

# Simultaneous Water Quality Monitoring and Fecal Pollution Source Tracking in the Colorado Big Thompson Water Project

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## ABSTRACT

Inadequate drinking water and sanitation are considered two of the world's major causes of preventable morbidity and mortality, and international bodies such as the World Health Organization (WHO) estimate that 50,000 deaths per day are due to water-related diseases. While the majority of waterborne illness occurs in the developing world, water quality problems also abound in the developed world. The detection, isolation, and identification of waterborne pathogens continue to be expensive, difficult, and labor intensive. To alleviate the issues with waterborne pathogen testing, indicator microorganisms are commonly used to determine the relative risk from the possible presence of pathogenic microorganisms in a sample. Historically, the coliform, thermotolerant coliform group, enterococci, and *Clostridium perfringens* have been the bacterial indicators used to detect fecal contamination based on the rationale that these indicator organisms are indigenous to feces, and their presence in the environment is therefore indicative of fecal pollution. However, there are major problems with the current use of indicator bacteria to detect fecal pollution. Many of these bacteria are routinely isolated from soil and water environments that have not been impacted by fecal pollution. Also, while the persistence of these bacteria in water distribution systems is comparable to that of some bacterial pathogens, the relationship between bacterial indicators and the presence of enteric (intestinal) viruses and protozoa is poor. Due to the limitations of these bacterial indicators and problems with their rapid detection, there remains an acute need to develop water quality test procedures to identify fecal contamination quickly and to simultaneously determine the source of that pollution so that corrective actions could be initiated. Such methods should also be capable of assessing water quality in large-scale water transfer projects. The overall goal of this project, therefore, is to develop integrated detection methods that will simultaneously determine the presence of fecal pollution and the source of that pollution within six hours. The methods will be based upon rapid identification of F-specific RNA bacteriophages (FRNA phages, or phages), followed by characterization of the phages to determine their source (which allows for an indication of the source of fecal pollution). FRNA phages have attracted interest as useful alternatives to bacterial indicators because their morphology and survival characteristics closely resemble the human enteric gastrointestinal viruses, and they have been used to reliably detect the presence and source of fecal pollution in water.

**Keywords:** Drinking water, sanitation, fecal contamination, indicator microorganisms, FRNA bacteriophages, rapid detection, microbial source tracking



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# Simultaneous Water Quality Monitoring and Fecal Pollution Source Tracking in the Colorado Big Thompson Water Project

## 1 Justification of Work Performed

The overall goal of this project was to develop two integrated detection methods that would simultaneously determine the presence of fecal pollution and also the source of that pollution within six hours. The methods were based on rapid identification of FRNA phages followed by characterizing the phages to determine their source (which allows for an indication of the source of fecal pollution).

The specific aims of the project were to: 1) identify bacterial hosts from human, avian, and livestock fecal waste (the main sources of fecal water pollution) that are better suited than currently-used bacterial hosts to support the growth of FRNA phages isolated from the same waste source; 2) develop two rapid methods to characterize FRNA phages (also allowing for identification of the fecal pollution source) based on an immunological method (using a Lateral Flow Device) and a molecular-based method (Reverse Transcriptase Real Time Polymerase Chain Reaction); and 3) use the newly developed methods to assess water quality in the Colorado-Big Thompson Water Project (CBTWP).

### 1.1 Specific Aim 1.

We did not identify any new bacterial hosts (bacteriophages are viruses that reproduce inside a bacterial host) that were better suited to support the growth of the FRNA phages used in this study. As such, we focused our efforts on two commonly used bacterial strains: *Escherichia coli* (*E. coli*) C3000 and *E. coli* F<sub>amp</sub>. Both strains were able to support the growth of all FRNA phages used in this study. It was decided that *E. coli* F<sub>amp</sub> would be used for all phage propagation and enumeration work, because this strain is resistant to ampicillin and streptomycin (antibiotics) and would therefore be useful when assessing water samples from environmental sources – background bacterial flora from these sources could be decreased by incorporating antibiotics into the growth media.

### 1.2 Specific Aim 2.

A novel method for concentrating FRNA phages from large volumes of water has been developed. The method is based on using anionic exchange resins, which bind the negatively-charged phages in water. This method has been shown to concentrate FRNA phages from each serogroup (phage groups classified by differing antigens, which stimulate immune response) in as much as 40 liters of water. The method has been incorporated with the downstream detection methods described below.

Two rapid methods for detection of FRNA phages were developed. The first method was the development of a lateral flow device (LFD) to detect serogroup I FRNA phages. These LFDs were able to detect the presence of serogroup I FRNA phages in spiked water samples and also FRNA phages that were naturally present in environmental water. Based on these results, we have begun to produce a LFD that will be capable of detecting all four of the serogroups of FRNA phages. Currently, high quality polyclonal antisera (serum containing antigens) from phages representing each serogroup are being commercially produced, and these antisera will be used to construct the LFDs.

The second rapid method was based on the design of serogroup-specific PCR primers for use in a reverse transcriptase, real time polymerase chain reaction assay (RT-RT-PCR). This



assay (an analysis test) has been completed and evaluated using various concentrations of FRNA phages that were spiked into drinking water. The assay has been fully integrated with the anionic resin concentration step described above, and can deliver sensitive results within six hours from sampling to result.

## **2 Review of Methods Used**

### **2.1 Bacterial strains**

Bacterial strains used in this study were *E. coli* F<sub>amp</sub> (ATCC 700891), and *E. coli* C3000, and these strains served as hosts for the FRNA phages. Bacterial strains were maintained at -80°C (-112°F) in 20% glycerol. Prior to all experiments, frozen cultures were streaked onto tryptic soy agar (a culture medium) supplemented with 15µg ml<sup>-1</sup> each of ampicillin and streptomycin and incubated at 37°C (98.6°F) overnight. Overnight cultures were created by inoculating (placing a substance in it that will reproduce) 10 ml of tryptic soy broth supplemented with 15µg ml<sup>-1</sup> each of ampicillin and streptomycin with several isolated colonies from a tryptic soy agar plate and incubated with shaking for 24 hours.

### **2.2 FRNA bacteriophage strains**

FRNA phages MS2 (group I), GA (group II), Qβ (group III) and SP (group IV) were obtained from the American Type Culture Collection (MS2, Qβ) or from Scientific Methods Inc. (Granger, In) (GA, SP) and maintained at -80°C (176°F) in 20% glycerol. Working stocks of FRNA phages were kept in tryptic soy broth at 4°C (39.2°F) and protected from excess light exposure.

### **2.3 FRNA bacteriophage propagation**

Phages MS2 (group I), GA (group II), Qβ (group III), and SP (group IV) were individually propagated in tryptic soy broth that was supplemented with 15µg ml<sup>-1</sup> of ampicillin and streptomycin. Exponential phase cultures (0.2-0.3 O.D.<sub>600nm</sub>) of *E. coli* F<sub>amp</sub> (ATCC 700891) were infected at a multiplicity of infection (MOI) of 0.1 and incubated with shaking for 24 hours. Infected cultures were treated with chloroform at a final concentration of 1%. Cultures were centrifuged at 4°C (39.2°F) in a Beckman Coulter J2-MC centrifuge at 6000 × g for 25 min., and the supernatant (separated part) was decanted into a sterile vessel followed by filtration through a 0.22µm low protein-binding filter and stored at 4°C (39.2°F).

### **2.4 FRNA bacteriophage plaque assay**

Plaque assays to determine phage titer (concentration) were performed prior to all experiments by individually mixing 100µl of an overnight culture of *E. coli* F<sub>amp</sub> and 100µl of serial dilutions (10 fold) of the phages (MS2, Qβ, GA, and SP) in four ml of molten top layer agar (TSB 30 g/L, Bacto-Agar 0.6%), at 55°C (131°F). The mixture was pour plated onto a 100mm × 15mm tryptic soy agar plate and allowed to solidify. The plates were incubated overnight at 37°C (98.6°F).

### **2.5 Capture assays**

Small and large volume capture assays were conducted to evaluate the ability of an anionic exchange resin to concentrate the FRNA phages. For small volume assays, 50 mls of sterile tap water were individually spiked with approximately 10<sup>4</sup> PFU ml<sup>-1</sup> of MS2, Qβ, GA, or SP. One gram of anionic exchange resin Amberlite® IRA 900 was added to each sample, which

was continuously mixed. Aliquots (subsamples) of water were removed from the water samples at 15 minute intervals (starting at time 0) for a period of 120 minutes. The concentration of FRNA phages in the aliquots was determined by plaque assay as described above. In a separate study, 50 mls of tap water was spiked with approximately  $10^0$ ,  $10^1$ ,  $10^2$ , or  $10^3$  PFU  $\text{ml}^{-1}$  of MS2, Q $\beta$ , GA, or SP. One gram of anionic exchange resin Amberlite® IRA 900 was added. Aliquots were obtained at 0 and 60 minutes and assayed for phage by plaque assay. For large volume capture assays, 10 liters of tap water was added to a 20 l sterile carboy (a rigid container) and individually spiked with approximately  $10^0$ ,  $10^1$ ,  $10^2$ , or  $10^3$  PFU  $\text{ml}^{-1}$  of MS2, Q $\beta$ , GA, or SP. One and a half grams of anionic exchange resin Amberlite® IRA 900 was added. Aliquots were obtained at 0 and 60 minutes and assayed for phage by plaque assay.

## 2.6 RNA isolation

FRNA phage RNA isolation was performed using a modified method of the Qiagen easy viral RNA isolation kit protocol. Anionic resin beads (with phages attached) were added to a 15 ml Falcon tube. Approximately 1.2 ml of viral lysis buffer (a compound that resists changes in its acidity) was added to the tube, and after a 10-minute incubation with mixing, an equal volume of ethanol was added. The resin bead-lysis buffer suspension was transferred to a 5 ml syringe that had been modified by attachment of a 200  $\mu\text{l}$  filter pipette tip to the bottom of the syringe. The lysis buffer was separated from the resin beads by placing the syringe into a new 15 ml Falcon tube and pushing the syringe plunger down. Alternatively, the syringe-Falcon tube assembly was capped and centrifuged at  $5,000 \times g$  for 5 minutes to separate the liquid from the resin beads. The lysis buffer entered the Falcon tube while the resin remained in the syringe. The rest of the RNA isolation protocol was accomplished as per manufacturer's instructions. An ethanol precipitation step was performed to increase the concentration of the isolated RNA. Sixty microliters of purified RNA was added to 140  $\mu\text{l}$  of cold ethanol on ice in a 1.7 ml microfuge tube. Twenty microliters of 5M NaOAc (pH 5.4) was added and the mixture was incubated at  $-80^\circ\text{C}$  ( $-112^\circ\text{F}$ ) for 30 minutes. The mixture was then centrifuged at  $16,000 \times g$  at  $4^\circ\text{C}$  ( $39.2^\circ\text{F}$ ) for 30 minutes. The supernatant (separated liquid) was aspirated and the pellet was allowed to dry for 15 minutes at room temperature then re-suspended in 10  $\mu\text{l}$  of sterile deionized water and stored at  $-20^\circ\text{C}$  ( $-4^\circ\text{F}$ ) until use.

## 2.7 Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Identification of FRNA phages MS2, Q $\beta$ , GA, and SP by RT-PCR was performed using primers sets specific for each subgroup (Table 1) and a Qiagen One Step RT-PCR kit (Qiagen). Thermal cycling conditions included a reverse transcriptase step at  $45^\circ\text{C}$  ( $113^\circ\text{F}$ ) for 30 minutes, 1 cycle of  $95^\circ\text{C}$  ( $203^\circ\text{F}$ ) for 15 minutes, followed by 40 cycles of  $95^\circ\text{C}$  ( $203^\circ\text{F}$ ) for 1 minute,  $60^\circ\text{C}$  ( $140^\circ\text{F}$ ) for 30 seconds,  $72^\circ\text{C}$  ( $161.6^\circ\text{F}$ ) for 1 minute, and a final extension step of  $72^\circ\text{C}$  ( $161.6^\circ\text{F}$ ) for 10 minutes. Amplicons were identified by agarose gel analysis on a 1.5% agarose gel run at 100V for 60 minutes, and visualized using sybersafe DNA gel stain and UV light.

Table 1. Primer Sequences used in the reverse transcriptase, real time PCR assays

Primer	Sequence	T <sub>m</sub> °C	Product Size
Subgroup I			
Forward	5'CGTGGTTCCATACTGGAGGT3'	60	
Reverse	5'CTTTCGAGCACACCCACC3'	60	177 bp
Subgroup II			
Forward	5'GGTTCAAGTTGCGGGATG3'	60	
Reverse	5'GAAAACAAACCGTTGCCG3'	60	184 bp
Subgroup III			
Forward	5'CCGCGTGGGGTAAATCC3'	62	
Reverse	5'TTACGATTGCGAGAAGGCTG3'	61	113 bp
Subgroup IV			
Forward	5'CGTGGAAGCATGCCTGT3'	59	
Reverse	5'TTCCAGCCRGGCTCGAT3'	63	182 bp

### 2.8 Reverse Transcriptase Real Time (RT-RT-PCR)

FRNA detection was also accomplished by RT-RT-PCR. RT-RT-PCR was performed using a Qiagen One Step RT-PCR kit and SYBR® Green I Nucleic Acid Stain. Real Time RT-PCR was performed in an Applied Biosystems Step One Plus™ instrument in MicroAmp™ 96 well 0.1ml plates. To each 15 µl reaction, 2.5 µl of H<sub>2</sub>O, 3µl of five × One-Step PCR buffer, 0.6 µl of dNTP mix (10 µM), 0.3 µl of SYBR Green (10×), 1.5 µl of RNase inhibitor (1U µl<sup>-1</sup>), 1.5 µl of primers (100 nM final concentration), 0.6 µl of One Step RT-PCR enzyme mix, and one µl of template RNA were added. Thermal cycling conditions were 45°C (113°F) for 30 minutes then 95°C (203°F) for 15 minutes, followed by 40 cycles of 95°C (203°F) for one minute, 60°C (140°F) for 30 seconds, and 72°C (161.6°F) for 1 minute. Melt curve analysis cycling conditions were 95°C (203°F) for 15 seconds, 60°C (140°F) one minute + 0.3°C (32.54°F) for 0.1 minute 35 max, and 95°C (203°F) for 15 seconds. Melt curve analysis was conducted using One Step v2.0 software Applied Biosystems with threshold, baseline start and baseline end settings on default auto.

### 2.9 Development of Lateral Flow Devices

Lateral flow devices (LFDs) were produced to determine if these assays could affect rapid detection of the FRNA phages. MS2 specific antibodies were used to produce MS2 specific lateral flow devices. Ten liter samples of tap water were placed into 20 l carboys and individually seeded with MS2 to final concentrations of 10<sup>2</sup>, 10<sup>4</sup>, and 10<sup>6</sup> PFU/ml, and 1.5 g of Amberlite IRA 900 resin beads were added to each sample. The carboys were placed onto stir plates and agitated. Following a capture period of 120 minutes, the resin beads were collected and added to a flask containing 200 mls of a log phase culture of *E. coli* F<sub>amp</sub>. The flask was incubated at 37°C (98.6°F) with shaking at 250 rotations per minute (RPM), and samples were withdrawn at 12 and 24 hours. A drop of the suspension was filtered through a 0.2 µm filter and dropped onto the LFD.

## 2.10 Testing of environmental water

An environmental water sample was obtained and phages present in the sample were captured as described above. Following capture, the resin beads were suspended in a broth culture containing host cells and enriched at 37°C, with shaking at 250 RPM for 12 hours.

## 3 Discussion of Results and their Significance

### 3.1 Small volume capture assays

Four FRNA phages (MS2, GA, SP and Qbeta) representing each of the four FRNA serogroups were investigated for their ability to bind to the anionic exchange resin. Two experiments were conducted. In the first experiment, the phages were individually seeded into 50 ml samples of water at a final concentration of  $10^4$  PFU/ml. One gram of anionic exchange resin beads was added to each sample, and aliquots of water were withdrawn at 15 minute intervals for a total of 2 hours and assayed for the presence of each phage. The results are shown in Figure 1. The concentration of each phage in the water samples decreased over time, indicating that the phages were binding to the resin beads.

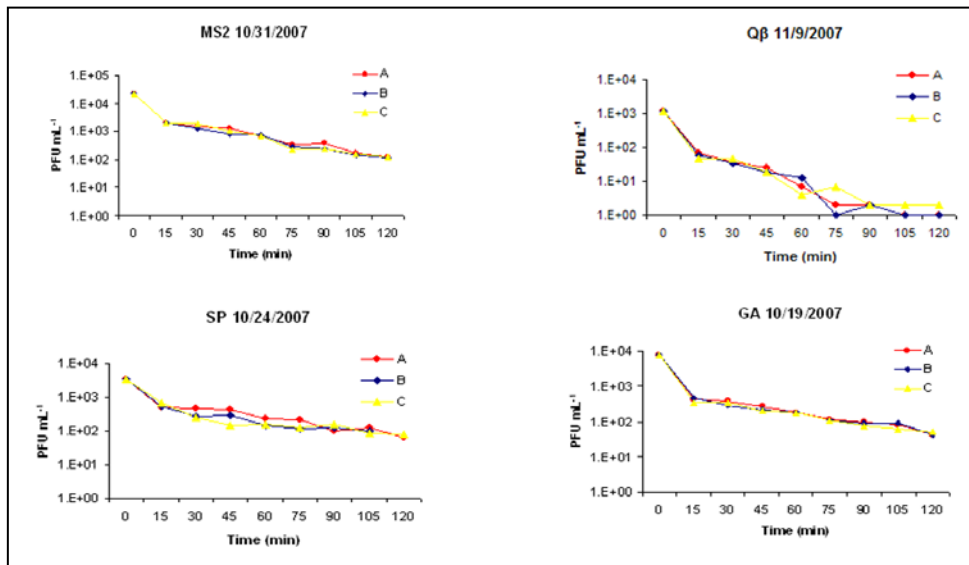


Figure 1. Anionic exchange resin based capture of FRNA phages MS2, Qβ, SP, and GA. Each capture experiment (represented by a different letter in the legend) was completed in triplicate.

An analysis of the FRNA phage capture over time indicated that at 60 minutes, greater than (>) 94% of all phages were captured (Table 2). This time represented an adequate trade-off between the time to capture and the percent of phages captured. Therefore, the 60 minute capture time was used for all subsequent experiments.

Table 2. Capture efficiency of the anionic exchange resin as a function of FRNA phage capture over time

	<b>MS22</b>	<b>GA</b>	<b>Qbeta</b>	<b>SP</b>
15 min	90.8%	94.7%	95.1%	82.9%
30 min	93.1%	95.7%	96.8%	90.2%
45 min	95.3%	97.1%	98.3%	91.5%
<b>60 min</b>	<b>96.7%</b>	<b>97.8%</b>	<b>99.3%</b>	<b>94.6%</b>
75 min	98.7%	98.6%	99.7%	95.6%
90 min	98.5%	98.8%	99.8%	96.2%
105 min	99.3%	99.0%	99.9%	96.9%
120 min	99.4%	99.4%	99.9%	98.0%

The second experiment was conducted to ascertain whether the capture efficiency of the resin differed when various concentrations of FRNA phage were seeded into the water samples. Ten fold serial dilutions of each phage were seeded into individual 50 ml samples of water, followed by the addition of 1 g of anionic exchange resin to each sample. Samples were agitated for 1 hour. Aliquots were withdrawn from each sample at zero and 60 minutes and assayed for FRNA phage by plaque assay. The results are shown in Figure 2. The capture efficiency of the resin increased as the concentration of each FRNA phage decreased.

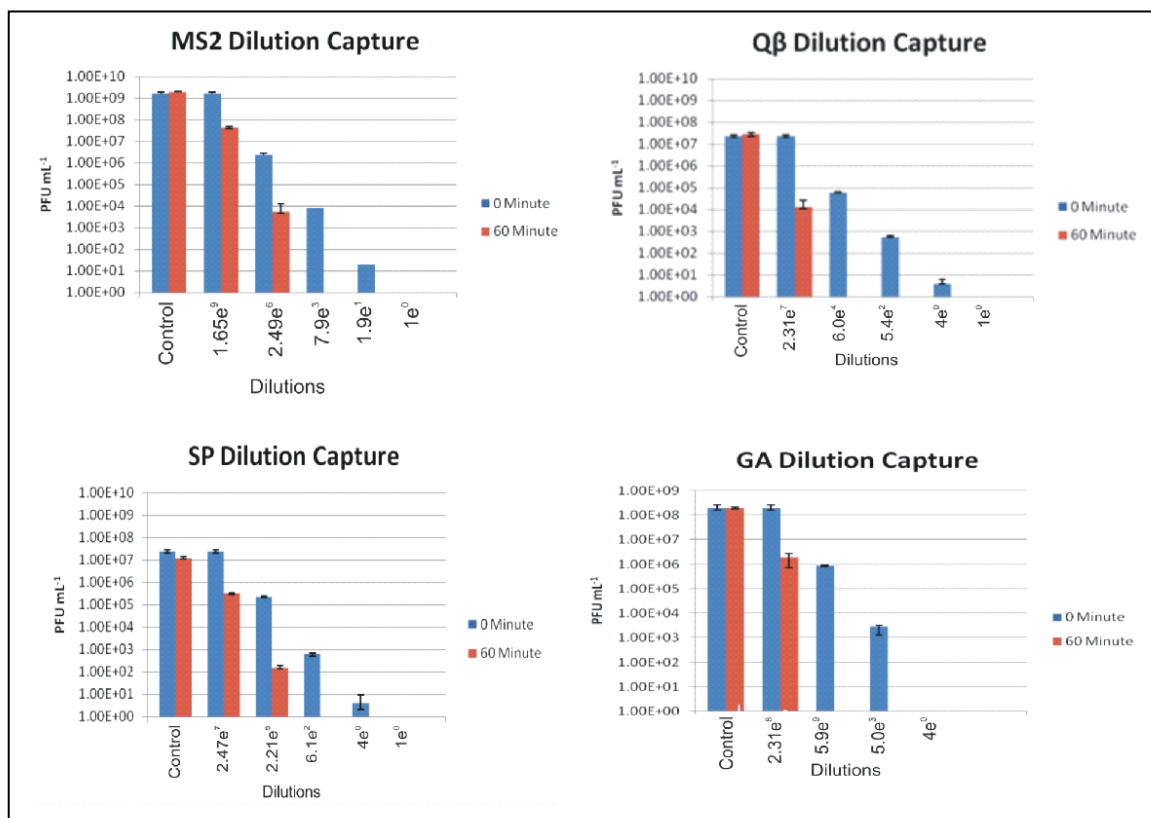


Figure 2. Anionic exchange resin based capture of FRNA phages MS2, Qbeta, SP and GA in 50 ml samples of water at different initial phage concentrations. The blue bar represents the phage concentration in the water samples at zero minutes (control), and the red bar indicates the concentration of each phage at 60 minutes. The absence of a red bar at a given concentration indicates that the phage concentration was below the detection limit of the plaque assay.

### 3.2 Large volume capture assay

The results of the small volume capture assays formed the basis for the experiments conducted using larger volumes of water. For these studies, FRNA phages at different concentrations were spiked into 10 liters of water. The efficiency of the resin to capture phages after 60 minutes of capture time was evaluated. The 60 minute time frame was developed from the small volume capture work, during which 60 minutes of capture represented a good compromise between time of capture and capture efficiency (Table 1). The results of the large volume capture are shown in Figure 3. As with the small volume capture results, the resin beads were more efficient at removing phages at lower concentrations.

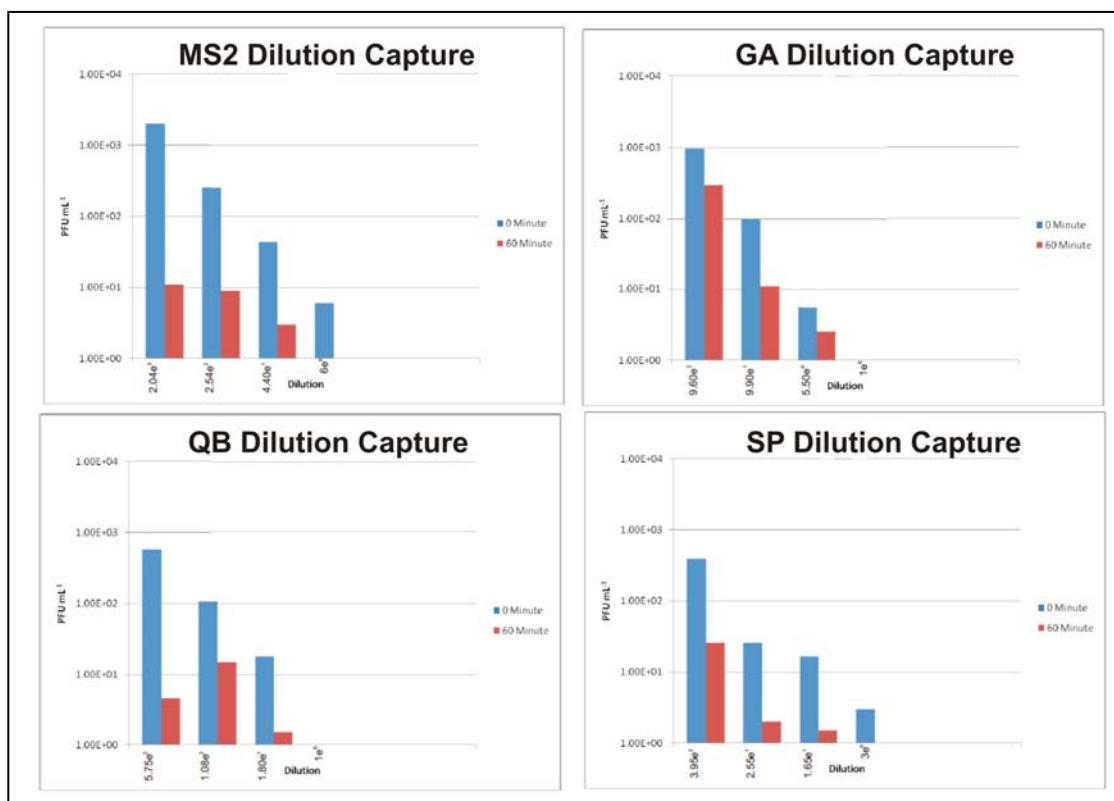


Figure 3. Anionic exchange resin based capture of FRNA bacteriophages MS2, QB, SP and GA in large (10 liter) samples of water at different initial phage concentrations. The blue bar represents the phage concentration in the water samples at zero minutes (control), and the red bar indicates the concentration of each phage at 60 minutes. The absence of a red bar at a given concentration indicates that the phage concentration was below the detection limit of the plaque assay.

The results of the large volume capture assay indicated that the resin was an acceptable method of rapidly concentrating the phages from large volumes of water, and it was also clear that the capture method would integrate well with downstream detection methods. The next step was to design and evaluate two detection methods including polymerase chain reaction and lateral flow detection.

### 3.3 RNA isolation

RNA isolation was performed using a modified method of the Qiagen easy viral RNA isolation kit protocol. For example, a major problem in isolating the viral nucleic acid is the fact that most commercially available kits are designed to isolate nucleic acid from liquid or semi-liquid samples, but our samples are solid (anionic resin beads with phages attached). To address this challenge, a flow through system was developed as a method to isolate bacteriophage RNA directly from the anionic resin bound phage. The process is shown in Figure 4. Briefly, the anionic exchange resin beads were collected and placed into a 15 ml Falcon tube to which lysis buffer (Qiagen QIAamp Viral RNA Mini Kit) was added. After a 10 minute incubation step (per manufacturer's instructions), an equal volume of ethanol was added and the bead-lysis buffer-ethanol suspension was transferred to a 5 ml syringe that had been modified such that it could fit

into a 15 ml Falcon tube, and to contain a filter pipette tip on its end. The newly constructed spin column containing the bead-lysis buffer-ethanol suspension was inserted into a new 15 ml Falcon tube followed by centrifugation at 5,000 x g for 5 minutes; alternatively, the syringe plunger was pushed down and used to separate the lysis-buffer-ethanol mixture from the resin beads. Both methods worked equally well, and during either approach, the lysis buffer-ethanol mixture was separated from the beads and deposited into the bottom of the Falcon tube. In contrast, the resin beads stayed in the spin column, because the filter in the pipette tip prevented the beads from being drawn into the Falcon tube. The lysis buffer-ethanol mixture containing the phage RNA was subsequently purified using the QIAamp Viral RNA Mini Kit (Qiagen) as per manufacturer's instructions.

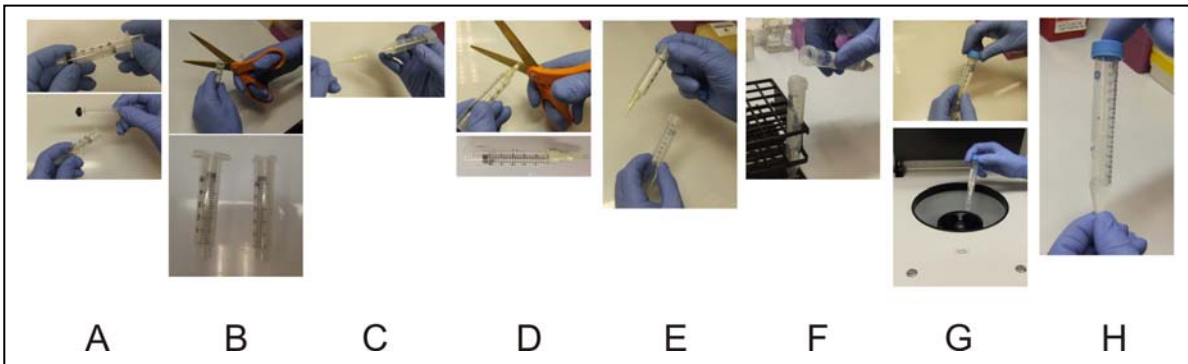


Figure 4. Isolation of bacteriophage RNA from anionic resin beads. (A) The plunger is removed from a 5 ml syringe. (B) The plastic edge around the syringe is trimmed to allow it to fit into a 15 ml Falcon tube. (C) A 100 µl pipette tip (with a filter) is attached to the bottom of the syringe. (D) The pipette tip is trimmed, and the spin column is wrapped in foil and autoclaved. (E) The spin column is inserted into a 15 ml Falcon tube to create the spin column assembly. (F) The bead-lysis buffer-ethanol suspension is poured into the spin column assembly. (G) The assembly is capped and centrifuged at 5,000 × g for 5 minutes. (H) The spin column is removed from the assembly, and RNA isolation proceeds.

#### 3.4 *Reverse Transcription, Real Time Polymerase Chain Reaction (RT-RT-PCR) of FRNA bacteriophages*

Following RNA purification, RT-RT-PCR was accomplished. Identification of FRNA bacteriophages MS2, Qbeta, GA and SP by RT-RT-PCR was performed using primers sets specific for each phage (Table 1). The results showed that we could detect extremely low concentrations ( $10^0$  (MS2, Qβ),  $10^1$  (GA) and  $10^2$  (SP)) PFU/ml of FRNA phages in the water (Figure 5). This assay is currently being further optimized and will be expanded to include other phages that are also excellent indicators of water quality. The goal will be to develop a multiplex RT-RT-PCR assay for up to seven different bacteriophages as a method to detect and track the source of fecal contamination in water.



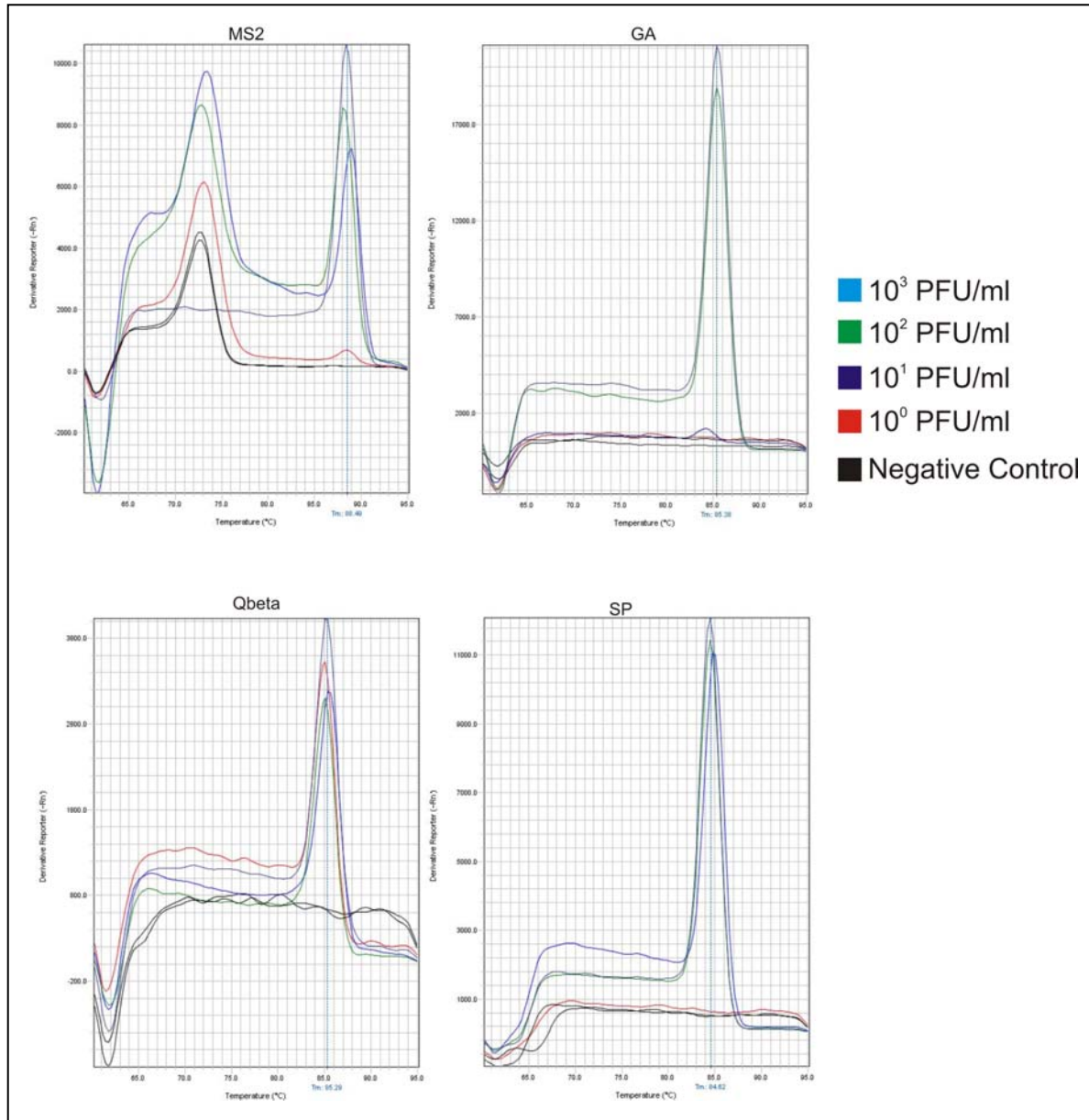


Figure 5. Reverse Transcriptase Real Time PCR of phages MS2, GA, Q $\beta$ , and SP. The assay could detect as few as  $10^0$  PFU/ml of water.

Following reverse transcriptase PCR, FRNA phage amplicons (double stranded DNA) were visualized by agarose gel electrophoresis (a separation method). The results (Figure 6) indicated that agarose gel visualization was more sensitive in some cases and less sensitive in others at detecting the FRNA phage amplicons, as the RT-RT-PCR assay. For example, agarose gel electrophoresis was less sensitive at detecting MS2 and Q $\beta$  amplicons as the RT-RT-PCR assay but was equally sensitive at detecting GA and more sensitive at detecting SP. The results probably reflect the fact that different PCR primers were used in the reverse transcriptase/agarose gel electrophoresis assay than in the RT-RT-PCR assay.

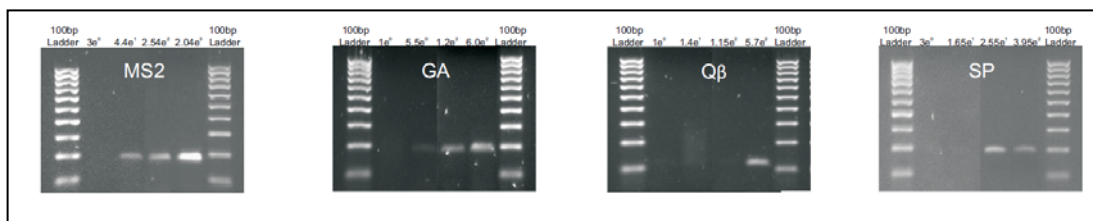


Figure 6. Agarose gel electrophoresis of MS2, GA, Q $\beta$  and SP FRNA bacteriophage cDNA isolated from anionic exchange resin beads and amplified by reverse transcriptase PCR

### 3.5 Detection of FRNA phages in environmental water by Lateral Flow Assay

A field based assay was developed to detect the FRNA bacteriophages. Following capture using the anionic resin beads, the beads were recovered and directly suspended into a broth culture containing a suitable host for the FRNA phages followed by enrichment for 12 hours at 37°C with shaking at 250 RPM. After the enrichment, an aliquot of the phage lysate (broken-down cell) was removed and filtered through a 0.22  $\mu$ m syringe filter, and 200  $\mu$ l was placed on newly developed lateral flow devices (LFD) that were developed to detect group I FRNA phages. The LFD (capable of detecting group I FRNA phages) was designed as a proof of concept device to determine whether these devices could be used in the field to detect FRNA phages. The results (Figure 7) indicate that, after an enrichment, a positive test result was observed. The low intensity of the band (T) is due to the low sensitivity of the LFD (which we are currently trying to improve) and not a low concentration of FRNA phages in the sample. Also, since group I FRNA phages are known to be found in animal waste, this result provides preliminary information that suggests that this environmental sample contained animal fecal material. Future lateral flow devices will be developed to include 4 lines (one for each serotype of FRNA phage). This will enable simultaneous detection of all serogroups of FRNA phages (and therefore the presence of fecal material), and it will also allow us to determine the source of the fecal contamination, since different FRNA serotypes are associated with different sources (animal or human) of fecal waste.

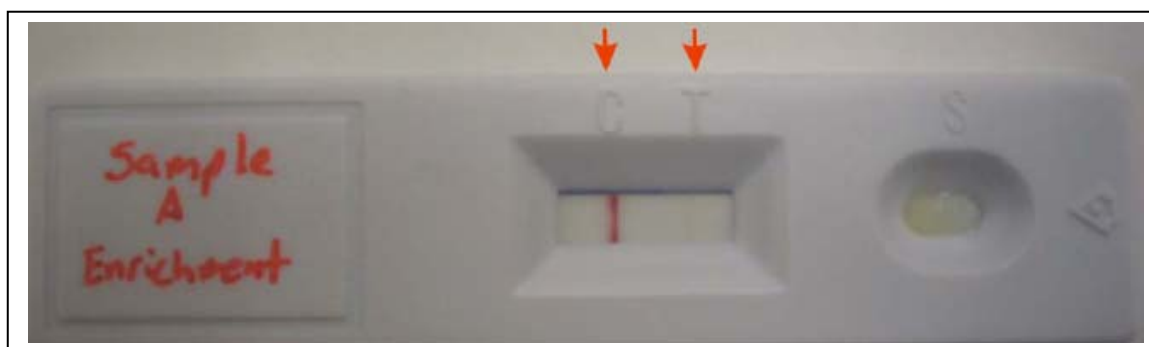


Figure 7. Lateral flow detection of group I FRNA phages in the environmental water sample. The arrows indicate the quality control (C) and test (T) lines.

#### **4 Principal Findings, Conclusions, and Recommendations**

The anionic exchange resin efficiently concentrated all of the FRNA phages from the tap water regardless of whether the volume of the water was small (50 mls) or large (10, 20 liters). The efficiency of capture was greater than 90% for all viruses. The flow through nucleic acid isolation method worked well and permitted nucleic acid isolation from all phages. The RT-RT-PCR method also worked well and allowed for detection of as low as  $10^0$  PFU/ml from water samples. A lateral flow device was developed for group I FRNA phages and was shown to be a viable way of detecting the FRNA phages following capture by the anionic exchange resins and an enrichment step.

Many potential applications are envisioned from the outcome of this study. For example, the anionic exchange resin beads could be packaged along with a disposable water collection container and the flow through nucleic acid isolation kit. In this scenario, multiple samples of water could be collected and transported back to the lab, where the anionic exchange resin beads would be used to concentrate the FRNA bacteriophages followed by the flow through nucleic acid isolation and RT-PCR. Alternatively, a pump-based, portable water sampling device could be used to concentrate FRNA phages from water directly in the field, and the resin beads could be collected and enriched in a suitable bacterial host followed by lateral flow detection of the FRNA phages directly in the field. Future research is currently focused on developing a lateral flow device to detect all four serogroups of FRNA bacteriophages and on refining the RT-RT-PCR assay to make it more sensitive. The resin capture-RT-RT-PCR assay will be evaluated for robustness during a three week experiment in South Africa in January, 2009.