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

for

ANTHROPOGENIC VIRUS SURVEY: 1998-2000

Task 28
MWRA Harbor and Outfall Monitoring Project

submitted to

MASSACHUSETTS WATER RESOURCES AUTHORITY
Environmental Quality Department
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COMBINED WORK/QUALITY ASSURANCE PROJECT PLAN (CW/QAPP)

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ANTHROPOGENIC VIRUS STUDY: 1998-2000
Task 28
MWRA Harbor and Outfall Monitoring Project

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CONTENTS

CONTENTS (Continued)

CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

1.0 PROJECT NAME

MWRA Harbor and Outfall Monitoring Project
Task 28
Anthropogenic Virus Survey

2.0 PROJECT REQUESTED BY

Massachusetts Water Resources Authority

3.0 DATE OF REQUEST

November 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, pre- and 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 (Poliovirus, Coxsackie Virus, and Echovirus); polymerase chain reaction (PCR) testing for Hepatitis A Virus, Rotavirus, and pan-enterovirus screen; male-specific bacteriophages, and somatic bacteriophages.

The distribution and total number of samples that will be collected by MWRA and Battelle staff annually from 1998-2000 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 will include:

- quantification of cultivatable strains of viruses (Poliovirus, Coxsackie Virus, and Echovirus) using the Buffalo Green Monkey Kidney (BGMK) cell method, with results reported as most probable number (MPN) total cultivatable virus density;

- additional screening of strains not readily detected by the BGMK method (Hepatitis A Virus and Rotavirus) using the polymerase chain reaction (PCR) method, with results reported individually for each;

Table 1. Total Samples to be Collected Annually, 1998-2000

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.
(2 surveys × 5 stations/survey = 10 and 4 surveys with 9 samples/survey = 36)

Table 2. Monitoring Parameters for Anthropogenic Virus Surveys

Parameter	Sample Container	Preservation	Analysis Method	Holding Time	Units
Cultivatable Viruses	Filtered in field	4°C (cooler with ice)	BGMK	elute within 48 hrs, indefinite at -20°C	MPN/L
Viral Genome Screening	Filtered in field	4°C (cooler with ice)	PCR	elute within 48 hrs, indefinite at -20°C	presence/ absence
Bacteriophages	Filtered in field	4°C (cooler with ice)	SM 9211 D	elute within 48 hrs, indefinite at -20°C	PFU/mL
Fecal coliform	sterile plastic specimen cup or filter in field if holding time would be exceeded	4°C (cooler with ice)	SM 9222 D or	6 hrs	fecal coliforms per 100 mL
			SM9222E	72 hrs	
Enterococcus	sterile plastic specimen cup or filter in field if holding time would be exceeded	4°C (cooler with ice)	SM 9230 C	6 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
Transmissometry[a, f]	*in-situ*	NA	probe[b]	NA	m-1
Secchi Disk	*in-situ*	NA	[e]	NA	m
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
BGMK Buffalo Green Monkey Kidney cell method
PCR Polymerase Chain Reaction method
SOP Standard Operating Procedure
MPN Most Probable Number
PFU Plaque Forming Unit
PSU Practical Salinity Unit
SM Standard Methods
mS micro Sieman

- pan-enterovirus PCR screen for enteroviruses;
- enumeration of both male-specific and somatic bacteriophages; and
- enumeration of fecal coliform and *Enterococcus* bacteria densities (by MWRA).

Concentration of surface (0 to 12 inches deep) receiving water samples for virus analyses will be performed in the field using microporous filtration. Filters will be transported on ice in coolers to the Waterborne Disease Laboratory at UNH for subsequent elution, 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. If transport to the laboratory within a 6-hr holding time is not possible, initial processing of indicator bacteria analysis will be done by MWRA in the field.

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 1998-2000. 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.

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;
- 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.

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.

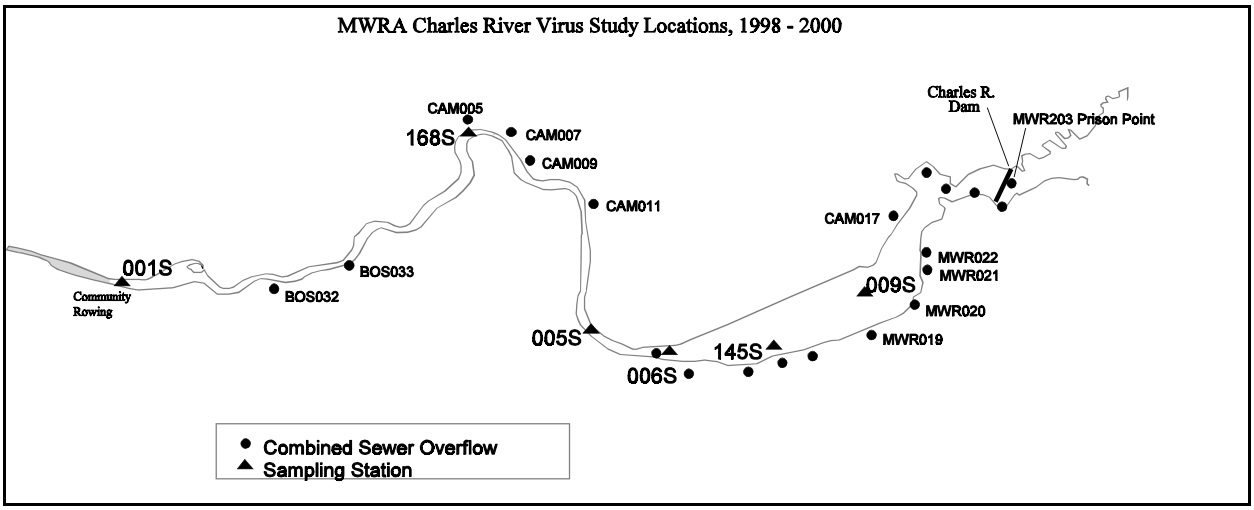

Figure 2. Charles River Sampling Locations.

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)
COTT-FRM INF	NA	NA	Cottage Farm Influent
COTT-FRM EFF	NA	NA	Cottage Farm Effluent

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). Wet weather sampling will proceed after a storm of sufficient size to activate the Cottage Farm Treatment Facility. If possible, sampling will be done during facility activation. The facility operator will notify Kelly Coughlin, MWRA Biologist, of an impending activation. Ms. Coughlin will communicate with treatment plant operators and notify Dr. Rex, MWRA's Anthropogenic Virus Survey Area Manager. Once the facility is activated, a designated MWRA representative will notify Battelle and Dr. Margolin of the impending sampling event. The locations indicated below will be sampled, including one sample of CSO influent and one sample of CSO effluent (treated, dechlorinated).

Sampling will be conducted by two sampling teams. The first team will collect samples from land at Stations 001S (Community Rowing), 168S (CAM005), and 005S (Magazine Beach). The second team will sample stations 006S, 145S and 009S from boat.

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, ten wastewater samples will be collected for virus analysis. These will include influent and primary and secondary effluents from the Deer Island Treatment Plant. Sampling and analysis will continue in 1999 and 2000. 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 will be collected before and after the outfall is commissioned. 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.

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

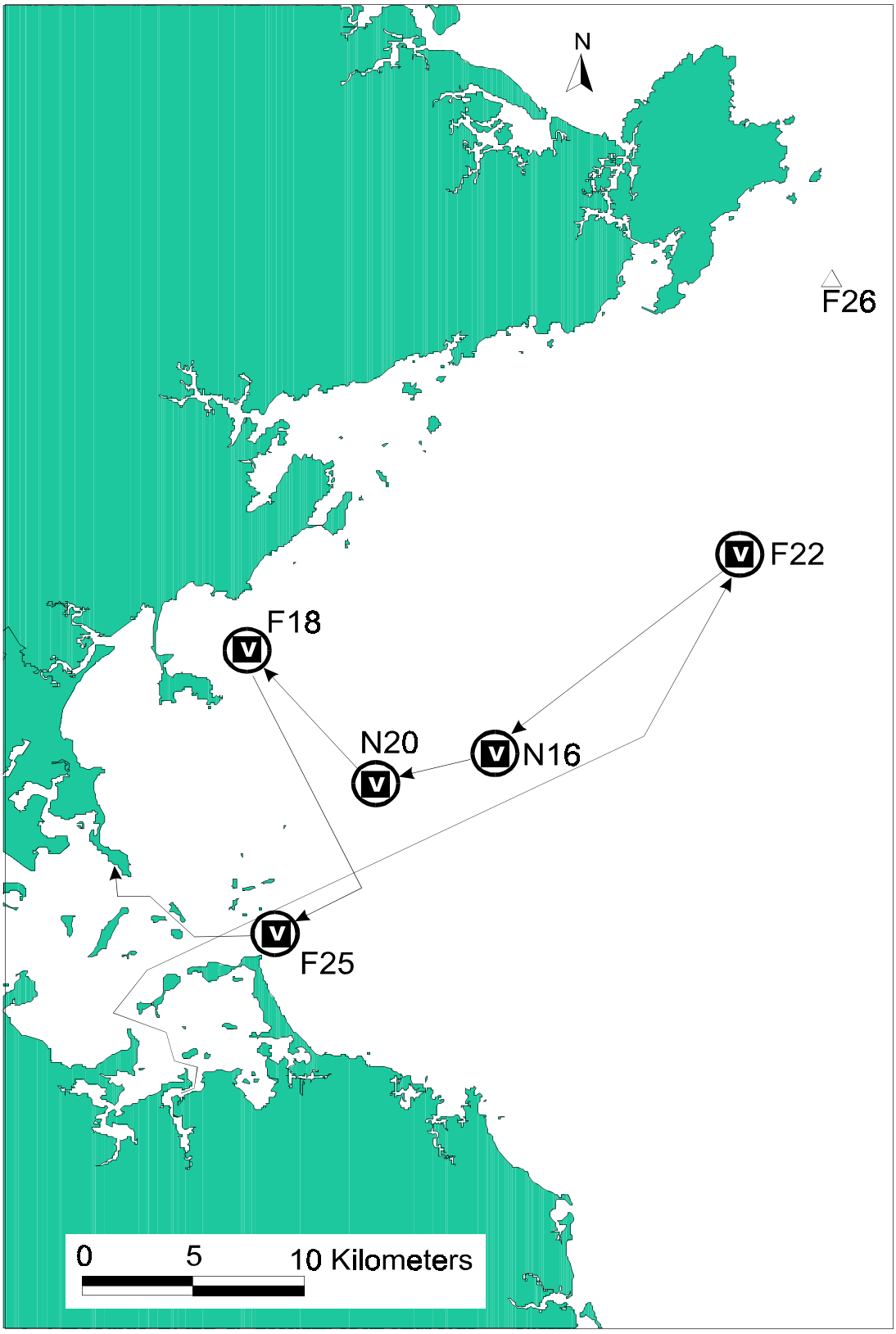

Figure 3. Massachusetts Bay Sampling Locations (virus sampling locations are circled.)

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.

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, 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**.**

Samples will be delivered to the MWRA Central Laboratory within six hours of collection 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. If transport to the laboratory within a 6-hr holding time is not possible, initial processing of indicator bacteria analyses will be done by MWRA in the field.

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. Virus recovery efficiency testing will be covered under a separate task order to be issued by MWRA.

9.0 SCHEDULE OF ACTIVITIES AND DELIVERABLES

Sampling activities associated with the Anthropogenic Virus Survey (Task 28) are scheduled in 1998, 1999, and 2000. 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
AV981	25-Feb-98
AV982	22-Apr-98
AV983	16-Jun-98
AV984	18-Aug-98
AV985	06-Oct-98
AV986	15-Dec-98
AV991	23-Feb-99
AV992	21-Apr-99
AV993	15-Jun-99
AV994	17-Aug-99
AV995	05-Oct-99
AV996	13-Dec-99
AV001	22-Feb-00
AV002	19-Apr-00
AV003	13-Jun-00
AV004	15-Aug-00
AV005	03-Oct-00
AV006	11-Dec-00

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 Dr. Rex 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 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 Dr. Andrea Rex 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 Dr. Rex. 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. 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. Carl Albro is the Battelle Field Manager responsible for all Battelle field collections. Ms. Heather Trulli, Battelle's Laboratory Manager, is responsible for overseeing all laboratory activities in the contract. Ms. Ellie Baptiste-Carpenter is Battelle's Database Manager. Dr. Kristen Tilton 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.

10.2 FIELD PROGRAM

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

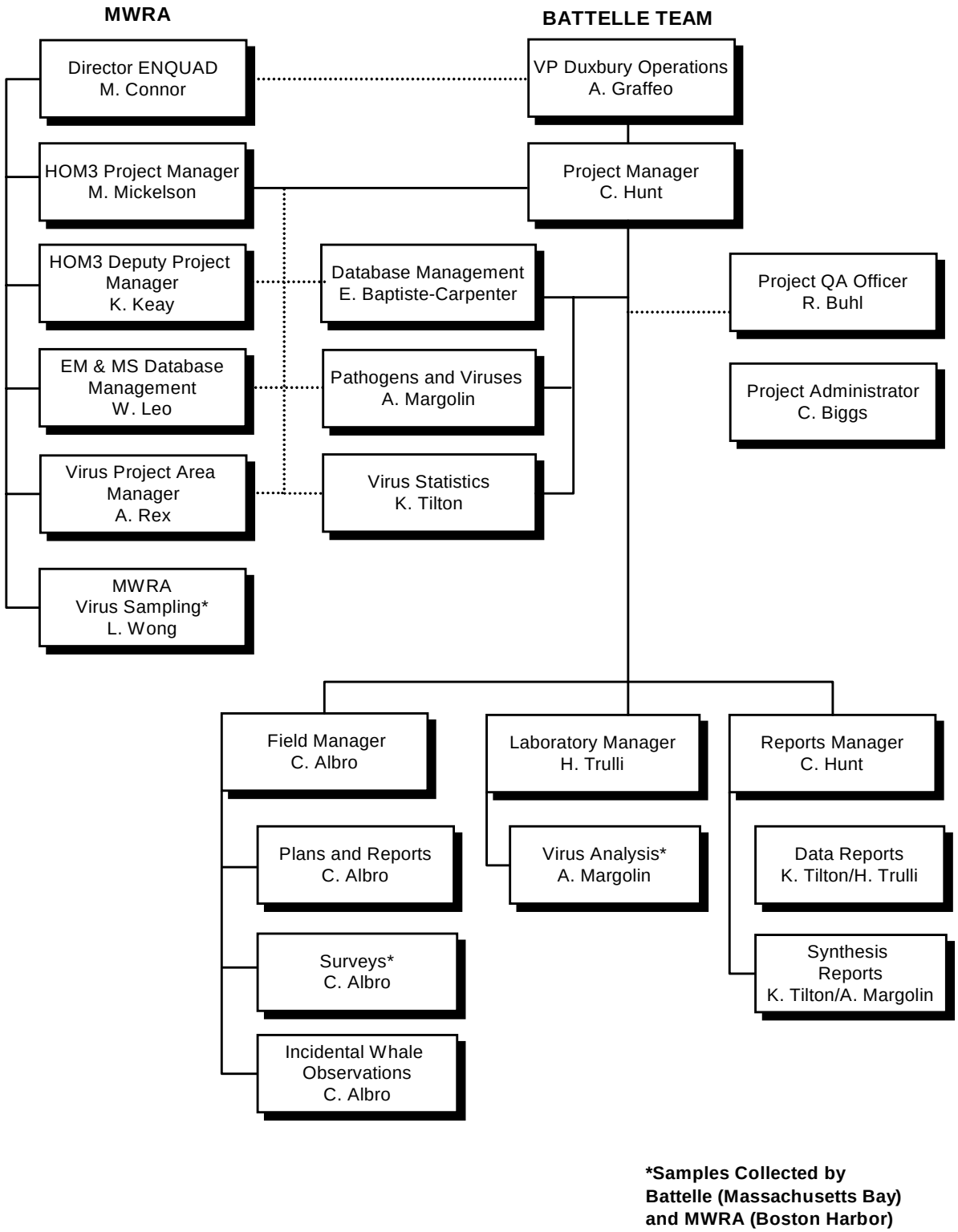

Figure 4. Organizational Structure for the Anthropogenic Virus Study Area

10.3 LABORATORY ANALYSIS PROGRAM

Analyses of viral 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 Lisa Wong.

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 and will provide the final virus data to Dr. Tilton 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 QUALITY CONTROL/QUALITY ASSURANCE

11.1 NAVIGATIONAL AND HYDROGRAPHIC INFORMATION

The definitions for precision, accuracy, completeness, comparability, and representativeness are provided in the Quality Management Plan (Battelle, 1998). The application of these measures of data quality are described below.

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

Anthropogenic virus measurements methods are the subject of continuing development and assessment. EPA is in the process of developing information on this (see Appendix I). As such appropriate data quality objectives are under evaluation. Therefore precision, accuracy, and efficiency necessary under this program and corrective actions are the subject of continuing method development.

In the examination of a particular water, a preliminary evaluation of virus recovery efficiency should be done. To accomplish this, virus recovery efficiencies will be determined by analyzing samples prepared in the field and by analyzing samples prepared at the bench scale. A total of six field scale and two bench scale samples will be collected and analyzed annually.

For the field-prepared virus-recovery efficiencies, a sample volume of 150 L of surface (approximately 12 inches deep) water will be collected by pump into large pre-sterilized garbage barrels. A known concentration of bacteriophage (MS2) will be added to each sample. The pH of the sample will be adjusted to 3.3 to 3.7 by the addition of 2.0 N HCl, and $AlCl_3$ added to adjust to 0.001 to 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). The recovery efficiency will be determined by comparing the concentration of seeded virus to the concentration of virus recovered.

In previous years, only five liter carboy samples were collected to estimate recovery efficiencies. The primary advantage of conducting the field-prepared sampling is that the virus recovery efficiencies are based on volumes of water that are representative of sample volumes (150 L). MS2 was selected as the seeding phage due to its morphological and behavioral similarities to the human viruses most likely to be found in receiving water samples. Additional benefits of seeding with bacteriophage rather than Poliovirus in the field include the ease of obtaining samples, ease of enumerating phage, and non-pathogenicity.

For the bench scale-prepared virus recovery efficiencies, an additional five-gallon sample will be collected in a carboy at the time of field-prepared virus recovery efficiencies. This carboy will be placed on deck and covered to shield against effects of sunlight. Upon return to the UNH laboratory, a known quantity of bacteriophage and Poliovirus I will be added to the sample. The sample will be processed and analyzed as referenced in sections 12.4 (Anthropogenic Virus Sampling and Analysis Procedure) and 12.5.6 (Bacteriophage Sampling and Analysis Procedures) to determine the recovery efficiencies of bacteriophage and Poliovirus 1, respectively. The recovery efficiencies will be determined by comparing the concentration of seeded virus to the concentration of virus recovered.

The bench scale sampling will yield estimates of bacteriophage and Poliovirus recovery efficiencies based on much smaller sample volumes (five liter). These samples will provide data upon which a ratio of recovery efficiencies of phage versus Poliovirus I can be determined. This ratio could also be used as a benchmark to help evaluate the filtration efficiency in the field.

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.

11.3.4 Polymerase Chain Reaction Screening

To ensure quality control the following reactions will be run:

1. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) control provided by Perkin-Elmer (or laboratory equivalent)
2. primers, master mix, reverse transcriptase (RT) and taq polymerase (TP) - this reaction will be run for each set of virus primers
3. primers, master mix, RT, TP, Rotavirus
4. primers, master mix, RT, TP, Hepatitis A Virus
5. primers, master mix, RT, TP, pan-enterovirus
6. primers, master mix, RT, TP, samples from surveys

Reaction 1 will ensure that the enzymes, nucleotide precursors and buffers provided by Perkin-Elmer do work according to their specification. This will be demonstrated by amplification of a segment of nucleic acid provided by Perkin-Elmer. Visualization of this amplified band and all amplified bands in the experimental reactions will occur by gel electrophoresis and subsequent ethidium-bromide staining. Amplified bands will appear as pink lines in the gel upon examination with UV light. Band size will be evaluated by comparison to known lengths of nucleic acid, known as a DNA ladder, provided by Perkin-Elmer. Quality control procedures will be performed per manufacturer’s method manual. If quality control procedures are not met, analysis will be repeated.

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

will be evaluated by this procedure and if demonstrated negative, used for the experimental reactions.

Reactions 3, 4 and 5 will act as positive controls for Rotavirus, Hepatitis A Virus and pan-enterovirus screening.

Reactions 6-n will contain one each of the concentrated samples collected from the sampling stations.

11.3.5 Coliphage

A positive control for the male specific, MS-2 (C 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.

11.3.6 Fecal Coliform and *Enterococcus*

Replicate laboratory analyses will be done for all samples. 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. 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 Kelly Coughlin, MWRA Biologist. Ms. Coughlin will communicate with treatment plant operators and notify Dr. Rex when the CSO facility discharges. If rainfall criteria appear likely to be satisfied by the forecasted weather pattern, the MWRA Project Area Manager (Dr. Rex) 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*) following MWRA Standard Operating Procedures. 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 #
Dr. Andrea Rex	**MWRA Program Manager - Harbor Monitoring**	(617) 241-6510	(617) 333-0662	
Kelly Coughlin	MWRA staff	(617) 241-6518	(617) 983-3291	
Microbiology Lab	MWRA staff	(617) 539-4300	NA	
Rob Rabideau	MWRA boat Captain	(617) 242-6000 x4762	(508) 664-1731	(617) 429-6982 (car phone)
Greg Alessandro	MWRA Somerville Marginal CSO facility	(617) 389-1685 (617) 389-6605	NA	
Greg Alessandro	MWRA Commercial Point CSO facility	(617) 265-8344 (617) 389-1685 (617) 389-6605	NA	
Lisa Wong	MWRA Central Laboratory	(617) 539-4331	(508) 664-4760	
Dr. Aaron Margolin	UNH Principal Investigator	(603) 862-2250	(603) 679-8387	(800) 225-0256
Bob Mooney	UNH staff	(603) 862-2250	available from UNH PI	
Jason Bellner	UNH staff	(603) 862-2250	available from UNH PI	
Derek Hodgkins	UNH staff	603-862-2250	available from UNH PI	
Heather Trulli	Battelle Laboratory Manager	(781) 934-0571	available from Battelle	
Carl Albro	Battelle Field Manager	(781) 934-0571	available from Battelle	

12.2 MASSACHUSETTS BAY SAMPLING BY BATTELLE

12.2.1 Navigation and Hydrographic Profiling

Vessel positioning during sampling operations will be accomplished with the BOSS navigation system. This system consists of a Northstar DGPS interfaced to the BOSS 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
- SeaBird CTD interface deck unit
- SeaBird SBE-9 CTD system (the Ocean Sensors 0S200-CTD will be used as a backup) 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.

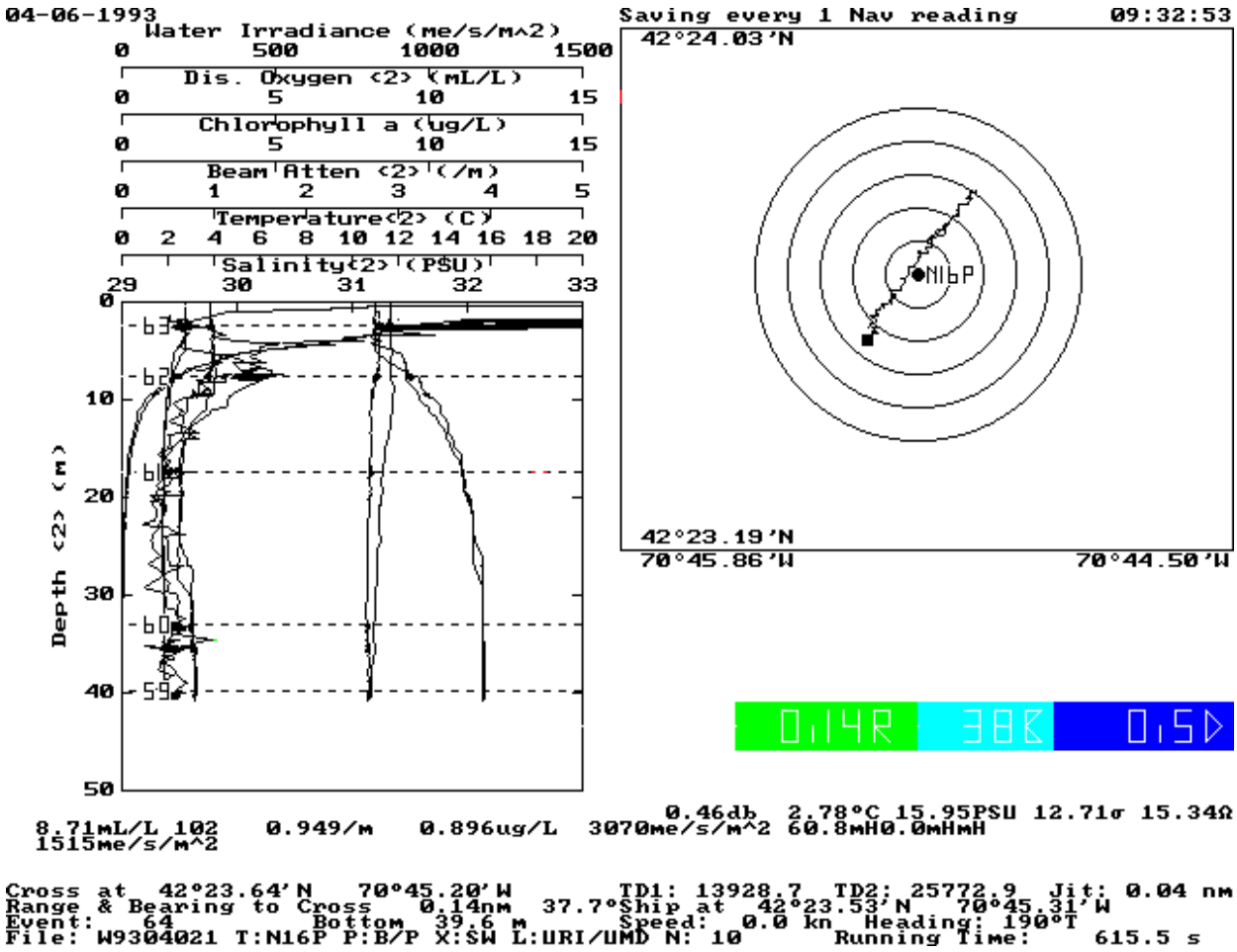

Figure 5. Sample NAVSAM Data Acquisition Screen

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 six 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 will be used for the enumeration of fecal coliform, and m-*Enterococcus* agar will be used for counting *Enterococcus* colonies.

If it is not possible to transport the water samples to the laboratory within the 6-hour time, initial processing of samples will be done in the field by MWRA. For fecal coliform tests, two 10-mL aliquots of water will be filtered through a membrane filtration setup per MWRA's SOP. The filters will be transferred to M-ST holding medium (Standard Methods 9222E Delayed-Incubation Fecal Coliform Procedure). The test will be completed in the laboratory by transferring the filter to M-FC medium, and completing the test per MWRA's SOP (MWRA, 1996b).

For *Enterococcus*, two 10-mL aliquots of water will be filtered in the field, and the filters placed on m-*Enterococcus* agar. Incubation will be delayed until the filters are returned to the laboratory.

MWRA staff will perform initial processing of samples in the field using MWRA's equipment and supplies.

12.4 ANTHROPOGENIC VIRUS SAMPLING PROCEDURES

Virus collection, elution, concentration and detection techniques will be those referenced in Standard Methods for the Examination of Water and Wastewater, 19th edition, section 9510 C and 9510 G (APHA *et al.*, 1995), except as noted. Negatively charged micro-wound filters will be used in the collection process and viruses will be evaluated using the BGMK Cell Line.

12.4.1 Virus Collection

For natural water samples, both freshwater and seater, a sample volume of 30 to 40 gallons water will be collected using a pump. Samples will be collected in large pre-sterilized garbage barrels in which the pH will be adjusted to between 3.3 and 3.7 by the addition of 2.0 N HCl (6.0 N HCl may be used at locations where the buffering capacity of seawater is high). 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, 40L of sample will be collected into presterilized barrels. If wastewater is chlorinated, sample will be collected into barrels 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. A Peel Internal gear pump with maximum flow rate of 10 gal/min will be used in Massachusetts Bay.

As sample is collected, $AlCl_3$ will be added to each 30 to 40 gallon sample to yield a solution concentration of between 0.001 and 0.0001 molar. The final pH of each sample will be adjusted to fall between 3.3 and 3.7 and the sample mixed thoroughly prior to filtering. The pump will then be used to pump the treated water through a microporous filter at a rate no faster than four gallons per minute. The entire volume of water will be passed through the filter. A flow meter will be placed directly after the filter to measure the total volume of water filtered and to ensure that a flow rate of four gallons per minute is not exceeded. Once filtration is completed, the filter will be removed from its housing, placed in a labeled plastic bag, and stored chilled at 4°C until elution. Volumes are documented on field data sheets.

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 and allowed to remain for 15-30 minutes. Following bleaching, water from the pending sampling site will be mixed with sodium thiosulfate and used to flush the entire system to neutralize all the bleach. Once-through contact is sufficient to neutralize chlorine. After bleaching the tubing will be flushed for a minimum of five minutes with non-chlorinated water.

12.5 VIRUS LABORATORY ANALYSIS

12.5.1 Virus Elution

Viruses will be eluted from filters within 48 hrs using 3.0% beef extract, 90 mM glycine at pH 9.0. For this procedure, filters will be placed in a filter housing at the lab. To this, between 800-1000 mL of beef extract eluent will be added to the filter. The pH of the mixture will be checked and adjusted back to 9.0 if needed. The filter will be agitated for ten minutes using an up and down motion which will allow the beef extract to flow inwards and outwards continuously. After ten minutes, the filter will be removed and the eluent transferred to a 1-liter flask containing a stir bar.

12.5.2 Virus Concentration by Organic Flocculation

The pH of the eluent will then be adjusted to 3.5 using 1 N HCl. The mixture will be allowed to mix for 30 minutes and then transferred to four 250-mL centrifuge flat bottom bottles. The floc will be collected by centrifugation at 10,000 X g 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 20-30 mL of a 0.1 N solution of sodium phosphate buffer (dibasic), pH 9.0. The resuspension process will be done by pipetting the floc up and down in a 10-25 mL pipet for no more than ten minutes. After the first minute of resuspension, the pH of the solution should be checked and if needed, adjusted back to 9.0. After ten minutes of resuspension, the solution will be returned to pH 7.0-7.4 using 1 N HCl. To remove any undissolved particles, the sample will be centrifuged for 10 minutes at 10,000 X g. The supernatant will be carefully removed and divided into two aliquots, one aliquot having twice as much as the other, i.e., 2/3 in one and 1/3 in the other. The samples will be labeled and stored at -20°C for analysis by cell culture. The 2/3 sample is the primary sample used for analysis. The 1/3 sample is archived as a backup sample.

12.5.3 Detection of Virus Using the BGMK Cell Line

Concentrated water samples will be defrosted and brought to 37°C prior to application to cells. The sample is split as follows: two-thirds of the sample is for active evaluation (12 mL for cell culture and 3 mL for PCR assay) and one-third of the sample is archived. Four flasks containing confluent monolayers of 75 cm^2 of BGMK cells will be inoculated with one mL of the sample per 25 cm^2 of cells, or three mL of the sample. Once samples have been inoculated, they will be incubated at 37°C for one to two hours with rocking every 15 minutes. Samples will then be observed for potential toxicity to the BGMK cell line. Potential toxicity will be determined by microscopically examining the cells to determine their state of health. Sample toxicity will be confirmed if detrimental effects are observed (e.g., cell rounding, floating, or death). Samples found to exhibit toxicity to the BGMK cell line will be inoculated for only one hour, whereas samples with no observed toxicity will be inoculated for two hours.

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

second passage, one mL of the supernatant/lysate will be place on confluent monolayers of BGMK cells housed in 25 cm^2 flasks and then examined daily for 10-14 days.

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

12.5.4 Detection of Virus Using PCR

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

12.5.5 Calculation of Virus Concentration

12.5.5.1 Total Cultivatable Viruses Using the BGMK Cell Line

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

For a given dilution yielding both positive and negative cultures,

$$\text{MPN}/L \quad \frac{P}{\sqrt{NQ}}$$

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

Dilution is accounted for by multiplying MPN/L by the reciprocal of the dilution factor.

12.5.5.2 PCR

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

12.5.6 Bacteriophage Sampling and Analysis Procedures

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

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

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 six 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. Lisa Wong	Dr. Aaron Margolin
Deer Island	4 Lewisburg 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). 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, "VI1" 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
EN	Enterococcus	MWRA	60
FE	Fecal coliform	MWRA	60
VI	Virus analysis	UNH	60

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	☐	☐	☐	☐
	WF9810 71WW1	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
FOR
MISCELLANEOUS SAMPLES

PAGE: 1 OF 1

SAMPLE LOC.	SAMPLE ID	DATE COLLECTED	TIME	SAMPLE LOCATION DESCRIPTION	PLANT	TYPE / TESTS	/ PRESERVATIVE / BOTTLE
						G C CG GS/ 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. The same SAMPLE_ID will be used for virus and indicator bacteria samples from the same station (and depth, if applicable).

Subsequent coding in the UNH lab would then indicate subsample type and number, as follows:

- TCV = total cultivatable virus
- HAV = Hepatitis A Virus
- ROT = Rotavirus
- PAN = pan-enterovirus screen
- PHA = bacteriophage

Virus samples filtered 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. Analytical procedures and data management will be documented on individual data sheets used for analyses of cultivatable viruses, PCR screening, and bacteriophage assays (Figures 9 through 13). After processing, the remaining eluted sample will be archived frozen (-20°C) until the end of the study.

Transfer of bacteriological samples will be documented on the chain-of-custody forms, which will be signed and dated by both the person relinquishing the samples as well as the recipient. One sheet of the multiple-page form will be retained by the 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.

Enteric Virus Sample Data Sheet

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

Primary Passage

Dilution Factor: _____

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

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

Confirmation

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

Figure 9. Enteric Virus Sample Data Sheet

PCR Sample Data Sheet

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

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

Final Results:

Virus: _____ Nucleic acid present in sample: _____

Virus: _____ Nucleic acid present in sample: _____

Virus: _____ Nucleic acid present in sample: _____

Figure 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

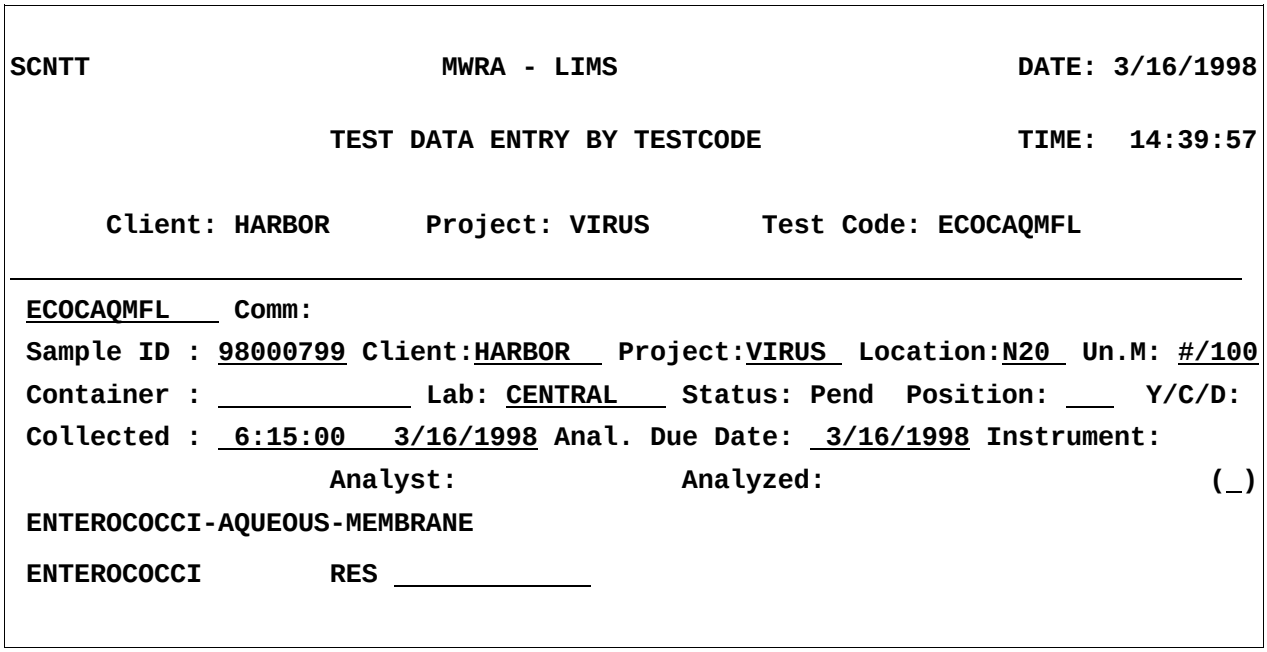

Figure 12. MWRA LIMS Entry Screen. The following fields are not used for this study: 'container', 'position', 'Y/C/D', and 'Instrument'. The date and time the sample was filtered are entered into the 'analyzed' field. The result is entered in the 'RES' field.

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

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

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

Matrix
DW-Drinking Water
DTW-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								

CALCULATION OF RESULTS:
Colonies / 100mL = (100 x Plate Count (PC)) / Volume (mL) x Dilution Factor (DF)

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

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

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.

Proper function of navigational equipment will be checked by confirmation of readings at surveyed reference point within the last three months 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).

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 AND REDUCTION

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.

Experimental results will be processed and summarized in tabular and/or graphical form. Summary graphs and tables will be prepared and reviewed by the Senior Scientist (Drs. Margolin and Tilton) to observe noteworthy trends or inconsistencies. Summary graphs and tables will be maintained for subsequent use in preparing annual reports.

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

15.2 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 Annual Virus Data Reports

15.2.1 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. 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.

15.2.2 Annual Virus Data Reports

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

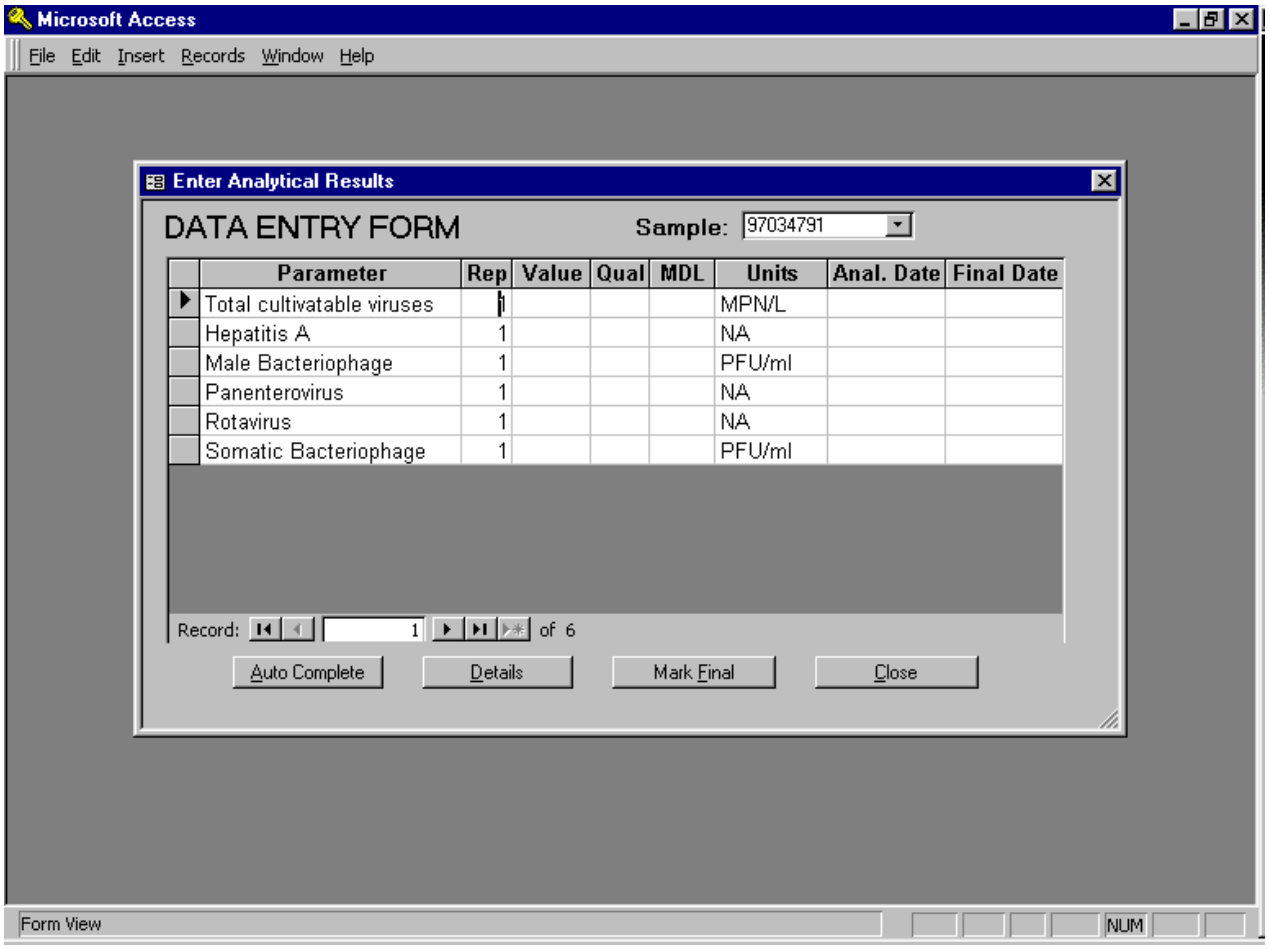

Figure 14. Data Loading Application

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

Parameter	Param_Code	Unit_Code	Anal_Lab_ID	Meth_Code
Total cultivatable viruses	CULTV	MPN/L	UNHV	BGMK
Panenterovirus	PANENT	NA	UNHV	PCR
Hepatitis A	HEPA	NA	UNHV	PCR
Rotavirus	ROTAV	NA	UNHV	PCR
Male Bacteriophage	MPHAGE	PFU/L	UNHV	SM9211D
Somatic Bacteriophage	SPHAGE	PFU/L	UNHV	SM9211D
Fecal Coliform	MWRA22	#/100 ml	DIL	MFFC
Enterococcus	ECOCC2	#/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	PCR	Polymerase chain reaction
METH_CODE	SM9211D	Plaque count for bacteriophages (Modified AHPA 1989, Section 9211 D)
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	MPN/L	Most probable number per liter
UNIT_CODE	PFU/L	Plaque forming units per liter
UNIT_CODE	#/100 ml	Number of bacteria per 100 mL
VAL_QUAL	0	Absent
VAL_QUAL	1	Present

sample analyses plus QC data. The contents of the Virus Data Reports will include an executive summary, introduction, objectives, methods, results, discussion and conclusions sections.

16.0 DATA VALIDATION

Data validation is the responsibility of those immediately responsible for overseeing and/or performing analyses, data entry, data reduction, and data reporting. The HOM3 Quality Management Plan (Battelle, 1998) describes data validation procedures in the laboratory. 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. Tilton) who will determine the appropriate corrective action (e.g., reanalysis or data reported with qualifiers).

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

- 100% of data that are hand-entered into a database or spreadsheet will be verified for accuracy either by (1) printing the spreadsheet and proofreading against the original hand entry or by (2) duplicate entry into the database and comparison of the entries to detect any differences. These tasks will be carried out by two people and documented for each data set.

- All manual calculations will be checked for accuracy by a second staff member.

- Calculations performed by software will be checked by the technical staff member at a frequency sufficient to ensure the accuracy of the calculations. All data-reduction algorithms will be verified prior to final data submission.

- Subsets of the analytical data will be reviewed by in-house or subcontractor data validators. The data will be reviewed for adherence to analytical protocols and to pre-established criteria (e.g., for holding times, equipment calibration, laboratory duplicates, blank contamination).

- Database staff will check the received data and associated documentation for completeness, freedom from errors, and technical reasonableness.

- All new software developed for this task will be validated before entry of data.

As part of data synthesis efforts Dr. Tilton will be responsible for validation of all data generated by UNH to ensure that the data are accurate, complete, and scientifically reasonable. 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.

17.0 PERFORMANCE AND SYSTEM AUDITS

This project will be monitored by the Project QA Officer. Tabular and graphic data reported in deliverables generated by UNH will be reviewed by the Project QA Officer. Raw data will be reviewed for traceability, accuracy, completeness, and proper documentation by the UNH QA Officer (Dr. Margolin).

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

Audits of the subcontractor laboratory data-collection programs will be the responsibility of the subcontractor. During the time work is in progress, an inspection will be conducted by the subcontractor QA Officer or their designee to evaluate the laboratory data-production process. All data must be reviewed by the UNH 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.

The Battelle QA Officer will conduct an initiation audit and, as needed, a laboratory inspection to access compliance with the Quality Management Plan and this CW/QAPP. Performance audits, procedures used to determine quantitatively the accuracy of the total measurement system or its components, will be the responsibility of the subcontractor laboratory and may include internal performance evaluation samples and participation in external certification programs.

18.0 CORRECTIVE ACTION

Identification of problems regarding technical performance is the responsibility of all staff members working on this project. Responsibility for overall conduct of the project, including schedule, costs, and technical performance lies with the Battelle Project Manager. The Project Manager is responsible for identifying and resolving problems that (1) have not been addressed promptly or successfully at a lower level, (2) influence other components of the project, (3) require changes in this CW/QAPP, or (4) require consultation with 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 Area Manager, the Battelle Field Manager, and the Project Senior Scientists. 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, and annual virus data 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 synthesis reports prepared under Task 33 will describe methods used in the surveys and any deviations from the CW/QAPP, report results, and provide a discussion of the data. Two reports will be prepared. The 1999 report will discuss results obtained between 1995 and 1998. The report prepared in 2000 will cover results through 2000. A detailed outline for each report will be submitted for MWRA approval. Draft and final reports will be developed.

20.0 REFERENCES

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APPENDIX I

SOP and Quality Assurance Manual for EPA Information Collection Rules

STANDARD OPERATING PROCEDURE MANUAL

&

QUALITY ASSURANCE MANUAL

for the

INFORMATION COLLECTION RULE

for the laboratory of:

Dr. Aaron B. Margolin
Waterborne Disease Laboratory
Department of Microbiology
University of New Hampshire
Biological Sciences Bldg.
Durham, New Hampshire 03824

updated: 4/17/96

I. **All procedures used, listed here or not, are those detailed by the August 1995 Draft ICR. All media, chemicals and equipment are prepared or used according to manufactures directions.**

II. Personnel:
- A. Principal Analysts
  1. Minimum of a bachelors degree in the life sciences and three years experience in cell culture and animal virus analyses
- B. Analyst
  1. Minimum of two years of full time college with a major in life science and six months of full-time bench experience in cell culture and animal virus analyses
- C. Technician
  1. High School Diploma or equivalent and three months experience in filter extraction of virus samples and sample processing

III. Laboratory Facilities
- A. Laboratory is kept clean at all times
- B. Filter extraction, cell culture, and virus work is done in separate rooms
- C. Inoculation of samples is done in a separate hood from virus work
- D. All biological hoods are inspected annually by the University

IV. Laboratory Safety
- A. The lab follows all biosafety procedures prescribed by NIH for a biosafety level 2
- B. Lab doors are kept closed when the lab is not staffed
- C. Vestibule doors are kept closed at all times
- D. Lab coats are worn
- E. Mouth pipetting is not allowed in the lab
- F. Food and drink, other than that which is being analyzed, is not allowed in any and all parts of the lab
- G. Biosafety rules will be those prescribed by the University of New Hampshire Biohazard Committee
- H. All contaminated material is autoclaved for a minimum of 1 hour prior to disposal according to the University Biohazard Committee disposal rules

V. Laboratory Equipment and Supplies
- A. pH Meters
  1. calibrated daily and recorded in the log
  2. pH buffer aliquots are used only once
  3. electrodes are maintained according to the manufacture's recommendations and any service to the meter or electrode is recorded on the comment section of the log sheet

4. pH buffer solutions are dated upon receipt and when opened and discarded upon expiration

B. Light Microscope and Inverted Microscope
   1. should be kept clean and in workable condition at all times

C. Biological Hood
   1. hood is inspected annually by the University annually
   2. hood is wiped down prior to use with 0.1% chlorine and upon completion of work
   3. hood is on at all times when in use

D. Temperature Monitoring
   1. glass/mercury, dial thermometers or continuous recording devices are used to monitor equipment
   2. units are graduated in 0.5 ° C increments or less
   3. mercury columns in glass thermometers are not separated
   4. calibration at the temperature used of each thermometer is checked annually against a reference NIST thermometer kept in Dr. Margolin's office or upon replacement of existing thermometer
      a. calibration results are recorded in calibration log and results are initialed

E. Freezers, Refrigerators, Incubators, Water Baths
   1. all devices are visually inspected to insure no leakage of fluids
   2. water baths should contain water and be cleaned regularly to insure no growth of bacteria or fungi
   3. all devices contain a thermometer or have a digital temperature readout
   4. temperatures are recorded daily in the log and initialed
   5. temperatures:
      a. REVCO - between -70 and -80 ° C
      b. chest freezer - between -15 and -20° C
      c. refrigerators - 4 +/- 1° C
      d. incubators - 36.5 +/- 1° C
      e. water baths - set by operators as needed
      f. drying oven - 100° C

F. Pump and Polypropylene Container should be kept clean and in working order for preparation and evaluation of QC samples.
   1. Polypropylene container must be autoclavable

G. Nitrogen tanks housed in the cell culture and filter elution room must be kept stable by either using a base or clamps to an immovable object

H. Scales
   1. scales are calibrated twice a year
   2. should be kept clean at all times
   3. calibrated with ANSI/ASTM Class 2 weights at 1 gm, 10 gm and 100 gm kept in Dr. Margolin's office
   4. calibration

a. using white glove, place weight on scale and allow to equilibrate
b. record weight
c. remove weight and repeat procedure two more times
d. use the average of the three trials/weight to calibrate the scale
e. record calibration correction factor in log book and initial

I. Autoclaves
1. autoclave cycle tapes are placed in log book upon completion of each autoclave cycle
2. spore strips are used monthly to validate autoclave
3. all items being autoclaved have at least one piece of autoclave tape placed on them

J. Sterilization Hot Air Oven
1. maintains a temperature of at least 170° C
2. temperature and time for sterilization is recorded for each use

K. Analytical Media
1. commercial media and chemicals are dated upon receipt and when first opened
2. only analytical reagent or ACS grade chemicals are used for the preparation of media
3. use commercial dehydrated or liquid media for propagation of tissue culture cells as indicated in this manual
4. commercial media and chemicals are discarded on manufactures' expiration date
5. laboratory prepared media are discarded by the expiration dates indicated in the Virus Monitoring Protocol
6. each lot of medium is checked for sterility before use
   a. inoculate 1 ml portions of the material to be tested for sterility into tubes containing 9 ml of thioglycolate broth
   b. incubate at 36.5° C
   c. examine the broth daily for seven days to determine growth of contaminating organisms
   d. incubate at least 1 500 ml bottle of media at 36.5° C for 1 week prior to use
   e. record all results on the log sheet and initialize
   f. lot numbers of media and chemicals are recorded, date of preparation, type of medium, lot number, sterilization procedure, pH and tech initials are recorded for media prepared in the laboratory

L. Automatic pipettes
1. calibrated annually by the University and returned to indicated volumes
2. use only cotton plugged pipette tips

VI. Sterilization and Disinfection

A. Autoclavable glassware, plasticware and equipment are sterilized by autoclaving at 121° C for one hour or, if appropriate, by dry heat at 170° C for at least one hour

B. Non-autoclavable supplies are disinfected with 0.1% chlorine (pH 6-7) for 30 minutes

C. Contaminated material is sterilized by autoclaving at 121° C for at least one hour

D. glassware is washed in the wash room

E. All surfaces are disinfected with 0.1% chlorine, pH 6-7 before and after each use and after any spill or other contamination

VII. Working solutions

5.1.2 Thiosulfate (2%)

5.1.2.1 A stock solution of 2% thiosulfate is prepared by dissolving 100 g of $Na_2S_2O_3$ in a total of 5000 ml of reagent grade water. The solution is autoclaved for 30 minutes at 121°C.

5.1.2.2 2% thiosulfate is stored at or below room temperature for up to six months.

5.1.3 Hydrochloric acid (HCl)

5.1.3.1 Solutions of 0.1, 1 and 5 M HCl are prepared by mixing 50, 100 or 50 ml of concentrated HCl with 4950, 900 or 50 ml of reagent grade water, respectively. Solutions of HCl are self-sterilizing and should be prepared at least 24 hours prior to use.

5.1.3.2 Solutions of HCl are stored at or below room temperature for up to six months.

5.1.4 Sodium Hydroxide (NaOH)

5.1.4.1 Solutions of 1 M and 5 M NaOH are prepared by dissolving 4 or 20 g of NaOH in a final volume of 100 ml of reagent grade water, respectively. Solutions of NaOH are self-sterilizing and should be prepared at least 24 hours prior to use.

5.1.4.2 Solutions of NaOH are stored in polypropylene containers at room temperature for up to three months.

5.1.5 Beef Extract, 1.5%

5.1.5.1 Buffered 1.5% beef extract is prepared by dissolving 30 g of beef extract V powder and 7.5 g of glycine (final glycine concentration = 0.05 M) in 1.9 liters of reagent grade water. The pH is adjusted to 9.5 with 1 or 5 M NaOH and the final volume is brought to 2 liters with reagent grade water. The solution is autoclaved at 121°C for 15 min.

5.1.5.2 Solutions of 1.5% beef extract are stored for one week at 4°C or for up to 18 months at -20°C.

Each new lot of beef extract should be screened prior to use. Screening is done by spiking 1 liter of beef extract solution with 1 ml of a diluted QC sample containing 200 pfu/ml. Assay the spiked sample according to the Organic Flocculation and Total Culturable Virus Assay procedures. Use a single passage with undiluted sample and sample diluted 1:5 and 1:25 along with an equivalent positive control. The mean recovery of poliovirus for 3 trials should be at least 50%.

5.1.6 Sodium Phosphate, 0.15 M

5.1.6.1 A solution of 0.15 M sodium phosphate is prepared by dissolving 40.2 g of sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$) in a final volume of 1000 ml of reagent grade water. The pH is checked to ensure that it is between 9.0 - 9.5 and adjusted with 1 M NaOH, if necessary. The solution is autoclaved at 121°C for 15 minutes.

5.1.6.2 Solutions of 0.15 M sodium phosphate are stored at or below room temperature for up to six months.

5.1.7 Washing Solution

5.1.7.1 Washing solution is prepared by dissolving 8.5 g of NaCl in a final volume of 980 ml of reagent grade water. The solution is autoclaved at 121°C for 15 minutes and cooled to room temperature. 20 ml of bovine serum is added and the solution is mixed thoroughly.

5.1.7.2 The wash solution is stored at 4°C for up to three months or at -20°C for up to 18 months.

5.1.8 Chlorine, 0.1%

5.1.8.1 A solution of 0.1% chlorine (HOCl) is prepared by adding 19 ml of household bleach to 900 ml of reagent grade water, adjusting the pH of the solution to 6-7 with 1 M HCl and bringing the final volume to 1 liter with reagent grade water. Solutions of 0.1% chlorine are self-sterilizing.

5.1.8.2 Solutions of 0.1% chlorine are stored at or below room temperature for up to one month.

5.1.9 Iodine, 0.5%

5.1.9.1 A solution of 0.5% iodine is prepared by dissolving 5 g I_2 in 1000 ml of 70% ethanol. Solutions of 0.5% iodine are self-sterilizing.

5.1.9.2 Solutions of 0.5% iodine are stored at room temperature for up to six months.

Cell Culture

MEDIA FOR CELL CULTURE (ICR RELATED)

	15L
MEM	70.5g
L-15	111g
HEPES	63.675g
L-glutamine	4.38g
Sodium bicarbonate	11.25g
Non-essential aa's	150ml

- constant stirring in about 12L while adding ingredients, until solution is clear

- QS to 15L

- pH to 7.4 with Hcl or NaOH

- aliquot to sterile bottles with positive pressure through a sterilized filter apparatus

Check sterility with inoculation into thioglycolate broth and by incubation of at least one bottle for seven days at 37° C

Store at 4° C

(10 X) Phosphate Buffered Saline (PBS)

(for one liter)

Formula	pH 7.2-7.3
NaCl	80.0 gm
KCl	2.0 gm
Phenol Red (Na salt)	0.05 gm
Na_2HPO_4	20.0 gm
KH_2PO_4	4.0 gm
Deionized water	QS to 1 L

Preparation:

1. Into a clean 1-2 liter flask, add all ingredients
2. Mix with stir bar until dissolved
3. Remove stir bar
4. Sterilize by filtration (0.22 μ Millipore GSWP) or autoclave for 15-20 minutes on slow exhaust
5. Check for sterility with thioglycolate broth
6. Store at -20° C in 500 ml volumes
7. Working solution should be stored at 4° C

Or if using DulBecco's concentrated phosphate buffered saline (Sigma D-5652)

1. Add 4.75 gm to a clean 1 liter flask
2. QS to 500 ml
3. Mix well and transfer to 500 ml bottle
4. Autoclave at 121° C for 15-20 minutes
5. Store at -20° C
6. Working solution and powder should be stored at 4° C

TABLE I

ANTIBIOTICS USEFUL FOR ELIMINATION OF CONTAMINANTS FROM MAMMALIAN CELL CULTURES[a]

Antibiotic	Antibiotic Spectrum	Recommended Concentration[b]	Stability in Tissue Culture Media (37°C)[c]
Amphotericin B (deoxycholate complex)	Fungi and yeasts	2.5 mcg/ml	3 days
Ampicillin	Gram-positive and Gram-negative bacteria	100 mcg/ml	3 days
Cephalothin	Gram-positive and Gram-negative bacteria	100 mcg/ml	3 days
Chloramphenicol	Gram-negative bacteria	5 mcg/ml	5 days
7-Chlortetracycline hydrochloride	Gram-positive and Gram-negative bacteria	10 mcg/ml	1 day
6-Dimethyl-7-chlortetracycline hydrochloride	Gram-positive and Gram-negative bacteria	5 mcg/ml	5 days
Dihydrostreptomycin sulfate	Gram-positive and Gram-negative bacteria	100 mcg/ml	5 days
Erythromycin	Gram-positive bacteria and Mycoplasma	100 mcg/ml	3 days
Gentamicin sulfate	Gram-positive and Gram-negative bacteria and Mycoplasma	50 mcg/ml	5 days
5-Hydroxytetracycline	Gram-positive and Gram-negative bacteria	5 mcg/ml	3 days
Kanamycin sulfate	Gram-positive and Gram-negative bacteria and Mycoplasma	100 mcg/ml	3 days
Lincomycin	Gram-positive bacteria	100 mcg/ml	4 days
Neomycin sulfate	Gram-positive and Gram-negative bacteria	50 mcg/ml	5 days
Neomycin sulfate	Gram-positive and Gram-negative bacteria	50 mcg/ml	5 days
Nystatin	Fungi and yeasts	50 mcg/ml	3 days
Paromomycin sulfate	Gram-positive and Gram-negative bacteria	100 mcg/ml	5 days
Polymyxin B sulfate	Gram-negative bacteria	50 mcg/ml	5 days
Potassium benzylpenicillin (penicillin G)	Gram-positive bacteria	100 units/ml	3 days
Potassium phenoxymethylpenicillin (penicillin V)	Gram-positive bacteria	100 units/ml	3 days
Tetracycline hydrochloride	Gram-positive and Gram-negative bacteria and Mycoplasma	10 mcg/ml	4 days
Tylosin tartrate	Gram-positive bacteria and Mycoplasma	100 mcg/ml	2 days
Viomycin sulfate	Gram-positive and Gram-negative bacteria	50 mcg/ml	5 days
Streptomycin sulfate	Gram-positive and Gram-negative bacteria	100 mcg/ml	5 days

[a] For further information consult Perlman and co-workers (3,4) and Schafer (5).
[b] Concentration effective in controlling "light" infection and not noticeably toxic to Earle's L-929 cells or Eagle's KB cells in serum containing media.
[c] Length of time in which at least 10% of initial antibiotic activity could be demonstrated in studies up to 5 days incubation at 37°C in serum supplemented Eagle's MEM medium.

(Product Nos. T 8154 and T 6146)

CELL COUNTING AND CELL VIABILITY: USE OF TRYPAN BLUE STAIN (0.4%) AND THE HEMOCYTOMETER TO DETERMINE TOTAL CELL COUNTS AND VIABLE CELL NUMBER

Trypan Blue is one of several stains recommended for use in dye exclusion procedures for viable cell counting. This method is based on the principle that live (viable) cells do not take up certain dyes, whereas dead (non-viable) cells do. Staining facilitates the visualization of cell morphology.

NOTE: Trypan Blue has a greater affinity for serum proteins than for cellular protein. If the background is too dark, cells should be pelleted and resuspended in protein-free medium or salt solution prior to counting.

1) Prepare a cell suspension in a balanced salt solution (e.g., Hanks' Balanced Salts [HBSS]; Product No. H 2513).
2) Transfer 0.5 ml of 0.4% Trypan Blue solution (w/v) to a test tube. Add 0.3 ml of HBSS and 0.2 ml of the cell suspension (dilution factor = 5) and mix thoroughly. Allow to stand for 5 to 15 minutes.

   NOTE: If cells are exposed to Trypan Blue for extended periods of time, viable cells, as well as non-viable cells, may begin to take up dye.

3) With the cover-slip in place, use a Pasteur pipette or other suitable device to transfer a small amount of Trypan Blue-cell suspension mixture to both chambers of the hemocytometer. Carefully touch the edge of the cover-slip with the pipette tip and allow each chamber to fill by capillary action. Do not overfill or underfill the chambers.
4) Starting with chamber 1 of the hemocytometer, count all the cells in the 1 mm center square and four 1 mm corner squares (see Diagram II). Non-viable cells will stain blue. Keep a separate count of viable and non-viable cells.

   NOTE: Count cells on top and left touching middle line of the perimeter of each square. Do not count cells touching the middle line at bottom and right sides (see Diagram III).

5) Repeat this procedure for chamber 2.

   NOTE: If greater than 10% of the cells appear clustered, repeat entire procedure making sure the cells are dispersed by vigorous pipetting in the original cell suspension as well as the Trypan Blue-cell suspension mixture. If less than 200 or greater than 500 cells (i.e., 20-50 cells/square) are observed in the 10 squares, repeat the procedure adjusting to an appropriate dilution factor.

6) Withdraw a second sample and repeat count procedure to ensure accuracy.
7) CELL VIABILITY (%) = total viable cells (unstained) ÷ total cells (stained and unstained) × 100.

   Ex: If the average count per 10 squares of unstained (viable) cells is 27.5, the total viable cells = [1.9 × 10^5] ÷ [2.3× 10^5 (total cells)] × 100 = 83% cell viability.

8) CELL COUNTS—Each square of the hemocytometer, with cover-slip in place, represents a total volume of 0.1 mm^3 or 10^{-4} cm^3. Since 1 cm^3 is equivalent to approximately 1 ml, the subsequent cell concentration per ml (and the total number of cells) will be determined using the following calculations:

   CELLS PER ml = the average count per square × dilution factor × 10^4 (count 10 squares)

   Ex: If the average count per square is 45 cells × 5 × 10^4 = 2.25 × 10^6 cells/ml.

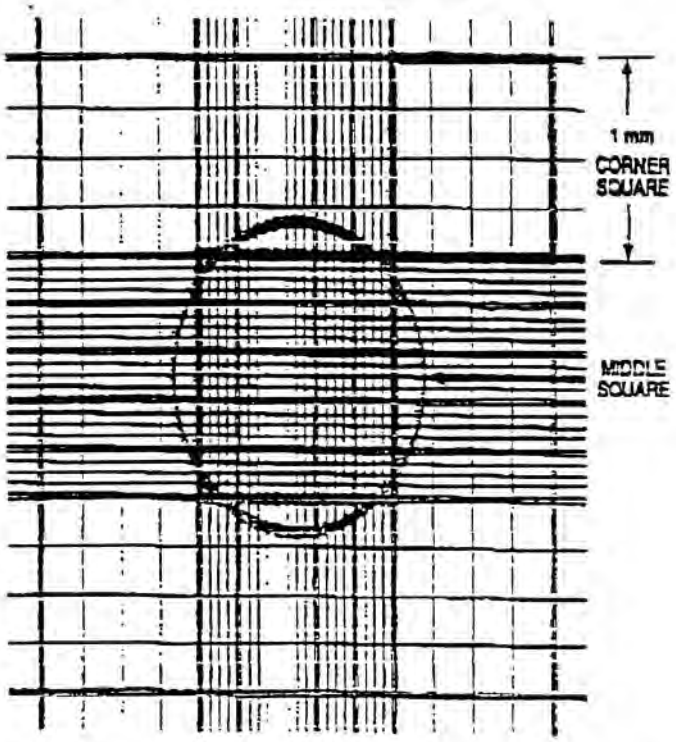
   TOTAL CELLS = cells per ml × the original volume of fluid from which cell sample was removed.

   Ex: 2.25 × 10^6 (cells/ml) × 10 ml (original volume) = 2.25 × 10^7 total cells.

Trypan blue formulation:

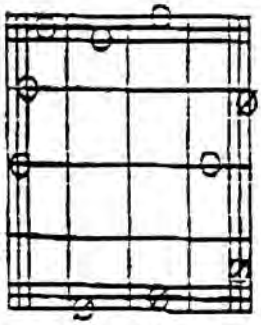
0.2 gm trypan blue
49.8 ml PBS
mix on stir plate until dissolved

DIAGRAM II
STANDARD HEMOCYTOMETER CHAMBER



The circle indicates the approximate area covered at 100× microscope magnification (10× ocular and 10× objective). Include cells on top and left touching middle line ( ). Do not count cells touching middle line at bottom and right ( ). Count 4 corner squares and middle square in both chambers (one chamber represented here).

DIAGRAM III
CORNER SQUARE (ENLARGEMENT)



Count cells on top and left touching middle line ( ). Do not count cells touching middle line at bottom and right ( ).

SAFETY MANUAL

POLICE = 911 **FIRE = 911** **AMBULANCE = 911**

1. All emergencies will be reported to the University Police, Fire or Ambulance.

2. If the emergency is life threatening or requires medical attention, contact the University Ambulance.

3. In the vestibule outside of the lab, on either side of the lab exists a fire blanket, emergency shower and a fire extinguisher, know their location.

4. There is no EATING, SMOKING, DRINKING, MOUTH PIPETTING of any type at any time in the lab.

5. Lab coats should be worn at all times while in the lab.

6. When necessary, use disposable gloves.

7. This laboratory follow the UNIVERSITY SYSTEMS OF NEW HAMPSHIRE HAZARDOUS MATERIAL MANAGEMENT PLAN contained in this book.

8. There is a First Aid Kit in the kitchen.

9. Eye washes are at each sink.

10. The MSDS of each chemical are kept in Bob Mooney's office and a copy is on file at the Fire Department.

vember 4, 1993

Policy on the Use of Hazardous Materials at the University of New Hampshire

The primary responsibility for using hazardous materials safely and in a manner nsistent with all applicable laws, regulations, and standards, resides in the user of : hazardous material. In general, a hazardous material is defined as any substance pable of causing harm to human beings through direct contact or indirectly through :ruption of the environment. Such materials include certain chemicals, radioactive aterials, and infectious agents. More practically, a hazardous material is defined as :zardous if it appears on a list of hazardous materials in a law, regulation or andards, or has a characteristic or property identified as hazardous by a law, gulation, or standard.

A significant number of activities at the University require the use of hazardous aterials. Hazardous materials exist in many departments and in many buildings of ıe University. The manner in which the University manages the uses of these ıaterials is of considerable interest to governmental agencies, as well as to the eneral public.

Hazardous materials are used by faculty, staff, and students of the University in ıe normal course of educational research service and support activities. All users of azardous materials must not only be aware of the hazards but also knowledgeable in ıethods that control the hazards to themselves, others around them, and the onsequences of releases of that material into the environment.

The management of hazardous materials at the University of New Hampshire equires the collective actions of the users, the chairperson, or program manager, ınder whose authority the user acts, the Advisory Committee on Radioactive and Hazardous Materials, and the Administration of the University. In matters involving ıazardous materials, the Administration acts through the Office of Environmental Health and Safety. The office was established to provide expert advice and technical services to the users of hazardous materials. The staff of this office investigates all incidents or unplanned events involving hazardous materials that occur. The staff reports its findings to the user: the chairperson, or program manager, and the Advisory

[illegible] that uses hazardous materials or causes the users to conduct a self-audit for the purposes of establishing compliance with laws, regulations, and standards. The staff reports these findings to the Advisory Committee. The Advisory Committee will take the necessary actions to ensure compliance with government regulations and the control of hazards to persons, property, or the environment, via the Office of Environmental Health and Safety.

The Advisory Committee on Radioactive and Hazardous Materials (ACRHM) consists of three Subcommittees: Radiation Safety, Biohazards, and Chemical Safety. The Biohazards Subcommittee serves as the Institutional Biohazards Committee as required by the National Institutes of Health. The membership of the Radiation Safety and Biohazards Subcommittees is formed from the faculty of the University, since the dominant use of these hazardous materials is in teaching and research. The Chemical Safety Subcommittee includes not only teaching and research activities, but also service and support activities, including the activities of contractors while on the properties of the University. Therefore, the membership of this Subcommittee reflects the activities of several service and support departments.

Provost and Vice President for Academic Affairs

Vice President for Finance and Administration

Vice President for Research and Public Service

UNIVERSITY SYSTEM OF NEW HAMPSHIRE

HAZARDOUS MATERIALS MANAGEMENT PLAN

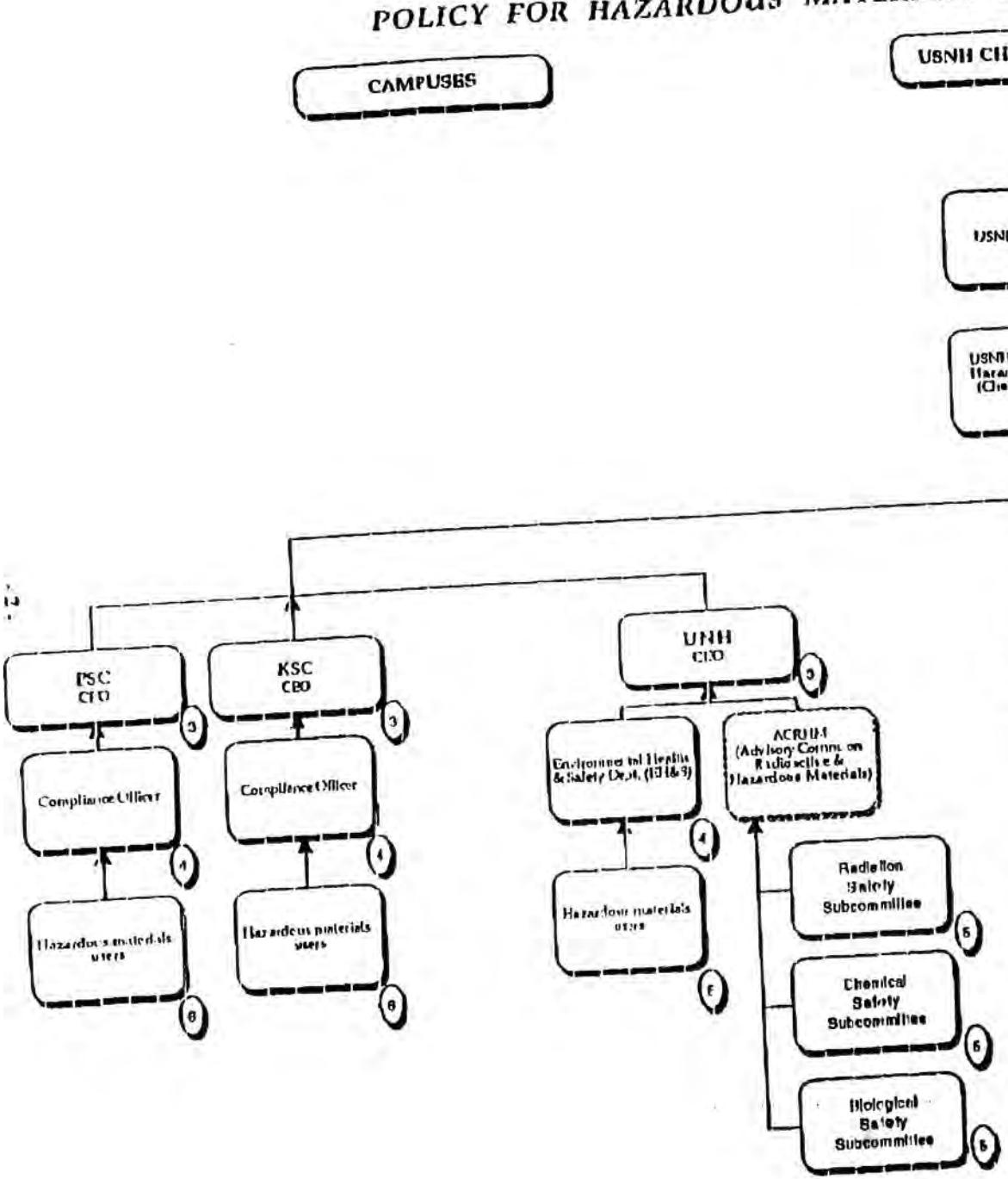
Approved by the
Capital & Strategic Planning Committee
of the USNH Board of Trustees
November 3, 1994

USNH

HAZARDOUS MATERIALS MANAGEMENT PLAN

Table of Contents

USNH
POLICY FOR HAZARDOUS MATERIALS MANAGEMENT



Notes

1. Responsible for prudent management of hazardous materials in compliance with applicable laws.
2. Responsible for:
   - coordination of compliance efforts
   - annual reports to the Chancellor, and
   - any proposed policy changes.
3. Responsible for:
   - establishment and administration of hazardous materials management policies, and
   - ensuring proper representation on the USNH Task Force
4. Responsible for:
   - implementation of policy
   - coordination of compliance efforts
   - training and guidance to users
5. Responsible for taking necessary actions to ensure compliance with government regulations and the control of hazards to persons, property or the environment, via the office of EH&S.
6. Responsible for safe use of hazardous materials in compliance with all laws.

III. DETAILED POLICIES OF THE USNH TASK FORCE ON HAZARDOUS MATERIALS

A. INVENTORY MANAGEMENT

A critical objective of this Plan is to enable the Compliance Officer to know the types, amounts and locations of hazardous materials. The information should be as current and as accessible as possible. In any event, the Compliance Officer will use this information to demonstrate compliance with those regulations that require that the campus to be considered a single business entity with campus-wide regulatory limits.

Therefore, the most important effort on the part of the User is the maintenance, at least annually of a written inventory of all hazardous materials in storage or use during the prior twelve months. This Plan requires Users to maintain an inventory in order to complete the Self-Audit Worksheet. The USNH Task Force on Hazardous Materials is currently reviewing software programs to facilitate this process on a USNH-wide basis. During the initial phases of the Plan (until the software is fully implemented) Users may maintain inventories using desktop computers or manual records. The software is expected to be available on the USNH network, and will allow Compliance Officers to access the information for purposes of monitoring both waste minimization efforts and facility thresholds.

B. HAZARDOUS WASTE MINIMIZATION

Location	No Waste	Very Small Waste (<100 kg/mo)	Small Quantity (<1000 kg/mo)	Full Quantity (>1000 kg/mo)
UNH				X
UNH Satellite:			X	
Heidelberg-Harris			X	
Jackson Lab			X	
Manchester			X	
Keene State				
Plymouth State:			X	
Dept. of Chemistry			X	
Physical Plant				

In 1984, the US Congress passed the Hazardous and Solid Waste Amendments to the Resource Conservation and Recovery Act of 1976 (RCRA). These amendments state that elimination and reduction of hazardous waste amounts has priority over disposal through incineration, treatment etc. In May, 1993, the US EPA published Interim Final Guidelines for Waste Minimization. These regulations require full quantity generators such as UNH to:

a. document a program to reduce the volume and toxicity of hazardous waste;
b. document actual changes of volume and toxicity achieved by comparison with a base year.

According to these regulations, each User of hazardous materials regulated by RCRA at UNH needs to be instructed that minimization is required of them including written documentation of specific actions taken and amounts of hazardous waste reduced. These instructions must be approved by the three Vice Presidents at UNH who previously signed the Policy on the Use of Hazardous Materials.

D. MANDATORY TRAINING REQUIREMENTS FOR USERS OF CERTAIN HAZARDOUS MATERIALS, cont.

The UNH Office of Environmental Health and Safety will be responsible for developing and acquiring training materials necessary to achieve the mandatory training requirements. EH&S will provide services to campus Compliance Officers and Users.

Mandatory training requirements contained in pending and future legislation will be accomplished within the time period established by the enforcing agency.

Each Compliance Officer will maintain records of all mandatory training received by Users. This process shall be retroactive to all past training that is used as evidence of current compliance. Users must determine their individual training requirements, based on the hazardous materials they are in contact with. EH&S is currently developing minimum training requirements for all Users of hazardous materials. Minimum requirements will differ based on types of uses and will be MANDATORY for continued use of applicable hazardous materials.

Law: Occupational Safety and Health Act of 1970 (US Department of Labor)
and all subsequent revisions to date (9/22/94)

1910	General Industry Standards
1910.96	Ionizing Radiation
1910.252	Welding, Cutting and Brazing
1910.134	Respiratory Protection
1910.133	Personal Protective Equipment (eye and face protection)
1910.1000	Air Contaminants
1910.1001	Asbestos
1910.1025	Lead
1910.1003-.1017	OSHA Cancer-Causing Chemicals
1910.1200	Hazard Communication
1910.1030	Bloodborne Pathogens
1910.1450	Exposure to Hazardous Chemicals in Laboratories
	This section takes the place 1910.1000 for chemicals used in education or research laboratory operations. Pilot Plant and Quality Assurance Laboratories are not covered by this standard.

1926	Construction Industry Standards
1926.103	Respiratory Protection
1926.350	Gas Welding and Cutting
1926.53	Ionizing Radiation

1926	Construction Industry Standards, cont.
1926.55	Gases, Vapors, Fumes, Dusts and Mists
1926.58	Asbestos
1926.59	Hazard Communication
1926.62	Lead
1926.63	Cadmium
1926.65	Hazardous Waste Operations and Emergency Response
1926.102	Eye and Face Protection
1926.103	Respiratory Protection
1926.150	Fire Protection
1926.151	Fire Prevention
1926.152	Flammable and Combustible Liquids
1926.153	LP-GAS
1926.350	Gas Welding and Cutting
1926.1103-.1117	Cancer Causing Chemicals
1926.1128	Benzine
1926.1145	Acrylonitrile
1926.1148	Formaldehyde

Law: Resource Conservation and Recovery Act of 1976
Regulation: NH Hazardous Waste Rules Env-Wm 110, 211-216, 351-353, 400-1000

F. LIFECYCLE COST ACCOUNTING FOR PROJECTS USING HAZARDOUS MATERIALS

All materials used at UNH/KSC/PSC have a lifecycle cost. The components of this lifecycle include selection, transportation, storage, use/processing and disposal of the material.

It is the responsibility of the User of hazardous materials to identify whether the current or proposed use of hazardous materials is ordinary (normal, routine) or extraordinary. Persons applying for research funding will identify to the Office of Sponsored Research (OSR) whether it is an ordinary use or extraordinary use and include in their proposal to the sponsor the total cost of the project over its life, including commissioning and decommissioning. Processes requiring extraordinary control measures should not be conducted until all lifecycle costs are identified and future funds committed. EH&S is responsible for the training of OSR personnel as to the ordinary and extraordinary uses, and implications to costs that should be included in proposals.

Some projects involving hazardous materials require extraordinary measures be taken in order to provide an acceptable level of safety and compliance with laws and regulations. Most projects involving hazardous materials can be controlled using normal or routine measures.

For example, an employee transferring gasoline from a dispenser into a portable fuel container by observing routine control measures can accomplish this process safely and in compliance with all laws and regulations. Avoiding spills, avoiding overfilling, choice of materials for the container, and not smoking all achieve a level of safety that is acceptable, hence a Lifecycle Cost Accounting Review is unnecessary.

On the other hand, a chemical fume hood is considered a normal and routine control measure for the protection of laboratory workers from airborne hazardous materials. However, recent EPA action of the Clean Air Act Amendments may define each fume hood as a source of hazardous air pollutants. The installation and maintenance of air pollution control devices should be evaluated for total lifecycle costs.

The indirect cost recovery provides funding for ordinary control measures. Costs associated with extraordinary control measures should be evaluated; these may be recoverable as direct costs.

G. EMERGENCY ACTION PLAN FOR USERS OF HAZARDOUS MATERIALS.

A spill or other unplanned release of hazardous material is a foreseeable event. Therefore, each User must be capable of handling hazardous materials under normal and abnormal or unplanned conditions of use. It is the responsibility of the User to control any unplanned event. The User should only handle hazardous materials in physical forms and amounts that he or she is capable of controlling under ALL CONDITIONS.

The initial response to a non-routine event is the responsibility of the User. This could include: use of personal protective equipment, exhaust systems, chemical neutralizing supplies, liquid absorbents, etc. It is also the responsibility of the User to ensure that procedures for each potential event are clearly delineated and available for easy reference.

A User is not expected to be capable of handling unplanned events caused by deliberate acts of vandalism or by unforeseeable natural disasters such as earthquakes, hurricanes (flooding) or tornadoes.

An emergency therefore is any event which results in loss of control by the User due to incompetence, vandalism or natural disaster.

Interior spaces in which hazardous materials are stored, transported or used should not be located near wetlands, bodies of water, or stormwater drains. A spill or release of hazardous materials into these receiving waters is a reportable spill.

G. EMERGENCY ACTION PLAN FOR USERS OF HAZARDOUS MATERIALS, cont.

Once an emergency has ended:

1. The Compliance Officer will permit the User to remediate the area if the release or spill is of a scale capable of remediation by the User.

2. If the release or spill is a scale beyond the ability, equipment and supplies of the User, the Compliance Officer or designated individual will ensure that a contractor is hired to conduct the remediation and elimination of any hazard;

3. The building or area will not be opened for general use until the emergency is ended or over, and the Compliance Officer has determined that contamination is under control in specified areas and there is no risk of continuing release or spill.

Thorough planning for foreseeable unplanned events will limit declared emergencies to acts of vandalism, gross negligence, and natural disasters. In the absence of planning, any abnormal event becomes an emergency, resulting in costly interruptions to normal activities and adverse public and employee relations.

IV. PROCEDURES FOR MONITORING COMPLIANCE WITH LAWS AND REGULATIONS, Cont.

B. RESPONSIBILITIES OF USERS, cont.

9. Identify actions and schedules of such actions which will lead to compliance if compliance is not the present condition;

10. Within 90 days after completing the User Self-Audit, if compliance cannot be achieved, cease using all hazardous materials and transfer, recycle, or arrange for the disposal of such materials; and

11. Contain or declare emergencies (as defined) and follow appropriate procedures as outlined in the Emergency Action Plan.

C. SELF-AUDIT PROCESS

The Compliance Officers have identified all departments with Users of hazardous materials, and have classified them as Cycle 1 or Cycle 2 departments. Cycle 1 departments are those with significant amounts of hazardous materials, whose self-audit process will begin at the end of calendar 1994. Cycle 2 departments will begin their self-audit process at the end of calendar 1995.

Annually, at the conclusion of each calendar year, each User of hazardous materials will be required to complete a User Self-Audit Worksheet. This Worksheet will consist of a series of questions. Each User will be given thirty days to complete the Worksheet. The Worksheets will be returned to the Compliance Officer at UNH, KSC, and PSC.

The Worksheet will be reviewed by the Compliance Officer for accuracy and completeness. The Compliance Officer will at his or her discretion telephone or visit with the User to verify information or correct errors of omission or fact.

Within sixty days or March 1, the Compliance Officer will prepare a summary report to the President or the appropriate Committee. The Task Force on Hazardous Materials will then assemble information from all three campuses in a report to the Chancellor.

This System Report will identify three groups of Users of hazardous materials:

Group A– Users in compliance with laws and regulations;

Group B– Users not in compliance but for whom a plan of compliance is in place and if necessary funds ($) have been identified to purchase training, supplies equipment or upgrade facilities. A compliance date will be established;

Group C– Users for whom compliance is not achievable through funding or for whom funding is not available. These Users will be expected to cease handling hazardous materials within ninety days of the completion of the Worksheet. As necessary, all hazardous materials will be transferred to another user or disposed of or recycled. These actions will be documented in a close-out plan.

The System Report will also address campus projects which have been identified by the User as requiring a Lifecycle Cost Accounting Review. All projects involving long-lived radioactive materials must undergo a Lifecycle Cost Accounting Review.

Campuses approving Users of hazardous materials that require a Lifecycle Cost Accounting Review guarantee funding of the project including all decommissioning costs.

The System Report will be completed by June 1.

Note: The first two cycles of this Audit process will be the most time consuming for both the Users and the Compliance Officers. Users maintaining current inventories of hazardous materials will have an advantage over those Users who choose to conduct an annual inventory.

References to Local, State and Federal Laws, Regulations and Standards

These definitions represent a compilation of many definitions derived from local, state and federal laws, regulations and standards, as noted below. The intent of these definitions is to be inclusive and comprehensive; that is, all materials defined as hazardous in any applicable source are included.

Hazardous Chemical Substances:

NFPA Flammable and Combustible Liquids Code (NFPA 30, NFPA 45)
National Building Codes: Boca 1993 12th Edition Sections 307, 417, 418, 419, 904
US 29 CFR 1910.1000 air contaminants
US 29 CFR 1910.1200 hazard communication standard
US 29 CFR 1910.1450 laboratory standard
US 29 CFR 1910.106 flammable and combustible liquids
US 29 CFR 1910.120 hazardous waste operations and emergency response
US 29 CFR 1910.1001 - specific chemical standards
NH RSA 277A Right to Know Law
NH RSA 125C Clean Air Act and Amendments
US 40 CFR Resource Conservation and Recovery Act
US 40 CFR Toxic Substances Control Act
US 49 CFR Hazardous Materials Transportation Regulations

Radioactive Materials:

US 10 CFR Parts 19 and 20
NH He-P 2000 Rules for the Control of Radiation

Hazardous Biological Agents:

NH Part Env-Wm 2604 Infectious Waste
US 42 CFR Part 72 Interstate Shipment of Etiologic Agents
US 42 CFR Part 71 Importation of Etiological Agents, Hosts and Vectors
US 49 CFR 173.386 Etiologic Agents; definition and scope / Infectious Substances Human
US 29 CFR 1910.120 (a) HAZWOPER
US 29 CFR 1910.1030 BLOODBORNE PATHOGENS
US Federal Register July 5, 1994 Part IV NIH Guidelines for Research Involving Recombinant DNA Molecules
Classification of Etiologic Agents on the Basis of Hazard, 4th Ed. 1974, US DHEW:PHS Atlanta, GA 30333
Biosafety in Microbiological and Biomedical Laboratories, 3rd Ed. May, 1993, US DHHS: GPO
National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses, October 3, 1974, National Cancer Institute NIH 75-790
Biohazards Safety Guide, 1974, National Institutes of Health, US DHEW
Biosafety in the Laboratory (Prudent Practices for the Handling and Disposal of Infectious Materials) National Research Council, National Academy Press, 1989.
Benenson, Abram S. (Ed) Control of Communicable Diseases in Man. American Public Health Association (current edition)
American Academy of Pediatrics. Report of the Committee on Infectious Diseases (Red Book-current edition)

EXHIBIT C

USNH
SELF AUDIT WORKSHEET
USERS OF HAZARDOUS MATERIALS

ATTACHMENT 3- Hazardous Biological Agents
(Complete one for each room in which hazardous materials are stored)

NIH/CDC CLASSIFICATION	
Name	Aaron Mangelin
Bldg/Room#	Bio Sci 235
Campus	UNH

Part A: General Information

Category of Biological Agents	YES	NO
Infectious Agents	X	
Recombinant DNA	X	
In live culture	X	
In tissue culture		X
type of tissue		X
fragments only	X	
Human blood or blood products	X	
Poisonous plants		X
Poisonous animals		X
Poisonous plants		X
Infected laboratory animals		X
Infected wild/feral animals		X

As a User of Hazardous Biological Agents, have you been immunized or should you be immunized to reduce the risk of injury from the agents handled/processed? Y/N

EXHIBIT C

USNH
SELF AUDIT WORKSHEET
USERS OF HAZARDOUS MATERIALS

ATTACHMENT 3- Hazardous Biological Agents
(Complete one for each room in which hazardous materials are stored)

Name: MARGOLIN, AARON
Building/Room: Bio Sci 835
Campus: UNH

PART B: Questionnaire -Hazardous Biological Agents

1. Is access to the laboratory limited or restricted when experiments are in progress?	X		
2. Are work surfaces decontaminated daily?	X		
3. Are decontamination procedures in writing and easily referenced?	X		
4. Are work surfaces decontaminated after any spill of viable materials?	X		
5. Is mouth pipetting of ANY SUBSTANCE prohibited?	X		
6. Are mechanical pipetting devices available, calibrated and in good repair?	X		
7. Is eating, drinking, smoking and application of cosmetics/lip balm prohibited in the work area?	X		
8. Do all workers wash their hands after handling HBA?	X		
9. Is a handwash sink with decontaminant and drying device available and in good repair?	X		
10. Are all procedures planned and conducted to prevent or minimize the production of aerosols?	X		
11. Are contaminated wastes placed in a leak proof, durable container for transport outside the laboratory?	X		
12. Are waste containers clearly labeled?	X		
13. Are all laboratory surfaces (not just work surfaces) accessible and cleanable?	X		
14. Are all laboratory bench tops impervious to water and resistant acids, alkalis, solvents and mild heat?	X		
15. Is an insect and rodent control program in place?	X		
16. Is there an area outside the laboratory for the consumption of food and drink?	X		
17. Is protective clothing such as lab coats, gowns, smocks or uniforms worn ONLY in the Laboratory?	X		
18. Is protective clothing removed and decontaminated after a spill or other contamination incident?		X	
19. Are street clothes prohibited from the laboratory when handling HBA?	X		
20. Are hypodermic needles and syringes used only for in vivo or animal injection purposes and not as a substitute for a mechanical pipetting device?	X		
21. Are Biological Safety Cabinets (Class 1 or 2) or other equally effective ventilated enclosure used whenever high concentrations of HBA are generated or large volumes of HBA are made?	X		
22. Is the Biological Safety Cabinet certified for safe operation every 6 months?	X		
23. Is a hazard warning sign incorporating the universal biosafety symbol posted on the access door to the laboratory?	X		
24. Is an autoclave available in the laboratory for the decontamination of HBA-contaminated objects?		X	
25. Is Ethylene Oxide used for the decontamination of HBA-contaminated objects?	X		X
26. Are only workers trained in aseptic technique permitted to handle HBA?			
... rooms/hoods protected by high-efficiency particulate filters?			

EXHIBIT C

USNH
SELF AUDIT WORKSHEET
USERS OF HAZARDOUS MATERIALS

ATTACHMENT 3- Hazardous Biological Agents
(Complete one for each room in which hazardous materials are stored)

Name A. Margolin
Building/Room Bio Sc. 833
Campus UNH

PART B: Questionnaire -Hazardous Biological Agents

	YES	NO	N/A
28. Is the laboratory under negative pressure with respect to uncontrolled areas (e.g. corridors, offices ETC.)?	X		
29. Are all shipments of HBA from the laboratory labeled and packaged in accordance with DOT and PHS regulations?	X		
30. Have all workers been trained and have they practiced the cleanup of a spill involving HBA in a "what if" exercise?	X		

USER CERTIFICATION
I CERTIFY THAT I POSSESS AND USE HAZARDOUS BIOLOGICAL AGENTS IN COMPLIANCE WITH ALL LAWS AND REGULATIONS REGARDING ACQUISITION, STORAGE, PROCESSING AND DISPOSAL AS REQUIRED BY NEW HAMPSHIRE LAW.

SIGNATURE Aaron Margolin

DATE 4/96

Massachusetts Water Resources Authority
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www.mwra.com
617-242-6000