylococcus* (2 samples), *Giardia* (one sample), and *Cryptosporidium* (one sample). One sample, from a City of Davis park had two positives, *Staphylococcus* and *Giardia*. Thus pathogens were detected in 10 of the 49 samples. The park sample is listed as soil which means that a lawn area was washed with dechlorinated or unchlorinated water and the drainage was sampled. *Staphylococcus* was also detected in washdown from a residential driveway in San Diego. Adenovirus was detected by washing down a Davis park and a Davis residential lawn, in a drain in Sacramento County, and in two drains leading to beaches in San Diego County. Given that 20 percent of the samples were positive for at least one pathogen, one would suspect that the indicator organisms might provide some information, also. However, there appears to be no correlation between either the presence or the concentration of indicator organisms and the presence of pathogens. Note that the samples having the highest concentrations of indicator organisms are, with one exception, non-detects for pathogens. The one high concentration sample in which pathogens were detected was the Davis park soil sample that was positive for *Staphylococcus*. *Staphylococcus* were not detected in any of the drain samples and after a period of non-detects in the field investigations was dropped from the list being monitored.

In one case a positive was detected for *Salmonella* when the indicator organism concentrations were very low. In another case, *Staphylococcus* was detected when total and fecal coliforms were very low but *Enterococcus* was over 100 MPN/100mL.

All of the non-drain samples had significant total coliform, fecal coliform and/or *Enterococcus* concentrations. Most probable number values as high as 5,000,000 total coliforms per 100 mL and fecal coliform values up to 200,000 were found in soil wash samples. If parks were managed in the same manner as beaches, many would be posted on a regular basis, or perhaps permanently.

However, the lack of correlation between the presence of pathogens and the presence of indicator organisms particularly stands out. A conclusion that can be derived from these results is that basing regulatory actions for runoff and drainage on indicator organism MPN values may well lead to faulty conclusions and misguided responses.

Table 16.

Summary of baseline experiment results. Values for PCR data for viruses, bacteria and protozoa can be read as: blank = non detect, 0 = detected in undiluted sample only, 1, 2, ...n = detected at the nth fold dilution

| Location | County | Weather | Surface Type | Date | Indicator Organisms, MPN/100mL | | | | | | Viruses | | | | Bacteria | | | Protozoa | | | |
|-----------------------|--------|---------|--------------|----------|--------------------------------|----------------|----------------|---------------------|------------|-------------|-------------|-----------|------|------|----------|------------|----------|----------|-------------|--|--|
| | | | | | Total Coliform | Fecal Coliform | <i>E. coli</i> | <i>Enterococcus</i> | Adenovirus | Enterovirus | Hepatitis A | Rotavirus | EHEC | ETEC | Shigella | Salmonella | Staphyl. | Giardia | Cryptospor. | | |
| Aliso Creek | Or | Dry | Drain | 6/20/99 | = | 3000 | = | 80 | = | 20 | = | 130 | | | | | | | | | |
| Amundale Barranca | SB | Wet | Drain | 10/30/99 | | | | | | | | | | | | | | | | | |
| Atascadero Creek | SB | Wet | Drain | 10/30/99 | = | 30000 | = | 900 | | | = | 300 | | | | | | | | | |
| Camino Park Barranca | SB | Wet | Drain | 10/30/99 | | | | | | | | 0 | | | | | | | | | |
| Davis (park) 1 | Yolo | Dry | Paved | 11/15/98 | = | 20000 | = | 20000 | | | | 3 | | | | | | | | | |
| Davis (park) 2 | Yolo | Dry | Paved | 12/19/98 | = | 200000 | = | 200000 | | | | | | | | | | | | | |
| Davis (residence) | Yolo | Dry | Soil | 3/6/99 | = | 50000 | = | 50 | | | | 0 | | | | | | | | | |
| Davis (park) | Yolo | Dry | Soil | 3/20/99 | = | 5000000 | = | 50 | | | | | | | | | | 1 | 2 | | |
| Davis (roof) | Yolo | Dry | Roof | 4/13/99 | = | 2000 | = | 90 | | | | | | | | | | | | | |
| Elk Grove (pond) | Sac | Dry | Drain | 5/9/99 | = | 200 | = | 50 | | | | | | | | | | | | | |
| Encinitas (Moonlight) | SD | Dry | Drain | 1/4/99 | = | 5000 | = | 100 | | | = | 100 | | | | | | | | | |
| Encinitas (firehouse) | SD | Dry | Paved | 7/31/99 | = | 901 | < | 4 | | | = | 23 | | | | | | | | | |
| Encinitas (firehouse) | SD | Dry | Soil | 7/31/99 | = | 2883 | < | 4 | | | = | 30631 | | | | | | | | | |
| Encinitas (Moonlight) | SD | Dry | Drain | 7/31/99 | > | 16000 | = | 9000 | | | = | 1044 | 1 | | | | | | | | |
| Huntington Beach | Or | Dry | Drain | 8/2/99 | = | 140 | = | 80 | | | = | 14 | | | | | | | | | |
| Huntington Beach | Or | Dry | Paved | 8/2/99 | = | 500 | < | 2 | | | = | 13 | | | | | | | | | |

Table 16. continued

| Location | County | Weather | Surface Type | Date | Indicator Organisms, MPN/100mL | | | | Viruses | | | | Bacteria | | | Protozoa | |
|-------------------------------|--------|---------|--------------|---------|--------------------------------|----------------|----------------|---------------------|------------|-------------|-------------|-----------|----------|------|-----------------|-------------------|-----------------|
| | | | | | Total Coliform | Fecal Coliform | <i>E. coli</i> | <i>Enterococcus</i> | Adenovirus | Enterovirus | Hepatitis A | Rotavirus | EHEC | ETEC | <i>Shigella</i> | <i>Salmonella</i> | <i>Staphyl.</i> |
| Huntington Beach | Or | Dry | Soil | 8/2/99 | = 1600 | < 2 | | = 17000 | | | | | | | | | |
| Irvine (drain) | Or | Dry | Drain | 8/1/99 | = 80000 | = 1700 | | | | | | | | | | | |
| Irvine (wash station) | Or | Dry | Paved | 8/1/99 | = 30 | < 2 | | | | | | | | | | | |
| Irvine (wash station) | Or | Dry | Soil | 8/1/99 | = 50 | < 2 | | | | | | | | | | | |
| Kearney Mesa (truck wash) | SD | Dry | Paved | 7/31/99 | | | | = 42 | | | | | | | | | |
| Kearney Mesa (truck wash) | SD | Dry | Soil | 7/31/99 | | | | > 2005 | | | | | | | | | |
| Laguna Niguel (park) | Or | Dry | Paved | 6/20/99 | = 5000 | = 20 | = 20 | = 22000 | | | | | | | | | |
| Laguna Niguel (park) | Or | Dry | Soil | 6/20/99 | = 280000 | = 16000 | = 9000 | = 500000 | | | | | | | | | |
| Laguna Niguel (senior center) | Or | Dry | Roof | 6/20/99 | < 20 | < 20 | < 20 | < 20 | | | | | | | | | |
| Malibu Creek 1 | LA | Dry | Drain | 9/11/99 | < 2 | < 2 | | < 1 | | | | | | 1 | | | |
| Malibu Creek 2 | LA | Dry | Drain | 9/11/99 | < 2 | < 2 | | < 1 | | | | | | | | | |
| Malibu Creek 3 | LA | Dry | Drain | 9/11/99 | = 30 | < 2 | | < 1 | | | | | | | | | |
| Port Hueneme | Ven | Dry | Drain | 6/21/99 | = 900 | = 50 | | | | | | | | | | | |
| Port Hueneme | Ven | Dry | Paved | 6/21/99 | = 500 | = 8 | | | | | | | | | | | |
| Port Hueneme | Ven | Dry | Roof | 6/21/99 | = 240 | = 240 | | | | | | | | | | | |
| Port hueneme | Ven | Dry | Soil | 6/21/99 | > 1600 | > 1600 | | | | | | | | | | | |
| Redondo Beach 1 | LA | Dry | Drain | 9/13/99 | = 230 | = 8 | | = 300 | | | | | | | | | |
| Redondo Beach 2 | LA | Dry | Drain | 9/13/99 | = 110 | = 8 | | = 50 | | | | | | | | | |

Table 16. continued

| Location | County | Weather | Surface Type | Date | Indicator Organisms, MPN/100mL | | | | Viruses | | | | Bacteria | | | Protozoa | | | |
|--------------------------|--------|---------|--------------|---------|--------------------------------|----------------|----------------|---------------------|------------|-------------|-------------|-----------|----------|------|-----------------|-------------------|-----------------|----------------|--------------------|
| | | | | | Total Coliform | Fecal Coliform | <i>E. coli</i> | <i>Enterococcus</i> | Adenovirus | Enterovirus | Hepatitis A | Rotavirus | EHEC | ETEC | <i>Shigella</i> | <i>Salmonella</i> | <i>Staphyl.</i> | <i>Giardia</i> | <i>Cryptospor.</i> |
| Redondo Beach 3 | LA | Dry | Drain | 9/13/99 | = | 140 | = | 13 | | | = | 50 | | | | | | | |
| Sacramento (Riverbend) | Sac | Dry | Drain | 5/9/99 | = | 300 | = | 70 | | | | 2 | | | | | | | |
| Sacramento Airport | Sac | Dry | Drain | 5/9/99 | = | 50 | = | 30 | | | | | | | | | | | |
| San Diego (Solano Beach) | SD | Dry | Drain | 1/4/99 | > | 16000 | > | 16000 | | | > | 2005 | | | | | | | |
| San Diego (Del Mar) | SD | Dry | Drain | 1/5/99 | = | 5000 | = | 800 | | | = | 500 | | | | | | | 0 |
| San Diego (La Jolla) | SD | Dry | Drain | 1/5/99 | = | 9000 | = | 3000 | | | = | 700 | 2 | | | | | | |
| San Diego (Ravina) | SD | Dry | Drain | 6/19/99 | = | 130000 | = | 2000 | | | > | 2005 | | | | | | | |
| San Diego (residence) | SD | Dry | Paved | 6/19/99 | < | 2 | < | 2 | | | = | 164 | | | | | | 1 | |
| San Diego (residence) | SD | Dry | Soil | 6/19/99 | = | 140000 | = | 110000 | | | > | 2005 | | | | | | | |
| San Diego (residence) | SD | Dry | Roof | 6/19/99 | < | 2 | < | 2 | | | = | 75 | | | | | | | |
| San Diego (Del Mar) | SD | Dry | Drain | 8/1/99 | > | 1600 | = | 9000 | | | = | 1044 | 2 | | | | | | |
| Santa Monica (Ashland) 1 | LA | Dry | Drain | 9/12/99 | = | 24000 | = | 500 | | | < | 1 | | | | | | | |
| Santa Monica (Ashland) 2 | LA | Dry | Drain | 9/12/99 | = | 24000 | = | 1300 | | | < | 1 | | | | | | | |
| Santa Monica (Ashland) 3 | LA | Dry | Drain | 9/12/99 | = | 24000 | = | 230 | | | < | 1 | | | | | | | |
| Simi Valley (park) | Ven | Wet | Drain | 11/1/99 | = | 24000 | < | 2 | | | < | 2 | | | | | | | |
| Simi Valley (residence) | Ven | Wet | Drain | 11/1/99 | | | | | | | | | | | | | | | |

Field Investigations

Results of the 97 field investigations are presented in Table 17. Pathogens were detected in 12 samples with one sample having two positives, making a total of 13 positive detections. *Salmonella* was the most commonly detected pathogen, with four positives in Malibu Creek under dry conditions, one each in bmp effluents at Rincon and June Way during a rain events and one each at Leucadia (concrete lined drain) and Encinitas (drain discharge at Moonlight Beach) during dry weather. Enterotoxigenic *E. coli* (ETEC) was detected in wet weather samples taken at El Camino and Montiel and in the bmp effluent at the Filmore site. Adenovirus was detected in one of six dry-weather Culver samples. Because of a protocol error by the commercial laboratory carrying out the indicator organism analysis a large number of data were lost. The laboratory used dilutions that would detect violations, as per Table 3, rather than determining actual MPN/100 mL values. When pathogens were detected there were usually significant numbers of at least one indicator group. However, there were significant numbers of indicators in most samples and the samples with the very highest numbers of indicators were not associated with detection of pathogens.

What can be said is that urban drainage occasionally contains pathogens and there seems to be little reason to believe that the presence of pathogens is statistically correlated with the presence of indicator organisms. The presence of organisms such as *Salmonella* and *E. coli* ETEC in urban drainage is not surprising because there are animal sources of both (birds, lizzards, and mammals for *Salmonella* and cattle and some other mammals for *E. coli* ETEC). Detection of human viruses, such as adenovirus, is a definite indication of human contamination.

Table 17.

Summary of field investigation results. Values for PCR data for viruses, bacteria and protozoa can be read as: blank = non detect, 0 = detected in undiluted sample only, 1, 2, ...n = detected at the nth fold dilution.

| Location | | | | Indicators, MPN/100 mL | | | Viruses | | | | Bacteria | | | | Protozoa | | |
|---------------|--------|------------------|---------|------------------------|----------------|--------------|---------|--------|--------|-----------|----------|------|----------|---------|----------|---------|--------|
| | County | Hours since rain | Date | Total Coliform | Fecal Coliform | Enterococcus | Adeno | Entero | Hep. A | Rotavirus | EHEC | ETEC | Shigella | Salmon. | Staph | Giardia | Crypto |
| Altadena/210 | LA | raining | 2/24/01 | = 17,000 | = 2,600 | | | | | | | | | | | | |
| Altadena/210 | LA | raining | 3/4/01 | | | | | | | | | | | | | | |
| Altadena/210 | LA | raining | 4/7/01 | | | | | | | | | | | | | | |
| Altadena/210 | LA | raining | 4/20/01 | = 240 | = 13 | = 461 | | | | | | | | | | | |
| Calabasas/101 | LA | >72 | 1/23/01 | = 2,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Calabasas/101 | LA | >72 | 2/5/01 | = 13,000 | < 2,000 | = 23,000 | | | | | | | | | | | |
| Calabasas/101 | LA | raining | 2/12/01 | = 8,000 | = 8,000 | = 8,000 | | | | | | | | | | | |
| Calabasas/101 | LA | >72 | 3/19/01 | < 2,000 | < 2,000 | = 2,300 | | | | | | | | | | | |
| Calabasas/101 | LA | >72 | 5/21/01 | = 50,000 | = 1,100 | = 1,300 | | | | | | | | | | | |
| Calabasas/101 | LA | >72 | 6/1/01 | | | | | | | | | | | | | | |
| Chavez/101 | LA | 1-72 | 3/1/01 | = 300,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Chavez/101 | LA | >72 | 3/27/01 | < 2,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Chavez/101 | LA | 1-72 | 5/14/01 | = 11,000 | = 7,000 | = 3,000 | | | | | | | | | | | |
| Culver/405 | OC | 1-72 | 1/25/01 | = 13,000 | = 2,000 | < 2,000 | | 1 | | | | | | | | | |
| Culver/405 | OC | 1-72 | 2/22/01 | < 2,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Culver/405 | OC | 1-72 | 3/1/01 | = 80,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Culver/405 | OC | >72 | 3/27/01 | < 2,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Culver/405 | OC | 1-72 | 4/23/01 | 1 = 130,000 | = 1,300 | = 2,300 | | | | | | | | | | | |
| Culver/405 | OC | >72 | 5/7/01 | = 30,000 | = 800 | = 13,000 | | | | | | | | | | | |
| El Camino/78 | SD | 1-72 | 3/8/01 | = 4,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| El Camino/78 | SD | >72 | 3/22/01 | < 2,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| El Camino/78 | SD | 1-72 | 4/5/01 | > 1,600 | = 50 | = 80 | | | | | | | | | | | |
| El Camino/78 | SD | raining | 4/10/01 | = 17,000 | = 5,000 | = 23,000 | | | | | | 1 | | | | | |
| El Camino/78 | SD | >72 | 4/30/01 | = 3,000 | = 230 | = 300 | | | | | | | | | | | |
| El Camino/78 | SD | >72 | 5/30/01 | | | | | | | | | | | | | | |
| Encinitas/5 | SD | 1-72 | 3/8/01 | = 2,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Encinitas/5 | SD | >72 | 3/22/01 | < 2,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Encinitas/5 | SD | 1-72 | 4/5/01 | > 1,600 | = 240 | = 130 | | | | | | | | | | | |

Table 17.
Summary of field investigation results. continued

| Location | | | | Indicators, MPN/100 mL | | | Viruses | | | | Bacteria | | | | Protozoa | | |
|--------------|------------------|---------|----------|------------------------|----------------|---------------|---------|--------|--------|-----------|----------|------|----------|---------|----------|---------|--------|
| County | Hours since rain | Date | | Total Coliform | Fecal Coliform | Entero-coccus | Adeno | Entero | Hep. A | Rotavirus | EHEC | ETEC | Shigella | Salmon. | Staph | Giardia | Crypto |
| Encinitas/5 | SD | raining | 4/10/01 | = 90,000 | = 8,000 | = 3,000 | | | | | | | | | | | |
| Encinitas/5 | SD | >72 | 4/30/01 | = 7,000 | = 300 | = 5,000 | | | | | | | | | | | |
| Encinitas/5 | SD | >72 | 5/30/01 | | | | | | | | | | | 1 | | | |
| Filmore/210 | LA | raining | 2/24/01 | = 7,000 | = 900 | | | | | | | | | | | | |
| Filmore/210 | LA | raining | 3/4/01 | = 8,000 | = 8,000 | | | | | | | | | | | | |
| Filmore/210 | LA | raining | 4/7/01 | | | | | | | | | 1 | | | | | |
| Filmore/210 | LA | raining | 4/20/01 | = 24,000 | = 900 | = 260 | | | | | | | | | | | |
| June Way/60 | LA | raining | 2/19/01 | | | < 2,000 | | | | | | | | | | | |
| June Way/60 | LA | raining | 4/7/01 7 | | | = 200,000 | | | | | | | | 1 | | | |
| Lakewood/405 | LA | >72 | 1/23/01 | = 23,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Lakewood/405 | LA | 1-72 | 2/15/01 | = 22,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Lakewood/405 | LA | 1-72 | 2/22/01 | = 50,000 | < 2,000 | = 4,000 | | | | | | | | | | | |
| Lakewood/405 | LA | >72 | 5/7/01 | = 50,000 | = 8,000 | = 70,000 | | | | | | | | | | | |
| Las Posas/34 | VC | >72 | 2/5/01 | = 70,000 | < 2,000 | = 240,000 | | | | | | | | | | | |
| Las Posas/34 | VC | raining | 2/12/01 | = 30,000 | < 2,000 | = 5,000 | | | | | | | | | | | |
| Las Posas/34 | VC | >72 | 3/19/01 | = 50,000 | < 2,000 | = 4,000 | | | | | | | | | | | |
| Las Posas/34 | VC | >72 | 5/21/01 | = 1,600,000 | = 500 | = 7,000 | | | | | | | | | | | |
| Las Posas/34 | VC | >72 | 6/1/01 | | | | | | | | | | | | | | |
| Leucadia/5 | SD | 1-72 | 3/8/01 | < 2,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Leucadia/5 | SD | >72 | 3/22/01 | < 2,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Leucadia/5 | SD | >72 | 3/22/01 | < 2,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Leucadia/5 | SD | 1-72 | 4/5/01 | | | | | | | | | | | | | | |
| Leucadia/5 | SD | raining | 4/10/01 | = 240,000 | = 7,000 | = 8,000 | | | | | | | | | | | |
| Leucadia/5 | SD | >72 | 4/30/01 | = 13,000 | = 500 | = 5,000 | | | | | | | | | | | |
| Leucadia/5 | SD | >72 | 5/30/01 | | | | | | | | | | | 1 | | | |

Table 17.
Summary of field investigation results. continued

| Location | | | | Indicators, MPN/100 mL | | | | | Viruses | | | | Bacteria | | | | Protozoa | |
|------------------|--------|------------------|---------|------------------------|----------------|--------------|-------|--------|---------|-----------|------|------|----------|---------|-------|---------|----------|--|
| | County | Hours since rain | Date | Total Coliform | Fecal Coliform | Enterococcus | Adeno | Entero | Hep. A | Rotavirus | EHEC | ETEC | Shigella | Salmon. | Staph | Giardia | Crypto | |
| Malibu Creek/101 | LA | >72 | 1/23/01 | < 2,000 | < 2,000 | < 2,000 | | | | | | | | 0 | | | | |
| Malibu Creek/101 | LA | >72 | 2/5/01 | < 2,000 | < 2,000 | = 23,000 | | | | | | | | 0 | | | | |
| Malibu Creek/101 | LA | raining | 2/12/01 | = 8,000 | = 8,000 | = 8,000 | | | | | | | | | | | | |
| Malibu Creek/101 | LA | >72 | 3/19/01 | < 2,000 | < 2,000 | < 2,000 | | | | | | | | | | | | |
| Malibu Creek/101 | LA | >72 | 5/21/01 | = 5,000 | = 26 | = 800 | | | | | | 0 | 0 | | | | | |
| Malibu Creek/101 | LA | >72 | 6/1/01 | | | | | | | | | | | 1 | | | | |
| Montiel/15 | SD | 1-72 | 3/8/01 | = 17,000 | = 4,000 | < 2,000 | | | | | | | | | | | | |
| Montiel/15 | SD | >72 | 3/22/01 | 4,000 | < 2,000 | < 2,000 | | | | | | | | | | | | |
| Montiel/15 | SD | >72 | 3/22/01 | 4,000 | < 2,000 | < 2,000 | | | | | | | | | | | | |
| Montiel/15 | SD | 1-72 | 4/5/01 | > 1,600 | = 500 | = 50 | | | | | | | | | | | | |
| Montiel/15 | SD | raining | 4/10/01 | = 170,000 | = 230 | = 5,000 | | | | | | 1 | | | | | | |
| Montiel/15 | SD | >72 | 4/30/01 | = 11,000 | = 230 | = 900 | | | | | | | | | | | | |
| Montiel/15 | SD | >72 | 5/30/01 | | | | | | | | | | | | | | | |
| Pico drain/10 | LA | >72 | 1/23/01 | = 50,000 | < 2,000 | < 2,000 | | | | | | | | | | | | |
| Pico drain/10 | LA | 1-72 | 2/15/01 | = 170,000 | = 2,000 | < 2,000 | | | | | | | | | | | | |
| Pico drain/10 | LA | 1-72 | 2/22/01 | = 170,000 | = 4,000 | < 2,000 | | | | | | | | | | | | |
| Pico drain/10 | LA | 1-72 | 3/1/01 | = 50,000 | = 7,000 | < 2,000 | | | | | | | | | | | | |
| Pico drain/10 | LA | 1-72 | 4/23/01 | = 50,000 | = 2,300 | = 1,700 | | | | | | | | | | | | |
| Pico drain/10 | LA | >72 | 5/7/01 | | | | | | | | | | | | | | | |
| Pico drain/10 | LA | 1-72 | 5/14/01 | | | | | | | | | | | | | | | |

Table 17.
Summary of field investigation results. continued

| Location | | | | Indicators, MPN/100 mL | | | Viruses | | | | Bacteria | | | | Protozoa | | |
|-------------|------------------|---------|---------|------------------------|----------------|--------------|---------|--------|--------|-----------|----------|------|----------|---------|----------|---------|--------|
| County | hours since rain | Date | | Total Coliform | Fecal Coliform | Enterococcus | Adeno | Entero | Hep. A | Rotavirus | EHEC | ETEC | Shigella | Salmon. | Staph | Giardia | Crypto |
| Orcas/210 | LA | raining | 2/24/01 | = 7,000 | = 2,300 | | | | | | | | | | | | |
| Orcas/210 | LA | raining | 3/4/01 | = 50,000 | = 2,300 | | | | | | | | | | | | |
| Orcas/210 | LA | raining | 4/7/01 | | | | | | | | | | | | | | |
| Orcas/210 | LA | raining | 4/20/01 | = 800 | = 500 | = 548 | | | | | | | | | | | |
| Rincon/210 | LA | raining | 2/10/01 | | | < 2,000 | | | | | | | | | | | |
| Rincon/210 | LA | raining | 2/19/01 | | | < 2,000 | | | | | | | | | | | |
| Rincon/210 | LA | raining | 2/24/01 | | | < 2,000 | | | | | | | | | | | |
| Rincon/210 | LA | raining | 4/7/01 | | | = 200,000 | | | | | | | | 1 | | | |
| Riverside | RC | 1-72 | 2/22/01 | < 2,000 | < 2,000 | = 2,000 | | | | | | | | | | | |
| Riverside | RC | >72 | 3/27/01 | = 80,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Spruce/91 | RC | 1-72 | 3/1/01 | = 23,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Spruce/91 | RC | >72 | 3/13/01 | < 2,000 | | = 8,000 | | | | | | | | | | | |
| Spruce/91 | RC | >72 | 3/13/01 | = 17,000 | | = 11,000 | | | | | | | | | | | |
| Spruce/91 | RC | >72 | 3/13/01 | < 2,000 | | = 2,300 | | | | | | | | | | | |
| Spruce/91 | RC | >72 | 3/13/01 | < 2,000 | | = 2,300 | | | | | | | | | | | |
| Spruce/91 | RC | >72 | 3/27/01 | < 2,000 | < 2,000 | < 2,000 | | | | | | | | | | | |
| Spruce/91 | RC | >72 | 4/16/01 | = 2,300 | = 17 | = 500 | | | | | | | | | | | |
| Spruce/91 | RC | >72 | 4/16/01 | = 500 | = 13 | = 500 | | | | | | | | | | | |
| Spruce/91 | RC | >72 | 4/16/01 | = 300 | = 2 | = 300 | | | | | | | | | | | |
| Spruce/91 | RC | >72 | 4/16/01 | = 1,700 | = 50 | = 300 | | | | | | | | | | | |
| Spruce/91 | RC | >72 | 5/7/01 | = 800 | = 110 | = 110 | | | | | | | | | | | |
| Turnbull/60 | LA | raining | 2/10/01 | | | < 2,000 | | | | | | | | | | | |
| Turnbull/60 | LA | raining | 2/19/01 | 1 | | < 2,000 | | | | | | | | | | | |
| Turnbull/60 | LA | raining | 2/24/01 | | | < 2,000 | | | | | | | | | | | |
| Turnbull/60 | LA | raining | 4/7/01 | | | = 200,000 | | | | | | | | | | | |

DISCUSSION

Examination of the data in Tables 16 and 17 will surely lead to the conclusion that indicator organisms are ubiquitous in the urban environment. They are found in runoff from most paved and unpaved surfaces, including roofs, grassy areas in parks, parking lots, and residential driveways. Concentrations vary greatly but in some cases are very high. For example, washing park soil in Davis resulted in a total coliform MPN of 5,000,000 /100 mL and washing park soil in Laguna Niguel resulted in a total coliform of 280,000, a fecal coliform MPN of 16,000 per 100 mL, an *E. coli* MPN of 9,000/100 mL, and an *Enterococcus* MPN of 500,000 per 100 ML. The Davis park sample was positive for *Staphylococcus* and *Giardia* but no pathogens were detected in the Laguna Niguel sample. Washing residential roofs resulted in samples which exceeded the recreational water geometric mean limits for total coliforms in one case and for the *Enterococcus* in another. The source of indicator organisms in the baseline samples is not clear, but it is very unlikely that human wastes are a significant factor. Based on the literature, there is a possibility that indicator organisms are resident in the soil [Chao and Feng, 1990; Francy *et al.*, 2000; Hardina and Fujioka, 1991; LeChevallier *et al.*, 1991; Solo-Gabriele *et al.*, 2000]. Birds, wild animals, and domestic animals may be significant sources. In California, and particularly in Southern California, there are significant periods between storms during which contamination may accumulate on soil and paved surfaces. Thus washing dry surfaces with a set volume of water (100 L in these experiments) may result in maximum concentrations being observed. Washing for a set period of time prior to sampling may have resulted in lower concentrations.

The indicator organism data from the field investigations (Table 17) generally follow the pattern of the baseline investigations. Most of the drains had significant concentrations of indicator organisms. The highest indicator organism MPNs for samples taken during periods of

precipitation. High indicator organism concentration is not correlated with detection of pathogens in the field investigation samples. For example, the Las Posas/34 samples included the highest observations for total coliforms and *Enterococcus*, included both dry and wet weather samples and had no positives for pathogens. The field investigation indicator organism data was badly damaged by the failure of the commercial laboratory doing the testing to analyze the proper dilutions. However, there are enough data between the remaining field investigations and the baseline studies to provide confidence in the conclusion that the indicator organisms are relatively ubiquitous in urban drainage and are not likely to be strong indicators of the presence of pathogens and even less of recent human contamination.

Detection Limits

The above conclusion must be made with the caveat that detection limits for pathogens using PCR appear to be unsatisfactorily high in some cases, as indicated in Table 15. Detection limits are rarely estimated for environmental samples and thus developing a general frame of reference that would allow verification of the approach to determining detection limits is not possible. However, we do not actually know what is present below the detection limit. The answer is that the actual value may be any concentration up to the limit. For many of the environmental samples evaluated in Table 15 the detection limits were quite satisfactory. Thus the general conclusions developed in the baseline and field investigations appear to be valid. Additional work to provide consistently satisfactory detection limits for environmental samples is a great need. Detection limit objectives should reflect known infectious doses (Table 1) and knowledge of the incidence of disease resulting from swimming and other recreational activities.

Pathogen Sources

The pathogens detected in the baseline and field investigation studies fall into two groups, those that are almost certainly of human origin and those that are most likely of non-human origin. It is important to note that pathogens were detected in only 10 of the 49 baseline samples and only 12 of the 97 field investigation samples. In one of the baseline study and one of the field investigations samples two pathogens were detected, making a total of 24 pathogen detections in the 146 samples. Six of the baseline study samples and one of the field investigation samples contained human viruses. Four of the baseline study samples positive for viruses were drains and hence similar to the field investigations. Thus there does not appear to be a deviation in the data in terms of the phase of the investigations. Six of the seven virus detections were adenovirus and one was enterovirus. In either case, the viruses must be presumed to be of human origin. Of the six positive viral samples in the baseline studies, five were from storm drains and one was from a residential lawn. All but one of the baseline viral positives were obtained by washing down dry surfaces while sixth was found in a wet storm drain sample at Camino Park Barranca in Santa Barbara County. The field investigation viral positive was taken at the Culver/405 site under dry weather conditions. Thus the source of the human viruses does not appear to have been leaking sewers or other sources of human wastes.

Of the 17 pathogen detections classified as most likely non-human in origin, 9 were *Salmonella*, 4 were *E. coli* ETEC, two were *Staphylococcus*, one was *Giardia* and one was *Cryptosporidium*. Both the *Giardia* and the *Cryptosporidium* positives were in the baseline studies, one in a drain onto a beach in Del Mar and the other in a lawn sample from a Davis park. All of the pathogens detected, the two bacteria found in 14 samples and the two protozoans, are commonly found in both domestic and wild animals. *Salmonella* is resident in both the bird and

reptile populations and *E. coli* ETEC is common in a number of urban mammals, although transmission from animals to humans is not considered to be a significant route of infection. *Staphylococcus aureus*, an opportunistic pathogen, is commonly found in animals and is a normal resident of the human respiratory tract from the first weeks following birth.

Significance of Microorganisms in Urban Drainage

In summary, indicator organisms are ubiquitous in urban drainage while human pathogens are sometimes found in urban drainage. Based on the MPN data available from the baseline studies and the field investigations there does not appear to be a correlation of any kind between the number of indicator organisms and the presence of pathogens. The fact that indicator organisms are present in soil and pavement from parks, drainage from parking lots, roofs, and residential lawns presents interesting issues in terms of regulation of water quality. Based on these investigations there is good evidence that public health is not protected by using the common indicators (total coliforms, fecal coliforms, fecal streptococci, *E. coli* and *Enterobacter*) in this context. However, pathogens are found in urban drainage. Whether there is greater danger from swimming in surf receiving urban drainage, from rolling around on park grass, or from chewing blades of park grass, is unknown. The detection limit problems associated with PCR analysis make defining the problem more difficult. However, regulators must begin to recognize that new approaches to protecting public health and recreational waters must be investigated. Decisions based on detection of indicator organisms often result in significant costs and may very often have no public health benefit.

Of the organisms selected for the study, the viruses and infective *Shigella* exclusively affect humans, and implicitly must have human sources. All the other bacteria and protozoa may

have multiple non-human sources. No samples were found to contain infective *Shigella*, so the viruses appear to be the best indicators of human contamination.

Human waste may not necessarily contain these viruses, and could contain any of the non-human source pathogens. Therefore, only those samples that were positive for viruses were conclusive for presence of human contamination and no sample is conclusive for the absence. The field study locations were sampled repeatedly, and viruses are common enough in human waste that at least some of those samples would be expected to contain viruses if a site is subject to chronic human contamination. Incidental contamination is virtually impossible to detect.

Of the samples collected from drains in both study phases, the sites where collected can be categorized as having drainage predominantly from highway uses, predominantly from city uses, and a mixture of the two. Of 18 samples tested, none of those from highway sites were positive for viruses. One of 53 samples from mixed use sites was found to contain the viruses, but 5 of 25 samples from city sites were positive for viruses. Additionally, one of the city sites, three of the mixed use sites, and two of the highway sites produced samples in which a pathogenic bacteria or protozoa were found.

The data may also be categorized by hydrologic regime, as coming from more than 72 hrs since last rain (dry), 1 to 72 hrs since last rain (recent rain), and raining at the time of collection (wet). Fifty-four samples were collected during dry conditions. Four of these were found to contain viruses. Two of the 18 samples taken from sites having received recent rain contained viruses. In the 24 samples tested that were collected wet, no viruses were found.

There were six exclusively highway drainage sites sampled, with 22 total samples. Two samples, each from a different site, tested positive for bacteria, none were positive for viruses. The city sites, relatively remote from highways, were sampled almost exclusively during the dry

season. Those samples were collected during the summer and fall before the rainy season collection on the highways. Five of the 28 samples were collected after recent rains, one of which was positive for virus. Four of the city sites sampled under dry conditions tested positive for viruses, and one each was positive for bacteria and protozoa.

The mixed drainage sites were sampled mostly during dry and recent rain conditions. Only six samples, each from a different site, were taken from these sites during rains and neither viruses nor bacterial/protozoan pathogens were detected. Thirteen sites with mixed drainage sampled during dry conditions with a total of 46 samples. Six of the 46 samples tested positive for bacteria, representing 3 sites; none of the samples tested positive for viruses. Ten of the mixed drainage sites were sampled after recent rains. One of the 23 samples was found to contain viruses.

These results support the belief that highway facilities are not a significant source of human contamination of either urban drainage or storm drainage. Consideration should be given to setting a low priority on monitoring these facilities for microbial contamination.

Suggested Directions

Four specific research directions should be undertaken for the purposes of protecting the biological quality of and developing appropriate standards for recreational waters.

- (1) Improving the detection limits of molecular methods for analysis of environmental samples and the ability to provide quantitative results using these methods,
- (2) Further study of field sites where highway runoff and drainage from surrounding areas are mixed to establish whether highway runoff has been a contributor to pathogen detections,

(3) Development of improved understanding of the contributions of urban drains to the microbial quality of the surf zone, and

(4) Development of improved indicators of human contamination.

Detection limits have been discussed above and there is no need of further discussion here. The question of developing better understanding of the contribution of urban drains to the microbial quality of the surf zone results from the fact that the principal recreational waters in Southern California are at beaches. Urban drains are believed to be a major source of surf zone contamination. As Most storm drains in Southern California are quite small and the dry weather flow either is non-existent or does not reach the surf zone under at low tide, but disappears into the sand. The fate of microorganisms in the drainage is unknown and there is not any information available on conditions in the sand. The most extensive investigations of the impacts of drainage on surf zone water quality were conducted by Grant and his associates [2001]. Their work was focused on a tidal marsh fed by storm drains that was hypothesized impact indicator organism levels in the surf zone at Huntington Beach. The results were inconclusive.

The concept of using indicators of contamination is valid but there is obviously great difficulty in finding appropriate organisms. If human contamination is the principal concern viruses would be a suitable choice because of their host specificity. If satisfactory detection limits can be established the necessity of having a large number of organisms present may be negated. Improvements in PCR methodology may result in human viruses becoming a suitable indicator of human contamination. Because of their host specificity, the presence of human viruses would indicate a high probability of contamination by human wastes. Viruses travel through soil and sand more efficiently than the larger bacteria and protozoa, which is attractive in

in detecting sources such as leaking sewers. The difficulty of determining if the source of the nucleic acid sequence detected in PCR analysis was from an active or inactive virus would not be an issue for an indicator because in either case evidence of contamination would exist.

Consideration of viruses as indicators leads to another question about regulation of the biological quality of urban drainage. The principal concern has been contamination with sewage, although there are cases where contamination by animals (e.g. runoff from pastures, horse race tracks, and feed lots) is also a major issue. Sewage contamination of storm drains occurs through overflows in collection systems, leaks that migrate to the drains, and failed septic tank/leach fields used for non-sewered areas. Stormwater overflows generally occur because of high infiltration and inflow rates in municipal collection systems, or, in older communities, because a portion of the sanitary sewer system is combined with the storm drainage system. Both problems can be addressed through collection system improvements which will be expensive but quite possibly less costly than treating stormwater flows. Similarly, leaking collection systems and failed on-site systems must be corrected. Given that most contamination of stormwater by human waste can be stopped through infrastructure improvements and maintenance, the question of the impact of animals must be considered. At the beach there are often enormous numbers of shore birds, each of which is defecating at will. Handrails, picnic tables, and walkways are all contaminated by these birds and yet there is little concern about the public health implications. Few parks are free of dog manure, despite campaigns to pick up after pets, and sandboxes around play equipment attract cats. These animal sources may be a principal factor in both the indicator organism counts and the bacterial and protozoan pathogens found in both the baseline and field studies. Thought needs to be given to the public health

implications of domestic and wild animals in urban settings, the actual risk versus the perceived risk, and whether treatment of urban drainage is appropriate.

CONCLUSIONS

- Significant concentrations of indicator organisms are nearly ubiquitous in urban drainage.
- Pathogens can be found in urban drainage but there does not appear to be a relationship between the presence of pathogens and the concentration or presence of indicator organisms.
- Based on the number of pathogen detections in this study, 12 of 97 samples in the field investigations and 10 of 49 samples in the baseline studies, contact with pathogenic organisms in the urban environment is not a rare event.
- The most commonly detected pathogens in stormwater are those for which the principal reservoirs are domestic and/or wild animals.
- Molecular methods of detection of environmental pathogens, such as PCR, are very promising but inhibition and interferences associated with environmental samples can result in unacceptably high detection limits.
- Human viruses may be more suitable indicators of recent contamination with human wastes than coliforms, fecal coliforms, *E. coli*, or *Enterococcus* counts.
- Consideration should be given to both the probable origins of indicators and pathogens in setting policy and developing strategies for protecting recreational waters.
- With respect to control of waterborne disease, money used for improving sanitary sewers and wastewater treatment probably is more wisely invested than money used for treatment of urban drainage.

- Highway facilities, including park and rides and maintenance stations, do not appear to be a significant source of pathogens in urban drainage.

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Table A-1

Highway Drain Workbook Data - Gross characteristics

| | | | | Gross characteristics | | | | | | |
|-----------|------------------|---|--------|-----------------------|-------|------------------|-----------------|-----------|----------|------|
| Sample No | Location | | County | Since Rain (hr) | Type | Date & Time | Sample Vol. (L) | Hard ness | EC, (µS) | pH |
| a-23-1 | Calabasas/101 | a | LA | >72 hrs | mixed | 1/23/01 6:00 AM | 40 | 900 | 3,310 | 8.30 |
| a-5-2 | Calabasas/101 | a | LA | >72 hrs | mixed | 2/5/01 10:00 AM | 40 | 894 | 3,310 | 8.08 |
| a-12-2 | Calabasas/101 | a | LA | raining | mixed | 2/12/01 9:00 AM | 40 | <54 | 990 | 7.93 |
| a-19-3 | Calabasas/101 | a | LA | >72 hrs | mixed | 3/19/01 10:00 AM | 40 | 894 | 3,325 | 7.87 |
| a-21-5 | Calabasas/101 | a | LA | >72 hrs | mixed | 5/21/01 10:00 AM | 20 | 894 | 3,125 | 8.12 |
| a-1-6 | Calabasas/101 | a | LA | >72 hrs | mixed | 6/1/01 10:00 AM | 20 | 896 | 3,045 | 8.10 |
| b-23-1 | Malibu Creek/101 | b | LA | >72 hrs | mixed | 1/23/01 7:00 AM | 40 | 715 | 2,150 | 8.00 |
| b-5-2 | Malibu Creek/101 | b | LA | >72 hrs | mixed | 2/5/01 6:30 AM | 40 | 450 | 2,190 | 7.82 |
| b-12-2 | Malibu Creek/101 | b | LA | raining | mixed | 2/12/01 8:00 AM | 40 | <54 | 1,700 | 8.05 |
| b-19-3 | Malibu Creek/101 | b | LA | >72 hrs | mixed | 3/19/01 6:30 AM | 40 | 894 | 3,430 | 7.81 |
| b-21-5 | Malibu Creek/101 | b | LA | >72 hrs | mixed | 5/21/01 10:30 AM | 20 | 894 | 2,900 | 8.05 |
| b-1-6 | Malibu Creek/101 | b | LA | >72 hrs | mixed | 6/1/01 10:20 AM | 20 | 596 | 2,905 | 8.04 |
| c-23-1 | Pico drain/10 | c | LA | >72 hrs | mixed | 1/23/01 10:00 AM | 40 | 269 | 1,160 | 7.50 |
| c-15-2 | Pico drain/10 | c | LA | 1-72 hrs | mixed | 2/15/01 11:00 AM | 40 | 358 | 1,055 | 8.37 |
| c-22-2 | Pico drain/10 | c | LA | 1-72 hrs | mixed | 2/22/01 10:30 AM | 40 | 358 | 1,270 | 8.48 |
| c-1-3 | Pico drain/10 | c | LA | 1-72 hrs | mixed | 3/1/01 11:30 AM | 40 | 269 | 964 | 8.24 |

Table A-1 continued

| Sample No | Location | | | Gross characteristics | | | | | | |
|-----------|---------------|-----|--------|-----------------------|-------|------------------|-----------------|----------|----------------|------|
| | | | County | Since Rain (hr) | Type | Date & Time | Sample Vol. (L) | Hardness | EC, (μ S) | pH |
| c-23-4 | Pico drain/10 | c | LA | 1-72 hrs | mixed | 4/23/01 12:00 PM | 20 | 269 | 1,140 | 8.49 |
| c-7-5 | Pico drain/10 | c | LA | >72 hrs | mixed | 5/7/01 11:30 AM | | | | |
| c-14-5 | Pico drain/10 | c | LA | 1-72 hrs | mixed | 5/14/01 10:30 AM | | | | |
| d-23-1 | Lakewood/405 | d | LA | >72 hrs | mixed | 1/23/01 11:00 AM | 40 | 900 | 10,620 | 7.20 |
| d-15-2 | Lakewood/405 | d | LA | 1-72 hrs | mixed | 2/15/01 11:30 AM | 40 | 538 | 6,550 | 7.88 |
| d-22-2 | Lakewood/405 | d | LA | 1-72 hrs | mixed | 2/22/01 11:30 AM | 40 | 894 | 12,200 | 7.28 |
| d-7-5 | Lakewood/405 | d | LA | >72 hrs | mixed | 5/7/01 11:00 AM | 20 | 179 | 3,610 | 7.60 |
| e23-10-2 | Rincon/210 | e23 | LA | raining | sole | 2/10/01 6:15 AM | 20 | 358 | 98 | 8.06 |
| e23-19-2 | Rincon/210 | e23 | LA | raining | sole | 2/19/01 2:35 PM | 20 | <54 | 123 | 7.85 |
| e23-24-2 | Rincon/210 | e23 | LA | raining | sole | 2/24/01 3:00 PM | 20 | <54 | 84 | 7.81 |
| e23-7-4 | Rincon/210 | e23 | LA | raining | sole | 4/7/01 7:00 AM | 20 | <54 | 61 | 7.55 |
| e6-10-2 | Turnbull/60 | e6 | LA | raining | sole | 2/10/01 7:45 AM | 20 | 448 | 300 | 8.23 |
| e6-19-2 | Turnbull/60 | e6 | LA | raining | sole | 2/19/01 10:45 AM | 20 | <54 | 255 | 8.20 |
| e6-24-2 | Turnbull/60 | e6 | LA | raining | sole | 2/24/01 12:45 PM | 20 | <54 | 134 | 8.22 |
| e6-7-4 | Turnbull/60 | e6 | LA | raining | sole | 4/7/01 7:00 AM | 20 | <54 | 150 | 7.87 |
| e8-19-2 | June Way/60 | e8 | LA | raining | sole | 2/19/01 3:35 PM | 20 | <54 | 133 | 8.25 |
| e8-7-4 | June Way/60 | e8 | LA | raining | sole | 4/7/01 7:00 AM | 20 | <54 | 52 | 8.33 |
| f-25-1 | Culver/405 | f | OC | 1-72 hrs | mixed | 1/25/01 10:00 AM | 40 | 179 | 1,070 | 8.70 |

Table A-1 continued

| Sample No | Location | | | Gross characteristics | | | | | | |
|---------------|------------------|-----------|----|-----------------------|-----------------|------------------|-------------|-----------------|------------|----------|
| | | | | County | Since Rain (hr) | Type | Date & Time | Sample Vol. (L) | Hard ness | EC, (μS) |
| f-22-2 | Culver/405 | f | OC | 1-72 hrs | mixed | 2/22/01 12:30 PM | 40 | 179 | 1,070 | 10.26 |
| f-1-3 | Culver/405 | f | OC | 1-72 hrs | mixed | 3/1/01 1:00 PM | 40 | 179 | 1,258 | 9.38 |
| f-27-3 | Culver/405 | f | OC | >72 hrs | mixed | 3/27/01 1:30 PM | | 179 | 1,373 | 10.44 |
| f-23-4 | Culver/405 | f | OC | 1-72 hrs | mixed | 4/23/01 10:00 AM | 18 | 179 | 1,245 | 9.28 |
| f-7-5 | Culver/405 | f | OC | >72 hrs | mixed | 5/7/01 10:00 AM | 20 | 179 | 1,510 | 8.49 |
| g-5-2 | Las Posas/34 | g | VC | >72 hrs | mixed | 2/5/01 8:00 AM | 40 | 450 | 2,360 | 8.16 |
| g-12-2 | Las Posas/34 | g | VC | raining | mixed | 2/12/01 7:00 AM | 40 | 90 | 430 | 7.73 |
| g-19-3 | Las Posas/34 | g | VC | >72 hrs | mixed | 3/19/01 7:30 AM | 40 | 894 | 3,525 | 7.98 |
| g-21-5 | Las Posas/34 | g | VC | >72 hrs | mixed | 5/21/01 11:30 AM | 20 | 450 | 2,275 | 8.30 |
| g-1-6 | Las Posas/34 | g | VC | >72 hrs | mixed | 6/1/01 11:00 AM | | 596 | 2,760 | 6.23 |
| h1-24-2 | Filmore/210 | h1 | LA | raining | sole | 2/24/01 10:33 AM | 20 | <54 | 148 | 7.77 |
| h1-4-3 | Filmore/210 | h1 | LA | raining | sole | 3/4/01 5:00 PM | 20 | <54 | 182 | 7.34 |
| h1-7-4 | Filmore/210 | h1 | LA | raining | sole | 4/7/01 3:00 AM | | <54 | 134 | 7.18 |
| h1-20-4 | Filmore/210 | h1 | LA | raining | sole | 4/20/01 11:47 PM | 18 | <54 | | 7.57 |
| h2-24-2 | Orcas/210 | h2 | LA | raining | sole | 2/24/01 12:30 PM | 20 | <54 | 120 | 7.50 |
| h2-4-3 | Orcas/210 | h2 | LA | raining | sole | 3/4/01 6:00 PM | 20 | <54 | 102 | 7.63 |
| h2-7-4 | Orcas/210 | h2 | LA | raining | sole | 4/7/01 4:15 AM | | <54 | 263 | |
| h2-20-4 | Orcas/210 | h2 | LA | raining | sole | 4/20/01 11:25 PM | 20 | <54 | 48 | 7.47 |
| h3-24-2 | Altadena/210 | h3 | LA | raining | sole | 2/24/01 12:15 PM | 20 | <54 | 23 | 7.81 |
| h3-4-3 | Altadena/210 | h3 | LA | raining | sole | 3/4/01 7:30 PM | 20 | <54 | 122 | 7.34 |

Table A-1 continued

| Sample No | Location | | | Gross characteristics | | | | | | |
|-----------|--------------|----|--------|-----------------------|-------|------------------|-----------------|-----------|----------|-------|
| | | | County | Since Rain (hr) | Type | Date & Time | Sample Vol. (L) | Hard ness | EC, (µS) | pH |
| h3-7-4 | Altadena/210 | h3 | LA | raining | sole | 4/7/01 5:30 AM | 20 | <54 | 16 | 6.93 |
| h3-20-4 | Altadena/210 | h3 | LA | raining | sole | 4/20/01 10:15 PM | 20 | <54 | 30 | 7.52 |
| i-22-2 | Riverside | l | RC | 1-72 hrs | mixed | 2/22/01 8:00 AM | 20 | 90 | 520 | 10.03 |
| i-27-3 | Riverside | l | RC | >72 hrs | mixed | 3/27/01 9:30 AM | 40 | 90 | 548 | 9.54 |
| k-1-3 | Spruce/91 | k | RC | 1-72 hrs | mixed | 3/1/01 8:30 AM | 40 | 90 | 508 | 8.40 |
| k1-13-3 | Spruce/91 | k | RC | >72 hrs | mixed | 3/13/01 9:30 AM | 40 | 90 | 712 | 10.46 |
| k2-13-3 | Spruce/91 | k | RC | >72 hrs | mixed | 3/13/01 10:05 AM | 40 | 90 | 548 | 10.17 |
| k3-13-3 | Spruce/91 | k | RC | >72 hrs | mixed | 3/13/01 10:50 AM | 40 | 90 | 568 | 10.23 |
| k4-13-3 | Spruce/91 | k | RC | >72 hrs | mixed | 3/13/01 11:18 AM | 40 | <54 | 540 | 10.27 |
| k-27-3 | Spruce/91 | k | RC | >72 hrs | mixed | 3/27/01 9:00 AM | | 90 | 624 | 10.35 |
| k1-16-4 | Spruce/91 | k | RC | >72 hrs | mixed | 4/16/01 9:00 AM | 20 | 90 | 644 | 10.50 |
| k2-16-4 | Spruce/91 | k | RC | >72 hrs | mixed | 4/16/01 9:10 AM | 20 | 90 | 642 | 10.72 |
| k3-16-4 | Spruce/91 | k | RC | >72 hrs | mixed | 4/16/01 9:20 AM | 20 | 90 | 666 | 10.69 |
| k4-16-4 | Spruce/91 | k | RC | >72 hrs | mixed | 4/16/01 9:25 AM | 20 | 90 | 702 | 10.60 |
| k-7-5 | Spruce/91 | k | RC | >72 hrs | mixed | 5/7/01 1:00 PM | 20 | 269 | 1,115 | 8.10 |
| l-1-3 | Chavez/101 | l | LA | 1-72 hrs | mixed | 3/1/01 10:00 AM | 40 | 450 | 2,965 | 7.29 |
| l-27-3 | Chavez/101 | l | LA | >72 hrs | mixed | 3/27/01 11:00 AM | 40 | 450 | 2,950 | 7.37 |
| l-14-5 | Chavez/101 | l | LA | 1-72 hrs | mixed | 5/14/01 10:00 AM | 20 | 330 | 3,145 | 8.15 |

Table A-1 continued

| Sample No | Location | County | | Gross characteristics | | | | | | |
|----------------|--------------|--------|----|-----------------------|-------|------------------|-----------------|----------|----------|-------|
| | | | | Since Rain (hr) | Type | Date & Time | Sample Vol. (L) | Hardness | EC, (µS) | pH |
| sd1-8-3 | Montiel/15 | sd 1 | SD | 1-72 hrs | mixed | 3/8/01 9:30 AM | 40 | 90 | 736 | 9.75 |
| sd1-22-3n | Montiel/15 | sd 1 | SD | >72 hrs | mixed | 3/22/01 10:00 AM | 40 | 269 | 1,310 | 9.30 |
| sd1-22-3s | Montiel/15 | sd 1 | SD | >72 hrs | mixed | 3/22/01 10:00 AM | 40 | 269 | 1,340 | 9.20 |
| sd1-5-4 | Montiel/15 | sd 1 | SD | 1-72 hrs | mixed | 4/5/01 10:00 AM | 20 | 90 | 618 | 8.36 |
| sd1-10-4 | Montiel/15 | sd 1 | SD | raining | mixed | 4/10/01 6:00 AM | 20 | <54 | 294 | 7.88 |
| sd1-30-4 | Montiel/15 | sd 1 | SD | >72 hrs | mixed | 4/30/01 9:30 AM | 20 | 269 | 1,365 | 9.27 |
| sd1-30-5 | Montiel/15 | sd 1 | SD | >72 hrs | mixed | 5/30/01 9:00 AM | 20 | 179 | 2,645 | 8.23 |
| sd2-8-3 | El Camino/78 | sd 2 | SD | 1-72 hrs | mixed | 3/8/01 11:00 AM | 40 | 358 | 1,560 | 7.76 |
| sd2-22-3 | El Camino/78 | sd 2 | SD | >72 hrs | mixed | 3/22/01 10:30 AM | 40 | 541 | 2,840 | 7.80 |
| sd2-5-4 | El Camino/78 | sd 2 | SD | 1-72 hrs | mixed | 4/5/01 10:30 AM | 40 | 715 | 2,393 | 7.83 |
| sd2-10-4 | El Camino/78 | sd 2 | SD | raining | mixed | 4/10/01 6:30 AM | 20 | 269 | 1,248 | 7.77 |
| sd2-30-4 | El Camino/78 | sd 2 | SD | >72 hrs | mixed | 4/30/01 10:00 AM | 20 | 894 | 2,468 | 8.07 |
| sd2-30-5 | El Camino/78 | sd 2 | SD | >72 hrs | mixed | 5/30/01 10:00 AM | 20 | 450 | 2,535 | 7.87 |
| sd3-8-3 | Leucadia/5 | sd 3 | SD | 1-72 hrs | mixed | 3/8/01 11:30 AM | 40 | 179 | 1,490 | 10.45 |
| sd3-22-3n | Leucadia/5 | sd 3 | SD | >72 hrs | mixed | 3/22/01 11:30 AM | 40 | 269 | 1,550 | 10.50 |
| sd3-22-3s | Leucadia/5 | sd 3 | SD | >72 hrs | mixed | 3/22/01 11:30 AM | 40 | 269 | 1,643 | 10.30 |
| sd3-5-4 | Leucadia/5 | sd 3 | SD | 1-72 hrs | mixed | 4/5/01 11:00 AM | | | | |
| sd3-10-4 | Leucadia/5 | sd 3 | SD | raining | mixed | 4/10/01 7:15 AM | 20 | 90 | 594 | 7.71 |

Table A-1 continued

| Sample No | Location | County | | Gross characteristics | | | | | | |
|-----------|-------------|--------|----|-----------------------|-------|------------------|-----------------|----------|----------|-------|
| | | | | Since Rain (hr) | Type | Date & Time | Sample Vol. (L) | Hardness | EC, (µS) | pH |
| sd3-30-4 | Leucadia/5 | sd 3 | SD | >72 hrs | mixed | 4/30/01 10:30 AM | 10 | 269 | 1,585 | 9.92 |
| sd3-30-5 | Leucadia/5 | sd 3 | SD | >72 hrs | mixed | 5/30/01 10:30 AM | 15 | 269 | 1,750 | 10.21 |
| sd4-8-3 | Encinitas/5 | sd 4 | SD | 1-72 hrs | mixed | 3/8/01 12:00 PM | 40 | 925 | 4,710 | 7.75 |
| sd4-22-3 | Encinitas/5 | sd 4 | SD | >72 hrs | mixed | 3/22/01 12:15 PM | 40 | 894 | 5,390 | 8.48 |
| sd4-5-4 | Encinitas/5 | sd 4 | SD | 1-72 hrs | mixed | 4/5/01 11:30 AM | 40 | 1,073 | 5,265 | 7.87 |
| sd4-10-4 | Encinitas/5 | sd 4 | SD | raining | mixed | 4/10/01 7:30 AM | 20 | 179 | 1,380 | 7.54 |
| sd4-30-4 | Encinitas/5 | sd 4 | SD | >72 hrs | mixed | 4/30/01 11:00 AM | 20 | 894 | 4,575 | 8.36 |
| sd4-30-5 | Encinitas/5 | sd 4 | SD | >72 hrs | mixed | 5/30/01 10:45 AM | 20 | 894 | 5,395 | 7.86 |

Table A-2
Highway Drain Workbook Data - Viruses

| | | Indicators, MPN/100 mL | | | | | | Viruses | | | | | | | | | |
|--------|------------------|---------------------------|---------|----------------|-------|---------------|--------|---------------|----------------------|--------------|---------------------------|--------------------------|----------------------------|--------|--------|--------|--------|
| | | Total Coliform | | Fecal Coliform | | Entero coccus | | Indicator Lab | Virus Field Recovery | | | | | MPD | | | |
| | | | | | | | | | Marker organism | Marker titer | Minimum positive dilution | Conce ntrate Volume (µL) | Maximum inhibited dilution | A. ADE | B. ENV | C. HAV | E. ROT |
| | | | | | | | | | | MPD | CV | | | | | | |
| a-23-1 | Calabasas/101 | = | 2,000 | < | 2,000 | < | 2,000 | Siliker | D. BOV ENT | 316,000 | 4 | | | | | | |
| a-5-2 | Calabasas/101 | = | 13,000 | < | 2,000 | = | 23,000 | | D. BOV ENT | 316,000 | 4 | | | | | | |
| a-12-2 | Calabasas/101 | = | 8,000 | = | 8,000 | = | 8,000 | | D. BOV ENT | 316,000 | 2 | 170 | 0 | | | | |
| a-19-3 | Calabasas/101 | < | 2,000 | < | 2,000 | = | 2,300 | | D. BOV ENT | 100,000 | 4 | 50 | | | | | |
| a-21-5 | Calabasas/101 | = | 50,000 | = | 1,100 | = | 1,300 | | D. BOV ENT | 100,000 | 4 | 120 | 0 | | | | |
| a-1-6 | Calabasas/101 | | | | | | | | D. BOV ENT | 100,000 | 4 | 80 | 0 | | | | |
| b-23-1 | Malibu Creek/101 | < | 2,000 | < | 2,000 | < | 2,000 | | D. BOV ENT | 316,000 | 2 | | | | | | |
| b-5-2 | Malibu Creek/101 | < | 2,000 | < | 2,000 | = | 23,000 | | D. BOV ENT | 316,000 | 4 | | | | | | |
| b-12-2 | Malibu Creek/101 | = | 8,000 | = | 8,000 | = | 8,000 | | D. BOV ENT | 316,000 | | 70 | 0 | | | | |
| b-19-3 | Malibu Creek/101 | < | 2,000 | < | 2,000 | < | 2,000 | | D. BOV ENT | 100,000 | 4 | 100 | | | | | |
| b-21-5 | Malibu Creek/101 | = | 5,000 | = | 26 | = | 800 | | D. BOV ENT | 100,000 | 4 | 120 | 0 | | | | |
| b-1-6 | Malibu Creek/101 | | | | | | | | D. BOV ENT | 100,000 | 4 | 90 | 0 | | | | |
| c-23-1 | Pico drain/10 | = | 50,000 | < | 2,000 | < | 2,000 | | D. BOV ENT | 316,000 | 4 | | | | | | |
| c-15-2 | Pico drain/10 | = | 170,000 | = | 2,000 | < | 2,000 | | D. BOV ENT | 316,000 | 4 | 90 | 0 | | | | |
| c-22-2 | Pico drain/10 | = | 170,000 | = | 4,000 | < | 2,000 | | D. BOV ENT | 316,000 | 4 | 90 | 0 | | | | |
| c-1-3 | Pico drain/10 | = | 50,000 | = | 7,000 | < | 2,000 | | D. BOV ENT | 316,000 | | 100 | 1 | | | | |

Table A-2 Continued

| | | Indicators, MPN/100 mL | | | | | Viruses | | | | | | | | | |
|----------|------------------|---------------------------|--------|----------------|-------|--------------|---------|---------------|----------------------|--------------|---------------------------|---------------------------|----------------------------|--------|--------|--------|
| | | Total Coliform | | Fecal Coliform | | Enterococcus | | Indicator Lab | Virus Field Recovery | | | | MPD | | | |
| | | | | | | | | | Marker organism | Marker titer | Minimum positive dilution | Concentration Volume (µL) | Maximum inhibited dilution | A. ADE | B. ENV | C. HAV |
| c-23-4 | Pico drain/10 | = | 50,000 | = | 2,300 | = | 1,700 | D. BOV ENT | 100,000 | 4 | 110 | 0 | | | | |
| c-7-5 | Pico drain/10 | | | | | | | | | | | | | | | |
| c-14-5 | Pico drain/10 | | | | | | | | | | | | | | | |
| d-23-1 | Lakewood/40 5 | = | 23,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 316,000 | | | 0 | | | | |
| d-15-2 | Lakewood/40 5 | = | 22,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 316,000 | | 90 | 0 | | | | |
| d-22-2 | Lakewood/40 5 | = | 50,000 | < | 2,000 | = | 4,000 | D. BOV ENT | 316,000 | | 100 | 0 | | | | |
| d-7-5 | Lakewood/40 5 | = | 50,000 | = | 8,000 | = | 70,000 | D. BOV ENT | 100,000 | 3 | 90 | 0 | | | | |
| e23-10-2 | Rincon/210 | | | | | < | 2,000 | D. BOV ENT | 316,000 | 4 | 90 | | | | | |
| e23-19-2 | Rincon/210 | | | | | < | 2,000 | D. BOV ENT | 316,000 | 4 | 90 | 1 | | | | |
| e23-24-2 | Rincon/210 | | | | | < | 2,000 | D. BOV ENT | 316,000 | 2 | 100 | | | | | |
| e23-7-4 | Rincon/210 | | | | | = | 200,000 | D. BOV ENT | 100,000 | 4 | 80 | 0 | | | | |
| e6-10-2 | Turnbull/60 | | | | | < | 2,000 | D. BOV ENT | 316,000 | 1 | 150 | 0 | | | | |
| e6-19-2 | Turnbull/60 | | | | | < | 2,000 | D. BOV ENT | 316,000 | 2 | 180 | 0 | | | | |
| e6-24-2 | Turnbull/60 | | | | | < | 2,000 | D. BOV ENT | 316,000 | 2 | 110 | 1 | | | | |
| e6-7-4 | Turnbull/60 | | | | | = | 200,000 | D. BOV ENT | 100,000 | 4 | 75 | 0 | | | | |
| e8-19-2 | June Way/60 | | | | | < | 2,000 | D. BOV ENT | 316,000 | 4 | 95 | 0 | | | | |
| e8-7-4 | June Way/60 | | | | | = | 200,000 | D. BOV ENT | 100,000 | 4 | 85 | 0 | | | | |
| f-25-1 | Culver/405 | = | 13,000 | = | 2,000 | < | 2,000 | D. BOV ENT | 316,000 | 4 | | | 1 | | | |
| f-22-2 | Culver/405 | < | 2,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 316,000 | | 90 | 0 | | | | |

Table A-2 Continued

| | | Indicators, MPN/100 mL | | | | | | Viruses | | | | | | | | |
|---------------|------------------|---------------------------|-----------|----------------|-------|--------------|---------|---------------|----------------------|--------------|---------------------------|---------------------------|----------------------------|--------|--------|--------|
| | | Total Coliform | | Fecal Coliform | | Enterococcus | | Indicator Lab | Virus Field Recovery | | | | MPD | | | |
| | | | | | | | | | Marker organism | Marker titer | Minimum positive dilution | Concentration Volume (µL) | Maximum inhibited dilution | A. ADE | B. ENV | C. HAV |
| f-1-3 | Culver/405 | = | 80,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 316,000 | | 120 | 1 | | | | |
| f-27-3 | Culver/405 | < | 2,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 100,000 | | #N/A | | | | | |
| f-23-4 | Culver/405 | = | 130,000 | = | 1,300 | = | 2,300 | D. BOV ENT | 100,000 | 4 | 110 | 0 | | | | |
| f-7-5 | Culver/405 | = | 30,000 | = | 800 | = | 13,000 | D. BOV ENT | 100,000 | | 80 | 0 | | | | |
| g-5-2 | Las Posas/34 | = | 70,000 | < | 2,000 | = | 240,000 | D. BOV ENT | 316,000 | 3 | | 1 | | | | |
| g-12-2 | Las Posas/34 | = | 30,000 | < | 2,000 | = | 5,000 | D. BOV ENT | 316,000 | 4 | 100 | 2 | | | | |
| g-19-3 | Las Posas/34 | = | 50,000 | < | 2,000 | = | 4,000 | D. BOV ENT | 100,000 | 3 | 80 | | | | | |
| g-21-5 | Las Posas/34 | = | 1,600,000 | = | 500 | = | 7,000 | D. BOV ENT | 100,000 | 4 | 125 | 0 | | | | |
| g-1-6 | Las Posas/34 | | | | | | | D. BOV ENT | 100,000 | | | | | | | |
| h1-24-2 | Filmore/210 | = | 7,000 | = | 900 | | | D. BOV ENT | 316,000 | 3 | 100 | 1 | | | | |
| h1-4-3 | Filmore/210 | = | 8,000 | = | 8,000 | | | D. BOV ENT | 316,000 | | 105 | 0 | | | | |
| h1-7-4 | Filmore/210 | | | | | | | | #N/A | #N/A | 70 | | | | | |
| h1-20-4 | Filmore/210 | = | 24,000 | = | 900 | = | 260 | D. BOV ENT | 100,000 | 4 | 100 | 0 | | | | |
| h2-24-2 | Orcas/210 | = | 7,000 | = | 2,300 | | | D. BOV ENT | 316,000 | 3 | 110 | 0 | | | | |
| h2-4-3 | Orcas/210 | = | 50,000 | = | 2,300 | | | D. BOV ENT | 316,000 | | 110 | 0 | | | | |
| h2-7-4 | Orcas/210 | | | | | | | | | | | | | | | |
| h2-20-4 | Orcas/210 | = | 800 | = | 500 | = | 548 | D. BOV ENT | 100,000 | 4 | 110 | 0 | | | | |
| h3-24-2 | Altadena/210 | = | 17,000 | = | 2,600 | | | D. BOV ENT | 316,000 | 3 | 120 | 0 | | | | |
| h3-4-3 | Altadena/210 | | | | | | | D. BOV ENT | 316,000 | | 110 | 0 | | | | |

Table A-2 continued

| | | Indicators, MPN/100 mL | | | | | | Viruses | | | | | | | | |
|---------|--------------|---------------------------|---------|----------------|-------|--------------|--------|---------------|----------------------|--------------|---------------------------|---------------------------|----------------------------|--------|--------|--------|
| | | Total Coliform | | Fecal Coliform | | Enterococcus | | Indicator Lab | Virus Field Recovery | | | | MPD | | | |
| | | | | | | | | | Marker organism | Marker titer | Minimum positive dilution | Concentration Volume (µL) | Maximum inhibited dilution | A. ADE | B. ENV | C. HAV |
| h3-7-4 | Altadena/210 | | | | | | | D. BOV ENT | 100,000 | 4 | 75 | 0 | | | | |
| h3-20-4 | Altadena/210 | = | 240 | = | 13 | = | 461 | D. BOV ENT | 100,000 | 4 | 60 | 0 | | | | |
| i-22-2 | Riverside | < | 2,000 | < | 2,000 | = | 2,000 | D. BOV ENT | 316,000 | 3 | 100 | 0 | | | | |
| i-27-3 | Riverside | = | 80,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 100,000 | 3 | 90 | 0 | | | | |
| k-1-3 | Spruce/91 | = | 23,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 316,000 | | 80 | 0 | | | | |
| k1-13-3 | Spruce/91 | < | 2,000 | | | = | 8,000 | D. BOV ENT | 100,000 | | 150 | 0 | | | | |
| k2-13-3 | Spruce/91 | = | 17,000 | | | = | 11,000 | D. BOV ENT | 100,000 | 2 | 150 | 0 | | | | |
| k3-13-3 | Spruce/91 | < | 2,000 | | | = | 2,300 | D. BOV ENT | 100,000 | 2 | 150 | 0 | | | | |
| k4-13-3 | Spruce/91 | < | 2,000 | | | = | 2,300 | D. BOV ENT | 100,000 | 2 | 140 | 0 | | | | |
| k-27-3 | Spruce/91 | < | 2,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 100,000 | | #N/A | | | | | |
| k1-16-4 | Spruce/91 | = | 2,300 | = | 17 | = | 500 | D. BOV ENT | 100,000 | 4 | 100 | | | | | |
| k2-16-4 | Spruce/91 | = | 500 | = | 13 | = | 500 | D. BOV ENT | 100,000 | 3 | 75 | | | | | |
| k3-16-4 | Spruce/91 | = | 300 | = | 2 | = | 300 | D. BOV ENT | 100,000 | 4 | 65 | | | | | |
| k4-16-4 | Spruce/91 | = | 1,700 | = | 50 | = | 300 | D. BOV ENT | 100,000 | 4 | 90 | | | | | |
| k-7-5 | Spruce/91 | = | 800 | = | 110 | = | 110 | D. BOV ENT | 100,000 | 4 | 125 | 1 | | | | |
| l-1-3 | Chavez/101 | = | 300,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 316,000 | | 100 | 1 | | | | |
| l-27-3 | Chavez/101 | < | 2,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 100,000 | | 90 | | | | | |
| l-14-5 | Chavez/101 | = | 11,000 | = | 7,000 | = | 3,000 | D. BOV ENT | 100,000 | 4 | 115 | 0 | | | | |

Table A-2 continued

| | | Indicators, MPN/100 mL | | | | | | Viruses | | | | | | | | |
|----------------|--------------|---------------------------|---------|----------------|-------|--------------|--------|---------------|----------------------|--------------|---------------------------|---------------------------|----------------------------|--------|--------|--------|
| | | Total Coliform | | Fecal Coliform | | Enterococcus | | Indicator Lab | Virus Field Recovery | | | | MPD | | | |
| | | | | | | | | | Marker organism | Marker titer | Minimum positive dilution | Concentration Volume (µL) | Maximum inhibited dilution | A. ADE | B. ENV | C. HAV |
| sd1-8-3 | Montiel/15 | = | 17,000 | = | 4,000 | < | 2,000 | D. BOV ENT | 316,000 | 1 | 65 | | | | | |
| sd1-22-3n | Montiel/15 | | 4,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 100,000 | | 60 | 0 | | | | |
| sd1-22-3s | Montiel/15 | | 4,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 100,000 | | 80 | | | | | |
| sd1-5-4 | Montiel/15 | > | 1,600 | = | 500 | = | 50 | D. BOV ENT | 100,000 | 2 | 40 | | | | | |
| sd1-10-4 | Montiel/15 | = | 170,000 | = | 230 | = | 5,000 | D. BOV ENT | 100,000 | 4 | 100 | 0 | | | | |
| sd1-30-4 | Montiel/15 | = | 11,000 | = | 230 | = | 900 | D. BOV ENT | 100,000 | 4 | 70 | | | | | |
| sd1-30-5 | Montiel/15 | | | | | | | D. BOV ENT | 100,000 | 4 | 100 | 0 | | | | |
| sd2-8-3 | El Camino/78 | = | 4,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 316,000 | 4 | 70 | 0 | | | | |
| sd2-22-3 | El Camino/78 | < | 2,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 100,000 | 4 | 80 | 0 | | | | |
| sd2-5-4 | El Camino/78 | > | 1,600 | = | 50 | = | 80 | D. BOV ENT | 100,000 | 4 | 55 | | | | | |
| sd2-10-4 | El Camino/78 | = | 17,000 | = | 5,000 | = | 23,000 | D. BOV ENT | 100,000 | 4 | 55 | 0 | | | | |
| sd2-30-4 | El Camino/78 | = | 3,000 | = | 230 | = | 300 | D. BOV ENT | 100,000 | 4 | 130 | | | | | |
| sd2-30-5 | El Camino/78 | | | | | | | D. BOV ENT | 100,000 | 4 | 70 | 0 | | | | |
| sd3-8-3 | Leucadia/5 | < | 2,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 316,000 | | 100 | | | | | |
| sd3-22-3n | Leucadia/5 | < | 2,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 100,000 | | 60 | | | | | |
| sd3-22-3s | Leucadia/5 | < | 2,000 | < | 2,000 | < | 2,000 | D. BOV ENT | 100,000 | | 70 | | | | | |
| sd3-5-4 | Leucadia/5 | | | | | | | | | | | | | | | |
| sd3-10-4 | Leucadia/5 | = | 240,000 | = | 7,000 | = | 8,000 | D. BOV ENT | 100,000 | 4 | 70 | 0 | | | | |

Table A-2 continue

| | | Indicators, MPN/100 mL | | | | | | Viruses | | | | | | | | |
|----------|-------------|---------------------------|--------|----------------|-------|--------------|-------|---------------|----------------------|--------------|---------------------------|---------------------------|----------------------------|--------|--------|--------|
| | | Total Coliform | | Fecal Coliform | | Enterococcus | | Indicator Lab | Virus Field Recovery | | | | MPD | | | |
| | | | | | | | | | Marker organism | Marker titer | Minimum positive dilution | Concentration Volume (µL) | Maximum inhibited dilution | A. ADE | B. ENV | C. HAV |
| sd3-30-4 | Leucadia/5 | = | 13,000 | = | 500 | = | 5,000 | | D. BOV ENT | 100,000 | 4 | 80 | | | | |
| sd3-30-5 | Leucadia/5 | | | | | | | | D. BOV ENT | 100,000 | 3 | 100 | 0 | | | |
| sd4-8-3 | Encinitas/5 | = | 2,000 | < | 2,000 | < | 2,000 | | D. BOV ENT | 316,000 | 4 | 150 | 0 | | | |
| sd4-22-3 | Encinitas/5 | < | 2,000 | < | 2,000 | < | 2,000 | | D. BOV ENT | 100,000 | 4 | 80 | | | | |
| sd4-5-4 | Encinitas/5 | > | 1,600 | = | 240 | = | 130 | | D. BOV ENT | 100,000 | 4 | 55 | | | | |
| sd4-10-4 | Encinitas/5 | = | 90,000 | = | 8,000 | = | 3,000 | | D. BOV ENT | 100,000 | 4 | 100 | 0 | | | |
| sd4-30-4 | Encinitas/5 | = | 7,000 | = | 300 | = | 5,000 | | D. BOV ENT | 100,000 | 4 | 100 | | | | |
| sd4-30-5 | Encinitas/5 | | | | | | | | D. BOV ENT | 100,000 | 4 | 85 | | | | |

Table A-3
Highway Data Workbook - Bacteria and Protozoa

| Sample No | Location | Bacteria & Protozoa | | | | | | | | |
|-----------|------------------|--------------------------------|----------------------------------|----------------------------|---------|---------|---------|----------|---------|---------|
| | | Concentrat e Volume (µL) | Maximum inhibited dilution | Maximum positive dilutions | | | | | | |
| | | | | F. EHEC | G. ETEC | H. Shig | I. Salm | J. Staph | K. Giar | L. Cryp |
| a-23-1 | Calabasas/101 | | | | | | | | | |
| a-5-2 | Calabasas/101 | | | | | | | | | |
| a-12-2 | Calabasas/101 | | 0 | | | | | | | |
| a-19-3 | Calabasas/101 | 140 | | | | | | | | |
| a-21-5 | Calabasas/101 | 140 | 0 | | | | | | | |
| a-1-6 | Calabasas/101 | 115 | 0 | | | | | | | |
| b-23-1 | Malibu Creek/101 | | | | | | 0 | | | |
| b-5-2 | Malibu Creek/101 | | | | | | 0 | | | |
| b-12-2 | Malibu Creek/101 | | 1 | | | | | | | |
| b-19-3 | Malibu Creek/101 | 125 | 0 | | | | | | | |
| b-21-5 | Malibu Creek/101 | 170 | | | 0 | | 0 | | | |
| b-1-6 | Malibu Creek/101 | 100 | 0 | | | | 1 | | | |
| c-23-1 | Pico drain/10 | | | | | | | | | |
| c-15-2 | Pico drain/10 | | 0 | | | | | | | |
| c-22-2 | Pico drain/10 | 35 | | | | | | | | |
| c-1-3 | Pico drain/10 | 60 | 0 | | | | | | | |
| c-23-4 | Pico drain/10 | 115 | 0 | | | | | | | |
| c-14-5 | Pico drain/10 | | | | | | | | | |
| d-23-1 | Lakewood/405 | | | | | | | | | |
| d-15-2 | Lakewood/405 | | | | | | | | | |
| d-22-2 | Lakewood/405 | 35 | | | | | | | | |
| d-7-5 | Lakewood/405 | 115 | 0 | | | | | | | |
| e23-10-2 | Rincon/210 | | | | | | | | | |
| e23-19-2 | Rincon/210 | | | | | | | | | |
| c-7-5 | Pico drain/10 | | | | | | | | | |
| e23-24-2 | Rincon/210 | 45 | | | | | | | | |
| e23-7-4 | Rincon/210 | 95 | 0 | | | | 1 | | | |

Table A-3 continued

| Sample No | Location | Bacteria & Protozoa | | | | | | | | |
|---------------|------------------|--------------------------------------|----------------------------------|----------------------------|---------|--------|---------|----------|---------|---------|
| | | Concentrat e Volume (μ L) | Maximum inhibited dilution | Maximum positive dilutions | | | | | | |
| | | | | F. EHEC | G. ETEC | H.Shig | I. Salm | J. Staph | K. Giar | L. Cryp |
| e6-10-2 | Turnbull/60 | | 1 | | | | | | | |
| e6-19-2 | Turnbull/60 | | 0 | | | | | | | |
| e6-24-2 | Turnbull/60 | 40 | | | | | | | | |
| e6-7-4 | Turnbull/60 | 110 | 0 | | | | | | | |
| e8-19-2 | June Way/60 | | | | | | | | | |
| e8-7-4 | June Way/60 | 80 | 0 | | | | 1 | | | |
| f-25-1 | Culver/405 | | | | | | | | | |
| f-22-2 | Culver/405 | 85 | | | | | | | | |
| f-1-3 | Culver/405 | 70 | | | | | | | | |
| f-27-3 | Culver/405 | 90 | | | | | | | | |
| f-23-4 | Culver/405 | 60 | 0 | | | | | | | |
| f-7-5 | Culver/405 | 65 | | | | | | | | |
| g-5-2 | Las Posas/34 | | 0 | | | | | | | |
| g-12-2 | Las Posas/34 | | 1 | | | | | | | |
| g-19-3 | Las Posas/34 | 130 | 0 | | | | | | | |
| g-21-5 | Las Posas/34 | 70 | 0 | | | | | | | |
| g-1-6 | Las Posas/34 | | | | | | | | | |
| h1-24-2 | Filmore/210 | 50 | | | | | | | | |
| h1-4-3 | Filmore/210 | 140 | 0 | | | | | | | |
| h1-7-4 | Filmore/210 | 90 | | | 1 | | | | | |
| h1-20-4 | Filmore/210 | 80 | 0 | | | | | | | |
| h2-24-2 | Orcas/210 | 20 | | | | | | | | |
| h2-4-3 | Orcas/210 | 60 | 0 | | | | | | | |
| h2-7-4 | Orcas/210 | | | | | | | | | |
| h2-20-4 | Orcas/210 | 110 | 0 | | | | | | | |
| h3-24-2 | Altadena/210 | 40 | | | | | | | | |
| h3-4-3 | Altadena/210 | 35 | 0 | | | | | | | |

Table A-3 continued

| Sample No | Location | Bacteria & Protozoa | | | | | | | | |
|-----------|--------------|--------------------------------------|----------------------------------|----------------------------|---------|---------|---------|----------|---------|---------|
| | | Concentrat e Volume (μ L) | Maximum inhibited dilution | Maximum positive dilutions | | | | | | |
| | | | | F. EHEC | G. ETEC | H. Shig | I. Salm | J. Staph | K. Giar | L. Cryp |
| h3-7-4 | Altadena/210 | 95 | 0 | | | | | | | |
| h3-20-4 | Altadena/210 | 110 | 1 | | | | | | | |
| i-22-2 | Riverside | 90 | | | | | | | | |
| i-27-3 | Riverside | 100 | | | | | | | | |
| k-1-3 | Spruce/91 | 100 | | | | | | | | |
| k1-13-3 | Spruce/91 | 100 | | | | | | | | |
| k2-13-3 | Spruce/91 | 80 | | | | | | | | |
| k3-13-3 | Spruce/91 | 100 | | | | | | | | |
| k4-13-3 | Spruce/91 | 90 | | | | | | | | |
| k-27-3 | Spruce/91 | 90 | | | | | | | | |
| k1-16-4 | Spruce/91 | 133 | 0 | | | | | | | |
| k2-16-4 | Spruce/91 | 140 | | | | | | | | |
| k3-16-4 | Spruce/91 | 125 | | | | | | | | |
| k4-16-4 | Spruce/91 | 125 | | | | | | | | |
| k-7-5 | Spruce/91 | 143 | 0 | | | | | | | |
| l-1-3 | Chavez/101 | 110 | 1 | | | | | | | |
| l-27-3 | Chavez/101 | 90 | 0 | | | | | | | |
| l-14-5 | Chavez/101 | 135 | 1 | | | | | | | |
| sd1-8-3 | Montiel/15 | 90 | | | | | | | | |
| sd1-22-3n | Montiel/15 | 140 | | | | | | | | |
| sd1-22-3s | Montiel/15 | 140 | | | | | | | | |
| sd1-5-4 | Montiel/15 | 100 | 0 | | | | | | | |
| sd1-10-4 | Montiel/15 | 115 | | | 1 | | | | | |
| sd1-30-4 | Montiel/15 | 165 | | | | | | | | |
| sd1-30-5 | Montiel/15 | 75 | 1 | | | | | | | |
| sd2-8-3 | El Camino/78 | 90 | 0 | | | | | | | |
| sd2-22-3 | El Camino/78 | 125 | 0 | | | | | | | |

Table A-3 continued

| Sample No | Location | Bacteria & Protozoa | | | | | | | | |
|----------------|--------------|--------------------------------|----------------------------------|----------------------------|---------|---------|---------|----------|---------|---------|
| | | Concentrat e Volume (µL) | Maximum inhibited dilution | Maximum positive dilutions | | | | | | |
| | | | | F. EHEC | G. ETEC | H. Shig | I. Salm | J. Staph | K. Giar | L. Cryp |
| sd2-5-4 | El Camino/78 | 125 | 0 | | | | | | | |
| sd2-10-4 | El Camino/78 | 135 | 0 | | 1 | | | | | |
| sd2-30-4 | El Camino/78 | 150 | 1 | | | | | | | |
| sd2-30-5 | El Camino/78 | 60 | 0 | | | | | | | |
| sd3-8-3 | Leucadia/5 | 75 | 0 | | | | | | | |
| sd3-22-3n | Leucadia/5 | 135 | 0 | | | | | | | |
| sd3-22-3s | Leucadia/5 | 140 | | | | | | | | |
| sd3-5-4 | Leucadia/5 | | | | | | | | | |
| sd3-10-4 | Leucadia/5 | 150 | 1 | | | | | | | |
| sd3-30-4 | Leucadia/5 | 180 | 0 | | | | | | | |
| sd3-30-5 | Leucadia/5 | 60 | 0 | | | | 1 | | | |
| sd4-8-3 | Encinitas/5 | 75 | 0 | | | | | | | |
| sd4-22-3 | Encinitas/5 | 130 | | | | | | | | |
| sd4-5-4 | Encinitas/5 | 100 | | | | | | | | |
| sd4-10-4 | Encinitas/5 | 80 | 0 | | | | | | | |
| sd4-30-4 | Encinitas/5 | 70 | 0 | | | | | | | |
| sd4-30-5 | Encinitas/5 | 75 | 0 | | | | 1 | | | |

Table A-4
UC Riverside Sample Details

| <u>Sample date</u> | <u>sample #</u> | <u>location</u> | <u>time</u> | <u>weather</u> | <u>appearance</u> | <u>volume (L)</u> | <u>pH</u> | <u>EC (uS)</u> | <u>hardness</u> | <u>pellet mass(g)</u> |
|--------------------|-----------------|------------------|-------------|---|----------------------------|-------------------|-----------|----------------|-----------------|-----------------------|
| 1/23/2001 | a-23-1 | calabasas | 6am | clear,dry | clear,colorless | 40 | 8.3 | 3310 | 900 | |
| 1/23/2001 | b-23-1 | malibu cr. | 7am | clear,dry | clear,colorless | 40 | 8 | 2150 | 715 | |
| 1/23/2001 | c-23-1 | pico drain | 10am | clear,dry | clear,colorless | 40 | 7.5 | 1160 | 269 | |
| 1/23/2001 | d-23-1 | lakewood\405 | 11am | clear,dry | brown, clear | 40 | 7.2 | 10620 | 900 | |
| 1/25/2001 | f-25-1 | culver/405 | 10am | rain last 24hrs | clear, slight tint | 40 | 8.7 | 1070 | 179 | 0.2 |
| 2/5/2001 | b-5-2 | malibu cr. | 6:30am | clear,dry | clear,colorless | 40 | 7.82 | 2190 | 450 | 0.4 |
| 2/5/2001 | g-5-2 | las posas | 8:00am | clear,dry | brown,muddy | 40 | 8.16 | 2360 | 450 | 0.72 |
| 2/5/2001 | a-5-2 | calabasas | 10:00am | clear,dry | clear, colorless | 40 | 8.08 | 3310 | 894 | 0.68 |
| 2/10/2001 | e23-10-2 | bmp Rincon | 6:15am | raining | dirty | 20 | 8.06 | 98 | 358 | 0.84 |
| 2/10/2001 | e6-10-2 | bmp Turnbull | 7:45am | raining | very dirty | 20 | 8.23 | 300 | 448 | 2.34 |
| 2/12/2001 | g-12-2 | las posas | 7:00am | heavy raining | lot of mud | 40 | 7.73 | 430 | 90 | 2.14 |
| 2/12/2001 | b-12-2 | malibu creek | 8:00am | heavy raining | brown, muddy | 40 | 8.05 | 1700 | <54 | 2.16 |
| 2/12/2001 | a-12-2 | calabasas | 9:00am | heavy raining | slightly yellow and turbid | 40 | 7.93 | 990 | <54 | 0.88 |
| 2/15/2001 | i-15-2 | riverside | 9:00am | rain 72 h ago | slightly yellow and turbid | 40 | 9.03 | 500 | 90 | 0.42 |
| 2/15/2001 | c-15-2 | pico drain | 11:00am | rain 72 h ago | moderately muddy | 40 | 8.37 | 1055 | 358 | 1.36 |
| 2/15/2001 | d-15-2 | lakewood\405 | 11:30am | rain 72 h ago | brown, slightly turbid | 40 | 7.88 | 6550 | 538 | 0.59 |
| 2/15/2001 | f-15-2 | culver/405 | 13:00 | rain 72 h ago | slightly yellow and turbid | 40 | 8.97 | 1725 | 269 | 0.84 |
| 2/19/2001 | e6-19-2 | bmp Turnbull | 10:45am | raining | dirty | 20 | 8.2 | 255 | <54 | 2.54 |
| 2/19/2001 | e23-19-2 | bmp Rincon | 14:35 | raining | dirty | 20 | 7.85 | 123 | <54 | 1.16 |
| 2/19/2001 | e8-19-2 | bmp June Way | 15:35 | raining | dirty | 20 | 8.25 | 133 | <54 | 1.64 |
| 2/22/2001 | i-22-2 | riverside | 8:00am | rain 72 h ago | clear, slightly yellow | 20 | 10 | 520 | 90 | 0.52 |
| 2/22/2001 | c-22-2 | pico drain | 10:30am | rain 72 h ago | slightly turbid and yellow | 40 | 8.48 | 1270 | 358 | 0.37 |
| 2/22/2001 | d-22-2 | lakewood\405 | 11:30am | rain 72 h ago | moderately dirty | 40 | 7.28 | 12200 | 894 | 0.32 |
| 2/22/2001 | f-22-2 | culver/405 | 12:30pm | rain 72 h ago | slightly turbid and yellow | 40 | 10.3 | 1070 | 179 | 0.06 |
| 2/24/2001 | h1-24-2 | bmp filmore | 10:33am | heavy raining | dirty | 20 | 7.77 | 148 | <54 | 0.99 |
| 2/24/2001 | h2-24-2 | bmp orcas | 12:30pm | heavy raining | moderately dirty | 20 | 7.5 | 120 | <54 | 0.54 |
| 2/24/2001 | h3-24-2 | bmp altadena | 12:15pm | heavy raining | slightly dirty | 20 | 7.81 | 23 | <54 | 1.22 |
| 2/24/2001 | e6-24-2 | bmp Turnbull | 12:45pm | heavy raining | dirty | 20 | 8.22 | 134 | <54 | 1.48 |
| 2/24/2001 | e23-24-2 | bmp Rincon | 15:00 | heavy raining | moderate dirty | 20 | 7.81 | 84 | <54 | 0.76 |
| 3/1/2001 | site I | riverside | | 1st dry day after 5 days of rain | NA | NA | NA | NA | NA | NA |
| 3/1/2000 | k-1-3 | Spruce | 8:30am | 1st dry day after 5 days of rain | slightly yellow and turbid | 40 | 8.4 | 508 | 90 | 0.33 |

Table A-4 continued

| | | | | | | | | | |
|------------------------|--------------------|---------|---|------------------------------------|-----------|-----------|-----------|-----------|-----------|
| 3/1/2000 I-1-3 | Chavez | 10:00am | 1st dry day after 5 days of rain | slightly yellow and turbid | 40 | 7.29 | 2965 | 450 | 1.61 |
| 3/1/2000 c-1-3 | pico drain | 11:30am | 1st dry day after 5 days of rain | slightly turbid | 40 | 8.24 | 964 | 269 | 1.57 |
| 3/1/2001 site d | lakewood405 | | 1st dry day after 5 days of rain | NA | NA | NA | NA | NA | NA |
| 3/1/2000 f-1-3 | culver/405 | 13:00 | 1st dry day after 5 days of rain | slightly turbid, remarkably yellow | 40 | 9.38 | 1258 | 179 | 0.39 |
| 3/4/2001 h1-4-3 | bmp filmore | 17:00 | raining | dirty | 20 | 7.34 | 182 | <54 | 0.56 |
| 3/4/2001 h2-4-3 | bmp orcas | 18:00 | raining | dirty & debris | 20 | 7.63 | 102 | <54 | 0.9 |
| 3/4/2001 h3-4-3 | bmp altadena | 19:30 | raining | dirty | 20 | 7.34 | 122 | <54 | 3.21 |
| 3/8/2001 SD1-8-3 | Montiel | 9:30 | rain 2-5 days ago | slightly yellow and turbid | 40 | 9.75 | 736 | 90 | 0.14 |
| 3/8/2001 SD2-8-3 | El Camino | 11:00 | rain 2-5 days ago | slightly yellow and turbid | 40 | 7.76 | 1560 | 358 | 0.55 |
| 3/8/2001 SD3-8-3 | Leucadia | 11:30 | rain 2-5 days ago | slightly yellow and turbid | 40 | 10.5 | 1490 | 179 | 0.84 |
| 3/8/2001 SD4-8-3 | Encinitas | 12:00 | rain 2-5 days ago | slightly yellow and turbid | 40 | 7.75 | 4710 | 925 | 1.29 |
| 3/13/2001 K1-13-3 | Spruce | 9:30am | clear, dry | green, slightly turbid | 40 | 10.5 | 712 | 90 | 0.32 |
| 3/13/2001 K2-13-3 | Spruce | 10:05am | clear, dry | green, slightly turbid | 40 | 10.2 | 548 | 90 | 0.71 |
| 3/13/2001 K3-13-3 | Spruce | 10:50am | clear, dry | green, slightly turbid | 40 | 10.2 | 568 | 90 | 0.47 |
| 3/13/2001 K4-13-3 | Spruce | 11:18am | clear, dry | green, slightly turbid | 40 | 10.3 | 540 | <54 | 0.23 |
| 3/19/2001 A-19-3 | Calabassas | 10:00am | clear, dry | clear | 40 | 7.87 | 3325 | 894 | 0.23 |
| 3/19/2001 B-19-3 | Malibu creek | 6:30am | clear, dry | clear | 40 | 7.81 | 3430 | 894 | 1.07 |
| 3/19/2001 G-19-3 | Las Posas | 7:30am | clear, dry | clear | 40 | 7.98 | 3525 | 894 | 0.51 |
| 3/19/2001 G-19-3 | Las Posas | | | | | | | | |
| 3/19/2001 HTP-P-19-3 | POTW | 12:00pm | purge sample reactor | clear | 40 | 1.73 | 17200 | 179 | 0.4 |
| 3/22/2001 SD1-22-3N | Montiel | 10:00am | cloudy, dry | Yellow, quite clear | 40 | 9.3 | 1310 | 269 | 0.33 |
| 3/22/2001 SD1-22-3S | Montiel | | | | 40 | 9.2 | 1340 | 269 | 0.3 |
| 3/22/2001 SD2-22-3 | El Camino | 10:30am | cloudy, dry | clear | 40 | 7.8 | 2840 | 541 | 0.13 |
| 3/22/2001 SD3-22-3N | Leucadia | 11:30am | cloudy, dry | Slightly brownish | 40 | 10.5 | 1550 | 269 | 0.28 |
| 3/22/2001 SD3-22-3S | Leucadia | | | | 40 | 10.3 | 1643 | 269 | 0.22 |
| 3/22/2001 SD4-22-3 | Encinitas | 12:15pm | sun&clouds, dry | Clear | 40 | 8.48 | 5390 | 894 | 0.62 |
| 3/27/2001 K-27-3 | Spruce | 9:00am | clear, dry | many algae | 40 | 10.4 | 624 | 90 | 0.58 |
| 3/27/2001 I-27-3 | Riverside | 9:30am | clear, dry | many algae | 40 | 9.54 | 548 | 90 | 0.33 |
| 3/27/2001 L-27-3 | Chavez | 11:00am | clear, dry | dirty | 40 | 7.37 | 2950 | 450 | 0.92 |
| 3/27/2001 F-27-3 | Culver | 13:30 | clear, dry | algae | 40 | 10.4 | 1373 | 179 | 0.79 |

Table A-4 continued

| | | | | | | | | | | |
|-----------|------------|------------|---------|-------------------------------|---------------------------------------|-----------|------|-------|------|------|
| 4/5/2001 | SD1-5-4 | Montiel | 10:00am | scattered showers pst 24 h | very yellow and dirty | 20 | 8.36 | 618 | 90 | 1.08 |
| 4/5/2001 | SD2-5-4 | El Camino | 10:30am | scattered showers pst 24 h | slightly yellow and dirty | 40 | 7.83 | 2393 | 715 | 0.53 |
| 4/5/2001 | SD3-5-4 | Leucadia | 11:00am | scattered showers pst 24 h | Not enough water to sample | NA | NA | | NA | NA |
| 4/5/2001 | SD4-5-4 | Encinitas | 11:30am | scattered showers pst 24 h | clear | 40 | 7.87 | 5265 | 1073 | 0.48 |
| 4/7/2001 | E6-7-4 | Turnbull | ~7:00am | raining | dark brown and dirty | 20 | 7.87 | 150 | <54 | 7.2 |
| 4/7/2001 | E8-7-4 | June Way | ~7:00am | raining | dark brown and dirty | 20 | 8.33 | 52 | <54 | 3.57 |
| 4/7/2001 | E23-7-4 | Rincon | ~7:00am | raining | moderate brown and dirty | 20 | 7.55 | 61 | <54 | 1.04 |
| 4/7/2001 | H1-7-4 | Filmore | 3:00am | raining | moderate brown and dirty | 20 | 7.18 | 134 | <54 | 1.2 |
| 4/7/2001 | H2-7-4 | Orcas | 4:15am | raining | dark brown and dirty | 20 | | 263 | <54 | NA |
| 4/7/2001 | H3-7-4 | Altadena | 5:30am | raining | moderate brown and dirty | 20 | 6.93 | 16 | <54 | 1.26 |
| 4/10/2001 | SD1-10-4 | Montiel | 6:00am | tail of rain storm | slightly dirty | 20 | 7.88 | 294 | <54 | 1.02 |
| 4/10/2001 | SD2-10-4 | El Camino | 6:30am | tail of rain storm | slightly dirty | 20 | 7.77 | 1248 | 269 | 2.31 |
| 4/10/2001 | SD3-10-4 | Leucadia | 7:15am | tail of rain storm | slightly dirty | 20 | 7.71 | 594 | 90 | 1.96 |
| 4/10/2001 | SD4-10-4 | Encinitas | 7:30am | tail of rain storm | slightly dirty | 20 | 7.54 | 1380 | 179 | 1.5 |
| 4/11/2001 | HTP-P-11-4 | POTW | 10:00am | purge sample reactor | slightly turbid, no color | 20 | 2.11 | 16670 | 179 | 1.91 |
| 4/11/2001 | HTP-F-11-4 | POTW | 10:00am | feed sample reactor | moderate brown and dirty | 20 | 7.7 | 1623 | 179 | 3.41 |
| 4/16/2001 | K1-16-4 | Spruce | 9:00am | sun | slightly turbid, no color | 20 | 10.5 | 644 | 90 | 0.74 |
| 4/16/2001 | K2-16-4 | Spruce | 9:10am | sun | slightly turbid, no color | 20 | 10.7 | 642 | 90 | 0.7 |
| 4/16/2001 | K3-16-4 | Spruce | 9:20am | sun | slightly turbid, no color | 20 | 10.7 | 666 | 90 | 0.7 |
| 4/16/2001 | K4-16-4 | Spruce | 9:25am | sun | slightly turbid, no color | 20 | 10.6 | 702 | 90 | 0.74 |
| 4/20/2001 | HTP-P-20-4 | POTW | 10:00am | NA | colorless, slightly turbid | 20 | 2 | 17840 | 179 | 1.98 |
| 4/20/2001 | HTP-F-20-4 | POTW | 10:05am | NA | moderately dirty | 20 | 7.24 | 1620 | 179 | 3.13 |
| 4/20/2001 | H1-20-4 | Filmore | 23:47 | raining | dark brown and dirty | 20 | 7.57 | | <54 | 1.85 |
| 4/20/2001 | H2-20-4 | Orcas | 23:25 | raining | quite dirty | 20 | 7.47 | 48 | <54 | 0.78 |
| 4/20/2001 | H3-20-4 | Altadena | 22:15 | raining | dark brown and dirty | 20 | 7.52 | 29.6 | <54 | 1.81 |
| 4/23/2001 | C-23-4 | pico drain | 11:30am | rain 48-60 h ago | Clear | 20 | 8.49 | 1140 | 269 | 0.66 |

Table A-4 continued

| | | | | | | | | | | |
|-----------|---------------|--------------|---------|-------------------------|---|----|------|------|-----|------|
| 4/23/2001 | F-23-4 | Culver | 10:00am | rain 48-60 h ago | Slightly brown and turbid | 20 | 9.28 | 1245 | 179 | 0.95 |
| 4/25/2001 | HTP-P-25-4 | POTW | 7:00am | NA | moderately dirty | 20 | 7.45 | 8600 | 90 | 1.32 |
| 4/25/2001 | HTP-F-25-4 | POTW | 7:05am | NA | moderately dirty | 20 | 7.09 | 1628 | 179 | 3.01 |
| 4/30/2001 | SD1-30-4 | Montiel | 9:30am | dry, morning clouds | Somewhat turbid and yellow | 20 | 9.27 | 1365 | 269 | 0.5 |
| 4/30/2001 | SD2-30-4 | El Camino | 10:00am | dry, morning clouds | Clear, colorless | 20 | 8.07 | 2468 | 894 | 0.71 |
| 4/30/2001 | SD3-30-4 | Leucadia | 10:30am | dry, morning clouds | Turbid, brown | 20 | 9.92 | 1585 | 269 | 1.4 |
| 4/30/2001 | SD4-30-4 | Encinitas | 11:00am | dry, morning clouds | Clear, colorless | 20 | 8.36 | 4575 | 894 | 1.14 |
| 5/7/2001 | F-7-5 | Culver | 10:00am | hot and sunny | turbid and orange/red | 20 | 8.49 | 1510 | 179 | 0.97 |
| 5/7/2001 | D-7-5 | Lakewood | 11:00am | hot and sunny | Turbid, green/dirty | 20 | 7.6 | 3610 | 179 | 1.11 |
| 5/7/2001 | C-7-5 | pico drain | 11:30am | hot and sunny | HIGH TIDE, NO SAMPLING | NA | NA | NA | NA | NA |
| 5/7/2001 | K-7-5 | Spruce | 13:00 | hot and sunny | Clear | 20 | 8.1 | 1115 | 269 | 0.4 |
| 5/11/2001 | HTP-AIN-11-5 | POTW | NA | NA | Clear | 14 | 7.04 | 13.3 | <54 | 0.46 |
| 5/11/2001 | HTP-AOUT-11-5 | POTW | NA | NA | Clear | 12 | 6.92 | 2.3 | <54 | 0.41 |
| 5/14/2001 | L-14-5 | Chavez | 10:00am | local rain two days ago | Clear, solids settled on bottom | 20 | 8.15 | 3145 | 330 | 0.95 |
| 5/14/2001 | C-14-5 | Pico drain | 10:30am | local rain two days ago | DRAIN IS CLOSED, NO WATER, NO MORE SAMPLES | NA | NA | NA | NA | NA |
| 5/14/2001 | HTP-P-14-5 | POTW | 11:30am | NA | NA | 20 | 7.56 | 7260 | 90 | 1.58 |
| 5/21/2001 | A-21-5 | Calabassas | 10:00am | Morning clouds, dry | Clear | 20 | 8.12 | 3125 | 894 | 0.42 |
| 5/21/2001 | B-21-5 | Malibu Creek | 10:30am | Morning clouds, dry | Very clear | 20 | 8.05 | 2900 | 894 | 0.57 |
| 5/21/2001 | G-21-5 | Las Posas | 11:30am | Morning clouds, dry | Turbid and brown | 20 | 8.3 | 2275 | 450 | 1.74 |
| 5/30/2001 | SD1-30-5 | Montiel | 9:00am | Sunny | turbid, yellow/brown | 20 | 8.23 | 2645 | 179 | 0.69 |
| 5/30/2001 | SD2-30-5 | El Camino | 10:00am | Sunny | slightly turbid, no color | 20 | 7.87 | 2535 | 450 | 0.72 |
| 5/30/2001 | SD3-30-5 | Leucadia | 10:30am | Sunny | turbid, dark redish | 20 | 10.2 | 1750 | 269 | 0.87 |
| 5/30/2001 | SD4-30-5 | Encinitas | 10:45am | Sunny | clear | 20 | 7.86 | 5395 | 894 | 0.41 |
| 6/1/2001 | A-1-6 | Calabassas | 10:00am | Sunny | clear | 20 | 8.1 | 3045 | 896 | 0.66 |
| 6/1/2001 | B-1-6 | Malibu Creek | 10:20am | Sunny | clear | 20 | 8.04 | 2905 | 596 | |
| 6/1/2001 | G-1-6 | Las Posas | 11:00am | Sunny | very dirty | 20 | 6.23 | 2760 | 596 | 0.86 |
| 6/4/2001 | F-4-6 | Culver | 9:30am | Sunny | Turbid, red/brown | 20 | 9.26 | 1118 | 90 | 1 |

Table A-4 continued

| | | | | | | | | | |
|--------------------|-----------|---------|-------|-------------------------------|----|------|------|------|------|
| 6/4/2001 D-4-6 | Lakewood | 10:30am | Sunny | Quite clear, slightly brown | 20 | 7.94 | 2585 | 90 | 0.65 |
| 6/4/2001 L-4-6 | Chavez | 11:30am | Sunny | Turbid, yellow/green | 20 | 8.07 | 2965 | 300 | 0.91 |
| 18-Jun I-18-6 | Riverside | 8.45am | Sunny | Very turbid, brown | 20 | 10.1 | 664 | 90 | 0.67 |
| 6/18/2001 K-18-6 | Spruce | 9:10am | Sunny | Slightly turbid, yellow green | 20 | 8.7 | 492 | 90 | 0.74 |
| 6/20/2001 SD2-20-6 | El Camino | 10:00am | Sunny | Clear | 20 | 7.57 | 2670 | 596 | 0.92 |
| 6/20/2001 SD3-20-6 | Leucadia | 11:00am | Sunny | Turbid, yellow brown | 20 | 9.7 | 1960 | 269 | 1.05 |
| 6/20/2001 SD4-20-6 | Encinitas | 11:30am | Sunny | Very clear | 20 | 7.81 | 5640 | 1192 | 0.51 |

Table A-5
Sample Site Locations

| Code | Name location | Direction | Description |
|--|---------------|---|---|
| <i>URS, Steve Kummerfeld; W (714) 433-7774; C (714) 299-4593; (714) 835-6886</i> | | | |
| E6 | Turnbull | 60 W, exit at Hacienda Boulevard North between the 57 and 605 fwy. Turn left on Gale, turn South on Turnbull Canyon Road, turn left into fenced gate street just after fwy overpass. Walk for 500 ft, and go through opening in fence, and cross the wooden bridge spanning the culvert. | This bmp is very similar to the one at Rincon (E23). It collects water running down from the 60 fwy. |
| E8 | June Way | 60 W between the 605 and 710, exit at Wilcox Ave, turn left on Pomona Bld, park at June Way road, cross the street towards the fwy. | |
| E23 | Rincon | 210 W, just after intersection with the 2 fwy, exit Ocean View Boulevard south, turn left at Montrose Ave, turn left at Rincon Ave and go straight until reaching the cul-de-sac at the end. | This site collects water from a few acres area with freeway only. Caltrans. Channel comes to surface, very simple bmp: water is screened through something like fish-nets. Then the water goes onto the street in residential area. |
| <i>Lawkranda, Steve Ohtmer;</i> | | | |
| H1 | Fillmore | 210 W, exit at Paxton, turn to your right at the off-ramp's end at follow the road until the T-intersection (in front, a land-fill is located). At the intersection, turn right, pass one stop sign, and continue till the end of the cul-de-sac. There is a gate and gravel path to the bmp. | |
| H2 | Orcas | 210 W, exit at Wheatland, turn left on Foothill Blvd (parallel to the 210), after a few blocks turn left into Orcas Ave., which is a small street. Just before going under the fwy, go through the gate at your left. | This bmp collects water running down from the slope of the 210 fwy. Relatively small, its major purpose seems to be removal of large particles by e.g., filtration. |
| H3 | Altadena | 210 W, exit at Arroyo Blvd, go right and directly go right again into the Caltrans maintenance station. This station is located next to the off-ramp you were just on. | This bmp probably treats run-off from the premises of the maintenance station. Bmp is constructed wetland in combination with a rock filter. |

Table A-6
Baseline Study - Gross Characteristics

| No | Location | Type | Date | Sample Volume (L) | Volume to 1MDS (L) | Wash off area (sq m) | Gross characteristics | | | |
|-----|-------------------------------|-------|------------|-------------------|--------------------|----------------------|-----------------------|------|-----|-------------|
| | | | | | | | Hardness | SS | pH | Filtrate pH |
| D1 | Davis (park) 1 | Paved | 11/16/1999 | 31 | 31 | 400 | | | | |
| D2 | Davis (park) 2 | Paved | 12/20/1999 | 20 | 20 | 400 | | | | |
| E1 | Encinitas (Moonlight) | Drain | 1/5/2000 | 40 | 40 | | | | | |
| SD1 | San Diego(Solano Beach) | Drain | 1/5/2000 | | | | | | | |
| SD2 | San Diego (Del Mar) | Drain | 1/6/2000 | 40 | 40 | | | | | |
| SD3 | San Diego (La Jolla) | Drain | 1/6/2000 | 40 | 40 | | | | | |
| D3 | Davis (residence) | Soil | 3/7/2000 | 37 | 37 | 400 | | | | |
| D4 | Davis (park) | Soil | 3/21/2000 | 40 | 40 | 600 | | | | |
| D5 | Davis (roof) | Roof | 4/14/2000 | 40 | 40 | 400 | | | | |
| EG1 | Elk Grove (pond) | Drain | 5/10/2000 | 40 | 40 | | | | | |
| S5 | Sacramento (Riverbend) | Drain | 5/10/2000 | 40 | 40 | | | | | |
| S4 | Sacramento Airport | Drain | 5/10/2000 | 40 | 40 | | | | | |
| SD7 | San Diego (Ravina) | Drain | 6/20/2000 | 40 | 40 | | | | | |
| SD5 | San Diego (residence) | Paved | 6/20/2000 | 20 | 20 | 200 | | | | |
| SD4 | San Diego (residence) | Roof | 6/20/2000 | 20 | 20 | 450 | | | | |
| SD6 | San Diego (residence) | Soil | 6/20/2000 | 20 | 20 | 200 | | | | |
| LN4 | Aliso Creek | Drain | 6/21/2000 | 40 | 40 | | | | | |
| LN2 | Laguna Niguel (park) | Paved | 6/21/2000 | 20 | 20 | 200 | | | | |
| LN3 | Laguna Niguel (park) | Soil | 6/21/2000 | 40 | 40 | 2000 | | | | |
| LN1 | Laguna Niguel (senior center) | Roof | 6/21/2000 | 20 | 20 | 150 | | | | |
| PH4 | Port Hueneme | Drain | 6/22/2000 | 40 | 40 | | | | | |
| PH2 | Port Hueneme | Paved | 6/22/2000 | 40 | 40 | 225 | | | | |
| PH1 | Port Hueneme | Roof | 6/22/2000 | 15 | 15 | 225 | | | | |
| PH3 | Port hueneme | Soil | 6/22/2000 | 20 | 20 | 120 | | | | |
| E2 | Encinitas (firehouse) | Paved | 8/1/2000 | 40 | 40 | 400 | 46 | 230 | 7.5 | 4.8 |
| E3 | Encinitas (firehouse) | Soil | 8/1/2000 | 40 | 40 | 400 | 12 | 128 | | 4.7 |
| E4 | Encinitas (Moonlight) | Drain | 8/1/2000 | 40 | 40 | | 128 | 2.5 | | 4.5 |
| KM1 | Kearney Mesa (truck wash) | Paved | 8/1/2000 | 40 | 40 | 400 | 50 | 201 | | 4.6 |
| KM2 | Kearney Mesa (truck wash) | Soil | 8/1/2000 | 30 | 30 | 400 | 260 | 3390 | | 4 |
| I3 | Irvine (drain) | Drain | 8/2/2000 | 40 | 40 | | 46 | 4 | | 4.3 |
| I1 | Irvine (wash station) | Paved | 8/2/2000 | 40 | 40 | 400 | 14 | 555 | | 4.4 |
| I2 | Irvine (wash station) | Soil | 8/2/2000 | | | 240 | | | | |

Table A-6 continued

| No | Location | Type | Date | Sample Volume (L) | Volume to 1MDS (L) | Wash off area (sq m) | Gross characteristics | | | |
|-----|--------------------------|-------|------------|-------------------|--------------------|----------------------|-----------------------|------|----|-------------|
| | | | | | | | Hardness | SS | pH | Filtrate pH |
| KM3 | San Diego (Del Mar) | Drain | 8/2/2000 | 40 | 40 | | 172 | 4 | | 4.8 |
| HB3 | Huntington Beach | Drain | 8/3/2000 | 40 | 40 | | 1200 | | | 4.7 |
| HB1 | Huntington Beach | Paved | 8/3/2000 | 40 | 40 | 300 | 14 | 68 | | 4.2 |
| HB2 | Huntington Beach | Soil | 8/3/2000 | 20 | 20 | 100 | 200 | 303 | | 3.85 |
| MC1 | Malibu Creek 1 | Drain | 9/12/2000 | 40 | 40 | | 1040 | 3.4 | | 5.1 |
| MC2 | Malibu Creek 2 | Drain | 9/12/2000 | 40 | 40 | | 680 | 3.2 | | 5.2 |
| MC3 | Malibu Creek 3 | Drain | 9/12/2000 | 40 | 40 | | 960 | 3.2 | | 3.2 |
| SM1 | Santa Monica (Ashland) 1 | Drain | 9/13/2000 | 40 | 40 | | 1940 | 9.4 | | 5.5 |
| SM2 | Santa Monica (Ashland) 2 | Drain | 9/13/2000 | 40 | 40 | | 1740 | 9.3 | | 4.3 |
| SM3 | Santa Monica (Ashland) 3 | Drain | 9/13/2000 | 40 | 40 | | 1800 | 12.2 | | 5.5 |
| RB1 | Redondo Beach 1 | Drain | 9/14/2000 | 40 | 40 | | 460 | 3.3 | | 4.2 |
| RB2 | Redondo Beach 2 | Drain | 9/14/2000 | 40 | 40 | | 380 | 3.6 | | 4.9 |
| RB3 | Redondo Beach 3 | Drain | 9/14/2000 | 40 | 40 | | 720 | 7.5 | | 5.3 |
| SB2 | Amundale Barranca | Drain | 10/31/2000 | 40 | 40 | | 1800 | | | 5.2 |
| SB1 | Atascadero Creek | Drain | 10/31/2000 | 40 | 40 | | 350 | | | 5.4 |
| SB3 | Camino Park Barranca | Drain | 10/31/2000 | 40 | 40 | | 2800 | | | 4.7 |
| SV2 | Simi Valley (park) | Drain | 11/2/2000 | 40 | 40 | | 850 | | | 5.3 |
| SV1 | Simi Valley (residence) | Drain | 11/2/2000 | 40 | 40 | | 840 | | | 5.5 |

Table A-7
Baseline Study - Indicator Organisms

| Sample No | Location | Indicators, MPN/100 mL | | | | E Coli | Enterococcus | Indicator Lab |
|-----------|---------------------------|------------------------|----------------|---|---------|-----------|--------------|---------------|
| | | Total Coliform | Fecal Coliform | | | | | |
| D1 | Davis (park) 1 | = | 2.0E+04 | = | 2.0E+04 | | | UCD |
| D2 | Davis (park) 2 | = | 2.0E+05 | = | 2.0E+05 | | | UCD |
| E1 | Encinitas (Moonlight) | = | 5.0E+03 | = | 1.0E+02 | | = 1.0E+02 | Julie |
| SD1 | San Diego (Sola no Beach) | > | 1.6E+04 | > | 1.6E+04 | | > 2.0E+03 | Julie |
| SD2 | San Diego (Del Mar) | = | 5.0E+03 | = | 8.0E+02 | | = 5.0E+02 | Julie |
| SD3 | San Diego (La Jolla) | = | 9.0E+03 | = | 3.0E+03 | | = 7.0E+02 | Julie |
| D3 | Davis (residence) | = | 5.0E+04 | = | 5.0E+01 | | | UCD |
| D4 | Davis (park) | = | 5.0E+06 | = | 5.0E+01 | | | UCD |
| D5 | Davis (roof) | = | 2.0E+03 | = | 9.0E+01 | | | UCD |
| EG1 | Elk Grove (pond) | = | 2.0E+02 | = | 5.0E+01 | | | UCD |
| S5 | Sacramento (Riverbend) | = | 3.0E+02 | = | 7.0E+01 | | | UCD |
| S4 | Sacramento Airport | = | 5.0E+01 | = | 3.0E+01 | | | UCD |
| SD7 | San Diego (Ravina) | = | 1.3E+05 | = | 2.0E+03 | | > 2.0E+03 | Julie |
| SD5 | San Diego (residence) | < | 2.0E+00 | < | 2.0E+00 | | = 1.6E+02 | Julie |
| SD4 | San Diego (residence) | < | 2.0E+00 | < | 2.0E+00 | | = 7.5E+01 | Julie |
| SD6 | San Diego (residence) | = | 1.4E+05 | = | 1.1E+05 | | > 2.0E+03 | Julie |
| LN4 | Aliso Creek | = | 3.0E+03 | = | 8.0E+01 | = 2.0E+01 | = 1.3E+02 | Sierra |

Table A - 7 continued

| Sample No | Location | Indicators, MPN/100 mL | | | | | | | | |
|-----------|-------------------------------|------------------------|---------|----------------|---------|--------|---------|---------------|---------|---------------|
| | | Total Coliform | | Fecal Coliform | | E Coli | | Entero coccus | | Indicator Lab |
| LN2 | Laguna Niguel (park) | = | 5.0E+03 | = | 2.0E+01 | = | 2.0E+01 | = | 2.2E+04 | Sierra |
| LN3 | Laguna Niguel (park) | = | 2.8E+05 | = | 1.6E+04 | = | 9.0E+03 | = | 5.0E+05 | Sierra |
| LN1 | Laguna Niguel (senior center) | < | 2.0E+01 | < | 2.0E+01 | < | 2.0E+01 | < | 2.0E+01 | Sierra |
| PH4 | Port Hueneme | = | 9.0E+02 | = | 5.0E+01 | | | | | Ventura Co |
| PH2 | Port Hueneme | = | 5.0E+02 | = | 8.0E+00 | | | | | Ventura Co |
| PH1 | Port Hueneme | = | 2.4E+02 | = | 2.4E+02 | | | | | Ventura Co |
| PH3 | Port hueneme | > | 1.6E+03 | > | 1.6E+03 | | | | | Ventura Co |
| E2 | Encinitas (firehouse) | = | 9.0E+02 | < | 3.6E+00 | | | = | 2.3E+01 | San Elijo |
| E3 | Encinitas (firehouse) | = | 2.9E+03 | < | 3.6E+00 | | | = | 3.1E+04 | San Elijo |
| E4 | Encinitas (Moonlight) | > | 1.6E+04 | = | 9.0E+03 | | | = | 1.0E+03 | Julie |
| KM1 | Kearney Mesa (truck wash) | | | | | | | = | 4.2E+01 | Julie |
| KM2 | Kearney Mesa (truck wash) | | | | | | | > | 2.0E+03 | Julie |
| I3 | Irvine (drain) | = | 8.0E+04 | = | 1.7E+03 | | | | | IRWD |
| I1 | Irvine (wash station) | = | 3.0E+01 | < | 2.0E+00 | | | | | IRWD |
| I2 | Irvine (wash station) | = | 5.0E+01 | < | 2.0E+00 | | | | | IRWD |
| KM3 | San Diego (Del Mar) | > | 1.6E+03 | = | 9.0E+03 | | | = | 1.0E+03 | Julie |

Table A-7 continued

| Sample No | Location | Indicators, MPN/100 mL | | | | E Coli | Enterococcus | Indicator Lab | |
|-----------|--------------------------|------------------------|----------------|---|---------|--------|--------------|---------------|-------------|
| | | Total Coliform | Fecal Coliform | | | | | | |
| HB3 | Huntington Beach | = | 1.4E+02 | = | 8.0E+01 | | = | 1.4E+01 | CSDOC |
| HB1 | Huntington Beach | = | 5.0E+02 | < | 2.0E+00 | | = | 1.3E+01 | CSDOC |
| HB2 | Huntington Beach | = | 1.6E+03 | < | 2.0E+00 | | = | 1.7E+04 | CSDOC |
| MC1 | Malibu Creek 1 | < | 2.0E+00 | < | 2.0E+00 | | < | 1.1E+00 | Silliker |
| MC2 | Malibu Creek 2 | < | 2.0E+00 | < | 2.0E+00 | | < | 1.1E+00 | Silliker |
| MC3 | Malibu Creek 3 | = | 3.0E+01 | < | 2.0E+00 | | < | 1.1E+00 | Silliker |
| SM1 | Santa Monica (Ashland) 1 | = | 2.4E+04 | = | 5.0E+02 | | < | 1.1E+00 | Silliker |
| SM2 | Santa Monica (Ashland) 2 | = | 2.4E+04 | = | 1.3E+03 | | < | 1.1E+00 | Silliker |
| SM3 | Santa Monica (Ashland) 3 | = | 2.4E+04 | = | 2.3E+02 | | < | 1.1E+00 | Silliker |
| RB1 | Redondo Beach 1 | = | 2.3E+02 | = | 8.0E+00 | | = | 3.0E+02 | Silliker |
| RB2 | Redondo Beach 2 | = | 1.1E+02 | = | 8.0E+00 | | = | 5.0E+01 | Silliker |
| RB3 | Redondo Beach 3 | = | 1.4E+02 | = | 1.3E+01 | | = | 5.0E+01 | Silliker |
| SB2 | Amundale Barranca | | | | | | | | Aquatic Bio |
| SB1 | Atascadero Creek | = | 3.0E+04 | = | 9.0E+02 | | = | 3.0E+02 | Aquatic Bio |
| SB3 | Camino Park Barranca | | | | | | | | Aquatic Bio |
| SV2 | Simi Valley (park) | = | 2.4E+04 | < | 2.0E+00 | | < | 2.0E+00 | Pat-Chem |
| SV1 | Simi Valley (residence) | | | | | | | | Pat-Chem |

Table A-8
Baseline Study - Viruses

| No | Location | Virus Field Recovery | | Viruses | | | Maximum positive dilutions | | | |
|-----|-------------------------------|----------------------|--------------|---------------------------|-------------------------|----------------------------|----------------------------|--------|--------|------|
| | | Marker organism | Marker titer | Minimum positive dilution | Concentrate Volume (µL) | Maximum inhibited dilution | | | | |
| | | | | MPD | CV | | Adeno | Entero | Hep. A | Rota |
| D1 | Davis (park) 1 | | | | 100 | | 3 | | | |
| D2 | Davis (park) 2 | | | | 100 | 0 | | | | |
| E1 | Encinitas (Moonlight) | | | | 100 | | | | | |
| SD1 | San Diego(Solano Beach) | | | | 100 | | | | | |
| SD2 | San Diego (Del Mar) | | | | 100 | | | | | |
| SD3 | San Diego (La Jolla) | | | | 100 | | | 2 | | |
| D3 | Davis (residence) | | | | 100 | | 0 | | | |
| D4 | Davis (park) | | | | 100 | 0 | | | | |
| D5 | Davis (roof) | | | | 100 | 0 | | | | |
| EG1 | Elk Grove (pond) | | | | 100 | 0 | | | | |
| S5 | Sacramento (Riverbend) | | | | 100 | | 2 | | | |
| S4 | Sacramento Airport | | | | 100 | | | | | |
| SD7 | San Diego (Ravina) | | | | 100 | | | | | |
| SD5 | San Diego (residence) | | | | 100 | | | | | |
| SD4 | San Diego (residence) | | | | 100 | 0 | | | | |
| SD6 | San Diego (residence) | | | | 100 | 0 | | | | |
| LN4 | Aliso Creek | | | | 100 | | | | | |
| LN2 | Laguna Niguel (park) | | | | 100 | 0 | | | | |
| LN3 | Laguna Niguel (park) | | | | 100 | 0 | | | | |
| LN1 | Laguna Niguel (senior center) | | | | 100 | 0 | | | | |
| PH4 | Port Hueneme | | | | 100 | | | | | |
| PH2 | Port Hueneme | | | | | 0 | | | | |
| PH1 | Port Hueneme | | | | 100 | 0 | | | | |
| PH3 | Port hueneme | | | | 100 | | | | | |
| E2 | Encinitas (firehouse) | D. BOV ENT | 100000 | 3 | 400 | 1 | | | | |
| E3 | Encinitas (firehouse) | D. BOV ENT | 100000 | 2 | 500 | 0 | | | | |
| E4 | Encinitas (Moonlight) | D. BOV ENT | 100000 | 4 | 350 | | 1 | | | |
| KM1 | Kearney Mesa (truck wash) | D. BOV ENT | 100000 | 2 | 500 | 0 | | | | |

Table A-8 continued

| No | Location | Virus Field Recovery | | Viruses | | | Maximum positive dilutions | | | |
|-----|---------------------------|----------------------|--------------|---------------------------|-------------------------|----------------------------|----------------------------|--|--|--|
| | | Marker organism | Marker titer | Minimum positive dilution | Concentrate Volume (μL) | Maximum inhibited dilution | | | | |
| KM2 | Kearney Mesa (truck wash) | D. BOV ENT | 100000 | 3 | 400 | 1 | | | | |
| I3 | Irvine (drain) | D. BOV ENT | 100000 | 3 | 550 | 0 | | | | |
| I1 | Irvine (wash station) | D. BOV ENT | 100000 | 4 | 550 | 0 | | | | |
| I2 | Irvine (wash station) | D. BOV ENT | 100000 | | | | | | | |
| KM3 | San Diego (Del Mar) | D. BOV ENT | 100000 | 4 | 420 | | 2 | | | |
| HB3 | Huntington Beach | D. BOV ENT | 100000 | 2 | 320 | | | | | |
| HB1 | Huntington Beach | D. BOV ENT | 100000 | 4 | 440 | 0 | | | | |
| HB2 | Huntington Beach | D. BOV ENT | 100000 | 4 | 490 | 2 | | | | |
| MC1 | Malibu Creek 1 | D. BOV ENT | 25000 | 0 | 200 | | | | | |
| MC2 | Malibu Creek 2 | D. BOV ENT | 25000 | 3 | 200 | | | | | |
| MC3 | Malibu Creek 3 | D. BOV ENT | 25000 | 1 | 150 | | | | | |
| SM1 | Santa Monica (Ashland) 1 | D. BOV ENT | 25000 | #N/A | 400 | 0 | | | | |
| SM2 | Santa Monica (Ashland) 2 | D. BOV ENT | 25000 | #N/A | 450 | 0 | | | | |
| SM3 | Santa Monica (Ashland) 3 | D. BOV ENT | 25000 | #N/A | 300 | 0 | | | | |
| RB1 | Redondo Beach 1 | D. BOV ENT | 25000 | 0 | 500 | | | | | |
| RB2 | Redondo Beach 2 | D. BOV ENT | 25000 | 0 | 500 | | | | | |
| RB3 | Redondo Beach 3 | D. BOV ENT | 25000 | 0 | 500 | | | | | |
| SB2 | Amundale Barranca | D. BOV ENT | 100000 | 3 | 475 | | | | | |
| SB1 | Atascadero Creek | D. BOV ENT | 100000 | 3 | 450 | | | | | |
| SB3 | Camino Park Barranca | D. BOV ENT | 100000 | 3 | 500 | | 0 | | | |
| SV2 | Simi Valley (park) | D. BOV ENT | 100000 | 4 | 350 | | | | | |
| SV1 | Simi Valley (residence) | D. BOV ENT | 100000 | 4.30103 | 250 | | | | | |

Table A-9
Baseline Study - Bacteria and Protozoa

| No | Location | Concentrate Volume, µL | Maximum inhibited dilution | Maximum positive dilutions | | | | | | |
|-----|-------------------------------|------------------------|----------------------------|----------------------------|---------|---------|---------|----------|---------|---------|
| | | | | F. EHEC | G. ETEC | H. Shig | I. Salm | J. Staph | K. Giar | L. Cryp |
| D1 | Davis (park) 1 | 100 | | | | | | | | |
| D2 | Davis (park) 2 | 100 | 0 | | | | | | | |
| E1 | Encinitas (Moonlight) | 100 | | | | | | | | |
| SD1 | San Diego(Solano Beach) | | | | | | | | | |
| SD2 | San Diego (Del Mar) | 100 | | | | | | | | 0 |
| SD3 | San Diego (La Jolla) | 100 | | | | | | | | |
| D3 | Davis (residence) | 100 | | | | | | | | |
| D4 | Davis (park) | 100 | 0 | | | | | 1 | 2 | |
| D5 | Davis (roof) | 100 | 0 | | | | | | | |
| EG1 | Elk Grove (pond) | 100 | 0 | | | | | | | |
| S5 | Sacramento (Riverbend) | 100 | 0 | | | | | | | |
| S4 | Sacramento Airport | 100 | 0 | | | | | | | |
| SD7 | San Diego (Ravina) | 100 | | | | | | | | |
| SD5 | San Diego (residence) | 100 | | | | | | 1 | | |
| SD4 | San Diego (residence) | 100 | 0 | | | | | | | |
| SD6 | San Diego (residence) | 100 | 0 | | | | | | | |
| LN4 | Aliso Creek | 100 | | | | | | | | |
| LN2 | Laguna Niguel (park) | 100 | 0 | | | | | | | |
| LN3 | Laguna Niguel (park) | 100 | 0 | | | | | | | |
| LN1 | Laguna Niguel (senior center) | 100 | 0 | | | | | | | |
| PH4 | Port Hueneme | 100 | | | | | | | | |
| PH2 | Port Hueneme | 100 | 0 | | | | | | | |
| PH1 | Port Hueneme | 100 | 0 | | | | | | | |
| PH3 | Port hueneme | 100 | | | | | | | | |
| E2 | Encinitas (firehouse) | 100 | 1 | | | | | | | |
| E3 | Encinitas (firehouse) | 100 | 2 | | | | | | | |
| E4 | Encinitas (Moonlight) | 100 | 1 | | | | | | | |

Table A-9 continued

| No | Location | Concentrate Volume, μ L | Maximum inhibited dilution | Maximum positive dilutions | | | | | | |
|-----|---------------------------|-----------------------------|----------------------------|----------------------------|---------|---------|---------|----------|---------|---------|
| | | | | F. EHEC | G. ETEC | H. Shig | I. Salm | J. Staph | K. Giar | L. Cryp |
| KM1 | Kearney Mesa (truck wash) | 100 | 2 | | | | | | | |
| KM2 | Kearney Mesa (truck wash) | 100 | 2 | | | | | | | |
| I3 | Irvine (drain) | 100 | 1 | | | | | | | |
| I1 | Irvine (wash station) | 100 | 2 | | | | | | | |
| I2 | Irvine (wash station) | | | | | | | | | |
| KM3 | San Diego (Del Mar) | 100 | 1 | | | | | | | |
| HB3 | Huntington Beach | 100 | 2 | | | | | | | |
| HB1 | Huntington Beach | 100 | 2 | | | | | | | |
| HB2 | Huntington Beach | 100 | 2 | | | | | | | |
| MC1 | Malibu Creek 1 | 100 | | | | | 1 | | | |
| MC2 | Malibu Creek 2 | 100 | 0 | | | | | | | |
| MC3 | Malibu Creek 3 | 100 | 0 | | | | | | | |
| SM1 | Santa Monica (Ashland) 1 | 100 | | | | | | | | |
| SM2 | Santa Monica (Ashland) 2 | 100 | | | | | | | | |
| SM3 | Santa Monica (Ashland) 3 | 100 | 0 | | | | | | | |
| RB1 | Redondo Beach 1 | 100 | 0 | | | | | | | |
| RB2 | Redondo Beach 2 | 100 | 0 | | | | | | | |
| RB3 | Redondo Beach 3 | 100 | 1 | | | | | | | |
| SB2 | Amundale Barranca | 100 | 0 | | | | | | | |
| SB1 | Atascadero Creek | 100 | 0 | | | | | | | |
| SB3 | Camino Park Barranca | 100 | 1 | | | | | | | |
| SV2 | Simi Valley (park) | 100 | 1 | | | | | | | |
| SV1 | Simi Valley (residence) | 100 | 1 | | | | | | | |