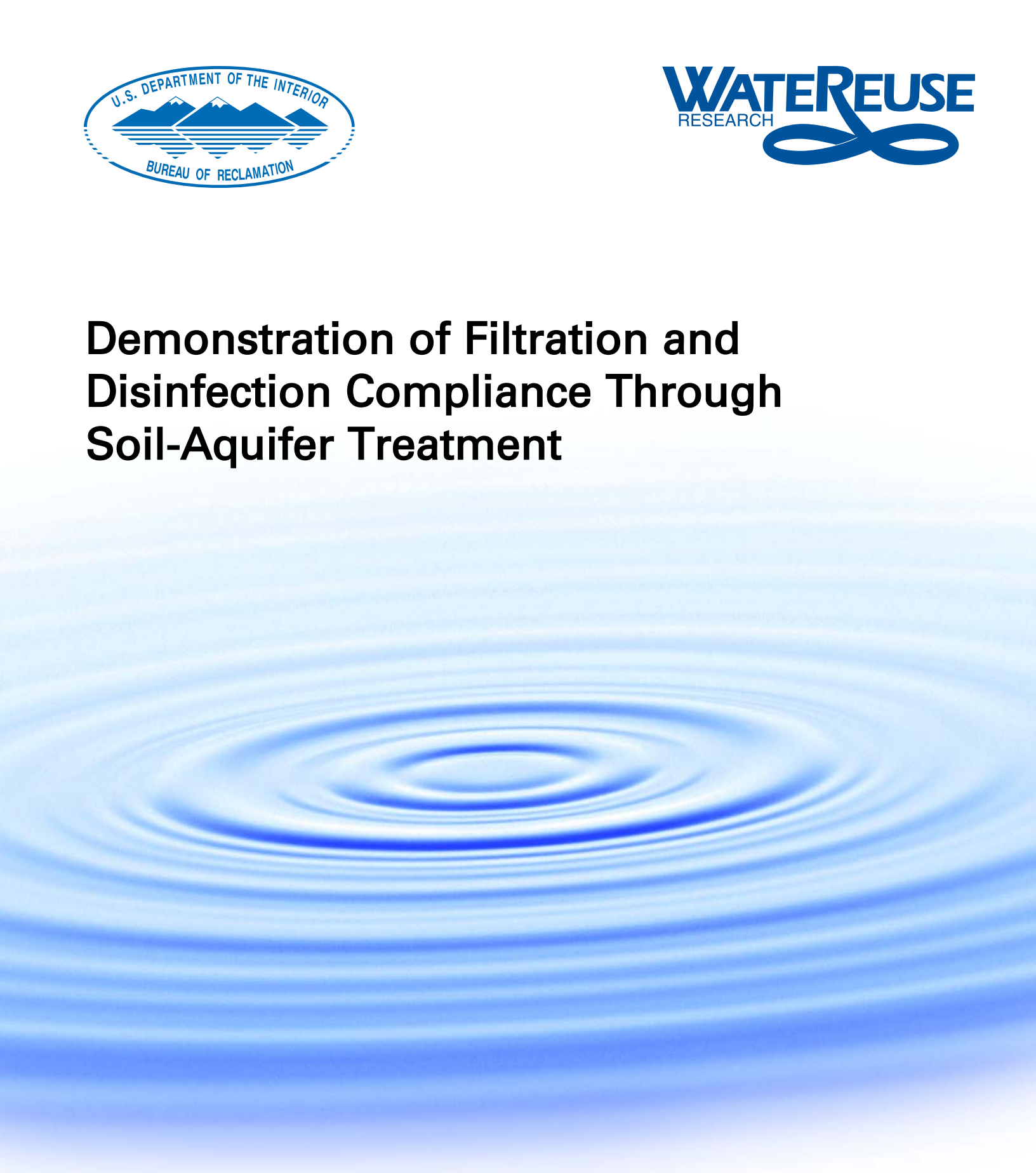
Demonstration of Filtration and Disinfection Compliance Through Soil-Aquifer Treatment



WateReuse Research Foundation

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About the WateReuse Research Foundation

The mission of the WateReuse Research Foundation is to conduct and promote applied research on the reclamation, recycling, reuse, and desalination of water. The Foundation's research advances the science of water reuse and supports communities across the United States and abroad in their efforts to create new sources of high-quality water through reclamation, recycling, reuse, and desalination while protecting public health and the environment.

The Foundation sponsors research on all aspects of water reuse, including emerging chemical contaminants, microbiological agents, treatment technologies, salinity management and desalination, public perception and acceptance, economics, and marketing. The Foundation's research informs the public of the safety of reclaimed water and provides water professionals with the tools and knowledge to meet their commitment of increasing reliability and quality. The Foundation's funding partners include the Bureau of Reclamation, the California State Water Resources Control Board, the California Energy Commission, and the California Department of Water Resources. Funding is also provided by the Foundation's subscribers, water and wastewater agencies, and other interested organizations.

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Stephen Hogg
City of Fresno, CA

Rosa Lau-Staggs
City of Fresno, CA

Dean K. Uota
City of Dinuba, CA

Andrew Salveson
Nicola Fontaine
Steve Swanback
Erin Mackey
Carollo Engineers, Inc.

Rick Danielson
Robert Cooper
BioVir Laboratories

Cosponsors

Bureau of Reclamation
City of Fresno, CA
City of Dinuba, CA
Carollo Engineers

WateReuse Research Foundation
Alexandria, VA

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For more information, contact:

WateReuse Research Foundation
1199 North Fairfax Street, Suite 410
Alexandria, VA 22314
703-548-0880
703-548-5085 (fax)
www.WateReuse.org/Foundation

WateReuse Research Foundation Project Number: WRRF-10-10
WateReuse Research Foundation Product Number: 10-10-1

ISBN: 978-1-934183-92-2

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Acronyms

AWT	advanced wastewater treatment
BioVir	BioVir Laboratories, Inc.
CCR	California Code of Regulations
CDPH	California Department of Public Health
CL	confidence level
Dinuba	The City of Dinuba, California
EC	electrical conductivity
EPA	Environmental Protection Agency
FID	Fresno Irrigation District
Fresno	The City of Fresno, California
gal/min	gallons per minute
MFT	multiple tube fermentation test
MGD	million gallons per day
MS2	male-specific type 2 coliphage
MPN	most probable number
MW	monitoring well
NPDES	National Pollutant Discharge Elimination System
NTU	nephelometric turbidity units
PBMS	performance-based measurement system
pfu	plaque-forming units
PSD	particle size distribution
QAO	Quality Assurance Office(r)
R11	Reclamation Well No. 11, City of Fresno
RW#1	Reclamation Well No. 1, City of Dinuba
RWRF	Regional Wastewater Reclamation Facility
RWQCB	Regional Water Quality Control Board
SAT	soil-aquifer treatment
SWRCB	State Water Resources Control Board
TDS	total dissolved solids
UVT	ultraviolet transmittance
WDR	Waste Discharge Requirement
WWRF	wastewater reclamation facility

Foreword

The WateReuse Research Foundation, a nonprofit corporation, sponsors research that advances the science of water reclamation, recycling, reuse, and desalination. The Foundation funds projects that meet the water reuse and desalination research needs of water and wastewater agencies and the public. The goal of the Foundation's research is to ensure that water reuse and desalination projects provide high-quality water, protect public health, and improve the environment.

An Operating Plan guides the Foundation's research program. Under the plan, a research agenda of high-priority topics is maintained. The agenda is developed in cooperation with the water reuse and desalination communities, including water professionals, academics, and Foundation subscribers. The Foundation's research focuses on a broad range of water reuse research topics including the following:

- Definition of and addressing emerging contaminants
- Public perceptions of the benefits and risks of water reuse
- Management practices related to indirect potable reuse
- Groundwater recharge and aquifer storage and recovery
- Evaluation and methods for managing salinity and desalination
- Economics and marketing of water reuse

The Operating Plan outlines the role of the Foundation's Research Advisory Committee (RAC), Project Advisory Committees (PACs), and Foundation staff. The RAC sets priorities, recommends projects for funding, and provides advice and recommendations on the Foundation's research agenda and other related efforts. PACs are convened for each project and provide technical review and oversight. The Foundation's RAC and PACs consist of experts in their fields and provide the Foundation with an independent review, which ensures the credibility of the Foundation's research results. The Foundation's Project Managers facilitate the efforts of the RAC and PACs and provide overall management of projects.

Low-cost and -impact treatment techniques, such as soil-aquifer treatment (SAT), are needed for the production of reclaimed water. Although it has long been assumed that SAT provides a substantial barrier to pathogens, detailed studies have been limited by insufficient analytical techniques. A new, high-volume virus enumeration technique for evaluating log removal of organisms present in very low numbers was field tested and used to demonstrate the efficacy of SAT to remove microorganisms at the wastewater treatment facilities of both the City of Fresno and the City of Dinuba, in California. This new enumeration method was successfully field validated through a series of spiking studies at Fresno and Dinuba. This validated method can be used worldwide to evaluate, monitor, and permit SAT systems. Virus sampling results of the SAT influent and effluent during this project showed that the SAT process is capable of achieving approximately 5-log reduction of male-specific (MS2) coliphage as well as reducing total coliforms and turbidity. These results indicate that SAT was effective at removing microorganisms from the percolated secondary wastewater.

Richard Nagel
Chair
WateReuse Research Foundation

G. Wade Miller
Executive Director
WateReuse Research Foundation

Acknowledgments

This project was funded by the WateReuse Research Foundation in cooperation with the Bureau of Reclamation, the City of Fresno, CA, the City of Dinuba, CA, and Carollo Engineers.

The project team would like to thank the engineering and laboratory staffs at the City of Fresno and the City of Dinuba.

Principal Investigator
Steve Hogg, City of Fresno, CA

Project Team
Rosa Lau-Staggs, City of Fresno, CA
Dean K. Uota, City of Dinuba, CA
Andrew Salveson, Carollo Engineers
Nicola Fontaine, Carollo Engineers
Steve Swanback, Carollo Engineers
Erin Mackey, Carollo Engineers
Rick Danielson, BioVir Laboratories
Robert Cooper, BioVir Laboratories
Carl Carlucci, California Department of Public Health
Randy Barnard, California Department of Public Health
Kurt Souza, California Department of Public Health

Participating Agencies
The City of Fresno, CA
The City of Dinuba, CA

Project Advisory Committee
Peter Fox, Arizona State University
Ron LeBlanc, Bureau of Reclamation
Naoko Munakata, Sanitation Districts of Los Angeles County
Jeff Stone, Consultant

Executive Summary

Utilities currently rely upon advanced wastewater treatment (AWT) facilities, typically filtration and disinfection, to produce high-quality effluent as an alternative water supply. However, construction of additional AWT facilities and the added operational costs are often economic barriers to implementation. Further, AWT energy use adds to the already burgeoning climate change issue. Therefore, low-cost and -impact treatment techniques are needed for the production of reclaimed water. One such technique is percolation, also known as soil-aquifer treatment (SAT). Although it has long been assumed that SAT provides a substantial barrier to pathogens, detailed studies have been limited by insufficient analytical techniques.

The cities of Fresno, CA (Fresno), and Dinuba, CA (Dinuba), currently percolate unfiltered secondary wastewater effluent into the ground as a disposal method under Waste Discharge Requirements (WDR). Both utilities would like to capture this percolated water through extraction wells and reuse it for various applications. Using SAT for reclamation and reuse instead of constructing new filtration and disinfection facilities at both sites would save these cities many millions of dollars.

Project Objectives

This project had two main objectives:

1. Validate a high-volume, analytical technique with increased sensitivity to determine log reduction of indigenous male-specific type 2 (MS2) coliphage through the SAT process. Testing was performed with samples from both Fresno and Dinuba.

2. Demonstrate that unfiltered secondary wastewater percolated into the ground is capable of meeting California tertiary recycled water filtration (low turbidity) and disinfection (5-log virus as measured by MS2 coliphage reduction and low-level coliform criteria) requirements. The regulatory targets for this project were selected to match those set by California Code of Regulations (CCR) Title 22 for filtration and disinfection of tertiary recycled water in California.

Although this project directly targets California regulations, the collection of particle size distribution (PSD) data, total coliform data, *Escherichia coli* data, and coliphage data allows for the determination of performance in accordance with other reuse regulations. The validated, high-volume, low-level virus enumeration method can be used worldwide to evaluate and monitor SAT disinfection performance.

High-Volume, Low-Level Virus Enumeration Analytical Technique Validation

Unfiltered secondary effluent typically has a relatively small concentration of coliphage. The United States Environmental Protection Agency (EPA) Method 1601 is by design a presence/absence test, but with multiple volumes at various dilutions it becomes a most probable number (MPN) test (similar to the coliform test). Method 1601 can thus measure the concentration of coliphage in 1 liter (L) samples, which often results in nondetected concentrations in unfiltered secondary effluent that has gone through SAT. Therein lies the

problem—the low concentration of organisms in the SAT influent (upstream of where the SAT occurs) combined with nondetected SAT effluent samples does not allow for quantification of log reduction through the SAT process with current methods (i.e., high log removal may be achieved but cannot be reliably measured). Such a technical difficulty requires a method modification to accommodate larger volumes to increase the detection sensitivity and thereby quantify virus (coliphage) log reduction through SAT.

To this end, BioVir Laboratories, Inc. (BioVir), has developed a method for validation in the presence/absence format of Method 1601 to an MPN format in sample volumes greater than 100 L. Validation of the high-volume method consisted of analyzing three 100 L volumes per sample so that a minimum 5-log reduction of coliphage in percolated water matrices could be documented and quantified. The validation procedure followed EPA Method 1601 for Tier 1 of EPA's performance-based measurement system (PBMS). Based on the PBMS, the California Department of Public Health (CDPH) requested that 10 replicates each of spiked reagent water and spiked field-sampled water with associated method blanks be analyzed. The spiked reagent water testing was completed by BioVir prior to this project.

The field-sample water testing was validated through a series of spiking studies at the Fresno and Dinuba sites. The goal was to achieve at least three positive results out of 10 samples collected during the spiking studies with an MS2 coliphage range of 1–3 plaque-forming units (pfu) per 300 L.

The high-volume, low-level virus enumeration analytical technique was successfully field validated at both the Fresno and Dinuba sites. At Dinuba, 6 of the 10 samples were positive. At Fresno, 3 of the 10 samples were positive. The technique showed the increased sensitivity required to demonstrate 5-log reduction of indigenous MS2 coliphage through SAT. Because of the presence of indigenous coliphage at Dinuba, a lower detection limit could not be determined.

Demonstration of Filtration and Disinfection Compliance Through SAT

The validated, high-volume, low-level virus enumeration analytical technique was used at both sites to evaluate the log reduction of indigenous MS2 coliphage that can be achieved through SAT. Unfiltered secondary effluent (SAT influent) and percolated and extracted water (SAT effluent) were repeatedly sampled for total and fecal coliforms and indigenous MS2 coliphage at both sites during the study.

Coliform analysis of the SAT influent and effluent by the traditional MPN method at Fresno and Dinuba resulted in an average of 5.56-log and 4.37-log reduction of total coliforms, respectively. The average SAT effluent coliform levels at Fresno and Dinuba were <2.0 MPN/100 milliliters (mL) and 4.8 MPN/100 mL, respectively. Sampling with the validated, high-volume method resulted in approximately 6-log removal of MS2 coliphage through SAT at Fresno and approximately 5-log removal at Dinuba. One of the 18 SAT effluent samples at Fresno and 2 of the 7 SAT effluent samples at Dinuba had MS2 levels greater than the analytical method measurement range and could not provide a log removal (therefore, data from these samples were not used in the analysis). The turbidity levels of the SAT effluent at Fresno were consistently less than 2.0 nephelometric turbidity units (NTU). At Dinuba, with the exception of two samples (2.50 NTU and 2.33 NTU), all of the SAT effluent turbidity levels were less than 2.0 NTU.

At Fresno, it was demonstrated that the SAT process was capable of meeting the CCR Title 22 tertiary recycled water filtration (turbidity at or below 2.0 NTU) and disinfection (5-log virus reduction and low-level coliform criteria of 2.2 MPN/100 mL, 7-day median) requirements. The data set for Fresno presented a more compelling case for full virus reduction credit than the data set for Dinuba, which indicated substantial virus reduction but did not definitively meet treatment criteria. Further testing would be needed to confirm that virus removal and SAT effluent coliform levels to meet treatment requirements could be attained consistently.

As part of the study, water chemistry data (chloride, sulfate, sodium, electrical conductivity, nitrate, and total dissolved solids) were collected to determine the influence of the secondary effluent on the extracted wastewater and demonstrate nondilution with surrounding groundwater. In addition, turbidity and PSD data were collected to characterize the samples. At both sites, the SAT effluent chemistry results were similar to the SAT influent and, for almost all parameters evaluated, very different from the upgradient potable groundwater. These data strongly support the assumption that there is no significant dilution with local groundwater during percolation and extraction (i.e., the observed log reduction in microorganisms was due to SAT, not dilution).

It is important to note that although the data from this study have value to other wastewater treatment plants, it is only directly applicable to the sites from which it was collected (Fresno or Dinuba) as it is dependent on water quality, the site-specific hydrology, and infiltration basin operational strategy.

Chapter 1

Introduction

Water is a scarce resource within the United States and the world. Recent years of drought conditions have exacerbated this issue, making water a major economic and environmental concern. Utilities currently rely upon advanced wastewater treatment (AWT) facilities, typically filtration and disinfection, to produce high-quality effluent as an alternative water supply. However, construction of additional AWT facilities and the added operational costs are often economic barriers to implementation. Further, AWT energy use adds to the already burgeoning climate change issue.

There is a significant need for reclaimed water treatment techniques that are low-cost and -impact. One such option is soil-aquifer treatment (SAT), where unfiltered secondary wastewater effluent is allowed to percolate into an existing groundwater source. This groundwater is later extracted for reuse. Although it has long been assumed that the percolation process is an environmental buffer to pathogens, detailed studies have been limited by insufficient analytical techniques. This project was designed to demonstrate the validity of a new, low-level, virus enumeration analytical technique and use this method to better quantify the true log removal of virus through SAT. Further, this project also examines the reduction in coliform bacteria and the improvement in water quality as measured by turbidity and particle size distribution (PSD).

1.1 Background

The City of Fresno, CA (Fresno), currently percolates approximately 68 million gallons per day (MGD) of unfiltered secondary effluent into the ground, and the City of Dinuba, CA (Dinuba), percolates 2.3 MGD of its unfiltered secondary effluent. Both utilities would like to capture this percolated water through extraction wells and reuse it for various applications.

Fresno has disposed of secondary treated effluent via percolation/evaporation ponds since approximately 1891. It intends to use reclaimed water in the future for irrigation and other approved reuse applications. Dinuba has disposed of secondary treated effluent via percolation/evaporation ponds since approximately 1922 and intends to use reclaimed water in the future for landscape irrigation and industrial cooling.

1.1.1 Regulatory Requirements

The Fresno-Clovis Regional Wastewater Reclamation Facility (RWRF) and the City of Dinuba Wastewater Reclamation Facility (WWRF) are both regulated by Waste Discharge Requirements (WDRs) issued by the California Regional Water Quality Control Board (RWQCB). WDRs are state permits issued to land dischargers that are not subject to federal jurisdiction nor the National Pollutant Discharge Elimination System (NPDES).

The State Water Resources Control Board (SWRCB) oversees the nine RWQCBs in the state. The SWRCB, the RWQCBs, and the California Department of Public Health (CDPH) have regulatory authority over projects using recycled water. The CDPH is the primary state

agency responsible for public health; whereas the SWRCB and RWQCBs are the primary state agencies charged with protection, coordination, and control of water quality. Generally, the CDPH interprets the state regulations applicable to recycling and makes recommendations on individual projects to the RWQCB. The RWQCB issues the permits for recycling projects.

The primary regulation governing recycled water use is California Code of Regulations (CCR) Title 22. Title 22 requirements are established and administered by the CDPH. Opportunities to use recycled water are maximized if the effluent is treated to the "disinfected tertiary" level, which allows the water to be used for unrestricted irrigation.

Disinfected tertiary recycled water refers to filtered wastewater that has been subsequently disinfected and does not exceed the following total coliform bacterial counts:

- 2.2 most probable number (MPN)/100 milliliters (mL) based on a 7-day median
- 23 MPN/100 mL in more than one sample in any 30-day period
- 240 MPN/100 mL on any day

Disinfection can be achieved by either

- A chlorine disinfection process that provides a contact time value of not less than 450 milligrams per minute per liter at all times, with a modal hydraulic contact time of at least 90 minutes, based on peak dry weather design flow; or

- A disinfection process that, when combined with the filtration process, has been demonstrated to inactivate or remove 99.999% of the plaque-forming units (pfu) of F-specific bacteriophage (coliphage) male-specific type 2 coliphage (MS2), or polio virus, in the wastewater. A virus that is at least as resistant to disinfection as polio virus may be used for purposes of the demonstration.

1.1.1.1 Fresno

The Fresno-Clovis RWRF operates under WDR Order No. 5-01-254. The RWRF has a rated capacity of 88 MGD. Current flows are roughly 68 MGD. The RWRF is an activated sludge facility that produces un-disinfected secondary effluent. Approximately 10% of the effluent is delivered to neighboring farmland for restricted irrigation of feed, fodder, and fiber crops. The majority of the effluent is discharged to 1750 acres of percolation/evaporation ponds. Approximately 20 to 45% of the percolated effluent is extracted by a network of on-site wells and delivered to canals owned by the Fresno Irrigation District (FID) for agricultural irrigation during the growing season.

Currently, there are no restrictions on the use of the extracted groundwater discharged to the FID canals. However, Provision H.19 of Fresno's WDR requires the RWRF to evaluate the degree of SAT provided by the current percolation and extraction operation. As stated previously, one purpose of this project is to determine the level of filtration and virus removal achieved and if the level of treatment is equivalent to Title 22 disinfected tertiary effluent.

1.1.1.2 Dinuba

The Dinuba WWRF operates under WDR Order No. 95-200. The WWRF is an activated sludge facility that produces un-disinfected secondary effluent. It has a rated capacity of 3.0 MGD. Current flows are approximately 2.3 MGD. The effluent is discharged to on-site percolation/evaporation ponds that cover 110 acres.

In 2008, Dinuba constructed a municipal golf course adjacent to the WWRF. It has investigated alternatives to producing disinfected tertiary recycled water to irrigate the golf course. Upgrading the WWRF to treat the secondary effluent with aboveground filtration and disinfection was found to be cost-prohibitive. Extraction of the percolated effluent is a much less costly alternative. However, unless Title 22 equivalency can be demonstrated, Dinuba would be required to further treat the extracted water.

1.1.2 Previous SAT Work

To demonstrate treatment performance and obtain CDPH approval in accordance with the listed standards, both utilities have previously investigated the reduction of indigenous coliform and virus (coliphage) through their respective SAT processes. The majority of work on this topic from Fresno is documented by Fresno and Schmidt (2003).

This work for Fresno examined the secondary effluent quality, infiltration ponds, extracted wastewater, and other sampling locations. The results indicated that the SAT process contributes to the removal of microbial constituents; the secondary effluent (SAT influent) coliform samples (both total and fecal) were consistently greater than 1600 MPN/100 mL, and the extracted well water (SAT effluent) coliform samples were consistently <2 MPN/100 mL. Virus (coliphage) results were less conclusive. The low level of influent samples combined with nondetected effluent samples at a low concentration did not allow for quantification of log reduction through the SAT process. Such a technical difficulty requires a method modification to accommodate larger volumes to increase the detection sensitivity and thereby quantify virus (coliphage) log reduction through SAT. Following the research by Fresno, Dinuba tested the water quality through its SAT process and found complete removal of coliphage and coliform down to the detection limit. As in Fresno, analytical limitations did not allow for full quantification of coliphage reduction at Dinuba to demonstrate the required 5-log reduction of virus.

1.2 Project Objectives

Although complete removal of coliphage and coliform were demonstrated in the previous work, the low sensitivity of the coliphage analytical technique did not allow quantification of a full 5-log reduction of coliphage through SAT. Thus, the two central objectives of this work were as follows:

- Demonstrate and validate a new, more sensitive, high-volume, low-level method for virus enumeration.
- Determine if the extracted water in Fresno and Dinuba (post-SAT) meets Title 22 filtration and microbiological disinfection requirements.

1.3 Related Research

1.3.1 The Efficacy of Infiltration

There have been a number of related investigations of virus removal through riverbank filtration in Europe (Schijven et al., 1999; Hoetzl and Reichert, 1996; Peters, 1998). Over 6-log removal of spiked MS2 and PRD1 bacteriophage through riverbank filtration was measured. After 10 days of travel time, MS2 and PRD1 removal were shown to be equivalent (Peters, 1998). As part of another riverbank filtration project (Hoetzl and Reichert, 1996), a 1- to 2-log reduction of a range of viruses was witnessed over a distance of only 20 meters (m). No viruses were detected beyond 100 m.

Fox et al. (2001) also looked at SAT for reuse. This work attempted to utilize indigenous coliphages as indicators of pathogen transport and process performance during SAT. Field studies demonstrated that such indigenous coliphages could be used as a surrogate for human viruses under actual recharge conditions. The relative absence of coliphages in monitoring wells using high-volume testing methods was consistent with the rate of coliphage removal observed during bacteriophage tracer studies. Extrapolation of tracer study data suggested a 7-log reduction of coliphages should occur within 100 feet of subsurface travel.

Shortcomings of the high-volume testing method (insufficient volumes could be recovered to allow precise measurements of log reduction) and the low concentrations of coliphages in the infiltration basins did not allow for an accurate quantification of virus removal through SAT. It should also be noted that the SAT systems studied by Fox et al. (2001) were either filtered and disinfected (Rio Hondo and San Gabriel sites) or disinfected (Tucson site) prior to infiltration. Fresno and Dinuba do not filter or disinfect prior to infiltration, an important difference.

1.3.2 Enumeration of Virus in Percolated Water

Previously, the United States Environmental Protection Agency (EPA) Method 1601 had been validated to demonstrate the presence/absence of virus (coliphage) in 1 liter(L) samples of water (EPA, 2003). However, 1 L samples typically do not have coliphage concentrations large enough to demonstrate the required 5-log reduction at the monitoring well. Such a technical difficulty requires a method modification to accommodate larger volumes to increase the detection sensitivity. To this end, BioVir Laboratories, Inc. (BioVir), has already validated a large volume (33.3 L), MPN grab-sample method both in the laboratory-simulated matrix and in a utility well water matrix. The primary objective of this project is to extend this work with higher volumes to further increase analytical sensitivity.

1.4 Project Approach

The technical approach to this project was broken into four tasks, as follows:

1. **Document infiltration basin operational strategies**. The operational approaches and known hydrogeology for both the Fresno and Dinuba infiltration sites were characterized to provide context for interpretation of the SAT performance data (Fox et al. [2001] indicated that the operational strategy and underlying hydrogeology for a particular wastewater infiltration basin can impact SAT performance).

2. **Demonstrate nondilution during infiltration and extraction.** The impact of dilution was investigated at both the Fresno and Dinuba sampling sites to substantiate whether dilution impacted SAT performance.

3. **Validate new, low-level, virus enumeration technique.** A high-volume filtration technique for the detection of extremely low concentrations of coliphage virus in percolated water was field validated. This technique is required to demonstrate a minimum of 5-log reduction of virus for "disinfected tertiary" reuse applications.

4. **Characterize the log removal of microorganisms through SAT.** After the analysis method was validated, the next step of the study was to sample and compare the concentrations measured in the field to that of the unfiltered secondary effluent to demonstrate the efficacy of SAT on removal of microorganisms.

Extracted percolated water was sampled for indigenous total and fecal coliforms and MS2 coliphage at both the Fresno RWRF and the Dinuba WWRF. The coliphage samples were analyzed using the validated, high-volume, low-level virus enumeration method. Unfiltered, undisinfected secondary effluent was sampled just prior to percolation to develop a baseline level of indigenous total and fecal coliforms and MS2 coliphage at both the Fresno RWRF and the Dinuba WWRF. These prepercolation data were then compared to the extracted percolated water to calculate the log reduction of all microorganisms.

Chapter 2

Materials and Methods

2.1 Sampling Sites

2.1.1 Fresno

MS2 coliphage, total coliform, and *Escherichia coli* (*E. coli*) sampling was done at two sites in Fresno: the secondary effluent at the RWRF (grab sampled at the secondary clarifier) and a reclamation well (R11). Water quality sampling was taken at three sites in Fresno, including the two previously mentioned sites and a monitoring well upgradient of the percolation ponds (MW-10B). The relative locations of these sites and regional hydrogeology are discussed in Section 3.1.

2.1.2 Dinuba

MS2 coliphage, total coliform, and *E. coli* sampling was done at two sites in Dinuba: the secondary effluent at the WWRF and a reclamation well (RW#1). The relative locations of these sites and regional hydrogeology are discussed in Section 3.2.

Dinuba pilot RW#1 had not been in operation for some time and did not have a pump installed at the start of the project. For this work, a pump was installed, and the well was inspected, chlorinated, and flushed prior to sampling. A video survey of the well was conducted, and it was found that the well perforations were partially plugged. Consequently, the well was flushed continuously for 2 days to draw from all formations within reach of the well. Flushing of the well for 2 days and sampling for chlorine residual ensured that chlorination of the well did not affect sampling. After the flushing was complete, MS2 coliphage, total coliform, and *E. coli* samples were collected.

2.2 Water Quality Assessment Procedure

Water quality parameters for all the water sources evaluated were assessed using standard laboratory methods in certified laboratories. Table 2.1 lists the analytical methods employed for this project and the laboratory that performed the services. The methods for the historic water quality data taken prior to this project are not included.

The particle size sampling was done to provide additional qualitative data as to the high quality of percolated and extracted water. PSD analysis was done with the Model 770 AccuSizer, Particle Sizing Systems, Inc.

The virus enumeration method is described in more detail in the next section.

Table 2.1. Analytical Methods Employed

Parameter	Method(s)	Laboratory
Total Coliform	Colilert® (SM 9223 B) and MPN (SM 9221C)	Fresno
E. coli	Colilert® (SM 9223 B)	Fresno
Low-Level MS2 Coliphage	BioVir Modification of EPA Method 1601	BioVir
MS2 Coliphage	Kott (1966)	BioVir
Turbidity	EPA 180.1	Fresno
Particle Counts	Model 770 AccuSizer, Particle Sizing Systems, Inc.	Equipment owned by Carollo Engineers
UVT	UV/Vis Spectrophotometer	Equipment owned by Carollo Engineers
Electrical Conductivity	SM 2510 B	Fresno
Total Dissolved Solids	SM 2540 C	Fresno
Sulfate	EPA 300.0	Fresno
Chloride	EPA 300.0	Fresno
Sodium	EPA 200.7	Fresno
Potassium	EPA 200.7	Fresno

Note. MS2 = male-specific type 2; UVT = ultraviolet transmittance

2.3 High-Volume, Low-Level MS2 Coliphage Enumeration Procedure

A new virus enumeration method developed by BioVir was field tested at both Fresno and Dinuba. The full, detailed protocol is included in Appendix A. A brief summary of the method is included here.

MS2 coliphage virus was enumerated using a method modification of EPA Method 1601, *Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure* (EPA, 2001). EPA Method 1601 is designed for the detection of bacteriophage, including male-specific coliphage and somatic coliphage. However, this method modification is only for detecting MS2 coliphage. This new method is a presence/absence–based method originally designed for 1 L volumes. In this modified method format, large volumes of water (e.g., 300 L for this work) are passed through negatively charged microporous filters, which catch the viruses by electrostatic attraction. Each filter is then directly assayed, without virus extraction, in an MPN format.

Upon receipt at the lab, the individual filters and any residual water are each collected separately in an appropriate container (bag or vessel). Appropriate volumes of growth media with supplements are added to the filter container along with the bacterial host (*E. coli*) to grow the coliphage present. Following overnight incubation, the presence of the target coliphage is confirmed by placing a portion of the sample culture from each filter sample onto a plate with host bacteria (spot plate). The presence of coliphage on the spot plate test confirms the presence of coliphage from that specific filter sample. The number of positive filters within a set of filters is recorded, and the MPN is calculated. The MPN method allows

for the estimation of the coliphage concentration from the original volume of the sample. The MPN approach of this method is based on that described by Kott (1966).

2.4 Method Validation Requirements

The validation procedure followed EPA Method 1601 for Tier 1 of EPA's performance-based measurement system (PBMS). The acceptability goal of at least three positive results out of 10 samples with a coliphage spike in the 1–3 plaque-forming units (pfu) range per 300 L was taken directly from EPA Method 1601, Section 14.0 Method Performance (EPA, 2001). The acceptability criteria are based upon a statistical analysis the EPA performed with the labs that conducted the validation study for this method. In addition, it is the goal that was specified by the CDPH. On the basis of the PBMS, the CDPH requested that 10 replicates each of spiked reagent water and spiked field-sampled water with associated method blanks be analyzed.

The goal is to meet the criteria of at least three positive results out of 10 samples with a coliphage spike in this range, as described previously. The results of the well field samples can be used to demonstrate the validity of this large volume MPN format for the detection of low levels of coliphage in percolated water and further used to evaluate if 5-log reduction can be achieved through measurement of secondary effluents from both utilities.

2.5 Reagent Grade Water Method Validation

The method validation in spiked reagent grade water was completed prior to this project by BioVir, with 10 samples averaging 1–3 pfu per 300 L (Danielson et al., 2008).

2.6 Extracted Water Method Validation

Extracted water sampling consisted of collecting and filtering multiple 300-L utility well field samples on filters on-site at both Fresno and Dinuba. BioVir spiked the utility water samples at 1–3 pfu/300 L in portable tanks, filtered through a pilot size filter in the field for coliphages, and the sample filters were transported to BioVir for final measurements by the modified EPA 1601 MPN method.

2.6.1 Matrix Spike Test at Fresno

On September 8, 2010, BioVir conducted a set of matrix spike (spike) tests at R11. The sample concentration method used three 5 in. NanoCeram electropositive filters run in parallel to process a total of 300 L (80 gallons) for each spike. Seed MS2 bacteriophage (ATCC 15597b) was prepared in quintuplicate the day before the test at BioVir and transported on ice to the site. Two of the MS2 seed aliquots were assayed on the day of seed preparation (September 7, 2010) to provide an MS2 concentration estimate so the proper volume would be added during the spike tests.

Based on the previous day's assay (September 7, 2010) to estimate the proper volume for spiking, the MS2 concentration was estimated to be at about 79 pfu per spike dose, far above the expected 2–3 pfu/dose. Consequently, the spiking solution was diluted at the Fresno lab to reduce the concentration to the target level (2–3 pfu/dose). The filter equipment was set up and connected to the sample port at RW#11. An initial 300 L sample was processed without

MS2 addition to act as a nonspike background control. Then, 13 matrix spike samples were processed. Approximately 150 L was allowed to pass through the filters prior to the addition of the spike suspension, and this was followed by the remaining volume for that filter set (about another 150 L). At the end of the 300 L filter run, the flow was shut off and the filters disconnected from the sampling rig and placed on ice in coolers for transport back to BioVir. A 15th sample was collected and spiked with approximately 100 pfu of MS2 to act as a positive control.

On October 6, 2010, BioVir conducted the second spike test at Fresno at R11 using the same methodology as Test 1. For this test, based on the previous assay (October 5, 2010) to estimate the proper spiking volume, the MS2 concentration was estimated to be at about 2 pfu per spike dose. Ten matrix spike samples were processed. Results for both tests are detailed in Chapter 3.

2.6.2 Matrix Spike Test at Dinuba

On September 29, 2010, BioVir conducted the spike test at RW#1. As with the Fresno tests, the filter concentration method used three 5in. NanoCeram electropositive filters run in parallel to process a total of 300 L for each run. Seed MS2 bacteriophage (ATCC 15597b) was prepared in quintuplicate the day before the spike at BioVir and transported on ice to the site. Two of the MS2 seed aliquots were assayed on the day of preparation (September 28, 2010) to provide an estimate for the proper volume to be added during the spiking tests.

The laboratory confirmed that the spike concentration average from two randomly chosen MS2 seed aliquots was about 0.7 pfu per spike portion, lower than the expected 2–3 pfu/dose. Consequently, in order to be sure that enough spike was present (~2 pfu per spike), two of the field MS2 seed aliquots were combined and used for each spiking exercise.

The filter equipment was set up and connected to the sample port at RW#1, which was modified to provide sufficient pressure to conduct the test. An initial 300 L sample was processed without the spike to act as a nonspiked background control. Ten matrix spike samples were then processed. Approximately 150 L was allowed to pass through the filters prior to the addition of the spike suspension, and this was followed by the remaining volume for that filter set (about another 150 L). At the end of the 300 L run, the flow was shut off and the filters disconnected from the sampling rig and placed on ice in coolers for transport back to the lab. An additional sample was collected and spiked with approximately 100 pfu of MS2 coliphage to act as a positive control. Results are detailed in Chapter 3.

2.7 Background Coliphage and Coliform Testing

Sampling of secondary effluent and extracted water was conducted to determine a baseline level of microorganisms to allow comparison of SAT influent and effluent. Coliphage results were used to calculate virus log reductions, and coliform results were used to determine reductions and whether SAT met Title 22 coliform requirements at both sites.

2.7.1 Testing at Fresno

Sampling to determine background levels for coliphage was conducted at R11 on 6 days using the high-volume, low-level virus enumeration technique. Sampling consisted of filtering 300 L of extraction well water through three filters for each sample. The filters were

transported to BioVir for analysis by the modified EPA 1601 MPN method. Samples were also taken from R11 for total coliform and *E. coli* analysis. Enumeration was done using SM 9221C and the Colilert method (SM 9223B; American Public Health Association, 1992).

The secondary effluent was also sampled for MS2 coliphage, total coliform, and *E. coli*. Coliphage samples from the secondary effluent were not concentrated but were enumerated by the MPN method of Kott (1966). Analysis methods for indicator bacteria were as described previously. Results are detailed in Chapter 3.

2.7.2 Testing at Dinuba

Sampling to determine the background levels for coliphage was conducted at RW#1 on 2 days. For each sample collected, two filter units with three filters each were connected to the spigot on the well pipe. Because the well pipe was an open system, there was insufficient back pressure for a full 300 L sample through a single filter set during the first sampling event. Running one sample at 0.5 gallon per minute (gal/min) through all six filters required 3 to 4 hours. Piping on the well was reconfigured to provide adequate back pressure for the next sampling event. Samples were analyzed by the modified EPA 1601 MPN method. Samples were also taken from the extraction well for total coliform and *E. coli* analysis. Enumeration was done using SM 9221C and the Colilert method (SM 9223B; American Public Health Association, 1992).

The secondary effluent was also sampled for MS2 coliphage, total coliform, and *E. coli*. Coliphage samples from the secondary effluent were not concentrated but were enumerated by the MPN method of Kott (1966). Analysis methods for indicator bacteria were as described previously. Results are detailed in Chapter 3.

Chapter 3

Results and Analysis

The results and analysis for the Fresno and Dinuba sites are presented and discussed separately. Although the data from this study do have value to other wastewater treatment plants, they are only directly applicable to the sites from which they were collected (Fresno or Dinuba, as applicable) as it is dependent on water quality, the site-specific hydrogeology, and infiltration basin operational strategy.

3.1 Fresno

3.1.1 Infiltration Basin Operational Strategies and Hydrogeologic Conditions

Currently, Fresno has 1750 acres of percolation ponds that receive 68 MGD of unfiltered secondary effluent. In August 2010, 782 acres of these ponds were being used. Secondary clarifier effluent flows to a series of canals or ditches and is evenly distributed to the ponds. Pond levels, freeboard, and erosion are checked and recorded daily. When ponds require rehabilitation, the inlet valve is closed, and the water is left to percolate or it is pumped out. Once the pond is empty, it is allowed to dry for 30 to 40 days. Any sediment buildup is removed, and rubble is replaced to reduce erosion. If necessary, the bottom of the pond is then leveled to promote drainage away from the inlet valve. After rehabilitation, the pond bottom is ripped with a slip plow to reduce compaction. It is then ready to be put back into service when needed. There is no set standard for selecting ponds to be put in service. Typically, ponds are rehabilitated every 3 years.

The RWRF lies within a semiarid region in the Fresno Hydrologic Area (No. 551.30), which is within the Kings River Basin. Local soils consist of unconsolidated alluvial deposits of interbedded layers of sand, gravel, silt, sandy clay, clay, and localized cobble zones. Soils in the upper 5 feet are described as well-sorted sands with good permeability.

Figure 3.1 charts the area geography. Area groundwater comprises the north portion of an essentially closed groundwater basin (Tulare Lake Basin) and flows southwesterly under unconfined conditions from the foothills east of Fresno westward to a northwest-trending line through Kerman and Raisin City. West of that line, groundwater occurs under both unconfined and semiconfined conditions.

Groundwater in the RWRF vicinity occurs within three zones: a shallow, unconfined zone consisting of the upper 50 feet of the aquifer; a semiconfined lower zone, the top of which is about 200 feet below the water table; and a deep zone below the confining bed. Fresno uses monitoring wells at all three depths to track groundwater quality. The shallow zone is monitored by A-series wells, the upper deep zone by B-series wells, and the lower deep zone, below the confining bed, by C-series wells.

Figure 3.2 illustrates the locations of the monitoring well network. Fresno monitors area groundwater by this network, which is currently composed of 24 wells around the RWRF. Many of the well sites have two to three wells in series (a shallow A-series well, an upper deep B-series well, and a lower deep C-series well).

Figure 3.1. The Fresno-Dinuba study area.

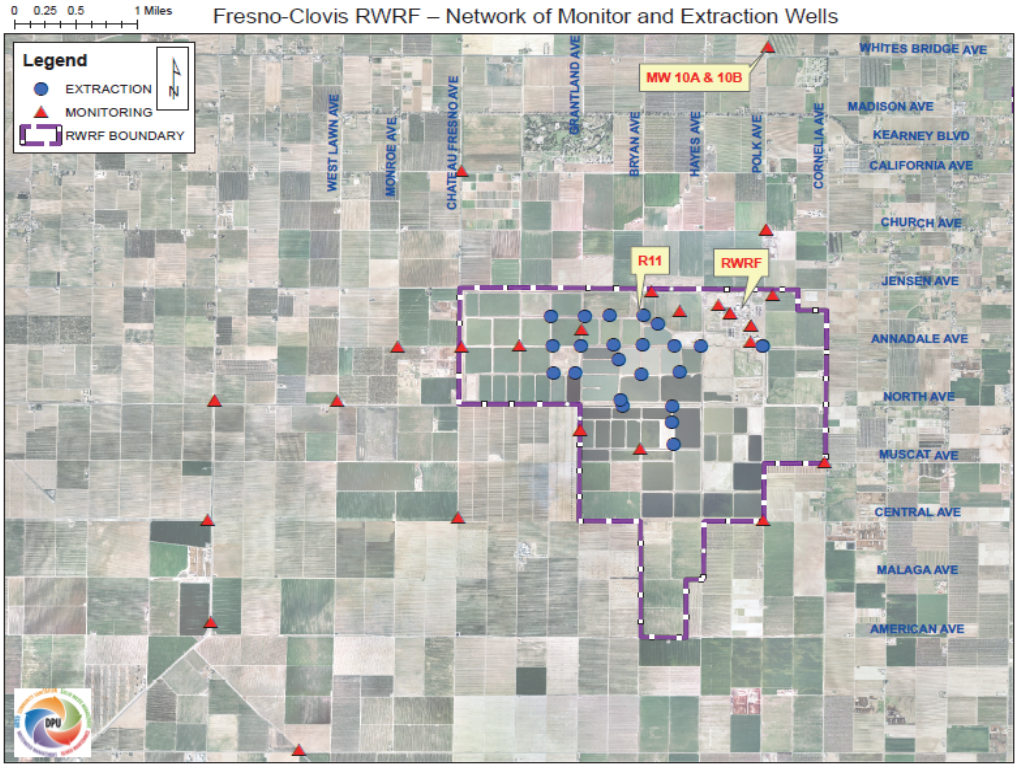

Figure 3.2. Fresno monitoring well network.

The approximate location of R11, which is the extraction well studied as part of this project, is shown on the map to the west of the RWRF. It is 260 ft deep, with a perforation interval of 203 to 253 ft.

The upgradient groundwater quality is measured at two wells, MW-10A and MW-10B, which are located approximately 10,000 ft north of the RWRF. The background (upgradient) groundwater quality is highly variable because of the presence of dairies and other land uses that affect water quality. The deep B-series monitoring wells, including MW-10B, are similar to the extraction well in depth and perforation interval. The upgradient water data are meant to represent groundwater unaffected by secondary effluent.

3.1.2 Demonstration of Nondilution During Infiltration and Extraction

An important assumption in this work is that the extracted water is not diluted; that is, the measured reduction in bacteria and virus is based on treatment and not dilution.
Figures 3.3 through 3.8 show comparisons of the MW-10B (upgradient groundwater), RWRF secondary effluent, and R11 water qualities. The extracted well water average electroconductivity (EC), total dissolved solids (TDS), sodium, chloride, nitrate, and sulfate levels were in the same range as the RWRF secondary effluent (Table 3.1). The levels measured in the upgradient groundwater well, MW-10B, were very low compared to the other two sampling locations, with the exception of nitrate. The differences between nitrate levels are likely due to biological activity.

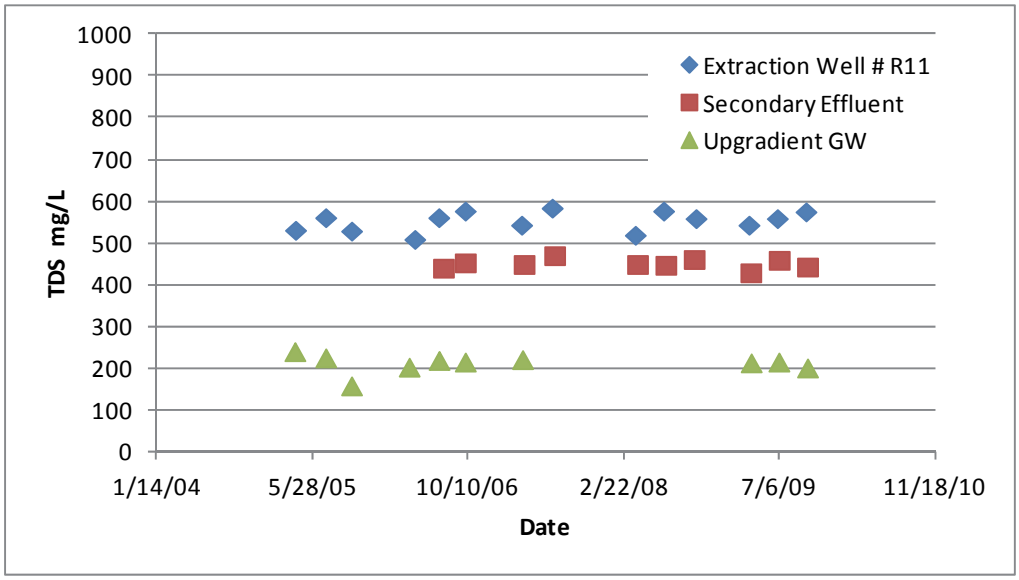

Figure 3.3. Fresno water quality comparison—TDS.

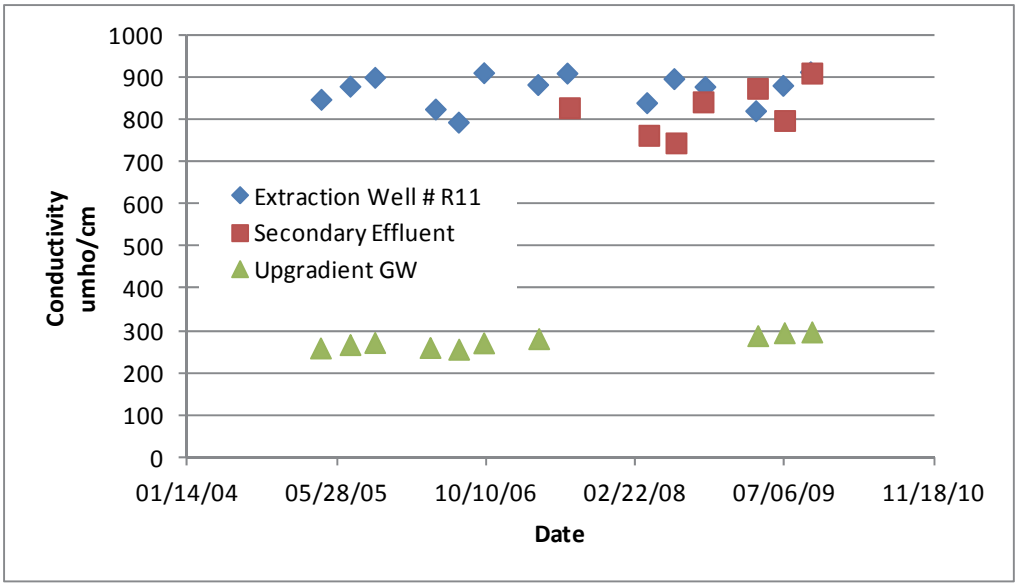

Figure 3.4. Fresno water quality comparison—EC.

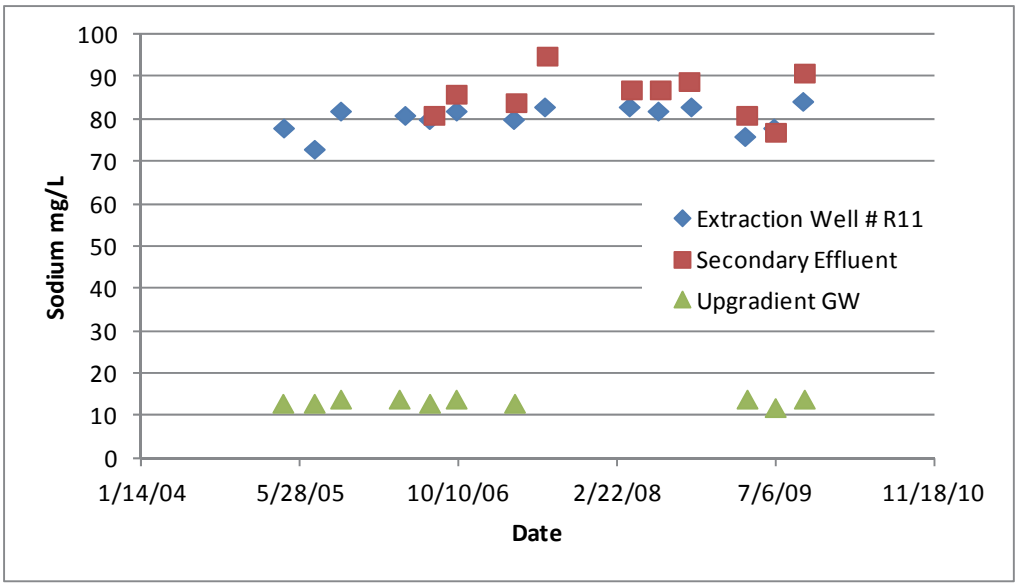

Figure 3.5. Fresno water quality comparison—sodium.

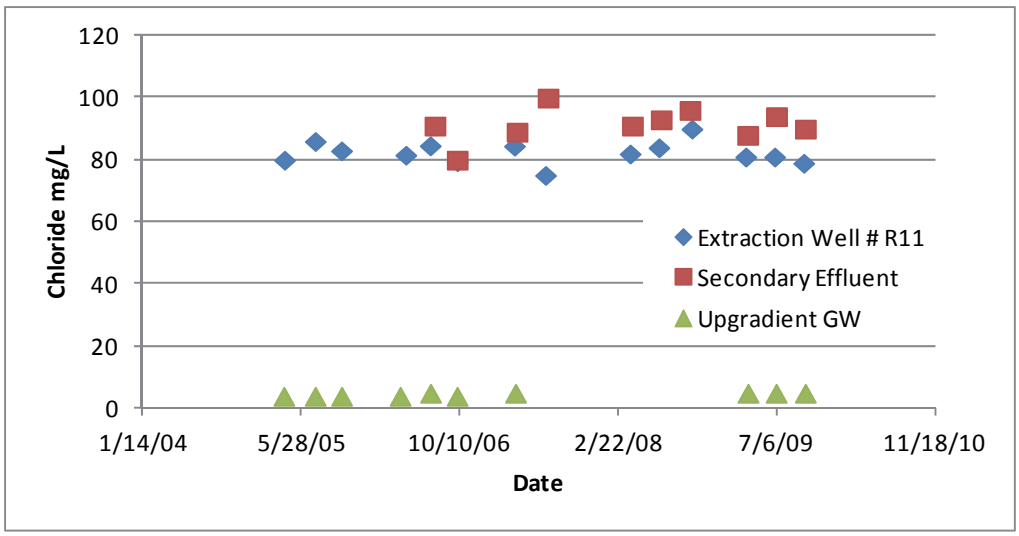

Figure 3.6. Fresno water quality comparison—chloride.

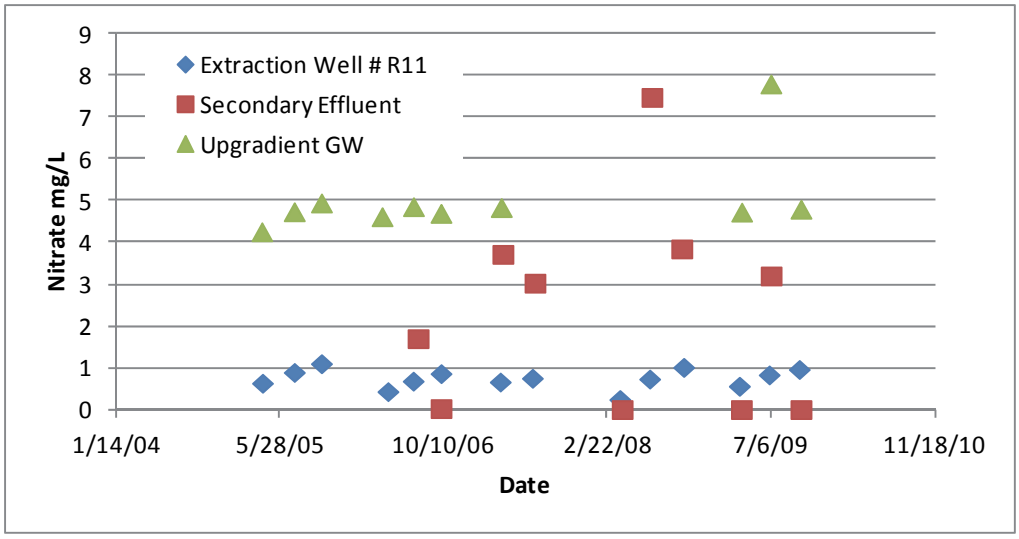

Figure 3.7. Fresno water quality comparison—nitrate.

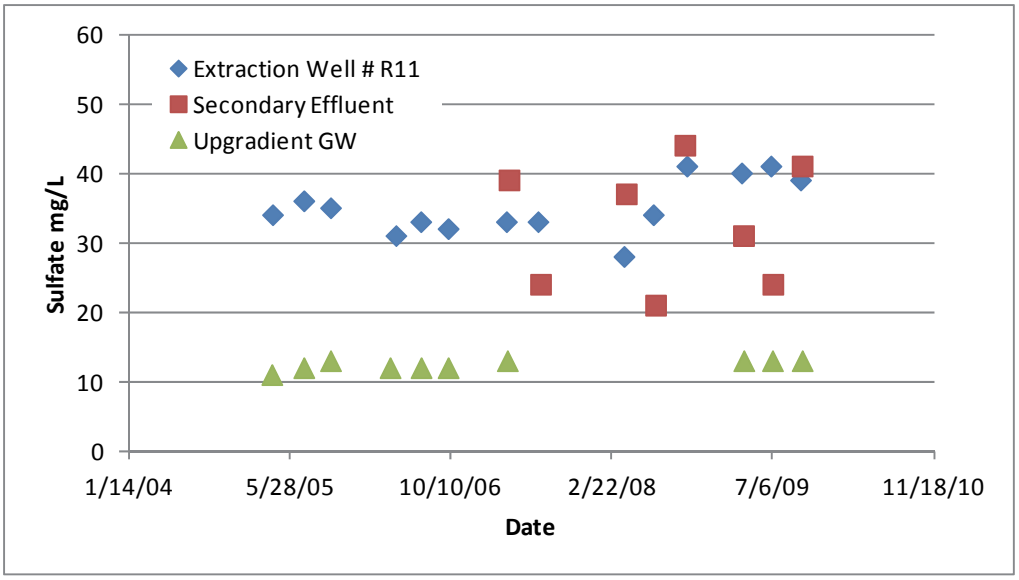

Figure 3.8. Fresno water quality comparison—sulfate.

Table 3.1. Water Quality Characteristics—Fresno

Parameter	R11	Secondary Effluent	Upgradient Groundwater
Conductivity, µmho/cm	868	822	274
TDS, mg/L	552	449	210
Sodium, mg/L	80	86	13
Chloride, mg/L	91	82	4.5
Nitrate, mg/L	0.7	2.3	5.0
Sulfate, mg/L	35	33	12

Note. µmho/cm = micromhos per centimeter; mg/L = milligrams per liter; TDS = total dissolved solids

To confirm a statistically significant difference between the average concentrations of each of the three water types, Student t-tests at a 99% confidence level (CL), assuming unequal variances, were run. The data evaluated spanned 2005–2009 for R11 and the upgradient groundwater and 2006–2009 for the secondary effluent. Student t-test is a standard statistical method for comparing "populations." Unequal variances were assumed because the variances (scatter) of the data sets were very different; for example, there was much less variance in the upgradient groundwater data than in the R11 data, as illustrated in Figure 3.3. A 99% CL means that there is a 1% chance that the test will find a difference between the two sets of data where there is none. The results are presented in Table 3.2.

As expected, at a 99% CL (probability or "p-value" ≤ 0.01), the average concentrations of the constituents evaluated were very different between the upgradient groundwater and both the secondary wastewater and R11 water, with the exception of nitrate. There was no trend in the comparison between the secondary effluent and the extraction well. The average conductivity, TDS, and nitrate concentrations were significantly different between the extraction well and the secondary effluent; the chloride, sodium, and sulfate levels were not significantly different.

Table 3.2. T-test Analysis of Water Quality Differences Among Sampling Locations for Fresno

Samples Compared	p-value	Different at a 99% confidence level?
R11 vs. Secondary Effluent		
Conductivity	9.24×10^{-6}	Yes
TDS	1.12×10^{-14}	Yes
Sodium	0.0106	No
Chloride	0.0241	No
Nitrate	2.88×10^{-11}	Yes
Sulfate	0.827	No
R11 vs. Upgradient Groundwater		
Conductivity	7.07×10^{-23}	Yes
TDS	1.24×10^{-24}	Yes
Sodium	6.98×10^{-21}	Yes
Chloride	4.02×10^{-12}	Yes
Nitrate	7.43×10^{-24}	Yes
Sulfate	4.78×10^{-12}	Yes
Secondary Effluent vs. Upgradient Groundwater		
Conductivity	1.01×10^{-50}	Yes
TDS	1.78×10^{-33}	Yes
Sodium	7.70×10^{-80}	Yes
Chloride	1.81×10^{-12}	Yes
Nitrate	0.0298	No
Sulfate	1.01×10^{-21}	Yes

Note. p-value = probability; TDS = total dissolved solids

The significant differences in TDS and conductivity between secondary effluent and the extraction well water could potentially be due to dissolution of minerals. The differences between nitrate levels are likely due to biological activity.

These data support the conclusion that there was no dilution during infiltration and extraction. In other words, these data strongly support the conclusion that any significant drop in microbial counts between the secondary effluent that is percolated into the ground and the extraction wells is not due to dilution but to the SAT process.

3.1.3 High-Volume, Low-Level Coliphage Enumeration Method Validation

As described previously, the acceptability goal of at least three positive results out of 10 samples with a coliphage spike in the 1–3 pfu range per 300 L must be demonstrated to validate the method. This was also the goal specified by the CDPH.

The high-volume, low-level virus enumeration technique was successfully validated at Fresno (Table 3.3). In the first sampling event (9/8/2010), the coliphage were spiked at the wrong concentration. However, a second test event (10/6/2010) was successful.

In the first sampling event, the laboratory measurement of the spike the day before the sampling event was approximately tenfold higher than expected. Two additional travel spike controls were conducted upon return to the lab and were consistent with the calculated theoretical concentration. The stock phage concentration, from which the spike was made,

was confirmed to be at the expected level. It appears that the dilution conducted at the city lab was unnecessary and that the diluted spike was below detection. The positive control was expected to have three out of three positive filters, but resulted in a single positive filter. Therefore, the positive control, which was also estimated based on the original assay, was far below the expected concentration.

During the second sampling event, the minimum required 3 of the 10 samples were positive (Table 3.3). The nonspike background control was negative, and the positive control was positive for all three filters.

3.1.4 Removal of Microorganisms Through SAT

Both the extraction well water and secondary effluent from the RWRF were tested for the presence of indigenous MS2 coliphage (Table 3.4). The average MS2 concentration in the secondary effluent was 2611 ± 453 MPN/100 mL. No MS2 was detected in most of the extraction well field samples; only one sample was greater than 0.0004 MPN/100 mL. The "greater than" value does not provide enough information to determine a 5-log reduction for that sample. Therefore, this sample was not used in the data analysis. In the future, if a sample is "greater than" using the high-volume, low-level method, there should be a resample and analysis carried out with a smaller sample volume to determine concentration. Collection of a 1 gallon grab sample at the time of sampling with the high-volume, low-level method would allow additional testing to be conducted in the event of a "greater than" result.

The MS2 concentration was calculated as an MPN, which is also used for total coliform measurements and is familiar to the industry. MPN results are not an absolute measure but an "index" number. Therefore, traditional parametric statistics do not apply to MPN results. Because the present method MPN is based on a "three-tube, one-dilution" format, there is not a traditional table readily available. Instead of using an MPN table, the result is calculated from the Thomas Formula found in Standard Methods Section 9221.C (APHA, 1992).

The 5-log virus reduction required by CDPH was demonstrated within the upper 95% CL of the test. At the method detection limit (0.0004/100 mL), the estimated 95% CL is 0.00013 to 0.0012 MPN (BioVir). The estimated 95% CL can be added to the MPN indices reported. When compared to the upper range of the 95% CL (0.0012), the average concentration of phage from the secondary effluent needed to be 120 MPN/100 mL (LOG 120 – LOG 0.0012 = 5) in order to demonstrate a 5-log reduction. At Fresno, both the average secondary effluent phage concentration (2611) and the lower 95% CL concentration (1723 MPN/100 mL) were over tenfold more than 120 MPN/100 mL.

Simply put, the mean data set for log reduction through percolation and extraction exceeds 6.0 for the RWRF.

Table 3.3. MS2 Virus Enumeration Test Results for Fresno

Date	MPN Result	MPN/100 mL	Comment
9/8/2010	0/3	<0.0004	Nonspike background
	0/3	<0.0004	Spiked 0.07 pfu/300 L
	0/3	<0.0004	Spiked 0.07 pfu/300 L
	0/3	<0.0004	Spiked 0.07 pfu/300 L
	0/3	<0.0004	Spiked 0.07 pfu/300 L
	0/3	<0.0004	Spiked 0.07 pfu/300 L
	0/3	<0.0004	Spiked 0.07 pfu/300 L
	0/3	<0.0004	Spiked 0.07 pfu/300 L
	0/3	<0.0004	Spiked 0.07 pfu/300 L
	0/3	<0.0004	Spiked 0.07 pfu/300 L
	0/3	<0.0004	Spiked 0.07 pfu/300 L
	0/3	<0.0004	Spiked 0.07 pfu/300 L
	0/3	<0.0004	Spiked 0.07 pfu/300 L
	0/3	<0.0004	Spiked 0.07 pfu/300 L
	(1/3)	0.0004	Positive control
10/6/2010	0/3	<0.0004	Nonspike background
	(1/3)	**0.0004**	**Spiked = 2.2 pfu / 300 L**
	0/3	<0.0004	Spiked = 2.2 pfu / 300 L
	0/3	<0.0004	Spiked = 2.2 pfu / 300 L
	0/3	<0.0004	Spiked = 2.2 pfu / 300 L
	(1/3)	**0.0004**	**Spiked = 2.2 pfu / 300 L**
	0/3	<0.0004	Spiked = 2.2 pfu / 300 L
	0/3	<0.0004	Spiked = 2.2 pfu / 300 L
	(2/3)	**0.0012**	**Spiked = 2.2 pfu / 300 L**
	0/3	<0.0004	Spiked = 2.2 pfu / 300 L
	0/3	<0.0004	Spiked = 2.2 pfu / 300 L
	(3/3)	>0.0017	Positive control

Note. L = liter; mL = milliliter; MPN = most probable number; MS2 = male-specific type 2; pfu = plaque-forming units

The coliform data tell a similar story. These data indicate that there was more than 5.5-log difference in total coliform concentrations between the secondary effluent and the extracted well water. The concentration of *E. coli* was reduced in excess of 5-log as well (Table 3.5). No *E. coli* was found in the extracted water. Total coliforms were not detected (that is, <2.0 MPN/100 mL) in the extracted water using the traditional MPN method. Total coliforms were detected at low concentrations using the Colilert method (Table 3.5).

Table 3.4. MS2 Coliphage Results for Fresno

Secondary Effluent (2009-2010)		
		MPN/100 mL
Mean		2611
Standard Error		453
Median		1781
Mode		3000
Standard Deviation		2562
Range		9000
Count		32
R11		
Date	**Sample #**	**MPN/100 mL**
7/16/2010	1	<0.0004
	2	<0.0004
	3	<0.0004
	4	<0.0004
4/5/2011	1	<0.0001
	2	<0.0001
	3	<0.0001
4/18/2011	1	<0.0001
	2	<0.0001
	3	<0.0001
5/17/2011	1	0.00038
	2	0.00013
	3	>0.00057
5/24/2011	1	<0.00011
	2	<0.00011
	3	<0.00011
5/31/2011	1	<0.0001
	2	0.0001

Note. mL = milliliter; MPN = most probable number; MS2 = male-specific type 2

Two methods were used to measure coliform concentrations, the Colilert method and the more common MPN method. The MPN method, performed on 50 mL samples, relies on organism growth. Therefore, if damaged or weakened organisms are present they will not be enumerated, which may result in an underestimation of the actual coliform density. The Colilert method, performed on 100 mL samples, simultaneously detects total coliforms and *E. coli*. It detects whether the enzymes specific to total coliforms and *E. coli* are present (e.g., the total coliform enzyme *β*-galactosidase and the *E. coli* enzyme *β*-glucuronidase). The Colilert method does not rely on growth of the organisms, resulting typically in higher counts, and so is a more conservative measure than MPN. Both methods support the hypothesis that SAT can achieve the Title 22 total coliform requirement of 2.2 MPN/100 mL (based on a 7-day median) at the RWRF.

Besides total coliform and *E. coli*, PSD, UV transmittance (UVT), and turbidity were measured. Of the 21 samples analyzed, an estimated average of 94% (with a range of 73 to

99%) of particles was removed through SAT. The low turbidity (average 0.27 NTU, range 0.14 to 0.54 NTU) and high UVT (89 to 91%) of the extraction well water indicate high water quality. The PSD data are shown graphically in Appendix B. Turbidity and UVT results for each sample are contained within the PSD graphs.

These data, combined with the microbiological results, support the conclusion that SAT is able to provide full "disinfected tertiary" treatment to RWRF secondary effluent.

Table 3.5. Coliform Results for Fresno

Sample	*E. coli*	Total Coliform	
	Colilert (MPN/100 mL)	Colilert (MPN/100 mL)	MPN (MPN/100 mL)
Secondary effluent	47,900	3,076,000	500,000
	173,000	1,019,000	500,000
	148,000	960,000	1,100,000
	379,000	1,274,000	800,000
Average	**186,975**	**1,582,250**	**725,000**
R11	<1.0	<1.0	<2.0
	<1.0	<1.0	<2.0
	<1.0	3.1	<2.0
	<1.0	1.0	<2.0
Average	**<1.0**	**2.1**	**<2.0**
Log difference between the secondary effluent and extraction well	**5.27**	**5.89**	**5.56**

Note. All < values, which indicate that the number was below the method detection limit (MDL), were estimated to be equal to the MDL to allow a conservative estimate of the average concentration.
mL = milliliter; MPN = most probable number

3.2 Dinuba

3.2.1 Infiltration Basin Operational Strategies and Hydrogeologic Conditions

Dinuba sends 2.3 MGD of unfiltered secondary effluent to 110 acres of percolation ponds. It does not use all 110 acres at one time. Pond usage is rotated in a manner similar to Fresno's process. The water then filters vertically and horizontally through more than 110 feet of alluvial soil. Area groundwater is monitored by a groundwater monitoring well network currently composed of seven wells in the vicinity of the WWRF (Figure 3.9). Dinuba has nine active groundwater supply wells located within the city limits on the northwest, southeast, and southwest sides of town. The beneficial uses of the underlying groundwater are municipal and domestic, industrial, and agricultural supply.

Figure 3.1, shown previously, maps the study area. Figure 3.9 contains a map of the WWRF, percolation ponds, monitoring wells, and extraction well. As a point of reference, the WWRF is located on the southwest edge of town, outside the city limits.

Soils in the area are predominantly high-permeability Delhi sandy loams. The geologic log from the drilling of the extraction well that was used for this project RW#1, indicates that

above 180 feet, the soil consists of silty fine-to-medium sand, sandy clay, and fine-to-medium sand.

RW#1 was installed as part of a previous study conducted from July to September 2006. This study was performed to characterize the water quality and observe aquifer conditions. Dinuba has not used the well for some time and needed to rent and install a well pump for this work. RW#1 is a 12-in.-diameter well, sealed to a depth of 110 ft, with perforations from 135 to 235 ft. The standing water level in the well is approximately 40 ft below the ground surface.

Monitoring well #6 (MW6), which is located in the vicinity of RW#1, was used to approximate the water quality of RW#1 because RW#1 was not in recent service prior to this study. MW6 is similar in depth and perforation interval to RW#1.

3.2.2 Demonstration of Nondilution During Infiltration and Extraction

Figures 3.10–15 show comparisons of the water quality at MW6 (used to approximate the water quality at RW#1), the WWRF secondary effluent, and Dinuba's drinking water wells. The drinking water well data represent the detected average reported in Dinuba's 2007 and 2009 water quality reports. The drinking water data are meant to represent groundwater unaffected by secondary effluent.

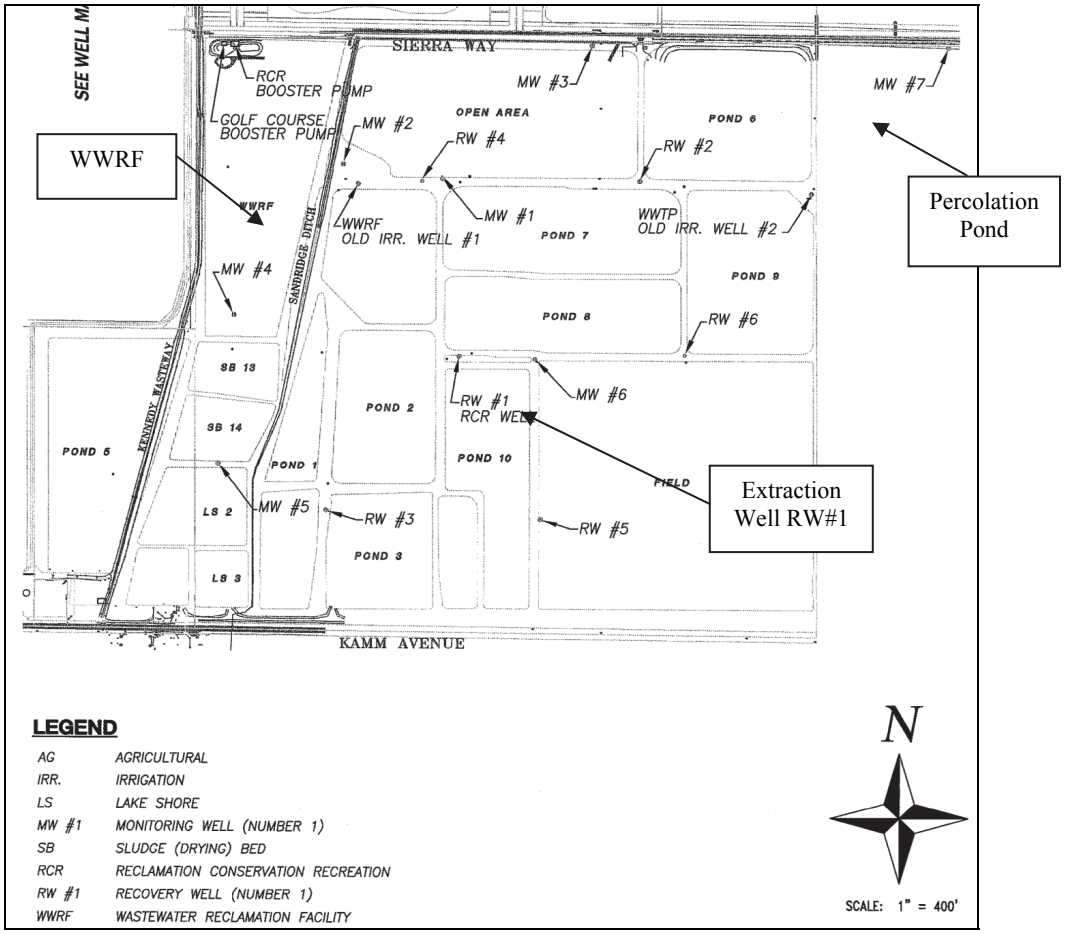

Figure 3.9. Map of the Dinuba study site.

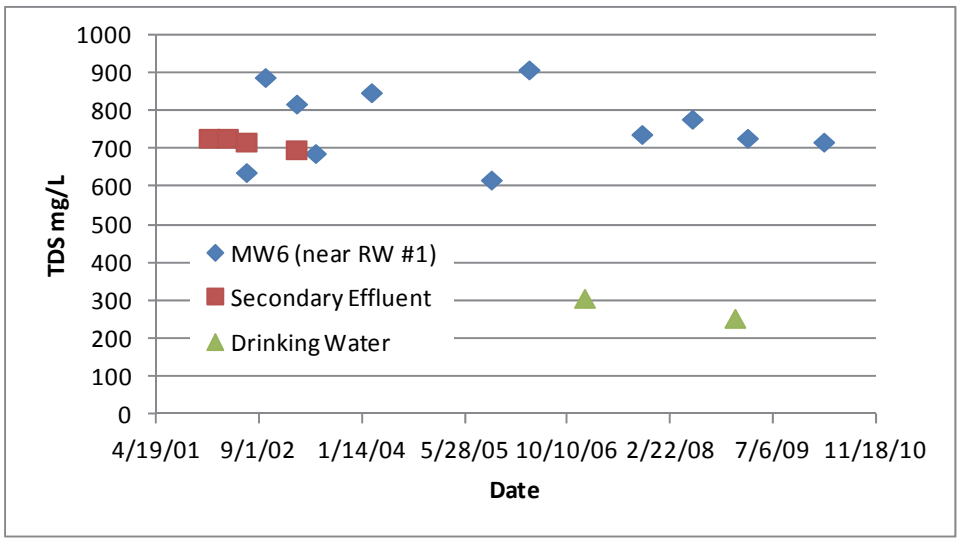

Figure 3.10. Dinuba water quality comparison—TDS.

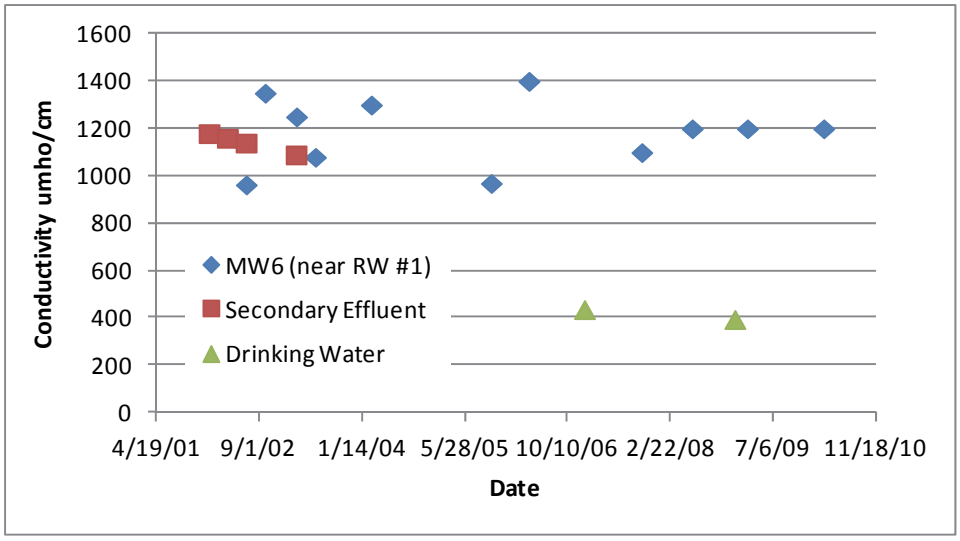

Figure 3.11. Dinuba water quality comparison—EC.

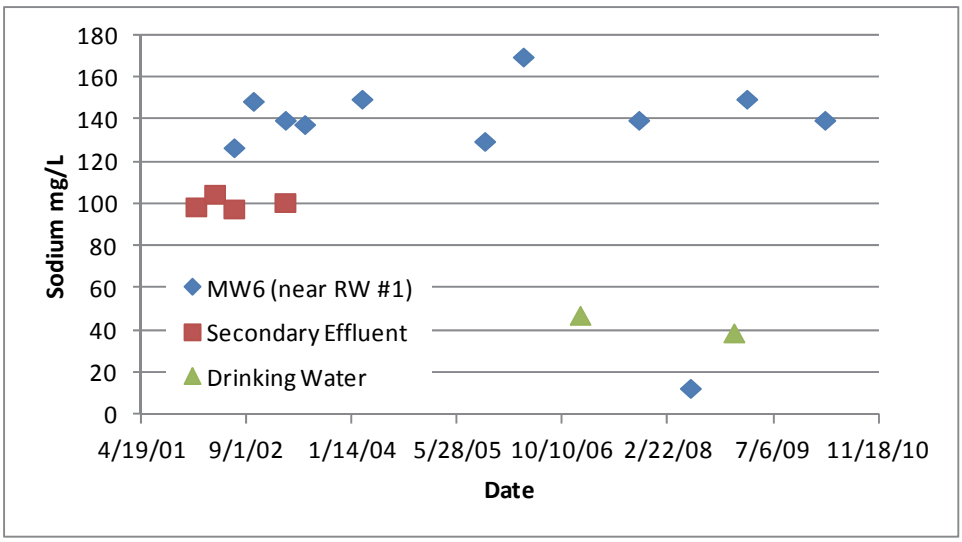

Figure 3.12. Dinuba water quality comparison—sodium.

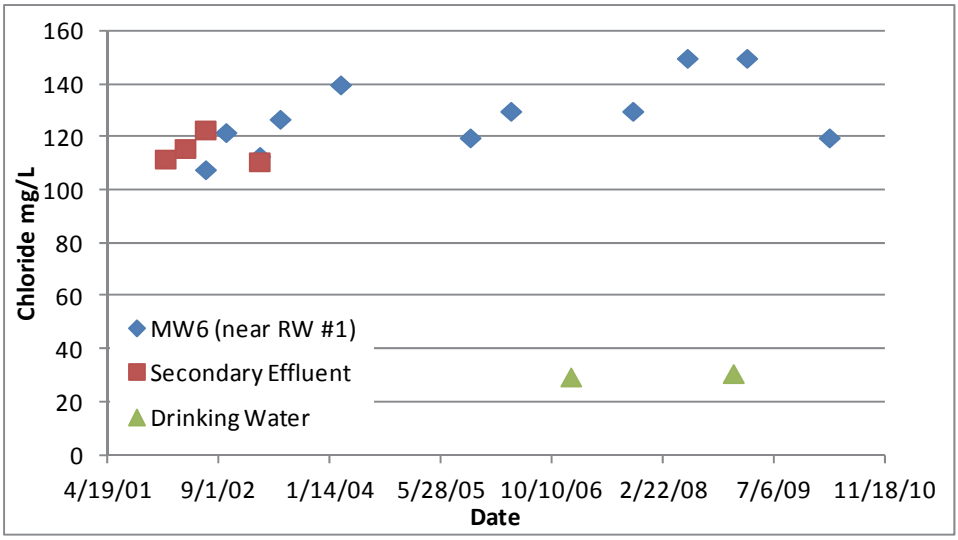

Figure 3.13. Dinuba water quality comparison—chloride.

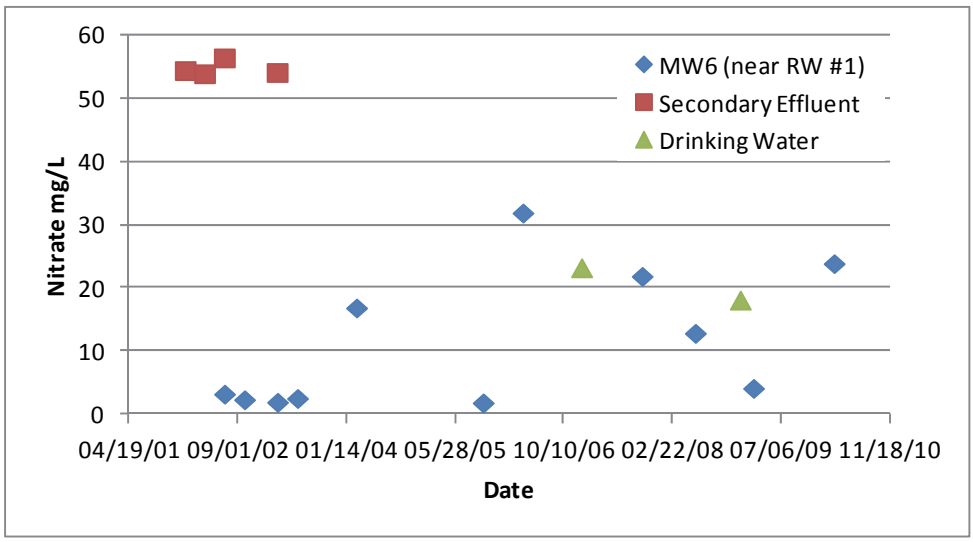

Figure 3.14. Dinuba water quality comparison—nitrate.

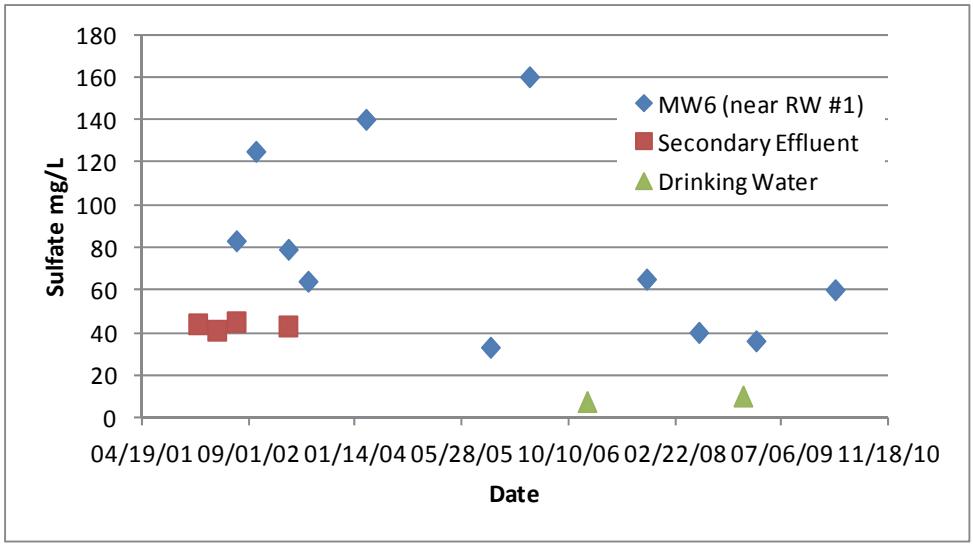

Figure 3.15. Dinuba water quality comparison—sulfate.

The extracted well water average TDS, EC, and chloride levels were in the same range as the WWRF secondary effluent (Table 3.6). The extracted well average sodium and sulfate were greater than the sodium and sulfate in both the secondary effluent and drinking water. In the case of nitrate, the concentration in the extracted well water was lower than for either the WWRF secondary effluent or the upgradient, drinking water wells. The limited data set precluded further statistical analysis.

Table 3.6. Water Quality Characteristics—Dinuba

Parameter	Well MW6 (near RW#1)	Secondary Effluent	Drinking Water
Conductivity, µmho/cm	1183	1143	418
TDS, mg/L	763	720	282
Sodium, mg/L	132	101	43
Chloride, mg/L	128	116	31
Nitrate, mg/L	11	55	21
Sulfate, mg/L	80	43	9

Note. Mg/L = milligrams per liter; µmho/cm = micromhos per centimeter; TDS = total dissolved solids

Taken as a whole, this data set supports the conclusion that there is no significant dilution, though the smaller number of data points precludes this conclusion being as well supported as in the case of Fresno.

3.2.3 High-Volume, Low-Level Coliphage Enumeration Method Validation

The high-volume, low-level virus enumeration technique was successfully validated at Dinuba. As stated in Section 3.1.3, the goal to validate the method was to achieve at least three positive results out of 10 samples collected during the spiking studies. For Dinuba, 6 of the 10 samples collected were positive (Table 3.7).

For this analysis, the positive control was positive. In addition, the nonspiked background control was also positive for indigenous coliphage, indicating that some small concentration of coliphage was present in the well water. The total number of positive samples was much higher than what was expected given the matrix spike concentration. The assay on the remaining travel controls gave a result of about 2 pfu/spike dose. The relatively high number of positive samples and coliphage present in the nonspiked background control indicated nonspiked MS2 coliphage were present in the well water. The presence of coliphage in the nonspiked background control does not invalidate the sampling event. The background coliphage concentration detected was not high enough to interfere with the matrix spike. However, the lower detection limit of the method could not be determined because of the presence of the background coliphage.

Background sampling conducted before the matrix spiking resulted in nondetected coliphage. The presence of coliphage in the background during the matrix spike testing illustrates the importance that background phage should be mitigated prior to validation testing. In this case, because of previous sampling it was assumed that there was no indigenous coliphage.

Table 3.7. MS2 Virus Enumeration Test Results for Dinuba

Date	MPN Result	MPN/100 mL	Comment
9/29/2010	(1/3)	0.0004	Nonspiked background control
	0/3	<0.0004	Spiked 2.1 pfu/300 L
	(1/3)	**0.0004**	**Spiked 2.1 pfu/300 L**
	(1/3)	**0.0004**	**Spiked 2.1 pfu/300 L**
	(2/3)	**0.0012**	**Spiked 2.1 pfu/300 L**
	0/3	<0.0004	Spiked 2.1 pfu/300 L
	0/3	<0.0004	Spiked 2.1 pfu/300 L
	0/3	<0.0004	Spiked 2.1 pfu/300 L
	(2/3)	**0.0012**	**Spiked 2.1 pfu/300 L**
	(2/3)	**0.0012**	**Spiked2.1 pfu/300 L**
	(2/3)	**0.0012**	**Spiked 2.1 pfu/300 L**
	(2/3)	0.0012	Positive control

Note. L= liter; mL = milliliter; MPN = most probable number; MS2 = male-positive type 2; pfu = plaque-forming units

3.2.4 Removal of Microorganisms Through SAT

Both the extraction well water and secondary effluent from the WWRF were tested for the presence of indigenous MS2 coliphage (Table 3.8). The objective of this analysis is to demonstrate a 5-log reduction, not to demonstrate that the well water is phage free. The test method employed is very sensitive, so positive findings are not surprising. In this case, two of the seven samples had concentrations above the measurement range. These "greater than" values do not provide enough information to determine a 5-log reduction for all cases. Therefore, these samples were not used in the data analysis. A > 0.0017 MPN/100 mL may indicate that: (a) the method is too sensitive relative to the concentration of coliphage present; or (b) the well is positioned too close to the injection source; or (c) the soil type is not sufficient to remove viruses by 5-log.

In the future, if a sample is "greater than" using the high-volume, low-level method, then there should be a resample and analysis carried out with a smaller sample volume to determine coliphage concentration. Collection of a 1 gal grab sample at the time of sampling with the high-volume, low-level method would allow additional testing to be conducted in the event of a "greater than" result.

The average coliphage concentration in the secondary effluent was 784 MPN/100 mL, and the extraction well field samples ranged from <0.0004 to >0.0017 MPN/100 mL. This suggests but cannot definitely substantiate an approximate 5-log difference between the secondary effluent and the extracted well water.

Table 3.8. MS2 Coliphage Results for Dinuba

Secondary Effluent (2009-2010)		
		MPN/100 mL
Mean		784
Standard Error		354
Median		306
Standard Deviation		866
Range		1898
Count		6
R11		
Date	**Sample #**	**MPN/100 mL**
9/23/2010	0/6	<0.0004
10/12/2010	3/3	>0.0017
	3/3	>0.0017
	1/3	0.0004
	0/3	<0.0004
	1/3	0.0004
	1/3	0.0004

Note. mL = milliliter; MPN = most probable number; MS2 = male-positive type 2

There was an average 4.6-log difference in total coliform concentrations between the secondary effluent and the extracted well water enumerated by the lauryl tryptrose broth–multiple-tube fermentation test (LTB-MFT) method. (Table 3.9). Only one sample set of *E. coli* data was collected, so no firm conclusions can be made regarding removal of these organisms. It appears that the extracted well water may not meet the Title 22 coliform requirement for tertiary disinfected water (2.2 MPN/100 mL, 7-day median). Additional testing should be conducted to determine whether further treatment is needed at this site.

In addition to total coliform, PSD, UVT, and turbidity were measured. Of the 17 samples analyzed, an estimated average of 79% (with a range of 0 to 99%) of particles was removed through SAT. The turbidity (average 1.27 NTU, range 0.78 to 2.50 NTU) and high UVT (85 to 90%) of the extraction well water indicate high water quality. The maximum turbidity values measured at two discrete time points (2.33 and 2.50 NTU) may not meet the Title 22 turbidity 24-hour average limit of 2.0 NTU or less. The turbidity of the remaining 15 samples was less than 2.0 NTU. The PSD data are shown graphically in Appendix B. Turbidity and UVT results for each sample are contained within the PSD graphs.

Table 3.9. Coliform Results for Dinuba

Sample	*E. coli* Colilert (MPN/100 mL)	Total Coliform Colilert (MPN/100 mL)	Total Coliform LTB-MFT (MPN/100 mL)
Secondary effluent	31,000	122,000	80,000
	Not analyzed	Not analyzed	220,000
	Not analyzed	Not analyzed	220,000
	Not analyzed	Not analyzed	300,000
	Not analyzed	Not analyzed	230,000
Average	**--**	**--**	**210,000**
R11	<1.0	435.2	8.0
	Not analyzed	Not analyzed	4.0
	Not analyzed	Not analyzed	8.0
	Not analyzed	Not analyzed	2.0
	Not analyzed	Not analyzed	2.0
Average	**--**	**--**	**4.8**
Log difference between the secondary effluent and extraction well	**--**	**--**	**4.64**

Note. LTB-MFT = lauryl tryptrose broth–multiple tube fermentation test mL = milliliter; MPN = most probable number.

Chapter 4

Conclusions

High-Volume, Low-Level Virus Enumeration Analytical Technique Validation

BioVir's high-volume, low-level virus enumeration method can be used for evaluation of log removal of male-specific coliphage present in very low numbers. This method was successfully field validated through a series of spiking studies at the Fresno and Dinuba sites. At Fresno, 3 of the 10 samples were positive. At Dinuba, 6 of the 10 samples were positive, in part because of the presence of indigenous coliphage; a lower detection limit could not be determined at this site. The technique showed the increased sensitivity required to demonstrate 5-log reduction of indigenous MS2 coliphage through SAT. This method is a valuable monitoring tool that could allow regulators to evaluate and permit SAT systems for removal of microorganisms worldwide.

Demonstration of Filtration and Disinfection Compliance Through SAT

The 5-log reduction of virus required by the CDPH was generally achieved with the SAT process, although several samples had levels higher than the measurable range, and log reductions could not be calculated for these samples. Indigenous MS2 coliphage removal was used to demonstrate virus removal performance. It was substantiated that there was no significant dilution of specific secondary effluent constituents following percolation into the groundwater; therefore, measured performance was due to treatment and not dilution.

Specifically, the SAT process at Fresno met the CCR Title 22 tertiary recycled water disinfection requirements (5-log virus reduction and low-level coliform criteria) for 17 out of 18 MS2 coliphage samples and all 4 coliform samples; the last MS2 coliphage sample had levels outside the measurable range and was inconclusive. Coliform sampling of the SAT influent and effluent at Fresno resulted in a 5.56-log reduction of total coliforms. The average SAT effluent coliform concentration was less than 2.0 MPN/100 mL with the LTB-MFT method. Sampling at Fresno with the validated high-volume, low-level virus enumeration method resulted in approximately 6-log removal of MS2 coliphage through SAT.

In addition to total coliform and *E. coli*, PSD, UVT, and turbidity were measured. Of the 21 samples analyzed, it was estimated that an average of 94% (with a range of 73 to 99%) of particles was removed through SAT. The low turbidity (average 0.27 NTU, range 0.14 to 0.54 NTU) and high UVT (89 to 91%) of the extraction well water indicate high water quality, particularly in contrast to the secondary effluent percolation ponds. These data, combined with the microbiological results, support the conclusion that SAT is able to provide full "disinfected tertiary" treatment to RWRF secondary effluent at Fresno.

The data set at Dinuba was not as conclusive. Two of the seven samples in the Dinuba dataset were "greater than"; further testing would be needed to confirm that 5-log removal could consistently be attained. Coliform sampling of the SAT influent and effluent at Dinuba resulted in a 4.37-log reduction of total coliforms. The average SAT effluent coliform concentration (4.8 MPN/100 mL with the LTB-MFT method) would not have met Title 22

requirements for the days sampled during this project. Sampling at Dinuba resulted in approximately 5-log removal of MS2 coliphage through SAT.

In addition to total coliform, PSD, UVT, and turbidity were measured. Of the 17 samples analyzed, it was estimated that an average of 79% (with a range of 0 to 99%) of particles was removed through SAT. The turbidity (average 1.27 NTU, range 0.78 to 2.50 NTU) and high UVT (85 to 90%) of the extraction well water indicate high water quality. The maximum turbidity values measured at two discrete time points (2.33 and 2.50 NTU) may not meet the Title 22 turbidity 24-hour average limit of 2.0 NTU or less. The turbidity of the remaining 15 samples was less than 2.0 NTU. Further testing would be needed at Dinuba to demonstrate that the SAT process can provide "disinfected tertiary" treatment.

It is important to note that although the data from this study have value to other wastewater treatment plants, it is only directly applicable to the sites from which it was collected (Fresno or Dinuba) as it is dependent on water quality, the site-specific hydrology, and infiltration basin operational strategy.

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Appendix A

High-Volume, Low-Level Virus Enumeration Protocol

BioVir Laboratories, Inc. SOP. VIII.7 PMBS Modification of EPA Method 1601: High Volume Filtration/Concentration of Bacteriophage from Water: Male-Specific Coliphage in an MPN Format

1 Scope and Application

1.1 This protocol is a method modification of EPA Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure (April 2001). Method 1601 is a presence/absence–based method originally designed for 1-L volumes. In this modified method format, large volumes of water (e.g., 100 L) are passed through negatively charged, microporous filters, which catch the viruses by electrostatic attraction. Each filter is then directly assayed, without virus extraction, in an MPN format.

1.2 EPA Method 1601 is designed for the detection of bacteriophage, including male-specific and somatic coliphage. However, this method modification (Danielson et al., 2008; Danielson et al., 2010) is only for detecting MS2 coliphage. It includes the growth of the host *E. coli*, the enrichment broth, and spot-plate confirmation agar. The MPN method allows for the estimation of the phage concentration from the original volume of the sample. The MPN approach of this method is based on that described by Kott (1966).

2 Summary of Method

2.1 Water is passed through multiple, negatively charged, microporous filters running in parallel. The volume of water used will be dependent upon the water quality of the source.

2.2 Upon receipt at the lab, the individual filters and any residual water are each collected separately in an appropriate container (bag or vessel).

2.3 Appropriate volumes of growth media with supplements are added to the filter container along with a bacterial host to grow the bacteriophage.

2.4 Following overnight incubation, the presence of the target bacteriophage is confirmed by placing a portion of the sample culture from each filter sample onto a plate with host bacteria (spot plate).

2.5 The presence of bacteriophage on the spot plate test confirms the presence of bacteriophage from that specific filter sample.

2.6 The number of positive filters within a set of filters is recorded, and the MPN is calculated.

3 Definition

EPA	Environmental Protection Agency
IDC	Initial demonstration of capability
MPN	Most probable number
MS2	Male-specific type 2 bacteriophage
ODC	Ongoing demonstration of capability
TSB	Trypticase soy broth

4 Interferences

4.1 Extremes in pH can inhibit binding of the viruses onto the filters. With the NanoCeram filters, stay within a range of pH 6 to pH 9.

4.2 The presence of disinfectants can lead to a false negative result. Apply an appropriate neutralizer for the disinfectant in-line while collecting the sample.

4.3 Water with high turbidity can lead to premature clogging of the filter; multiple filters may have to be employed to process a specific volume of water.

4.4 Organic compounds in the water may compete for virus binding sites, leading to lower recovery rates or possibly false negative results.

5 Safety

5.1 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and materials, and while operating sterilizing equipment. Please refer to the BioVir Laboratories, Inc., Biological Safety Manual.

5.2 Mouth pipetting is prohibited.

6 Equipment and Supplies

6.1 Refer to EPA Method 1601 for all but the following:

6.2 Argonide 5-in. NanoCeram Filter (Argonide, Cat# P2.5-5)

6.3 Filter housing for 5-in. filter (Argonide, Cat# H2.5-5C, or equivalent)

6.4 Hose assembly for filtration unit (see Figure 1, Appendix XIII.7 A)

6.5 Water flow meter/totalizer (ABB-Kent, McMaster Carr, or equivalent)

6.6 Scale that can measure 500 grams (g) to 1 kilogram (kg).

6.7 Containers for filter incubation may include 1 qt and 1 gal Ziploc or WhirlPak bags, one each per filter (various sources); beakers or jars to accommodate filter plus sufficient volume of media to cover the filter.

7 Reagents and Standards

7.1 Purity of Reagents: reagent grade chemicals shall be used in all tests. Unless otherwise indicated, reagents shall conform to the specifications of the Committee on Analytical

Reagents of the American Chemical Society. The microbiological growth media must be of microbiological grade.

7.2 Whenever possible, use commercial culture media as a means of quality control.

7.3 Purity of Water: Reagent grade water (RGW) conforming to Specification D1193, Reagent water conforming Type II, Annual Book of ASTM Standards

7.4 For all reagents, refer to Method 1601.

8 Sample Collection, Preservation, and Storage

8.1 See the appendix attached, XIII.7.A, "High-Volume Bacteriophage Sample Collection."

9 Quality Control

9.1 Quality control (QC) on laboratory equipment as described in Section 9.0 in Method 1601 will be followed.

9.2 General QC requirements as described in Section 9.0 in Method 1601 will be followed. This includes the performance of IDC, ODC, matrix spikes; method blanks, and positive controls.

10 Calibration and Standardization

10.1 General QC requirements as described in Section 10.0 in Method 1601 will be followed.

11 Procedure

11.1 This procedure is a modification of Section 12.0 of Method 1601.

11.2 The same media and reagents are used; however, the volumes can vary dependent upon the volume of water collected with the filters. Typically, the 5 in. filter in a 1 qt Ziploc or WhirlPak bag will require 500 mL.

11.3 The filters will be cultured in a vessel (e.g., 1 qt bag). Tare the vessel on a scale that has a range of 500 g to 1 kg.

11.4 Collect the excess water from the filter unit into the tared vessel. Record the weight.

11.5 Alternatively, express the residual water from the filter and housing with a peristaltic pump.

11.6 With gloved hand, place each filter unit into a separate vessel. If using a 1 qt bag, the filter will fit on its side.

11.7 Based upon the volume of excess water (from the measurement of the weight), add an additional volume of RGW for a total of 475– 500 mL. If the excess water is at 475–500 mL, no additional RGW may be required. Note: the filter must be FULLY immersed in the water.

11.8 Based on the reagent/media additions specified in Method 1601 (flow chart #4), add the media/reagents and bacterial host in proportion to the final volume.

11.8.1 Example: to 500 mL of water add

11.8.1.1 6.25 mL magnesium chloride (MgCl)

11.8.1.2 2.5 mL *E. coli* host F_{amp}

11.8.1.3 25 mL of 10x TSB

11.8.1.4 5.0 mL of stock amp/strep antibiotic solution

11.8.1.5 Alternatively, with the exception of the *E. coli* host, all the above ingredients may be premixed with the RGW in a carboy and portioned into the bags. The *E. coli* host can then be added to each individual filter in the container.

11.9 In order to protect against leakage of the 1 qt bag, carefully place the 1 qt bag with filter into a 1 gal bag. Place the bagged filter into a leakproof pan.

11.10 Incubate 16–24 hours at 35° C.

11.11 Prepare confirmatory spot plates as described in Section 7.3.4 of EPA 1601. Label the spot plate in a grid pattern for three spots per sample. Each spot represents one filter within that set of three filters for each sample. Up to three samples (nine spots) along with positive and negative controls can be accommodated on a plate.

11.12 After incubation, carefully mix the contents of filter and bag by gentle kneading.

11.13 One by one, carefully open a bag and withdraw 10 L of suspension from the bag.

11.14 Spot the 10-L volume onto the prelabeled spot plate. Allow the 10 L to absorb into the spot plate before inverting the plate.

11.14.1 NOTE: Be very careful not to juggle the plates excessively as the drops may run into the other areas of the grid. This may lead to false positive spot counts.

11.15 Incubate 16–24 h at 35° C.

11.16 Lysis zone formation (typically a circular zone of clearing around the spot colony; see Figure 1) indicates confirmation for coliphage. If the spot contains an intact lawn of bacteria indistinguishable from the background lawn of bacteria, this indicates a negative result. Refer to Section 12.3.9 of 1601 for other possible outcomes.

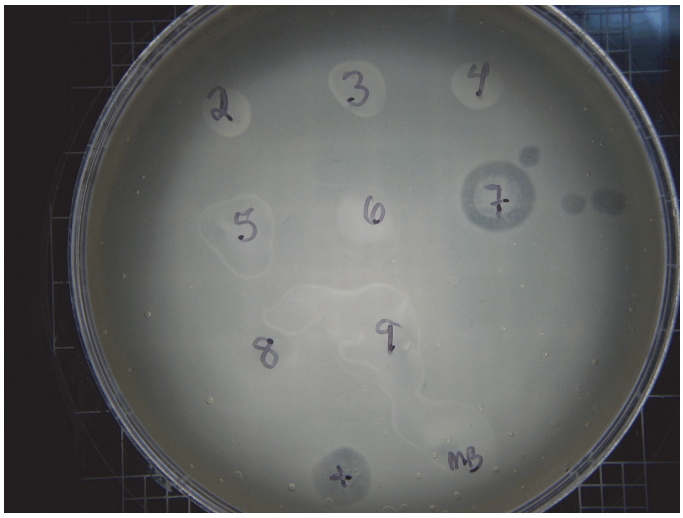

Figure 1. Typical Clear Zone Lysis (#7 and Positive Control).

12 Data Analysis and Calculations

12.1 Refer to Section 13.0 of EPA 1601.

13 Method Performance

13.1 Refer to Section 14.0 of EPA 1601.

13.2 Single laboratory method modification of this procedure was performed in accordance with the EPA PBMS. Reagent grade water spikes were performed and met the requirement of IDC for F+ coliphage, 3 positive responses in 10 300-L spiked samples. The criteria of 3/10 spiked samples was requested by the CDPH.

14 Reporting Results

14.1 Results are expressed as MPN per volume analyzed.

15 Laboratory Precision and Accuracy

15.1 Precision with the MPN methods will be determined as described in Standard Methods, Section 9020 (APHA, 1992). Duplicate analyses will be made on samples of raw sewage and any other water source showing detectable indicator counts. Each set of duplicates will be analyzed by the same analyst, and all analysts performing the MPN method will be included in the intralaboratory precision determination check. The mean range of the logarithm counts (R) will be used to calculate a control limit of 3.27 × R. Thereafter, 10% of routine samples will be analyzed in duplicate if enough sample has been submitted. If the range of duplicates is greater than the control limit, then laboratory variability will be considered excessive. The range criteria will be updated quarterly using the most recent set of 15 duplicate results.

15.2 One individual will be assigned primary responsibility for all MPN analyses, although interanalyst variability determinations will be conducted as needed if more than one individual conducts these analyses.

15.3 The accuracy of microbiological data will be determined by comparing the results of split, performance evaluation, or both. The statistical method for setting acceptable range criteria used for precision evaluations (Standard Methods, Section 9020) could also be used for setting acceptable accuracy criteria for interlaboratory split sample

analyses of each of the positive test waters. Alternative statistical measures will be discussed and agreed upon in consultation with the external laboratory.

16 Corrective Actions

16.1 When there is a departure from documented policies, procedures, and quality control measures, the Quality Assurance Office (QAO) shall be notified. If the QAO is not available, consult with the laboratory manager or laboratory director.

16.2 Corrective actions are initiated or recommended by the QAO, laboratory manager, or laboratory director. Implementation of corrective actions can be authorized by the QAO, laboratory manager, or laboratory director.

16.3 If the data set is associated with QC measurements that are out of control, the technician or analyst is to immediately note the deviation on the bench sheets and associate the data point with either footnote to link to the explanation. The "Client Contact Required/Client Complaints" form is prepared and turned in at the front office. A customer service representative shall contact the client representatives and inform them of the test outcome. This form is to remain with all of the paperwork for that sample.

16.4 The QAO, laboratory manager, or laboratory director shall review the "Client Contact Required/Client Complaints" form and initial that a review of the incident occurred. There will be a determination if there are corrective measures that can be implemented to reduce the risk of similar QC failures in the future (documented on the "Client Contact Required/Client Complaints" form).

17 Contingencies for Handling Out-of-Control or Unacceptable Data. To the extent possible, samples shall be reported only if all QC measures are acceptable. If a QC measure is found to be out of control, and the data are to be reported, all samples associated with the failed QC measure shall be reported with the appropriate data qualifier(s).

18 Pollution Prevention

18.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

18.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

19 Waste Management

19.1 All glassware, pipettes, and transfer materials that have been in contact with a sample are presumed to be contaminated and must be sterilized before disposal.

19.2 Cultured material contains amplified microorganisms and must be sterilized prior to disposal.

19.3 All contaminated materials, after they have been sterilized, may be discarded with the regular solid waste.

20 Regulatory Compliance Notification Procedures

20.1 At this time there are none for this method.

21 Invalidation

21.1 Invalidation can occur when media QC samples fail.

21.2 A positive reaction in the negative control with positive results on the spot plate is an invalidation. However, a positive reaction in the negative control with no spots on the spot plate may not necessarily invalidate the test. Check with controls performed on the media to confirm.

21.3 A negative reaction on the spot plate for the positive control will invalidate the test. Check the stock host culture to be sure that it is sensitive to the MS2 phage. If not, start a new host culture from the long-term deep freezer (-80° C).

22 References

22.1 Danielson, R. E.; Truscott, J. R. , Cooper, R. C. Validation of Large Volume MPN Techniques Using a Modification of US EPA Method 1601: Detecting Low Concentrations of MS2 Coliphage from Secondary Effluent Substrata Infiltration Systems. 110th General Meeting of the American Society for Microbiology, San Diego, CA. Poster Q2409. May 2010.

22.2 Danielson, R. E.; Truscott, J. R.; Hogg, S.; Staggs, R.; Adams, T. Validation of a Large Volume Most-Probable-Number Technique for US EPA Method 1601: Coliphage by a Two-Step Enrichment Procedure. Water Quality Technology Conference, Cincinnati, OH, 2008.

22.2 Kott, Y. Estimation of low numbers of *Escherichia coli* bacteriophage by use of the most probable number method. *Appl. Microbiol.* **1966,** 14. 141–144.

22.4 US EPA Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-Step Enrichment Procedure. EPA 821-R-01-030. April 2001.

VIII.7.A High-Volume Bacteriophage Sample Collection

1 Filtration unit is made up of three filtration units arranged to process water in parallel. The influent connection is a ¾-in. garden hose type connector. There are three primary parts to the filter unit:

a. The influent hose

b. The three filter units

c. The effluent hose and flow meter

2. Determine flow rate. A time-keeping device with seconds will be necessary.

a. The small tick marks on the far right wheel of the flow meter represent 0.1-gallon values.

b. Connect the influent hose to the well sample tap. Open influent hose valve on the filter unit all the way.

c. If they are not connected, connect the outlet of the influent hoses (marked in GREEN) to the inlet of the flow meter hoses (marked in RED). If they are connected, proceed to the next step. See Figure 1.

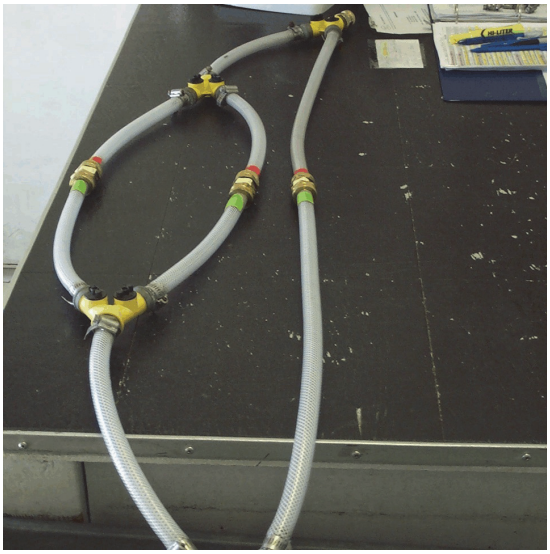

Figure 1. Illustration of how to connect the influent and well sample tap hoses.

d. Turn on the water at the well-head tap and adjust the flow to approximately 2 gal/min (two numbers on the tick-mark wheel per minute).

e. Turn off the flow of water at the influent hose valve of the filter unit, not the well-head tap.

f. Record a unique sample number on each of the filter units on the sample label. Record on the data sheet the sample number, date, and time.

3. Collect a sample.

a. Record the value from the flow meter onto the sample collection data sheet, including the value from the small tick marks. Calculate the target total volume to pass 80 gal through the system. Record the other information as well (e.g., date, time, location).

b. Disconnect the influent hoses (GREEN) from the flow meter hoses (RED) at the YELLOW connection point.

c. Remove the three filter units from their individual bags; KEEP THE BAGS for cooling and shipping the filters. Connect the outlet of the influent hose to the inlet of the filter set. Be sure the filter is oriented in the direction of the flow arrow on the filter unit. See Figure 2.

Figure 2. Illustration of the correct influent hose–filter set connections.

d. Connect the outlet of the filter set to the inlet of the flow meter.

e. Gradually turn on the filter unit influent hose valve to fully open.

f. After about 1 min of flow, double check the flow rate and adjust as necessary to achieve 2 gal/min.

g. Total run time should be close to 40 min or so. Option: Set a timed alarm to sound after 30 min and observe the flow meter to track when 80 gal is achieved.

h. At 80 gal, turn off the flow at the well tap. Disconnect the influent hose from the well tap.

i. Disconnect the filter set inlet hose from the influent outlet hose and screw on a blank cover.

Repeat this step with the filter set outlet hose and the flow meter inlet hose; screw on a blank cover on the filter set outlet hose.

4. Shipping Samples

Precool the filter set by placing it into iced water or a refrigerator for 1 h. Place the precooled filter set into the travel cooler with double-bagged ice. Place field data sheets in double Ziploc bags in the cooler. Seal and ship by overnight courier to the laboratory:

BioVir Laboratories, Inc.
685 Stone Road, Unit 6
Benicia, CA 94510
(800) 442-7342

Appendix B
Particle Size Distribution Plots

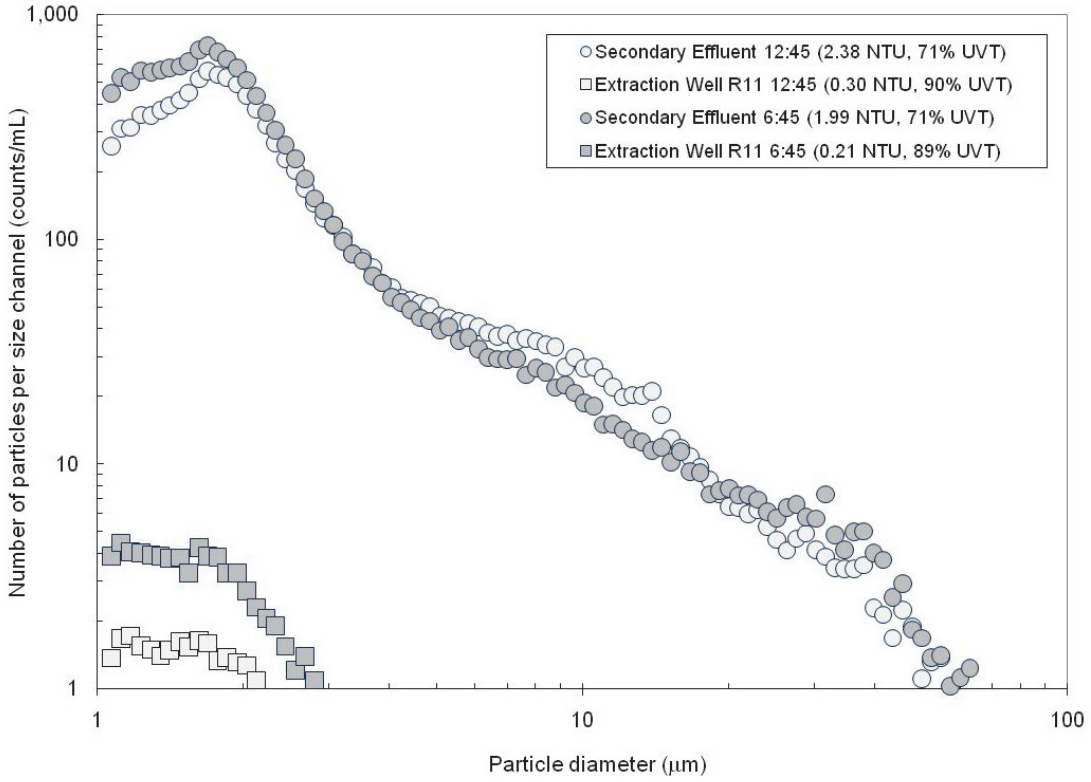

Figure B.1. Fresno water quality comparison—PSDs. Sampled on 7/15/10.

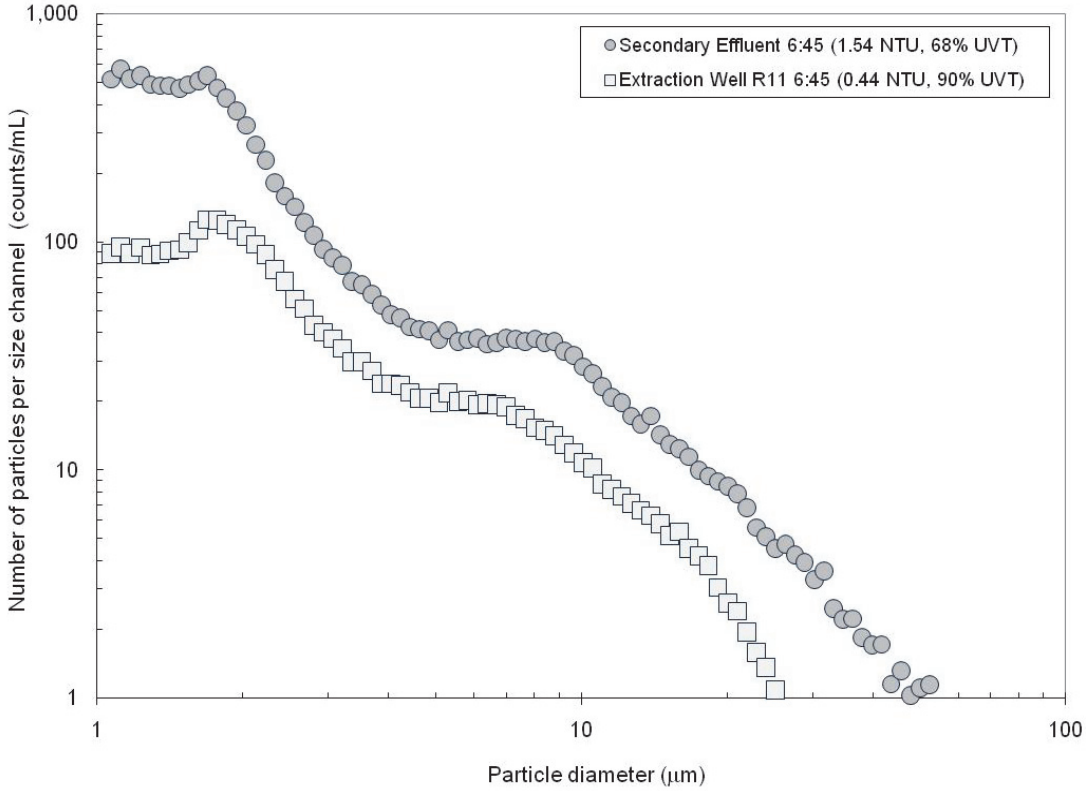

Figure B.2. Fresno water quality comparison—PSDs. Sampled on 9/8/10.

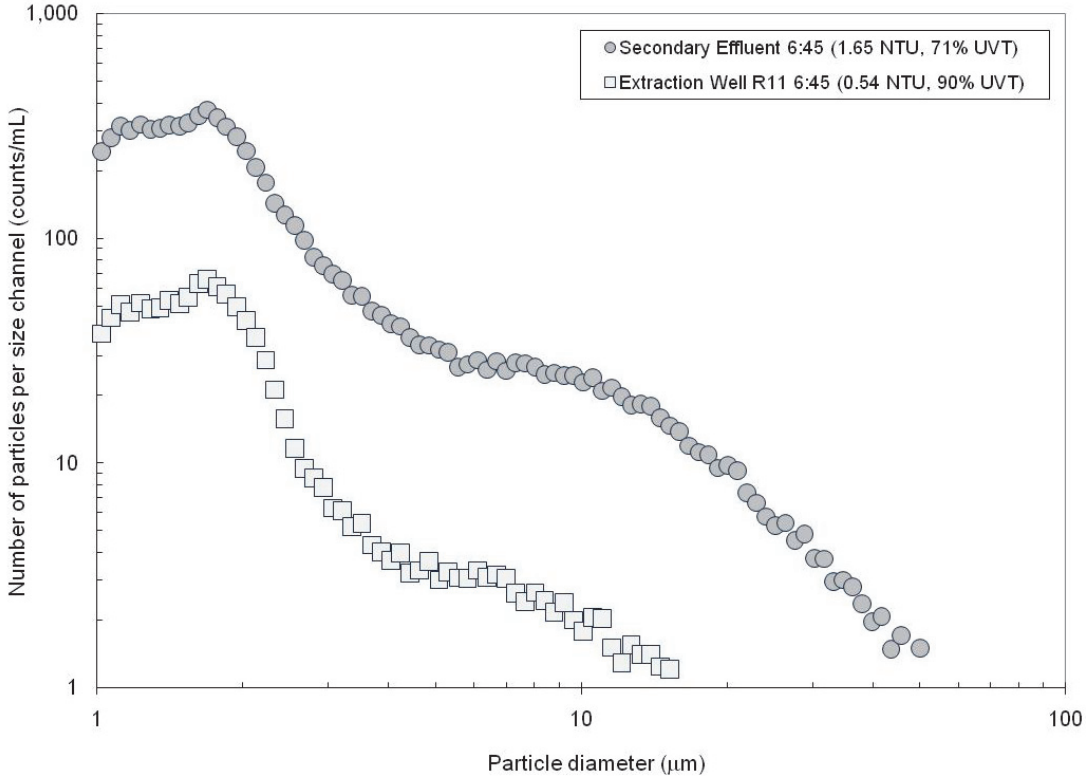

Figure B.3. Fresno water quality comparison—PSDs. Sampled on 10/6/10.

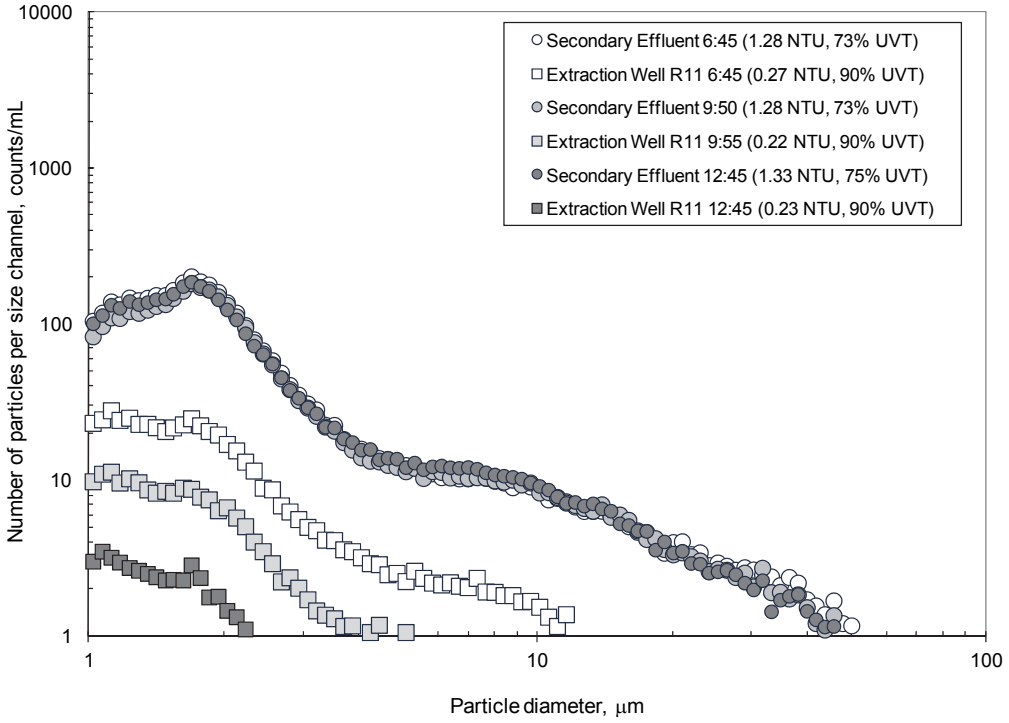

Figure B.4. Fresno water quality comparison—PSDs. Sampled on 10/20/10.

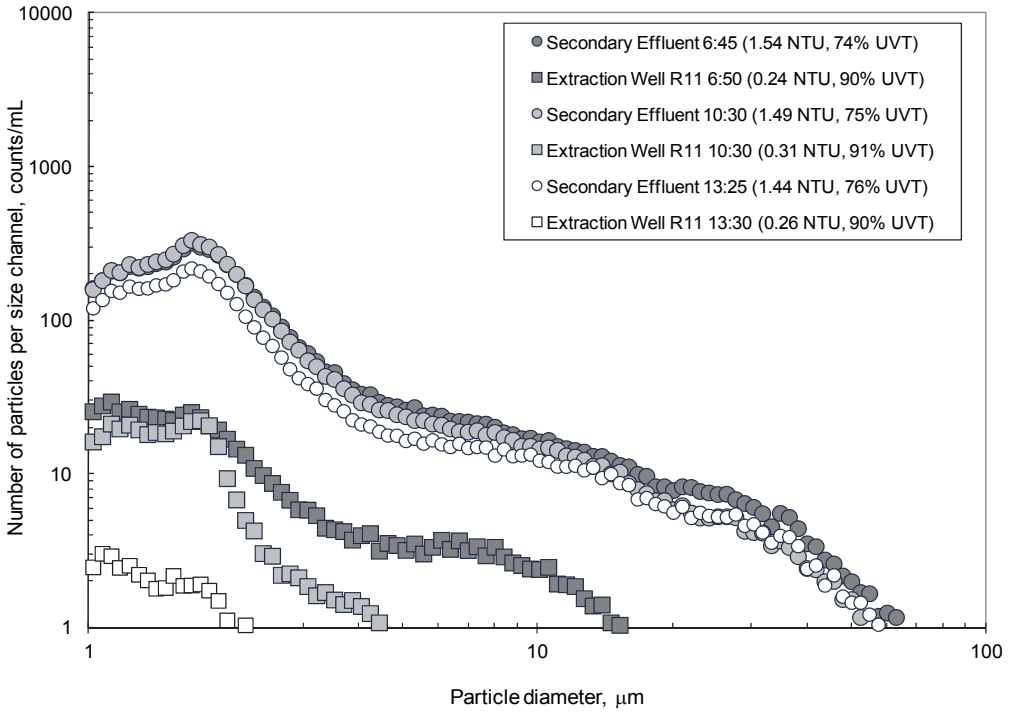

Figure B.5. Fresno water quality comparison—PSDs. Sampled on 10/26/10.

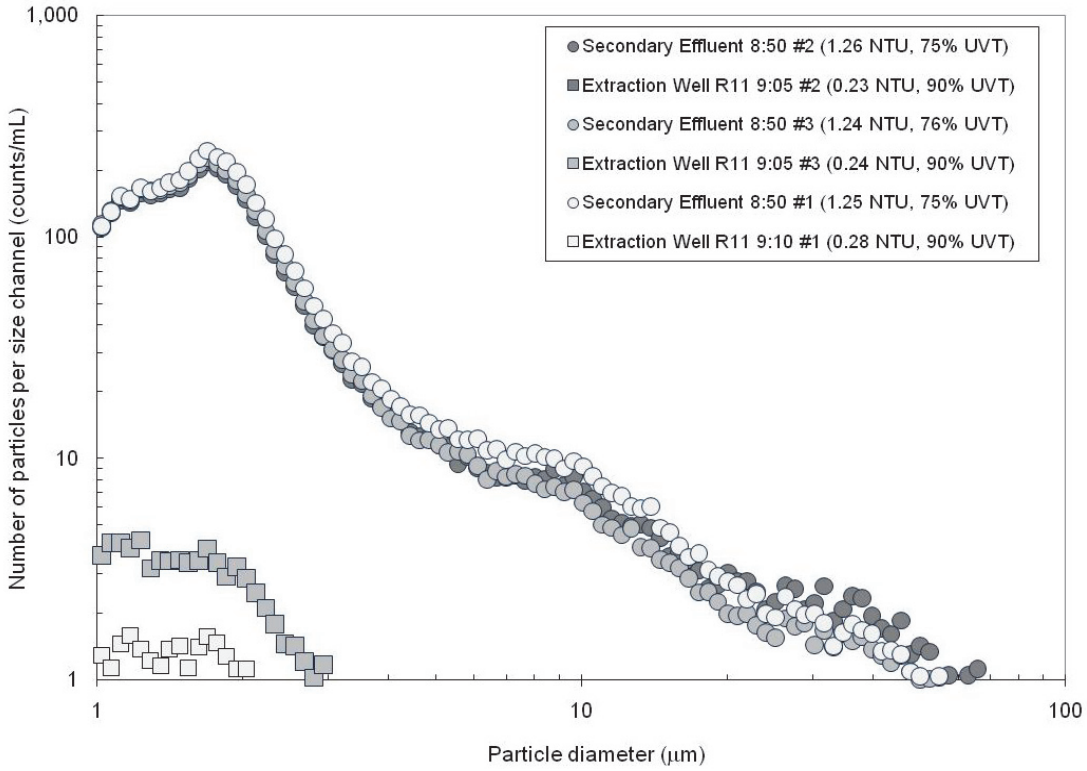

Figure B.6. Fresno water quality comparison—PSDs. Sampled on 4/18/11.

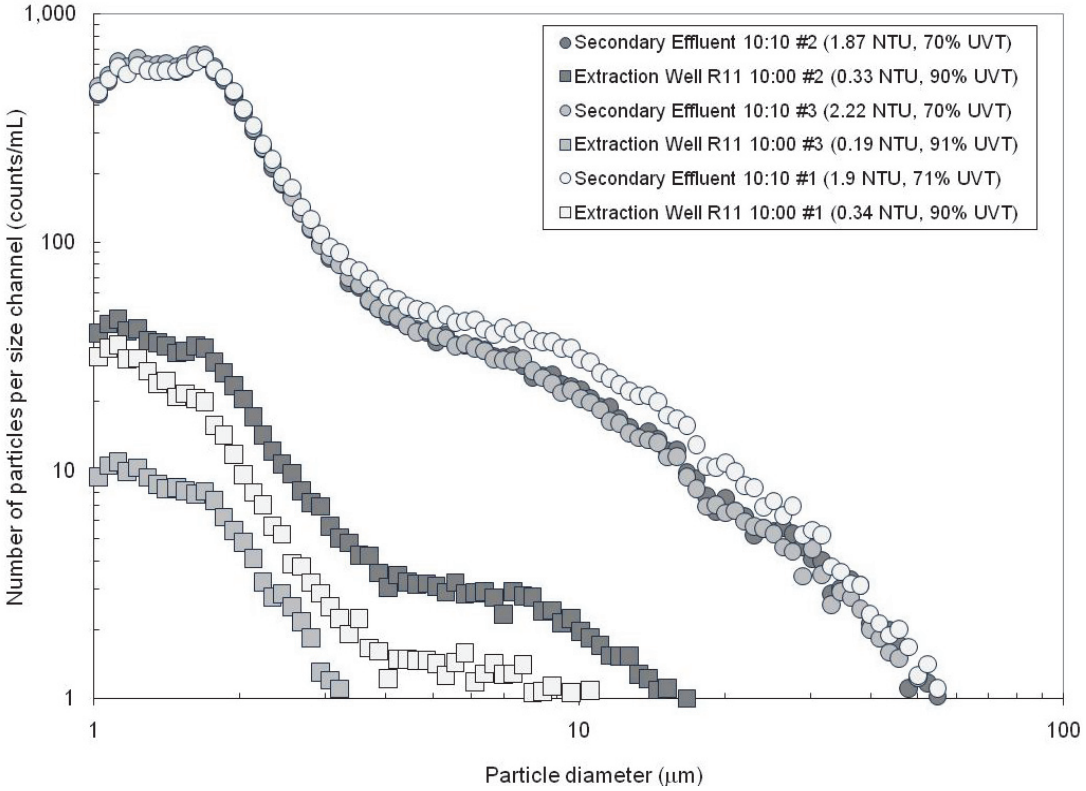

Figure B.7. Fresno water quality comparison—PSDs. Sampled on 5/17/11.

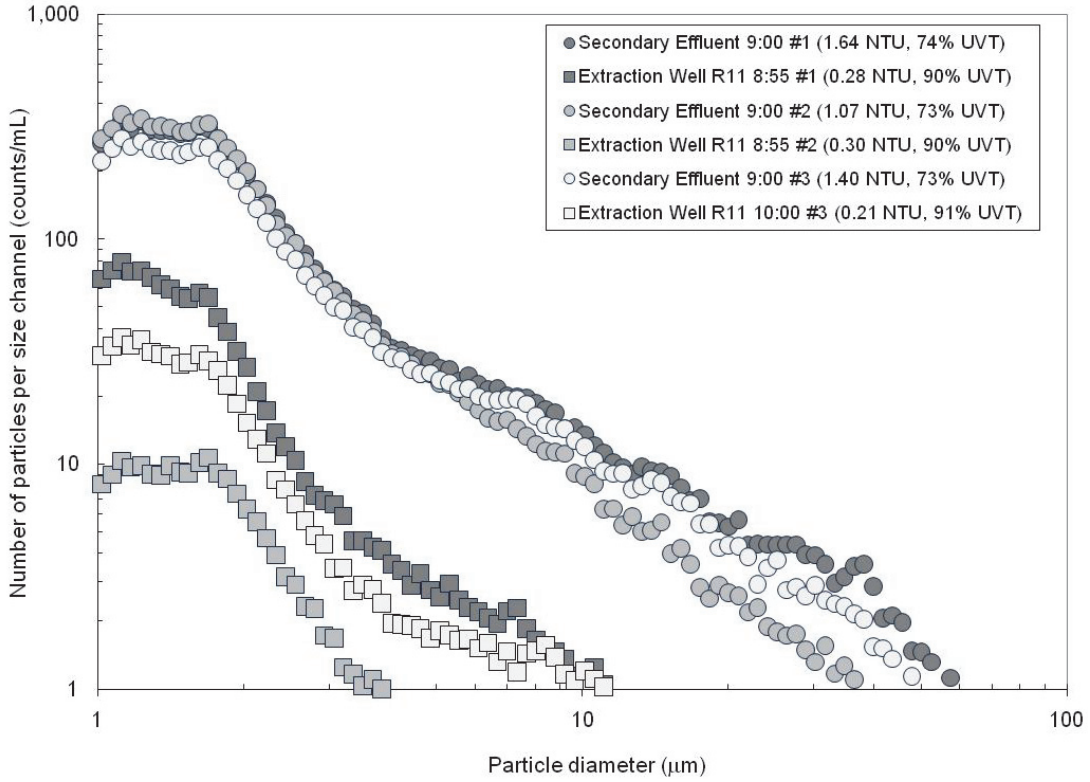

Figure B.8. Fresno water quality comparison—PSDs. Sampled on 5/24/11.

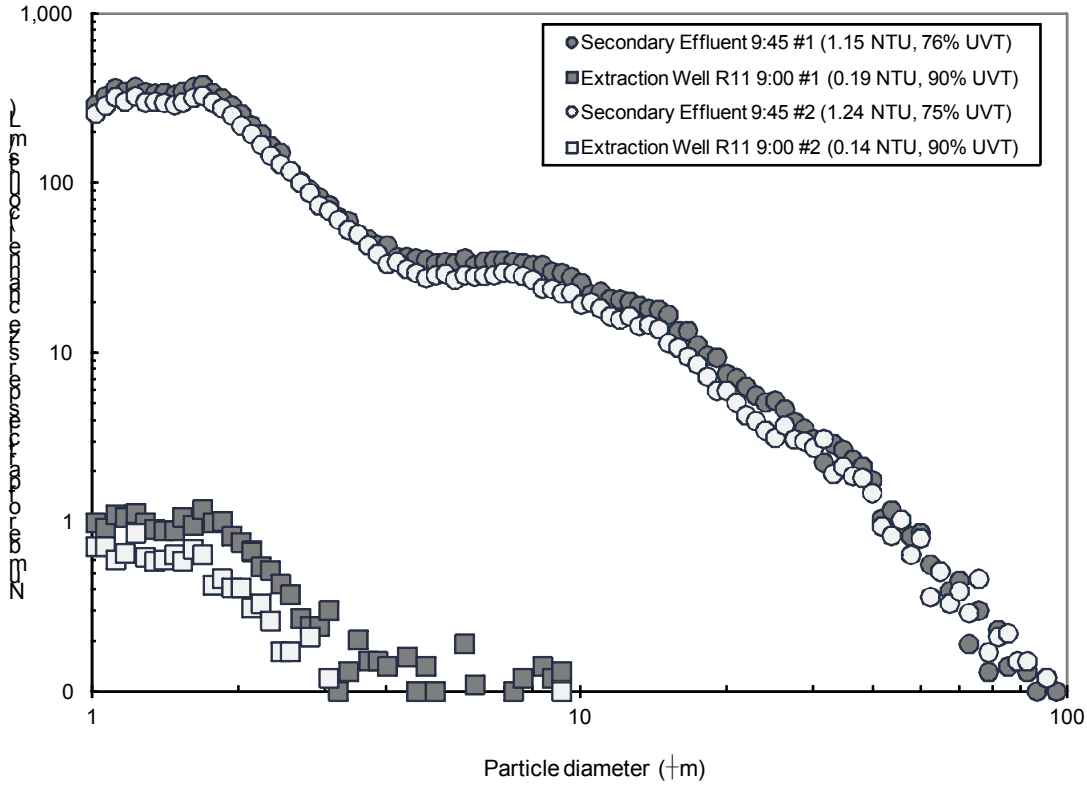

Figure B.9. Fresno water quality comparison—PSDs. Sampled on 5/31/11.

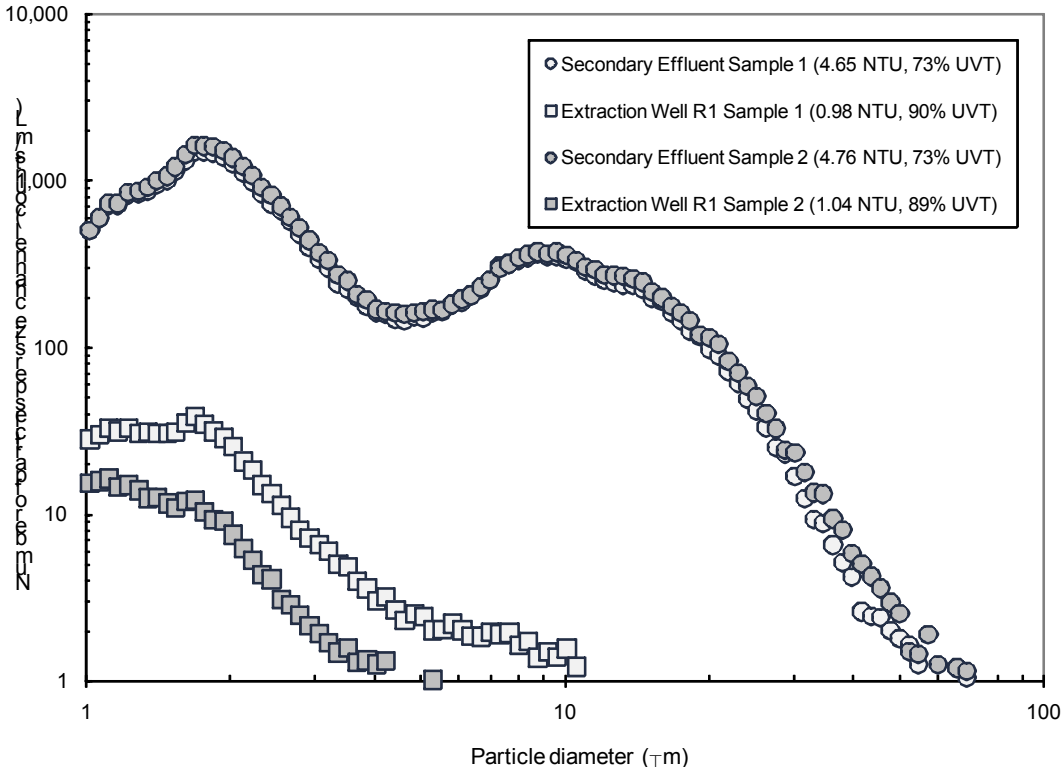

Figure B.10. Dinuba water quality comparison—PSDs. Sampled on 9/23/10.

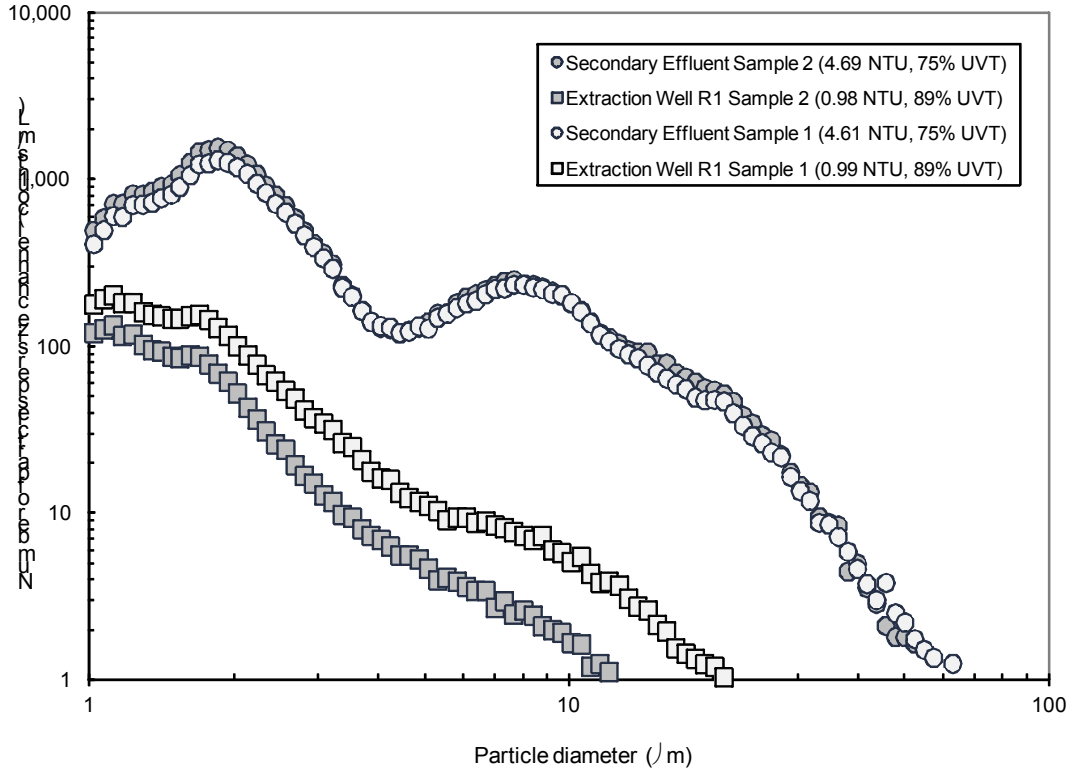

Figure B.11. Dinuba water quality comparison—PSDs. Sampled on 9/29/10.

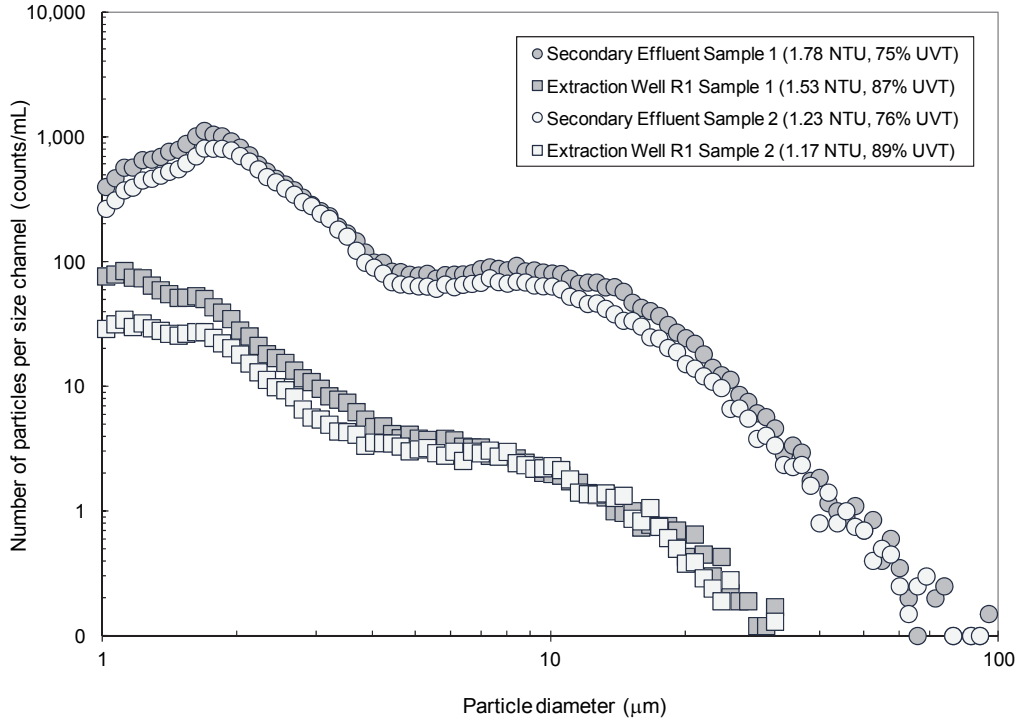

Figure B.12. Dinuba water quality comparison—PSDs. Sampled on 10/8/10.

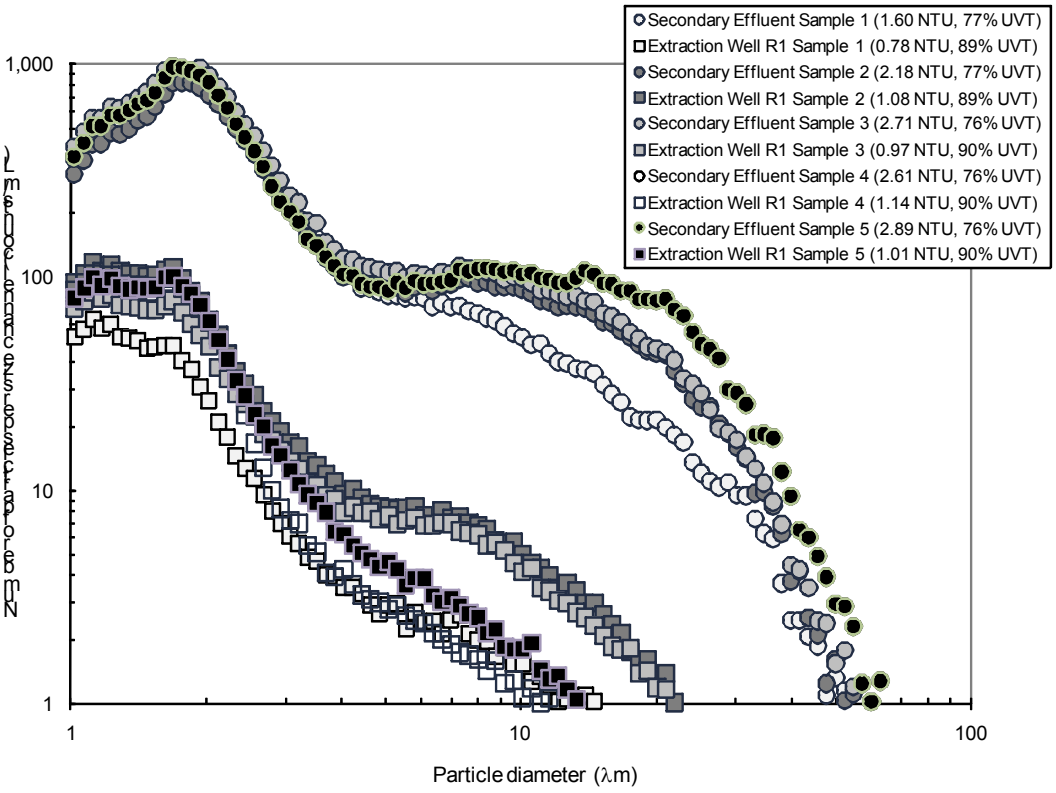

Figure B.13. Dinuba water quality comparison—PSDs. Sampled on 10/12/10.

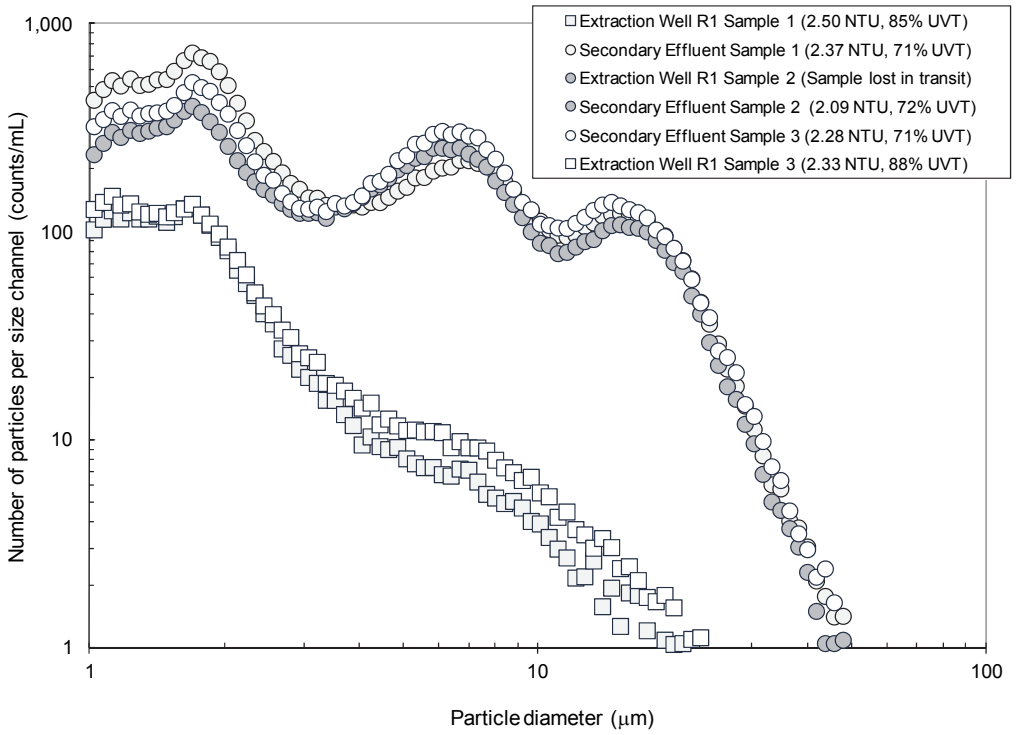

Figure B.14. Dinuba water quality comparison—PSDs. Sampled on 5/10/11.

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WATEREUSE RESEARCH

1199 North Fairfax Street, Suite 410
Alexandria, VA 22314 USA
(703) 548-0880
Fax (703) 548-5085
E-mail: Foundation@WateReuse.org
www.WateReuse.org/Foundation