COMBINED WORK/QUALITY ASSURANCE PROJECT PLAN (CW/QAPP)

for

ANTHROPOGENIC VIRUS SURVEY: 1995-1997

Task 28
MWRA Harbor and Outfall Monitoring Project

submitted to

MASSACHUSETTS WATER RESOURCES AUTHORITY
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CONTENTS

1.0 PROJECT NAME 1

2.0 PROJECT REQUESTED BY 1

3.0 DATE OF REQUEST 1

4.0 DATE OF PROJECT INITIATION 1

5.0 PROJECT MANAGEMENT 1

6.0 QUALITY ASSURANCE (QA) MANAGEMENT 1

7.0 PROJECT DESCRIPTION 2
- 7.1 Objectives and Scope 2
- 7.2 Data Usage 2
- 7.3 Technical Approach 3
  - 7.3.1 Criteria for Selection of Sampling Locations 3
  - 7.3.2 Selection of Sampling Locations 3
  - 7.3.3 Criteria for Sampling 9
  - 7.3.4 Field Program 10

8.0 PROJECT FISCAL INFORMATION 12

9.0 SCHEDULE OF ACTIVITIES AND DELIVERABLES 12

10.0 PROJECT ORGANIZATION AND RESPONSIBILITIES 12
- 10.1 Project Management 12
- 10.2 Field Program 12
- 10.3 Laboratory Analysis Program 14
- 10.4 Data Management and Reporting 14

11.0 QUALITY CONTROL/QUALITY ASSURANCE 14
- 11.1 Cultivatable Enteric Viruses 14
  - 11.1.1 Virus Recovery Efficiencies 14

CONTENTS
(Cont'd)

11.1.2 Cell Sensitivity 15
11.1.3 Media, Beef Extract, and Positive and Negative Controls 15
11.2 Polymerase Chain Reaction Screening 15
11.3 Coliphage 16
11.4 Fecal Coliform and *Enterococcus* 16

12.0 SAMPLING AND ANALYTICAL PROCEDURES 16
12.1 Mobilization and Sampling Logistics 17
12.1.1 Weather Forecasting 17
12.1.2 Mobilization Procedures 17
12.2 Anthropogenic Virus Sampling and Analysis Procedures 19
12.2.1 Virus Collection 19
12.2.2 Disinfection of Field Equipment 20
12.2.3 Virus Elution 20
12.2.4 Virus Concentration by Organic Flocculation 20
12.2.5 Detection of Virus Using the BGMK cell line. 20
12.2.6 Detection of Virus Using PCR 21
12.2.7 Calculation of Virus Concentration 22
12.2.7.1 Total Cultivatable Viruses Using the BGMK Cell Line 22
12.2.7.2 PCR 22
12.3 Bacteriophage Sampling and Analysis Procedures 22
12.4 Indicator Bacteria Sampling and Analysis Procedures 23
12.5 Field Measurements of Water Quality Parameters 23

13.0 SAMPLE CUSTODY PROCEDURES 23

14.0 CALIBRATION PROCEDURES AND PREVENTATIVE MAINTENANCE 27

15.0 DOCUMENTATION, DATA REDUCTION, AND REPORTING 27
15.1 Data Collection and Reduction 27
15.2 Result Reporting 31
15.2.1 Harbor Studies Database 31
15.2.2 Annual Virus Data Reports 31

16.0 DATA VALIDATION 32

CONTENTS
(Cont'd)

17.0 PERFORMANCE AND SYSTEM AUDITS 32

18.0 CORRECTIVE ACTION 33

19.0 REPORTS 34

20.0 REFERENCES 34

LIST OF TABLES

1 MWRA Anthropogenic Virus Survey Sampling Locations 4
2 Monitoring Parameters for Anthropogenic Virus Surveys 11
3 MWRA CSO Virus Survey Points of Contact 18

LIST OF FIGURES

1	Anthropogenic Virus Survey Sampling Locations	6
2	Anthropogenic Virus Survey Team	13
3	Field Sample Data Sheet	24
4	Virus Sample Chain of Custody Record	25
5	Bacteriological Sample Chain of Custody Record	26
6	Enteric Virus Sample Data Sheet	28
7	PCR Sample Data Sheet	29
8	Bacteriophage Sample Data Sheet	30

1.0 PROJECT NAME

MWRA Harbor and Outfall Monitoring Project
Task 28
Anthropogenic Virus Survey

2.0 PROJECT REQUESTED BY

Massachusetts Water Resources Authority

3.0 DATE OF REQUEST

November 2, 1994

4.0 DATE OF PROJECT INITIATION

November 9, 1994

5.0 PROJECT MANAGEMENT

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6.0 QUALITY ASSURANCE (QA) MANAGEMENT

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Ms. Debra McGrath, ENSR Project QA Director

7.0 PROJECT DESCRIPTION

7.1 Objectives and Scope

The following objectives have been identified for this task:

- identify the potential for hazards to public health due to anthropogenic viruses in Combined Sewer Overflow (CSO) receiving water or shellfish;
- identify sources of anthropogenic viruses in receiving water;
- develop baseline data for evaluation of potential improvements in water quality resulting from CSO remediation and wastewater treatment plant (WWTP) outfall relocations; and
- provide correlation between anthropogenic viruses, viral indicators, and fecal coliform contamination.

The scope of this task consists of sampling of receiving waters influenced by CSO and WWTP discharges, and does not at present include sampling of shellfish tissues. A demonstration that viruses are present in the water column in the vicinity of shellfish resources implies that a hazard also exists from shellfish consumption. However, a definitive demonstration of risk from shellfish will require tissue analyses, which may be considered at a later time after initial results for the receiving waters are available for review. Identification of sources will primarily be demonstrated by the proximity of positive results to known discharges, with the potential for confirmatory sampling to be conducted in the future.

Task 28 includes sampling during both wet and dry weather, with the collection of ten samples for viral analysis during each type of sampling. The final objective for demonstrating a correlation between the presence of viruses and fecal coliforms will be fulfilled by concurrent collection of grab samples for bacteriological analysis.

7.2 Data Usage

Data collected under this task will be used to assess the baseline potential risks to humans from recreational activities in Boston Harbor and its tributaries. These baseline data will be used to assess potential benefits to water quality in the harbor from WWTP outfall relocation and CSO system improvements. Water quality data will also be used to determine whether shellfish sampling should be conducted to assess additional risk posed by consumption of shellfish. Finally, data will support the correlation between fecal bacteria indicators, viral indicators (bacterial phages and nucleic acid assays), and cultivatable viruses.

7.3 Technical Approach

7.3.1 Criteria for Selection of Sampling Locations

Sampling locations were selected to provide data on areas where the highest potential for health risk exists. In addition, a pilot study conducted by the MWRA in 1989 (Rex, 1989) provided initial data on where anthropogenic viruses may be present. The following criteria were considered during selection of sampling locations:

- revisit sampling stations which produced positive screening results during the 1989 pilot study;
- provide coverage in proximity to bathing beaches; and
- provide coverage in areas of shellfish resources (both currently viable shellfish beds as well as potentially productive shellfish habitat).

Assuming that viral densities are positively correlated with fecal coliform densities, the existing MWRA data for CSO receiving water segments (MWRA, 1994) were reviewed to identify areas of highest fecal coliform loading from CSOs, stormwater discharges, and upstream sources. Further consideration was given to the following in the selection of sampling locations:

- proximity to significant potential sources (CSOs, storm drains, WWTP discharges, non-point sources);
- relative discharge volume, discharge quality, and discharge frequency;
- mixing characteristics of the discharge; and
- background water quality.

7.3.2 Selection of Sampling Locations

Based on the study objectives and criteria presented above for sampling location selection, the sampling locations listed in Table 1 were identified for inclusion in Task 28 field studies. Each of these proposed locations is discussed in detail below.

Charles River at Stony Brook (MWR 023). The Lower Charles River Basin is designated as Class B waters, with uses defined as fishable/swimmable. The principal exposure pathway for human health risk is therefore from ingestion of water during primary and secondary contact recreation, which primarily consists of sailboarding and boating. Based on data collected between 1988 and 1993, existing water quality in this segment does not meet the swimming

TABLE 1

MWRA ANTHROPOGENIC VIRUS SURVEY SAMPLING LOCATIONS

MWRA Location Code	Coordinates		Location Description
	North Latitude	West Longitude	
145S	42° 20.98'	71° 05.58'	Charles River at Stony Brook
014S	42° 22.23'	71° 03.09'	Mouth of Charles River
052S	42° 23.63'	71° 04.55'	Mystic River Below Earhart Dam
146S	42° 20.40'	70° 57.37'	Deer Island Outfall 002 Plume
081S	42° 17.64'	70° 57.50'	Nut Island Outfall 102 Plume
047S	42° 16.83'	71° 00.42'	Off Wollaston Beach Storm Drains 7 & 8
110S	42° 17.47'	71° 02.49'	Middle of Pine Neck Creek
041S	42° 17.98'	71° 02.60'	Old Colony Yacht Club
036S	42° 19.59'	71° 02.75'	Carson Beach Bathhouse
075S	42° 20.74'	71° 03.35'	Fort Pt. Channel at Broadway Bridge

standard of 200 fecal coliform colonies per 100 ml under either dry or wet conditions. The boating standard of 1000 fecal coliform colonies per 100 ml is exceeded after rainfall events.

This segment produced one of three positive results (out of a total of 25 samples) during the 1989 pilot study using the nucleic acid probe screening assay. This sample (V25) was taken approximately 335 meters (m) downstream of the discharge from MWR023, which discharges baseflow, combined sewage, and stormwater from Stony Brook through the Fens Gatehouse (Figure 1). Several other CSOs are located along the basin, primarily on the southern bank. The largest is the Cottage Farm CSO Facility (60 percent of CSO flow in this segment), however, this discharge is disinfected by chlorination prior to discharge. Future CSO flows (1997 planning year) are expected to decrease approximately 28 percent compared to existing flows for a 3-month storm (MWRA, 1994).

The discharge from MWR023 is considered the largest single source of most pollutants to the lower Charles River. During a 3-month storm (1.84 inches of rainfall), MWR023 discharges approximately 340,000 m^3 of stormwater and creek baseflow and approximately 38,000 m^3 of combined sewage (MWRA, 1994). Dry weather flows do not contain any stormwater or combined sewage flows. The maximum estimated dilution factor of this discharge by Charles River water (1.5:1) is believed to occur within about 250 m of the discharge (Ayuso and Adams, 1994). Model simulations for the 3-month rainfall event predict that the boating standard in this reach of the river will be exceeded for a period up to 40 hours. While this exceedance was predicted to occur almost immediately just upstream of MWR023 (Boston University boathouse), there appeared to be a time lag of up to 16 hours further downstream (Community Boat House).

Based on these data, sampling at MWRA Station 145S will be conducted to reflect river concentrations after initial dilution and to provide continuity with the existing MWRA Receiving Water Monitoring database.

Mouth of Charles River. The Inner Harbor is designated SB - fishable/swimmable. As this area includes the main shipping channels, potential hazards to human health are associated primarily with recreational boating. Both the swimming and boating standards are exceeded after rainfall events.

Water quality in this receiving water segment is influenced by flows from the Charles and Mystic Rivers, as well as a number of untreated CSOs and storm sewers discharging directly into it. These discharges include drainage from Charlestown, East Boston, and the North End. The largest discharge is the Prison Point Facility (MWR203), which chlorinates and discharges downstream of the Charles River Dam. This facility has been noted to discharge after about 0.25 inch of rainfall, whereas several others discharge after about 0.15 inch (MWRA, 1994). Future CSO flows are expected to decrease by 45% of existing conditions for a 3-month storm, and more flows will receive treatment (MWRA, 1994).

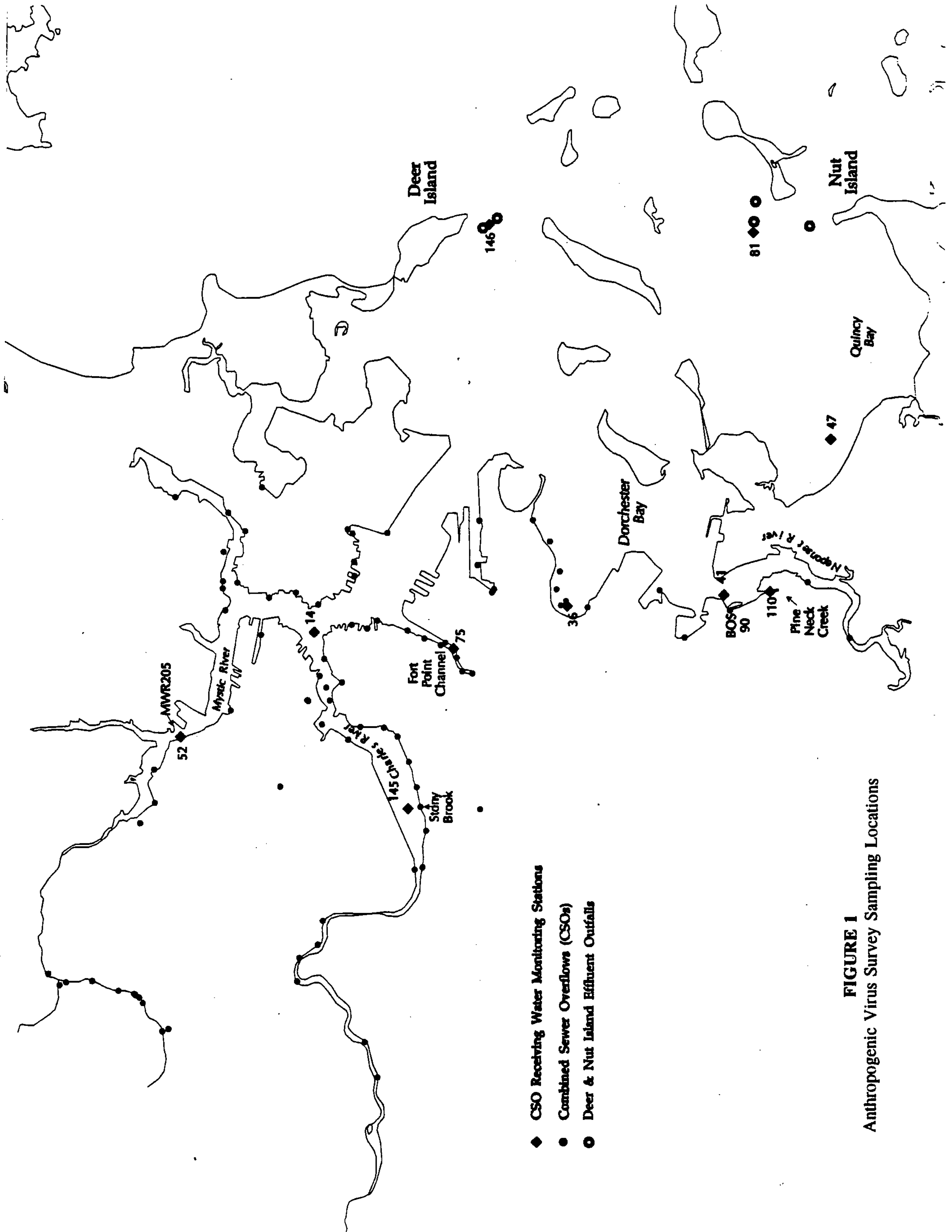

FIGURE 1
Anthropogenic Virus Survey Sampling Locations

MWRA Monitoring Station 014S, located in the upper Inner Harbor at the confluence of the Charles and Mystic Rivers (Figure 1), will be occupied during the virus surveys to provide baseline data on the effects of CSO discharges as well as the contributions from the two rivers.

Lower Mystic River. The lower Mystic River (tidal segment below Amelia Earhart Dam to the upper Inner Harbor) is classified as SB - fishable/swimmable with restricted shellfishing in approved areas. Much of the adjacent waterfront is industrial in nature, and the river segment experiences a high volume of shipping. Wet weather conditions result in bacterial counts in excess of the swimming standard, and the boating standard can be exceeded during larger storms (MWRA, 1994).

Two CSOs are located along this segment of the river, with the principal CSO contributor being the Somerville Marginal CSO facility which discharges below the Earhart Dam at MWR205. The Somerville Marginal facility utilizes screening and chlorination to treat CSO flows prior to discharge. Monitoring during 1992 indicated that overflows from these two CSOs required about 0.1 inch of rainfall (MWRA, 1994). It should be noted that the facility may discharge above the dam at SOM007A during high tides.

MWRA monitoring station 052S, located just below the Earhart Dam, will be sampled during Task 28 to assess the influence from the CSOs and upstream sources (Figure 1).

Fort Point Channel. Fort Point Channel is on the south side of the Inner Harbor and separates South Boston from the north end. It is also classifies as SB with restricted shellfishing, although there are no identifiable shellfish resources within this segment (MWRA, 1994). Potential risks to human health are associated primarily with recreational boating. The swimming standard is exceeded under both dry and wet weather conditions, and the boating standard is exceeded after storms.

This segment is heavily impacted by untreated CSO flows and stormwater. It is the receiving water body for the largest untreated CSO in the system, BOS070, which discharges at the head of the channel. It has been shown to overflow with only 0.1 inch of rainfall, with other CSOs in the segment discharging with rainfall amounts of between 0.4 inch and 0.8 inch (MWRA, 1993). These other discharges are subject to control by tidal stage. Dilution of CSO flow from BOS070 is approximately 10:1 (Ayuso and Adams, 1994). Residence time in the channel has been estimated to be between 1 and 2.5 days, with approximately 6 days required for a return to background levels after a 3-month storm.

Sampling will be conducted at MWRA Monitoring Station 075S, located at the head of Fort Point Channel. This sampling location is influenced by flows from five CSO discharges, and is adjacent to the discharge from BOS070.

Northern Dorchester Bay. Northern Dorchester Bay is designated as Class SB with restricted shellfishing in approved areas. The potential principal exposure pathways for human health risk are therefore from ingestion of water during primary and secondary contact recreation, and from consumption of shellfish taken from local beds. Shellfishing in Northern Dorchester Bay is, at present, prohibited. Swimming areas are located in Pleasure Bay and Carson Beach, which both also have shellfish beds. Both the swimming and shellfish standards are met under dry weather conditions, and the boating standard is met even after 3-month storm discharges.

Seven untreated CSOs discharge subtidally into this segment, with rainfall minima required to produce discharges ranging from 0.15 inch to over an inch. Bacterial levels in the water column require approximately four days to return to background after a 3-month storm (Ayuso and Adams, 1994). CSO discharges are expected to be eliminated along the beaches under future planning scenarios, with the swimming standard expected to be violated for about one day at Carson Beach (MWRA, 1994).

Based on the heavy recreational uses and information on existing and future discharge scenarios, sampling will be conducted off Carson Beach at MWRA Sampling Station 036S.

Southern Dorchester Bay. Southern Dorchester Bay is designated as Class SB with restricted shellfishing in approved areas. The principal exposure pathways for human health risk are therefore from ingestion of water during primary and secondary contact recreation, and from consumption of shellfish taken from local beds. Several heavily used beaches are located in this segment, as are numerous shellfish beds of various designations. The swimming standard is not always met under dry weather conditions near Tenean Beach in the southern reach of this segment, probably due to the influence of the Neponset River and contamination from Pine Neck Creek (MWRA, 1991; Rex, 1993).

Three CSOs discharge into this segment, including the discharge from the Fox Point and Commercial Point CSO facilities (BOS 089 and BOS 090, respectively). Although both facilities chlorinate prior to discharge, additional stormwater flows enter downstream of the facilities (MWRA, 1994). During 1992 CSO monitoring, BOS089 discharged after about 0.18 inch of rain had fallen, while BOS090 required 0.38 inch of rain. The third CSO in this segment (BOS088), which serves as a bypass discharge for Fox Point, did not discharge during 1992 monitoring (MWRA, 1993). The CSO plan calls for building separate storm and sanitary sewers in this area, eliminating CSO flows.

The positive screening result produced in sample #V16 during 1989 was taken approximately 150 meters from BOS089. MWRA Monitoring Station 041S, located off of the Old Colony Yacht Club, will be sampled to assess the influence from the two CSO facilities and the potential for upstream influence from the Neponset River.

One additional station will be sampled within this area of Boston Harbor. Dry weather sampling performed by Boston Water and Sewer Commission has indicated that sewage may be contaminating separate storm drains discharging into Pine Neck Creek (BWSC, 1991; 1993). MWRA Monitoring Station 110S (Figure 1) will also be sampled to assess water quality conditions in this small tributary of Dorchester Bay.

Wollaston Beach. Sampling will be performed off Wollaston beach at MWRA Monitoring Station 047S. This station will be included in the program to assess the potential influence of wet weather discharges from storm drains 7 and 8, located just southeast of Wollaston Yacht Club (Figure 1).

Deer Island and Nut Island Effluent Discharge Areas. Sampling in the vicinity of the effluent discharges from the two WWTPs will be conducted to support the objective for development of baseline water quality and risks associated with existing conditions. Future sampling under this task will demonstrate the degree of potential improvements in water quality in Boston Harbor attributable to the relocation of these two discharges. While the precise locations for sampling of these discharges will vary depending on wind and tidal conditions, the general vicinity of sampling for each outfall is listed in Table 1.

7.3.3 Criteria for Sampling

The following were considered during development of sampling logistics:

- antecedent rainfall;
- rainfall depth and intensity;
- time lag prior to initiation of sampling;
- maximum allowable sampling window after rainfall event; and
- tidal stage.

The sampling program includes both dry and wet weather sampling. In the case of dry weather sampling intended to quantify baseline water quality conditions, recent rainfall and resultant CSO or stormwater discharges would potentially influence water quality and thus should be avoided. Conversely, significant amounts of antecedent rainfall could potentially result in reduced densities of viruses discharged by CSOs due to in-system dilution. Antecedent rainfall criteria were therefore established for this sampling program, consisting of no more than 0.1 inch of rain during the previous 48 hours and rainfall not to exceed 0.5 inch during the previous 72 hours.

A minimum rainfall criterion was necessary to ensure that sufficient CSO discharges would occur to warrant mobilization and sampling. Based on information discussed earlier for each receiving water segment and available data on CSO responses to rainfall, a minimum rainfall criterion of 0.5 inch was established for a wet weather sampling event. Ideally, this rainfall total should occur within a 6-hour period to provide sufficient intensity to result in widespread CSO discharges.

Sampling for wet weather events should be initiated after the onset of major CSO discharges (i.e., Somerville Marginal, Prison Point, Fox Point, and Commercial Point). Since sampling locations have been selected in close proximity to these discharges, a prolonged lag time after onset of discharge would be unnecessary. Sampling will therefore be conducted as soon as logistics permit. In an effort to sample as synoptically as possible, every effort will be made to complete sampling within 36 hours of the onset of discharges.

7.3.4 Field Program

The anthropogenic virus survey will provide a comprehensive assessment of human enteric viruses, virus indicators, and fecal bacteria in the CSO receiving waters. Each survey will collect data at 10 stations for the parameters listed in Table 2. Sampling and analyses will include:

- quantification of cultivatable strains of viruses (polio, coxsackie, and echovirus) using the Buffalo Green Monkey Kidney (BGMK) cell method, with results reported as most probable number (MPN) total cultivatable virus density;
- additional screening of strains not readily detected by the BGMK method (hepatitis A, rotavirus, and Norwalk agent) using the polymerase chain reaction (PCR) method, with results reported individually for each;
- enumeration of both male-specific and somatic bacteriophages; and
- quantification of fecal coliform and *Enterococcus* bacteria densities.

Concentration of subsurface (0 to 12 inches) receiving water samples for virus analyses will be performed in the field using microporous filtration. Filters will be transported on ice in coolers to the Water Borne Disease Laboratory at UNH for subsequent elution, concentration, and analysis. Subsurface grab samples for bacteriological analyses will be taken directly into pre-sterilized 200 ml specimen cups and transported on ice in coolers to the MWRA Central Laboratory on Deer Island.

Specific details on sampling and analytical protocols are provided in Section 12. Surveys will be coordinated with the MWRA Harbor Water Quality Monitoring Program, which will provide a second vessel and sampling support.

TABLE 2

Monitoring Parameters for Anthropogenic Virus Surveys

Parameter	Sample Container	Preservation	Analysis Method	Holding Time	Units
Cultivatable Viruses	Filtered in field	4°C (cooler with ice)	BGMK	elute within 48 hrs, indefinite at -20°C	MPN/mL
Viral Genome Screening	Filtered in field	4°C (cooler with ice)	PCR	elute within 48 hrs, indefinite at -20°C	presence/ absence
Bacteriophages	Filtered in field	4°C (cooler with ice)	SM 9211 D	elute within 48 hrs, indefinite at -20°C	PFU/mL
Fecal coliform	sterile plastic specimen cup	4°C (cooler with ice)	SM 9222 D	6 hrs	fecal coliforms per 100 mL
Enterococcus	sterile plastic specimen cup	4°C (cooler with ice)	SM 9230 C	6 hrs	enterococci per 100 mL
Temperature	in-situ	NA	probe	MWRA SOP	°C
pH	in-situ	NA	probe	MWRA SOP	standard pH units
Conductivity	in-situ	NA	probe	MWRA SOP	mS/cm
Salinity	in-situ	NA	probe	MWRA SOP	‰
Dissolved Oxygen	in-situ	NA	probe	MWRA SOP	mg/L
Turbidity	in-situ	NA	probe	MWRA SOP	NTU

Notes:
NA Not applicable
BGMK Buffalo Green Monkey Kidney cell method
PCR Polymerase Chain Reaction method
SOP Standard Operating Procedure
MPN Most Probable Number
PFU Plaque Forming Unit
NTU Nephelometric Turbidity Units

8.0 PROJECT FISCAL INFORMATION

Task 28 is being carried out under the Harbor and Outfall Monitoring contract (Contract No. S186) between MWRA and ENSR Consulting and Engineering.

9.0 SCHEDULE OF ACTIVITIES AND DELIVERABLES

Sampling activities associated with Task 28 are scheduled in 1995, 1996, and 1997, tentatively in May of each year. Exact dates will be determined as the study progresses, subject to the criteria established for sampling.

Deliverables associated with each sampling event include survey plans which will be submitted to the MWRA two weeks prior to initiation of each survey. Survey reports will be submitted one month after each survey. Annual Virus Data Reports will be submitted three months after the conclusion of sampling activities for the year (tentatively in August).

10.0 PROJECT ORGANIZATION AND RESPONSIBILITIES

10.1 Project Management

The project organization is shown in Figure 2. Dr. Michael Mickelson is the MWRA Project Manager and Dr. Andrea Rex is the MWRA Project Area Manager for Task 28. They will be informed of all matters pertaining to work described in this CW/QAPP. Mr. Ken Keay is the MWRA Deputy Project Manager and will serve as a back-up to Dr. Mickelson and Dr. Rex. Ms. Wendy Leo is MWRA's EM & MS database manager.

Dr. James Blake is the ENSR Project Manager responsible for the overall performance of this project. Dr. James Bowen is the ENSR Technical Director responsible for the technical performance of this project. The Quality Assurance Director for the project is Ms. Debra McGrath. Mr. Stephen Cibik is the ENSR Project Area Manager and will have overall responsibility for the task. Ms. Therese Mounce is the ENSR Task Manager who will act as a back-up to Mr. Cibik and coordinate field activities. Dr. Aaron Margolin will lead the UNH technical team and will have overall responsibility for UNH operations.

10.2 Field Program

ENSR will schedule survey logistics and coordinate with the MWRA Harbor Water Quality Monitoring team through Dr. Rex. Dr. Margolin will act as Chief Scientist for each survey, and will communicate with the MWRA and ENSR Area Managers as needed. If a second team can be fielded (dictated by the availability of the MWRA vessel), one of Dr. Margolin's staff will

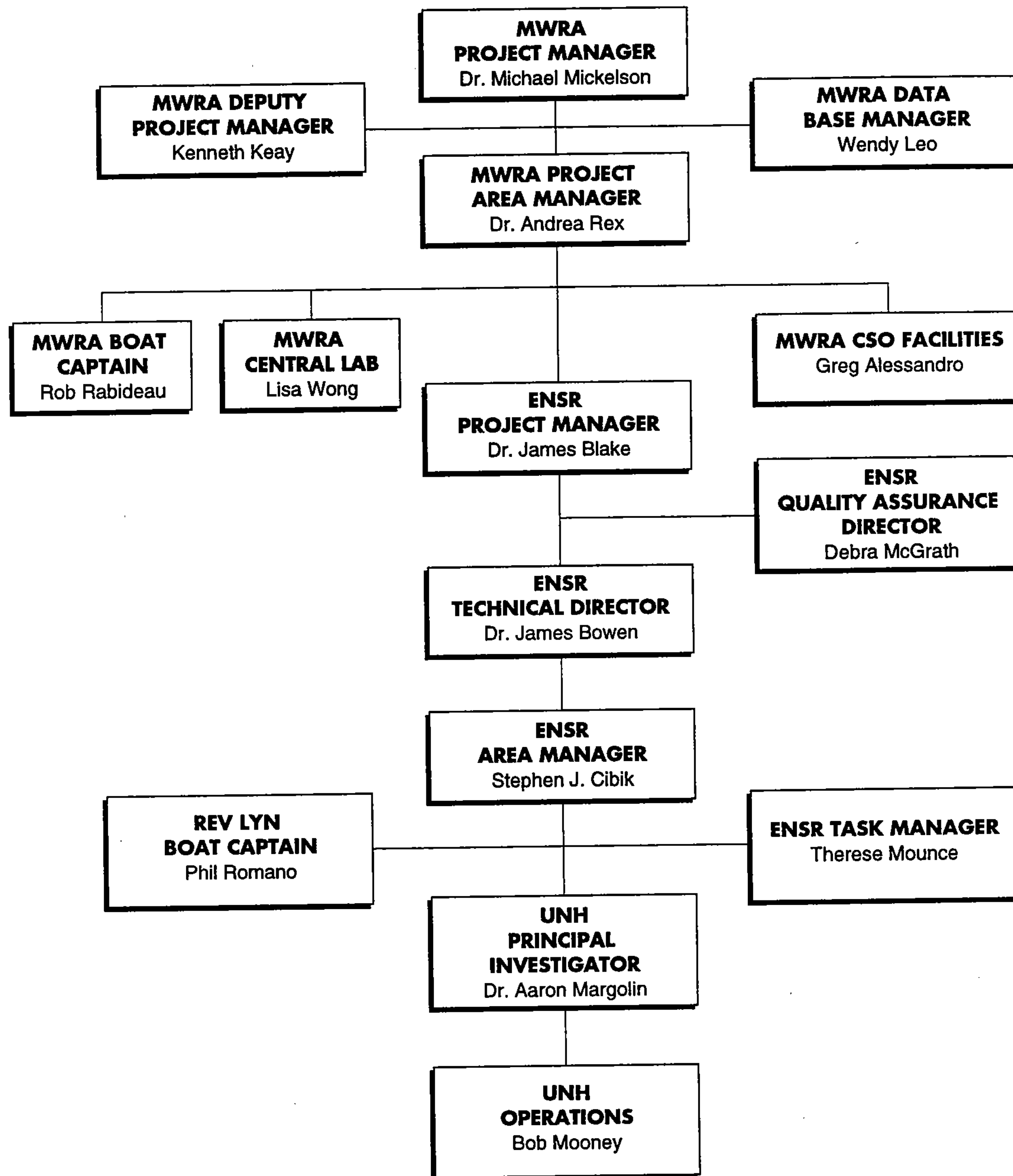

FIGURE 2
Anthropogenic Virus Survey Team

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act as the technical lead for the second team. Dr. Margolin will be responsible for ensuring the technical quality of the field sampling program.

10.3 Laboratory Analysis Program

Analyses of viral samples will be performed at the Water Borne Disease Laboratory at UNH, coordinated by Dr. Margolin. Bacteriological analyses will be performed at the MWRA Central Laboratory under the supervision of Lisa Wong.

10.4 Data Management and Reporting

UNH will prepare survey plans, survey reports, and virus data reports. UNH will be responsible for data management of their laboratory studies data, and will report data in both hard copy and electronic format to the ENSR Area Manager. ENSR will be responsible for overall project data management. Ms. Debra McGrath, ENSR's Project QA Director, will be responsible for QA review of the data reported under this task.

11.0 QUALITY CONTROL/QUALITY ASSURANCE

11.1 Cultivatable Enteric Viruses

11.1.1 Virus Recovery Efficiencies

In the examination of a particular water, a preliminary evaluation of virus recovery efficiency should be done. To accomplish this, additional 5-L carboy samples will be collected which are representative of the three matrices which are being investigated: non-saline receiving waters (Charles River station 145S), saline harbor water (Carson Beach Bathhouse station 036S), and chlorinated effluent plume (Deer Island station 146S). These carboys will be placed on deck and covered to shield against effects from sunlight.

Upon return to the UNH laboratory, a known quantity of test virus will be added to each sample to determine relative virus recovery efficiency. Test virus will be poliovirus I and will be produced on the BGMK cell line and enumerated using the plaque forming unit assay (PFU). This assay is the same as the one described in this document however, after inoculation, instead of the addition of maintenance media, media containing 1% agar is added, permitting solidification of the media. Cells are examined daily for the 10-14 days and plaques or clearing zones are counted. The number of plaques times the dilution factor equals the original concentration of virus.

11.1.2 Cell Sensitivity

All BGMK cells will be challenged monthly with predetermined concentration of virus. If 50% or more of the original virus titer is not detected on the current cells, new cells with a lower passage number will be used.

11.1.3 Media, Beef Extract, and Positive and Negative Controls

All media and components will be made according to the manufacturer's recommendation, the current literature or those developed within the UNH laboratory. All media are checked prior to use for bacterial contamination. Beef extract is checked for virus recovery efficiency by seeding 1 liter of beef extract with a known amount of virus and then proceeding with organic flocculation and the rest of the procedure. For a lot of beef extract to be considered acceptable, at least 50% of the spike virus must be recovered. All cell culture assays will have two negative cultures and one positive culture. Negative cultures will be divided, one occurring in the beginning of the assay and one occurring at the end of the assay. The first negative control is to demonstrate the absence of virus in media and reagents prior to the start of the assay. The final negative control is to demonstrate the lack of virus in the media and reagents after inoculation.

11.2 Polymerase Chain Reaction Screening

To ensure quality control the following reactions will be run:

1) Reverse Transcription-Polymerase Chain Reaction (RT-PCR) control provided by Perkin-Elmer
2) primers, master mix, reverse transcriptase (RT) and taq polymerase (TP) - this reaction will be run for each set of virus primers
3) primers, master mix, RT, TP, rotavirus
4) primers, master mix, RT, TP, hepatitus A virus
5) primers, master mix, RT, TP, norwalk virus cDNA (copy DNA)
6-15) primers, master mix, RT, TP, samples 1-10

Reaction 1 will ensure that the enzymes, nucleotide precursors and buffers provided by Perkin-Elmer do work according to their specification. This will be demonstrated by amplification of a segment of nucleic acid provided by Perkin-Elmer. Visualization of this amplified band and all amplified bands in the experimental reactions will occur by gel electrophoresis and subsequent ethidium-bromide staining. Amplified bands will appear as pink lines in the gel upon examination with UV light. Band size will be evaluated by comparison to known lengths of nucleic acid, known as a DNA ladder, provided by Perkin-Elmer.

Reaction 2 will demonstrate that none of the reagents used in the reactions contain contaminating DNA segments which could be amplified and interpreted as false positive or that the primers themselves could cause amplification. Gel electrophoresis of this reaction (for each set of primers) should yield negative results. If positive results are obtained for any set of primers or reagents used, the primers and/or reagents will be discarded and a new set of primer or reagents will be evaluated by this procedure and if demonstrated negative, used for the experimental reactions.

Reactions 3, 4 and 5 will act as positive controls for rotavirus, hepatitis A virus and norwalk virus. Since norwalk virus cannot be grown in cell culture, cDNA (copy DNA) for norwalk virus will act as a positive control.

Reactions 6-15 will contain one each of the concentrated samples collected from the harbor.

11.3 Coliphage

A positive control for the male specific, MS-2, and a positive control for the somatic, θx174, will be run to ensure host sensitivity. For each assay negative controls, top agar and host with no virus or sample, will be run to ensure no contamination of reagents, agar, or host.

11.4 Fecal Coliform and *Enterococcus*

Replicate laboratory analyses will be done for all samples. Calculation of acceptable precision will be as in Standard Methods, 18th edition, Method 9020 B 4.

As a positive control, *E. coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 will be added to dilution buffer, filtered and cultured according to MWRA's SOP.

For negative controls, sterile buffer will be filtered, and the filters incubated on m-FC and m-*Enterococcus* media used according to MWRA's SOP. Absence of growth indicates non-contamination of buffer, filters and glassware.

12.0 SAMPLING AND ANALYTICAL PROCEDURES

This section details the specific mobilization procedures, sampling logistics, sampling protocols, and analytical techniques to be used to perform the investigation outlined in Section 7.3.

12.1 Mobilization and Sampling Logistics

Due to the weather-dependent nature of the anthropogenic virus survey task and the numerous participants, logistics are critical to meeting the task objectives. Mobilization and sampling logistics include weather forecasting, mobilization procedures, and reliable lines of communication. For wet weather sampling mobilization, a 24-hour standby notification will be issued if a determination is made based on weather observations that rainfall criteria will be met. This will be followed by a four to six-hour mobilization window once a decision has been made to conduct sampling. Lines of communication will be maintained during all phases of the survey.

12.1.1 Weather Forecasting

Each day during the annual sampling window (tentatively the month of May), the general weather outlook will be monitored by the ENSR Project Area Manager, Mr. Stephen Cibik. If rainfall criteria appear likely to be satisfied by the forecasted weather pattern, the MWRA Project Area Manager will be consulted and, if mutually agreed, the 24-hour standby notice will be issued. Within the 24-hour standby period, the forecast will be monitored on a frequent basis to support a determination that the mobilization notice be issued. Once mobilization has begun, weather patterns will continue to be monitored through initiation of sampling, and on an as-needed basis during sampling.

For dry weather surveys, conditions will be monitored on NOAA weather radio to ensure that no change from the dry conditions appears imminent. For weather conditions that require updates outside of normal working hours, ENSR will provide a contact person or persons to continue to monitor weather conditions and be available for consultation whenever necessary.

12.1.2 Mobilization Procedures

As indicated in the previous section, a 24-hour standby will be initiated by the MWRA and ENSR Project Area Managers based on the weather forecast. Once they have agreed that a survey should be undertaken, sampling teams and laboratories will be mobilized. The ENSR and MWRA Area Managers will determine whether the MWRA vessel is available for sampling, and in concert with the UNH Principal Investigator determine the availability of two sampling crews. These decisions will dictate whether the survey will require one or two days.

ENSR will contact Captain Phil Downey to prepare the *R/V QT* for sampling. The UNH Principal investigator will communicate with UNH sampling and laboratory staff. The MWRA Area Manager will be responsible for communicating with participating MWRA staff, including the MWRA Central Laboratory and Captain Rob Rabideau of the *R/V Plunger*. Telephone numbers for these participants are listed in Table 3. Confirmation of discharges from the MWRA CSO facilities at Somerville Marginal, Prison Point, Fox Point, and Commercial Point will be obtained by the ENSR Project Area Manager from Greg Alessandro.

TABLE 3

MWRA CSO Virus Survey
Points of Contact

Contact	Affiliation	Work Phone #	Home Phone #	Cellular/page #
Dr. Andrea Rex	MWRA Program Manager - Harbor Monitoring	(617) 241-6510	(617) 333-0662	
Kelly Coughlin	MWRA staff	(617) 241-6518	(617) 730-8389	
Microbiology Lab	MWRA staff	(617) 539-4300	NA	
Rob Rabideau	MWRA boat Captain	(617) 242-6000 x4762	(508) 664-1731	(617) 429-6982 (car phone)
Greg Alessandro	MWRA Somerville Marginal CSO facility	(617) 389-1685 (617) 389-6605	NA	
Greg Alessandro	MWRA Commercial Point CSO facility	(617) 265-8344 (617) 389-1685 (617) 389-6605	NA	
Lisa Wong	MWRA Central Laboratory	(617) 539-4331	(508) 664-1731	
Dr. Aaron Margolin	UNH Principal Investigator	(603) 862-2250	(603) 679-8387	(800) 225-0256 PIN # 1142740
Bob Mooney	UNH staff	(603) 862-2250	available from UNH PI	
Jason Bellner	UNH staff	(603) 862-2250	available from UNH PI	
Derek Hodgkins	UNH staff	603-862-2250	available from UNH PI	
Dr. James Blake	ENSR Project Manager	(508) 457-7900	available from ENSR Area Manager	
Mr. Stephen Cibik	ENSR Area Manager	(508) 635-9500 x3072	(508) 372-9036	
Ms. Therese Mounce	ENSR Task Manager	(508)635-3500 x3127	available from ENSR Area Manager	
Captain Phil Romano	RevLynn	NA	(617) 569-1726	(617) 945-6749

If two teams are fielded, sampling crews will assemble at the berths of the two research vessels. Sampling crew #1 will be assigned to the *R/V QT*, berthed at East Boston Pier #1 on South Bremen Street. This crew will sample the Deer Island Outfall #2 (146S), the Nut Island Outfall 102 plume (081S), Wollaston Beach (047S), Carson Beach (036S), Neponset River (041S), and Pine Neck Creek (110S, refer to Table 1 for station coordinates). Sampling crew #2 will be assigned to the *R/V Plunger*, berthed at Charlestown Navy Yard Pier No. 5. This crew will sample in the Charles River (145S), Inner Harbor (014S), Mystic River (052S), and Fort Point Channel (075S). The Carson Beach location (036S) may be sampled by crew #2 if conditions permit.

In the event that a wet weather survey is undertaken with only one sampling crew, these stations will be sampled on two successive days, with the first day covering the stations listed above for crew #1 and the second day completing the remaining stations.

Since the majority of sampling locations are close to shore adjacent to readily identifiable landmarks, sampling locations will be recorded using a combination of shoreline observations and GPS, which will be recorded in latitude and longitude. Wastewater treatment plant outfall plumes will be located visually if possible, and precise sampling locations recorded using the GPS navigation and visual bearings.

12.2 Anthropogenic Virus Sampling and Analysis Procedures

Virus collection, elution, concentration and detection techniques will be those referenced in Standard Methods for the Examination of Water and Wastewater, 18th edition, section 9510 C and 9510 G, except as noted. Negative - charged micro wound filters will be used in the collection process and viruses will be evaluated using the BGMK Cell Line.

12.2.1 Virus Collection

A sample volume of 150 L of subsurface (0 to 12 inches) water will be collected by pump into large pre-sterilized garbage barrels to which the pH will be adjusted to 3.5 by the addition of 1.0 N HCl. To this final volume, $AlCl_3$ will be added to yield a 0.0015 N solution. Sodium thiosulfate will also be routinely added as some of these outfalls may have a chlorine residual. The pump will then be turned on and the water will be pumped through the filter at no faster than 10 gallons per minute until the entire water sample has passed through the filter. To measure the actual volume of water filtered and to ensure that a flow rate of 10 gallons per minute is not exceeded, a flow meter will be placed directly after the filter to measure both gallons per minute and total gallons filtered. Once filtration is completed, the filter will be removed from its housing, placed in a labeled plastic bag, and held for elution chilled using "blue" ice packs and a cooler.

12.2.2 Disinfection of Field Equipment

All equipment will be decontaminated in the field using bleach according to Standard Methods for the Examination of Water and Wastewater, 18th edition, section 9510B. Bleach will be pumped through all hoses, housings and pumps and allowed to remain for 15-30 minutes. Following bleaching, water from the pending sampling site will be mixed with sodium thiosulfate and used to flush the entire system to neutralize all the bleach.

12.2.3 Virus Elution

Viruses will be eluted within 48 hrs using 3.0% beef extract, 90 mM glycine at pH 9.0. For this procedure, filters will be placed in a filter housing at the lab. To this, between 800-1000 ml of beef extract eluent will be added to the filter. The pH of the mixture will be checked and adjusted back to 9.0 if needed. The filter will be agitated for 10 minutes using an up and down motion which will allow the beef extract to flow inwards and outwards continuously. After 10 minutes, the filter will be removed and the eluent transferred to a one liter flask containing a stir bar.

12.2.4 Virus Concentration by Organic Flocculation

The pH of the eluent will then be adjusted to 3.5 using 1 N HCl for organic flocculation. The mixture will be allowed to mix for 30 minutes and then transferred to 4-250 ml centrifuge flat bottom bottles. The floc will be collected by centrifugation at 10,000 X g for 10 minutes. After centrifugation, the supernatant will be carefully poured out, taking care not to disturb the floc, and the supernatant suspended in a total volume of 20-30 ml of a 0.1 N solution of sodium phosphate buffer (dibasic), pH 9.0. The resuspension process will be done by pipetting the floc up and down in a 10-25 ml pipet for no more than 10 minutes. After the first minute of resuspension, the pH of the solution should be checked and if needed, adjusted back to 9.0. After 10 minutes of resuspension, the solution will be returned to pH 7.0-7.4 using 1 N HCl. To remove any undissolved particles, the sample will be centrifuged for 10 minutes at 10,000 X g. The supernatant will be carefully removed and divided into two aliquots, one aliquot having twice as much as the other, i.e., 2/3 in one and 1/3 in the other. The samples will be labeled and stored at -20°C for analysis by cell culture.

12.2.5 Detection of Virus Using the BGMK cell line.

Concentrated water samples will be defrosted and brought to 37°C prior to application to cells. Confluent monolayers of 75 cm^2 flasks of BGMK cells will be inoculated with 1 ml of the sample per 25 cm^2 of cells, or 3 ml of the sample. Once samples have been inoculated, they will be incubated at 37°C for 1-2 hours with rocking every 15 minutes. Samples will then be observed for potential toxicity to the BGMK cell line. Potential toxicity will be determined by microscopically examining the cells to determine their state of health. Sample toxicity will be

confirmed if detrimental effects are observed (e.g., cell rounding, floating, or death). Samples found to exhibit toxicity to the BGMK cell line will be inoculated for only one hour, whereas samples with no observed toxicity will be inoculated for two hours.

After inoculation, maintenance media (MEM with 2% fetal bovine serum) will be added and the flasks will be incubated at 37°C for 10-14 days. Flasks will be examined daily for cytopathogenic effects (CPE). CPE will be determined by examining the cells with an inverted microscope. CPE, characteristic of an unhealthy cell, include symptoms that can range from covert (not easily detected by the human eye) to overt (such as death of the cell). Other types of CPE include the detrimental effects noted above (i.e., rounding and floating). All flasks demonstrating CPE will be placed at -20°C and at the end of the 10-14 day incubation, all additional flasks not demonstrating CPE will also be frozen. To confirm the presence of virus and to detect virus which was not detected on the first passage, frozen cells (both those demonstrating CPE and those not demonstrating CPE) will then be removed from the freezer and thawed rapidly in a 37°C water bath. This freeze-thaw process will be repeated one more time to ensure complete cellular lysing. Cellular debris will be removed by centrifugation of the cellular supernatant and lysate at 10,000 x g for 10 minutes. Supernatant will be removed and 1 ml of each supernatant will be used as an inoculant for a second passage on BGMK cells. For the second passage, 1 ml of the supernatant/lysate will be place on confluent monolayers of BGMK cells housed in 25 cm^2 flasks and then examined daily for 10-14 days.

All samples which were positive for CPE on the first passage must demonstrate CPE on the second passage to be considered positive for virus. Those samples demonstrating CPE on the first passage but not on the second passage will be considered negative for virus. Those samples which were negative on the first passage but positive on the second passage will be passed to a third confluent monolayer of cells using the freeze-thaw technique described above. The presence of virus will be evaluated as just described.

12.2.6 Detection of Virus Using PCR

Detection of Hepatitis A virus, Norwalk virus and Rotavirus will be done by the Polymerase Chain Reaction (PCR). With this method, between 1-3 ml of the concentrated sample (post organic flocculation) will be purified by use of a sephadex/chelex spun column. The purified sample will then be processed according to the methods of Abbaszadegan et al. (1993). Briefly, between 40-100 ul of the sample will be added to each of the three tubes to which has been added the PCR reagents and the appropriate viral primers (HAV, Norwalk or Rotavirus). Samples will be allowed to cycle for 40 cycles in a Perkin Elmer thermal cycler. Results will be visualized by gel electrophoresis looking for the amplified segment of viral nucleic acid.

12.2.7 Calculation of Virus Concentration

12.2.7.1 Total Cultivatable Viruses Using the BGMK Cell Line

Total cultivatable virus concentration will be calculated by the most probable number of infectious units method (MPNIU). In this method, Thomas's equation is used to calculate the number of infectious units. The equation is as follows:

For a given dilution yielding both positive and negative cultures,

$$\text{MPN}/ml = \frac{P}{\sqrt{NQ}}$$

where P = total number of positive cultures
where N = total ml sample inoculated in all cultures
where Q = total ml sample in all negative cultures

12.2.7.2 PCR

PCR results will be reported as the presence or absence of amplified viral genome. There will be no quantification of the original concentration of viral nucleic acid.

12.3 Bacteriophage Sampling and Analysis Procedures

Bacteriophage will be assayed using the eluted sample prepared for cultivatable anthropogenic virus and PCR screening.

Phage will be detected according to Standard Methods for the Examination of Waters and Wastewater, Section 9211D with the following exceptions; both somatic and male specific phages will be screened and a double agar overlay will be used instead of the single agar plate method. In this method, sample is mixed in separate tubes containing melted top agar with either *E. coli* C or *E. coli* C-3000, which is in log phase. The mixture is then poured into a petri dish containing bottom agar. The agar is allowed to harden and the plates are inverted and incubated at 37^0 C for 16-24 hours. Plaques are then counted as clear zones. Male and somatic bacteriophages will be enumerated following the protocol in Standard Methods and will be reported as plaque forming units (PFU) per ml of receiving water. In brief, this method counts individual plaques, which are clearing zones obtained due to the death of bacteria in the confined area where the virus is replicating.

12.4 Indicator Bacteria Sampling and Analysis Procedures

Samples for analysis of fecal coliform and *Enterococcus* will be collected into sterile sample containers, stored in coolers with icepaks at <10°C, and analyzed within six hours of collection using Standard Methods membrane filtration procedures detailed in MWRA's Laboratory Standard Operating Procedures. M-FC agar with rosolic acid addition will be used for the enumeration of fecal coliform, and m-*Enterococcus* agar will be used for counting *Enterococcus* colonies.

12.5 Field Measurements of Water Quality Parameters

MWRA staff will perform and record field measurements of water depth, temperature, conductivity, salinity, dissolved oxygen and Secchi depth following MWRA Standard Operating Procedures. Daily calibration of field instruments will be according to MWRA's SOP.

13.0 SAMPLE CUSTODY PROCEDURES

Sample custody will be maintained through field log books, laboratory record book, virus field sample data sheets (Figure 3), and chain of custody forms for virus (Figure 4) and bacteriological samples (Figure 5). All original virus field sample data sheets will be kept in a project Sample Log notebook. The sampling crew chief will maintain custody of all samples on board the vessel. The sampling crew chief will record in the field log book event information such as station, location, sampling time, water depth, and weather and sea conditions (wind direction, sea state, etc).

Samples will be collected by trained field and laboratory personnel with complete sample identification filled in on the field sample data sheets and chain of custody forms. Sample log information and chain-of-custody forms will include the event and sample IDs. Event codes will utilize a five-character code including the task, year, and event number (e.g., V9501 = Virus survey sample taken in 1995 during the first sample round). Sample IDs will indicate the four-character station location (e.g., 075S for Fort Point Channel), one character visit number, and three-character sample code (DV1 or WV1 = dry weather or wet weather virus sample number 1, respectively; DB1 or WB1 = dry weather or wet weather bacteriological sample number 1, respectively). For example, 075S1DV = Station 075S, visit one, dry weather virus sample number 1. The visit number will only change if a station must be re-occupied on a subsequent occasion to complete sampling.

Subsequent coding would then indicate subsample type and number, as follows:

- TCV = total cultivatable virus
- HAV = hepatitis A virus
- ROT = rotavirus

Sampling Firm:		Sample Site*	
Samplers Name:		Sample ID*	

Sample Site & Sample ID will appear on your report for identification purposes.

Analysis Requested	1. ☐ *Giardia/Cryptosporidium* 2. ☐ Microscopic Particulate Analysis 3. ☐ Filtration Plant Performance Evaluation(includes 1 and 2) 4. ☐ Viruses

Water Type	☐ Raw ☐ Finished ☐ Other ____
Water Source	☐ Spring ☐ Infil. Gallery ☐ River ☐ Lake/Reserv./Pond ☐ Well - Type: ____

Treatment Chemicals	☐ Chlorine ☐ Alum	☐ $KMnO_4$ ☐ Carbon	☐ Polymer - Type: _ ☐Other ____
Filtration Type	☐ Rapid sand ☐ Pressure filter ☐ Cartridge	☐ Mixed media	☐ Slow sand ☐ Other ____

Sampling Data	Start	End
Date:		
Time:		
Turbidity:		
pH:		
Meter Reading (gallons/liters/cubic feet):		
Flow Rate (manual calibration):		

Total Volume Sampled:		Units: ☐ gallons ☐ liters ☐ cubic feet

LAB DATA (for lab use only)	Packed pellet volume: Color:	Initials/date
Date received:	Amt. pellet floated:	
Filter type:	Amt. packed pellet after flotation:	
Filter color:	Floc present (FES finished only):	
Giardia: Unconfirmed: Confirmed:	G/C Volume Assayed:	
Cryptosporidium: Unconfirmed: Confirmed:	Analyst/Date: Confirmed by:	
Chroococcales present:	Autofluorescence level - debris: organisms:	

Lab Sample No.: ____________________

FIGURE 3
Field Sample Data Sheet

Page ___ of ___

CHAIN OF CUSTODY RECORD

Submitted By:		Report To:
Phone: Fax:		Phone: Fax:
Project Name		Invoice To:
Job Site		
P.O. Number		Phone: Fax:

Sample Identification *	Sample Collection		Sample Type (check one)		Sample Matrix (check one)				Analysis Requested
	Date	Time	Grab	Composite	Water	Soil	Aerosol	Other	

* Sample ID should match ID written on the sample containers and data sheets. Sample ID will appear on the report for identification.

Relinquished By (signature)	Date/Time	Received By (signature)	Date/Time
Field Comments:		**Lab Comments:**	

White – To accompany samples   Yellow – Lab Copy   Pink – Sampler Copy

FIGURE 4
Virus Sample Chain of Custody

DATE:

MWRA CHAIN OF CUSTODY
FOR
MISCELLANEOUS SAMPLES

PAGE: 1 OF 1

SAMPLE LOC.	SAMPLE ID	DATE COLLECTED	TIME	SAMPLE LOCATION DESCRIPTION	PLANT	TYPE	/ TESTS	/ PRESERVATIVE	/ BOTTLE
						G C CG GS	/ FCOLSWMFL	/	/ P G S
						G C CG GS	/ ECOCAQMFL	/	/ P G S
						G C CG GS	/ FCOLSWMFL	/	/ P G S
						G C CG GS	/ ECOCAQMFL	/	/ P G S
						G C CG GS	/ FCOLSWMFL	/	/ P G S
						G C CG GS	/ ECOCAQMFL	/	/ P G S
						G C CG GS	/ FCOLSWMFL	/	/ P G S
						G C CG GS	/ ECOCAQMFL	/	/ P G S
						G C CG GS	/ FCOLSWMFL	/	/ P G S
						G C CG GS	/ ECOCAQMFL	/	/ P G S
						G C CG GS	/ FCOLSWMFL	/	/ P G S
						G C CG GS	/ ECOCAQMFL	/	/ P G S
						G C CG GS	/ FCOLSWMFL	/	/ P G S
						G C CG GS	/ ECOCAQMFL	/	/ P G S
						G C CG GS	/ FCOLSWMFL	/	/ P G S
						G C CG GS	/ ECOCAQMFL	/	/ P G S
						G C CG GS	/ FCOLSWMFL	/	/ P G S
						G C CG GS	/ ECOCAQMFL	/	/ P G S
						G C CG GS	/ FCOLSWMFL	/	/ P G S
						G C CG GS	/ ECOCAQMFL	/	/ P G S
						G C CG GS	/ FCOLSWMFL	/	/ P G S
						G C CG GS	/ ECOCAQMFL	/	/ P G S

COMMENTS: ______________________________

____

____

____

SAMPLED BY: ______________ DATE: ______________

RELINQUISHED TO: ______________ DATE: ______________

RECEIVED BY: ______________ DATE: ______________ (AT LAB)

FIGURE 5
Bacteriological Sample Chain of Custody Record

- NOR = Norwalk agent
- PHA = phage
- FCO = fecal coliform
- ENT = *Enterococcus*

Virus samples filtered in the field will be transported to the Water Borne Disease Laboratory at UNH by sampling personnel, who will be responsible for their custody from elution, storage, and analysis, minimizing the number of individuals involved in sample transfer. Analytical procedures and data management will be documented on individual data sheets used for analyses of cultivatable viruses, PCR screening, and bacteriophage assays (Figures 6 through 8). After processing, the remaining eluted sample will be archived frozen (-20°C) until the end of the study.

Transfer of bacteriological samples will be documented on the chain-of-custody forms, which will be signed and dated by both the person relinquishing the samples as well as the recipient. One sheet of the multiple-page form will be retained by the crew chief, who will forward the document to the Project Area Manager. The remaining sheets will accompany the samples to the laboratory for subsequent sample transfer.

14.0 CALIBRATION PROCEDURES AND PREVENTATIVE MAINTENANCE

All equipment associated with virus and bacteriological sampling and analyses (pumps, flowmeters, pH meters, analytical balances, and incubators) will be calibrated and maintained according to manufacturer's specifications. An equipment logbook will be maintained to document periodic maintenance of equipment. Proper function of navigational equipment will be checked by confirmation of readings at reference points (berthing area, Deer Island Light, and the radar reflector adjacent to the Nut Island Outfall 102) during each survey.

15.0 DOCUMENTATION, DATA REDUCTION, AND REPORTING

15.1 Data Collection and Reduction

To ensure accurate collection of data and a permanent record of all data the following procedures will be followed:

- all field data will be recorded in ink on field sample data sheets, field logbook, and chain-of-custody forms;
- all laboratory data will be recorded with permanent ink in a bound notebook or on standardized forms; and
- all QC data (precision, accuracy) will be recorded in laboratory notebooks.

Enteric Virus Sample Data Sheet

Client identification:	Type of sample:	Volume of sample:
Client id number:	Water type:	Type of test requested: Enteric viruses
Sample identification	Eluted & concentrated by:	Date eluted: Volume of eluent: ______ ml
UNH sample #:	Tissue culture tech:	Volume of concentrate: ______ ml
Logged in by:	Date of primary passage:	Date of confirmation passage:
Date Rec'd:	Date of completion:	Final results: MPNIU =

Primary Passage

Dilution Factor: ______

Day #	Flask 1	flask 2	flask 3	flask 4	Controls		
					(-)	(-)	(+)
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

(-) no CPE
1+ 25% CPE
2+ 50% CPE
3+ 75% CPE
4+ 100% CPE

Confirmation

Day #	Flask 1	flask 2	flask 3	flask 4	Controls		
					(-)	(-)	(+)
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

FIGURE 6

Enteric Virus Sample Data Sheet

PCR Sample Data Sheet

Client identification:	Type of sample:	Volume of sample:
Client id number:	Water type:	Type of test requested:
Sample identification	Eluted & concentrated by:	Date eluted:
UNH sample #:	PCR tech.	Volume of eluent: ______ ml Volume of concentrate: ______ ml
Logged in by:	Date of assay	
Date Rec'd.:	Date of completion:	

Gel electrophoresis lane #	Volume loaded onto gel in ul	Virus type/Amplification product present or absent	Virus type/Amplification product present or absent	Virus type/Amplification product present or absent	Controls		
					(-)	(-)	(+)
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Final Results:

Virus: ____________________ Nucleic acid present in sample: ________

Virus: ____________________ Nucleic acid present in sample: ________

Virus: ____________________ Nucleic acid present in sample: ________

FIGURE 7
PCR Sample Data Sheet

Bacteriophage Sample Data Sheet

Client identification:	Type of sample:	Volume of sample:
Client id number:	Water type:	Type of test requested: Bacteriophage
Sample identification	Eluted & concentrated by:	Date eluted:
UNH sample #:	Phage tech:	Volume of eluent: ____________ ml Volume of concentrate: ____________ ml
Logged in by:	Date of assay:	Final results male phage: ____________ PFU/
Date Rec'd.:	Date of completion:	Final results somatic phage: ____________ PFU/

Male phage

Dilution factor: ____________

Plate #	PFU	Controls		
		(-)	(-)	(+)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Somatic phage

Plate #	PFU	Controls		
		(-)	(-)	(+)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Volume of innoculant: ____________ ml

Size of plate: ____________ ml

FIGURE 8
Bacteriophage Sample Data Sheet

Experimental results will be processed and summarized in tabular and/or graphical form. Summary graphs and tables will be prepared and reviewed by the project directors to observe noteworthy trends or inconsistencies. Summary graphs and tables will be maintained for subsequent use in preparing annual reports.

Laboratory data books will have a carbon so that a file copy of raw data can be placed in safe storage in the event that the book is lost or destroyed. Summary data files will be put on IBM compatible PC floppy disks so that statistical analysis and data management can be done. Conventional statistical analyses such as students-t, Chi-Squared and ANOVA will be routinely performed as appropriate on data collected in this study. At the end of the project, all bound data books, and floppies will be stored by the Principal Investigator for at least 7 years.

15.2 Result Reporting

Two formats will be used to report the results of Task 28 to MWRA:

(1) Data submitted for inclusion in the Harbor Studies Database
(2) Data presented in Annual Virus Data Reports

15.2.1 Harbor Studies Database

Only data that have been designated as final by the Project Area Manager will be loaded into ENSR's copy of the Harbor Studies Database. All data will be loaded into the database by ENSR data management staff. Upon receipt, each diskette will be logged in and assigned a unique log in identifier. Any changes or additions to data, necessary for loading into the database, will be made using well-documented scripts that indicate the original values. The original diskette, scripts, and data-loading documentation will be filed at ENSR according to the log in identifier. The data sources notebook will contain the chain-of-custody forms and data entry information.

15.2.2 Annual Virus Data Reports

Virus Data Reports will be submitted to MWRA in both hard-copy and electronic forms. Included will be all sample collection information summarized from the Survey Reports from each sampling event. Data will be presented in tables containing the results of all individual sample analyses plus QC data.

16.0 DATA VALIDATION

All data reported for this project will be reviewed to check for errors in transcription, calculation, or computer input by the technical staff of the appropriate laboratory. The validation procedures that will be performed are:

- 100% of data that are hand-entered into a database or spreadsheet will be verified for accuracy either by (1) printing the spreadsheet and proofreading against the original hand entry or by (2) duplicate entry into the database and comparison of the entries to detect any differences. These tasks will be carried out by two people and documented for each data set.
- All manual calculations will be checked for accuracy by a second staff member.
- Calculations performed by software will be checked by the technical staff member at a frequency sufficient to ensure the accuracy of the calculations. All data-reduction algorithms will be verified prior to final data submission.
- Subsets of the analytical data will be reviewed by in-house or subcontractor data validators. The data will be reviewed for adherence to analytical protocols and to pre-established criteria (e.g., for holding times, surrogate recoveries, equipment calibration, laboratory duplicates, blank contamination).
- Database staff will check the received data and associated documentation for completeness, freedom from errors, and technical reasonableness.
- All new software developed for this task will be validated before entry of data.

The ENSR Project Area Manager will be responsible for validation of all data generated by ENSR to ensure that the data are accurate, complete, and scientifically reasonable. Subcontractors will be responsible for conducting similar data validations. As an additional data validation step, ENSR Project Area Manager will review all subcontractor data for technical reasonableness.

17.0 PERFORMANCE AND SYSTEM AUDITS

This project will be monitored by the Project QA Director. Tabular and graphic data reported in deliverables and associated raw data generated by ENSR will be reviewed by the Project QA Director or his/her designee. Raw data will be reviewed for traceability, accuracy, completeness, and proper documentation.

All deliverables generated during the course of this project will be submitted to an internal review prior to delivery of drafts to MWRA.

Audits of the subcontractor laboratory data-collection programs will be the responsibility of the subcontractor. During the time work is in progress, an inspection will be conducted by the subcontractor QA officer or their designee to evaluate the laboratory data-production process. All data must be reviewed by the Subcontractor QA Officer prior to submission to the ENSR Project Area Manager and must be accompanied by a signed QA statement that describes the types of audits and reviews conducted and any outstanding issues that could affect data quality.

Performance audits, procedures used to determine quantitatively the accuracy of the total measurement system or its components, will be the responsibility of subcontractor laboratories and may include internal performance evaluation samples and participation in external certification programs.

18.0 CORRECTIVE ACTION

Identification of problems regarding technical performance is the responsibility of all staff members working on this project. Responsibility for overall conduct of the project, including schedule, costs, and technical performance lies with the ENSR Project Manager. The Project Manager is responsible for identifying and resolving problems that (1) have not been addressed promptly or successfully at a lower level, (2) influence other components of the project, (3) require changes in this CW/QAPP, or (4) require consultation with ENSR management or with MWRA.

Technical problems relating to sample collection in the field (schedule changes, modifications to the sampling plan, etc.) will be resolved through discussion with the MWRA Area Manager, the ENSR Project Area Manager, and the UNH Principal Investigator. Problems relating to the overall successful completion of the project will be reported to the MWRA Task Manager in a timely manner for discussion and resolution between the ENSR and MWRA managers.

Identification of problems and corrective action at the laboratory level will be resolved by the laboratory staff. Issues that affect schedule, cost, technical performance, or data quality will be reported to the ENSR Project Area Manager or the ENSR Project Manager. They will be responsible for evaluating the overall impact to the project and for discussing corrective actions with the MWRA Task Manager.

A QA/QC Corrective Action Log will be maintained by the Project QA Director and submitted to MWRA at quarterly intervals. The log will include documentation of QA/QC activities as they

occur, descriptions of the methods and procedures recommended to prevent the problem from reoccurring, and verification that these actions have corrected the problem.

19.0 REPORTS

Reporting under this task will be through submittal of survey plans, survey reports, and annual virus data reports. Survey Plans will describe survey dates, vessel(s), participating personnel, anticipated schedule of operations, locations of proposed activities, and any deviations from the CW/QAPP known in advance of the survey. Survey reports will document actual survey dates and operations, interim results, problems encountered, corrective actions, and recommendations for potential modifications to the CW/QAPP. The annual virus report will describe methods used in the surveys and any deviations from the CW/QAPP, report results, and provide a discussion of the data.

20.0 REFERENCES

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