Combined work/quality assurance project plan (CW/QAPP)

for

Anthropogenic Virus Survey: 2000-2001

Massachusetts Water Resources Authority

Environmental Quality Department
Report ENQUAD MS-066

Citation: Stevens, KB, Campbell, JF, Margolin, AB, Abramson, SL, Trulli, W, Baptiste-Carpenter, EM. 2001. Combined Work/Quality Assurance Project Plan (CW/QAPP) for Anthropogenic Virus Survey: 2000-2001. Boston: Massachusetts Water Resources Authority. Report ENQUAD MS-066. 50 p.

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

for

Anthropogenic Virus Survey: 2000-2001

Task 28
MWRA Harbor and Outfall Monitoring Project
Contract No. S274

Submitted to

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March 2001
MS-066

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

for

ANTHROPOGENIC VIRUS SURVEY: 2000-2001

Task 28
MWRA Harbor and Outfall Monitoring Project
Contract No. S274

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Ms. Rosanna Buhl Battelle Project QA Officer	Date
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1.0 PROJECT NAME

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Task 28
MWRA Harbor and Outfall Monitoring Project

2.0 PROJECT REQUESTED BY

Massachusetts Water Resources Authority

3.0 DATE OF REQUEST

November 5, 1997

4.0 DATE OF PROJECT INITIATION

November 5, 1997

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7.0 PROJECT DESCRIPTION

7.1 Objectives and Scope

Water quality in Boston Harbor and Massachusetts Bay continues to be a major concern for members of the marine and drinking water communities, the general public, and regulators. These coastal resources are crucial to public health, economic development, and recreational opportunities. Boston Harbor and Massachusetts Bay are continually threatened by contaminants from treated and untreated domestic and industrial wastes, past discharges of sewage sludges, polluted surface waters and combined sewer overflows (CSOs). A wide variety of human enteric virus pathogens including those causing infectious hepatitis and acute gastroenteritis are associated with these sources of pollution.

The purpose of this work is to identify the potential for hazards to public health due to anthropogenic viruses in Combined Sewer Overflow (CSO) receiving water and wastewater treatment plant (WWTP) receiving water, including Boston Harbor, its tributary rivers, and Massachusetts Bay in the vicinity of the new outfall. The following objectives have been established for this project area:

- identify sources of anthropogenic viruses in receiving water;
- develop baseline data for evaluation of potential improvements in water quality resulting from CSO remediation and WWTP outfall relocations; and
- develop correlative data among bacterial sewage indicators, anthropogenic viruses, and viral indicators.

The scope of this task consists of sampling of receiving waters influenced by CSO and WWTP discharges, and treatment plant influents and effluents. Identification of sources will primarily be demonstrated by the proximity of positive results to known discharges. Confirmatory sampling may be conducted in the future.

The MWRA Harbor and Outfall Monitoring Project (Task 28) includes analysis of samples collected from Boston Harbor and in Massachusetts Bay. The collection of samples for viral analysis will be conducted by MWRA in Boston Harbor and its tributaries, and by Battelle in Massachusetts Bay. MWRA will be responsible for collection of bacterial indicators in each water body. The 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 potential risks to humans from recreational or shellfishing activities in Boston Harbor, its tributaries, and Massachusetts Bay. The data will be used to assess changes in levels of anthropogenic viruses and their indicators resulting from MWRA pollution abatement projects. Data collected in CSO and WWTP influent and effluent will help in understanding the effects of treatment on anthropogenic viruses in MWRA discharges. Finally, data will be used to increase the understanding of the relationships among fecal bacteria indicators, viral indicators (bacterial phages and nucleic acid assays), and cultivatable viruses.

7.3 Technical Approach

The general project design is presented below, however each area of study is discussed in greater detail in subsequent sections. Four areas of study are included in this project:

1. Boston Harbor and its tributary rivers, which focuses on the effects of combined sewer overflows and wet weather;

2. Charles River and the Cottage Farm CSO treatment facility, measuring wet weather impacts;

3. Deer Island Wastewater Treatment Plant, measuring effects of treatment on viruses;

4. Massachusetts Bay outfall study, post- discharge effects on presence of viruses and their indicators.

Surveys in the Boston Harbor area, Charles River and Cottage Farm CSO treatment facility, and Deer Island Wastewater Treatment Plant (1-3) will be conducted by MWRA. Surveys in Massachusetts Bay (4) will be conducted by Battelle. Samples delivered to the MWRA Central Laboratory will be analyzed for fecal coliforms and *Enterococcus*. Samples delivered to the University of New Hampshire (UNH) Waterborne Disease Laboratory will be analyzed for cultivatable strains of viruses[1] (Enteroviruses, Adenovirus 40/41, Rotavirus, and Astrovirus), male-specific bacteriophages, and somatic bacteriophages.

The distribution and total number of samples that will be collected by MWRA and Battelle staff annually from 2000-2001 are shown in Table 1.

7.4 Parameters Measured

The anthropogenic virus surveys will provide a comprehensive assessment of human enteric viruses, virus indicators, and fecal indicator bacteria in wastewater and receiving waters. Each survey will collect data for parameters listed in Table 2. Sampling and analyses[1] will include:

- detection of cultivatable strains of viruses (Enteroviruses [Poliovirus, Coxsackie Virus, and Echovirus], Adenovirus 40/41, Rotavirus, and Astrovirus) using the ICC-nPCR assay, with results reported as presence/absence ;
- detection of both male-specific and somatic bacteriophages; and
- numeration of fecal coliform and *Enterococcus* bacteria densities (by MWRA).

[1]The viral parameters monitored during Anthropogenic Virus Surveys was modified to the current regime in April, 2000. See Tilton, *et al*. (1999) for the parameters monitored during the first two years of the HOM3 program.

Table 1. Total Samples to be Collected Annually, 2000-2001.

Staff	Area of Study	# of Locations	Weather Condition (# Surveys)	# Samples
MWRA	Harbor and Rivers	6	Dry weather (3)	18
MWRA	Harbor and Rivers	6	Wet weather (3)	18
MWRA	Charles River and Cottage Farm	8	Wet weather (1)	8
MWRA	Charles River and Cottage Farm	6	Dry weather (1)	6
MWRA	Deer Island WWTP	1	-	10
Battelle	New outfall in Massachusetts Bay	5[a]	six surveys: Surface & Sub-pycnocline samples (surface only at 1 location)	46
TOTAL				**106**

[a] Samples from only one depth will be collected during surveys when no pycnocline is present.
Number of samples = (2 surveys × 5 stations/survey) + (4 surveys with 9 samples/survey) = 46

Table 2. Monitoring Parameters for Anthropogenic Virus Surveys.

Parameter	Sample Container	Preservation	Analysis Method	Holding Time	Units
Cultivatable Viruses (UNH)	4 L Cubitainer	4 ± 2°C (cooler with ice)	ICC-nPCR	process within 72 hrs, indefinite at -80°C	presence/absence
Bacteriophages (UNH)	1 L bottle	4 ± 2°C (cooler with ice)	EPA1601	process within 72 hrs, indefinite at -80°C	presence/absence
Fecal coliform (MWRA)	sterile plastic specimen cup	4 ± 2°C (cooler with ice)	SM 9222 D	12 hrs	fecal coliforms per 100 mL
Enterococcus (MWRA)	sterile plastic specimen cup	4 ± 2°C (cooler with ice)	SM 9230 C	12 hrs	enterococci per 100 mL
Temperature[a, f]	*in-situ*	NA	probe[b]	NA	°C
pH[f] (Harbor only)	*in-situ*	NA	probe	NA	standard pH units
Conductivity[a, f]	*in-situ*	NA	probe[b]	NA	mS/cm
Salinity[a,c, f]	*in-situ*	NA	probe[b]	NA	PSU
Dissolved Oxygen[a, f]	*in-situ*	NA	probe[b]	NA	mg/L
Secchi Depth	*in-situ*	NA	Secchi Disk[e]	NA	m
Transmissometry[a, f]	*in-situ*	NA	probe[b]	NA	m-1
Chlorine Residual[d]	sterile polypropylene container	NA	colorimetric method	NA	mg/L

Notes:
[a] Massachusetts Bay *in-situ* hydrographic and sensor data are described in Albro *et al*, 1998.
[b] Probes are described in Albro *et al*, 1998.
[c] Density is reported as a calculated value.
[d] Deer Island and Cottage Farm
[e] Lind, 1974
[f] Boston Harbor hydrographic data measured using Hydrolab Data Sonde 4.

NA Not applicable
PCR Polymerase Chain Reaction method
PSU Practical Salinity Unit
SM Standard Methods
mS micro Sieman

Surface receiving waters (0-12 inches) will be collected in sterile 4 L cubitainers for viral analyses and 1 L bottles for bacteriophage analyses. Containers will be transported on ice in coolers to the Waterborne Disease Laboratory at UNH for subsequent concentration and analysis.

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 as needed.

7.4.1 Boston Harbor and Tributary Rivers

As shown in Table 1, a total of three dry weather surveys and three wet weather surveys are planned for each year of 2000-2001. A total of 36 samples will be analyzed annually from viral surveys. Collection will occur during both warm and cool months.

7.4.1.1 Criteria for Selection of Sampling Locations

Sampling locations in the Boston Harbor area have been selected by MWRA to provide data on areas where the highest potential for health risk exists. 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 these sampling locations:

- revisit sampling stations which produced positive screening results during the 1989 pilot study;
- provide coverage in proximity to bathing beaches;
- provide coverage in areas of shellfish resources.

Assuming that viral densities are positively correlated with fecal coliform densities (CDC, 1991), the existing MWRA data for CSO receiving water segments (MWRA, 1994a,b) were reviewed to identify areas of highest fecal coliform loading from CSOs, storm water 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.

7.4.1.2 Sampling Locations

Based on the study objectives and criteria presented above for sampling location selection, the sampling locations listed in Table 3 were identified by MWRA for inclusion in the HOM3 Boston Harbor anthropogenic virus field studies. Each of these proposed locations is discussed in detail below.

Charles River at Stony Brook. 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, primarily sailboarding and boating. Data collected by MWRA between 1995-1997 show that existing water quality near the Stony

Brook outfall does not meet the swimming 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.

Table 3. MWRA Boston Harbor Anthropogenic Virus Survey Sampling Locations.

	Coordinates		
MWRA Location Code	**Latitude Degrees**	**Longitude Degrees**	**Location Description**
145S	42.3597	-71.0930	Charles River at Stony Brook
014S	42.3705	-71.0515	Mouth of Charles River
052S	42.3938	-71.0758	Mystic River Below Earhart Dam
019S	42.3590	-71.0448	Inner Harbor at Mouth of Fort Point Channel
036S	42.3265	-71.0458	Carson Beach Bathhouse
047S	42.2689	-71.0011	Wollaston Beach

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 storm water 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.

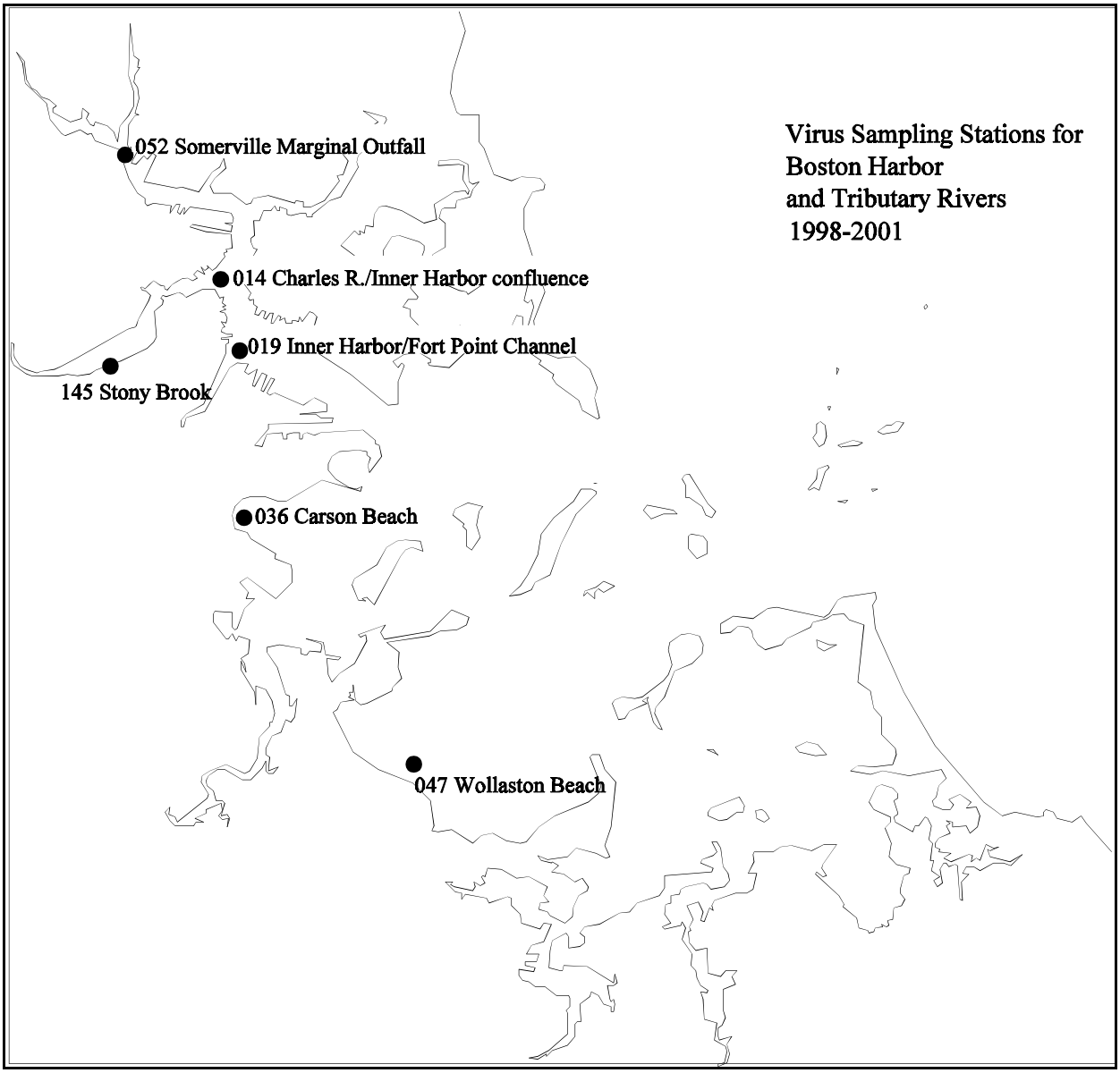

Figure 1. Boston Harbor and Tributary Rivers Sampling Locations.

The discharge from MWR023 is considered the largest single source of most pollutants to the lower Charles River (MWRA, 1994a). During a 3-month storm (1.84 inches of rainfall in 21 hours), MWR023 discharges approximately 340,000 m^3 of storm water and creek baseflow and approximately 38,000 m^3 of combined sewage (MWRA, 1994a). Dry weather flows do not contain any storm water 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.

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 of inner Boston Harbor, potential hazards to human health are associated primarily with recreational boating. Both the swimming and boating standards are sometimes exceeded after rainfall events (Leo *et al*., 1994).

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 (Leo *et al.,* 1994).

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 (Leo *et al.,* 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 (Leo *et al.,* 1994). 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 under HOM3 to assess the influence from the CSO 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 classified as SB. 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 storm water. 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 six days required for a return to background levels after a 3-month storm. The CSO Facility Plan calls for construction of a screening and chlorination facility to treat flows from BOS070.

During the first year of virus monitoring (1995), sampling was conducted at MWRA Monitoring Station 075S, located at the head of Fort Point Channel. In 1996, this location was changed to station 019S because of interference by construction of the Central Artery/Tunnel. 019S is at the mouth of Fort Point Channel.

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 located in Pleasure Bay and Carson Beach each 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 and storm water discharges are expected to be eliminated along the beaches under future planning scenarios (MWRA, 1994b).

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.

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 seven and eight, located just southeast of Wollaston Yacht Club.

7.4.1.3 Criteria for Sampling

Several criteria were considered during development of sampling logistics of the MWRA virus/fecal CSO sampling program: (Rex, 1989; Rex, 1993; Leo *et al.*, 1994)

- antecedent rainfall;
- rainfall depth and intensity;
- time lag prior to initiation of sampling;
- maximum allowable sampling window after rainfall event; and
- tidal stage (Rex, 1993).

MWRA will conduct sampling during both dry and wet weather. In the case of dry weather sampling which is intended to quantify baseline water quality conditions, rainfall and resultant CSO or storm water discharges can potentially influence water quality and are avoided. Conversely, significant amounts of

antecedent rainfall can result in reduced densities of viruses discharged by CSOs due to in-system dilution. Therefore, antecedent rainfall criteria have been established for this sampling program. This includes 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.

For wet weather sampling, minimum rainfall criterion was necessary to ensure that sufficient CSO discharges would occur to warrant mobilization and sampling. Based on information presented 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.

MWRA 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.

7.4.2 Charles River and Cottage Farm CSO Treatment Facility

Extensive virus monitoring of the Charles River and the Cottage Farm CSO Treatment Facility will be conducted to meet two objectives:

1. To determine the effect of untreated and disinfected CSO discharges on the presence of anthropogenic viruses in the Charles River.

2. To determine the relationship of conventional bacterial indicators and bacteriophage indicators to the presence of viruses in the Charles River and in Cottage Farm effluent.

Planned MWRA CSO remediation in the Charles River includes disinfection of almost all remaining CSO discharges into the river (proposed completion in 2001; MWRA, 1994b). There is concern regarding the ability of CSO disinfection to adequately reduce the viruses present in combined sewage, even if fecal coliform levels meet standards. MWRA has designed this study to discover whether or not pathogenic viruses are present at the control site upstream of CSOs, at sites affected by undisinfected CSO discharge, and at sites affected by disinfected CSO discharge. Previous sampling in the Charles River near the Stony Brook outfall has, like other locations in Boston Harbor and its tributary rivers, shown the presence of pathogenic viruses (Rex, 1993).

The virus sampling described here will measure anthropogenic viruses, bacteriophages and bacterial indicators during wet and dry weather:

- at a location upstream of all CSOs for background levels;
- in areas affected by untreated CSO;
- in locations affected by Cottage Farm treated CSO.

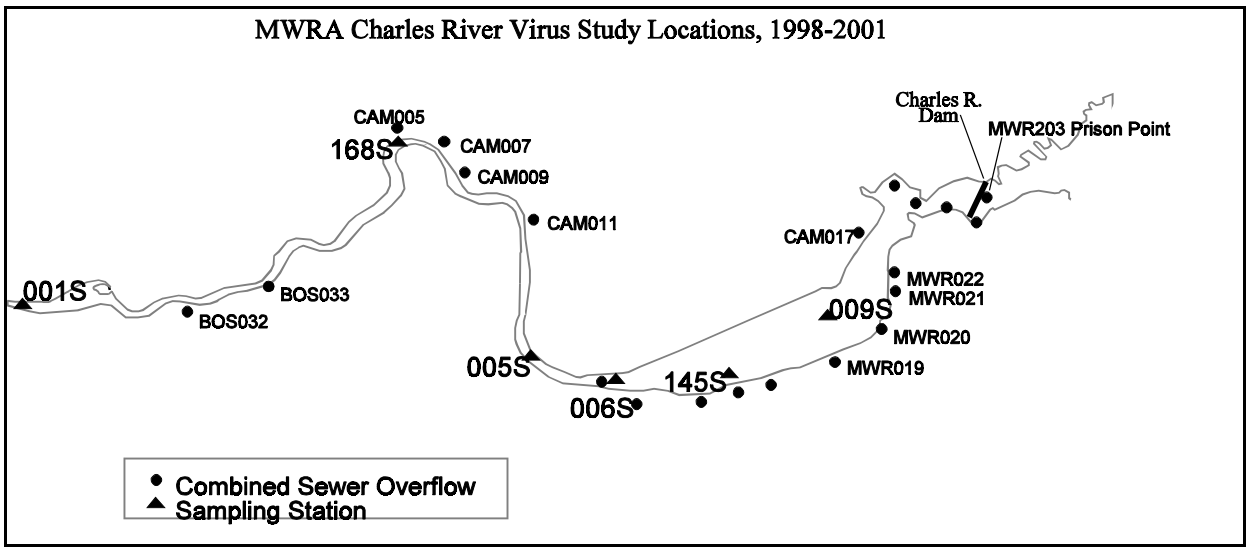

Figure 2. Charles River Sampling Locations.

In addition, anthropogenic viruses, bacteriophages, bacterial indicators and total chlorine residual will be measured during wet weather sampling events:

- in Cottage Farm influent;
- in Cottage Farm effluent; and
- at Cottage Farm plume.

Figure 2 illustrates the receiving water locations for the Charles River and Cottage Farm CSO Treatment Facility study. Sampling location parameters are provided in Table 4.

Table 4. Charles River and Cottage Farm Facility Sampling Locations.

Location Code	Latitude Degrees	Longitude Degrees	Location Description
001S	42.3590	-71.1742	Upstream of CSOs (Community Rowing Dock)
168S	42.3735	-71.1332	At CAM005 outfall
005S	42.3545	-71.1165	Magazine Beach (downstream of CAM005, CAM007, CAM009, CAM011, upstream of Cottage Farm)
145S	42.3497	-71.0930	Stony Brook outfall (MWR023)
009S	42.3575	-71.0822	Lower Basin
006S	42.3525	-71.1085	BU Bridge (just downstream of Cottage Farm MWR201 outfalls)
CF_ INF	NA	NA	Cottage Farm Influent
CF_ EFF	NA	NA	Cottage Farm Effluent

7.4.2.1 Sample Collection and Parameters Measured

During sampling events, MWRA will measure temperature, conductivity, salinity, dissolved oxygen, pH, turbidity, and secchi depth (*in situ*) at the Charles River and the Cottage Farm Facility sampling locations. Cottage Farm effluent samples will also be tested to determine the concentration of total chlorine residual in the effluent.

All Charles River and Cottage Farm Facility samples will be collected by MWRA staff. Samples will be delivered to the MWRA Central Laboratory within six hours of collection and to the University of New Hampshire (UNH) Waterborne Disease Laboratory via FedEx overnight. Sample analyses conducted at each laboratory are discussed in detail in sections 7.3 and 12.0.

7.4.2.2 Sampling Logistics

The number of virus samples to be analyzed at this study area includes eight during wet weather conditions and six during dry weather conditions (Table 1). A wet weather survey is triggered by at least 0.5 inches of rain falling within the previous day (as reported by the National Weather Service at Logan Airport). It is assumed that 0.5 inches of rain will trigger many CSO activations system-wide. Nicole Parilla O'Neill of Deer Island's Central Laboratory will monitor rainfall information and make the decision to mobilize the sampling team. Every reasonable effort will be made to conduct the sampling within 12 hours of the rainstorm. If there is sufficient preparation time, Kelly Coughlin (the MWRA Virus Project Area Manager), Battelle, and Dr. Margolin will be notified in advance by Ms. O'Neill that sampling will be conducted, otherwise they will be notified as soon as sampling is completed that samples are being shipped to UNH.

The wet weather sampling includes samples from the Cottage Farm facility, however, as of this writing, the facility is undergoing an upgrade, and virus sampling at this facility is suspended until modifications are complete. Once the upgrade is complete and virus sampling resumes, the facility operator will notify Ms. O'Neill or Ms. Coughlin by phone of the activation, and will then collect two 1-liter grab samples. Sterilized containers will be provided to the operator in advance, and samples will be collected during the activation from influent and effluent sampling locations. The samples will then be transported to the lab by MWRA's TRAC staff with other routine CSO samples. Custody will then be transferred to Ms. O'Neill. Every reasonable effort will be made to conduct wet weather sampling in the river and harbor during or at least within 12 hours of the activation at Cottage Farm. If for some reason the wet weather river sampling can not be conducted during or near to the time of activation, the Cottage Farm samples will be discarded. Sampling will be rescheduled for the next wet weather event.

7.4.3 Deer Island Wastewater Treatment Plant

The effectiveness of secondary sewage treatment processes at the Deer Island Treatment Plant to remove and/or inactivate infectious viruses and their indicators as well as the effects of discharging effluent into the waters of the Massachusetts Bay are of concern and need to be assessed.

MWRA has proposed reducing the level of chlorination of effluent after the new Massachusetts Bay outfall is commissioned because the long contact time available in the outfall tunnel will enable an increased level of disinfection. The levels of fecal coliform, an indicator of disinfection, are easily measured in effluent. However, there is a poorer understanding of how well viruses are inactivated during secondary treatment and disinfection. Also, there are concerns about the potential for any viruses remaining in effluent to affect endangered species or shellfish in the new outfall area. This study area will develop information to help assess these issues.

The three main objectives of this area of research are to assess:

1. the presence of anthropogenic viruses and their indicators in treated and untreated wastewater at MWRA's Deer Island Treatment Plant;

2. the effect of secondary treatment at the Deer Island WWTP on the presence of anthropogenic viruses and their indicators;

3. the effect of different levels of chlorination on recoverable viruses and their indicators in secondary treated effluent.

7.4.3.1 Sample Collection and Parameters Measured

In 1996 and 1997, MWRA sampled primary treated effluent and Deer Island pilot plant secondary-treated effluent, both undisinfected and with measured chlorine additions. In 1998 - 1999, ten wastewater samples were collected for virus analysis. Sampling and analysis will continue in 2000 and 2001. These will include influent and primary and secondary effluents from the Deer Island Treatment Plant. MWRA will conduct sampling and will determine collection dates. They will coordinate directly with Dr. Margolin for the virus analysis.

All Deer Island Treatment Plant samples will be collected by MWRA staff. Samples will be delivered to the MWRA Central Laboratory within six hours of collection and to the University of New Hampshire (UNH) Waterborne Disease Laboratory via FedEx overnight. Sample analyses conducted at each laboratory are discussed in detail in sections 7.3 and 12.0. In addition, MWRA will measure the chlorine residual (*in situ*) at the time of sampling. These data will be made available for virus evaluation. MWRA will supply an electronic file to UNH containing the SAMPLE_IDs used to label the virus samples during collection. This file will be available within two working days of the end of the sampling event.

7.4.4 New Outfall Location in Massachusetts Bay

The primary objective of this monitoring effort is to assess the presence of anthropogenic viruses and their indicators in areas of Massachusetts Bay which may be potentially impacted by discharges from the new outfall. To fulfill this objective, data have been collected since 1998. Effluent discharge at the outfall began in September 2000, and surveys conducted after this data will collect post-discharge data to discern any potential post outfall relocation effects. A major interest is whether the effluent discharge can potentially contaminate shellfish beds.

Figure 3 shows the sampling locations in Massachusetts Bay which have been selected for the outfall monitoring. Sampling location coordinates are provided in Table 5. Sample collections will be coordinated with other collections when feasible. Anthropogenic virus surveys in Massachusetts Bay will be conducted annually in February, April, June, August, October, and December (Table 6).

Station F22 is distant from nearshore sources of anthropogenic viruses and from the outfall, providing a clean control. Stations N20 and N16 are at opposite ends of the diffuser, and denote the area likely to have the highest effluent discharge and thus represent the potentially most contaminated area. F25 is near a potential shellfishing resource, and F18 provides a geographic sampling location to the north. Samples will be collected at the surface and subpycnocline (or mid depth) at each station, except for station F22. Only surface samples will be collected at F22 due to the depth of the water at this station.

Table 5. Massachusetts Bay Outfall Sampling Locations.

Station Number	Latitude Degrees	Longitude Degrees	Water Depth (m)
F22	42.48	-70.618	80
F25	42.322	-70.876	15
N20	42.382	-70.817	32
N16	42.394	-70.753	40
F18	42.442	-70.888	24

7.4.4.1 Sample Collection and Parameters Measured

All Massachusetts Bay samples will be collected by Battelle. Sample collection in Massachusetts Bay will be coordinated with other collections when feasible. As such, a designated Battelle representative will notify MWRA and Dr. Margolin of the impending sampling event.

During sampling events, *in situ* temperature, conductivity, turbidity, and dissolved oxygen and discrete samples for bacterial indicators and virus measurements listed in Table 2, will be conducted at the Massachusetts Bay outfall sampling locations. See Albro *et al.* (1998) for the methods and data reduction**.**

Fecal coliform and *Enterococcus* samples will be delivered to the MWRA Central Laboratory within twelve hours of collection; and virus samples to the University of New Hampshire (UNH) Waterborne Disease Laboratory via FedEx overnight. Sample analyses conducted at each laboratory are discussed in detail in sections 7.3 and 12.0.

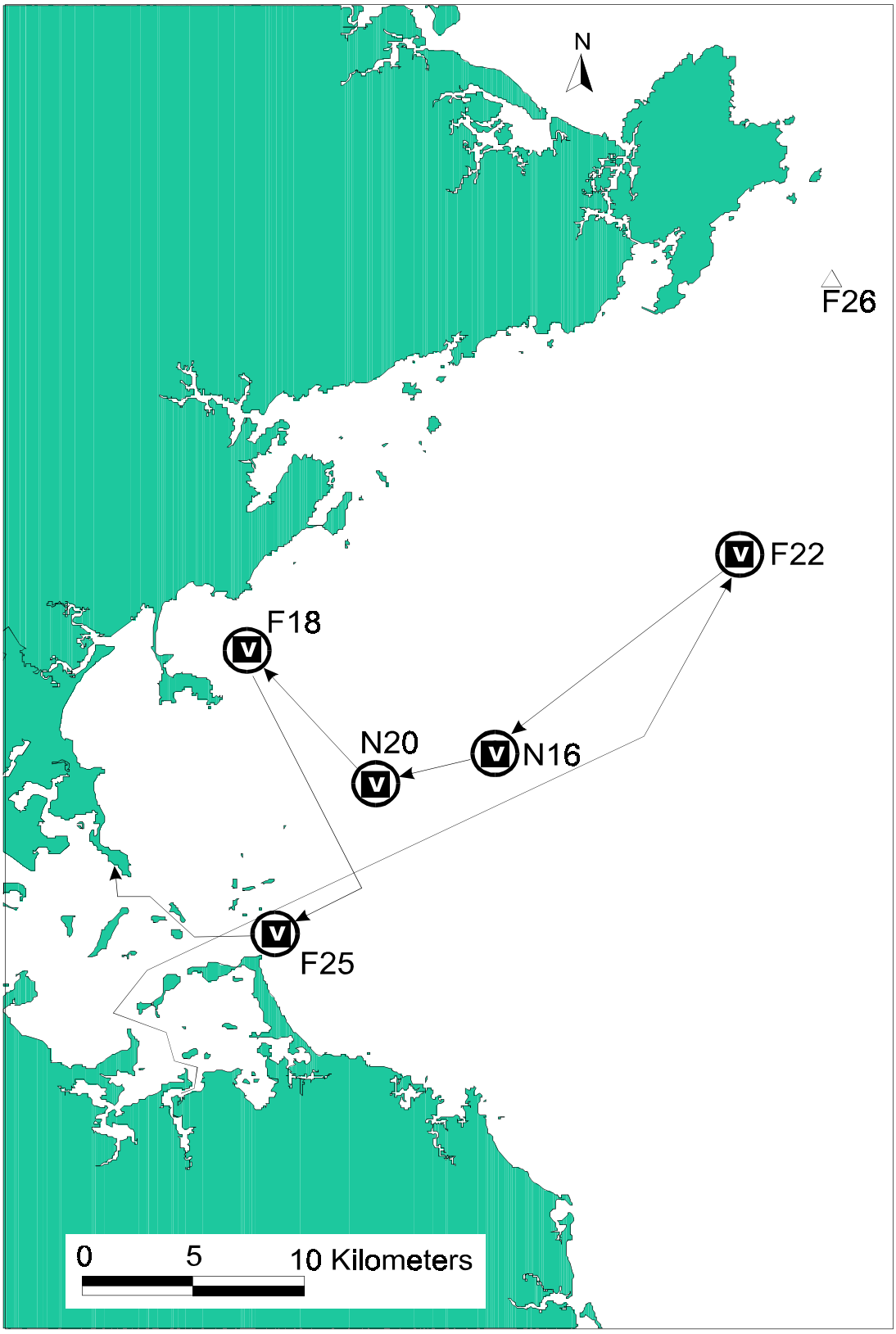

Figure 3. Massachusetts Bay Sampling Locations (virus sampling locations are circled).

8.0 PROJECT FISCAL INFORMATION

The Anthropogenic Virus Survey (Task 28) is being carried out under the Harbor and Outfall Monitoring contract (Contract No. S274) between MWRA and Battelle.

9.0 SCHEDULE OF ACTIVITIES AND DELIVERABLES

Sampling activities associated with the Anthropogenic Virus Survey (Task 28) described in this CW/QAPP are scheduled in 2000 and 2001. The planned survey schedule is shown in Table 6. Exact dates will be determined as the study progresses and will be subject to the criteria established for sampling.

Table 6. Master Schedule for Anthropogenic Virus Surveys.

SurveyID	Start Date
AV001	Feb-00
AV002	Apr-00
AV003	Jun-00
AV004	Aug-00
AV005	Oct-00
AV006	Dec-00
AV011	Feb-01
AV012	Apr-01
AV013	Jun-01
AV014	Aug-01
AV015	Oct-01
AV016	Dec-01

For the sample collections in Massachusetts Bay, survey plans 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. MWRA sample collections are controlled by Ms. Coughlin or her designated representative and are the responsibility of MWRA. MWRA will coordinate with Dr. Margolin or his designated representative regarding sample delivery dates. The Battelle Laboratory Manager and TPMC will be notified of each sampling event. Two synthesis reports will be delivered under Task 33.9.

10.0 PROJECT ORGANIZATION AND RESPONSIBILITIES

10.1 Project Management

Figure 4 presents the Project Management structure for the anthropogenic virus surveys and analysis. This represents the major tasks necessary to complete the scope of work. Dr. Michael Mickelson is the MWRA Project Manager, and Ms. Kelly Coughlin is the MWRA Anthropogenic Virus Project Area Manager. 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 Ms. Coughlin. Ms. Wendy Leo is MWRA's EM & MS Database Manager.

Dr. Carlton Hunt is the Battelle Project Manager and is responsible for the overall performance of this project. Ms. Jeanine Boyle is Battelle's Deputy Program Manager. The Battelle Quality Assurance Officer for the project is Ms. Rosanna Buhl. For this task, Ms. Buhl is responsible for reviewing data reports and QA Statements submitted by Dr. Margolin for completeness and adherence to the CW/QAPP. An initiation audit consisting of a review of laboratory procedures and personnel qualifications will be performed. The need for laboratory inspection will be based on the results of this audit. Mr. Wayne Trulli is the Battelle Field Manager responsible for all Battelle field collections. Ms. Deirdre Dahlen, Battelle's Laboratory Manager, is responsible for overseeing all laboratory activities in the contract. Ms. Ellie Baptiste-Carpenter is Battelle's Database Manager. Ms. Kristyn Stevens is the Technology Planning and Management Corporation (TPMC) Anthropogenic Virus Senior Scientist responsible for the synthesis reports. Dr. Aaron Margolin will lead the University of New Hampshire technical team and will have overall responsibility for UNH virus analyses, ensuring quality of virus analysis, and performing technical reviews of the anthropogenic virus synthesis report.

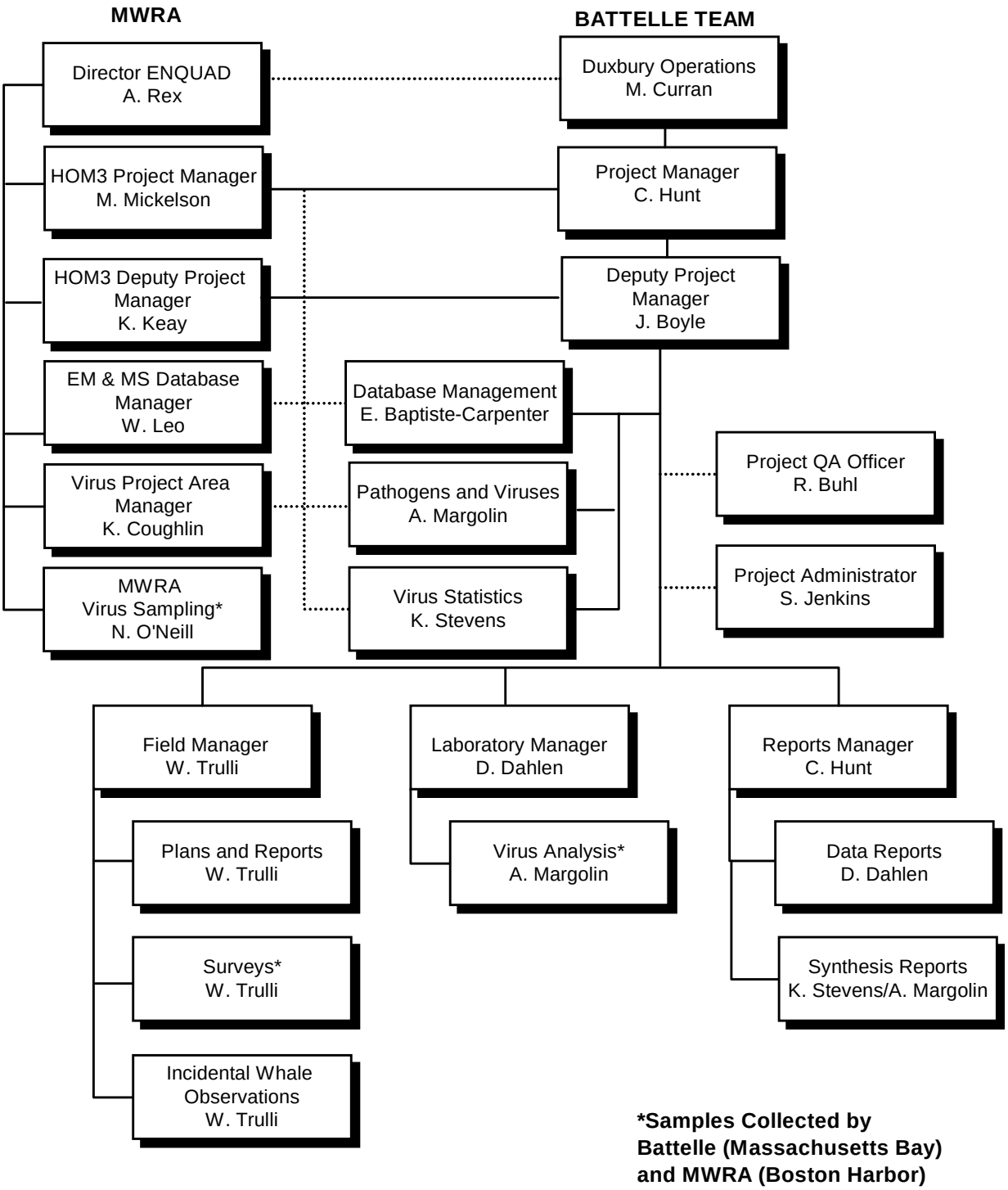

Figure 4. Organizational Structure for the Anthropogenic Virus Study Area.

10.2 Field Program

Battelle will schedule Massachusetts Bay survey logistics and coordinate with the MWRA Harbor Water Quality Monitoring team through Ms. Coughlin. MWRA is responsible for the Boston Harbor field effort and will operate under their standard procedures.

10.3 Laboratory Analysis Program

Analyses of viral and bacteriophage samples will be performed at the Waterborne Disease Laboratory at UNH, coordinated by Dr. Margolin. Bacteriological analyses will be performed at the MWRA Central Laboratory under the supervision of Nicole Parilla O'Neill.

10.4 Data Management and Reporting

For the Massachusetts Bay component of this study, Battelle will prepare all survey plans and survey 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 Battelle. MWRA will download bacteriological data from its LIMS into EM & MS and provide these data to Battelle in Oracle export format. Battelle will submit virus data reports to MWRA, also providing a copy to TPMC. MWRA will provide the final virus data to Kristyn Stevens at TPMC. Battelle will be responsible for overall project data management. Ms. Rosanna Buhl, Battelle's Project QA Officer, will be responsible for QA review of the data reported under this task.

11.0 DATA QUALITY REQUIREMENTS AND ASSESSMENTS

To ensure that all data generated during the conduct of surveys, analyses, and reporting are of the highest quality, data will be examined in terms precision, accuracy, completeness, comparability, and representativeness. These terms are defined in the HOM3 Quality Management Plan (Battelle 1998). The application of these measures of data quality is described below.

Accuracy - the extent of agreement between the measured value and the true value
Precision - the extent of agreement among independent, similar, or related measurements
Completeness - measure of the amount of data acquired relative to the amount of data required to fulfill the objectives and statistical criteria for the intended use of the data
Comparability - the extent to which data from one study can be compared directly to similar studies
Representativeness - the extent to which sample locations and measurements represent true systems

The comparability between the virus analytical methods used 1998-1999 and those presented here (2000-2001) is being tested through the re-analysis of archived sample aliquots for samples that were collected and analyzed in 1998 and 1999 using the previous methods. This exercise is described in Task 29, Task Order 30: Virus Sample Analysis by Revised Methods.

11.1 Navigational and Hydrographic Information

Refer to the Water Column Monitoring CW/QAPP (Albro *et al.*, 1998) for details concerning navigational data quality objectives.

11.2 Water Sampling

Refer to the Water Column Monitoring CW/QAPP (Albro *et al.*, 1998) for details concerning water sampling data quality objectives.

11.3 Laboratory Program

11.3.1 Virus Recovery Efficiencies

Beginning in 2000, the method of virus sample collection was modified to simplify operations in the field. Field-prepared virus-recovery efficiencies will not be required for 2000 and 2001. Refer to ENQUAD Report ms-47 (CW/QAPP for Anthropogenic Virus Survey: 1998-2000) for a description of virus recovery efficiency procedures (Tilton *et al*., 1998). Samples will be collected but not treated in the field. A sample volume of 4 L of surface (approximately 12 inches deep) water will be collected by pump or pre-sterilized Go-Flow bottle into a 4 L pre-sterilized plastic cubitainer. Under the new method, the following solutions are no longer added in the field as during previous surveys in 1998 and 1999: (1) bacteriophage (MS2) slurry, (2) 2.0 N HCl used to adjust pH between 3.3 and 3.7, and (3) $AlCl_3$ to adjust the concentration to between 0.001 and 0.0001 M $AlCl_3$. The sample will be collected and analyzed by the same techniques as referenced in Section 12.5.6 (Bacteriophage Sampling and Analysis Procedures).

11.3.2 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 tested and used.

11.3.3 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 one 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. If negative and positive controls perform as expected, the absence of extraneous contamination is demonstrated. If control samples fail, the control tests will be repeated until the expected results are achieved.

11.3.4 Coliphage

A positive control for the male specific, MS-2 © 3000), and a positive control for the somatic, θx174 (*E. coli* C), 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. If control samples fail, the control tests will be repeated until the expected results are achieved.

11.3.5 Fecal Coliform and *Enterococcus*

Replicate laboratory analyses will be done for all samples. Result values are averaged before being entered into LIMS. Calculation of acceptable precision will be as in Standard Methods, 19th edition, Method 9020 B 4 (APHA *et al.,* 1995).

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 (MWRA, 1996a,b).

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.

Analyses performed at MWRA will follow QC procedures, acceptance criteria, and corrective action defined by MWRA SOPs.

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 Boston Harbor Sampling (MWRA)

12.1.1 Sampling Logistics

MWRA is responsible for all Harbor sampling logistics and associated notification requirements. 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. For Combined Sewer Overflow (CSO) sampling, the CSO operator is given 1 L grab bottles in advance and is instructed to collect a sample upon CSO activation. River monitoring is initiated after the lab consults with Ms. Coughlin, and it is determined that enough rain has already fallen to mobilize. Lines of communication will be maintained during all phases of the survey. Telephone numbers for participants are listed in Table 7.

Since the majority of sampling locations in the harbor and rivers are close to shore adjacent to readily identifiable landmarks, sampling locations will be recorded by MWRA personnel using a combination of shoreline observations and GPS, which will be recorded in latitude and longitude.

12.1.2 Weather Forecasting

MWRA is responsible for weather forecasting and notifying the sampling team of mobilization. Each day during the annual sampling window, the general weather outlook will be monitored by Nicole Parilla O'Neill. Ms. O'Neill will communicate with treatment plant operators and notify Ms. Coughlin when the CSO facility discharges. If rainfall criteria appear likely to be satisfied by the forecasted weather pattern,

the MWRA Project Area Manager (Ms. Coughlin) 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.

12.1.3 Field Measurements of Water Quality Parameters

MWRA staff will perform and record field measurements of water depth, temperature, turbidity, pH, conductivity, salinity, dissolved oxygen, and secchi depth (*in situ*) in the receiving water following MWRA Standard Operating Procedures. Only total chlorine residual will be performed on facility samples (influent/effluent). Daily calibration of field instruments will be according to MWRA's SOP.

Table 7. MWRA CSO Virus Survey Points of Contact.

Contact	Affiliation	Work Phone #	Home Phone #	Cellular/page #
Kelly Coughlin	MWRA Project Area Manager	(617) 788-4717		
Nicole Parilla O'Neill	MWRA Central Laboratory	(617) 539-4327		
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)
Frank Cascarano	MWRA Cottage Farm CSO facility	(617) 491-8089	NA	(617) 403-5862
Bob Younis	MWRA Prison Point CSO facility	(617) 367-8432	NA	(617) 403-5882
Ed Mogan Ed Sullivan (Alternate)	MWRA Somerville Marginal CSO facility	(617) 389-1685 (617) 623-2050	(978) 474-4259	(617) 403-5863 (617) 403-5866
Ed Mogan Ed Sullivan (Alternate)	MWRA Commercial Point CSO facility	(617) 389-1685 (617) 623-2050	(978) 474-4259	(617) 403-5863 (617) 403-5866
Dr. Aaron Margolin	UNH Scientist	(603) 862-2252	(603) 772-2433	(603)770-0584
Justin Fontaine	UNH staff	(603) 862-3008	available from UNH PI	
Stacy Abramson	Battelle Virus Task Coordinator	(781) 952-5330	available from Battelle	
Deirdre Dahlen	Battelle Laboratory Manager	(781) 934-0571	available from Battelle	
Wayne Trulli	Battelle Field Manager	(781) 934-0571	available from Battelle	
Kristyn Stevens	TPMC Scientist	(781) 544-0477	available from TPMC	

12.2 Massachusetts Bay Sampling by Battelle

12.2.1 Navigation and Hydrographic Profiling

Vessel positioning during sampling operations will be accomplished with the NAVSAM navigation system. This system consists of a Northstar DGPS interfaced to the NAVSAM computer. The GPS receiver has six dedicated channels and is capable of locking onto six different satellites at one time. This capability ensures strong signal reception, and accurate and reliable positioning with 2-second updates.

The hydrographic profile sampling equipment and data acquisition equipment consists of the following:

- Battelle designed and fabricated winch with 150-m 9-conductor double-armored stainless steel cable and sheave
- Sea Bird 32 Carousel Water Sampling System or General Oceanics model 1015 rosette system
- 5- and 10-L GoFlo bottles
- Ocean Sensors CTD interface deck unit
- Ocean Sensors OS200 CTD system (two additional units as backups) with
  - SeaBird SBE-13 DO sensor which is a Beckman polarographic type that produces an oxygen-dependent electrical current and incorporates a thermistor for determination of membrane temperature;
  - SeaBird SBE 4-01/0 conductivity cell;
  - Paroscientific Digiquartz integral to the SBE-9 CTD which measures pressure;
  - SeaTech 20-cm-pathlength transmissometer that provides *in situ* measurements of optical beam transmission, which is related to the concentration of suspended matter in the water at the point of measurement;
  - WetStar *in situ* fluorometer;
  - Biospherical QSP-200L spherical quantum scalar irradiance sensor which measures underwater photosynthetically active radiation (PAR);
- Biospherical QSR 240 reference hemispherical quantum scalar irradiance sensor which measures on deck radiation conditions (e.g., due to atmospheric conditions)
- Data Sonic altimeter provides a measurement of underwater unit height from the bottom
- JRC JFV-120 dual-frequency color video echosounder to provide bathymetric measurements during vertical and horizontal profiling operations
- Computer with custom data acquisition software
- Barcode printer
- Hewlett-Packard PaintJet color printer

Battelle's software acquires data from all profile electronic sampling systems and navigation systems at the rate of four times per second. The software displays all of the information once per second on a color monitor. The screen is split to show sensor data on the left and navigation data on the right (Figure 5) Once the data are acquired, they are automatically written to a data file and logged concurrently with position data from the navigation system. The navigation portion of the display will show the position of

the vessel compared to the coastlines digitized from standard NOAA charts, navigation aids, preset sampling locations, and vessel track. A second monitor will be furnished to the helmsman as a steering display. During hydrocast operations, position fixes will be electronically recorded at 2-second intervals. Hard-copy printouts of position fixes will be made during discrete sampling events such as triggering of GoFlo bottles. During transit operations between stations, position fixes and deck irradiance will be electronically recorded at 5-minute intervals.

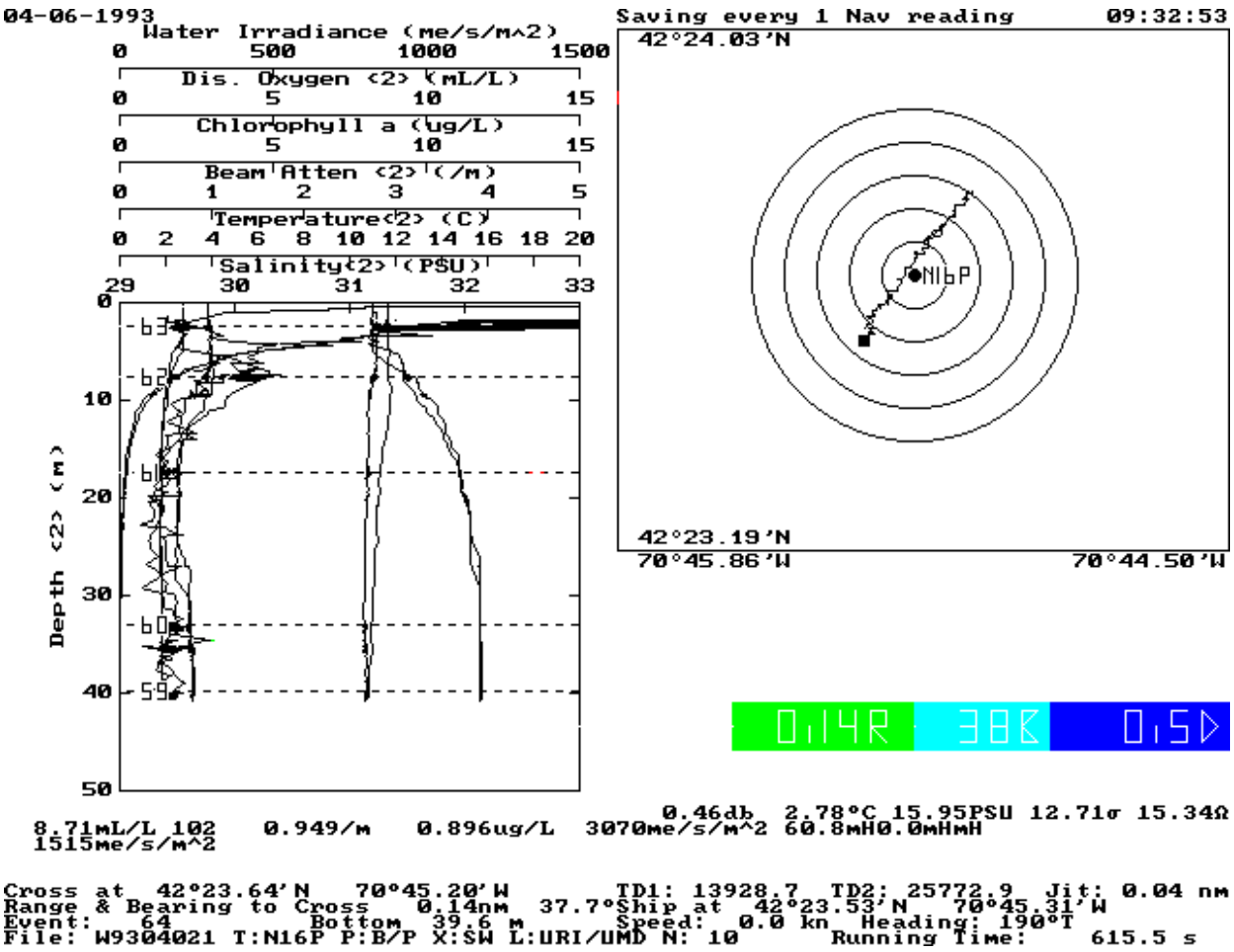

Figure 5. Sample NAVSAM Data Acquisition Screen.

12.3 Indicator Bacteria Sampling and Analysis Procedures

Samples for analysis of fecal coliform and *Enterococcus* will be collected into sterile pre-rinsed sample containers, stored in coolers with ice packs at <10°C, and analyzed within twelve hours of collection using Standard Methods membrane filtration procedures detailed in MWRA's Laboratory Standard Operating Procedures. Samples will be collected on the upstream side of the research vessel. M-FC agar with rosolic acid addition (SM 9222D) will be used for the enumeration of fecal coliform, and m-*Enterococcus* agar (SM 9230C) will be used for counting *Enterococcus* colonies. Bacterial samples will be filtered and analyzed following MWRA's SOP (MWRA, 1996b).

12.4 Anthropogenic Virus Sampling Procedures

Virus samples will be collected using a 4 L cubitainer and a 1 L bottle. Detection techniques will be those referenced in Chapron *et al*. (2000), except as noted.

12.4.1 Virus Collection

For natural water samples, both freshwater and seawater, a sample volume of 4 liters will be collected using a pre-sterilized pump or Go Flow bottles. Samples will be collected in pre-sterilized 4 L cubitainers (pH of those samples will not be adjusted to between 3.3 and 3.7 in the field, and $AlCl_3$ also will not be added as was done in previous years). Surface samples from Massachusetts Bay will be collected from the upper 30 cm at each station. Only surface samples will be collected if no pycnocline is present. If a pycnocline is present, samples will be collected from below the deepest extent of the pycnocline at the four stations furthest offshore. Only one sample will be collected at the station closest to shore. Volumes and samples times are documented on field data sheets.

Harbor and river samples will be collected from 0-12 inches (30 cm) below the surface using a Viton Gear pump, at a flow rate of 10 gal./min. For wastewater samples, 4 L of sample will be collected into pre-sterilized 4 L containers. If wastewater is chlorinated, samples will be collected into containers containing thiosulfate to dechlorinate the sample. Surface samples from Massachusetts Bay will be collected from the upper 30 cm at each station. Only surface samples will be collected if no pycnocline is present. If a pycnocline is present, samples will be collected from below the deepest extent of the pycnocline at the four stations furthest offshore. Only one sample will be collected at the station closest to shore. Pre-sterilized Go Flow bottles or a Peel Internal gear pump with maximum flow rate of 10 gal/min will be used to collect water samples in Massachusetts Bay. The samples will be stored chilled at 4°C until processing and elution. Samples will be shipped via FedEx overnight to the UNH analytical laboratory.

12.4.2 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, 19th edition, section 9510B (APHA *et al.,* 1995). Bleach will be pumped through all hoses, housings and pumps or applied to the inner surfaces of the Go Flow bottles and allowed to remain for 15-30 minutes. Following bleaching, a mixture of water and sodium thiosulfate will be used to flush the entire system to neutralize all the bleach. Once-through contact is

sufficient to neutralize chlorine. After bleaching, the pump tubing will be flushed for a minimum of five minutes with non-chlorinated water or Go Flow bottles will be completely flushed during deployment through the water column.

12.5 Virus Laboratory Analysis

12.5.1 Virus Concentration by Organic Flocculation

The 4 L grab sample is added to 60 g beef extract and mixed until homogeneous. The pH of the sample will then be adjusted to 3.5 using 1 M HCl. The mixture will be allowed to mix for 30 minutes and then transferred to four 1L centrifuge flat bottom bottles. The floc will be collected by centrifugation at 5,000 rpm for ten minutes. After centrifugation, the supernatant will be carefully poured out, taking care not to disturb the floc, and the floc suspended in a total volume of 10 ml of a 0.15 M solution of sodium phosphate buffer pH 9.5, which will then be adjusted to a neutral pH. To remove any undissolved particles, the sample will be centrifuged for 10 minutes at 10,000 rpm. The supernatant will be carefully removed and 6 ml syringed filtered (0.45 μm stacked filter with a 0.2 μm syringe filter, pretreated with 10 ml of 3% beef extract) for analysis by ICC-RT-PCR/nested PCR. The remaining supernatant will be archived at –80^{o} C.

12.5.2 Integrated Cell Culture

To the filtered samples add 10 µl Trypsin stock per 1 ml sample and incubate for 30 minutes at 37^{o} C. A Trypsin concentration of 10µg/ml is used for the BGMK cell line, while a concentration of 5µg/ml is used for the Caco-2 cell line. Following incubation, add 3 ml pre-activated sample to 75cm^2 flasks containing a confluent monolayer of the desired cell line. Incubate 90 minutes at 37^{o} C with rocking every 20 minutes. After incubation, add 5 ml trypsinized MEM (250 µl desired Trypsin stock/50 ml serum free MEM) to each flask and incubate for 5 days at 37^{o} C. Store at -80^{o} C until use.

12.5.3 Detection of Virus Using RT-PCR/PCR/nested PCR

Enterovirus, Rotavirus, and Astrovirus RNA will be detected in infected confluent monolayers of Caco-2 and BGMK cells by the RT-PCR/nested PCR method, and Adenovirus type 40/41 DNA will be detected by the PCR/nested PCR method. BGMK cells are utilized for Enterovirus and Adenovirus type 40/41 detection, while Caco-2 cells are utilized for Rotavirus and Astrovirus detection. The RT-PCR procedure to be used for Enterovirus detection was adapted from the procedure described by Grinde et al. (1996). The RT-PCR procedures for Rotavirus and Astrovirus are similar to that described by Grinde et al. (1996) for Enteroviruses but consist of slightly differing cycle temperatures and times in the PCR step. Adenovirus type 40/41 PCR cycle temperatures and times are exactly that of the Enterovirus PCR. Briefly, between 10-11 *u*l of the sample will be added to tubes containing PCR reagents and the appropriate viral primers (Astrovirus, Rotavirus, Adenovirus type 40/41, or Enterovirus). Samples will be allowed to cycle for 35 cycles in a Perkin Elmer thermal cycler.

The nested PCR will be performed immediately after the RT-PCR. One microliter of each RT-PCR mixture will be added to a new PCR tube containing PCR reagents and nested PCR primers. Nested

samples will be allowed to cycle for 35 cycles at varying temperatures and times. Results will be visualized by gel electrophoresis looking for the amplified segment of viral nucleic acid.

12.5.4 Nested PCR Results

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.

12.5.5 Bacteriophage Sampling and Analysis Procedures

Bacteriophage detection techniques will be those referenced in EPA Method 1601 (EPA, 2000). Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure. Separate from the 4 L grab sample, a 1 L grab sample will also be taken. Four hundred and fifty ml's of the sample will be placed into a 500 ml sterile bottle containing 50 ml of 10 X T-soy Broth. This will be done twice for each grab sample, so there will be one bottle for *E. coli* F-amp and one for *E. coli* CN-13. These bottles will then be allowed to warm to room temperature. After warming, a portion of the log cultures previously grown up will be added to the corresponding bottles, along with antibiotic (Streptomycin Sulfate and Ampicillin for F-amp, Nalidixic Acid for CN-13) and magnesium chloride. The bottles will then be incubated overnight at 37° C. After the overnight incubation, each enrichment is centrifuged at 9000 rpm for 10 minutes, and then spotted (100µl from each grab sample enrichment) on four separate T-soy agar plates containing lawns of corresponding bacteria. The T-soy plates will then be inverted and incubated overnight at 37° C. Results are generated by zones of clearing on the plate, which indicate presence or absence of male specific and/or somatic bacteriophages.

13.0 SAMPLE CUSTODY PROCEDURES

MWRA is responsible for collecting samples in Boston Harbor and its tributary rivers, in the Charles River and Cottage Farm locations, and at the Deer Island Waste Water Treatment Plant. Battelle is responsible for collecting samples in Massachusetts Bay. 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. MWRA will provide sampling containers and identification codes (LIMS SAMPLE_IDs) for fecal coliform and *Enterococcus* samples collected in Massachusetts Bay. Samples collected by Battelle will be transported to the MWRA Central Laboratory within twelve hours of sampling. Virus samples will be delivered to Dr. Aaron Margolin at the University of New Hampshire within the next day.

For the Massachusetts Bay samples, the custody of samples, and therefore the sample tracking and integrity, are assured through the following standard procedures, which are defined in Battelle SOP 6-010. MWRA sampling will follow internal MWRA procedures.

Sample custodians are designated at each analytical laboratory; the survey Chief Scientist is the field custodian.

Ms. Nicole Parrilla	Dr. Aaron Margolin
Deer Island	4 Louisburg Circle
Boston, MA 02152	Exeter, NH 03833
(617) 539-4331	(603) 862-2252
(617) 539-4300	(603) 778-4887

- Upon receipt, samples are inspected to verify that (1) integrity is intact (containers are sealed and intact), (2) the sample label and custody forms agree, (3) all shipped samples have been received, and (4) holding temperatures were maintained.

- Sample receipt and the receipt conditions are documented, as are any discrepancies, which are also communicated to the Laboratory Manager and the appropriate Senior Scientist immediately.

- Samples are logged into a formal sample receipt system to provide a permanent laboratory record; SAMPLE_IDs will be used throughout the laboratory analysis.

- Samples are stored in a limited access area according to the conditions specified in Section 12.0.

- Sample receipt and holding times are communicated to the laboratory manager who adds the samples to the laboratory schedule.

- The sample custodian retains custody of the samples until they are transferred from the holding location to the laboratory for analysis. The relinquishing of samples by the custodian and the receipt of sample by the analyst are documented.

- Internal laboratory documentation tracks sample custody location and storage conditions throughout processing and analysis.

- Sample archival and disposal are documented according to SOPs.

Battelle-Collected Samples

Sample custody will be maintained through field log books, laboratory record book, virus field sample data sheets (Figure 6), and chain-of-custody forms for virus (Figure 7) and bacteriological samples (Figure 8). Battelle will forward the virus samples and virus chain-of-custody to Dr. Margolin at UNH and bacteriological samples and bacteriological chain-of-custody to the MWRA laboratory. The MWRA laboratory forwards a copy of the chain-of-custody to the ENQUAD data staff. All original virus field sample data sheets will be kept in a project Sample Log notebook. The chief scientist will maintain custody of all samples on board the vessel. The chief scientist 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).

A unique eight character *Sample ID* which is a concatenation of a five character *Event ID* and a three-character hexadecimal number (*Marker No*) will identify samples collected in the field. The *Sample ID*

will identify the water collected in the collection bottles from a certain depth during a particular station on the specified survey. The five character *Event ID* will be unique to each survey, such as WF987, with "WF" indicating that it is a farfield water column survey, "98" indicating the survey year, and "7" signifying the seventh survey of the year (for surveys higher than nine, letters are used where A and C are equal to 10 and 11 respectively). The *Marker No* is a non-repeating number generated by the NAVSAM software during the closing of a collection bottle.

Each portion of a sample separated for analytical purposes will be assigned a unique *Bottle ID*, composed of the eight–character *Sample ID* plus a 3–character suffix designating the nature and replicate number. For example, "AV1" indicates that the subsample is the first replicate for Virus Analyses (see Table 8 for two letter codes). All data reporting will be keyed to Battelle's sample identification scheme.

Table 8. Analysis Codes used in *Bottle ID*.

Analysis ID	Description	Laboratory	Turn Around Time
FE	Bacteria	MWRA	60 Days
AV	Virus analysis	UNH	60 Days
PG	Phage analysis	UNH	60 Days

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* 3. ☐ Filtration Plant Performance Evaluation(includes 1 and 2) 2. ☐ Microscopic Particulate Analysis 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

			Initials/date
LAB DATA (for lab use only)	Packed pellet volume:	Color:	
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 6. Data Sheet.

MWRA Harbor and Outfall Monitoring Program
Contract No. S274
Chain-of-Custody Form

Today's Date : 3/6/98 11:17:11

Laboratory : University of New Hampshire

Chain-of-Custody # : WF981-VI-0002
Survey ID : WF981
Analysis ID : VI
Analysis Description : Virus analysis

4 Louisberg Circle
Exeter NH 03833
Dr. Aaron Margolin
603-778-4887 (Phone) 603-778-4887 (Fax)

Bottle ID :	Bottle ID :	Sampling Date :	Station ID :	Ck 1	Ck 2	Ck 3	Ck 4
	WF98104AWW1	2/1/98 01:41:00	F01	☐	☐	☐	☐
	WF98104CWW1	2/1/98 01:43:00	F01	☐	☐	☐	☐
	WF98106EWW1	2/3/98 13:49:35	F02	☐	☐	☐	☐
	WF98106EWW2	2/3/98 13:49:35	F02	☐	☐	☐	☐
	WF981071WW1	2/3/98 13:51:43	F02	☐	☐	☐	☐
	WF9810B1WW1	2/3/98 20:18:52	F27	☐	☐	☐	☐
	WF9810B1WW2	2/3/98 20:18:52	F27	☐	☐	☐	☐
	WF9810B3WW1	2/3/98 20:22:04	F27	☐	☐	☐	☐
	WF981108WW1	2/4/98 13:10:31	N16	☐	☐	☐	☐
	WF981108WW2	2/4/98 13:10:31	N16	☐	☐	☐	☐
	WF98110AWW1	2/4/98 13:12:06	N16	☐	☐	☐	☐
	WF98112EWW1	2/7/98 08:33:49	F24	☐	☐	☐	☐
	WF981130WW1	2/7/98 08:35:44	F24	☐	☐	☐	☐
	WF981146WW1	2/7/98 10:43:53	F25	☐	☐	☐	☐
	WF981148WW1	2/7/98 10:45:13	F25	☐	☐	☐	☐
	WF981155WW1	2/7/98 12:35:49	F31	☐	☐	☐	☐
	WF981156WW1	2/7/98 12:36:56	F31	☐	☐	☐	☐
	WF981162WW1	2/7/98 13:44:51	F30	☐	☐	☐	☐
	WF981163WW1	2/7/98 13:46:00	F30	☐	☐	☐	☐
	WF981173WW1	2/9/98 07:12:23	N04	☐	☐	☐	☐
	WF981175WW1	2/9/98 07:15:04	N04	☐	☐	☐	☐

Relinquished By / Date / Time / Company / Transport-Airbill #	Recieved By / Date / Time / Company

Figure 7. Virus Sample Chain of Custody Record.

DATE: MWRA CHAIN OF CUSTODY PAGE: 1 OF 1

FOR

MISCELLANEOUS SAMPLES

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

COMMENTS: ____________________

SAMPLED BY: ____________________ DATE: ____________

RELINQUISHED TO: ____________________ DATE: ____________

RECEIVED BY: ____________________ DATE: ____________ (AT LAB)

Figure 8. Bacteriological Sample Chain of Custody Record.

MWRA-Collected Samples

The MWRA LIMS SAMPLE_ID will be used. A different SAMPLE_ID will be used for each sample container (virus, bacteria, etc.). The BOTTLE_ID will be the same as the SAMPLE_ID.

Subsequent coding in the UNH lab would then indicate subsample type and number.

Virus samples collected in the field will be transported via FedEx to the Waterborne Disease Laboratory at UNH, who will be responsible for sample custody from elution, storage, and analysis. MWRA forwards copies of the chain-of-custody forms to Dr. Margolin at UNH and to the ENQUAD data staff. Analytical procedures and data management will be documented on individual data sheets used for analyses of cultivatable viruses and bacteriophage assays (Figures 9 through 13). After processing, the remaining concentrated sample will be archived frozen (-80°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 chief scientist, who will forward the document to the appropriate Laboratory Manager. The remaining sheets will accompany the samples to the laboratory for subsequent sample transfer. The MWRA central lab data sheet and data entry form for bacteriological data are shown in Figures 12 and 13.

SAMPLE ANALYSIS REQUEST FORM

Client:	Type of sample:	Sample condition upon arrival:
Client ID #:		
Sample ID:		A - acceptable; R-rejection
UNH #:		
Date Rec'd:		*(Describe reasons for sample rejection/integrity below)
Logged in by:		

Total Amount Rec'd:

Test (s) required:

[] Total Solids [] Fecal Coliforms [] Salmonella [] Helminth Ova

Phage by:

Medium to be tested: [] water; [] filter eluent; [] concentrated sample

[] Enumeration using double agar overlay;

[] Enumeration using ASTM method

[X] Enrichment procedure followed by plaque detection, qualitative (Presence/absence)

[] Enrichment procedure followed by plaque detection, quantitative (MPN)

Virus by:

[] plaque assay; [] MPN; []ICR Method; [] ICC-RT-PCR; [] ICC-PCR;

[X] nested ICC-RT-PCR; [X] nested ICC-PCR

Size and number of flasks to be used:

Size	Number	Cell Line
[] 25 cm^2		
[] 25 cm^2		
[X] 75 cm^2	1	BGM
[X] 75 cm^2	1	CaCo-2
[] other: _____		

PCR without the use of cells:

[] on concentrate; [] on filter efluent; [] on sample with no processing

Purification:

[] no purification; [] Quagen® colunm; [] paramagnetic beads

Expected turn around time:

Explanation of why sample integrity is in question:

Special handling/processing or testing instructions:

Figure 9. Sample Analysis Request Form.

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:	

Results:

Primer Used	1st round PCR - amplicon detected by gel electrophoresis	nested PCR - amplicon detected by gel electrophoresis	Comments
Panentero (Enterovirus)			
Adeno 40/41			
Rotavirus			
Asterovirus			

Figure 10. 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: Volume of eluent: _____ ml
UNH sample #:	Phage tech:	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 11. Bacteriophage Sample Data Sheet.

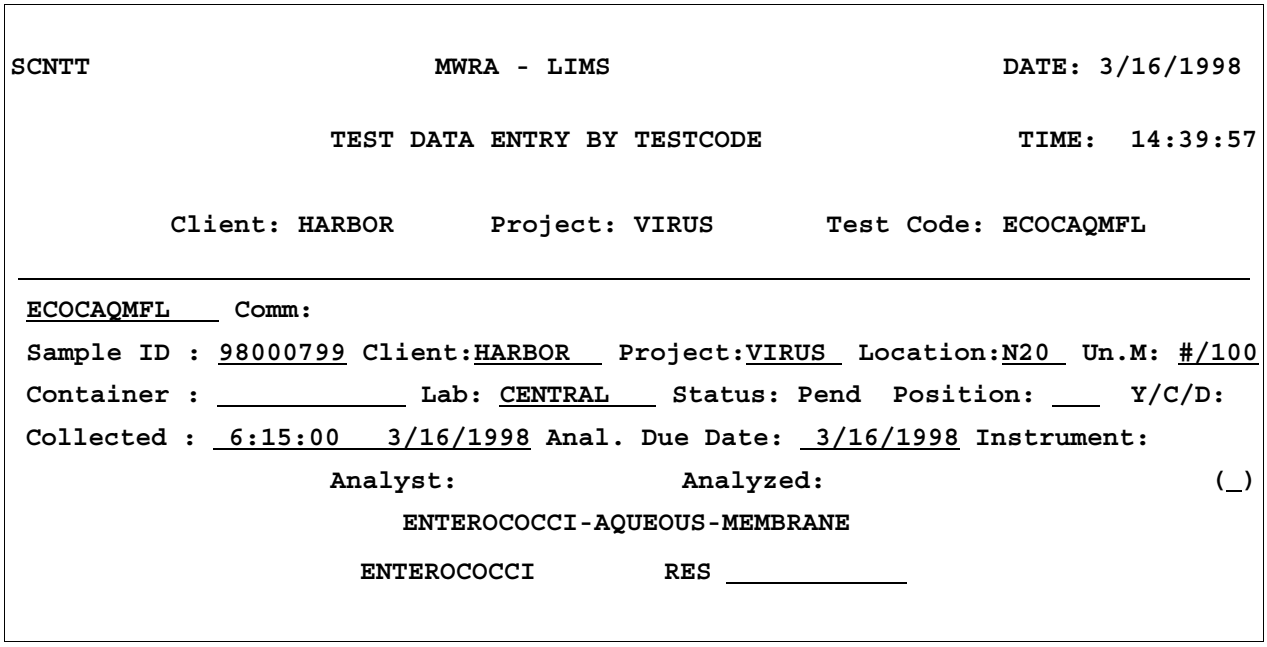

Figure 12. MWRA LIMS Entry Screen. The following fields are not used for this study: 'container', 'position', 'Y/C/D', and 'Instrument'. The date and time the sample was filtered are entered into the 'analyzed' field. The result is entered in the 'RES' field.

Fecal Coliforms and Enterococcus
Method: 9222D and 9230C
Ref.: Std Mtds 18th Ed

SOP: 10-ind-mfl-
LIMS: FCOL*MFL
SOP: 10-ind-mfl-
LIMS: ECOC*MFL

Container: Sterile, P or G
Preservation: Cool, 4°C
Max Hold Time: 6 hours

Matrix
DW-Drinking Water
DIW-Dilution Water
SW-Surface/Sea Water
WW-Wastewater

Entered By/Date:

Validated By/Date:

Date Samples Collected: _____
Project Name/Code/Area: _____

Filtration Start Time(s): _____
Incubation Times:
Time FCOL plates in 35°C: _____
Time FCOL plates in 44.5°C: _____
Time ECOC plates in 35°C: _____

Read Dates & Times:
Date/Time FCOL plates read: _____ By: _____
Date/Time ECOC plates read: _____ By: _____

Sample ID:																					
Location Code: _____ Time Collected: _____ Filtered By: _____																					
FCOL			ECOC			FCOL			ECOC			FCOL			ECOC			FCOL			ECOC		
Vol. (mL)	Dil. Factor (DF)	Plate Count (PC)	Vol. (mL)	Dil. Factor (DF)	Plate Count (PC)	Vol. (mL)	Dil. Factor (DF)	Plate Count (PC)	Vol. (mL)	Dil. Factor (DF)	Plate Count (PC)	Vol. (mL)	Dil. Factor (DF)	Plate Count (PC)	Vol. (mL)	Dil. Factor (DF)	Plate Count (PC)	Vol. (mL)	Dil. Factor (DF)	Plate Count (PC)	Vol. (mL)	Dil. Factor (DF)	Plate Count (PC)
Results						Results						Results						Results					
Circle Plate Counts (above) used in calculating AVERAGE						*Circle Plate Counts (above) used in calculating AVERAGE*						*Circle Plate Counts (above) used in calculating AVERAGE*						*Circle Plate Counts (above) used in calculating AVERAGE*					
FCOL AVERAGE: _____ #/100 mL			ECOC AVERAGE: _____ #/100 mL			FCOL AVERAGE: _____ #/100 mL			ECOC AVERAGE: _____ #/100 mL			FCOL AVERAGE: _____ #/100 mL			ECOC AVERAGE: _____ #/100 mL			FCOL AVERAGE: _____ #/100 mL			ECOC AVERAGE: _____ #/100 mL		

CALCULATION OF RESULTS:

$$\text{Colonies / 100mL} = \frac{\text{100 x Plate Count (PC)}}{\text{Volume (mL)}} \times \text{Dilution Factor (DF)}$$

Where: Volume (mL) = Volume added to funnel for filtration
Dilution Factor (DF) = $(\text{Vol}_{\text{sample}} + \text{Vol}_{\text{dilution water}}) / \text{Vol}_{\text{sample}}$

NOTE: Acceptable Plate Count Ranges: FCOL (20-60 colonies), ECOC (20-60), TCOL (20-80)

Figure 13. MWRA Central Laboratory Data Sheet.

14.0 CALIBRATION PROCEDURES AND PREVENTATIVE MAINTENANCE

14.1 Navigation Equipment

Proper function of navigational equipment will be checked by confirmation with readings taken at surveyed reference points within the previous year and spot-checking before leaving the dock and on return to the dock during each survey. See also the Water Column Monitoring CW/QAPP for details (Albro *et al.*, 1998).

14.2 Laboratory Equipment

Calibration of virus laboratory equipment will follow the EPA SOP and Quality Assurance Manual for the EPA Information Collection Rule (see Appendix I). All equipment associated with virus and bacteriological sampling and analyses (pumps, flow meters, pH meters, analytical balances, thermometers, and incubators) will be calibrated and maintained according to manufacturer's specifications. These are done on a daily basis. An equipment logbook will be maintained to document periodic maintenance of equipment.

15.0 DOCUMENTATION, DATA REDUCTION, AND REPORTING

All documentation will conform to the Battelle HOM3 Quality Management Plan.

- All original data are recorded in ink.
- Corrections are made by placing a single line through the incorrect entry.
- Corrections are initialed, dated, and justified at the time the correction is made.

15.1 Data Collection

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

- A survey log form will be generated for each station visited during the Massachusetts Bay surveys.
- All field data will be recorded in ink on field sample data sheets and field logbooks.
- All laboratory data will be recorded with permanent ink in a bound notebook or on standardized forms.
- All QC data (precision, accuracy) will be recorded in laboratory notebooks.

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 floppy disks so that statistical analysis and data management can be completed.

15.2 Data Reduction

Where quantitative viral and bacterial indicator data are available, preliminary analyses will be conducted including descriptive statistics such as sample size, mean, standard deviation, minimum, maximum, median, mean, 90th percentile, and the 10th percentile for all numeric variables in the study. Once descriptive statistics are completed, tests for normality and variance will be conducted to determine whether parametric (e.g., t-tests, ANOVAs, Pearson product moment correlations) or non-parametric statistical analyses are appropriate. If data are not normally distributed, a non-parametric test (e.g., Kruskal-Wallis, Wilcoxon Rank Sum) or transformation of the data may be warranted. Where qualitative (*i.e*. presence, absence) viral data area available, frequency determinations and logistic regressions will be conducted. In addition, if the number of observations is sufficient, Chi square analyses will also be performed.

All results will be presented in a graphical or tabular form with supporting text. Summary tables and graphics will be prepared by Kristyn Stevens (TPMC) and reviewed by the Senior Scientist (Dr. Margolin) to observe noteworthy trends or inconsistencies. Summary graphics and tables will be maintained for subsequent use in preparing annual reports and synthesis reports. At the end of the project, the Senior Scientist (Dr. Margolin) will store all bound data books and diskettes, for at least seven years.

15.3 Data Reporting

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

1. Data submitted for inclusion in the EM & MS Database
2. Data presented in virus data and synthesis reports

15.3.1 EM & MS Database

Only data that have been designated as final by the Senior Scientist (Dr. Margolin) will be loaded into Battelle's copy of the EM & MS Database. A data loading application will be generated for each virus sampling event (Figure 14). The application will be populated with the LIMS SAMPLE_IDs received from MWRA or the NAVSAM SAMPLE_IDs received from Battelle. Dr. Margolin will enter his data into the application and send final results back to Battelle in hard copy and diskette format. See *Albro et al.* (1998) for further detail on the loading application. Table 9 shows the parameters and codes that will be used by Battelle to store these data in EM & MS. The in-situ hydrographic monitoring parameters have previously been described in Albro *et al.* (1998). Table 10 describes each of these database codes. Bacterial results generated by MWRA from the same samples will be processed by MWRA and an electronic data set will be submitted to Battelle once the analyses are completed. The data from MWRA will be in the form of an Oracle export and the table structures will be consistent with EM & MS.

Upon receipt at Battelle, 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 Battelle according to the log in identifier. The data sources notebook will contain copies of the chain-of-custody forms and data entry information.

15.3.2 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. The contents of the virus synthesis reports will include an executive summary, introduction, objectives, methods, results, conclusions, discussion and recommendation sections.

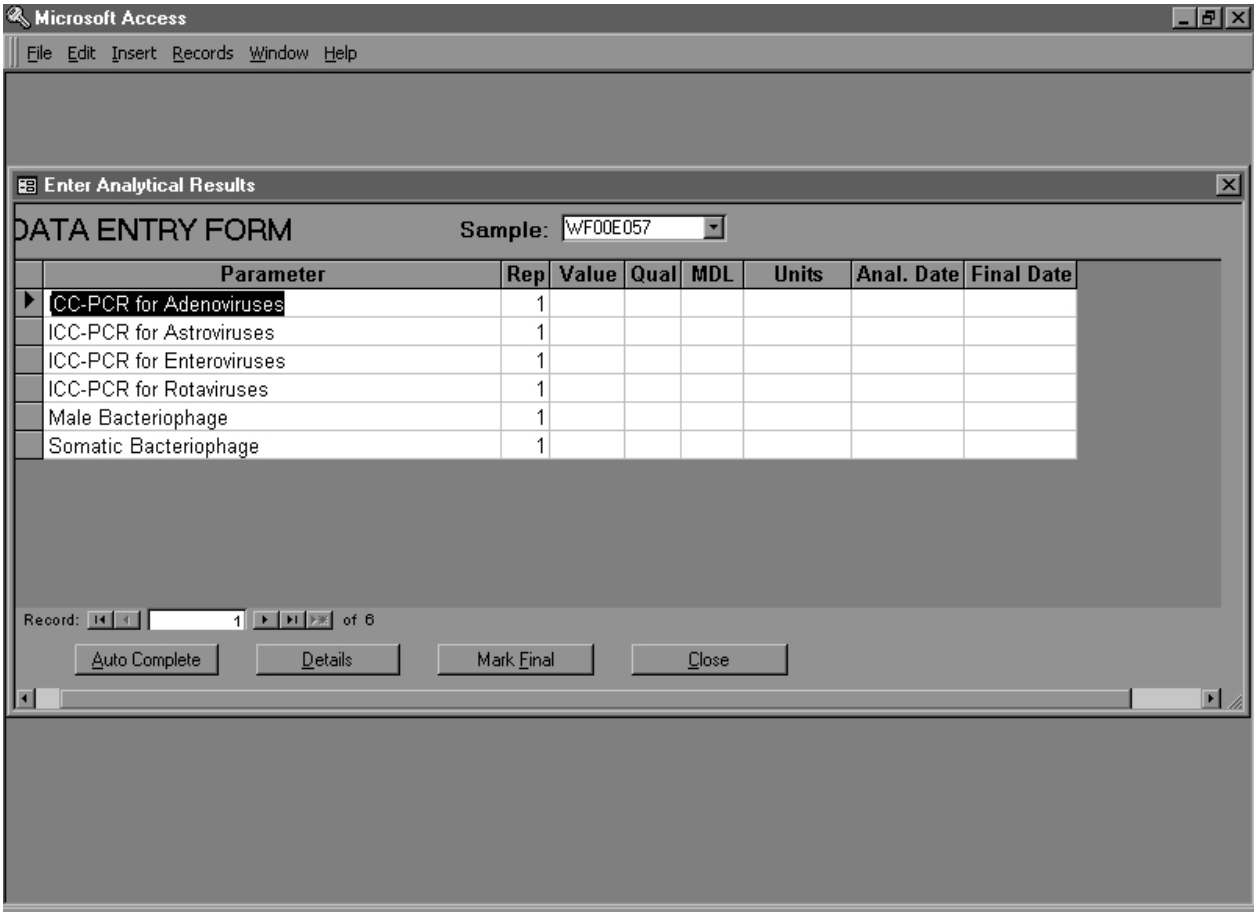

Figure 14. Data Loading Application.

Table 9. Parameters and Database Codes for the Anthropogenic Virus Survey.

Parameter	Param_Code	Unit_Code	Anal_Lab_ID	Meth_Code
Integrated Cell Culture-Polymerase Chain Reaction (ICC-PCR) for Adenoviruses	ICC_ADENO	NA	UNHV	BGMK
ICC-PCR for Astroviruses	ICC_ASTRO	NA	UNHV	CACO-2
ICC-PCR for Enteroviruses	ICC_ENTERO	NA	UNHV	BGMK
ICC-PCR for Rotavirus	ICC_ROTAV	NA	UNHV	CACO-2
Male Bacteriophage	MPHAGE	NA	UNHV	EPA 1601
Somatic Bacteriophage	SPHAGE	NA	UNHV	EPA 1601
Fecal Coliform	FCOL	#/100 ml	DIL	MFFC
Enterococcus	ECOC	#/100 ml	DIL	MFEC

Table 10. Descriptions of Database Codes.

Field Name	Code	Description
ANAL_LAB_ID	UNHV	University of New Hampshire
ANAL_LAB_ID	DIL	MWRA Central Lab
METH_CODE	BGMK	Buffalo Green Monkey Kidney cell method
METH_CODE	CACO-2	Calonic carcinoma cell culture method
METH_CODE	EPA1601	Two-step enrichment procedure for bacteriophage (EPA, 2000)
METH_CODE	MFFC	Membrane Filter Procedure (AHPA 1989, Section 9222D) for Fecal Coliform Bacteria
METH_CODE	MFEC	Membrane Filter Procedure (AHPA 1989, Section 9230C) for *Enterococcus*
UNIT_CODE	#/100 ml	Number of bacteria per 100 mL
VAL_QUAL	0	Absent
VAL_QUAL	1	Present
VAL_QUAL	A	Value above maximum detection limit, e.g. too numerous to count or beyond range of instrument
VAL_QUAL	As	Value above maximum detection limit and suspect/invalid, not fit for use
VAL_QUAL	B	Blank corrected, blank >= 5x MDL
VAL_QUAL	Bf	Blank corrected, blank >= 5x MDL, value reported < detect_limit
VAL_QUAL	D	Surrogate recovery < 50% or > 150%
VAL_QUAL	Ds	Surrogate recovery < 50% or > 150%, suspect/invalid, not fit for use
VAL_QUAL	E	Calibration Level Exceeded

Field Name	Code	Description
VAL_QUAL	ELs	Calibration exceeded, concentration reported from dilution, suspect/invalid, not fit for use
VAL_QUAL	Es	Calibration exceeded, suspect/invalid, not fit for use
VAL_QUAL	F	Abundance recorded for a fraction or portion of the sample collected
VAL_QUAL	I	Interferant from standard
VAL_QUAL	L	Analytical Concentration Reported From Dilution
VAL_QUAL	LE	Analytical concentration reported from dilution, calibration level exceeded
VAL_QUAL	LT	Analytical concentration reported from dilution, holding time exceeded
VAL_QUAL	Lq	Analytical concentration reported from dilution. May be invalid, under investigation (Do not use).
VAL_QUAL	Ls	Analytical concentration reported from dilution, suspect/invalid, not fit for use
VAL_QUAL	Lsx	Diluted, matrix interference, suspect/invalid, not fit for use
VAL_QUAL	P	Present but uncountable, value given is NULL
VAL_QUAL	S	Not surrogate corrected
VAL_QUAL	T	Holding time exceeded
VAL_QUAL	a	Not detected - value reported as negative or null
VAL_QUAL	aG	Not detected, value is null, co-eluting compound
VAL_QUAL	aGs	Not detected, value is null, co-eluting compound, suspect/invalid, not fit for use
VAL_QUAL	aGx	Not detected, value is null, co-eluting compound, matrix interference
VAL_QUAL	aL	Below MDL; value reported as negative or null, analytical conc. reported from dilution
VAL_QUAL	aLT	Not detected, analytical conc. reported from dilution, holding time exceeded
VAL_QUAL	aLs	Not detected, analytical conc. reported from dilution, suspect/invalid, not fit for use.
VAL_QUAL	aT	Not detected - value reported as negative or null, and holding time exceeded
VAL_QUAL	aq	Not detected - value reported as negative or null. May be invalid, under investigation (Do not use).
VAL_QUAL	as	Not detected - value reported as negative or null, and not fit for use
VAL_QUAL	asT	Not detected - value reported as negative or null, not fit for use, and holding time exceeded
VAL_QUAL	ax	not detected, value is null, matrix interference
VAL_QUAL	b	Not blank corrected, blank>=5x MDL
VAL_QUAL	bs	Not blank corrected, blank >=5x MDL, suspect/invalid, not fit for use
VAL_QUAL	e	Results not reported, value given is NULL. Explanation in COMMENTS field
VAL_QUAL	eq	Not reported, may be invalid, under investigation (Do not use).
VAL_QUAL	f	Value reported is below method detection limit

Field Name	Code	Description
VAL_QUAL	fG	Reported value below MDL and co-eluting compound interferes with peak of interest
VAL_QUAL	fL	Value reported is between zero and MDL, analytical conc. reported from dilution
VAL_QUAL	fT	Reported value below MDL and holding time is exceeded
VAL_QUAL	fq	VALUE reported is below method detection limit. May be invalid, under investigation (Do not use).
VAL_QUAL	fs	VALUE reported is below method detection limit, not fit for use
VAL_QUAL	fsT	Reported value is below MDL, suspect/invalid, not fit for use, and holding time is exceeded
VAL_QUAL	fsx	Value reported below detection limit, matrix interference, suspect/invalid, not fit for use
VAL_QUAL	fx	Below method detect limit, matrix interference
VAL_QUAL	g	Recovery outside data objectives
VAL_QUAL	gq	Recovery outside data objectives. May be invalid, under investigation (Do not use).
VAL_QUAL	h	Below the standard curve 0
VAL_QUAL	j	Estimated value
VAL_QUAL	jBS	Estimated, Blank corrected, blank > mdl by factor of 5 or greater, not surrogate corrected
VAL_QUAL	jS	Estimated, not surrogate corrected
VAL_QUAL	jp	Estimated value and bottles mislabeled
VAL_QUAL	m	Initial
VAL_QUAL	me	Initial, not reported
VAL_QUAL	ms	Initial value, not fit for use
VAL_QUAL	o	Value out of normal range judged fit for use by principal investigator
VAL_QUAL	p	Lab sample bottles mislabeled - caution data use
VAL_QUAL	q	Possibly suspect/invalid and not fit for use. Investigation pending.
VAL_QUAL	r	Precision does not meet data quality objectives
VAL_QUAL	s	Suspect/Invalid. Not fit for use.
VAL_QUAL	sT	Suspect/invalid, not fit for use and holding time is exceeded
VAL_QUAL	sv	Value is suspect/invalid and not fit for use, arithmetic mean of multiple results
VAL_QUAL	sx	Matrix interference, suspect/invalid, not fit for use
VAL_QUAL	v	Arithmetic mean
VAL_QUAL	x	Matrix interference

16.0 DATA VALIDATION

The data validation procedures for this project are defined in the HOM3 Quality Management Plan. As a part of data validation, each Laboratory Manager ensures that:

- Any data that are hand-entered (*i.e.*, typed) are 100% validated by qualified personnel prior to use in calculations or entry into the database.

- All manual calculations are performed by a second staff member to verify that calculations are accurate and appropriate.

- Calculations performed by software are verified at a frequency sufficient to ensure that the formulas are correct, appropriate, and consistent, and that calculations are accurately reported. All modifications to data reduction algorithms are verified prior to submission of data to the Authority.

Electronic data loading and transfer are swift and routine; data fields and formats are defined in the CW/QAPPs. Electronic submissions are loaded to temporary files prior to incorporation into the database, and are analyzed selectively using methods such as scatter plots, univariate and multivariate analyses, and range checks to identify suspect values. Routine system back-ups are performed daily.

Once data have been generated and compiled in the laboratory, senior project scientists review data to identify and make professional judgements about any suspicious values. All suspect data are reported with a qualifier. This data may not be used in calculations or data summaries without the review and approval of a knowledgeable Senior Scientist. No data measurements are eliminated from the reported data or database and data gaps are never filled based on other existing data. If samples are lost during shipment or analysis, it is documented in the data reports to the Authority and noted in the database. The methods used to identify suspect values for each type of data are defined in the CW/QAPP.

A series of reviews by technical personnel will be implemented to ensure that the data generated for Task 28 meet the data quality objectives. These reviews will include the following activities.

- Data and related project records will be reviewed by laboratory personnel at the end of each working day to ensure that analytical activities are completely and adequately documented.

- The Task Leaders will be responsible for reviewing analytical results and supporting documentation.

The results of QC sample analyses will be compared to pre-established criteria as a measure of data acceptability.

The review of quality control data is a critical step in the data validation process because quality control data that are within the QAPP acceptance criteria indicate that the sample processing and analysis systems are in control. Section 11.0 discusses the quality control program for anthropogenic virus work. The quality control procedures and any applicable corrective action for out-of-control quality control data and instrumentation calibrations are described in Section 11 and Appendix I. All quality control data that do not meet the data quality objectives will be flagged and brought to the attention of the Senior Scientist (Dr. Margolin) who will determine the appropriate corrective action (*e.g.*, re-analysis or data reported with qualifiers).

As part of data synthesis efforts, Ms. Stevens will be responsible for validation of all data generated by UNH to ensure that the data are accurate, complete, and scientifically reasonable. As stated above, all quality control data that do not meet standards will be brought to the attention of Dr. Margolin, Battelle, and MWRA. The MWRA will be responsible for conducting similar data validations of data generated in their laboratory. As an additional data validation step, the Senior Scientist will review all data for technical reasonableness. The Battelle Field Manager will be responsible for validation of the *in situ* water quality data and navigation data.

In summary, several documentation steps will be included in the data validation process. All data for the virus task will be extracted from the database by MWRA and delivered to both TPMC and the Senior Scientist (Dr. Margolin). In addition, TPMC will be included on the distribution list of data reports submitted to MWRA by Battelle. All data requests for the virus task will be submitted to Wendy Leo (MWRA), with Ken Keay and Kelly Coughlin copied.

17.0 PERFORMANCE AND SYSTEM AUDITS

The Battelle QA Officer for the Harbor and Outfall Monitoring Project is Ms. Rosanna Buhl. She will direct the conduct of at least one systems audit to ensure that Task 28 is carried out in accordance with this CW/QAPP. A systems audit will verify the implementation of the Quality Management Plan and this CW/QAPP for the work conducted in the Water Quality monitoring.

Tabular data reported in deliverables, and associated raw data generated by Battelle will be audited under the direction of the Project QA Officer. Raw data will be reviewed for completeness and proper documentation. For electronically acquired data (*e.g.*, navigational data), Ms. Buhl will verify that computer software used to process the data has been validated. Errors noted in data audits will be communicated to analysts and corrected data verified.

Audits of the data collection procedures at subcontractor laboratories will be the responsibility of the Subcontractor. Each subcontractor is fully responsible for the QA of the data it submits. Data must be submitted in CW/QAPP-prescribed formats; no other will be acceptable. 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 Battelle Database 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 and a QC narrative of activities.

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 Battelle Project Manager, Dr. Carlton Hunt. 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 Battelle 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 Project Area Manager (Ms. Kelly Coughlin), the Battelle Field Manager (Mr. Wayne Trulli), and the Project Senior Scientists (Dr. Aaron Margolin and Ms. Kristyn Stevens). Problems relating to the overall successful completion of the project will be reported to the MWRA Program and Project Area Manager in a timely manner for discussion and resolution between the Battelle 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 Battelle Laboratory Manager or the Battelle Project Manager. They will be responsible for evaluating the overall impact to the project and for discussing corrective actions with the MWRA Project Manager.

A QA/QC Corrective Action Log will be maintained by the Project QA Officer and submitted to MWRA at quarterly intervals. The log will include documentation of QA/QC activities, 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, data reports, and synthesis reports. Battelle 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. Battelle survey reports will document actual survey dates and operations, interim results, problems encountered, corrective actions, and recommendations for potential modifications to the CW/QAPP. Virus data reports will include data tables of the samples collected and reported, the qualifier codes used in the data report, and the virus, bacteriophage and bacteria survey data. Data quality considerations will also be noted.

The Anthropogenic Virus Synthesis report prepared under Task 33 will start with an introduction, which will provide a brief overview of current knowledge in the area of viral detection in waters impacted by fecal pollution and/or waste water treatment effluent. It will also serve as a reporting place for a small literature review of other studies that have investigated anthropogenic viral contributions to freshwater and marine ecosystems. A Materials and Methods section will also be included, detailing a description of the methods used for quantification of viruses, bacteria, and physiochemical data. This will also describe

any deviations from the CW/QAPP and include maps of sampling locations and rain gauge stations used for collecting rainfall data for Boston Harbor and Charles River.

Reporting of the results will be provided in tabular and/or graphical form for the four proposed areas: 1. Boston Harbor, 2) Charles River, 2a) Cottage Farm CSO Treatment Facility, 3) Deer Island Waste Water Treatment Plant, and 4) Massachusetts Bay. Conclusions resulting from the data analysis and related to the initial objectives from the introduction will be discussed. The last section of the synthesis report will include recommendations for future sampling and analysis, relevant references, and any extraneous appendices.

20.0 REFERENCES

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APPENDIX I

SOP and Quality Assurance Manual for EPA Information Collection Rules
Available upon request from the University of New Hampshire

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