REMOVAL OF THE HUMAN PATHOGEN *GIARDIA INTESTINALES* FROM GROUNDWATER

By

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To the faculty of Washington State University:

The members of the committee appointed to examine the thesis of COLLEEN FRANCES RUST find it satisfactory and recommend that it be accepted.

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Chair

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DEDICATION

This thesis is dedicated to Phillip Rust, my best friend, the love of my life, and my husband. Thank you for giving me unconditional love and support through the many trying days of our Masters programs. May we always work as a team and be blessed in our future life and careers.

REMOVAL OF THE HUMAN PATHOGEN *GIARDIA INTESTINALES* FROM GROUNDWATER

Abstract

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Cyst-forming protozoans such as *Giardia intestinales* can survive for extended periods of time in groundwater systems with temperatures of less than 10 °C, migrate significant distances, and are relatively resistant to standard municipal water system chlorination practices. Though dormant outside the host, as few as ten cysts can result in animal or human infection. Because pathogenic bacteria, viruses, and protozoans tend to be negatively charged in the pH range of most groundwaters, naturally occurring and modified materials such as surfactant-modified zeolites (SMZ) having net positive surface charges are suitable as barriers to impede pathogen migration in aquifer systems.

Three different SMZ formulations were prepared and characterized using zeolite from the St. Cloud mine in Winston, NM. Five different experimental runs were conducted under varied conditions (including use of raw zeolite and sand only) with a 10-cm wide barrier of SMZ to test the removal efficiency of SMZ for *G. intestinales* cysts and microsphere analogs. The model aquifer was filled with coarse silica sand to mimic realistic natural field conditions and potassium chloride (KCl), microspheres (*Giardia* analogs) and *Giardia intestinales* cysts were used as tracers. The arrival of the tracers down gradient of the SMZ barrier was compared to the arrival in the absence of the barrier to evaluate the effectiveness of each SMZ formulation.

The coarse-grained Cationic SMZ (1.4-2.4 mm) formulation was further tested in the field using water amended with microspheres to simulate *Giardia* cyst behavior. The field site was an existing multiple well site at the University of Idaho in Moscow. The wells were completed in the Lolo Basalt Formation, a highly heterogeneous and anisotropic fractured basalt aquifer system typical of the subsurface of most of eastern Washington and northeastern Oregon.

The SMZ pathogen field filter was installed directly in the well bore and the concentration of microspheres were measured before and after filtration. Pumping was continued over an extended period of time to test the lifetime of our prototype filter system. The goal of the project was to develop a prototype filter system for removing a multitude of human pathogens in drinking water.

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CHAPTER 1: INTRODUCTION

1.1 Significance of the Study

1.1.1 Waterborne Disease Outbreaks Associated with Groundwater

Microbial contamination of groundwater is a serious concern worldwide. For many countries, groundwater provides approximately 40% of the potable water used for human consumption. Cyst-forming protozoans such as *Giardia intestinales* can survive for extended periods of time in groundwater systems with temperatures of less than 10 °C, migrate significant distances, and are relatively resistant to standard municipal water system chlorination practices. Though dormant outside the host, as few as ten cysts can result in animal or human infection, called giardiasis (Akin and Jakubowski, 1986; Casemore et al., 1997; Jarroll, 1988). *G. intestinales* is the most frequently identified protozoan agent causing waterborne outbreaks of parasitic infections in humans in the world (EPA, 1998). The World Health Organization estimates approximately 200 million people world wide are infected each year (WHO, 1992).

In the United States, numerous waterborne outbreaks of giardiasis have been documented, prompting new EPA regulations requiring a maximum allowable concentration of zero *Giardia* cysts per liter in drinking water (Abbaszadegan, 1998; EPA, 1998). The sources of most waterborne outbreaks of giardiasis in the United States have been from unfiltered, chlorinated surface or groundwater systems. Subsequently in most U.S. outbreaks, filtration and chlorine disinfection were found to be inadequate and/or disrupted (EPA, 1998). During the period of 1971-1994, there were 127 different waterborne outbreaks of giardiasis in the United States that were associated with contaminated drinking water. Table 1 shows the determined source or reason for each outbreak. Eighty-one (74%) of these outbreaks were caused by insufficient treatment of surface water and the majority occurred in surface water systems that were

chlorinated but not filtered. Approximately 15% of the total giardiasis outbreaks occurred in groundwater systems that were insufficiently protected by filters and/or chlorine treatment (EPA, 1998).

Table 1: Causes of Waterborne Outbreaks of Giardiasis, 1971-94, USA (EPA, 1998)

Water Source, Treatment, or Deficiency	Outbreak Cases
Surface Water Source:	
Untreated	13
Chlorination Only	51
Filtered (includes outbreaks where filtration was by-passed)	17
Groundwater Source:	
Untreated	8
Chlorination Only	7
Filtration	1
Contamination of Distribution System or Storage	12
Use of Water not Intended for Drinking or Ingestion during Water Recreation or Other Water Activities	14
Insufficient Information	4
Totals	127

It is apparent that despite treatment of drinking water supplies by filtration and chlorination, giardiasis outbreaks were a frequent occurrence. Emphasis needs to be put on improving present treatment strategies, since current technologies do not eliminate all pathogens. It is important to develop a low maintenance, inexpensive system to effectively rid drinking water of the common gastrointestinal illness giardiasis which occurs from ingesting *Giardia* cysts. The goal of this project was to develop a prototype filter system for removing *Giardia* cysts and potentially other human pathogens present in drinking water.

1.2 Purpose and Objectives

There is a need for the development of a low maintenance, inexpensive system to treat drinking water from contamination by *G. intestinales* cysts. The overall purpose for this project was to develop and evaluate the ability of zeolite-based filter material for removal of the infectious human pathogen *G. intestinales* from contaminated drinking water. Specific objectives were the following:

1) Test the different Surfactant Modified Zeolite (SMZ) formulations in a model aquifer system to determine their ability to remove G. *intestinales* and microsphere analogs from water.

2) Test the most promising SMZ formulation at a well in the field using water amended with microspheres as analogs of *G. intestinales* cysts, and water containing naturally occurring *E. coli* bacteria, an indication of fecal coliform contamination.

1.3 *Giardia intestinales* (*G. intestinales*) and *Escherichia coli* (*E. coli*)

1.3.1 *Giardia intestinales* (*G. intestinales*)

G. intestinales is a binucleate flagellated protozoan parasite that causes the intestinal infection giardiasis in a variety of mammals, including humans, as well as birds and reptiles. The *Giardia* life cycle consists of an infective cyst (5-8 μm) and a trophozoite stage (10-12 μm long, 5-7 μm wide) (Figure 1) (Adam, 1991). A giardiasis infection can occur by ingestion of contaminated water with as few as 1-10 viable cysts (Akin and Jakubowski, 1986; Casemore et al., 1997; Jarroll, 1988).

After the cyst is initially ingested by the host, it excysts in the small intestine to form two trophozoites. The trophozoites divide by binary fission, attach to the epithelial tissue in the small intestine, and cause the symptoms of giardiasis. Symptoms of giardiasis include loose or explosive diarrhea, abdominal cramps and bloating, nausea, and decreased appetite, though some human cases are asymptomatic (Adam, 1991; Sagi et al., 1983). During infection, some trophozoites return to the cyst form and are excreted by the host. Infected humans may excrete from one to five million cysts per gram of feces (Bitton, 1999).

The surface of *Giardia* cysts are negatively charged (Gonzalez-Robles et al., 1989), have hydrophobic characteristics (Hayes, 2002; Drozd and Schwartzbrod, 1996; Dai, 2002), and calculated zeta potentials in waters at neutral pH were on average, -17 mV (Hsu and Huang, 2002). In addition, cysts are relatively resistant to external environments, while trophozoites do not survive outside of the host. *G. intestinales* cysts can survive for extended periods of time in surface or groundwater systems with temperatures of less than 10 °C; for example, cysts can survive more than 2 months at 4-8^{o}C, about 26 days at 21°C and less than 4 days at 37^{o}C

(Bingham et al., 1979; ICAIR Life Systems, 1984). Cysts can also migrate significant distances, and are relatively resistant to standard municipal water system chlorination practices.

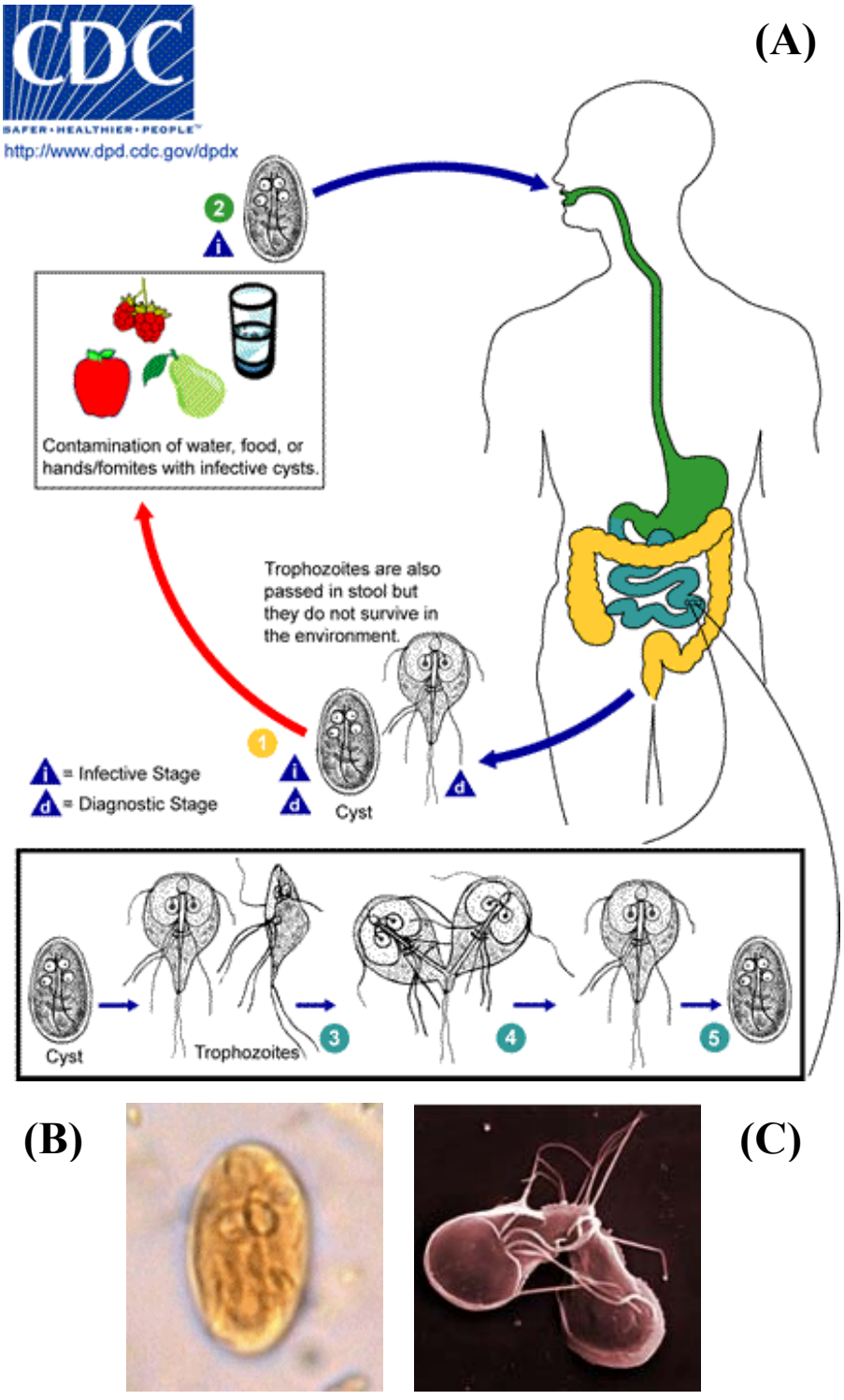


Figure 1: *G. intestinales* (a) Life Cycle (CDC, 2003); (b) Cyst Image (CDC, 2004); (c) Trophozoite Image (Ortega, 2005)

1.3.2 *Escherichia coli* (*E. coli*)

Escherichia coli (*E. coli*) are gram-negative, facultatively anaerobic bacteria that are representative of coliforms (Figure 2a-b). Several strains of *E. coli*, many of which are harmless, are found in the gastrointestinal tract of humans and warm-blooded animals. The pathogenic strain *E. coli* O157:H7 is the most noted human infectious strain. *E. coli* O157:H7 causes bloody diarrhea, and if left untreated can lead to kidney failure with observed 3-5% deaths in patients (Boyce et al., 1995) (Figure 2c). Twenty outbreaks of *E. coli* O157:H7 infections were reported in the United States between 1982 and 1993, leading to 1557 cases of infections in individuals, 358 hospitalizations and 19 deaths (Griffin, 1995). Fecal contamination of food and water is important in the transmission of this bacterial pathogen (Bitton, 1999).

The extent to which total coliforms (*E. coli*) are present in a water source can indicate the general quality of the water and the likelihood that the water is fecally contaminated. The Environmental Protection Agency (EPA) recommends testing for total coliforms (*E. coli*) as an indicator of fecal contamination of drinking water sources. Fecal indicator bacteria are easy to collect and analyze, relatively safe to handle, and are usually present when fecal pathogens such as *G. intestinales* are present, though this is not a definitive relationship. Higher levels of indicator bacteria *E. coli* typically suggest a greater level of contamination from fecal matter and a greater chance that other pathogenic microbes may be present (EPA, 2006).

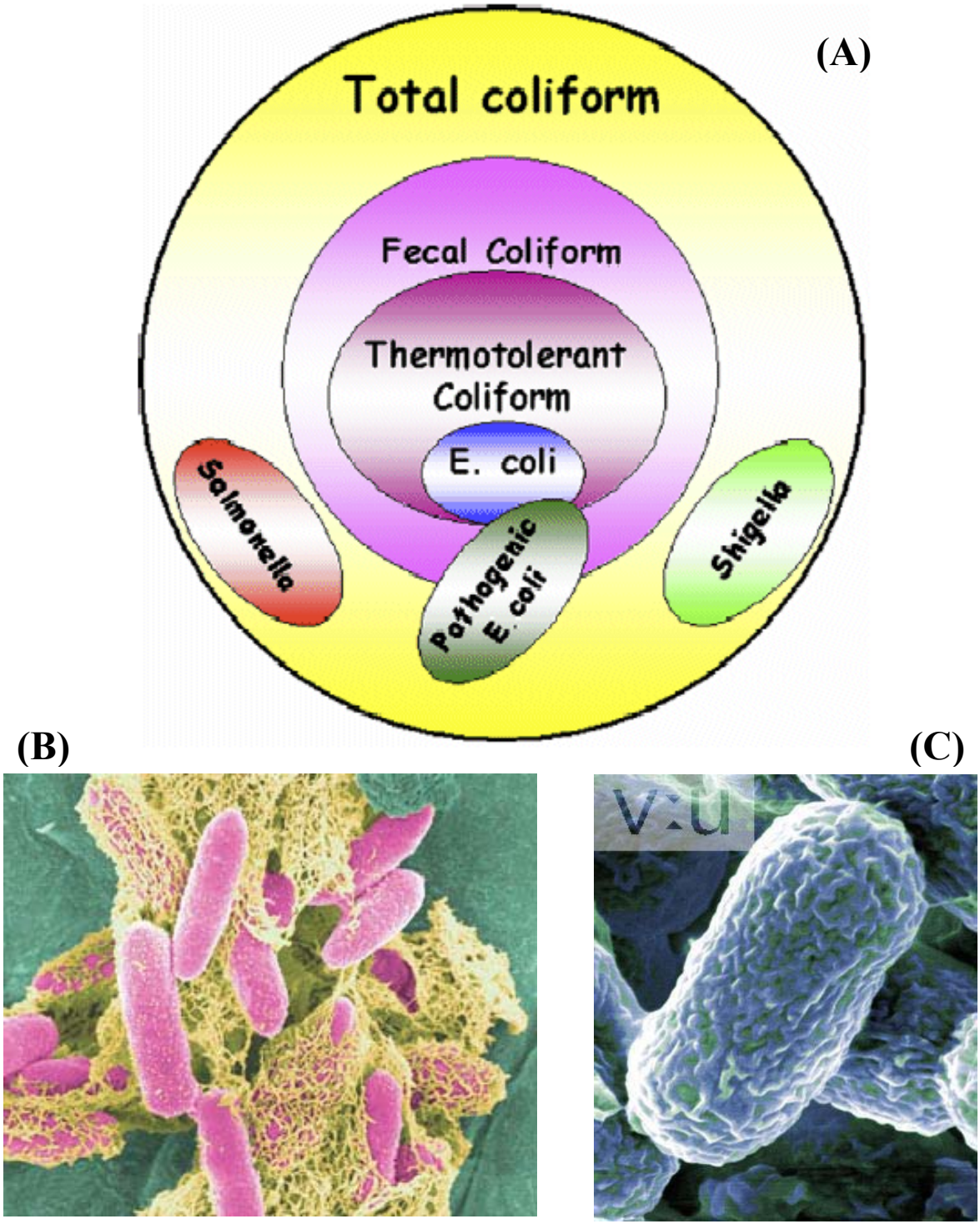


Figure 2: (a) Total coliform classification (Davis, 2006); (b) *E. coli* image (Derlet, 2006); (c) *E. coli* O157:H7 (Gaugler, 2006)

1.4 Previous Studies

Zeolites are naturally occurring hydrated alumniosilcates characterized as having cage-like structures, high surface areas (hundreds of m^2/g) and a net positive surface charge (Figure 4) (Bowman, 2005b). Zeolites share many of the surface properties of clay minerals, but display superior hydraulic properties including high specific surface area and increased cation exchange capacities (Schulze-Makuch et al., 2003; Sullivan et al., 1997). Such characteristics make zeolites an appropriate barrier for negatively charged *Giardia* cysts and microsphere analogs. Surfactant-modified zeolites (SMZ) have been extensively studied because of their potential use as environmental sorbents, and because of their potential use in water filtration. Surfactant-modified zeolites were shown to be capable of removing contaminants including, nonpolar organic compounds (Bowman et al., 2000; Cadena and Bowman, 1994; Li and Bowman, 1998; Neel and Bowman, 1992), transition metal cations (Bowman et al., 1995), inorganic anions and oxyanions (Haggerty and Bowman, 1994; Li and Bowman, 1997; Sullivan et al., 1998), and heavy metals (Bowman et al., 2000; Cadena and Bowman, 1994) from water in batch sorption studies.

In more recent studies, SMZ was further evaluated as an effective means of removing viruses and bacteria from groundwater. SMZ was shown to be effective in both the laboratory and the field at removing *E. coli* and the bacteriophage MS-2 from sewage water with a high success rate (*E. coli* 100%, MS-2 > 90%) (Schulze-Makuch et al., 2003; Schulze-Makuch et al., 2002). Removal of pathogenic parasites (*Cryptosporidium parvum* and *G. intestinales*) was also investigated in model aquifer tests by Lehner (2004) and batch experiments and column studies by Salazar (2004). Lehner (2004) used 4 μm and 10 μm polystyrene microspheres and *Bacillus subtilis*, a sporulating bacterium, as analogs for human infectious protozoan. Results proved that cationic SMZ was most effective at removing the microspheres (100%) whereas hydrophobic

SMZ was more efficient at removing the *B. subtilis* spores (75%). Batch studies by Salazar (2004) showed that cationic SMZ and hydrophobic SMZ preferentially removed *Cryptosporidium* analogs (5 μm microspheres) and *Giardia* analogs (10 μm microspheres) with 77.0(±0.8) to 82.3(±0.8) % and 98.8(±0.4) to 99.3(±0.1) % efficiency, respectfully. In support of these findings, cationic SMZ and hydrophobic SMZ in the column studies had similar removal efficiencies in the range of 79.7(±1.6) to 82.3(±0.8) % for 5 μm analogs and 99.1(±0.4) to 99.3(±0.1) % for 10 μm analogs.

In this study, we further studied cationic SMZ, hydrophobic SMZ and natural zeolite in the laboratory with viable *G. intestinales* cysts and microsphere analogs for removal efficiencies of each SMZ formulation. Then follow up by testing the most promising SMZ formulation at a well in the field using water amended with microspheres as analogs of *G. intestinales* cysts, and water containing naturally occurring *E. coli* bacteria, an indication of fecal coliform contamination.

CHAPTER 2: METHODOLOGY

2.1 Materials and Methods

2.1.1 Biosafety Level 2 Lab

The use of the human infectious agent *G. intestinales* requires a Bio-safety Level 2 Laboratory Facility set up according to regulations mandated by the Center for Disease Control and Prevention, the National Institutes of Health (CDC-NIH), and Washington State University. Standard microbiological practices were performed based on the policies and procedures from the CDC-NIH publication Biosafety in Microbiological and Biomedical Laboratories (1999) and Washington State University safety policies (Washington State University, 2005; WSU, 2005). A Hepa Filter Biosafety Cabinet was installed to decrease the risk of personal exposure due to aerosol and high splash potential during laboratory procedures. The entire model aquifer fit into the installed Hepa Filter Biosafety Cabinet.

2.1.2 Model Aquifer

A plexiglass model aquifer was used for all laboratory research experiments. A picture of the model aquifer is shown in Figure 3. The dimensions of the model aquifer were 53 x 29 x 2.2 cm. The model aquifer was packed with cleaned, sieved, silica sand with a grain size of 0.21-1.19 mm that was composed of 99.4% silicon dioxide (Table 2). The silica sand was washed 10 times with 10% bleach and rinsed 10 times with nano pure water in order to clean the sand. Once packed in the model aquifer, 10 liters of 0.005 M $CaCl_2$, 10 times the estimated pore water volume, was allowed to flow through the model aquifer before each run. The purpose was to wash off any excess surfactant on the barrier material and to allow the matrix chemistry and ionic strength of the model aquifer to be similar to that of natural water chemistries in the

Western U.S. (Bowman, 2005a). Three plastic piezometers (18 cm) were made and installed in the model aquifer for tracer injection and sample extraction. An injection piezometer was placed 10 cm from the SMZ barrier, and two sample piezometers were placed 5 cm before and 5 cm after the SMZ barrier to monitor the arrival of the *Giardia* cysts and analogs. Each piezometer was screened with a 20-25 μm filter to prevent sand from entering the sample during collection. Sample sizes of only 1 mL were collected to prevent disturbing the flow and transport during the experiment.

The hydraulic gradient was measured to be 0.07 and was kept constant by controlling the hydraulic head in the inflow and outflow reservoirs (Lehner, 2004). Flow rates varied for each material used within the model aquifer (Table 3). The flow rate for the conservative potassium chloride (KCl) tracer with fine cationic SMZ calculated from the injection well to well 1 was 0.71 cm/min. The flow rates for the microspheres and cysts, calculated from well 1 to well 2 for the cationic SMZ and raw zeolite and from the injection well to well 1 for the hydrophobic SMZ, ranged from 0.14 to 1.43 cm/min and 0.21 to 0.83 cm/min, respectfully.

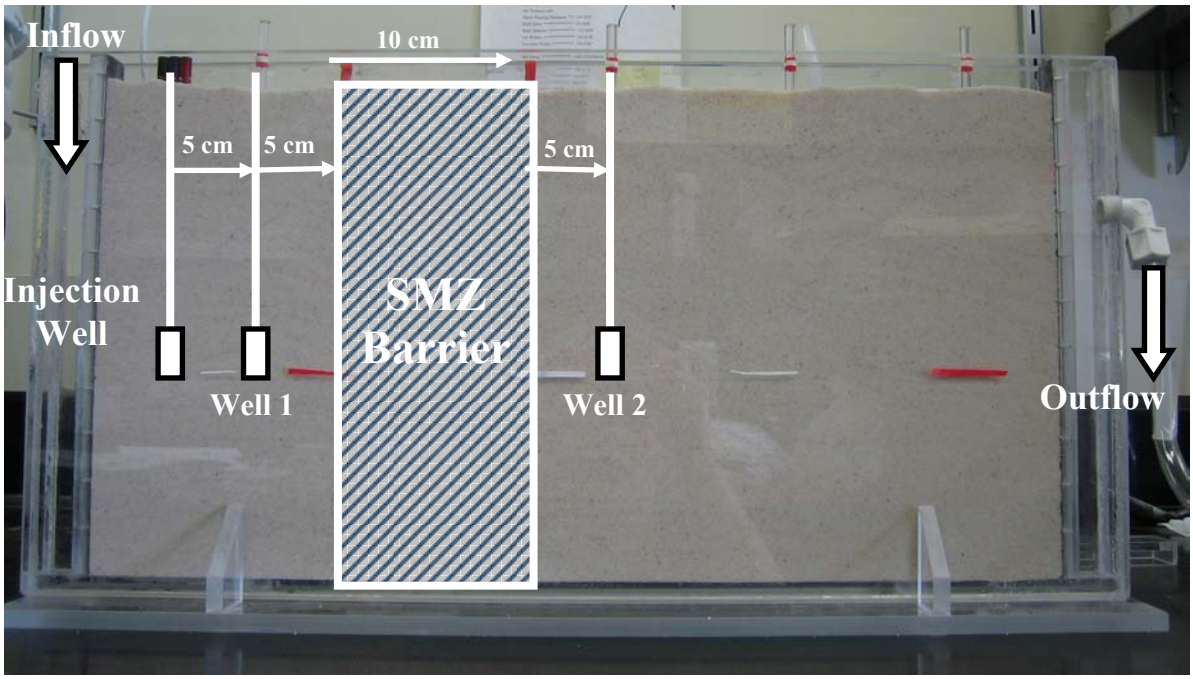


Figure 3: Plexiglass model aquifer set-up with a 0.07 gradient

Lane Mountain Company Silica Sand Ingredients (LM# 20/30)	
Chemical Analysis	**Percent (%)**
Silicon Dioxide	99.40
Aluminum Oxide	0.21
Iron Oxide	0.04
Titanium Dioxide	0.02
Calcium Oxide	0.10
Magnesium Oxide	0.00
Sodium Oxide	0.00
Potassium Oxide	0.06
L.O.I. (1200 °C)	0.20
Total	100.00

Table 2: Lane Mountain Company XRF results of silica sand used in model aquifer

Barrier Material	Tracer Type	Pore Water Velocity (cm/min)	Specific Discharge (cm/min)	Volumetric Discharge (cm^3/min)	Hydraulic Conductivity (cm/min)
Fine Cationic (0.4 - 1.4 mm)***	KCl	0.71	0.21	13.67	3.06
Fine Cationic (0.4 - 1.4 mm)**	Microspheres	Range from 0.14 to 1.43	Range from 0.04 to 0.43	Range from 2.68 to 27.37	Range from 0.60 to 6.13
Coarse Cationic (1.4 - 2.4 mm)**					
Raw Zeolite**					
Hydrophobic Try 1 (5 mL)***					
Fine Cationic (0.4 - 1.4 mm)**	Cysts	Range from 0.21 to 0.83	Range from 0.06 to 0.25	Range from 4.02 to 15.89	Range from 0.90 to 3.56
Coarse Cationic (1.4 - 2.4 mm)**					
Raw Zeolite**					
Hydrophobic Try 1 (5 mL)***					
Hydrophobic Try 2 (10 mL)***					

Table 3: Estimated values for materials used within model aquifer

Note: *Velocity from Injection well to Well 2, **Velocity from Well 1 to Well 2, ***Velocity from Injection well to Well 1, Porosity for all material was estimated to be 0.3, For independent flush experiments with 10 L of 0.005 M $CaCl_2$, the flow rate was between 0.04 cm/min and 0.10 cm/min.

2.1.3 Cationic SMZ, Hydrophobic SMZ, Raw Zeolite

Two formulations of SMZ, cationic and hydrophobic, and natural raw zeolite were tested to determine which of the three materials was more effective at removing *G. intestinales* and microsphere analogs from water (Table 3). Natural raw zeolites are naturally occurring hydrated alumniosilicates characterized by cage-like structures, high surface areas (hundreds of m^2/g) and

a net negative surface charge (Figure 4) (Bowman, 2005b). Natural raw zeolite material was used as the base material for the two formulations of SMZ (cationic and hydrophobic). The composition of the natural raw zeolite was 74% clinoptilolite, 10% feldspar, 10% quartz/cristobalite, 5% smectite, and 1% illite by weight. The natural raw zeolite was mined from St. Cloud Mining Co., in Winston, NM (Chipera and Bish, 1995; Sullivan et al., 1997). Dr. Robert Bowman and his graduate student Diane Agnew performed all the modifications of the natural raw zeolite at the New Mexico Institute of Mining and Technology. The cationic and hydrophobic SMZ formulations were produced by varying the concentration of hexadecyltrimethylammonium (HDTMA) on the surface of natural raw zeolite (Table 3). HDTMA is a common bulk-production surfactant used in hair conditioners and mouthwash. HDTMA molecules coat the zeolite surface and make up a bi-layer over the zeolite surface. HDTMA molecules consist of a 16-carbon chain tail group attached to a 3-methyl quaternary amine head group with a permanent 1+ charge (Sullivan, 1997) (Figure 6). The surfactant monomers are sorbed onto the raw zeolite and develop a stable monolayer or a bi-layer configuration depending on the amount of surfactant applied (Sullivan, 1997). With a positive bi-layer adsorption of negatively charged microbes and cysts was greatly improved (Figure 5). After surfactant loading, the coated zeolite was rinsed with de-ionized (DI) water for an extended period to remove excess surfactant from the material and allowed to air dry.

The raw zeolite had an internal cation exchange capacity (CEC) of 800 meq/kg and an external cation exchange capacity (ECEC) of 100 meq/kg, as determined using a method modified from that of Ming and Dixon (Bowman et al., 1999; Li and Bowman, 1997; Ming and Dixon, 1987). All details of the properties of the zeolite formulations used in laboratory experiments are tabulated in Table 3.

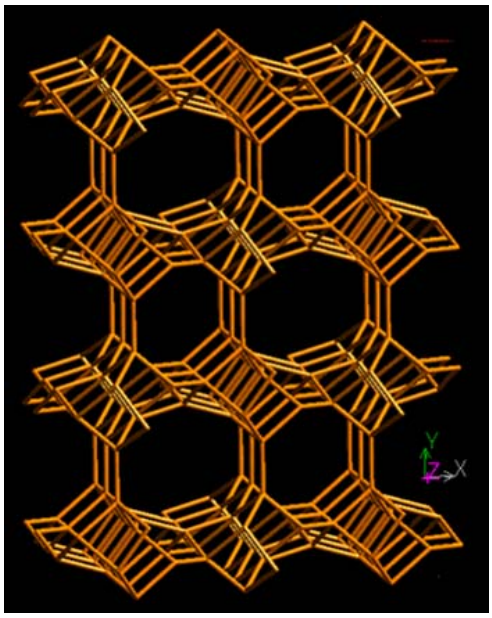


Figure 4: Diagram showing clinoptilolite with its 8- and 10-member channels (from International Zeolite Association, 2005)

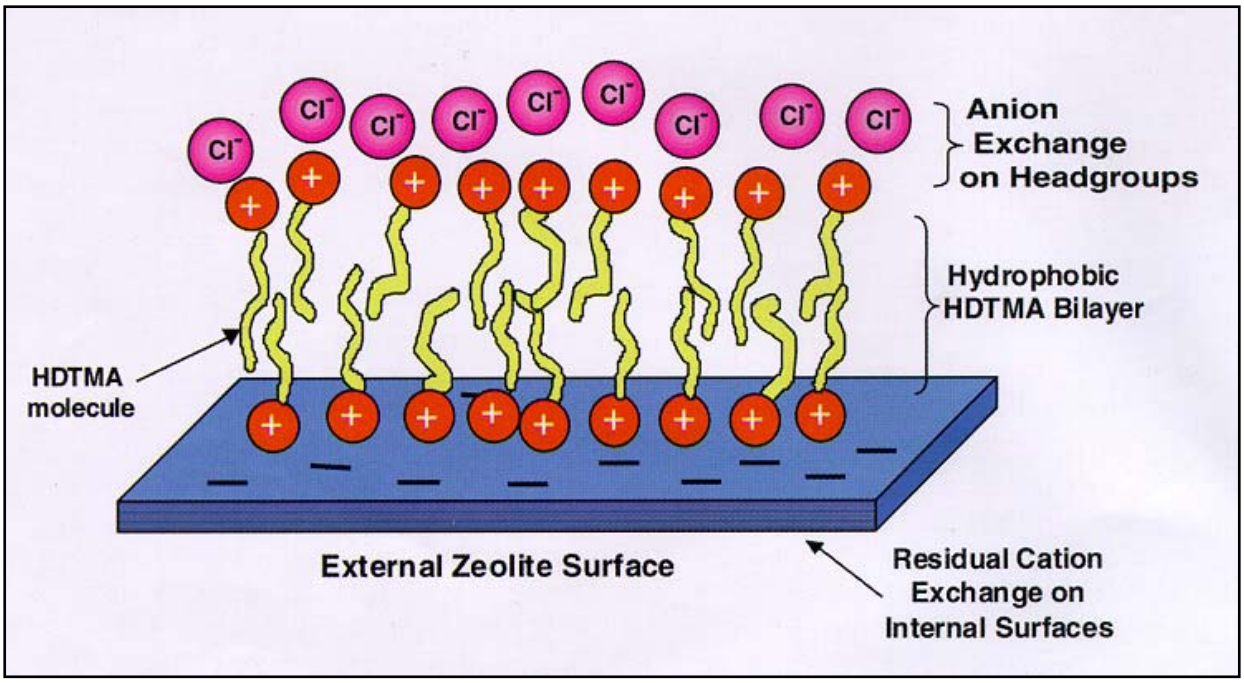


Figure 5: Schematic of the surface of the Surfactant-Modified Zeolite (SMZ) (from Schulze-Makuch et al., 2002)

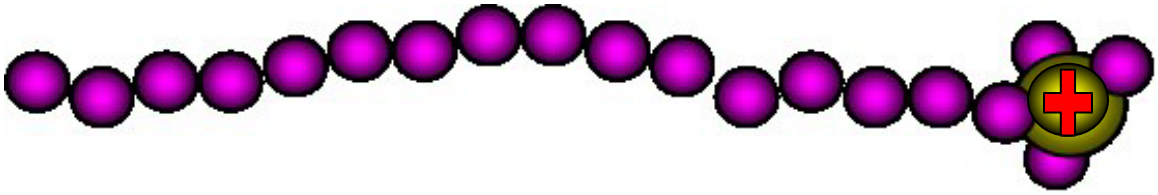


Figure 6: Schematic of a HDTMA molecule

Table 4: Details on Cationic Surfactant-Modified Zeolite (SMZ), Hydrophobic SMZ, and Natural Raw Zeolite

Sample Name	P2SMZ14-40 (Fine Cationic SMZ)	P2SMZ8-14 (Coarse Cationic SMZ)	Hydrophobic	Raw Zeolite
Zeolite Source	St. Cloud Mine, NM	St. Cloud Mine, NM	St. Cloud Mine, NM	St. Cloud Mine, NM
Mineralogy by weight	74% clinoptilolite 10% feldspar 10% quartz/cristobalite 5% smectite 1% illite	74% clinoptilolite 10% feldspar 10% quartz/cristobalite 5% smectite 1% illite	74% clinoptilolite 10% feldspar 10% quartz/cristobalite 5% smectite 1% illite	74% clinoptilolite 10% feldspar 10% quartz/cristobalite 5% smectite 1% illite
Mesh Size (US Standard Sieves)	14-40	8-14	8-14	8-14
Particle Size Range	0.4-1.4 mm	1.4-2.4 mm	1.4-2.4 mm	1.4-2.4 mm
External Cation Exchange Capacity (ECEC)	90-100 meq/kg	N/A	66.2 meq/kg (Salazar, 2004)	70-90 meq/kg (Sullivan, 1997)
External Surface Area	5.6 m^2/g	5.9 m^2/g	13.44 m^2/g (Salazar, 2004)	15.7 m^2/g (Schulze-Makuch et al., 2002; Sullivan et al., 1997)
HDTMA Loading	180 mmol/kg	140 mmol/kg	79.28 mmol/kg	0 mmol/kg

Note: External Cation Exchange Capacity (ECEC), Hexadecyltrimethylammonium (HDTMA)

2.1.4 Potassium Chloride (KCl) and Electrical Conductivity

Before any particle tracer test, an experimental run was done using a conservative 0.1 M potassium chloride (KCl) tracer. The breakthrough curve of the KCl tracer was used to estimate the arrival time for *G. intestinales* cysts (7-8 μm) and fluorescent polystyrene microspheres (6.0-7.9 μm). At time zero, 5 mL of the potassium chloride tracer was injected into the model aquifer and 3 mL samples were taken from two piezometers (Well 1). Samples were collected every 3 minutes from time 1 to 16 minutes and increased to 5 minutes from 20 to 80 minutes, 10 minutes from 80 to 120 minutes, and 30 minutes from 120 to 210 minutes at the end of the experiment. Samples were analyzed for electrical conductivity (EC) using a Thermo Orion conductivity meter (Orion 115). Each 3 mL sample was diluted in a 1:1 ratio with DI water to adequately cover the EC probe and acquire proper readings. Each EC reading was multiplied by 2 to obtain the original EC value of the 3mL samples taken during the experiment. The breakthrough curve was used to determine a shorter and more frequent sampling interval for the following *G. intestinales* cysts and fluorescent polystyrene microspheres experiments.

The following procedure was used:

(1) Calibrate electrode with two EC standard solutions (1413 μS, 84 μS)

(2) Dilute 3 mL samples with 3 mL of DI water (1:1 ratio)

(3) Test samples with electrode and record concentrations (μS)

(4) Plot EC concentrations versus time, for future sampling time schedule

2.1.6 *Giardia intestinales*

Viable *G. intestinales* cysts were used in all experiments to determine the interaction of protozoa with barrier materials. The viable cysts were purchased from Waterborne, Inc. (New Orleans, LA). Each 8 mL suspension of cysts was delivered in a storage solution of PBS with penicillin, streptomycin, gentamicin, and 0.01% Tween 20; this mixture allows the cysts to last for 6 months in 4^{o}C storage conditions. All cysts were from experimentally infected gerbils, later purified from feces by sucrose and Percoll density gradient centrifugation, to provide a total of about 5x10^{6} cysts per 8 mL sample.

Giardia samples were iodine-stained and counted on a Bright Line Hemacytometer (Hausser Scientific Company; Neubauer ruling). Counts provided a concentration of cysts per mL. A more detailed explanation of analysis and procedures are provided in Appendix A. Figure 7 and 8 illustrate the iodine-stain set up used for all laboratory samples.

Figure 7: Iodine-stain set up

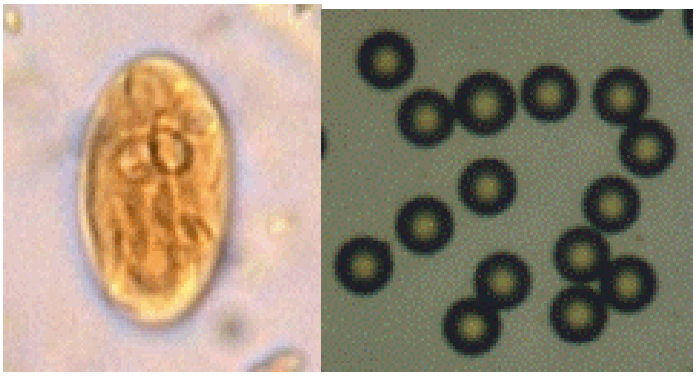


Figure 8: (a) Iodine-stained *Giardia* cyst (CDC, 2004); (b) Iodine-stained microspheres

2.1.7 Fluorescent Polystyrene Microspheres

Fluorescent polystyrene microspheres were used as analogs of *Giardia* cysts for each tested SMZ material. The fluorescent polystyrene microspheres were purchased from Gerlinde Kisker (Steinfurt, Germany). The microsphere size was 6.0 -7.9 µm with a mean particle size of 6.42 µm. The fluorescent microspheres were internally dyed with Nile Red which has excitation and emission wavelengths of 520 nm and 560 nm. Each 2 mL suspension of microspheres was delivered in a storage solution of DI water with 0.02% sodium azide, and required storage at 4^{o}C and protection from light. The concentration provided by Gerlinde Kisker for the 6.0 -7.9 µm Nile Red microspheres was 1% w/v = 10 mg/ ml.

Microsphere samples were iodine-stained and counted on a Bright Line Hemacytometer (Hausser Scientific Company; Neubauer ruling). Counts provided a concentration of microspheres per mL. A more detailed explanation of analysis and procedures are provided in Appendix A. Figure 7 and 8 illustrate the iodine-stain set up used for all laboratory samples.

2.2 Experiments

2.2.1 Transport Experiments

Transport experiments were conducted in the plexiglass model aquifer described in section 2.1.2. Each SMZ barrier (10 cm wide) was placed in between Well 1 and Well 2 with a 5 cm spacing (Figure 3). Once the model aquifer was packed, 10 liters of 0.005 M $CaCl_2$ were allowed to flow through before each run. During the laboratory run an additional 10 liters of 0.005 M $CaCl_2$ solution was allowed to flow through the model aquifer to create a hydraulic gradient. The hydraulic gradient was kept constant at 0.07 and flow rates ranged from 0.14 to 1.43 cm/min and 0.21 to 0.83 cm/min for microspheres and cysts, respectfully. For independent flush experiments with 10 L of 0.005 M $CaCl_2$, measured flow rates were between 0.04 cm/min and 0.10 cm/min. Flush rates were smaller due to the measurement over a longer distance (i.e. entire model aquifer, 53 cm).

In order to set up an appropriate sampling schedule for future runs, 0.1 M potassium chloride (KCl) was injected in the injection well. The breakthrough curve of the conservative tracer was used to estimate the arrival times for *G. intestinales* (7-8 μm) and fluorescent polystyrene microspheres (6.0-7.9 μm. Before each run, control samples were taken from Well 1 and Well 2 to determine if any particle contamination was present. Once the experiment was in progress, 1 mL water samples were collected from Well 1 and Well 2 at predetermined times with clean pipette tips with tube extensions. Small sampling size (1 mL) was meant to minimize the affect on the flow, and tube extensions were to ensure samples were taken from the piezometers inlets. After all water samples were collected, samples were stored at 4°C until analysis to protect the integrity of the samples.

Initial injection concentrations of *Giardia* cysts and microspheres were consistent for each laboratory experiment. There was a drop in concentrations observed at Well 1 before the tested barrier, probably due to dispersion and gravitational settling.

2.3 Data Analysis

The conservative tracer run, 0.1 M potassium chloride (KCl), was performed to provide baseline transport concentrations and times through cleaned, sieved, silica sand for *Giardia* cysts and microspheres. The breakthrough curve of the conservative tracer was used to estimate the arrival times for *G. intestinales* cysts (7-8 μm) and fluorescent polystyrene microspheres (6.0-7.9 μm) and compared with the following SMZ barrier runs.

All sample aliquots were iodine-stained and triplicate counts were completed for each individual sample time for both Well 1 and Well 2. For a more detailed explanation of *Giardia* cyst and microsphere counts refer to Appendix A. All *Giardia* cyst and microsphere arrivals were plotted with concentrations per mL versus time in minutes, to illustrate the effectiveness of each SMZ barrier tested.

The plots of *Giardia* cyst and microsphere arrivals were distinctly different than the traditional breakthrough curves of conservative tracers such as potassium chloride (KCl). Unlike the characteristic abrupt breakthrough peak followed by a gradual decrease as evident with conservative tracers (Figure 9, 10, 21 & 22), the spike-like presence of microspheres and *Giardia* cysts at later times in Well 2 occur throughout all experiments, except during hydrophobic runs. For this reason, all plots were considered to be arrival times of *Giardia* cysts and microspheres and not breakthrough curves. Similar erratic behavior after the SMZ barrier have occurred in Lehner's (2004) model aquifer experiments with the microspheres and *Cryptosporidium parvum* bacteria (*Giardia* cysts analogs). The initial arrival peaks and related concentrations were noted

in the following results. Removal rates (Table 5) represent the percent drop in the total sum of counts present within Well 1 samples versus the total sum of counts present within Well 2 samples.

Quantification of uncertainty was calculated for all triplicate counts made of each Well 1 and Well 2 water sample by using standard deviation (SDTEV) and 95% confidence interval (95% CI). The uncertainty about the triplicate count of each sample can be found in Appendix B. Standard deviation (STDEV) was calculated by the following formula;

$$\sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{(n-1)}} \quad (1)$$

where σ is the standard deviation, x is the sample mean average and n is the sample size. The 95 percentile confidence interval (95% CI) was calculated by the following formula;

$$\bar{x} \pm 1.96\left(\frac{\sigma}{\sqrt{n}}\right) \quad (2)$$

where x is the sample mean average, σ is the standard deviation, and n is the sample size. Average standard deviation (STDEV) was tabulated for all samples in Well 1 and Well 2 by averaging the standard deviation of each data point (triplicate count). In addition, the average 95% confidence interval was tabulated for all samples in Well 1 and Well 2 by averaging the 95% confidence interval of each data point (triplicate count). Both the average standard deviation and the average 95% confidence interval are noted below each appropriate arrival plot (e.g. Figure 9).

CHAPTER 3: RESULTS

Two formulations of SMZ, cationic and hydrophobic, and natural raw zeolite were tested to determine which material was more effective at removing *G. intestinales* and microspheres from groundwater. The ability of the three barriers at preventing pathogen migration was investigated through several laboratory model aquifer experiments. The coarse cationic SMZ formulation was further evaluated in a field test at the University of Idaho Groundwater Research Site with a prototype SMZ filter system.

For purposes of organization, only the arrival time results are presented for each laboratory run (Figures 9-19). Initial injection concentrations of *Giardia* cysts and microspheres were consistent for each laboratory experiment, though a drop in concentration was generally observed, at Well 1 before the tested barrier, probably due to dispersion and gravitational settling. Scales on arrival plots vary for each model aquifer experiment.

Individual concentrations (particles/mL) of *Giardia* cysts and microspheres for each sampling time were organized in color coded tables provided in Appendix B, which correspond to the graphed arrival results (Figures 9-19). For the standard deviation and 95% confidence interval of each individual sample, refer to Appendix B. Both the average standard deviation and 95% confidence interval were noted below each appropriate arrival plot (Figures 9-19).

3.1 Cationic SMZ (Fine & Coarse)

Two different grain sizes of the same cationic SMZ formulation, fine and course, were tested to determine which material was more effective at removing *G. intestinales* and microsphere analogs from groundwater. For both the fine and coarse cationic SMZ runs, microspheres and *Giardia* cysts appeared after the initial arrivals in Well 1 & 2 at various later times through out the remainder of the experiments (Figure 9-14).

3.1.1 Fine Cationic SMZ (0.4-1.4 mm)

The conservative potassium chloride (KCl) tracer breakthrough from the fine cationic SMZ run occurred at 7 minutes. In addition to the potassium chloride (KCl) tracer, bromine (Br_2) was also used in the sand only run. The bromine (Br_2) breakthrough occurred at 6 minutes. The appearance of both breakthroughs roughly one minute apart confirms the appropriate sampling scheme and both represent the travel time of a conservative tracer (Figure 9 & 10).

The microsphere arrival peaks were observed at 10 minutes in Well 1 and at 150 minutes in Well 2 (Figure 11). The *Giardia* cysts arrival peaks occurred at 10 minutes in Well 1 and then at 50 minutes in Well 2 (Figure 12). Both microspheres and *Giardia* cysts arrived at Well 1 at the same time, while at Well 2 the microspheres arrived 100 minutes later than the *Giardia* cysts. The removal rates observed for microspheres and *Giardia* cysts were 98.8% and 52.8 %, respectively.

3.1.2 Coarse Cationic SMZ (1.4-2.4 mm)

The microsphere arrival peaks were observed at 6 minutes in Well 1 and at two possible times in Well 2; 35 minutes or 65 minutes (Figure 13). The concentration at 35 minutes was 1.40 x 10^4 spheres per mL and the maximum concentration occurred at 65 minutes with 1.86 x 10^4 spheres per mL. *Giardia* cysts arrival peaks were at 4 minutes in Well 1 and then at 100 minutes in Well 2, shown in Figure 14. Arrivals at Well 1 for both microspheres and *Giardia* cysts occur a few minutes earlier than was observed with the previous fine cationic SMZ run. The removal rates observed for microspheres and *Giardia* cysts were 56.1% and 65.6 %, respectively.

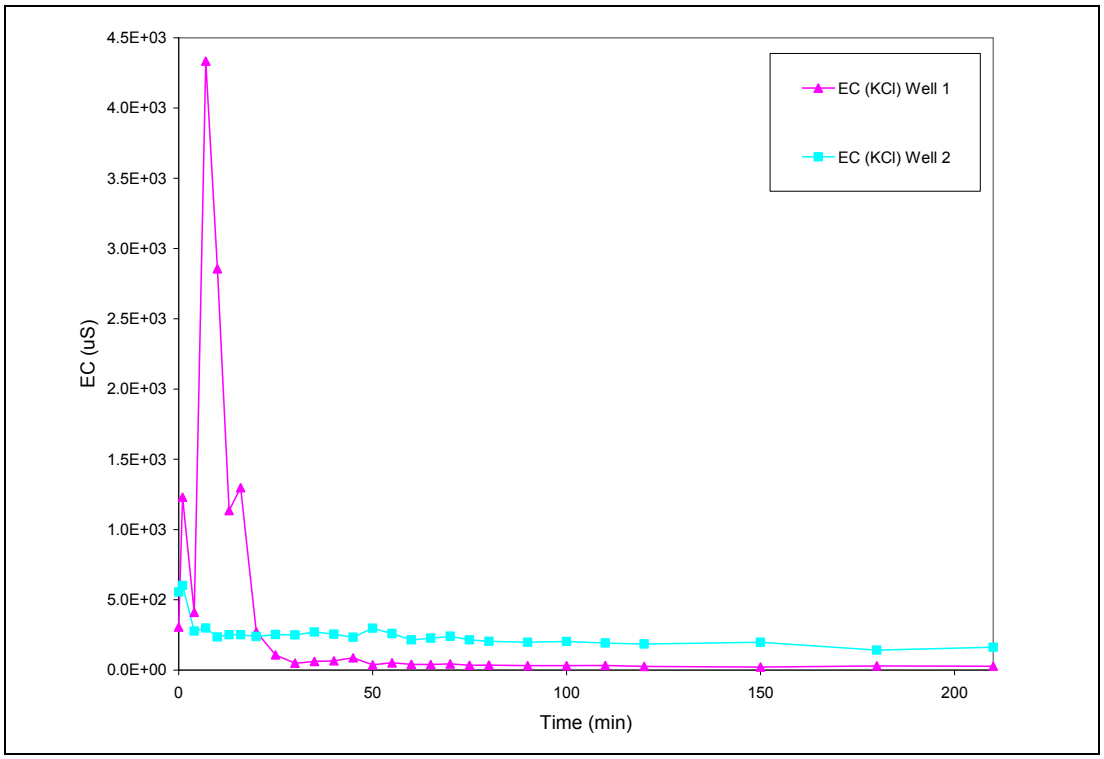


Figure 9: Electrical Conductivity (Potassium Chloride) of Fine Cationic SMZ experiment

Note: Ave. Standard Deviation of Well 1 was 1.0E+03 with an Ave. 95% Confidence Interval of 2.2E+02; Ave. Standard Deviation of Well 2 was 1.0E+02 with an Ave. 95% CI of 2.2E+01. For Standard Deviation and 95% CI of each triple count for each data point see Table B-1. Well 1 was located before barrier, Well 2 behind it.

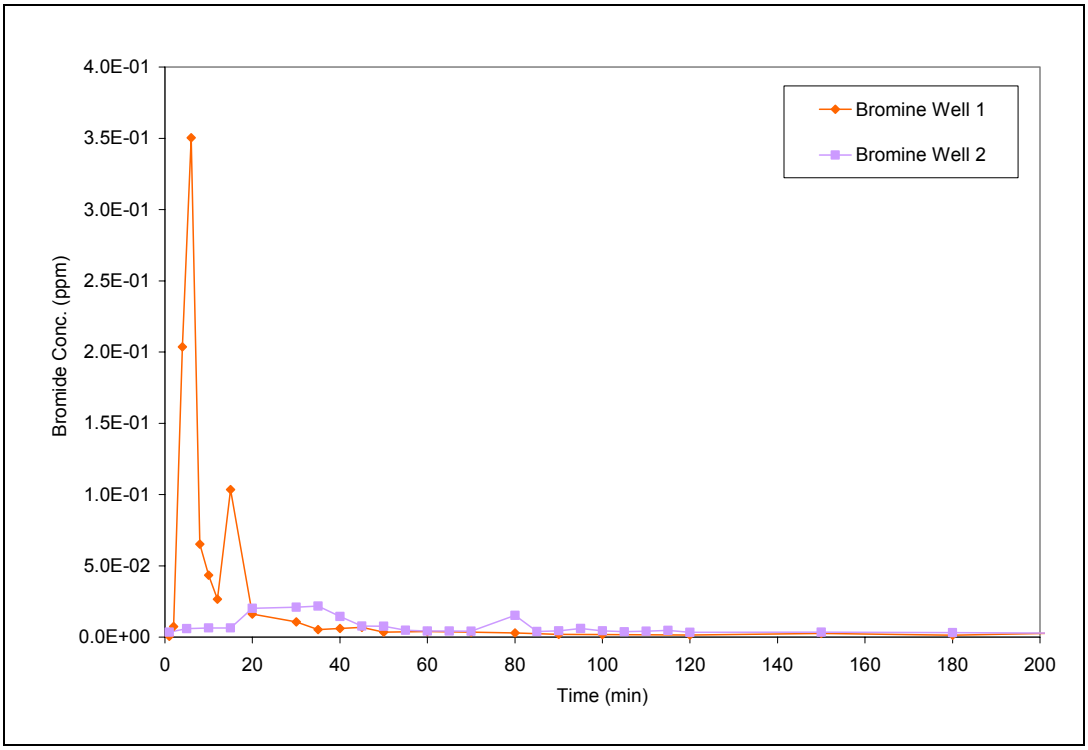


Figure 10: Bromine (Br_2) Concentrations of Sand only experiment

Note: Average Standard Deviation of Well 1 was 8.0E-02 with an Average 95% Confidence Interval of 1.8E-02; Ave. Standard Deviation of Well 2 was 5.5E-03 with an Ave. 95% CI of 1.1E-03. For Standard Deviation and 95% CI of each triple count for each data point see Table B-2.

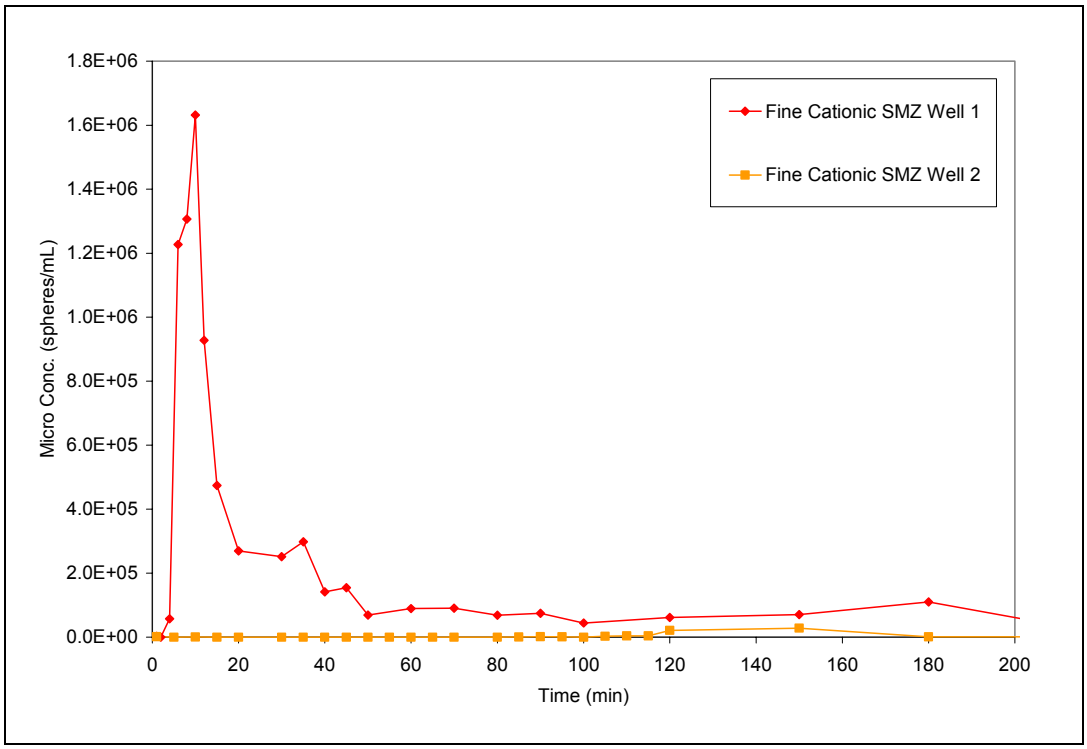


Figure 11: Microsphere Concentrations of Fine Cationic SMZ experiment

Note: Ave. Standard Deviation of Well 1 was 8.4E+04 with an Ave. 95% Confidence Interval of 9.5E+04; Ave. Standard Deviation of Well 2 was 5.7E+03 with an Ave. 95% CI of 6.4E+03. For Standard Deviation and 95% CI of each triple count for each data point see Table B-3.

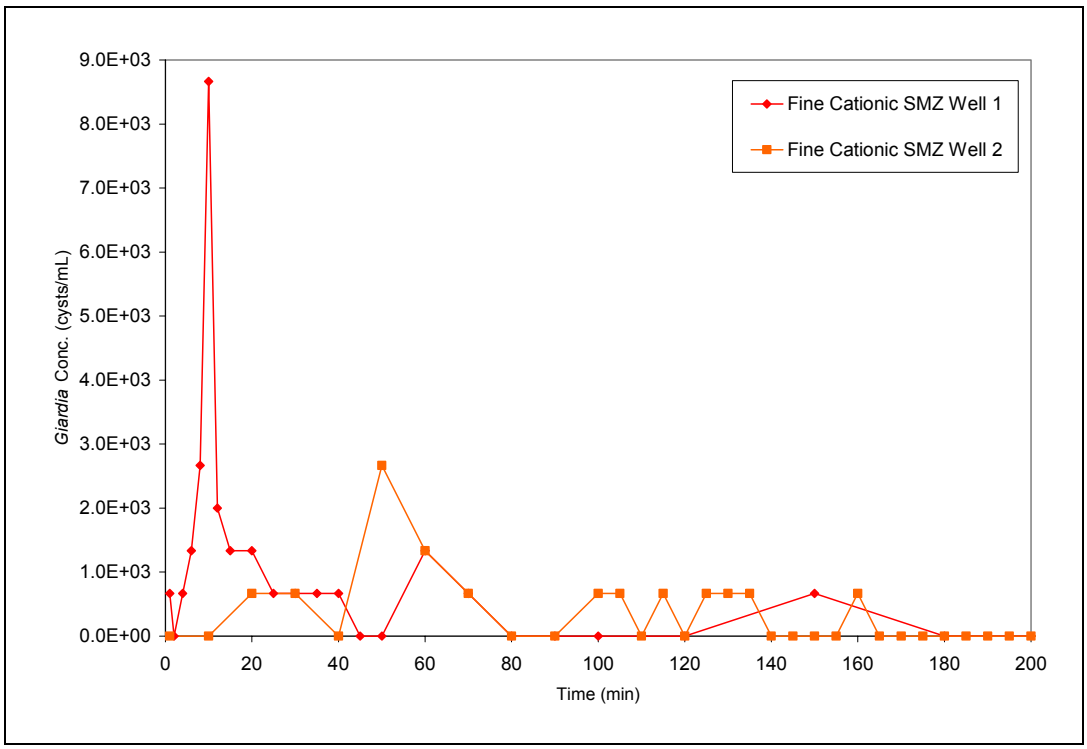


Figure 12: *G. intestinales* Cyst Concentrations of Fine Cationic SMZ experiment

Note: Ave. Standard Deviation of Well 1 was 1.1E+03 with an Ave. 95% Confidence Interval of 1.2E+03; Ave. Standard Deviation of Well 2 was 4.5E+02 with an Ave. 95% CI of 5.0E+02. For Standard Deviation and 95% CI of each triple count for each data point see Table B-4.

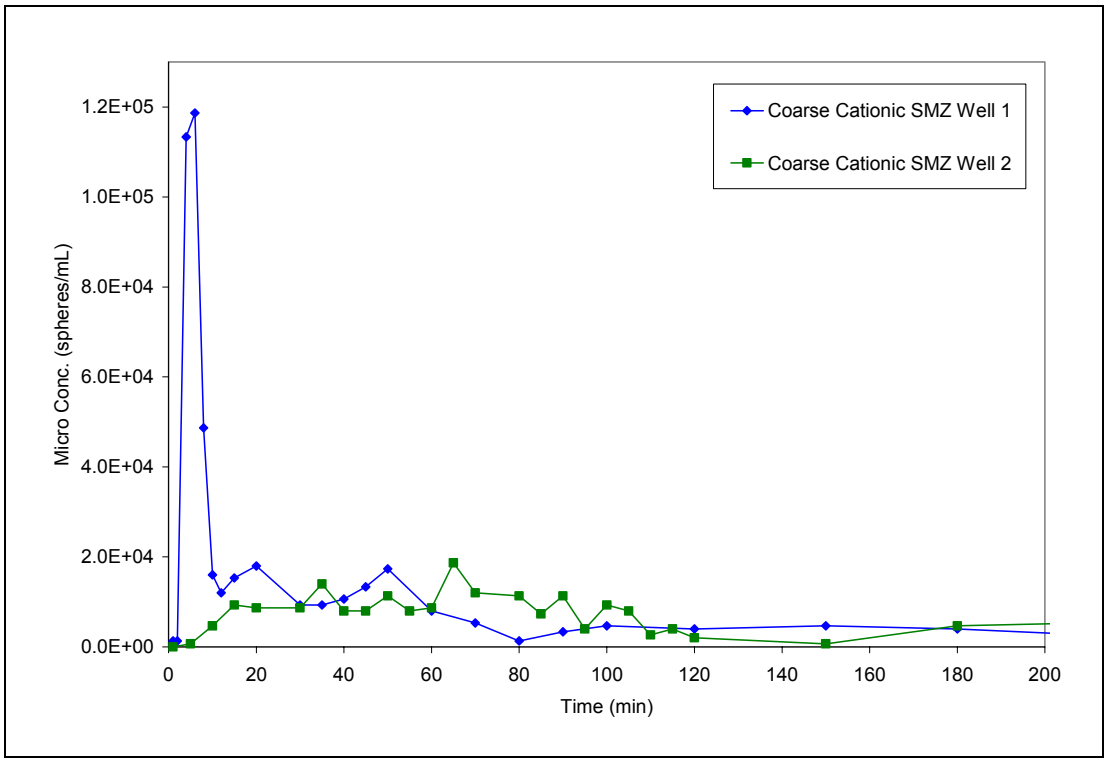


Figure 13: Microsphere Concentrations of Coarse Cationic SMZ experiment

Note: Ave. Standard Deviation of Well 1 was 5.8E+03 with an Ave. 95% Confidence Interval of 6.6E+03; Ave. Standard Deviation of Well 2 was 4.8E+03 with an Ave. 95% CI of 5.4E+03. For Standard Deviation and 95% CI of each triple count for each data point see Table B-5.

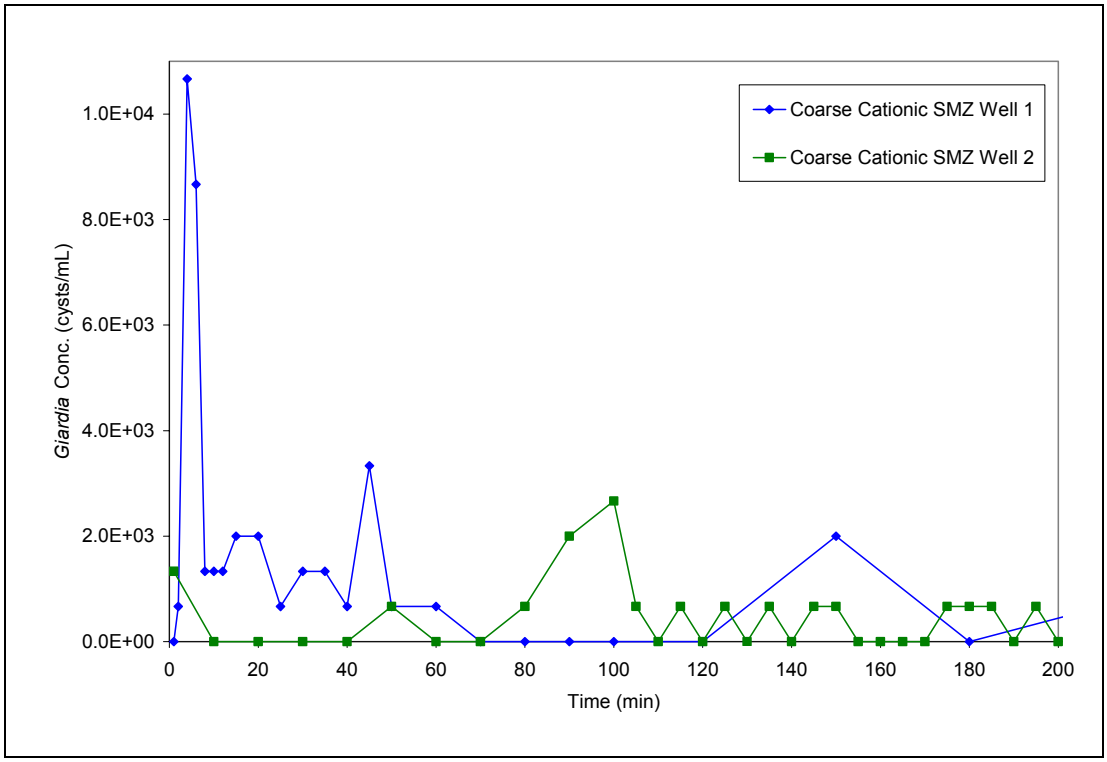


Figure 14: *G. intestinales* Cyst Concentrations of Coarse Cationic SMZ experiment

Note: Ave. Standard Deviation of Well 1 was 2.6E+03 with an Ave. 95% Confidence Interval of 2.9E+03; Ave. Standard Deviation of Well 2 was 5.1E+02 with an Ave. 95% CI of 5.8E+02. For Standard Deviation and 95% CI of each triple count for each data point see Table B-6.

3.2 Sand

Results of the experiment using sand only showed a *Giardia* cyst arrival peak at 6 minutes in Well 1 and then at 30 minutes in Well 2 (Figure 15). For the *Giardia* cysts, a removal rate 61.5% was observed by physical filtration by the silica sand alone. After the initial arrival peaks in Well 1 & 2, additional *Giardia* cysts showed up in both wells at later times through out the remainder of the experiment.

3.3 Natural Raw Zeolite

Results of experiments using raw zeolite as the barrier material showed the arrival of microspheres at 12 minutes in Well 1 and at 105 minutes in Well 2 (Figure 16). The unaltered natural raw barrier effectively removed 85.3% of the microspheres down gradient of the 10 cm barrier.

The *Giardia* cysts plot (Figure 17) illustrates two separate arrival peaks for both Well 1 and 2. For Well 1, two comparable arrival peaks appear at 50 minutes (2.0×10^3 cysts per mL) and later at 100 minutes (2.0×10^3 cysts per mL). In Well 2, two equivalent peaks appear at 130 minutes (1.33×10^3 cysts per mL) and at 140 minutes (1.33×10^3 cysts per mL). This arrival pattern was erratic in comparison to previous barrier experiments, and the data showed that the release of *Giardia* cysts were erratic throughout the experiment. The unaltered natural raw barrier was only 43.8% effective at removing *Giardia* cysts in comparison to its microsphere analogs with an 85.3% removal rate.

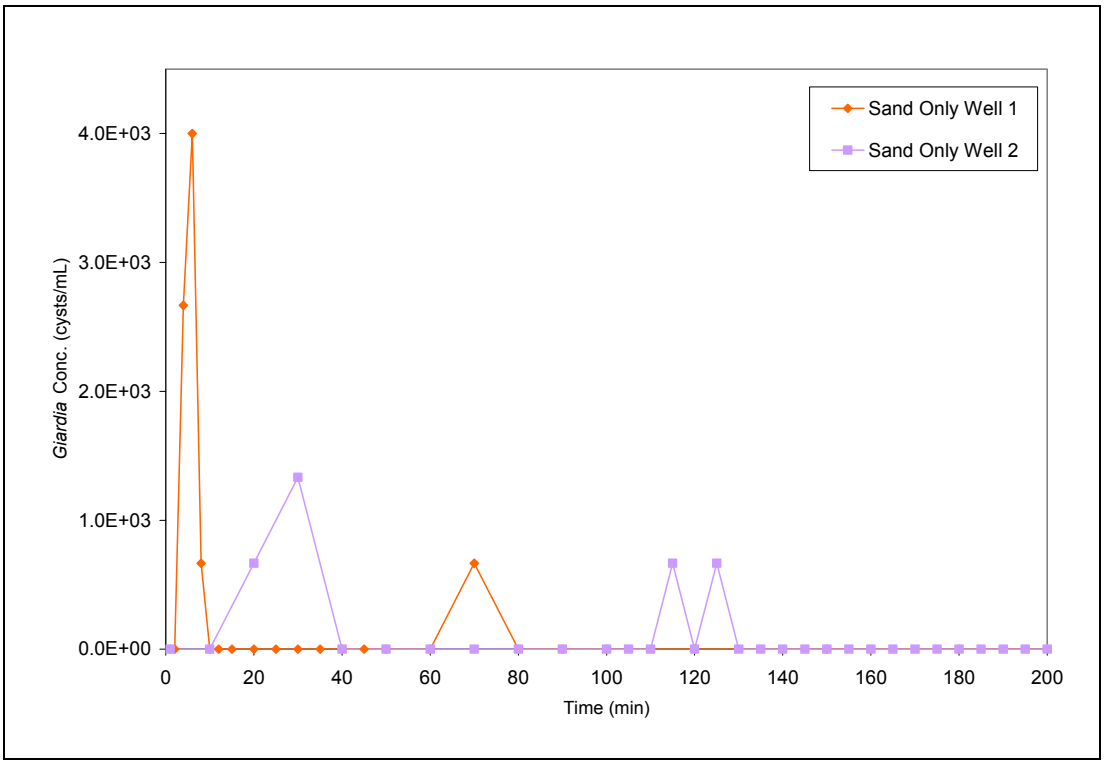


Figure 15: *G. intestinales* Cyst Concentrations of Sand only experiment

Note: Ave. Standard Deviation of Well 1 was 3.1E+02 with an Ave. 95% Confidence Interval of 3.5E+02; Ave. Standard Deviation of Well 2 was 1.5E+02 with an Ave. 95% CI of 1.7E+02. For Standard Deviation and 95% CI of each triple count for each data point see Table B-7.

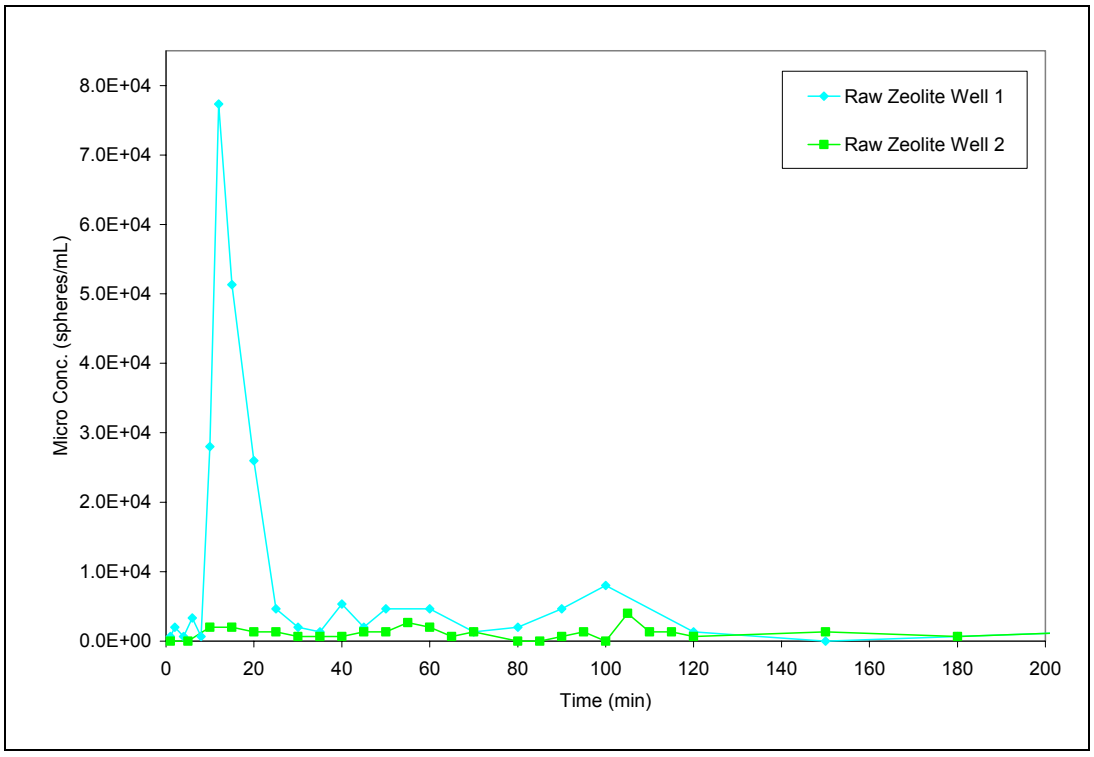


Figure 16: Microsphere Concentrations of Natural Raw Zeolite experiment

Note: Ave. Standard Deviation of Well 1 was 3.3E+03 with an Ave. 95% Confidence Interval of 3.7E+03; Ave. Standard Deviation of Well 2 was 1.5E+03 with an Ave. 95% CI of 1.7E+03. For Standard Deviation and 95% CI of each triple count for each data point see Table B-8.

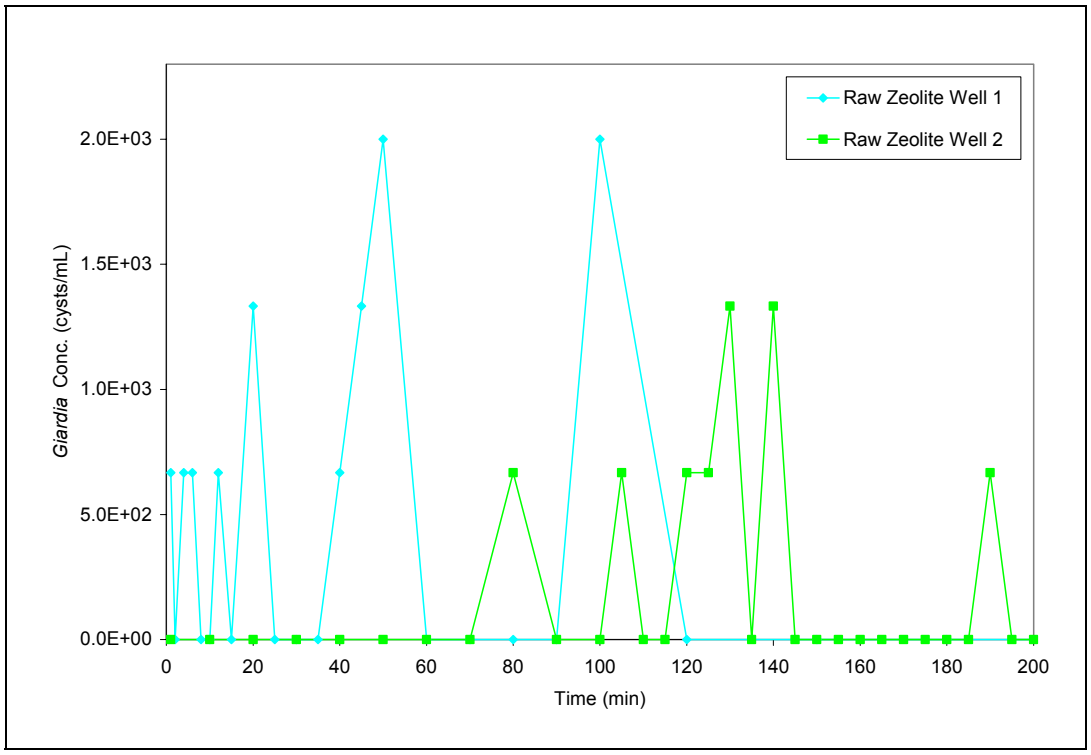


Figure 17: *G. intestinales* Cyst Concentrations of Natural Raw Zeolite experiment

Note: Ave. Standard Deviation of Well 1 was 6.7E+02 with an Ave. 95% Confidence Interval of 7.5E+02; Ave. Standard Deviation of Well 2 was 2.4E+02 with an Ave. 95% CI of 2.8E+02. For Standard Deviation and 95% CI of each triple count for each data point see Table B-9.

3.4 Hydrophobic SMZ

The effectiveness of hydrophobic SMZ barrier was tested with microspheres (6.0-7.9 μm) and viable *Giardia* cysts. The data indicated microsphere peaked at one minute in Well 1 with a peak concentration of 4.18×10^6 microspheres per mL and at 15 minutes in Well 2 with a peak concentration of 9.33×10^3 microspheres per mL (Figure 18). The hydrophobic barrier effectively removed 99.1% of the microspheres down gradient of the hydrophobic SMZ barrier.

For the hydrophobic SMZ and *Giardia* cysts run, a peak arrival was observed at 6 minutes in Well 1 with no *Giardia* cysts present in Well 2 at any time (Figure 19). *Giardia* cysts were removed at a rate of 100% by the emplaced barrier. To ensure the quality of data, a fourth separate cell count was completed for Well 2 water samples. All laboratory runs had a standard triplicate count done for each water sample collected during the experiment.

A second hydrophobic SMZ and *Giardia* cysts run was executed to validate findings. All laboratory runs had 5 mL of *Giardia* cysts (6.25×10^5 cysts per mL) injected into the injection well at time zero. For the second run, 10 mL of *Giardia* cysts (6.25×10^5 cysts per mL) were injected. The intention was to overload the new hydrophobic SMZ barrier and comprehensively test the removal rate of the barrier. An arrival peak was again observed at 6 minutes in Well 1 with no *Giardia* cysts present in Well 2 at any time (Figure 19). The hydrophobic SMZ barrier removed 100% of the *Giardia* cysts.

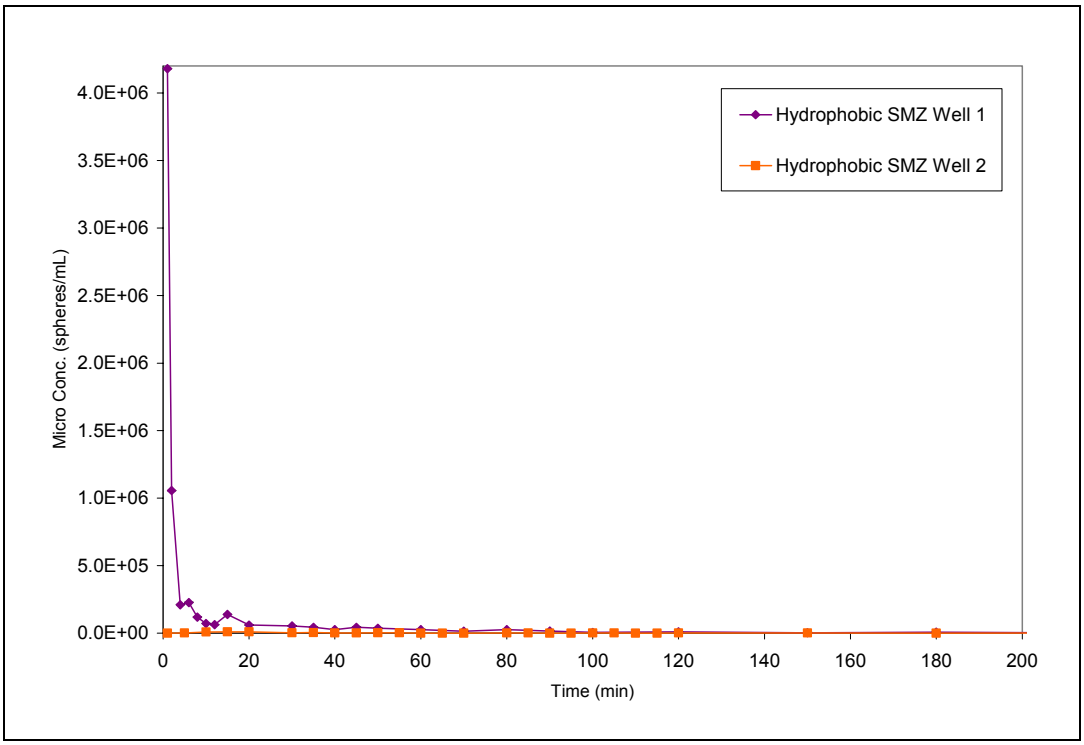


Figure 18: Microsphere Concentrations of Hydrophobic SMZ experiment

Note: Ave. Standard Deviation of Well 1 was 9.9E+04 with an Ave. 95% Confidence Interval of 1.1E+05; Ave. Standard Deviation of Well 2 was 1.5E+03 with an Ave. 95% CI of 1.7E+03. For Standard Deviation and 95% CI of each triple count for each data point see Table B-10.

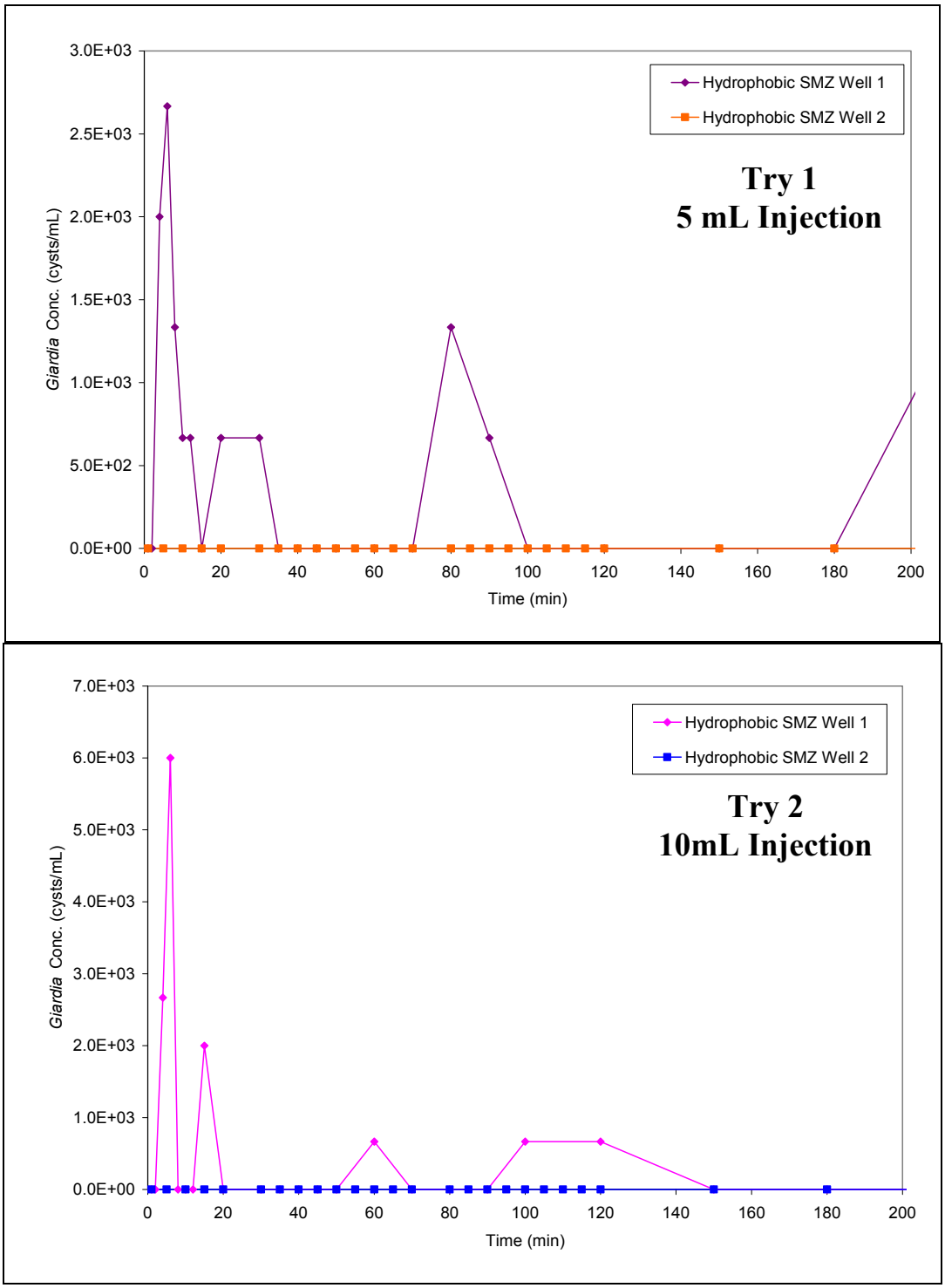


Figure 19: *G. intestinales* Cyst Concentrations of Hydrophobic SMZ experiment (Run 1& 2) (Run 1: 5 mL, Run 2: 10 mL *Giardia* cysts injection)

Run 1 Note: Ave. Standard Deviation of Well 1 was 5.1E+02 with an Ave. 95% Confidence Interval of 5.7E+02; Ave. Standard Deviation of Well 2 was 0.0E+00 with an Ave. 95% CI of 0.0E+00. For Standard Deviation and 95% CI of each triple count for each data point see Table B-11.

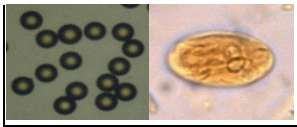
Run 2 Note: Ave. Standard Deviation of Well 1 was 4.8E+02 with an Average 95% Confidence Interval of 5.4E+02; Ave. Standard Deviation of Well 2 was 0.0E+00 with an Ave. 95% CI of 0.0E+00. For Standard Deviation and 95% CI of each triple count for each data point see Table B-12.

3.5 Removal rates of SMZ formulations

Removal rates for each formulation of SMZ through a 10 cm barrier from Well 1 to Well 2 were tabulated in Table 5. Removal rates (Table 5) represent the percent drop in the total number of particulates present within Well 1 samples versus the total number particulates present within Well 2 samples. Dilution due to dispersion, seen as a loss of concentration of particulates in later wells was not accounted for in the presented removal rates (Table 5). The tables in Appendix B list all concentrations observed and highlight, in yellow, were the maximum concentration observed in Well 1 and Well 2 during the duration of the experiment.

The highest removal rate observed for the 8 µm fluorescent microspheres was the hydrophobic SMZ with a 99.1% removal of microspheres (Figure 18). The highest removal rate observed for the viable 8 µm *Giardia* cysts was the hydrophobic SMZ with a 100% removal of cysts and the same 100% removal rate was observed for the second hydrophobic and *Giardia* cyst experiment (Figure 19).

Table 5: Total removal of SMZ formulations during the laboratory experiments

	**Hydrophobic**	**Fine Cationic**	**Coarse Cationic**	**Raw Zeolite**	**Sand Only (no barrier)**
8 um Microspheres (Fluorescent Polystyrene spheres)	**99.1%**	98.8%	56.1%	85.3%	*N/A*
8 um *Giardia* Cysts (Viable)	**100%**	52.8%	65.6%	43.8%	61.5%

Note: Dilution due to dispersion was not accounted for; total removals represent the percent drop in total counts of Well 1 samples versus Well 2 samples.

CHAPTER 4: DISCUSSION

4.1 Laboratory Discussion

Hydrophobic was most effective at removing *G. intestinales* cysts from the groundwater, while all other materials tested were just as effective as sand. Removal rates were not as high for cysts and microspheres as for bacteria and viruses in the previous experiments where SMZ had been used to remove *E. coli* and the bacteriophage MS-2 from sewage water (*E. coli 100%,* MS-2 > 90%) (Schulze-Makuch et al., 2003; Schulze-Makuch et al., 2002). The removal efficiency varied with the particular formulation of the SMZ used. Table 5 showed the removal rates from Well 1 to Well 2 (10 cm barrier) for each formulation of SMZ. Removal rates varied from 56.1% to 99.1% for the 8 μm microspheres and from 43.8% to 100% for the viable *Giardia* cysts. The SMZ filtration material with the highest removal rate, shown by model aquifer experiments, was the hydrophobic SMZ with a 100% removal rate for *Giardia* cysts and 99.1% removal rate for microspheres (Table 5). Many potential mechanisms for particle removal were evident based on laboratory results.

The most apparent reduction of particle concentration for all laboratory experiments was the removal rates from the Injection Well to Well 1. All injected particles passed through 10 cm of cleaned sieved silica sand before contact with the 10 cm barrier (Figure 3), by a forced gradient flow. It appears that the majority of microspheres and *Giardia* cysts were removed by gravitational settling and physical filtration by the sand. A large percentage of the initial concentration of microspheres and *Giardia* cysts were removed before arrival to Well 1. This reduction will need to be investigated in future studies. In order to answer whether sand was really so effective, another well would need to be installed between the Injection Well and Well

1, and a more careful quantification of initial concentrations of particles will need to be obtained before injection.

Results from the *Giardia* cysts with coarse cationic SMZ experiments and microspheres with hydrophobic SMZ (Figure 17 and 18), may indicate the possibility of preferred pathways in the sieved silica sand of the model aquifer. This interpretation was from the *Giardia* cyst and microsphere arrivals appearing a few minutes before the appearance of the conservative potassium chloride (KCl) tracer. Though heterogeneities in the sand may only play a small effect in comparison to other mechanisms such as gravitational settling and physical filtration.

Variation of removal rates may be affected by the chemical properties of the SMZ formulation, and its ability for adsorption. Cationic and hydrophobic SMZ differ by the amount of HDTMA surfactant loaded onto the surface of the raw zeolite. A higher concentration of HDTMA was loaded on cationic SMZ which produced a more positive charge, where as hydrophobic SMZ has a less positive charge but has a more hydrophobic character. The anion exchange and organic partitioning properties of both SMZ formulations encourage sorption, but absorption was reversible. This may be caused by collision with other particles, removal by water flow, or weak bonds of particles to the surface of the SMZ barrier. The continual presence of microspheres and *Giardia* cysts at later times in Well 2 occur throughout experiments, suggesting reversible sorption with the SMZ barriers. The only exception was the arrival plot of hydrophobic SMZ with *Giardia* cysts. The data showed that hydrophobic SMZ was most effective with irreversibly sorbing *Giardia* cysts, possibly due to the organic nature of the SMZ material. It is important to note that *Giardia* cysts cannot reproduce without a mammalian host, so none of the later Well 2 occurrences can be attributed to growth and replication of the cysts within the model aquifer.

After close inspection of arrival plots of both *Giardia* cyst and microsphere, a difference in removal rates between the *Giardia* cysts and microspheres demonstrated the two particles react differently to each SMZ barrier tested. This may be attributed to the difference in the surface properties of the cysts and microspheres. The microspheres were different than *Giardia* cysts because they do not have an organic protein layer or hydrophobic surface properties. Regardless, total removals listed in Table 5 showed that the size of the tracer was not important. Results showed *Giardia* cysts sorb 100% to the hydrophobic SMZ, suggesting irreversible sorption by electrostatic or hydrophobic forces between the two organic surfaces. Even though cationic SMZ and raw zeolite removed greater than 85% of microspheres from suspension, hydrophobic SMZ was most favorable for the organic surfaces of the *Giardia* cysts.

CHAPTER 5: PRELIMINARY FIELD TESTING

The coarse grained Cationic SMZ (1.4-2.4 mm) formulation was further tested at the University of Idaho Groundwater Research Site (UIGRS) with water amended with microspheres to simulate *Giardia* cyst behavior. The field site was an existing multiple well site at the University of Idaho in Moscow. The wells were completed in the Lolo Basalt Formation; a highly heterogeneous and anisotropic fractured basalt aquifer system typical of the subsurface of most of eastern Washington and northeastern Oregon.

The SMZ pathogen field filter was installed directly in the well bore and the concentrations of microsphere-amended groundwater were measured before and after filtration. Pumping was continued for an extended period of time, 18 hours, to test the lifetime of the prototype filter system. In addition, total coliforms and one indicator strain, *E. coli*, were enumerated before and after filtration. Tests and results were targeted at developing a prototype filter system for removing human pathogens in drinking water. Further details on the site and materials and methods are provided in Appendix C.

5.3 Experiments

All groundwater experiments were conducted in the known East fracture zone aquifer located at the UIGRS. Well V16D, was pumped while well T16D was used as the injection well. These wells were chosen because they both were completed in the East fracture zone aquifer and were close in proximity (approximately 9 m or 30 ft). Well V16D was utilized for pumping due to a higher well yield than well T16D.

A 9.525 cm (3.75 in) submersible water pump was used in the pumping/sampling well V16D. Discharge was maintained at a constant flow rate by an adjustable valve at the top of the well casing. Before the actual experiment using microspheres one tracer test was done using

bromide. During both tests, Well V16D was pumped at a constant rate of 0.92 liters per second (13 gpm). All water pumped from well V16D was discharged near Paradise creek.

5.3.1 Transport Experiments

Bromide (KBr) Tracer Test

On October 1st, 2005, a bromide tracer test was conducted to gain more information about the arrival times and sampling set-up for use in multi-tracer test. The conservative tracer, potassium bromide (1.24 M KBr), was injected into well T16D and samples were taken from the pumping well V16D. The injection line was weighted and lowered to the depth of 20.9 meters (68.6 feet) in the center of the well screen. At time zero, 4 liters of 1.24 M potassium bromide tracer was injected into well T16D. To ensure the entire amount of tracer was released, 2 liters of DI water was added to flush the tank and tubing.

Water was pumped from well V16D at a constant rate of 0.92 liters per second (13 gpm) and was discharged towards Paradise Creek. Samples were collected by hand in 15 mL test tubes directly from the discharge pipe for roughly one hour. Samples were analyzed later that day for bromide concentrations and an appropriate sampling schedule was set-up for multi-tracer test. No SMZ filter was used during this field test.

Multi-Tracer Test

On October 7th, 2005, the multi-tracer test was conducted with multiple sized microspheres. Figure 20 is a cross section of the tracer test set-up. For the multi-tracer test, the SMZ filter and electronic sampling pump were placed below the submersible water pump. The electronic sampling pump was secured 1.2 meters (4 feet) below the SMZ filter and the SMZ filter was secured 3.0 meters (10 feet) below the bottom of the submersible water pump. The 9.525 cm (3.75 in) submersible water pump was attached to 43 feet (13.1 meters) of pipe section

to ensure complete water submersion during the tracer test. All samples before the SMZ filter were taken at 18.3 meters (60 feet) below ground level, or 3.0 meters (10 feet) above the East Fracture Zone Aquifer.

The same injection well set-up was used as in the initial bromide tracer test. Three microsphere analogs (10, 6.0 – 7.9, 1 μm) were injected into well T16D during the multi-tracer test. They included Fluoresbrite® Yellow-Green Microspheres (10.0 μm), Nile Red Fluorescent polystyrene microspheres (6.0-7.9 μm), and Fluoresbrite® Polychromatic Red Microspheres (1.0 μm). The tracers were injected in a succession of three slugs, one hour apart starting with the combination of potassium bromide and 10 μm microspheres at time zero (Slug 1), followed by the 6.0 – 7.9 μm microspheres (Slug 2) and the 1 μm microspheres (Slug 3). To ensure the entire amount of each tracer was released, 1 liter of DI water was added to flush the tank and tubing after each injection. In addition to the injected tracers, samples were collected and analyzed for total coliforms and *E. coli* as mentioned in the previous section (Appendix E).

Bromide and microsphere samples were collected in 125 mL nalgene wide mouth bottles and total coliforms and *E. coli* were collected in 100 mL IDEXX sample bottles. The containers were pre-labeled and recorded as a time and location of sample (before vs. after filter). Additional information was recorded for each sample including the date and the sample collector's name. Samples were stored at room temperature and protected from light until they were analyzed. All total coliform and *E. coli* samples were analyzed the same day as the tracer test at the Moscow, ID Wastewater Treatment Plant laboratory.

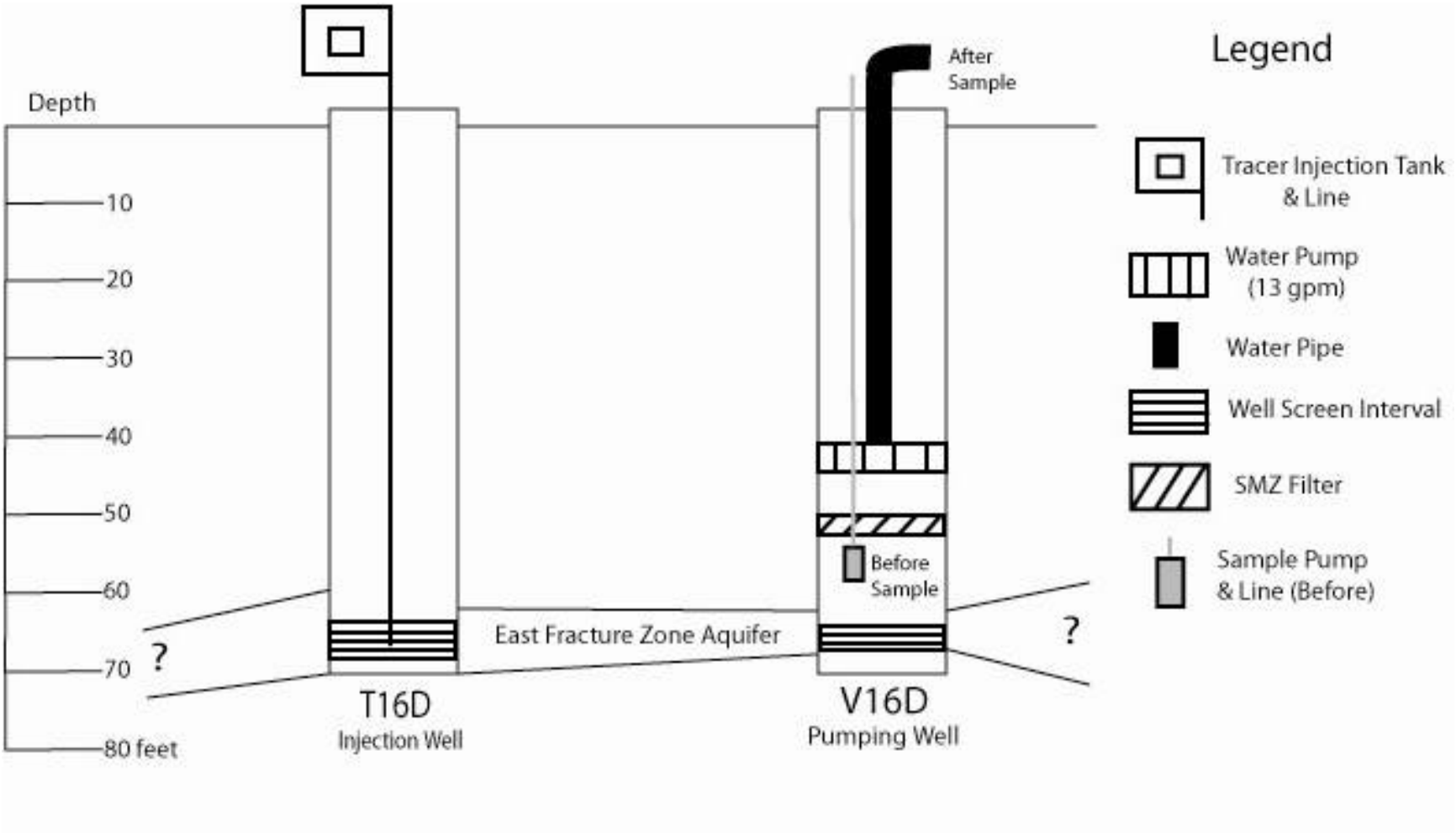


Figure 20: Cross section of the East fracture during Multi-Tracer test

Samples were collected before and after the SMZ filter for bromide, microspheres, total coliforms and *E. coli*. Background samples for total coliforms and *E. coli* were collected from Paradise Creek at a pre-designated location (see Figure C2). No background samples were taken for total coliforms and *E. coli* within the pumping well before the multi-tracer test. A varied time interval for the multi-tracer test was set-up based on information from the initial bromide tracer test. For the injected tracers, the first samples were collected at five minute intervals up to 240 minutes, then increased to ten minute intervals up to 360 minutes, fifteen minute intervals up to 420 minutes, a twenty minute interval until 540 minutes, a thirty minute interval until 720 minutes, and a one hour interval until the test ended at 840 minutes (October 7th 2005).

The total coliform and *E. coli* filter samples were collected at ten minute intervals for the duration of the sampling until 370 minutes. The background creek samples were collected every hour until sampling was completed at 420 minutes. Total coliform and *E. coli* enumeration costs limited the number of samples collected.

5.4 Data Analysis

Bromide concentrations (ppm) were analyzed by an Orion meter (Orion Model 290A) with a bromide electrode (9635BN model). A 25 mL aliquot was taken from each 125 mL water sample and mixed with 0.5 mL of ionic strength adjuster, to acquire proper readings. For samples collected during initial bromide tracer test, bromide concentrations were measured and recorded the same day as collected to determine a more representative sampling interval for the multi-tracer test. For samples collected during multi-tracer test, bromide concentrations were measured after they were filtered for the three microsphere tracer slugs. No decrease of the bromide concentration was observed.

The following procedure was used:

(1) Calibrate electrode with two Br standard solutions (1 ppm, 1000 ppm)

(2) Take a 25 mL aliquot from each 125 mL water sample

(3) Add 0.5 mL of ionic strength adjuster to each 25 mL aliquot

(4) Test samples with electrode and record concentrations (ppm)

(5) Plot Br concentrations versus time, for future sampling time schedule

Microspheres were filtered onto a black nucleopore filter and the entire filter was used for the enumeration of microspheres. Microspheres were visualized using a fluorescent microscope at a magnification of 100X (10X lens, 10X eyepieces). Polycarbonate, black membrane filters (0.4 μm pore size, 25 mm diameter) were used for ease of locating fluorescent microspheres under UV light (see Figure C8). De-ionized water was used to rinse the sides of the filter column to ensure all microspheres sticking to the sides of the glass were filtered (see Figure C7). Each filter was placed on a microscope slide, a drop of emersion oil was added to the filter, and a cover slip was placed on top of the filter. The cover slip was sealed to the slide with clear nail polish to prevent desiccation of the sample. The entire 125 mL water sample was filtered for analysis, providing concentrations of microspheres per 125 mL water sample. Distinguishing between the 1 μm microspheres and background material was difficult with the employed analysis techniques, making quantification unfeasible, therefore, the 1 μm microsphere concentrations were not counted for the final results. A more detailed explanation of analysis and procedures are provided in Appendix D.

Total coliforms and *E. coli* were analyzed the same day as collected using a standard fluorescent dye incubation procedure. Each 100 mL sample was mixed with an IDEXX Colilert reagent and placed in a sterile IDEXX Quanti-Tray. All trays were incubated at 35.5 °C for 24

hours and the numbers of positive wells were counted. If the well was yellow after 24 hours of incubation, then total coliforms were present. If the well fluoresced under UV light, then *E. coli* were present. A more detailed explanation of the analysis and procedures are provided in Appendix E.

5.5 Field Test Results

The coarse cationic SMZ was further tested in the field using a bromide tracer, three microsphere analogs (10, 6.0 – 7.9, 1 μm), and background total coliforms and *E. coli* present in the shallow aquifer system and neighboring Paradise Creek. All field test data are provided in Appendix F.

5.5.1 Initial Bromide Tracer Test Results

Bromide Tracer Test

On October 1st, 2005 a conservative tracer, potassium bromide (1.24M KBr), was injected in well T16D and samples were taken from the pumping well V16D. Bromide concentrations (ppm) versus time (minutes) were graphed for well V16D in Test-1 (Figure 21). Samples were collected for one hour and the peak concentration of bromide was not reached at the end of the test. The highest concentration measured for the initial bromide tracer test was 1.06 ppm which occurred at sixty one minutes. Arrival time of bromide occurred at thirty minutes. Thus, for the later test, monitoring was extended for 14 hours. No filter was emplaced during the initial bromide test.

5.5.2 Multi-Tracer Test Results with Cationic SMZ Coarse Filter Pack

On October 7th, 2005 a conservative potassium bromide tracer, and three different microsphere slugs (10, 6.0 – 7.9, 1 μm) were injected in well T16D. Initial concentrations for the

conservative potassium bromide tracer was 1.24 M, and the three different microsphere slugs (10, 6.0 – 7.9, 1 μm) were 4.55 x 10^7 particles per mL, 1%w/v, and 4.55 x 10^{10} particles per mL, respectfully. Samples were taken from the pumping well V16D before and after the emplaced cationic SMZ filter. In addition, samples were taken from Paradise Creek and the pumping well V16D for background total coliforms and *E. coli*.

Bromide Tracer Test

Bromide concentrations versus time for well V16D before and after the emplaced SMZ filter were plotted in Figure 22. Bromide was detected in samples at approximately twenty minutes after injection into well T16D. The peak concentration of bromide was 11.2 ppm and 12.0 ppm at forty five minutes before and after the SMZ filter, respectfully. After approximately one hundred and eighty minutes (3 hours), the slope of the breakthrough tapers off to a stable low concentration. Concentrations from both before and after the SMZ occurred at the same time with no retardation of the tracer as expected for a conservative tracer (Figure 22).

Microspheres

10, 6.0 - 7.9 - μm microspheres

The concentrations of microspheres versus time were plotted below for 10 μm microspheres (Figure 23) and for 6.0-7.9 μm microspheres (Figure 24). The peak arrivals for both microsphere tracers were unclear. For both the 10 μm and 6.0-7.9 μm microspheres, concentrations were higher after the SMZ filter than before the SMZ filter.

For 10 μm microspheres, peak concentrations occurred after the SMZ filter at zero minutes with 12 spheres per mL, 25 minutes with 11 spheres per mL, and at 185 minutes (3.08 hours) with 12 spheres per mL. In contrast, the highest peak concentration before the SMZ filter was at 75 minutes with 3 spheres per mL.

For the 6.0-7.9 μm microspheres, the peak concentrations after the SMZ filter were at 200 minutes with 3 spheres per mL, and at 630 minutes with 2 spheres per mL. In contrast, the highest peak concentrations before the SMZ filter were at various times throughout the experiment with only 1 sphere per mL.

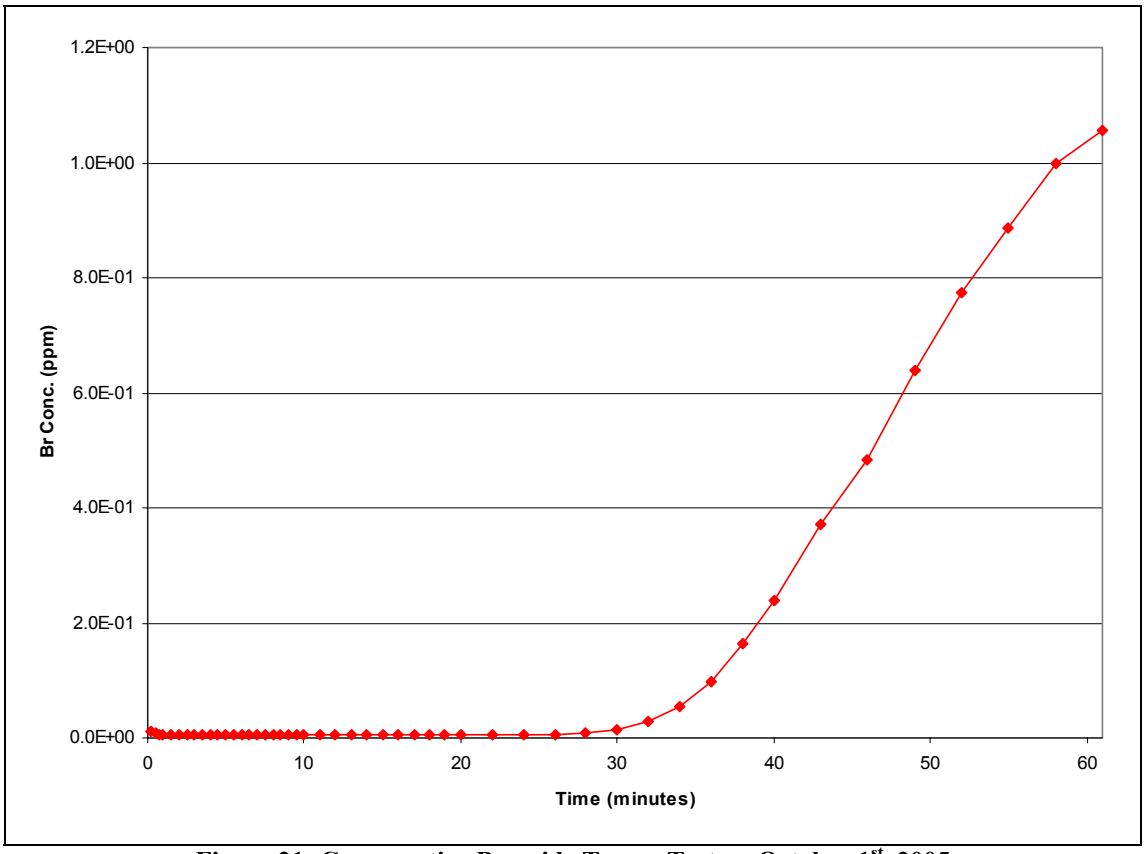


Figure 21: Conservative Bromide Tracer Test on October 1st, 2005

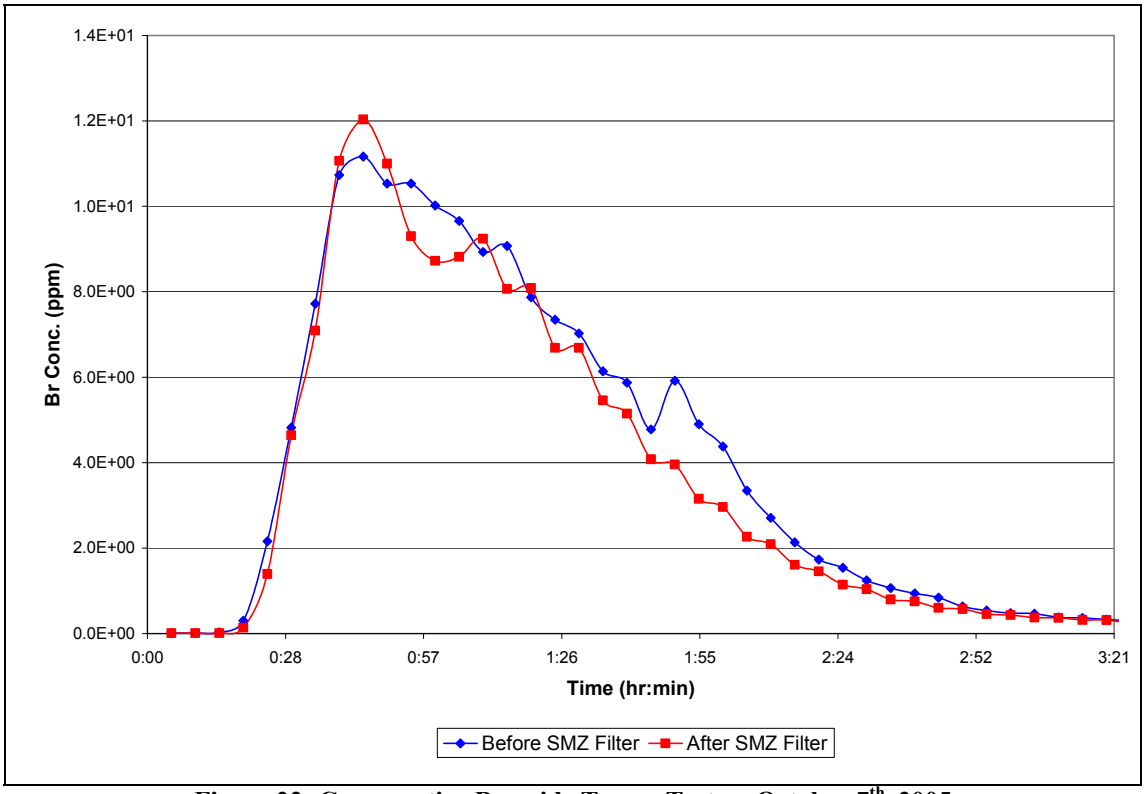


Figure 22: Conservative Bromide Tracer Test on October 7th, 2005

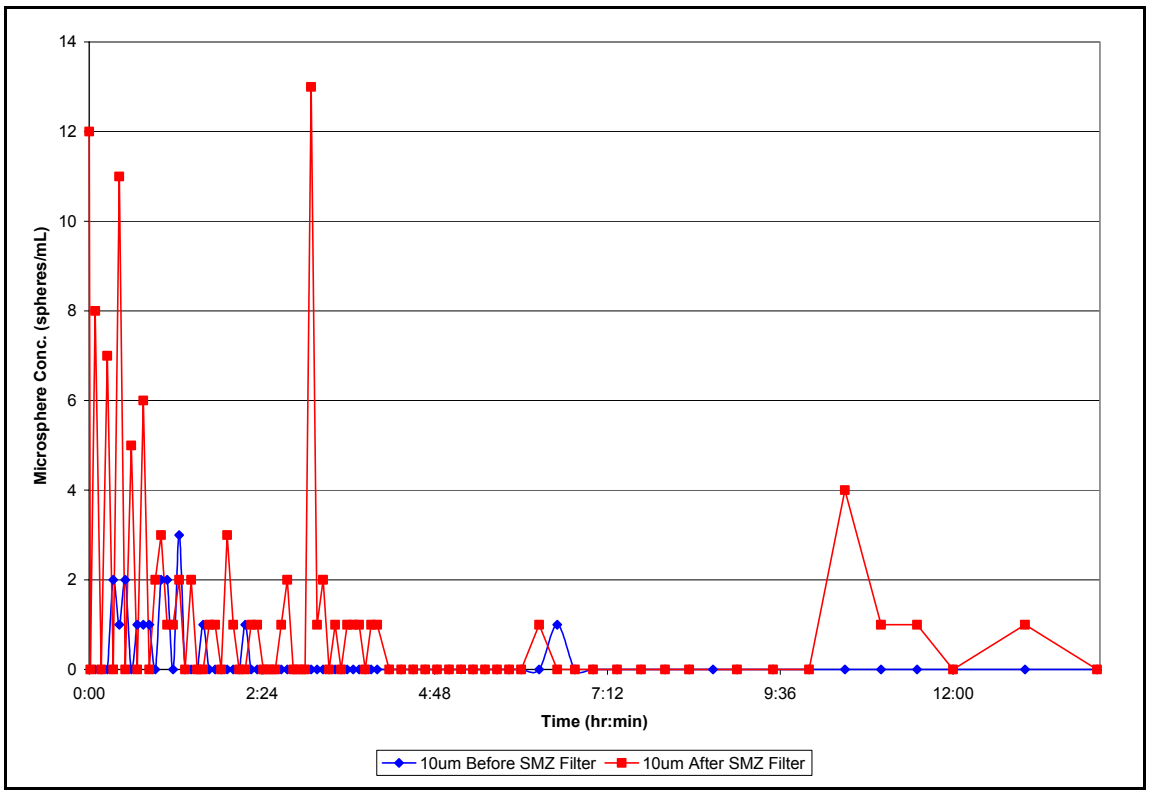


Figure 23: Field test arrivals for Injection 1 (10 um microspheres)

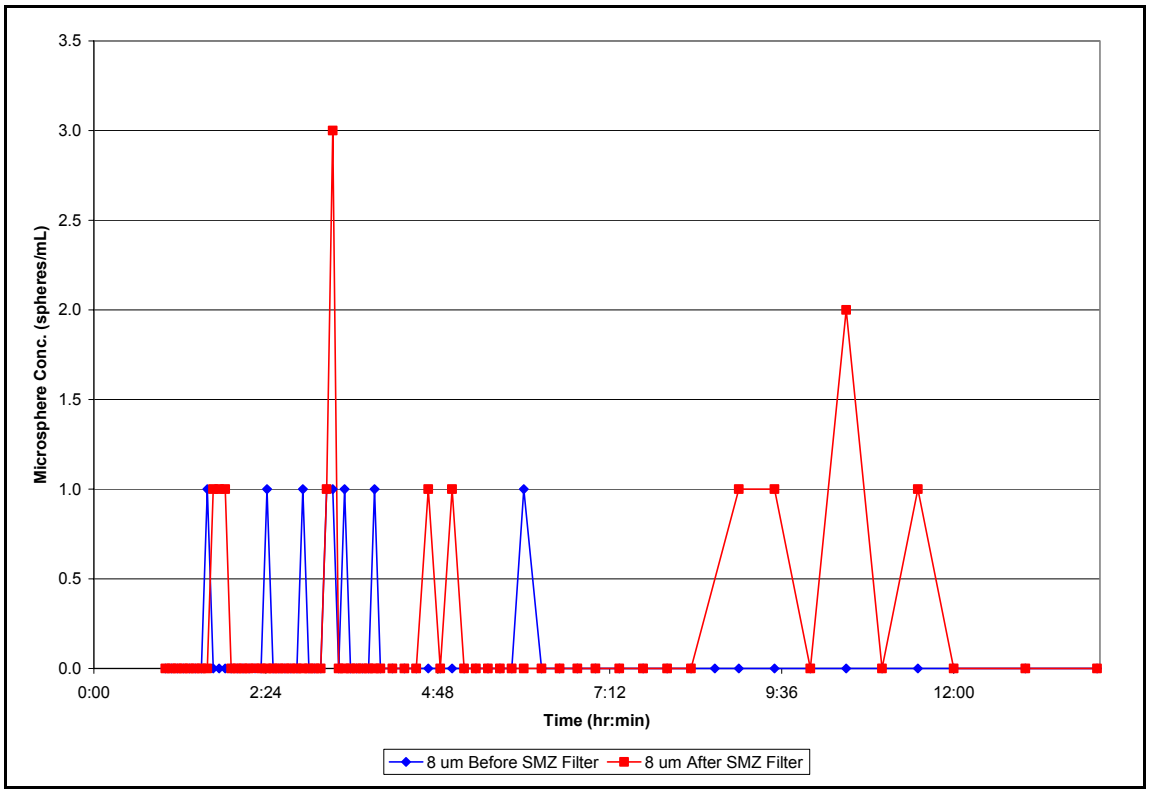


Figure 24: Field test arrivals for Injection 2 (6.0-7.9 um microspheres)

Total coliforms and *Escherichia coli* (*E. coli*)

In addition to the injected tracers, total coliforms and *E. coli* were enumerated from the background water present in the pumping well V16D and the neighboring Paradise Creek. Total coliforms were present in both the pumping well V16D and in Paradise Creek, although *E. coli* was only present in Paradise Creek.

Concentrations for total coliforms and *E. coli* in Paradise Creek during the multi-tracer test on October 7th 2005 are graphed on Figure 25. Every creek sample collected during the field test had the maximum possible concentration for the IDEXX colilert reagent Quanti-Trays® quantification method, 2419.6 bacteria per 100 mL. Given that the maximum concentration of the IDEXX Quanti-Trays® was reached, there were more bacteria present than quantifiable by the IDEXX method. For the concentrations of *E. coli*, peaks were observed at 180 and 240 minutes with 2419.6 bacteria per 100 mL. During the multi-tracer test, a rain runoff event occurred for the first time in roughly one month. The runoff event may have influenced the concentrations and graphed pattern observed in Paradise Creek during the multi-tracer test.

Only total coliforms were present in the pumping well V16D. Concentrations of total coliforms versus time for the pumping well V16D before and after the emplaced SMZ filter are graphed in Figure 26. Peak concentrations of total coliforms before the SMZ filter were observed at 30 minutes with 4.2 bacteria per 100 mL and later at 110 minutes with 5.3 bacteria per 100 mL. Peak concentrations after the SMZ filter were observed at 20 minutes with 3.1 bacteria per 100 mL and later at 330 minutes with 2.0 bacteria per 100 mL. Removal rate by the emplaced SMZ filter was found to be 51.6%, by comparing the sum of bacteria counted before and after the SMZ filter divided by the number of samples (Figure 27).

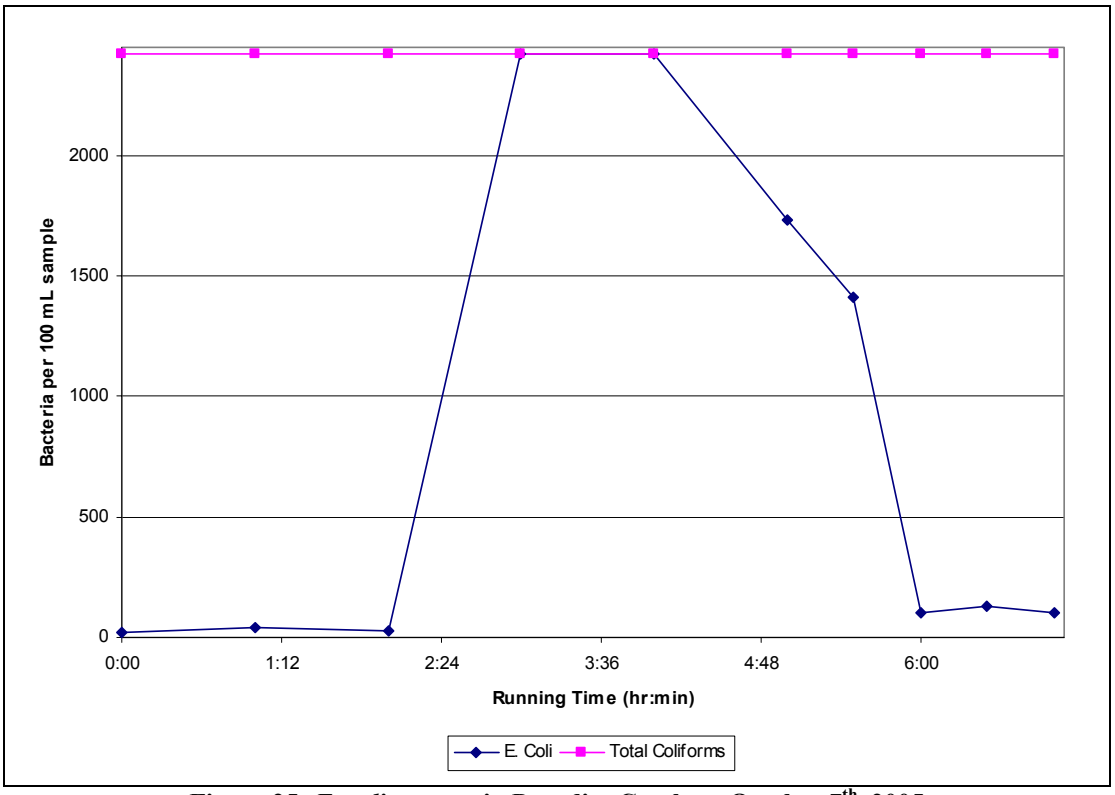


Figure 25: *E. coli* present in Paradise Creek on October 7th, 2005
(Note: Every Paradise creek sample had a max MPN # of total coliforms, 2419.6)

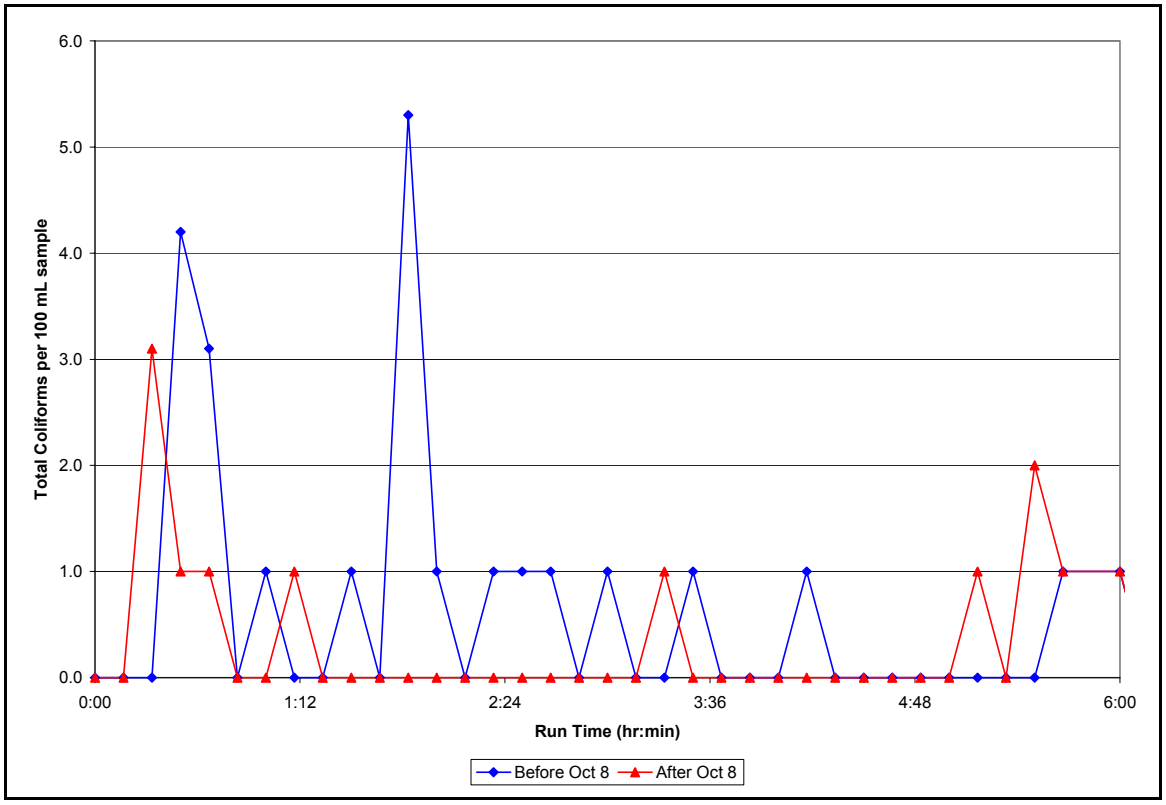


Figure 26: Field test arrivals for total coliforms

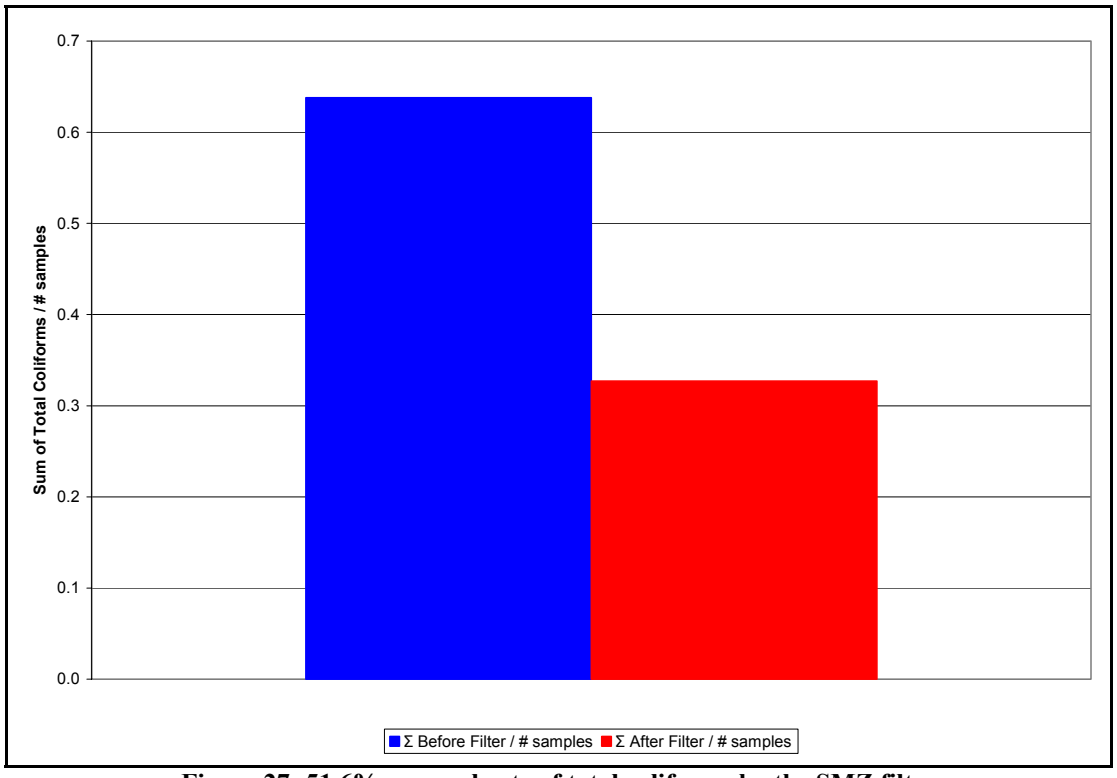


Figure 27: 51.6% removal rate of total coliforms by the SMZ filter
(Note: No *E. coli* was found within Well V16D)

5.6 Field Test Discussion

The field test of coarse Cationic SMZ was not as effective in the field as shown in earlier laboratory tests, as evident in Figure 16 and 17 with 86.9% and 76.2% removal rates, respectfully. This may be evidence of the effects from a high pumping rate (12.9 L/min) and a short residence time within the filter. One possible solution would be to lower the pumping rate and/or increase the length of the SMZ filter to increase the contact time between particulates and the filter pack. One other possible explanation of the particulate results would be that the pumped water was by-passing the filter due to a bad seal or traveling through preferred pathways within the SMZ filter. Nevertheless, typical commercial wells pump 20 to 1000 L/min and the thickness of the barrier would probably have to be increased for a longer contact time (Schulze-Makuch et al., 2003).

Arrival plots for both microsphere tracers (10, 6.0 – 7.9μm) were unclear. For both 10 μm and 6.0-7.9 μm microspheres, concentrations were consistently higher after the SMZ filter than before the SMZ filter (Figure 23 & 24). For the 10 μm microspheres (Slug 1), the presence of microsphere arrivals occurred at time zero before the conservative bromide tracer, and concentrations peaked throughout the experiment. For the 6.0-7.9 μm microspheres (Slug 2), microspheres occurred at the expected time after the initial injection, though peaks in concentrations still occurred throughout the field test. A few possible reasons for error could be the misidentification of microspheres, the presence of preferential flow paths, and reversible absorption within the East Fracture Aquifer. Alternatively, water may have was by-passed the filter due to a bad seal on the SMZ filter within the pumping well.

First, misidentification may have occurred for some of the filtered water samples. Some of the background material within the collected samples fluoresced under ultraviolet light and

was the same color as the pre-labeled microspheres. Figure 28 shows a few examples of background material that fluoresced under ultraviolet light. The pictures of the various microspheres were taken before counts were determined. Another possible error associated with the arrival plots was the possibility of preferential flow paths and reversible absorption of microspheres within the East Fracture Aquifer. A high pumping rate, coupled with preferential flow paths from injection well to pumping well, may account for the early appearance of 10 µm microspheres in samples. Plus, reversible absorption of microspheres within the fracture system would allow for a continual supply of microspheres throughout the 14 hour pumping test. Finally, a bad seal on the SMZ filter would explain the presence of higher concentrations of microspheres in samples collected after the SMZ filter. Regardless of the source of error, the field test should be repeated in the future.

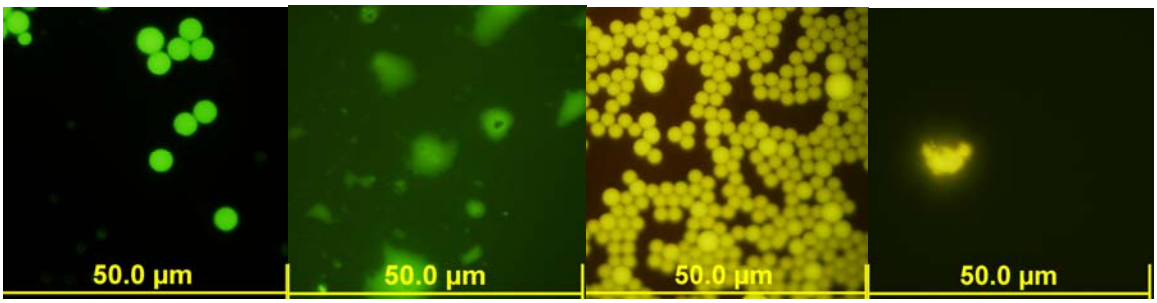


Figure 28: (a-d): Examples of background material that fluoresced under UV light
a) 10 µm spheres b) background c) 6.0-7.9 µm spheres d) background

Total coliforms and *E. coli* were enumerated from the background water collected from pumping well V16D and the neighboring Paradise Creek. Both samples were necessary because Li (1991) and Pardo (1993) found a reliable hydraulic connection between the shallow aquifer and the neighboring Paradise Creek. Total coliforms were present in all creek and pumping well samples, though none of the pumping well V16D samples were positive for *E. coli*. Every creek sample collected on October 7th, 2005 had the maximum possible concentration of total coliforms for the IDEXX colilert reagent Quanti-Trays® quantification method, 2419.6 bacteria

per 100 mL. However varying amounts of *E. coli* were present in the creek samples; with a peak concentration of 2419.6 bacteria per 100 mL occurring between the third and fourth hour of the field test. This unusually high concentration was probably due to a rain runoff event occurring during the field test. The area had not received rain for a full month and rain washed off the surrounding drainage basin, which is primarily used for agricultural processes.

Removal rates within the pumping well V16D were not as high as for bacteria and viruses in previous experiments where *E. coli* and the bacteriophage MS-2 where removed from sewage water with a high success rate (*E. coli 100%,* MS-2 > 90%) (Schulze-Makuch et al., 2003; Schulze-Makuch et al., 2002) A 51.6% removal rate was observed for total coliforms by the emplaced SMZ filter, though because no background samples were taken within the pumping well before the multi-tracer test, the percent removal may not be accurate. Apart from the unknown background counts, the low removal rate may be evidence of the effects from a high pumping rate (12.9 L/min), short residence time within the filter, or a bad seal on the SMZ filter. If the pumping rate was lowered or the residence time within the SMZ filter was increased, more bacteria may be removed by the combination of electrostatic and hydrophobic interactions with the SMZ. In the case of a bad seal, pumped water would not be forced to move completely through the filter pack allowing some water to circumvent the filter. Either way, the prototype filter system needs to be redesigned with use of hydrophobic SMZ to ensure a more successful future field test.

CHAPTER 6: CONCLUSIONS

Previous studies have shown that surfactant-modified zeolite (SMZ) was suitable as a barrier to impede bacteria and virus migration in aquifer systems. This study further evaluated the ability of SMZ to remove the important waterborne human pathogen *G. intestinale*s, which is an extremely infectious cyst-forming protozoan that can be transported in both ground and surface waters. The SMZ material is characterized as having high surface areas (hundreds of m^2/g), and a net positive surface charge appropriate for sorbing negatively charged *Giardia* cysts and microspheres.

Both laboratory and field tests were conducted. The laboratory study indicated that *Giardia* cysts and microspheres react differently to each SMZ material tested, raising the question of appropriate use of microspheres as bacteria or protozoan analogs. Removal rates of particles from Well 1 to Well 2 varied from 56.1% to 99.1% for the 8 μm microspheres and from 43.8% to 100% for the viable *Giardia* cysts showing that the size of the tracer was not important. The hydrophobic SMZ material tested had the highest removal rate for both *Giardia* cysts (100%) and microspheres (99.1%), while the other barriers were as good as sand. Field studies were less conclusive. Hydrophobic SMZ material needs to be further evaluated in the field at UIGRS with the prototype SMZ filter or a modified design to determine the lifetime of the filter, barrier thickness, effect of high flow rates through the filter, and use different microbial tracers.

Hydrophobic SMZ with a 100% removal rate of *Giardia* cysts could significantly reduce the potential for contamination of drinking water wells by pathogens. SMZ is economical (about $0.50/kg) (Schulze-Makuch et al., 2002) and is a simple method to treat contaminated water. Surfactant-modified zeolite could help eliminate microbial contamination of groundwater in areas not serviced by utilities, or in impoverished nations worldwide.

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APPENDIX A:

Enumeration of *Giardia* cysts and Fluorescent Polystyrene Microspheres in Laboratory Samples

In current literature, three methods for quantifying concentrations of *Giardia intestinales* in water samples are presently employed; (1) flow cytometry, (2) hemacytometry, and (3) filter membrane enumeration. For laboratory experiments, hemacytometry was utilized and for later field tests, filter membrane enumeration was utilized. Agnew (2006) thoroughly discusses the issue of adequately high concentrations counted on the grid of a hemacytometer for the concentrations measured to be statistically accurate based on EPA standards (Dufour, 1999). Even with the issue of low cyst concentrations, hemacytometry was the method chosen for all laboratory samples.

1) **Iodine-Stain Preparation (1:10 ratio):**

Iodine-stain was prepared by mixing 0.5 mL of Iodine concentrate and 5 mL of 70% iodine alcohol (ETOH) together using hollow Polypropylene Hub hypodermic needles and syringes. Once the iodine-stain was prepared, it was kept refrigerated to protect the integrity of the stain until later use for laboratory samples.

2) **Staining *Giardia* cysts (5-8 µm) and Microspheres (6~7.9 µm):**

Each laboratory sample tube was vortexed for one full minute to re-suspend the cysts in the test tube sample. Then 90 µL of the sample and 10 µL of the prepared iodine-stain were placed into one well of a 96 well plate with two separate pipettes (10 & 90 µL). Once in the well, the two solutions were mixed using the 90 µL pipette by pulling the sample in and out of the pipette 6 times to ensure even mixing.

3) Bright Line Counting Chamber (Hemacytometer):

A glass cover was placed on top of the hemacytometer and alined before the sample was pipetted onto the stage. The 90 µL pipette was used to gently pipette enough fluid to cover one of the two stages on the hemacytometer, and then was placed on the refractive light microscope. The slide was observed under 320 x magnification (40 x ocular lens and 8 x eyepieces) on a refractive light microscope. Counts were done on 5 grids starting from the top left-hand corner and following the direction shown by the arrow in Figure A1. Care was taken to ensure that the *Giardia* cysts and microspheres were not counted twice. Counts were written down as the number of cysts or microspheres present in each of the 5 grids counted. Triplicate counts were done for each sample collected, and an average of the 3 samples was calculated.

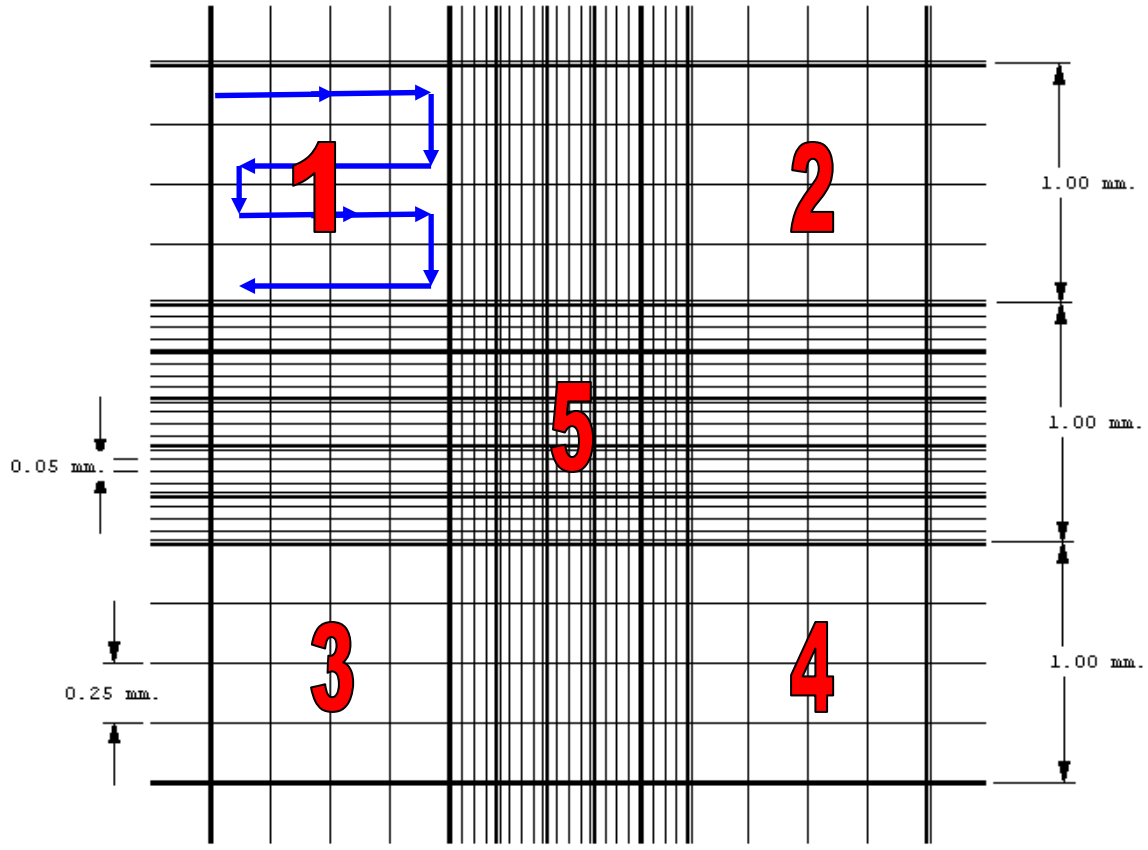

Figure A1: Counting grids on a hemacytometer

4) Clean Up:

To clean up after every individual count, both the hemacytometer and cover slip were rinsed with 70% Ethanol with the use of a Chem Wipe to blot off surfaces. Blotting with a Chem Wipe was important to avoid rubbing which could scratch the surface of the counting grid of the hemacytometer.

At the end of the day, the areas in the laboratory used for counts were cleaned and wiped down. All surfaces were wiped down with 10% bleach wash and then with 70% ethanol. Liberal amounts of bleach and ethanol were applied onto working surfaces and cleaned thoroughly. After use of gloves and paper towels, all were disposed of in a Biohazard bag and later autoclaved. The survival of *Giardia* cysts in the environment is significantly affected by temperature; survivability decreases as the temperature increases. Autoclaving contaminated materials at temperatures above boiling insured that all *Giardia* cysts were killed. Autoclaving materials for 10 minutes in water temperatures of 54 °C or by raising the water temperature to boiling temperature immediately killed the *Giardia* cysts.

5) Total concentration per mL was calculated by using the following formula:

$$\frac{\text{Counted cysts in 5 grids x dilution factor}}{\text{mm}^2 \text{ of counted area x chamber depths}} = \text{cysts / mL (Count 1, 2, 3)}$$

3 count Average (cysts / mL) X Sample Volume (mL) =Concentration cysts / mL

Concentration equations were provided by ProSciTech (Queensland, Australia) under company principles and practices for counting chambers/hemacytometers.

Example:

$$\frac{(1+0+0+1+0)\text{ cysts} \times 1:1}{5\text{ mm}^2 \times 0.1\text{ mm}} = \frac{2\text{ cysts}}{0.5\text{ mm}^3} = \frac{4\text{ cysts}}{\text{mm}^3} \times \frac{1000\text{ mm}^3}{1\text{ mL}} = 4000\text{ cysts per mL of sample (Count 1)}$$

$$\frac{(4000+2000+2000)\text{ cysts per mL}}{3\text{ counts}} = 2.67 \times 10^3\text{ cysts per mL}$$

APPENDIX B:

Laboratory Data

Table B1: Data for the Conservative Tracer Potassium Chloride (KCl) Experiment

Time (minutes)	Well 1: Electrical Conductivity Measurement (µS) (1413 standard solution)	Well 2: Electrical Conductivity Measurement (µS) (1413 standard solution)
0	3.1E+02	5.6E+02
1	1.2E+03	6.0E+02
4	4.1E+02	2.8E+02
7	4.3E+03	3.0E+02
10	2.9E+03	2.4E+02
13	1.1E+03	2.5E+02
16	1.3E+03	2.5E+02
20	2.7E+02	2.4E+02
25	1.1E+02	2.5E+02
30	4.9E+01	2.5E+02
35	6.1E+01	2.7E+02
40	6.6E+01	2.6E+02
45	8.7E+01	2.3E+02
50	3.7E+01	3.0E+02
55	5.2E+01	2.6E+02
60	4.1E+01	2.2E+02
65	3.9E+01	2.3E+02
70	4.3E+01	2.4E+02
75	3.4E+01	2.1E+02
80	3.5E+01	2.1E+02
90	3.1E+01	2.0E+02
100	3.2E+01	2.0E+02
110	3.3E+01	1.9E+02
120	2.6E+01	1.9E+02
150	2.2E+01	2.0E+02
180	3.0E+01	1.4E+02
210	2.8E+01	1.6E+02
STDEV=	**1.0E+03**	**1.0E+02**
95% Confidence Interval =	**2.2E+02**	**2.2E+01**

Table B2: Data for the Conservative Tracer Bromine (Br_2) Experiment

Time (minutes)	Well 1: Bromine Conc. (ppm)	Time (minutes)	Well 2: Bromine Conc. (ppm)
1	4.8E-04	**1**	3.4E-03
2	7.3E-03	**5**	5.9E-03
4	2.0E-01	**10**	6.5E-03
6	3.5E-01	**15**	6.5E-03
8	6.5E-02	**20**	2.0E-02
10	4.3E-02	**30**	2.1E-02
12	2.7E-02	**35**	2.2E-02
15	1.0E-01	**40**	1.4E-02
20	1.6E-02	**45**	7.8E-03
30	1.1E-02	**50**	7.6E-03
35	5.2E-03	**55**	4.8E-03
40	6.0E-03	**60**	4.2E-03
45	6.8E-03	**65**	4.3E-03
50	3.4E-03	**70**	4.1E-03
60	3.8E-03	**80**	1.5E-02
70	3.5E-03	**85**	3.8E-03
80	3.0E-03	**90**	4.4E-03
90	1.9E-03	**95**	6.0E-03
100	1.8E-03	**100**	4.4E-03
120	1.5E-03	**105**	3.8E-03
150	2.5E-03	**110**	4.1E-03
180	1.3E-03	**115**	4.7E-03
210	3.0E-03	**120**	3.3E-03
300	1.9E-03	**150**	3.4E-03
450	1.7E-03	**180**	3.1E-03
		210	0.0027
		240	0.0026
		270	0.0027
		300	0.0033
		350	0.0037
		400	0.0028
		450	0.0025
STDEV =	**8.0E-02**	**STDEV =**	**5.5E-03**
95% Confidence Interval =	**1.8E-02**	**95% Confidence Interval =**	**1.1E-03**

Table B3: Data for microspheres from the Fine Cationic SMZ (0.4 – 1.4 mm) Experiment

Time (minutes)	Well 1			Time (minutes)	Well 2		
	Average of 3 counts (spheres/mL)	STDEV	95% CI		Average of 3 counts (spheres/mL)	STDEV	95% CI
0-A	0.0E+00	0.0E+00	0.0E+00	**0-A**	3.9E+04	6.7E+04	7.6E+04
0-B	1.1E+04	9.5E+03	1.1E+04	**0-B**	4.0E+03	6.9E+03	7.8E+03
1	2.7E+03	4.6E+03	5.2E+03	**1**	2.0E+03	3.5E+03	3.9E+03
2	0.0E+00	0.0E+00	0.0E+00	**5**	0.0E+00	0.0E+00	0.0E+00
4	5.7E+04	1.4E+04	1.6E+04	**10**	6.7E+02	1.2E+03	1.3E+03
6	1.2E+06	5.5E+05	6.2E+05	**15**	0.0E+00	0.0E+00	0.0E+00
8	1.3E+06	1.5E+05	1.7E+05	**20**	0.0E+00	0.0E+00	0.0E+00
10	1.6E+06	2.8E+05	3.2E+05	**30**	0.0E+00	0.0E+00	0.0E+00
12	9.3E+05	2.6E+05	2.9E+05	**35**	0.0E+00	0.0E+00	0.0E+00
15	4.7E+05	1.1E+05	1.3E+05	**40**	0.0E+00	0.0E+00	0.0E+00
20	2.7E+05	1.3E+05	1.4E+05	**45**	0.0E+00	0.0E+00	0.0E+00
30	2.5E+05	8.2E+04	9.2E+04	**50**	0.0E+00	0.0E+00	0.0E+00
35	3.0E+05	2.9E+04	3.3E+04	**55**	0.0E+00	0.0E+00	0.0E+00
40	1.4E+05	3.7E+04	4.2E+04	**60**	0.0E+00	0.0E+00	0.0E+00
45	1.5E+05	4.0E+04	4.6E+04	**65**	0.0E+00	0.0E+00	0.0E+00
50	6.9E+04	4.9E+04	5.6E+04	**70**	0.0E+00	0.0E+00	0.0E+00
60	8.9E+04	6.6E+04	7.4E+04	**80**	0.0E+00	0.0E+00	0.0E+00
70	9.1E+04	4.9E+04	5.5E+04	**85**	6.7E+02	1.2E+03	1.3E+03
80	6.9E+04	4.4E+04	5.0E+04	**90**	2.0E+03	3.5E+03	3.9E+03
90	7.5E+04	3.9E+04	4.4E+04	**95**	1.3E+03	2.3E+03	2.6E+03
100	4.5E+04	3.9E+04	4.4E+04	**100**	0.0E+00	0.0E+00	0.0E+00
120	6.1E+04	1.2E+04	1.4E+04	**105**	2.7E+03	4.6E+03	5.2E+03
150	7.1E+04	4.1E+04	4.6E+04	**110**	4.0E+03	6.9E+03	7.8E+03
180	1.1E+05	2.7E+04	3.1E+04	**115**	4.0E+03	6.9E+03	7.8E+03
210	3.7E+04	9.0E+03	1.0E+04	**120**	2.1E+04	3.7E+04	4.2E+04
300	2.6E+04	2.1E+04	2.4E+04	**150**	2.9E+04	4.6E+04	5.2E+04
450	3.3E+04	2.5E+04	2.8E+04	**180**	1.3E+03	2.3E+03	2.6E+03
				210	1.3E+03	2.3E+03	2.6E+03
				240	6.7E+02	1.2E+03	1.3E+03
				270	2.7E+03	4.6E+03	5.2E+03
				300	5.3E+03	9.2E+03	1.0E+04
				350	6.7E+03	9.9E+03	1.1E+04
				400	2.0E+03	3.5E+03	3.9E+03
				450	0.0E+00	0.0E+00	0.0E+00
	Max concentration of each well during duration of experiment						

Table B4: Data for *G. intestinales* from the Fine Cationic SMZ (0.4 – 1.4 mm) Experiment

Time (minutes)	Well 1			Time (minutes)	Well 2		
	Average of 3 counts (cysts/mL)	STDEV	95% CI		Average of 3 counts (cysts/mL)	STDEV	95% CI
0-A	0.0E+00	0.0E+00	0.0E+00	**0-A**	0.0E+00	0.0E+00	0.0E+00
0-B	0.0E+00	0.0E+00	0.0E+00	**0-B**	0.0E+00	0.0E+00	0.0E+00
1	6.7E+02	1.2E+03	1.3E+03	**1**	0.0E+00	0.0E+00	0.0E+00
2	0.0E+00	0.0E+00	0.0E+00	**10**	0.0E+00	0.0E+00	0.0E+00
4	6.7E+02	1.2E+03	1.3E+03	**20**	6.7E+02	1.2E+03	1.3E+03
6	1.3E+03	1.2E+03	1.3E+03	**30**	6.7E+02	1.2E+03	1.3E+03
8	2.7E+03	1.2E+03	1.3E+03	**40**	0.0E+00	0.0E+00	0.0E+00
10	8.7E+03	1.0E+04	1.2E+04	**50**	2.7E+03	3.1E+03	3.5E+03
12	2.0E+03	2.0E+03	2.3E+03	**60**	1.3E+03	1.2E+03	1.3E+03
15	1.3E+03	2.3E+03	2.6E+03	**70**	6.7E+02	1.2E+03	1.3E+03
20	1.3E+03	1.2E+03	1.3E+03	**80**	0.0E+00	0.0E+00	0.0E+00
25	6.7E+02	1.2E+03	1.3E+03	**90**	0.0E+00	0.0E+00	0.0E+00
30	6.7E+02	1.2E+03	1.3E+03	**100**	6.7E+02	1.2E+03	1.3E+03
35	6.7E+02	1.2E+03	1.3E+03	**105**	6.7E+02	1.2E+03	1.3E+03
40	6.7E+02	1.2E+03	1.3E+03	**110**	0.0E+00	0.0E+00	0.0E+00
45	0.0E+00	0.0E+00	0.0E+00	**115**	6.7E+02	1.2E+03	1.3E+03
50	0.0E+00	0.0E+00	0.0E+00	**120**	0.0E+00	0.0E+00	0.0E+00
60	1.3E+03	1.2E+03	1.3E+03	**125**	6.7E+02	1.2E+03	1.3E+03
70	6.7E+02	1.2E+03	1.3E+03	**130**	6.7E+02	1.2E+03	1.3E+03
80	0.0E+00	0.0E+00	0.0E+00	**135**	6.7E+02	1.2E+03	1.3E+03
90	0.0E+00	0.0E+00	0.0E+00	**140**	0.0E+00	0.0E+00	0.0E+00
100	0.0E+00	0.0E+00	0.0E+00	**145**	0.0E+00	0.0E+00	0.0E+00
120	0.0E+00	0.0E+00	0.0E+00	**150**	0.0E+00	0.0E+00	0.0E+00
150	6.7E+02	1.2E+03	1.3E+03	**155**	0.0E+00	0.0E+00	0.0E+00
180	0.0E+00	0.0E+00	0.0E+00	**160**	6.7E+02	1.2E+03	1.3E+03
210	0.0E+00	0.0E+00	0.0E+00	**165**	0.0E+00	0.0E+00	0.0E+00
300	0.0E+00	0.0E+00	0.0E+00	**170**	0.0E+00	0.0E+00	0.0E+00
400	0.0E+00	0.0E+00	0.0E+00	**175**	0.0E+00	0.0E+00	0.0E+00
				180	0.0E+00	0.0E+00	0.0E+00
				185	0.0E+00	0.0E+00	0.0E+00
				190	0.0E+00	0.0E+00	0.0E+00
				195	0.0E+00	0.0E+00	0.0E+00
				200	0.0E+00	0.0E+00	0.0E+00
				220	0.0E+00	0.0E+00	0.0E+00
				240	0.0E+00	0.0E+00	0.0E+00
				260	6.7E+02	1.2E+03	1.3E+03
				280	0.0E+00	0.0E+00	0.0E+00
				300	0.0E+00	0.0E+00	0.0E+00
				350	0.0E+00	0.0E+00	0.0E+00
				400	0.0E+00	0.0E+00	0.0E+00

Table B5: Data for microspheres from the Coarse Cationic SMZ (1.4 – 2.4 mm) Experiment

Time (minutes)	Well 1			Time (minutes)	Well 2		
	Average of 3 counts (spheres/mL)	STDEV	95% CI		Average of 3 counts (spheres/mL)	STDEV	95% CI
0-A	0.0E+00	0.0E+00	0.0E+00	**0-A**	0.0E+00	0.0E+00	0.0E+00
0-B	0.0E+00	0.0E+00	0.0E+00	**0-B**	0.0E+00	0.0E+00	0.0E+00
1	1.3E+03	2.3E+03	2.6E+03	**1**	0.0E+00	0.0E+00	0.0E+00
2	1.3E+03	2.3E+03	2.6E+03	**5**	6.7E+02	1.2E+03	1.3E+03
4	1.1E+05	3.5E+04	4.0E+04	**10**	4.7E+03	1.2E+03	1.3E+03
6	1.2E+05	2.7E+04	3.1E+04	**15**	9.3E+03	9.5E+03	1.1E+04
8	4.9E+04	8.1E+03	9.1E+03	**20**	8.7E+03	3.1E+03	3.5E+03
10	1.6E+04	8.0E+03	9.1E+03	**30**	8.7E+03	5.0E+03	5.7E+03
12	1.2E+04	2.0E+03	2.3E+03	**35**	1.4E+04	5.3E+03	6.0E+03
15	1.5E+04	8.1E+03	9.1E+03	**40**	8.0E+03	5.3E+03	6.0E+03
20	1.8E+04	2.0E+03	2.3E+03	**45**	8.0E+03	6.9E+03	7.8E+03
30	9.3E+03	4.6E+03	5.2E+03	**50**	1.1E+04	4.2E+03	4.7E+03
35	9.3E+03	3.1E+03	3.5E+03	**55**	8.0E+03	5.3E+03	6.0E+03
40	1.1E+04	5.0E+03	5.7E+03	**60**	8.7E+03	5.0E+03	5.7E+03
45	1.3E+04	2.3E+03	2.6E+03	**65**	1.9E+04	1.2E+04	1.4E+04
50	1.7E+04	7.6E+03	8.6E+03	**70**	1.2E+04	1.6E+04	1.8E+04
60	8.0E+03	5.3E+03	6.0E+03	**80**	1.1E+04	7.6E+03	8.6E+03
70	5.3E+03	1.2E+03	1.3E+03	**85**	7.3E+03	4.2E+03	4.7E+03
80	1.3E+03	1.2E+03	1.3E+03	**90**	1.1E+04	8.1E+03	9.1E+03
90	3.3E+03	3.1E+03	3.5E+03	**95**	4.0E+03	5.3E+03	6.0E+03
100	4.7E+03	3.1E+03	3.5E+03	**100**	9.3E+03	6.1E+03	6.9E+03
120	4.0E+03	0.0E+00	0.0E+00	**105**	8.0E+03	8.7E+03	9.9E+03
150	4.7E+03	4.2E+03	4.7E+03	**110**	2.7E+03	1.2E+03	1.3E+03
180	4.0E+03	5.3E+03	6.0E+03	**115**	4.0E+03	2.0E+03	2.3E+03
210	2.7E+03	1.2E+03	1.3E+03	**120**	2.0E+03	2.0E+03	2.3E+03
300	4.7E+03	1.2E+03	1.3E+03	**150**	6.7E+02	1.2E+03	1.3E+03
450	4.7E+03	3.1E+03	3.5E+03	**180**	4.7E+03	6.4E+03	7.3E+03
				210	5.3E+03	4.6E+03	5.2E+03
				240	3.3E+03	5.8E+03	6.5E+03
				270	0.0E+00	0.0E+00	0.0E+00
				300	1.3E+03	2.3E+03	2.6E+03
				350	1.3E+03	2.3E+03	2.6E+03
				450	1.3E+03	1.2E+03	1.3E+03

Table B6: Data for *G. intestinales* from the Coarse Cationic SMZ (1.4 – 2.4 mm) Experiment

Time (minutes)	Well 1			Time (minutes)	Well 2		
	Average of 3 counts (cysts/mL)	STDEV	95% CI		Average of 3 counts (cysts/mL)	STDEV	95% CI
0-A	0.0E+00	0.0E+00	0.0E+00	**0-A**	2.0E+03	2.0E+03	2.3E+03
0-B	0.0E+00	0.0E+00	0.0E+00	**0-B**	6.7E+02	1.2E+03	1.3E+03
1	0.0E+00	0.0E+00	0.0E+00	**1**	1.3E+03	1.2E+03	1.3E+03
2	6.7E+02	1.2E+03	1.3E+03	**10**	0.0E+00	0.0E+00	0.0E+00
4	1.1E+04	1.8E+04	2.1E+04	**20**	0.0E+00	0.0E+00	0.0E+00
6	8.7E+03	1.3E+04	1.5E+04	**30**	0.0E+00	0.0E+00	0.0E+00
8	1.3E+03	2.3E+03	2.6E+03	**40**	0.0E+00	0.0E+00	0.0E+00
10	1.3E+03	1.2E+03	1.3E+03	**50**	6.7E+02	1.2E+03	1.3E+03
12	1.3E+03	2.3E+03	2.6E+03	**60**	0.0E+00	0.0E+00	0.0E+00
15	2.0E+03	3.5E+03	3.9E+03	**70**	0.0E+00	0.0E+00	0.0E+00
20	2.0E+03	3.5E+03	3.9E+03	**80**	6.7E+02	1.2E+03	1.3E+03
25	6.7E+02	1.2E+03	1.3E+03	**90**	2.0E+03	2.0E+03	2.3E+03
30	1.3E+03	2.3E+03	2.6E+03	**100**	2.7E+03	2.3E+03	2.6E+03
35	1.3E+03	2.3E+03	2.6E+03	**105**	6.7E+02	1.2E+03	1.3E+03
40	6.7E+02	1.2E+03	1.3E+03	**110**	0.0E+00	0.0E+00	0.0E+00
45	3.3E+03	5.8E+03	6.5E+03	**115**	6.7E+02	1.2E+03	1.3E+03
50	6.7E+02	1.2E+03	1.3E+03	**120**	0.0E+00	0.0E+00	0.0E+00
60	6.7E+02	1.2E+03	1.3E+03	**125**	6.7E+02	1.2E+03	1.3E+03
70	0.0E+00	0.0E+00	0.0E+00	**130**	0.0E+00	0.0E+00	0.0E+00
80	0.0E+00	0.0E+00	0.0E+00	**135**	6.7E+02	1.2E+03	1.3E+03
90	0.0E+00	0.0E+00	0.0E+00	**140**	0.0E+00	0.0E+00	0.0E+00
100	0.0E+00	0.0E+00	0.0E+00	**145**	6.7E+02	1.2E+03	1.3E+03
120	0.0E+00	0.0E+00	0.0E+00	**150**	6.7E+02	1.2E+03	1.3E+03
150	2.0E+03	3.5E+03	3.9E+03	**155**	0.0E+00	0.0E+00	0.0E+00
180	0.0E+00	0.0E+00	0.0E+00	**160**	0.0E+00	0.0E+00	0.0E+00
210	6.7E+02	1.2E+03	1.3E+03	**165**	0.0E+00	0.0E+00	0.0E+00
300	0.0E+00	0.0E+00	0.0E+00	**170**	0.0E+00	0.0E+00	0.0E+00
400	1.3E+03	2.3E+03	2.6E+03	**175**	6.7E+02	1.2E+03	1.3E+03
				180	6.7E+02	1.2E+03	1.3E+03
				185	6.7E+02	1.2E+03	1.3E+03
				190	0.0E+00	0.0E+00	0.0E+00
				195	6.7E+02	1.2E+03	1.3E+03
				200	0.0E+00	0.0E+00	0.0E+00
				220	0.0E+00	0.0E+00	0.0E+00
				240	0.0E+00	0.0E+00	0.0E+00
				260	0.0E+00	0.0E+00	0.0E+00
				280	0.0E+00	0.0E+00	0.0E+00
				300	0.0E+00	0.0E+00	0.0E+00
				350	0.0E+00	0.0E+00	0.0E+00
				400	0.0E+00	0.0E+00	0.0E+00

Table B7: Data for *G. intestinales* from the Sand Only Experiment

Time (minutes)	Well 1			Time (minutes)	Well 2		
	Average of 3 counts (cysts/mL)	STDEV	95% CI		Average of 3 counts (cysts/mL)	STDEV	95% CI
0-A	0.0E+00	0.0E+00	0.0E+00	**0-A**	0.0E+00	0.0E+00	0.0E+00
0-B	0.0E+00	0.0E+00	0.0E+00	**0-B**	0.0E+00	0.0E+00	0.0E+00
1	0.0E+00	0.0E+00	0.0E+00	**1**	0.0E+00	0.0E+00	0.0E+00
2	0.0E+00	0.0E+00	0.0E+00	**10**	0.0E+00	0.0E+00	0.0E+00
4	2.7E+03	1.2E+03	1.3E+03	**20**	6.7E+02	1.2E+03	1.3E+03
6	4.0E+03	3.5E+03	3.9E+03	**30**	1.3E+03	2.3E+03	2.6E+03
8	6.7E+02	1.2E+03	1.3E+03	**40**	0.0E+00	0.0E+00	0.0E+00
10	0.0E+00	0.0E+00	0.0E+00	**50**	0.0E+00	0.0E+00	0.0E+00
12	0.0E+00	0.0E+00	0.0E+00	**60**	0.0E+00	0.0E+00	0.0E+00
15	0.0E+00	0.0E+00	0.0E+00	**70**	0.0E+00	0.0E+00	0.0E+00
20	0.0E+00	0.0E+00	0.0E+00	**80**	0.0E+00	0.0E+00	0.0E+00
25	0.0E+00	0.0E+00	0.0E+00	**90**	0.0E+00	0.0E+00	0.0E+00
30	0.0E+00	0.0E+00	0.0E+00	**100**	0.0E+00	0.0E+00	0.0E+00
35	0.0E+00	0.0E+00	0.0E+00	**105**	0.0E+00	0.0E+00	0.0E+00
40	0.0E+00	0.0E+00	0.0E+00	**110**	0.0E+00	0.0E+00	0.0E+00
45	0.0E+00	0.0E+00	0.0E+00	**115**	6.7E+02	1.2E+03	1.3E+03
50	0.0E+00	0.0E+00	0.0E+00	**120**	0.0E+00	0.0E+00	0.0E+00
60	0.0E+00	0.0E+00	0.0E+00	**125**	6.7E+02	1.2E+03	1.3E+03
70	6.7E+02	1.2E+03	1.3E+03	**130**	0.0E+00	0.0E+00	0.0E+00
80	0.0E+00	0.0E+00	0.0E+00	**135**	0.0E+00	0.0E+00	0.0E+00
90	0.0E+00	0.0E+00	0.0E+00	**140**	0.0E+00	0.0E+00	0.0E+00
100	0.0E+00	0.0E+00	0.0E+00	**145**	0.0E+00	0.0E+00	0.0E+00
120	0.0E+00	0.0E+00	0.0E+00	**150**	0.0E+00	0.0E+00	0.0E+00
150	0.0E+00	0.0E+00	0.0E+00	**155**	0.0E+00	0.0E+00	0.0E+00
180	0.0E+00	0.0E+00	0.0E+00	**160**	0.0E+00	0.0E+00	0.0E+00
210	0.0E+00	0.0E+00	0.0E+00	**165**	0.0E+00	0.0E+00	0.0E+00
300	0.0E+00	0.0E+00	0.0E+00	**170**	0.0E+00	0.0E+00	0.0E+00
400	6.7E+02	1.2E+03	1.3E+03	**175**	0.0E+00	0.0E+00	0.0E+00
				180	0.0E+00	0.0E+00	0.0E+00
				185	0.0E+00	0.0E+00	0.0E+00
				190	0.0E+00	0.0E+00	0.0E+00
				195	0.0E+00	0.0E+00	0.0E+00
				200	0.0E+00	0.0E+00	0.0E+00
				220	0.0E+00	0.0E+00	0.0E+00
				240	0.0E+00	0.0E+00	0.0E+00
				260	0.0E+00	0.0E+00	0.0E+00
				280	0.0E+00	0.0E+00	0.0E+00
				300	0.0E+00	0.0E+00	0.0E+00
				350	0.0E+00	0.0E+00	0.0E+00
				400	0.0E+00	0.0E+00	0.0E+00

Table B8: Data for microspheres from the Raw Zeolite Experiment

Time (minutes)	Well 1			Time (minutes)	Well 2		
	Average of 3 counts (spheres/mL)	STDEV	95% CI		Average of 3 counts (spheres/mL)	STDEV	95% CI
0-A	0.0E+00	0.0E+00	0.0E+00	**0-A**	1.3E+03	1.2E+03	1.3E+03
0-B	2.0E+03	2.0E+03	2.3E+03	**0-B**	6.7E+02	1.2E+03	1.3E+03
1	6.7E+02	1.2E+03	1.3E+03	**1**	0.0E+00	0.0E+00	0.0E+00
2	2.0E+03	2.0E+03	2.3E+03	**5**	0.0E+00	0.0E+00	0.0E+00
4	6.7E+02	1.2E+03	1.3E+03	**10**	2.0E+03	3.5E+03	3.9E+03
6	3.3E+03	1.2E+03	1.3E+03	**15**	2.0E+03	3.5E+03	3.9E+03
8	6.7E+02	1.2E+03	1.3E+03	**20**	1.3E+03	1.2E+03	1.3E+03
10	2.8E+04	4.0E+03	4.5E+03	**25**	1.3E+03	2.3E+03	2.6E+03
12	7.7E+04	3.1E+03	3.5E+03	**30**	6.7E+02	1.2E+03	1.3E+03
15	5.1E+04	1.7E+04	1.9E+04	**35**	6.7E+02	1.2E+03	1.3E+03
20	2.6E+04	8.7E+03	9.9E+03	**40**	6.7E+02	1.2E+03	1.3E+03
25	4.7E+03	3.1E+03	3.5E+03	**45**	1.3E+03	1.2E+03	1.3E+03
30	2.0E+03	2.0E+03	2.3E+03	**50**	1.3E+03	1.2E+03	1.3E+03
35	1.3E+03	1.2E+03	1.3E+03	**55**	2.7E+03	4.6E+03	5.2E+03
40	5.3E+03	4.2E+03	4.7E+03	**60**	2.0E+03	3.5E+03	3.9E+03
45	2.0E+03	2.0E+03	2.3E+03	**65**	6.7E+02	1.2E+03	1.3E+03
50	4.7E+03	1.2E+03	1.3E+03	**70**	1.3E+03	1.2E+03	1.3E+03
60	4.7E+03	3.1E+03	3.5E+03	**80**	0.0E+00	0.0E+00	0.0E+00
70	1.3E+03	2.3E+03	2.6E+03	**85**	0.0E+00	0.0E+00	0.0E+00
80	2.0E+03	2.0E+03	2.3E+03	**90**	6.7E+02	1.2E+03	1.3E+03
90	4.7E+03	6.4E+03	7.3E+03	**95**	1.3E+03	1.2E+03	1.3E+03
100	8.0E+03	5.3E+03	6.0E+03	**100**	0.0E+00	0.0E+00	0.0E+00
120	1.3E+03	1.2E+03	1.3E+03	**105**	4.0E+03	3.5E+03	3.9E+03
150	0.0E+00	0.0E+00	0.0E+00	**110**	1.3E+03	2.3E+03	2.6E+03
180	6.7E+02	1.2E+03	1.3E+03	**115**	1.3E+03	1.2E+03	1.3E+03
210	1.3E+03	2.3E+03	2.6E+03	**120**	6.7E+02	1.2E+03	1.3E+03
300	2.7E+03	4.6E+03	5.2E+03	**150**	1.3E+03	2.3E+03	2.6E+03
400	1.3E+04	4.6E+03	5.2E+03	**180**	6.7E+02	1.2E+03	1.3E+03
				210	1.3E+03	1.2E+03	1.3E+03
				240	1.3E+03	1.2E+03	1.3E+03
				270	2.0E+03	3.5E+03	3.9E+03
				300	6.7E+02	1.2E+03	1.3E+03
				350	2.0E+03	0.0E+00	0.0E+00
				400	0.0E+00	0.0E+00	0.0E+00

Table B9: Data for *G. intestinales* from the Raw Zeolite Experiment

Time (minutes)	Well 1			Time (minutes)	Well 2		
	Average of 3 counts (cysts/mL)	STDEV	95% CI		Average of 3 counts (cysts/mL)	STDEV	95% CI
0-A	0.0E+00	0.0E+00	0.0E+00	**0-A**	0.0E+00	0.0E+00	0.0E+00
0-B	0.0E+00	0.0E+00	0.0E+00	**0-B**	0.0E+00	0.0E+00	0.0E+00
1	6.7E+02	1.2E+03	1.3E+03	**1**	0.0E+00	0.0E+00	0.0E+00
2	0.0E+00	0.0E+00	0.0E+00	**10**	0.0E+00	0.0E+00	0.0E+00
4	6.7E+02	1.2E+03	1.3E+03	**20**	0.0E+00	0.0E+00	0.0E+00
6	6.7E+02	1.2E+03	1.3E+03	**30**	0.0E+00	0.0E+00	0.0E+00
8	0.0E+00	0.0E+00	0.0E+00	**40**	0.0E+00	0.0E+00	0.0E+00
10	0.0E+00	0.0E+00	0.0E+00	**50**	0.0E+00	0.0E+00	0.0E+00
12	6.7E+02	1.2E+03	1.3E+03	**60**	0.0E+00	0.0E+00	0.0E+00
15	0.0E+00	0.0E+00	0.0E+00	**70**	0.0E+00	0.0E+00	0.0E+00
20	1.3E+03	1.2E+03	1.3E+03	**80**	6.7E+02	1.2E+03	1.3E+03
25	0.0E+00	0.0E+00	0.0E+00	**90**	0.0E+00	0.0E+00	0.0E+00
30	0.0E+00	0.0E+00	0.0E+00	**100**	0.0E+00	0.0E+00	0.0E+00
35	0.0E+00	0.0E+00	0.0E+00	**105**	6.7E+02	1.2E+03	1.3E+03
40	6.7E+02	1.2E+03	1.3E+03	**110**	0.0E+00	0.0E+00	0.0E+00
45	1.3E+03	2.3E+03	2.6E+03	**115**	0.0E+00	0.0E+00	0.0E+00
50	2.0E+03	3.5E+03	3.9E+03	**120**	6.7E+02	1.2E+03	1.3E+03
60	0.0E+00	0.0E+00	0.0E+00	**125**	6.7E+02	1.2E+03	1.3E+03
70	0.0E+00	0.0E+00	0.0E+00	**130**	1.3E+03	2.3E+03	2.6E+03
80	0.0E+00	0.0E+00	0.0E+00	**135**	0.0E+00	0.0E+00	0.0E+00
90	0.0E+00	0.0E+00	0.0E+00	**140**	1.3E+03	1.2E+03	1.3E+03
100	2.0E+03	3.5E+03	3.9E+03	**145**	0.0E+00	0.0E+00	0.0E+00
120	0.0E+00	0.0E+00	0.0E+00	**150**	0.0E+00	0.0E+00	0.0E+00
150	0.0E+00	0.0E+00	0.0E+00	**155**	0.0E+00	0.0E+00	0.0E+00
180	0.0E+00	0.0E+00	0.0E+00	**160**	0.0E+00	0.0E+00	0.0E+00
210	0.0E+00	0.0E+00	0.0E+00	**165**	0.0E+00	0.0E+00	0.0E+00
300	0.0E+00	0.0E+00	0.0E+00	**170**	0.0E+00	0.0E+00	0.0E+00
400	6.7E+02	1.2E+03	1.3E+03	**175**	0.0E+00	0.0E+00	0.0E+00
				180	0.0E+00	0.0E+00	0.0E+00
				185	0.0E+00	0.0E+00	0.0E+00
				190	6.7E+02	1.2E+03	1.3E+03
				195	0.0E+00	0.0E+00	0.0E+00
				200	0.0E+00	0.0E+00	0.0E+00
				220	0.0E+00	0.0E+00	0.0E+00
				240	0.0E+00	0.0E+00	0.0E+00
				260	0.0E+00	0.0E+00	0.0E+00
				280	0.0E+00	0.0E+00	0.0E+00
				300	0.0E+00	0.0E+00	0.0E+00
				350	0.0E+00	0.0E+00	0.0E+00
				400	0.0E+00	0.0E+00	0.0E+00

Table B10: Data for microspheres from the Hydrophobic SMZ Experiment

Time (minutes)	Well 1			Time (minutes)	Well 2		
	Average of 3 counts (spheres/mL)	STDEV	95% CI		Average of 3 counts (spheres/mL)	STDEV	95% CI
0-A	0.0E+00	0.0E+00	0.0E+00	**0-A**	0.0E+00	0.0E+00	0.0E+00
0-B	0.0E+00	0.0E+00	0.0E+00	**0-B**	0.0E+00	0.0E+00	0.0E+00
1	4.2E+06	1.7E+06	2.0E+06	**1**	0.0E+00	0.0E+00	0.0E+00
2	1.1E+06	2.5E+05	2.9E+05	**5**	1.3E+03	2.3E+03	2.6E+03
4	2.1E+05	1.1E+05	1.2E+05	**10**	8.0E+03	2.0E+03	2.3E+03
6	2.3E+05	3.1E+04	3.5E+04	**15**	9.3E+03	1.2E+03	1.3E+03
8	1.2E+05	5.1E+04	5.7E+04	**20**	1.0E+04	3.5E+03	3.9E+03
10	7.1E+04	2.4E+04	2.7E+04	**30**	2.7E+03	4.6E+03	5.2E+03
12	6.3E+04	6.1E+03	6.9E+03	**35**	4.7E+03	1.2E+03	1.3E+03
15	1.4E+05	2.3E+04	2.6E+04	**40**	2.0E+03	3.5E+03	3.9E+03
20	6.0E+04	3.2E+04	3.6E+04	**45**	2.0E+03	2.0E+03	2.3E+03
30	5.3E+04	6.4E+03	7.3E+03	**50**	2.7E+03	4.6E+03	5.2E+03
35	4.4E+04	5.3E+03	6.0E+03	**55**	3.3E+03	2.3E+03	2.6E+03
40	2.6E+04	3.5E+03	3.9E+03	**60**	6.7E+02	1.2E+03	1.3E+03
45	4.3E+04	3.1E+03	3.5E+03	**65**	0.0E+00	0.0E+00	0.0E+00
50	3.6E+04	1.8E+04	2.1E+04	**70**	6.7E+02	1.2E+03	1.3E+03
60	2.6E+04	9.2E+03	1.0E+04	**80**	1.3E+03	1.2E+03	1.3E+03
70	1.4E+04	2.0E+03	2.3E+03	**85**	1.3E+03	1.2E+03	1.3E+03
80	2.6E+04	1.7E+04	2.0E+04	**90**	0.0E+00	0.0E+00	0.0E+00
90	1.6E+04	7.2E+03	8.2E+03	**95**	6.7E+02	1.2E+03	1.3E+03
100	6.0E+03	3.5E+03	3.9E+03	**100**	1.3E+03	2.3E+03	2.6E+03
120	9.3E+03	9.2E+03	1.0E+04	**105**	1.3E+03	1.2E+03	1.3E+03
150	2.7E+03	1.2E+03	1.3E+03	**110**	0.0E+00	0.0E+00	0.0E+00
180	7.3E+03	6.1E+03	6.9E+03	**115**	0.0E+00	0.0E+00	0.0E+00
210	2.7E+03	1.2E+03	1.3E+03	**120**	1.3E+03	1.2E+03	1.3E+03
300	2.7E+03	3.1E+03	3.5E+03	**150**	2.0E+03	2.0E+03	2.3E+03
				180	6.7E+02	1.2E+03	1.3E+03
				210	1.3E+03	2.3E+03	2.6E+03
				240	6.7E+02	1.2E+03	1.3E+03
				270	0.0E+00	0.0E+00	0.0E+00
				300	6.7E+02	1.2E+03	1.3E+03
				360	0.0E+00	0.0E+00	0.0E+00

Table B11: Data for *G. intestinales* from the Hydrophobic SMZ Experiment (Run 1: 5 mL cyst injection)

Time (minutes)	Well 1			Time (minutes)	Well 2		
	Average of 3 counts (cysts/mL)	STDEV	95% CI		Average of 3 counts (cysts/mL)	STDEV	95% CI
0-A	0.0E+00	0.0E+00	0.0E+00	**0-A**	0.0E+00	0.0E+00	0.0E+00
0-B	0.0E+00	0.0E+00	0.0E+00	**0-B**	0.0E+00	0.0E+00	0.0E+00
1	0.0E+00	0.0E+00	0.0E+00	**1**	0.0E+00	0.0E+00	0.0E+00
2	0.0E+00	0.0E+00	0.0E+00	**5**	0.0E+00	0.0E+00	0.0E+00
4	2.0E+03	0.0E+00	0.0E+00	**10**	0.0E+00	0.0E+00	0.0E+00
6	2.7E+03	1.2E+03	1.3E+03	**15**	0.0E+00	0.0E+00	0.0E+00
8	1.3E+03	2.3E+03	2.6E+03	**20**	0.0E+00	0.0E+00	0.0E+00
10	6.7E+02	1.2E+03	1.3E+03	**30**	0.0E+00	0.0E+00	0.0E+00
12	6.7E+02	1.2E+03	1.3E+03	**35**	0.0E+00	0.0E+00	0.0E+00
15	0.0E+00	0.0E+00	0.0E+00	**40**	0.0E+00	0.0E+00	0.0E+00
20	6.7E+02	1.2E+03	1.3E+03	**45**	0.0E+00	0.0E+00	0.0E+00
30	6.7E+02	1.2E+03	1.3E+03	**50**	0.0E+00	0.0E+00	0.0E+00
35	0.0E+00	0.0E+00	0.0E+00	**55**	0.0E+00	0.0E+00	0.0E+00
40	0.0E+00	0.0E+00	0.0E+00	**60**	0.0E+00	0.0E+00	0.0E+00
45	0.0E+00	0.0E+00	0.0E+00	**65**	0.0E+00	0.0E+00	0.0E+00
50	0.0E+00	0.0E+00	0.0E+00	**70**	0.0E+00	0.0E+00	0.0E+00
60	0.0E+00	0.0E+00	0.0E+00	**80**	0.0E+00	0.0E+00	0.0E+00
70	0.0E+00	0.0E+00	0.0E+00	**85**	0.0E+00	0.0E+00	0.0E+00
80	1.3E+03	2.3E+03	2.6E+03	**90**	0.0E+00	0.0E+00	0.0E+00
90	6.7E+02	1.2E+03	1.3E+03	**95**	0.0E+00	0.0E+00	0.0E+00
100	0.0E+00	0.0E+00	0.0E+00	**100**	0.0E+00	0.0E+00	0.0E+00
120	0.0E+00	0.0E+00	0.0E+00	**105**	0.0E+00	0.0E+00	0.0E+00
150	0.0E+00	0.0E+00	0.0E+00	**110**	0.0E+00	0.0E+00	0.0E+00
180	0.0E+00	0.0E+00	0.0E+00	**115**	0.0E+00	0.0E+00	0.0E+00
210	1.3E+03	1.2E+03	1.3E+03	**120**	0.0E+00	0.0E+00	0.0E+00
300	0.0E+00	0.0E+00	0.0E+00	**150**	0.0E+00	0.0E+00	0.0E+00
390	0.0E+00	0.0E+00	0.0E+00	**180**	0.0E+00	0.0E+00	0.0E+00
				210	0.0E+00	0.0E+00	0.0E+00
				240	0.0E+00	0.0E+00	0.0E+00
				270	0.0E+00	0.0E+00	0.0E+00
				300	0.0E+00	0.0E+00	0.0E+00
				360	0.0E+00	0.0E+00	0.0E+00
				390	0.0E+00	0.0E+00	0.0E+00

Table B12: Data for *G. intestinales* from the Hydrophobic SMZ Experiment (Run 2: 10 mL cyst injection)

Time (minutes)	Well 1			Time (minutes)	Well 2		
	Average of 3 counts (cysts/mL)	STDEV	95% CI		Average of 3 counts (cysts/mL)	STDEV	95% CI
0-A	0.0E+00	0.0E+00	0.0E+00	**0-A**	0.0E+00	0.0E+00	0.0E+00
0-B	0.0E+00	0.0E+00	0.0E+00	**0-B**	0.0E+00	0.0E+00	0.0E+00
1	0.0E+00	0.0E+00	0.0E+00	**1**	0.0E+00	0.0E+00	0.0E+00
2	0.0E+00	0.0E+00	0.0E+00	**5**	0.0E+00	0.0E+00	0.0E+00
4	2.7E+03	1.2E+03	1.3E+03	**10**	0.0E+00	0.0E+00	0.0E+00
6	6.0E+03	5.3E+03	6.0E+03	**15**	0.0E+00	0.0E+00	0.0E+00
8	0.0E+00	0.0E+00	0.0E+00	**20**	0.0E+00	0.0E+00	0.0E+00
10	0.0E+00	0.0E+00	0.0E+00	**30**	0.0E+00	0.0E+00	0.0E+00
12	0.0E+00	0.0E+00	0.0E+00	**35**	0.0E+00	0.0E+00	0.0E+00
15	2.0E+03	2.0E+03	2.3E+03	**40**	0.0E+00	0.0E+00	0.0E+00
20	0.0E+00	0.0E+00	0.0E+00	**45**	0.0E+00	0.0E+00	0.0E+00
30	0.0E+00	0.0E+00	0.0E+00	**50**	0.0E+00	0.0E+00	0.0E+00
35	0.0E+00	0.0E+00	0.0E+00	**55**	0.0E+00	0.0E+00	0.0E+00
40	0.0E+00	0.0E+00	0.0E+00	**60**	0.0E+00	0.0E+00	0.0E+00
45	0.0E+00	0.0E+00	0.0E+00	**65**	0.0E+00	0.0E+00	0.0E+00
50	0.0E+00	0.0E+00	0.0E+00	**70**	0.0E+00	0.0E+00	0.0E+00
60	6.7E+02	1.2E+03	1.3E+03	**80**	0.0E+00	0.0E+00	0.0E+00
70	0.0E+00	0.0E+00	0.0E+00	**85**	0.0E+00	0.0E+00	0.0E+00
80	0.0E+00	0.0E+00	0.0E+00	**90**	0.0E+00	0.0E+00	0.0E+00
90	0.0E+00	0.0E+00	0.0E+00	**95**	0.0E+00	0.0E+00	0.0E+00
100	6.7E+02	1.2E+03	1.3E+03	**100**	0.0E+00	0.0E+00	0.0E+00
120	6.7E+02	1.2E+03	1.3E+03	**105**	0.0E+00	0.0E+00	0.0E+00
150	0.0E+00	0.0E+00	0.0E+00	**110**	0.0E+00	0.0E+00	0.0E+00
180	0.0E+00	0.0E+00	0.0E+00	**115**	0.0E+00	0.0E+00	0.0E+00
210	0.0E+00	0.0E+00	0.0E+00	**120**	0.0E+00	0.0E+00	0.0E+00
300	0.0E+00	0.0E+00	0.0E+00	**150**	0.0E+00	0.0E+00	0.0E+00
480	0.0E+00	0.0E+00	0.0E+00	**180**	0.0E+00	0.0E+00	0.0E+00
				210	0.0E+00	0.0E+00	0.0E+00
				240	0.0E+00	0.0E+00	0.0E+00
				270	0.0E+00	0.0E+00	0.0E+00
				300	0.0E+00	0.0E+00	0.0E+00
				360	0.0E+00	0.0E+00	0.0E+00
				480	0.0E+00	0.0E+00	0.0E+00

APPENDIX C:

Description of the University of Idaho Groundwater Research Site (UIGRS)

C.1 Description of the Test Field Site (UIGRS)

Field work was conducted at the University of Idaho Groundwater Research Site (UIGRS) in Moscow, Idaho. The UIGRS was located on the far western edge of University of Idaho campus in section 12, T39N R6W on Moscow West quadrangle on the Idaho-Washington border and roughly at Latitude: 46.73215 Longitude: -117.02486. The site location map (Figure C1) gives the location of the UIGRS within the city of Moscow, ID and the site plan view map (Figure C2) gives a more detailed description of the UIGRS. Paradise Creek and the Burlington Northern Railroad made the northern boundary, and Perimeter Drive made the eastern boundary of the field site. The site topography was generally flat near Paradise Creek and slopes upward into a hill towards the south and southwest. All wells were completed in the flat portion of the UIGRS.

Paradise Creek is one of the primary streams in the Pullman-Moscow Basin. Originating from Moscow Mountain, Paradise Creek is a tributary of the South Fork of the Palouse River. The U.S. Geological Survey had a gauging station roughly 30 meters (100 feet) east of the Perimeter Drive border of the UIGRS that operated from 1 Oct 1978 to 30 Sept 2005. The USGS gauging station (#13346800) is 775.25 meters (2,543.46 feet) above sea level (NGVD29 datum). Drainage area of Paradise creek is 45.8 square kilometers (17.7 square miles). Hydraulic data for Paradise Creek during the period of operation are listed in Table C1.

Paradise Creek Hydrologic Data	cms	L/sec	cfs	gal/min
Average Discharge for 27 yrs of record	0.20	204.84	7.23	3246.15
Maximum Discharge (9 Feb 1996)	27.16	27470.40	970	435336.00
Minimum Daily Flow (29 Nov 1987 & 10 Oct 2003)	0.00	1.13	0.04	17.95

Table C1: USGS Gauging Station #13346800 (USGS, 2004)
(**Note:** cubic meters per second, **cms**, cubic feet per second, **cfs**)

This site was selected for several reasons, including; (1) the presence of a well-documented fractured rock aquifer at shallow depths, (2) preexisting completed wells screened at the fracture of interest, and (3) close proximity to research facilities. Previous research conducted at this site included a site characterization by Li (1991), a groundwater microbial analysis by Zheng (1992), a study of the relationship between surface water and groundwater by Pardo (1993), and a fractured rock aquifer tracer study by Nimmer (1998).

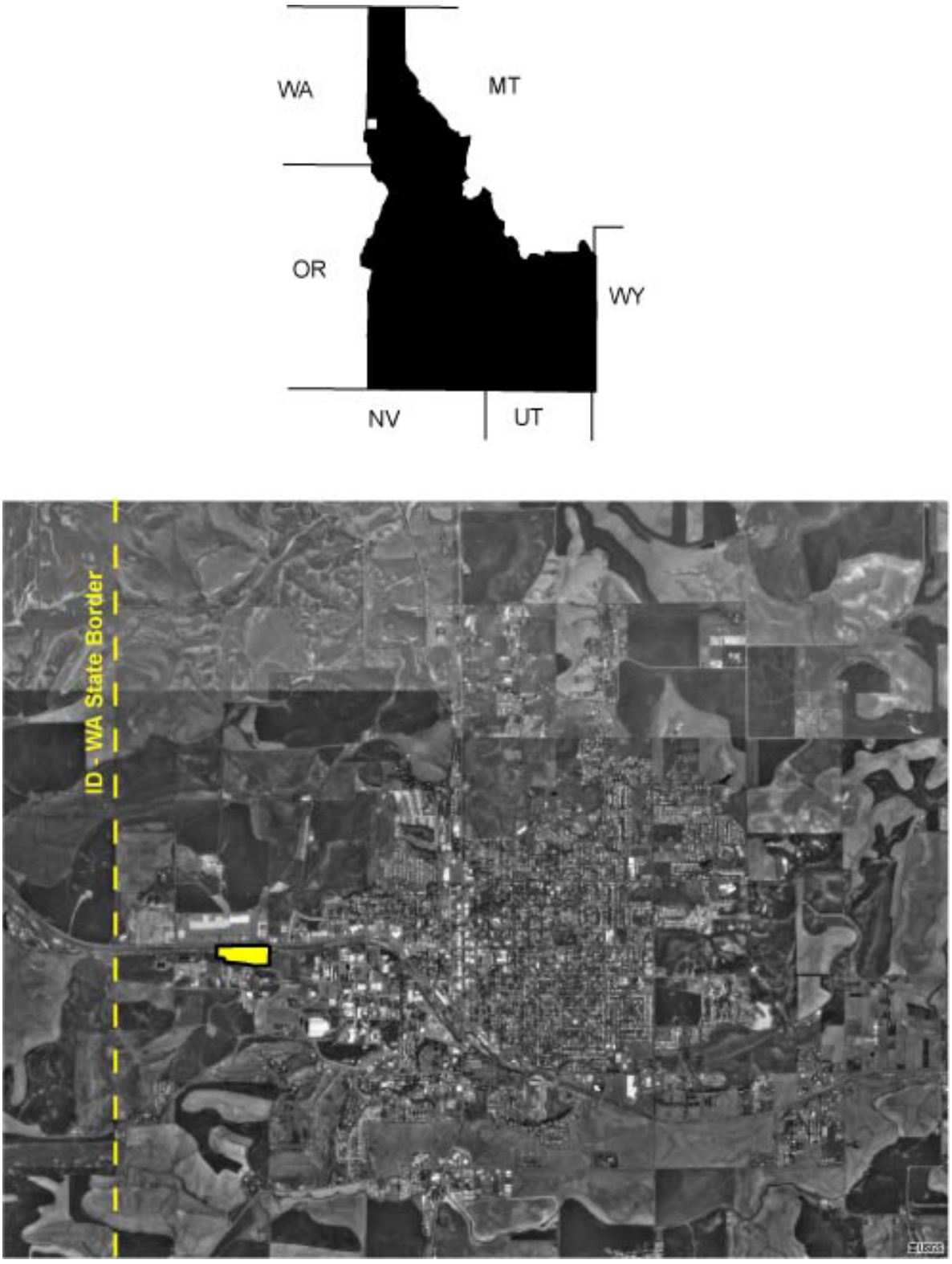

Figure C1: Location Map of the UIGRS (modified from Nimmer, 1998) (TerraServer USA, 5/22/1992)

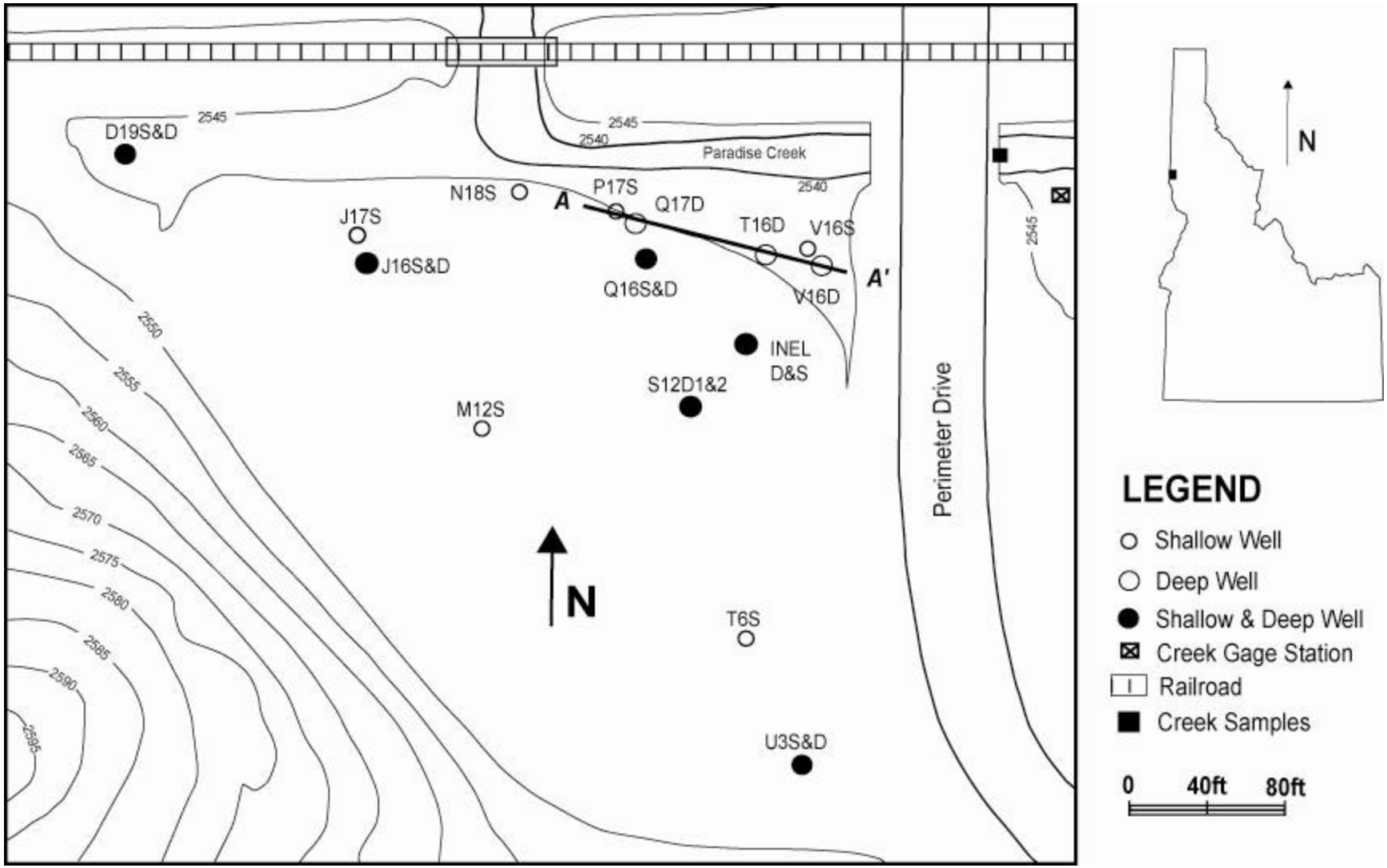

Figure C2: Plan view map of the UIGRS (modified from Li, 1991) A – A' is a cross section in Figure 10.

C.1.1 Regional Geology and Hydrogeology Setting

The UIGRS is located in the Moscow-Pullman Basin at the far eastern edge of the Columbia Plateau in an area known as the Palouse Region (Li, 1991; Provant, 1995). The Moscow-Pullman Basin from bottom to top is composed of the following three major units: Cambrian orthoquartzite with Cretaceous granitic intrusions create the crystalline basement rock (Li, 1991; Provant, 1995), miocene basalt flows with interbedded sediments cover the irregular surface of the crystalline basement rock (Lum II et al., 1990), and Pleistocene loess known as the Palouse Formation covers most of the Moscow-Pullman Basin (Hooper and Webster, 1982). Table C2 is a generalized stratigraphic section for the formations and basalt flows present in the Moscow-Pullman Basin.

Regional Stratigraphy and Lithology of the Moscow-Pullman Basin

The stratiography and lithology descriptions of the three major formations present in the Moscow-Pullman Basin; (1) the crystalline basement rock, (2) the Columbia River basalt group and (3) the Palouse Formation, are provide below as a literature summary. A generalized stratigraphic section is provided in Table C2.

(1) Crystalline Basement Rock

The crystalline basement rock outcrops at ground surface only around the edges of the basin to the northeast and southeast of Moscow, ID and to the northwest of Pullman, WA. The basin is bound to the northeast by the Idaho Batholith granite of the Palouse Range (or Moscow Mountain), to the southeast by granite and gneiss along the western edge of Paradise Ridge and Bald Butte, and to the northwest by Belt Supergroup metamorphics of Smoot Hill, Kamiak, and Randall Butte (Nimmer, 1998). The Moscow-Pullman Basin is open to the west and southwest.

(2) Columbia River Basalt Group

The Miocene basalt flows with interbedded sediments are of the Yakima Basalt Subgroup of the Columbia River Basalt Group. The two major formations are known as the Grande Ronde and Wanapum Basalts. The basalt formations were formed when large volumes of lava erupted from fissures in southeastern Washington and northeastern Oregon over millions of year's time in the Miocene (Li, 1991; Provant, 1995; Swanson et al., 1977, 1980). The two basalt formations are hydrologically and geochemically different from one another.

Grande Ronde Formation

The Grande Ronde Formation is the base basalt unit of the Columbia River Basalts in the Moscow-Pullman Basin that consists of many flows with interbedded sediments. The thickness of individual basalt flow averages from 12.2 to 24.4 meters (40 to 80 feet) although flows over 61.0 meters (200 feet) thickness have been found (Li, 1991; Lum II et al., 1990). The total thickness of the Grande Ronde Formation ranges from zero to over 762 meters (0 to 2500 feet), with increasing thickness from east to west (Li, 1991). The thickness of the Grande Ronde basalt is approximately 426.72 meters (1400 feet) under the UIGRS (Li, 1991).

Interbedded Sediments of the Columbia River Basalt Group

The first sedimentary interbed of the Latah Formation stratigraphically separates the Grande Ronde basalt from the Wanapum basalt, and is equivalent to the Vantage Member of the Miocene Ellensburg Formation (Li, 1991; Nimmer, 1998). This sedimentary interbed of the Latah Formation only ranges in thickness from 1.5 to 3 meters (5 to 10 feet) within the Pullman-Moscow Basin (Li, 1991), and are over 60.96 meters (200 feet) in thickness below the Moscow area (Nimmer, 1998).

Wanapum Formation

The Wanapum Formation is the top basalt unit of the Columbia River Basalts with only the Priest Rapids Member present in the Moscow-Pullman Basin. The Lolo flow of the Priest Rapids Member is the uppermost basalt unit in the basin with a range in thickness from 48.8 to 61.0 meters (160 to 200 feet) (Nimmer, 1998; Provant, 1995).

(3) Palouse Formation

Palouse Loess

The Palouse loess and associated alluvial sediments makes up the uppermost stratigraphic unit of the basin. The loess is composed of a silty loam with mostly quartz and feldspar composition and varies in thickness from 0 to 100 meters (0 to several hundred feet). The more recent alluvial sediments are derived from stream deposits and slope erosion of the loess hills and basalt outcrops (Li, 1991; Nimmer, 1998; Provant, 1995).

Regional Hydrogeology of Columbia River Basalt Flow

The two aquifers within the Moscow-Pullman basin are the lower aquifer in the Grande Ronde Formation and the upper aquifer in the Wanapum Formation. Both aquifers consist mainly of fractured basalt and some associated sediments. The lower Grande Ronde aquifer houses most municipal wells for the cities of Moscow, ID and Pullman, WA. Wells have depths ranging from 152.4 to 426.72 meters (500 to 1400 feet) with a depth to water between 76.2 to 91.44 meters (250 to 300 feet) (Nimmer, 1998).

The upper Wanapum aquifer houses many domestic wells and a limited number of municipal wells in the Moscow- Pullman area. Wells have depths ranging from 60.96 to 121.92 meters (200 to 400 feet) with a depth to water between 15.24 to 24.38 meters (50 and 80 feet) (Nimmer, 1998).

PERIOD	EPOCH	GROUP	SUBGROUP	FORMATION	K-Ar AGE (m-year)	MEMBER	STRATIGRAPHY	THICKNESS
Quaternary	Pleistocene/ Holocene					Stream Valley sediments	Alluvium	0-3 m (1-10 ft)
	Pleistocene			Palouse			Loess	0-76 m (0-250 ft)
Tertiary	Miocene	Columbia River Basalt Group	Yakima Basalt Subgroup	Wanapum Basalt	13.6-14.5	Priest Rapids	Lolo Flow with Rosalia Basalts	0-76 m (0-250 ft)
				Ellensburg interbeds		Vantage	Siltstone, claystone, tuffaceous rocks	1.5-3 m (5-10 ft)
				Grande Ronde Basalt	14.5-16.5		Many basalt flows	0-762 m (0-2500 ft)
Cretaceous					66-144		Granitic intrusives	
Cambrian					505-570		Orthoquartzite	

Table C2: Generalized Stratigraphic Section of the Moscow-Pullman Basin (Li, 1991).

C.1.2 UIGRS Geology and Hydrogeology Setting

The geology of the University of Idaho Groundwater Research Site (UIGRS) is similar to the regional geology of the Moscow-Pullman basin with a few differences in the stratigraphic thicknesses. The majority of the wells completed at the UIGRS were in the Lolo Flow of the Wanapum Formation and the overlying loess and alluvial sediments.

Local Stratigraphy and Lithology of the UIGRS

The Palouse loess and alluvial sediments are the uppermost stratigraphic units at the UIGRS. The Palouse loess consists of black soil, clay and silt and the alluvial sediments consists of sand and gravel. The total sediment thickness is up to 3.96 meters (13 feet) at the UIGRS site.

The Lolo basalt flow of the Priest Rapids Member of the Wanapum Formation is the only basalt flow at the UIGRS. The Lolo flow occurs at a depth of about 4.57 to 60.96 meters (15 to 200 feet) below ground surface at the UIGRS. The flow consists of mostly dense basalt with sub-horizontal fractures, numerous micro-fractures, vertical joints and vesicles due to emplacement and cooling of lava (Li, 1991; Nimmer, 1998). A majority of the wells at the UIGRS were completed in the sub-horizontal fractures in the upper third of the flow at depths ranging from 21.34 to 27.43 meters (70 to 90 feet) (Li, 1991; Nimmer, 1998).

East Fracture Lithology

Two major sub-horizontal fractures were identified by Li (1991) at the UIGRS site, and were named the East and West fracture zones (Figure C3). Significant lateral variation occurs with the fracture zones, and most sub-horizontal fractures do not extend more than a 100 meters (few hundred feet) (Li, 1991). Some vertical fractures cross sub-horizontal fractures forming complex systems resembling Z patterns. At the UIGRS, the East sub-horizontal fracture aquifer

was used for all field experiments and was located at a depth of around 18.29-24.38 meters (60-80 feet) (Figure C3).

C.1.3 UIGRS Geology and Hydrogeology Setting

The East fracture zone aquifer at the UIGRS was penetrated by four wells (Q17D, Q16D, T16D, and V16D). For all field tests, well T16D was used as the injection well and V16D was used as the pumping/sampling well. These wells were chosen because they were both completed within the East fracture zone (approximately 9 meter distance apart), and V16D was used as the pumping well because it had a higher well yield than T16D. The East fracture zone was 0.15 to 0.92 meters (0.5 to 3 feet) thick and was located approximately 19.50 to 21.33 meters (64 to70 feet) below land surface at wells T16D and V16D (Figure C4 & C5). The depth to water in wells T16D and V16D was about 1.5-2.1 meters (5-7 feet). The East fracture zone aquifer acted as a confined, heterogeneous and anisotropic aquifer with well yields from less than 0.44 liters per second (7 gpm) and up to 3.16 liters per second (50 gpm) and specific well yields between 3.73 to 0.67 liters per second per meter drawdown (0.3 to 3.25 gpm per foot of drawdown). Variability indicated a variable hydraulic conductivity of the East fracture zone aquifer. The East fracture zone aquifer was a good option for field tests because Li (1991) and Pardo (1993) found a reliable hydraulic connection between the shallow aquifer and the neighboring Paradise Creek. See Table C3 and Table C4 for more details on the injection well T16D and the pumping/sampling well V16D.

Table C3: Well Construction Information (Li, 1991)

Well #	Ground Elevation (ft)	Total Depth (ft)	Borehole Diameter (in)	Surface Casing Diameter (in)	PVC Liner Diameter (in)	Perforation (PVC)	Perf. Interval (ft)	Sand Pack Interval (ft)	Seal
T16D	2543.61	80	6	6	4	Hacksaw slots	65-69	59-70	C-B
V16D	2543.46	70	4	6	4	40-slot screen	65-67.5	63-70	C-B

C-B: Cement and Bentonite mix

Table C4: Groundwater Level and Well Yield Capacity Data at the UIGRS (Li, 1991)

Well #	Year Completed	Water Level Elevation Annual High (ft)	Water Elevation Annual Low (ft)	Max Well Yield (gpm)	Specific Well Yield (gpm/ft)	Datum Marker for Water Level	Datum Elevation (ft)
T16D	1988	2540.4	2536.7	7-10	0.3-0.4	Top of 6" casing	2545.24
V16D	1987	2540.7	2537	40-50	3.25	Top of 6" casing	2544.41

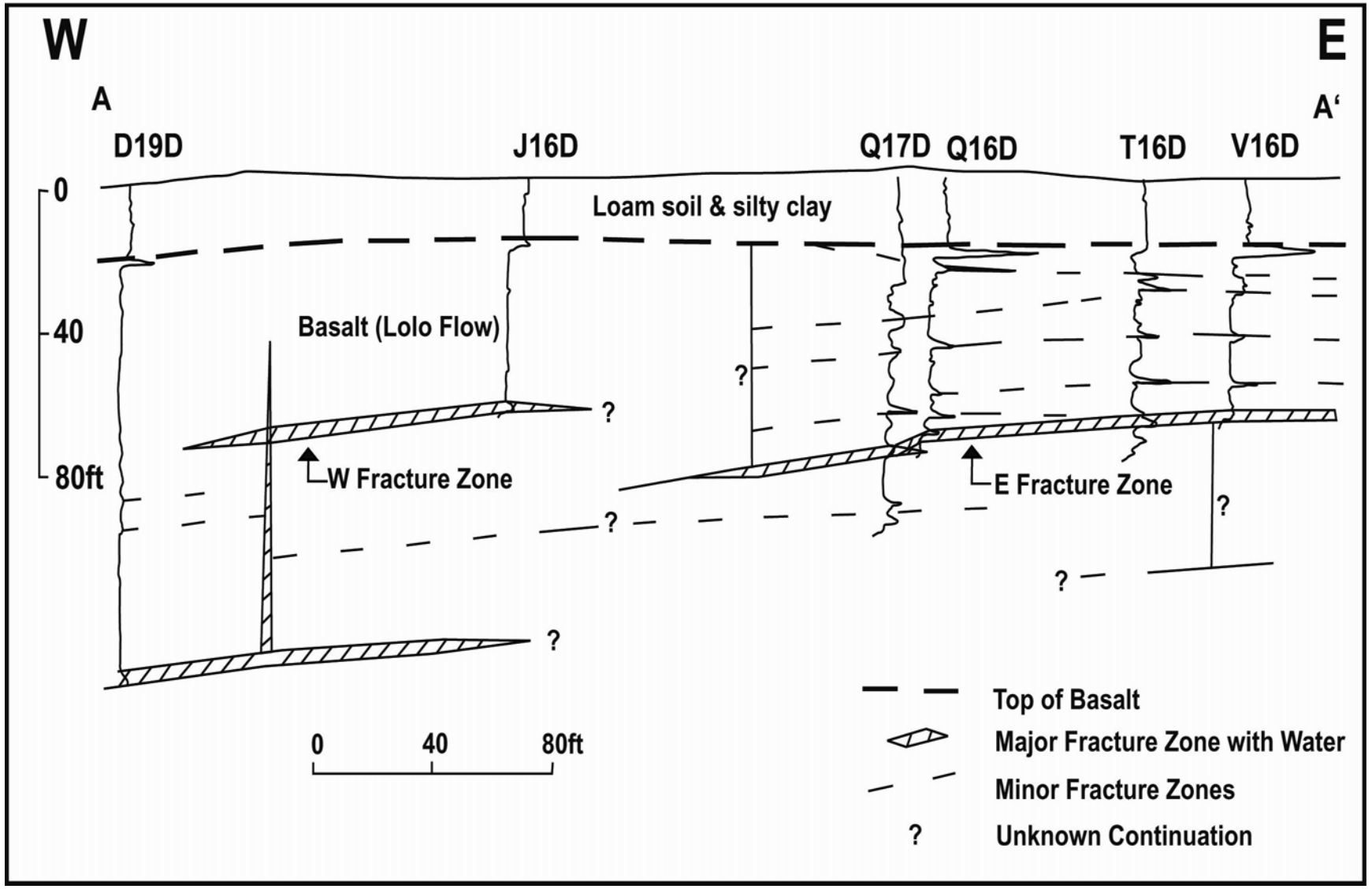

Figure C3: Cross-section A – A' with caliper logs along the north side of the UIGRS (modified from Li, 1991)

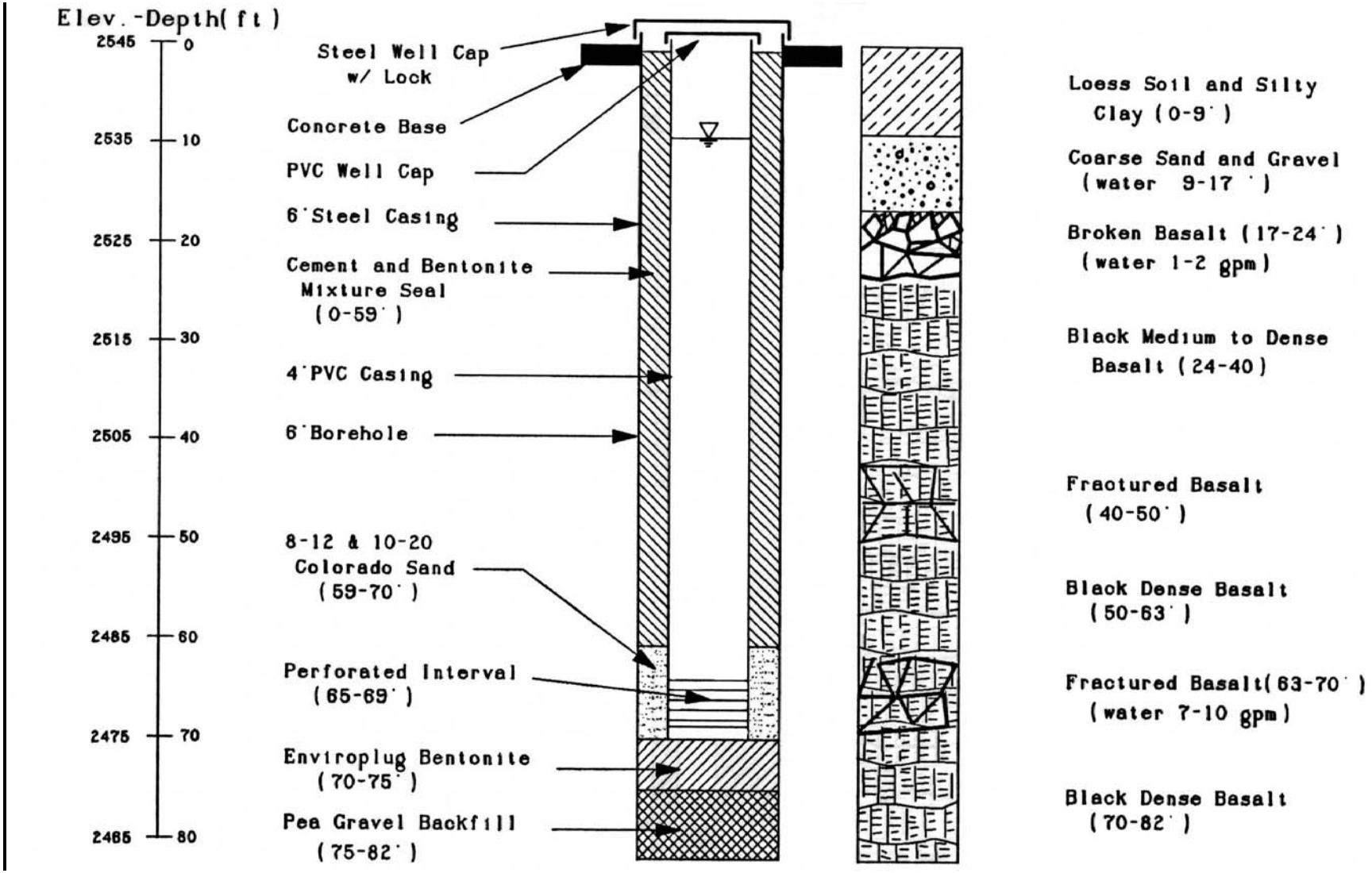

Figure C4: Construction and geology logs of well T16D (modified from Li, 1991)

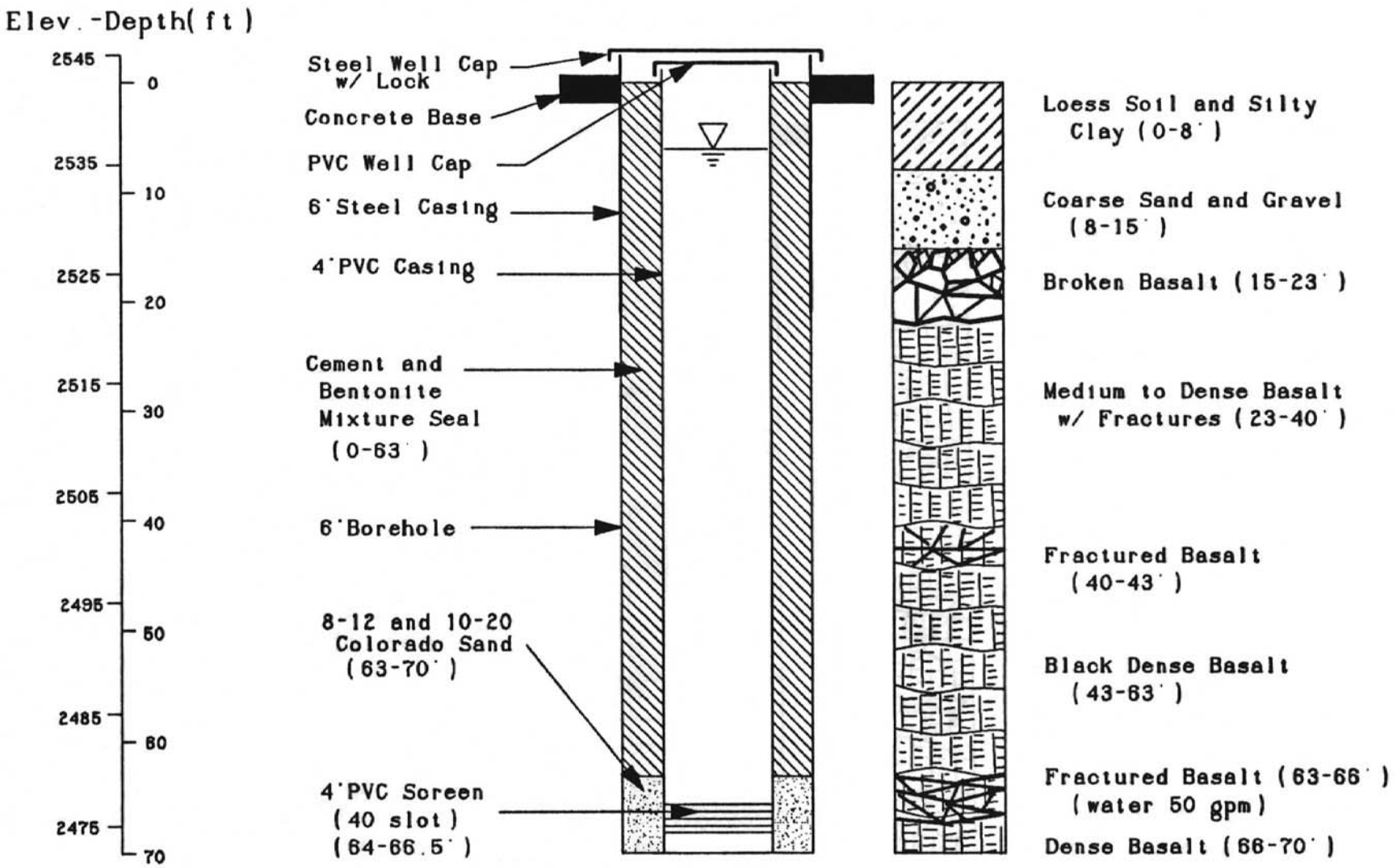

Figure C5: Construction and geology logs of well V16D (modified from Li, 1991)

C.2 Field Materials and Methods

C.2.1 SMZ Prototype Filter Pack

A SMZ prototype filter pack was constructed for use within the pumping well V16D for all tracer tests. Due to a short field season, the coarse cationic SMZ was used for all field tests, even though later laboratory tests showed hydrophobic SMZ would have been a better candidate.

The first SMZ prototype filter pack was designed and constructed by Diane Agnew, from New Mexico Tech in Socorro, NM. Due to complications during the first test run, the SMZ prototype filter design was later modified in Pullman, WA. The description below is of the final SMZ prototype filter pack used in the field test at the UIGRS. A schematic diagram is provided in Figure C6.

The SMZ filter is in the shape of a cylinder with dimensions of 104.14 cm (41 inches) long with a 11.43 cm (4.25 inch) radius. The main body of SMZ filter was made of a 25 μm polyester mesh material with two highly permeable nylon end screens. The nylon end screens were made of flexible nylon window screen plus a polyester active wear mesh for reinforcement and durability. The nylon end screens were highly permeable to allow maximum well yield through the SMZ filter. Rings of 7.62 cm (3 inch) PVC were used at the intersection of the nylon screens and 25 μm polyester mesh as a means to secure the fabric by clamping the materials with metal hose clamps. A system of four steal cables (two top and bottom) were attached to 0.79 cm (5/16 inch) bolts intersecting the rings of 7.62 cm (3 inch) PVC in order to support the filter while lowering it into place within well V16D and raising it after use. Through the middle of the SMZ filter, tubing for sampling plus the electrical cords for the peristaltic pump were placed and reinforced.

Before the field test, the SMZ filter was placed empty within the pumping/sampling well V16D and was gradually filled and packed with coarse cationic SMZ. Gradually filling the filter allowed for settling and created a snug fit in the 4 inch well bore in order to force all pumped water through the SMZ filter. Once filled and packed, the filter was closed by a draw string and sealed shut with duct tape to eliminate loss of SMZ material by high pumping rates. The filter was lowered by four steal cables to the water table and after saturation with water fell to the determined depth. After the four steal cables were secured to the well casing the water pump was replaced above the SMZ filter for the start of the field tests.

C.2.2 Tracers

Bromide

The conservative tracer, 1.24 M potassium bromide (KBr), was run at the UIGRS before the particle tracer test. Breakthrough curves of potassium bromide (KBr) tracer were used to estimate arrival times for three fluorescent polystyrene microsphere slugs (10, 6.0-7.9, and 1 μm). At time zero, 4 liters of 1.24 M potassium bromide tracer was injected into well T16D at a depth of 20.9 meters (68.6 feet) in the East fracture zone which was within the well's screened interval of 19.8-21.0 meters (65-69 feet). To ensure the entire amount of tracer was released into the fracture, 2 liters of DI water were added to flush the tank and tubing. A short time interval was chosen for the beginning of the field experiment and the time interval gradually increased for one hour of time. Samples were analyzed the same day for bromide concentration (ppm) using an Orion Model 290A meter with a 9635BN Combination Bromide Electrode. Data was used to construct a breakthrough curve for a more appropriate sampling time schedule for the following three fluorescent polystyrene microsphere slugs.

The following procedure was used:

(1) Calibrate electrode with two Br^- standard solutions (1 ppm, 1000 ppm)

(2) Dilute 15 mL samples to 25 mL with DI water

(3) Add 0.5 mL of ionic strength adjuster to each diluted 25 mL sample

(4) Test samples with electrode and record concentrations (ppm)

(5) Plot Br^- concentrations versus time, for future sampling time schedule

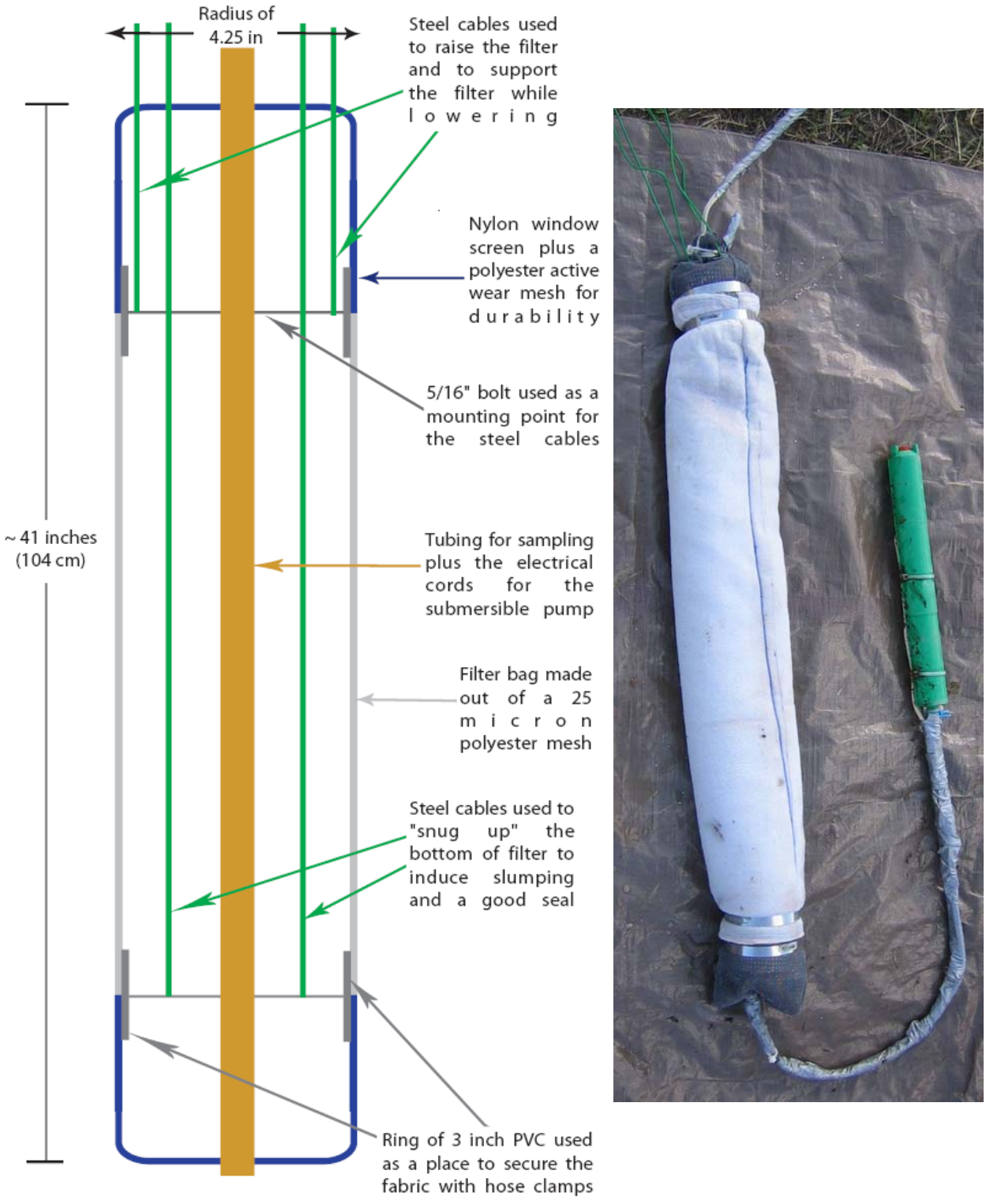

Figure C6: SMZ prototype filter (a) schematic design (Agnew, 2006); (b) photograph of SMZ filter

Fluorescent Polystyrene Particles (Microspheres)

Fluorescent polystyrene microspheres were used at the UIGRS as analogs for *G. intestinales* cysts. Microspheres have been used as analogs for groundwater microorganisms since the 1980s (Harvey and Harms, 2002). Protozoa-sized microspheres (2 to 15 μm) have been injected and recovered in many groundwater systems including a sandy aquifer at Cape Cod (Harvey et al., 1995), a heterogeneous granular aquifer in Idaho (Petrich et al., 1998), and a subsurface sediment and basalt in Idaho (Colwell et al., 1992).

Two different sized microspheres were purchased to best represent the possible size range of *Giardia* cysts (6.0-7.9 and 10μm). In addition, 1μm microspheres were included in the field experiment. The 10μm and 1μm fluorescent polystyrene microspheres were purchased from Polysciences, Inc (Warrington, PA) and the 6.0 -7.9 μm fluorescent polystyrene microspheres from Gerlinde Kisker (Steinfurt, Germany).

The Fluoresbrite® Yellow-Green Microspheres (10.0 μm) were internally dyed with a Yellow-Green color with excitation and emission wavelengths of 441 nm and 486 nm. The 2 mL of microspheres were delivered in a 2.65% aqueous suspension, with required storage at 4^{o}C and protection from light. The average diameter of the microspheres was 9.98μm with a standard deviation of 0.812 μm. The concentration provided by Polysciences, Inc. for the 10 μm Yellow-Green microspheres was 4.55 x 10^{7} particles per mL.

The Fluorescent polystyrene microspheres (6.0-7.9 μm) were internally dyed with a Nile Red color with excitation and emission wavelengths of 520 nm and 560 nm. The 2 mL of microspheres were delivered in a storage solution of DI water with 0.02% Sodium Azide, with required storage at 4^{o}C and protection from light. The concentration provided by Gerlinde Kisker

for the 6.0 -7.9 µm Nile Red microspheres was 1% w/v = 10 mg/ ml. The 6.0 -7.9 µm Nile Red microspheres were the same used in all laboratory experiments.

The Fluoresbrite® Polychromatic Red Microspheres (1.0 µm) were internally dyed with a Red color. They showed up as vivid orange under UV, bright red under a 475-490 nm filter, or yellow under a 545-610 nm filter. The 5 mL of microspheres were delivered in a 2.6% aqueous suspension, with required storage at 4^{o}C and protection from light. The average diameter of the microspheres was 0.923 µm with a standard deviation of 0.025 µm. The concentration provided by Polysciences, Inc. for the 1 µm Red microspheres was 4.55 x 10^{10} particles per mL.

Analysis was conducted by filtering each field sample through a black nucleopore filter, preparing a slide and counting the microspheres retained on the filter using a microscope with fluorescence capability (Figure C7 and C8). Polycarbonate, black membrane filters (0.4 µm pore size, 25 mm diameter) from Sterlitech (Kent, WA) were used for ease of locating fluorescent microspheres. Analysis provided a total concentration of microspheres per mL. A more detailed explanation of analysis and procedures are provided in the following Appendix D.

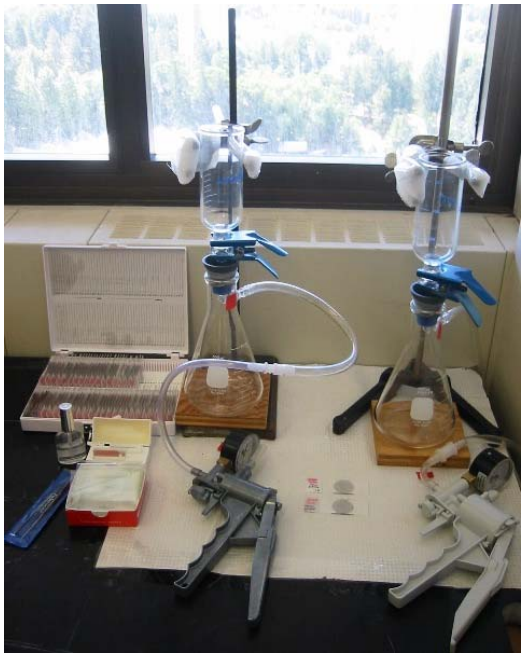

Figure C7: Filtration and enumeration set up for field samples

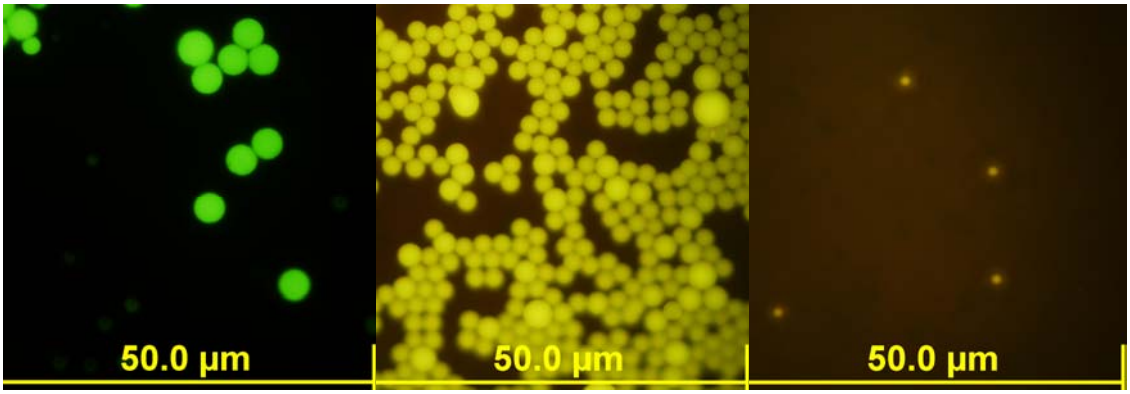

Figure C8: Microsphere slugs under UV light microscope at 40X (a) 10 µm Yellow-Green, (b) 6.0-7.9 µm Nile Red, (c) 1 µm Red

Escherichia coli (E. coli)

Bacteria are the most often used microbial groundwater tracer (Gerba, 1984; Harvey, 1997; Keswick et al., 1982), and the most commonly used bacteria are *Escherichia coli (E. coli)* (Wood and Enrlich, 1978). Advantages of using *E. coli* are that they are easy to grow and detect (Keswick et al., 1982). In the East fracture zone aquifer, Li (1991) and Pardo (1993) found a reliable hydraulic connection between the shallow aquifer and the neighboring Paradise Creek. Background counts of Paradise Creek showed a large source of *E. coli* present in the creek. Due to the known hydraulic connection and abundant *E. coli* bacteria, samples were taken before and after the SMZ filter to test the effectiveness of the SMZ with present *E. coli* bacteria. Samples were analyzed for total coliforms and *E. coli* by a standard fluorescent dye incubation procedure. A more detailed explanation of analysis and procedures are provided in Appendix E. Figure C2 shows the location of where all background creek samples were collected.

C.2.3 Field Instrumentation

Several field instruments were used during the tracer experiments including a water pump, water level measurement devices, and sampling equipment. Below are the details of the required field equipment at the UIGRS.

A Jacuzzi Sand Hander 9.525 cm (3.75 in) submersible water pump (Little Rock, AR) was used in the pumping/sampling well V16D for all experiments. The pump attached to 3.048 m sections of 3.175 cm inner diameter (ID) steel water pipes (10 foot sections of 1.25 ID). The water pump had a maximum discharge of 2.21 liters per second (35 gpm) which was controlled by an adjustable value at the top of the well casing. The pump was raised and lowered by a hand cranked winch and powered by a Honda EZ 3500 gas generator (AC 120/240 volts). The pump was set at 13.1 meters (43 ft =3 ft pump plus 4 sections of 10 ft pipe) below the top of the well casing and discharge was held constant at 0.92 liters per second (13 gpm) by the adjustable value at the top of the well casing.

A 12 volt peristaltic pump was used to sample below the SMZ filter and water pump. The sampling pump was submerged about 5.2 meters (17 feet) below the bottom of the water pump and SMZ filter, at roughly 18.3 meters (60 feet) below the top of the well casing. The sample pump was connected to 0.3175 cm (1/8 in) ID tubing and an electrical power cord that ran through the middle of the SMZ filter and up to ground surface where samples were collected. The pump had a maximum discharge rate of 0.189 liters per second (3 gpm).

All water levels were measured by hand using a Solinst Water Level Meter or electric sounder (e-tape). Water levels before and during the field tests were taken to ensure proper settings of the experiment's parameters.

All tracers were injected into well T16D through a 7.6 liter (2 gal) tank connected to 1.5875 cm (5/8 in) ID tubing. The inlet tubing attached to the tank was place roughly 1.2 meters (4 feet) directly over the well T16D with the outlet in the middle of the screened interval of the well at 20.4 meters (67 feet). The injection tubing was weighted at the outlet to limit bunching during injection. To ensure the entire amount of each tracer was released, 2 liters of DI water were added to flush the tank and tubing.

APPENDIX D:

Enumeration of microsphere slugs in field site samples

(10 µm Yellow-Green, 6.0-7.9 µm Nile Red, 1 µm Red)

1) **To assemble vacuum pump:**

The filtration set-up was assembled by connecting one end of a plastic hose to a hand pump and then the other end to a filtration flask. A glass frit (#5) was gently placed in the rubber stopper (#6) and the stopper placed into the top of the filtration flask. A black nano filter was placed in-between the glass frit (#5) and funnel (#1), detailed instructions are below. The glass funnel (#1) was finally attached to the frit (#5) with a metal clamp (Figure D1).

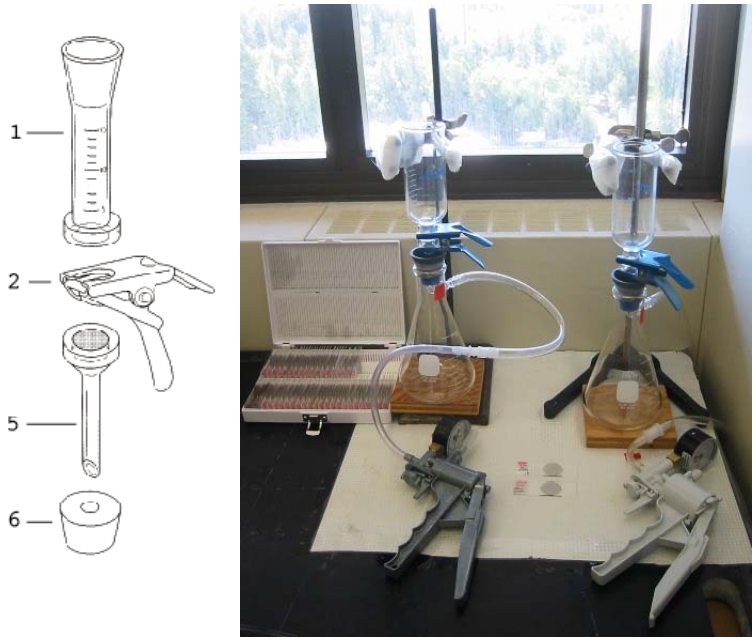

Figure D1: Schematic of filtration set-up

2) **Field Sample Filtration by a vacuum pump filter flask:**

Each field sample was filtered using the vacuum pump filter flask set up (Figure D1). The following steps were followed for each field water sample. First, the glass funnel (#1) was removed and a black nano filter was placed on the glass frit (#5). Forcipes were used to handle the filter to avoid contamination of the filter by hand oils or dirt, and each black filter was wetted with DI water and placed on the glass frit (#5), shiny side up. Once the filter was in place, the glass funnel (#1) was replaced and secured with metal clamps (#2) before filtration of each sample.

Each water sample was vortexed for one full minute to re-suspend particles within the sample. The entire sample (~125 mL) was filtered by hand vacuum and to ensure the entire sample was filtered, the sides of the funnel were washed with DI water. Once filtration was completed, the filtered water sample was rebottled and saved for later bromide concentration determinations. The black filter was removed from the glass frit (#5) with forceps and dried by gently shaking the filter back and forth before being placed on a glass slide with the shiny side up. Once on the slide, a drop of Type A immersion oil was placed onto the filter and a cover slide was placed on top of the filter and oil. The cover slide was sealed on both sides with clear nail polish, and the slide was labeled. Each slide was labeled with a Sample Name (Location, Time), amount filtered (~125 mL), date filtered, and the initials of the person who filtered the sample (CR) (Figure D2).

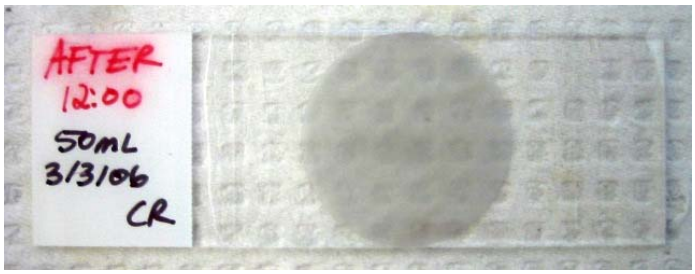

Figure D2: Image of finished slide after filtration

All slides were stored in a cool dry location until use. To prepare for the next filtration, the funnel was liberally washed with Nano Pure water to ensure no cross contamination of field samples. In addition, one vacuum pump filter flask was set up for before filter samples and one for after filter samples. This was to limit any cross contamination of samples before or after the SMZ filter.

3) <u>Field sample enumeration by a Fluorescent Microscope:</u>

A fluorescent microscope was used for enumeration, and images were captured using a cooled CCD camera and accompanying software. All pictures were taken within a 50 µm box with a scale bar in the lower bottom (Figure D3).

50 µm grid = (0.05 mm) ^{2} = 0.0025 mm^2
50 µm grid = 7068 gpf

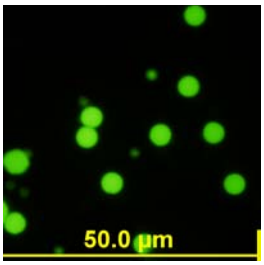

Figure D3: 50 µm grid used for all field samples

In order to locate fluorescent particles, plain light was used to focus on the slide before use of fluorescent light. Two phases were used to help enumerate fluorescent microspheres, phase 1 (10X) and phase 2 (40X). Due to low microsphere counts, the entire filter view area was counted (Figure D4). Pictures were taken of every particle on the filter that might have been a possible microsphere, and counts were accessed based on the captured images.

Inner diameter of funnel = 1.6 cm
Area of Circle = π r^2 = 2.01 cm^2 = 20.01 mm^2

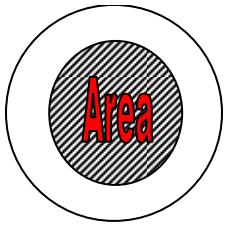

Figure D4: Enumerated black filter area

APPENDIX E:

Enumeration of Coliforms and *E. coli* with IDEXX Colilert reagent with Quanti-Trays in field site samples

IDEXX Colilert reagent is used around the world for the detection of coliforms and *E. coli* in water sources. Colilert is US EPA approved and is included in Standard Methods for Examination of Water and Wastewater. The IDEXX Quanti-Trays provide easy, rapid and accurate counts of coliforms, *E. coli* and enterococci. The IDEXX Quanti-Trays are semi-automated quantification methods based on the Standard Methods Most Probable Number (MPN) model. The Quanti-Tray® Sealer automatically distributes the sample/reagent mixture into separate wells. After incubation, the number of positive wells is converted to an MPN using Table E2 & E3 provided. IDEXX provides two types of trays, Quanti-Tray® which provides counts from one to 200/100 mL and Quanti-Tray/2000® which counts from one to 2,419/100 mL. (IDEXX, 2006)

IDEXX Colilert reagent with Quanti-Trays Details

Water Sample size: 100 mL
Time to results: 1 minute prep time, 24 hours incubation time
Read: Sample turns yellow after 24 hours of incubation when coliforms are present and fluoresces under a black light with the presence of *E. coli*
Trays: IDEXX Quanti-Tray®, Quanti-Tray®/2000

Quanti-Tray®
Sterile, disposable, 51-well trays designed for bacterial enumeration using Colilert®. Quanti-Tray has a maximum counting range of 200 per 100 mL without dilution. The Most Probable Number (MPN) table is provided below (Table E2).

Quanti-Tray/2000®
Sterile, disposable, 97-well trays designed for bacterial enumeration using Colilert®. Quanti-Tray/2000 has a maximum counting range of 2,419 per 100 mL without dilution. The MPN table is provided below (Table E3).

Table E1: Quantification Instructions

Step 1.
Add reagent to 100 mL sample and shake until all reagent dissolves into mixture.

Step 2.
Open the Quanti-Tray® (counts from 1-200) or Quanti-Tray®/2000 (counts from 1-2,419) by pulling the foil tab away from the well side. Avoid touching the inside of the foil or the tray.

Pour the reagent/sample mixture directly into Quanti-Tray® or Quanti-Tray®/2000. Allow foam to settle.

Step 3.
Place the sample-filled Quanti-Tray onto the rubber tray carrier of the Quanti-Tray Sealer with the well side (plastic) of the Quanti-Tray facing down to fit into the carrier.

Seal in Quanti-Tray® Sealer and place in incubator for 24 hours.

- **Step 4.**

Quanti-Tray-read results:
- Yellow wells = total coliforms
- Yellow/fluorescent wells = *E. coli*

Use the Most Probable Number (MPN) **Table E2** below.

Quanti-Tray/2000-read results:
- Yellow wells = total coliforms
- Yellow/fluorescent wells = *E. coli*

Use the Most Probable Number (MPN) **Table E3** below.

IDEXX, Inc. (2006) http://www.idexx.com/water/quantitray/

IDEXX
51-Well Quanti-Tray®
MPN Table

No. of wells giving positive reaction	MPN per 100 ml sample	95% Confidence Limits Lower	Upper
0	<1.0	0.0	3.7
1	1.0	0.3	5.6
2	2.0	0.6	7.3
3	3.1	1.1	9.0
4	4.2	1.7	10.7
5	5.3	2.3	12.3
6	6.4	3.0	13.9
7	7.5	3.7	15.5
8	8.7	4.5	17.1
9	9.9	5.3	18.8
10	11.1	6.1	20.5
11	12.4	7.0	22.1
12	13.7	7.9	23.9
13	15.0	8.8	25.7
14	16.4	9.8	27.5
15	17.8	10.8	29.4
16	19.2	11.9	31.3
17	20.7	13.0	33.3
18	22.2	14.1	35.2
19	23.8	15.3	37.3
20	25.4	16.5	39.4
21	27.1	17.7	41.6
22	28.8	19.0	43.9
23	30.6	20.4	46.3
24	32.4	21.8	48.7
25	34.4	23.3	51.2
26	36.4	24.7	53.9
27	38.4	26.4	56.6
28	40.6	28.0	59.5
29	42.9	29.7	62.5
30	45.3	31.5	65.6
31	47.8	33.4	69.0
32	50.4	35.4	72.5
33	53.1	37.5	76.2
34	56.0	39.7	80.1
35	59.1	42.0	84.4
36	62.4	44.6	88.8
37	65.9	47.2	93.7
38	69.7	50.0	99.0
39	73.8	53.1	104.8
40	78.2	56.4	111.2
41	83.1	59.9	118.3
42	88.5	63.9	126.2
43	94.5	68.2	135.4
44	101.3	73.1	146.0
45	109.1	78.6	158.7
46	118.4	85.0	174.5
47	129.8	92.7	195.0
48	144.5	102.3	224.1
49	165.2	115.2	272.2
50	200.5	135.8	387.6
51	> 200.5	146.1	infinite

IDEXX Sales and Technical Support
1-800-321-0207 or 1-207-856-0496
www.idexx.com/water

Table E2: Most Probable Number (MPN) table for Quanti-Tray®

IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

# Large Wells Positive	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.6	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6	435.2

09-63235-01

Table E3: Part 1: Most Probable Number (MPN) table for Quanti-Tray/2000®

IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

# Large Wells Positive	# Small Wells Positive																							
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.6	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.6	>2419.6

09-63235-01

Table E3: Part 2: Most Probable Number (MPN) table for Quanti-Tray/2000®

APPENDIX F:

UIGRS Field Test Data

Table F1: Data for the Conservative Bromide Tracer Test on October 1st, 2005

Time (minutes)	Bromide Concentration (ppm) (No SMZ Filter)
0.25	1.10E-02
0.5	8.80E-03
0.75	6.70E-03
1	6.43E-03
1.5	5.83E-03
2	5.70E-03
2.5	5.37E-03
3	5.27E-03
3.5	5.07E-03
4	5.33E-03
4.5	5.27E-03
5	5.17E-03
5.5	5.03E-03
6	4.93E-03
6.5	4.83E-03
7	5.40E-03
7.5	4.80E-03
8	4.90E-03
8.5	4.90E-03
9	4.97E-03
9.5	6.70E-03
10	6.03E-03
11	5.53E-03
12	5.43E-03
13	5.07E-03
14	5.40E-03
15	5.10E-03
16	5.33E-03
17	5.20E-03
18	6.93E-03
19	6.00E-03
20	5.57E-03
22	5.33E-03
24	5.93E-03
26	6.10E-03
28	8.77E-03
30	1.47E-02
32	2.79E-02
34	5.57E-02
36	9.77E-02
38	1.63E-01
40	2.40E-01
43	3.71E-01

46	4.84E-01
49	6.39E-01
52	7.73E-01
55	8.85E-01
58	9.98E-01
61	1.06E+00

Table F2: Data for the Conservative Bromide Tracer Test on October 7th, 2005

Before SMZ Filter		
Time	**Time (hr:min)**	**Bromide Conc. (ppm)**
8:00 AM	0-A	2.01E-02
8:05 AM	0-B	1.28E-02
9:00 AM	**Injection 1 (Br + 10 um)**	
9:05 AM	0:05	1.17E-02
9:10 AM	0:10	1.17E-02
9:15 AM	0:15	2.93E-02
9:20 AM	0:20	3.02E-01
9:25 AM	0:25	2.16E+00
9:30 AM	0:30	4.82E+00
9:35 AM	0:35	7.72E+00
9:40 AM	0:40	1.07E+01
9:45 AM	0:45	1.12E+01
9:50 AM	0:50	1.05E+01
9:55 AM	0:55	1.05E+01
10:00 AM	**Injection 2 (~8 um)**	
10:00 AM	1:00	1.00E+01
10:05 AM	1:05	9.66E+00
10:10 AM	1:10	8.93E+00
10:15 AM	1:15	9.07E+00
10:20 AM	1:20	7.87E+00
10:25 AM	1:25	7.34E+00
10:30 AM	1:30	7.03E+00
10:35 AM	1:35	6.14E+00
10:40 AM	1:40	5.87E+00
10:45 AM	1:45	4.78E+00
10:50 AM	1:50	5.92E+00
10:55 AM	1:55	4.90E+00
11:00 AM	**Injection 3 (1 um)**	
11:00 AM	2:00	4.38E+00
11:05 AM	2:05	3.35E+00
11:10 AM	2:10	2.71E+00
11:15 AM	2:15	2.13E+00
11:20 AM	2:20	1.73E+00
11:25 AM	2:25	1.54E+00
11:30 AM	2:30	1.24E+00
11:35 AM	2:35	1.06E+00
11:40 AM	2:40	9.37E-01
11:45 AM	2:45	8.38E-01
11:50 AM	2:50	6.33E-01
11:55 AM	2:55	5.41E-01

After SMZ Filter		
Time	**Time (hr:min)**	**Bromide Conc. (ppm)**
8:00 AM	0-A	7.40E-03
8:05 AM	0-B	8.50E-03
9:00 AM	**Injection 1 (Br + 10 um)**	
9:05 AM	0:05	8.20E-03
9:10 AM	0:10	8.93E-03
9:15 AM	0:15	9.63E-03
9:20 AM	0:20	1.37E-01
9:25 AM	0:25	1.39E+00
9:30 AM	0:30	4.64E+00
9:35 AM	0:35	7.09E+00
9:40 AM	0:40	1.11E+01
9:45 AM	0:45	1.20E+01
9:50 AM	0:50	1.10E+01
9:55 AM	0:55	9.30E+00
10:00 AM	**Injection 2 (~8 um)**	
10:00 AM	1:00	8.73E+00
10:05 AM	1:05	8.82E+00
10:10 AM	1:10	9.24E+00
10:15 AM	1:15	8.07E+00
10:20 AM	1:20	8.09E+00
10:25 AM	1:25	6.69E+00
10:30 AM	1:30	6.69E+00
10:35 AM	1:35	5.46E+00
10:40 AM	1:40	5.15E+00
10:45 AM	1:45	4.08E+00
10:50 AM	1:50	3.96E+00
10:55 AM	1:55	3.15E+00
11:00 AM	**Injection 3 (1 um)**	
11:00 AM	2:00	2.96E+00
11:05 AM	2:05	2.27E+00
11:10 AM	2:10	2.09E+00
11:15 AM	2:15	1.61E+00
11:20 AM	2:20	1.45E+00
11:25 AM	2:25	1.15E+00
11:30 AM	2:30	1.04E+00
11:35 AM	2:35	7.97E-01
11:40 AM	2:40	7.50E-01
11:45 AM	2:45	6.01E-01
11:50 AM	2:50	5.73E-01
11:55 AM	2:55	4.57E-01

12:00 PM	3:00	4.81E-01
12:05 PM	3:05	4.64E-01
12:10 PM	3:10	3.78E-01
12:15 PM	3:15	3.65E-01
12:20 PM	3:20	3.30E-01
12:25 PM	3:25	3.04E-01
12:30 PM	3:30	2.75E-01

12:00 PM	3:00	4.32E-01
12:05 PM	3:05	3.77E-01
12:10 PM	3:10	3.65E-01
12:15 PM	3:15	3.20E-01
12:20 PM	3:20	3.16E-01
12:25 PM	3:25	2.52E-01
12:30 PM	3:30	2.56E-01

Table F3: Data for the Three Microsphere Tracers on October 7th, 2005

Due to difficulty with quantification on a black filter with a fluorescent microscope, the one µm microspheres (Slug 3) were not counted. For the October 7th, 2005 field test, a bromide tracer, 10 and 6.0-7.9 µm microspheres, background total coliforms and *E. coli* were enumerated for the field test.

Before SMZ Filter (microspheres/125 mL)		
Time (hr:min)	**Slug 1 10µm Yellow/Green**	**Slug 2 6-7.9 µm Nile Red**
0-A	0	
0-B	0	
9:00 AM	**Injection 1(10 um)**	
0:05	0	
0:10	0	
0:15	0	
0:20	2	
0:25	1	
0:30	2	
0:35	0	
0:40	1	
0:45	1	
0:50	1	
0:55	0	
10:00 AM	**Injection 2 (~8 um)**	
1:00	2	0
1:05	2	0
1:10	0	0
1:15	3	0
1:20	0	0
1:25	0	0
1:30	0	0
1:35	1	1
1:40	0	0
1:45	0	0
1:50	0	0
1:55	0	0
11:00 AM	**Injection 3 (1 um)**	
2:00	0	0
2:05	0	0
2:10	1	0
2:15	0	0
2:20	0	0
2:25	0	1

After SMZ Filter (microspheres/125 mL)		
Time (hr:min)	**Slug 1 10µm Yellow/Green**	**Slug 2 6-7.9 µm Nile Red**
0-A	0	
0-B	0	
9:00 AM	**Injection 1 (10 um)**	
0:00	12	
0:01	0	
0:05	8	
0:10	0	
0:15	7	
0:20	0	
0:25	11	
0:30	0	
0:35	5	
0:40	0	
0:45	6	
0:50	0	
0:55	2	
10:00 AM	**Injection 2 (~8 um)**	
1:00	3	0
1:05	1	0
1:10	1	0
1:15	2	0
1:20	0	0
1:25	2	0
1:30	0	0
1:35	0	0
1:40	1	1
1:45	1	1
1:50	0	1
1:55	3	0
11:00 AM	**Injection 3 (1 um)**	
2:00	1	0
2:05	0	0
2:10	0	0
2:15	1	0

2:30	0	0
2:35	0	0
2:40	0	0
2:45	0	0
2:50	0	0
2:55	0	1
3:00	0	0
3:05	0	0
3:10	0	0
3:15	0	1
3:20	0	1
3:25	0	0
3:30	0	1
3:35	0	0
3:40	0	0
3:45	0	0
3:50	0	0
3:55	0	1
4:00	0	0
4:10	0	0
4:20	0	0
4:30	0	0
4:40	0	0
4:50	0	0
5:00	0	0
5:10	0	0
5:20	0	0
5:30	0	0
5:40	0	0
5:50	0	0
6:00	0	1
6:15	0	0
6:30	1	0
6:45	0	0
7:00	0	0
7:20	0	0
7:40	0	0
8:00	0	0
8:20	0	0
8:40	0	0
9:00	0	0
9:30	0	0
10:00	0	0
10:30	0	0
11:00	0	0
11:30	0	0
12:00	0	0
13:00	0	0
14:00	0	0

2:20	1	0
2:25	0	0
2:30	0	0
2:35	0	0
2:40	1	0
2:45	2	0
2:50	0	0
2:55	0	0
3:00	0	0
3:05	13	0
3:10	1	0
3:15	2	1
3:20	0	3
3:25	1	0
3:30	0	0
3:35	1	0
3:40	1	0
3:45	1	0
3:50	0	0
3:55	1	0
4:00	1	0
4:10	0	0
4:20	0	0
4:30	0	0
4:40	0	1
4:50	0	0
5:00	0	1
5:10	0	0
5:20	0	0
5:30	0	0
5:40	0	0
5:50	0	0
6:00	0	0
6:15	1	0
6:30	0	0
6:45	0	0
7:00	0	0
7:20	0	0
7:40	0	0
8:00	0	0
8:20	0	0
9:00	0	1
9:30	0	1
10:00	0	0
10:30	4	2
11:00	1	0
11:30	1	1
12:00	0	0
13:00	1	0
14:00	0	0

Table F4: Data for the Total Coliforms and *E. coli* of Paradise Creek

Running Time (hr:min)	Total Coliform (Yellow)		*E. Coli* (Fluorescent)		Total Coliforms (MPN #)	*E. Coli* (MPN #)
	# Large Wells	# Small Wells	# Large Wells	# Small Wells		
Practice 1	51	--	51	--	200.5	200.5
Practice 2	51	--	51	--	200.5	200.5
0:00:00	49	48	17	2	2419.6	22.8
1:00:00	49	48	26	4	2419.6	41.4
1:00 Double	49	48	25	2	2419.6	36.4
2:00:00	49	48	18	3	2419.6	25.6
2:00 Double	49	48	39	4	2419.6	78.9
3:00:00	49	48	49	48	2419.6	2419.6
4:00:00	49	48	49	48	2419.6	2419.6
5:00:00	49	48	49	45	2419.6	1732.9
5:30:00	49	48	49	43	2419.6	1413.6
6:00:00	49	48	41	10	2419.6	104.3
6:30:00	49	48	44	11	2419.6	129.6
6:30 Double	49	48	41	10	2419.6	104.3
7:00:00	49	48	42	8	2419.6	104.6
7:00 Double	49	48	26	11	2419.6	52.0

Table F5: Before SMZ filter data of Total Coliforms and *E. coli*

Before SMZ Filter				
Running Time (hr:min)	**Total Coliform (Yellow)** # Large Wells	***E. Coli* (Fluorescent)** # Large Wells	**Total Coliform (MPN #)**	***E. Coli* (MPN #)**
0:00:00	0	0	0.0	0.0
0:00 Double	0	0	0.0	0.0
0:10:00	0	0	0.0	0.0
0:20:00	0	0	0.0	0.0
0:30:00	4	0	4.2	0.0
0:40:00	3	0	3.1	0.0
0:50:00	0	0	0.0	0.0
1:00:00	1	0	1.0	0.0
1:00 Double	0	0	0.0	0.0
1:10:00	0	0	0.0	0.0
1:20:00	0	0	0.0	0.0
1:30:00	1	0	1.0	0.0
1:40:00	0	0	0.0	0.0
1:50:00	5	0	5.3	0.0
2:00:00	1	0	1.0	0.0
2:00 Double	0	0	0.0	0.0
2:10:00	0	0	0.0	0.0
2:20:00	1	0	1.0	0.0
2:30:00	1	0	1.0	0.0
2:40:00	1	0	1.0	0.0
2:50:00	0	0	0.0	0.0
3:00:00	1	0	1.0	0.0
3:10:00	0	0	0.0	0.0
3:20:00	0	0	0.0	0.0
3:30:00	1	0	1.0	0.0
3:40:00	0	0	0.0	0.0
3:50:00	0	0	0.0	0.0
4:00:00	0	0	0.0	0.0
4:10:00	1	0	1.0	0.0
4:20:00	0	0	0.0	0.0
4:30:00	0	0	0.0	0.0
4:40:00	0	0	0.0	0.0
4:50:00	0	0	0.0	0.0
5:00:00	0	0	0.0	0.0
5:10:00	0	0	0.0	0.0
5:20:00	0	0	0.0	0.0
5:30:00	0	0	0.0	0.0
5:40:00	1	0	1.0	0.0
6:00:00	1	0	1.0	0.0
6:10:00	0	0	0.0	0.0
6:10 Double	0	0	0.0	0.0

Table F6: After SMZ filter data of Total Coliforms and *E. coli*

After SMZ Filter				
Running Time (hr:min)	Total Coliform (Yellow) # Large Wells	*E. Coli* (Fluorescent) # Large Wells	Total Coliform (MPN #)	*E. Coli* (MPN #)
0:00:00	0	0	0.0	0.0
0:00 Double	0	0	0.0	0.0
0:10:00	1	0	1.0	0.0
0:20:00	0	0	0.0	0.0
0:30:00	2	0	2.0	0.0
0:40:00	1	0	1.0	0.0
0:50:00	1	0	1.0	0.0
1:00:00	0	0	0.0	0.0
1:00 Double	1	0	1.0	0.0
1:10:00	0	0	0.0	0.0
1:20:00	0	0	0.0	0.0
1:30:00	0	0	0.0	0.0
1:40:00	0	0	0.0	0.0
1:50:00	0	0	0.0	0.0
2:00:00	0	0	0.0	0.0
2:00 Double	1	0	1.0	0.0
2:10:00	0	0	0.0	0.0
2:20:00	0	0	0.0	0.0
2:30:00	3	0	3.1	0.0
2:40:00	1	0	1.0	0.0
2:50:00	1	0	1.0	0.0
3:00:00	0	0	0.0	0.0
3:10:00	0	0	0.0	0.0
3:20:00	1	0	1.0	0.0
3:30:00	0	0	0.0	0.0
3:40:00	0	0	0.0	0.0
3:50:00	0	0	0.0	0.0
4:00:00	0	0	0.0	0.0
4:10:00	0	0	0.0	0.0
4:20:00	0	0	0.0	0.0
4:30:00	0	0	0.0	0.0
4:40:00	0	0	0.0	0.0
4:50:00	0	0	0.0	0.0
5:00:00	0	0	0.0	0.0
5:10:00	0	0	0.0	0.0
5:20:00	0	0	0.0	0.0
5:30:00	1	0	1.0	0.0
5:40:00	0	0	0.0	0.0
6:00:00	0	0	0.0	0.0
6:10:00	0	0	0.0	0.0
6:10 Double	0	0	0.0	0.0