

Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents



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**United States Environmental Protection Agency
Office of Research and Development
Center for Environmental Solutions and Emergency Response
Homeland Security and Materials Management Division**

And

**Centers for Disease Control and Prevention
National Center for Emerging and Zoonotic Infectious Diseases**

Disclaimer

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Acronyms

% w/v	Percent weight over volume
APHIS	Animal and Plant Health Inspection Service
BMBL	Biosafety in Microbiological and Biomedical Laboratories
CBR	Chemical, Biological, and Radiological
CDC	U.S. Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
COC	Chain of custody
DEUF	Dead-end Ultrafiltration
DSRC	Distribution System Research Consortium
EPA	U.S. Environmental Protection Agency
ERLN	Environmental Response Laboratory Network
ESAM	Environmental Sampling and Analytical Methods
FBI	Federal Bureau of Investigation
FDA	Food and Drug Administration
g	Gram
Gal	gallon
GPS	Global Positioning System
HASP	health and safety plan
HazMat	Hazardous materials
HHS	Health and Human Services
HSMMMD	Homeland Security and Materials Management Division
HSPD	Homeland Security Presidential Directive
ICLN	Integrated Consortium of Laboratory Networks
kDa	kilodalton
L	liter
L/min	liters per minute
LRN	Laboratory Response Network
LRN-B	Laboratory Response Network Biological
mL	milliliter
mL/min	Milliliter per minute
NCEZID	National Center for Emerging and Zoonotic Infectious Diseases
NIAID	National Institute of Allergy and Infectious Diseases
NM	Nanometers
NRF	National Response Framework
NTU	Nephelometric Turbidity Units
ORD	Office of Research and Development
PPD	Presidential Policy Directive
PPE	personal protective equipment
psig	pounds per square inch gauge
QA / QC	quality assurance / quality control
SAP	sampling and analysis plan
SLTT	State, Local, Tribal, and Territorial
SOP	standard operating procedure
USDA	United States Department of Agriculture
USPS	United States Postal Service
WLA	Water Laboratory Alliance

Trademarked Products

Trademark	Holder	Location
Cole-Parmer [®]	Cole-Parmer [®]	Vernon Hills, IL
Fisher Scientific [™]	Thermo Fisher Scientific	Waltham, MA
Geotech Geopump [™]	Geotech Environmental Equipment, Inc.	Denver, CO
Masterflex [®]	Cole-Parmer [®]	Vernon Hills, IL
Omega [™]	Omega Engineering Inc	Norwalk, CT
Rexeed [™]	Asahi Kasei Medical Co., Ltd.	Tokyo, Japan
Smart Products [©]	Smart Products USA, Inc	Mills River, NC
US Plastics	U.S. Plastics Corp.	Lima, OH

Foreword

CDC and EPA have a long history of collaboration and innovation in environmental sampling, sample processing, and detection of pathogens, including bioterrorism agents. Both agencies have responsibilities for preventing, responding to, and remediating water-based public health incidents. Sample collection is the first step in successful analysis of contaminated water. Biothreat agents in water are particularly dangerous as exposure can occur through ingestion, cutaneous contact, and/or inhalation (e.g., via aerosols produced in showers) from a range of water sources, presenting challenges for environmental and epidemiological investigations. During an identified water contamination incident, sampling will occur continuously throughout the entirety of the response. Initial screening and agent identification, determining the extent of contamination, evaluation of decontamination efforts, and meeting the clearance goals are all dependent on the information produced from sampling. Due to such importance of sampling, there is a need for an efficient and standardized sampling protocol that allows for the collection of water with greater volumes than traditional sampling methods.

This protocol has been jointly developed by CDC and EPA and addresses water sample collection for biohazard incidents and situations, in addition to sampling during natural outbreaks, and intentional or accidental biothreat contamination. The protocol and methods can be implemented during pre- and post-decontamination phases of a response to an incident as well as for routine monitoring of water. The intended stakeholders and users include water utilities, waterborne outbreak environmental investigators, and emergency responders who may be called upon in a water response. In addition, the protocol was developed to account for the acceptability of samples by analytical laboratories such as the LRN and ERLN, thereby facilitating timely, high-throughput, and accurate water sample analysis. Finally, this protocol will also help in addressing the 2018 National Biodefense Strategy – Goal 2.1.7 – “Improve the ability to detect biohazardous agents in source and finished drinking water.”

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Overview of Federal Response to a Biothreat Contamination Incident

“Biological threats—whether naturally occurring, accidental, or deliberate in origin—are among the most serious threats facing the United States and the international community.” – The National Biodefense Strategy, 2018

During a response to a biothreat incident involving water contamination, both the Centers for Disease Control and Prevention (CDC) and the U.S. Environmental Protection Agency (EPA) will have various leading and supporting roles, many of which will change based on the phase of the response and resources available (see Figure 1 for response phases). Upon initial notification of an incident that requires federal assistance, CDC will typically lead the initial response in conjunction with the Federal Bureau of Investigation’s (FBI) criminal investigation (if applicable). This response entails the following:

- Examining and responding to public health effects from exposure and consumption of contaminated water.
- Supporting epidemiologic and surveillance activities.
- Helping to identify exposures pathways to support implementation of intervention strategies.
- Identifying, confirming, and completing strain-level characterization and confirmation of a biothreat agent through laboratory analysis.

CDC’s Laboratory Response Network (LRN) will process and analyze samples with the goal of identifying and confirming a biothreat agent that may be present. A biothreat agent is defined as a harmful biological agent, including bacterial, fungal, and viral pathogens, or a compound produced by a microorganism, such as a toxin [18 USC § 178(1)]. In some forms, biological agents can also be weaponized for use in bioterrorism or other crimes. During the initial response, EPA can support CDC with sampling through EPA contractors or special teams as well as provide technical expertise in developing sampling plans.

After the biothreat agent has been identified and contained and the criminal investigation releases the contaminated area, the response can transition into the remediation phase, where EPA becomes the primary agency for site and incident characterization, decontamination, and clearance. EPA will determine the extent of contamination through site characterization, a process that consists of developing comprehensive sampling plans and continued laboratory analysis. Once the contaminated areas and parts of the water distribution system are identified, EPA will plan for and coordinate decontamination. Through targeted sampling, EPA will continue to evaluate the effectiveness of decontamination, and to help determine a clearance strategy to transition into the recovery phase.

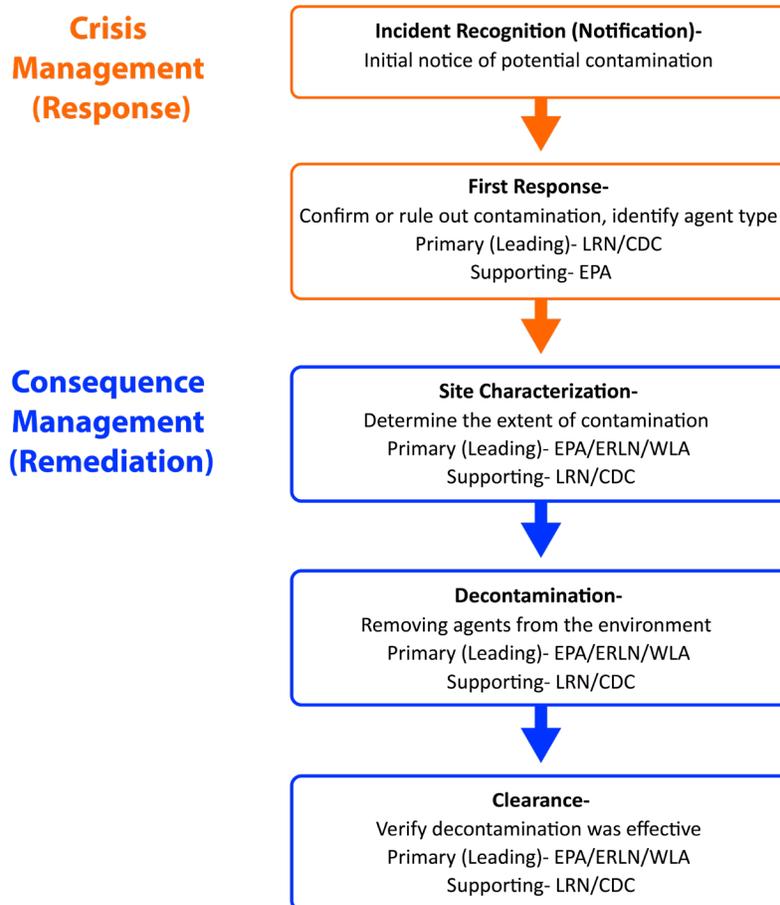


Figure 1. Response to a Water Based Biothreat Event Flow Chart Overview

Both CDC and EPA have respective laboratory networks that provide advanced laboratory response capabilities and capacity to evaluate a wide range of potential biological threats to water.

CDC's Laboratory Response Network (LRN)

Founded in 1999, the LRN is a network of state, local, federal, and international laboratories that provides rapid testing capacity to respond to chemical, biological, and radiological (CBR) threats and other public health emergencies. The LRN functions as a partnership among states, federal agencies, and various public health organizations. Participation in the LRN is voluntary and all member laboratories work under a single operational plan and adhere to strict policies of safety and security.

The objective of the LRN is to ensure an effective response to CBR terrorism by improving the nation's public health laboratory infrastructure.

The [LRN for biological threats \(LRN-B\)](#) is a component of the LRN that comprises sentinel-, reference-, and national level laboratories. Each laboratory level has various testing capabilities. The network includes local, state, and federal laboratories that perform routine diagnostic testing services and have the microbiology subspecialty capabilities to perform standardized protocol-driven steps in identifying infectious disease agents. LRN laboratories provide timely, accurate laboratory test results for various biological threats (e.g., anthrax, plague, tularemia) to inform public health decision making.

In the years since its creation, the LRN-B has played an instrumental role in improving domestic public health infrastructure by helping to boost laboratory capacity. Laboratories are now better equipped through increases in laboratory staffing and employing advanced analytical technologies to better respond to contamination events.

EPA's Environmental Response Laboratory Network (ERLN)

The [Environmental Response Laboratory Network \(ERLN\)](#) is EPA's national network of laboratories that can be accessed as needed to support large scale environmental responses. [EPA's Water Laboratory Alliance \(WLA\)](#), a nationwide network of laboratories that serves the water sector, is an integral part of the ERLN, but focuses solely on water matrices. With the threat of a CBR attack to the United States becoming more complex, the need for accurate, timely environmental testing capabilities becomes even more crucial. ERLN provides consistent analytical capabilities, capacities, and quality data in a systematic, coordinated response. ERLN integrates capabilities of existing public sector laboratories with accredited private sector laboratories to support environmental responses and provide federal, state, and local decision-makers with reliable, high-quality analyses of CBR samples taken in support of response and cleanup activities.

LRN/ERLN Collaboration

CDC's well-established LRN which includes private, state, and government laboratories, works to strengthen laboratory capacity by engaging in partnerships with the WLA network within the ERLN. Both networks use validated methods that allow for rapid detection and response between the laboratories. A biothreat incident will result in numerous samples, but with standardized protocols and testing capabilities, labs are able to provide surge capacity between networks.

Both LRN and ERLN also maintain relationships with other federal laboratory networks through the [Integrated Consortium of Laboratory Networks \(ICLN\)](#) for sample analysis in preparation for a major environmental CBR contamination incident.

Protocol for the Collection of Water Samples for Detection of Pathogens and Biothreat Agents

1.0 Purpose

This document describes methods for collecting and concentrating water samples in a field setting for waterborne pathogen and biothreat agent detection. The methods describe water sampling by concentrating large volumes of water in the field via dead-end ultrafiltration (DEUF) or via grab sampling when large volume methods are not feasible. DEUF concentrates microbiological contaminants by pushing large water volumes through a small pore size hollow fiber membrane to effectively capture protozoa, bacteria, viruses, and biotoxins larger than 10 to 30 kilodalton molecular weight depending on the filter¹. The samples produced through adhering to the protocols listed in this document will be acceptable for processing by the reference laboratories within the CDC's LRN and the EPA's ERLN, allowing for a streamlined response.

The guidance in this document provides recommendations and considerations for the entire sampling process, to include safety considerations when sampling in a low hazard or a high hazard incident (Section 3.0), choosing a sampling method and considerations for sampling strategies (Section 4.0), documentation activities when sampling (Section 5.0), sampling methods and preservation recommendations (Section 6.0), and guidance on packaging and shipping water samples (Section 7.0). Guidance is intended to support sampling for bacterial, fungal, viral, and other pathogens, including bioterrorism agents, and biotoxins for biological incident response, but can also be applied to sampling in support of routine and baseline monitoring, sampling in response to a contamination incident or outbreak, and sampling in support of remediation or decontamination efforts.

The intended users of this document are water utilities, sampling teams, and emergency responders who may be called upon in response to a large contamination/bioterrorism/outbreak incident. Sample acceptability by the analytical laboratories has been considered while developing this protocol because it can facilitate timely, high-throughput, and accurate water sample analysis. This document can also serve as a reference point for pertinent core capabilities of CDC, EPA, and their respective laboratory response networks during a waterborne outbreak after federal support is requested from State, Local, Tribal, and Territorial (SLTT) authorities.

2.0 Scope and Application

This document provides methods and information for collecting water samples suspected of containing viral, bacterial, parasitic, and other pathogens, including bioterrorism agents and biotoxins from a variety of water sources. This protocol provides water sample collection and concentration methods based on water source and equipment access.

The methods included are as follows:

- **Dead-end ultrafiltration (DEUF) from a non-pressurized source.** A pump draws up a large volume of water (10->100 L) which is then forced through the hollow-fiber ultrafilter.^{2,3}
- **Dead-end ultrafiltration (DEUF) from a pressurized source.** A hollow fiber ultrafilter is connected directly to a tap or valve in a pressurized pipe for which the water pressure drives a large volume of water (10-100L) through the filter.^{4,5}
- **Grab sample (1 liter).** This small volume sample can be sent to a lab for concentration.⁶

A list of common waterborne agents and their size range can be found in Table 1. For known waterborne agents smaller than 20 nanometers (nm), or 30,000 Daltons that will not be captured by the ultrafilter, grab sampling should be considered for the sampling method as the agents will need to be concentrated in the laboratory. In Section 4.0, the above methods are discussed further to help determine when one method may be preferable over another.

Table 1. Sizes of Common Waterborne Pathogens and Biothreat Agents

Pathogen Size	Waterborne Agents	
Protozoa (4-20 µm)	<ul style="list-style-type: none"> • <i>Acanthamoeba</i> spp. • <i>Cryptosporidium</i> spp. • <i>Entamoeba histolytica</i> 	<ul style="list-style-type: none"> • <i>Giardia</i> spp. • <i>Naegleria fowleri</i> • <i>Toxoplasma gondii</i>
Bacteria (0.2-5 µm)	<ul style="list-style-type: none"> • <i>Aeromonas</i> spp. • <i>Bacillus anthracis</i> • <i>Brucella</i> spp. • <i>Burkholderia mallei</i> • <i>Burkholderia pseudomallei</i> • <i>Campylobacter jejuni</i> • <i>Chlamydia psittaci</i> • <i>Coxiella burnetii</i> • <i>Elizabethkingia</i> • <i>Escherichia coli</i> • Pathogenic <i>Escherichia coli</i> • <i>Francisella tularensis</i> 	<ul style="list-style-type: none"> • <i>Legionella pneumophila</i> • <i>Leptospira</i> spp. • <i>Listeria monocytogenes</i> • Non-typhoidal <i>Salmonella</i> • <i>Pseudomonas aeruginosa</i> • Other <i>salmonellae</i> • <i>Salmonella Typhi</i> • <i>Shigella</i> spp. • <i>Staphylococcus aureus</i> • <i>Vibrio cholerae</i> • <i>Yersinia pestis</i> • <i>Yersinia enterocolitica</i>
Virus (0.02-0.2 µm)	<ul style="list-style-type: none"> • Adenovirus • Enteroviruses • Hepatitis A and E virus • Human coronavirus 	<ul style="list-style-type: none"> • Norovirus • Influenza H5N1 Virus • Rotavirus • Sapoviruses
Biotoxins (<1 – 150 kilodaltons (kDa))	<ul style="list-style-type: none"> • Botulinum (~149,000 Daltons) • Ricin (~65,000 Daltons) • mycotoxin (< 1,000 Daltons) • saxitoxin (< 1,000 Daltons) 	

3.0 Safety Considerations

3.1 Low Hazard Sampling Versus High Hazard Sampling

The hazard level of the sampling process is determined by the severity of the potential health risk associated with the presence of the suspected biological contaminant in the water body of interest. The hazard level dictates the requirements for the following sampling processes: 1) individual or agency responsible for collecting sample; 2) personal protective equipment (PPE) level; 3) federal shipping requirements; and 4) laboratory responsible for sample processing. The hazard level should be evaluated as the first step in the contaminant response and is assigned as either **low** or **high hazard**. The lead agency on scene commander assigns the hazard level.

3.1.1 Low Hazard - For the purpose of this document, Low Hazard refers to the sampling of water following unintentional, intentional, and natural water contamination incidents of microbiological organisms or biological toxins that have either resulted in or has the potential to result in waterborne disease. Low Hazard sampling incidents have a low association of health risks for exposure during sampling.

3.1.2 High Hazard - For the purpose of this document, High Hazard refers to the sampling of water following unintentional, intentional, and natural water biological contamination incidents that present a significant health threat to the individual sampling or exposed population. Characteristics of a high hazard include persistence, treatment resistance, host infectivity, etc. A pathogen in this category is not limited to risk from ingestion of contaminated water but can also include health impacts from additional exposure routes including inhalation and dermal/cutaneous contact.

Additionally, the hazard level can be categorized as high hazard if the FBI or other law enforcement agencies have suspected or identified a credible threat, or the water source has been deemed a significant risk to public health through credible identification of the contaminate/s and thus requiring emergency response. Any samples identified as high hazard should be communicated to the receiving laboratory to verify if samples will be accepted and to confirm appropriate biological safety levels and processing procedures are in place.

Specific pathogens, agents, and toxins can warrant a high hazard designation. The [Health and Human Services \(HHS\) and United States Department of Agriculture \(USDA\) Select Agent and Toxins List](#) identifies biological contaminants that pose a severe threat to public health and safety. Additional resources that provide information to aid in identifying high hazard biological contaminants are the [CDC Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)](#) (CDC 2020); [CDC's Bioterrorism Agents list](#); and [National Institute of Allergy and Infectious Diseases \(NIAID\) Emerging Infectious Diseases list](#).

3.2 General Safety Guidance

Proper safety precautions must be observed at all times when sampling. Safety guidance will vary based on information on the response and suspected contaminant. Event specific safety guidance will be determined by on site or response leadership based on the available information including, but not limited to, epidemiological data, the FBI intelligence assessment, and rapid field test results.

General safety guidelines for any environmental sampling for pathogens or biothreat agents include:

- No eating, drinking, or smoking on site
- Ensuring all sample collectors are familiar with sampling methods
- Proper usage of required PPE based on the Health and Safety Plan (Section 3.3)
- Sampling in pairs or teams if possible
- Avoiding direct contact with mucous membranes and open wounds, as well as ingestion or inhalation of water sample or water source
- Wash all skin in contact with water sample or water source with soap and clean water after sampling

3.3 Health and Safety Plans (HASP)

Health and safety plans account for potential hazards encountered by individuals sampling and will vary based on the suspected contaminant, extent of contamination, involved organizations, phase of response, and the individual sampling site.

At a minimum, Health and Safety Plans should include instructions or guidelines regarding:

- Names, titles, and contact information of samplers, involved agencies, and other key personnel

- PPE requirements and proper usage
- Decontamination methods for sampling equipment, surfaces, and personnel
- Exposure response plan
- Site specific risks or hazards associated with sampling
- Emergency response plan
- First aid considerations

3.4 Personal Protective Equipment (PPE)

Personal Protective Equipment (PPE) should be worn during all sampling activities. PPE level and usage requirements should be stated in the HASP, and will vary depending on the sampling site, routes of contaminant exposure, and suspected contaminant. PPE should be sufficient to protect samplers from contamination exposure in a wet environment. The recommended minimum PPE for all sampling events is disposable gloves. Gloves should be disposed between each sampling event to prevent cross contamination. Additional guidance on PPE selection can be found in [29 CFR 1910.120](#). Eye protection may be required in the HASP based on the target of interest or determined site hazard.

3.5 Site and Incident Characterization

Site characterization is information that is collected from the sampling site to identify potential safety hazards while sampling. Site characterization activities can include field testing, hazard assessments, and initial sample collection. More information on site characterization can be found in [EPA's Response Protocol Toolbox: Planning for and Responding to Drinking Water Contamination Threats and Incidents-Module 3: Site Characterization and Sampling Guide](#).

4.0 Sample Collection Methods and Considerations

4.1 Selection of Water Sampling Method

This section provides a general overview for large volume and grab sampling collection methods as well as guidelines for when to consider a specific method during a sampling event. As every sampling event is different, sampling methods can be modified as required based on water source, specific site conditions, or equipment limitations. Section 8.3 provides an array of resources for various sampling scenarios.

4.1.1 Dead-end Ultrafiltration from a Pressurized Source

DEUF from a pressurized source concentrates a water sample by using the pressure within a piped system, such as a water main or a building plumbing system, to push water through the ultrafilter and therefore does not require a pump. In this method, one end of a segment of tubing is attached to the pressured source (via hose bib or valve) and the other end is attached to the end port of a hollow fiber ultrafilter. Due to the potentially high pressure of the water source, the inlet pressure of the filter is controlled by maintaining a flow through the filter of <4 liters/min. If the flow begins to slow (*e.g.*, trickle of water passing through filter) or stop, signaling a potential clog, the water must be turned off immediately to prevent pressure increasing within the tubing. For drinking water or other types of water with minimal turbidity (< 5 NTU), 100 L or more can be filtered. At the end of this process, the filter is sealed and shipped to a laboratory where the filter is eluted to collect the captured microorganisms.

4.1.2 Dead-end Ultrafiltration from a Non-pressurized Source

DEUF from a non-pressurized source concentrates water by drawing the water through the ultrafilter using a peristaltic pump. Examples of such unpressurized sources are reservoirs or large containers of water collected from a pressurized source. Similar to pressurized source filtration, the inlet pressure of the filter controlled by maintaining a flow of <4 liters/min and immediately stopping the pump if the flow begins to slow (e.g., trickle of water passing through filter) or stop, signaling a potential clog. For surface water or other types of high turbidity water, 50 L is the maximum recommended sample volume. DEUF has been found to be effective for wastewater effluent samples and water samples having turbidity values of up to 80 NTU, but the maximum filterable volume will vary depending on water quality. Following filtration, the ultrafilter is similarly sealed and processed as described above in 4.1.1.

4.1.3 Grab Sampling

A 1-liter grab sample is collected following standard methods and shipped to a laboratory where it is directly assayed or subsequently concentrated (e.g., membrane filtration or centrifugation) and analyzed. This is the simplest water collection method; however, the relatively small sample volume will limit the ability of the sample to capture microorganisms present at low concentrations. If ultrafiltration cannot be performed in the field or if contaminants are suspected of being smaller than 20 nanometers (nm), or 30,000 Daltons, 1 L grab samples can be collected and transported to the laboratory for concentration and analysis.

4.2 Considerations for Sampling Plan

Developing an effective sampling and analysis plan (SAP) is necessary for obtaining an accurate representation of the site being sampled. Sampling and analysis plans provide sampling objectives and collection strategies that are based on numerous factors, including epidemiological and intelligence data available, contaminant of interest (if known), sampling location, frequency, field methods and procedures, and site characteristics. Sampling plans should be developed before sampling activities begin. Sampling plan strategies can also be adjusted based on the type of activity that warrants water testing. When the suspected contaminant is in a flowing water source (e.g., drinking water distribution system, stream), the contaminant may not be homogeneously distributed but instead concentrated in a plume or plug of the flowing water. To best capture the contaminant, a sampling strategy can filter water at a relatively low rate or intermittently over a representative period (e.g., 24 h). If a high sampling rate (e.g., >4 liters per minute) is needed to collect the desired sample, collecting the sample into a container, and then concentrating it is recommended.

Response types applicable to this document include, but are not limited to the following:

- **Water-associated outbreak initial response** – The primary sampling goal of an initial water response is identifying the pathogen and source of contamination to mitigate any public health threat. Sampling during this phase will be conducted from areas identified as having the highest likelihood of contamination, with subsequent samples taken from surrounding areas based on the information available to responders (e.g., suspected pathogen, suspected matrix, suspected route of exposure) and availability of resources (equipment, number of personnel, etc.). Sampling plans will typically evolve throughout the response, and as some pathogens may persist longer in various environments other than water, plans may include sampling of additional matrices (i.e., sediment, soil, and biofilms). Additional information on

sampling strategies for initial waterborne outbreak response sampling can be found in the [CDC Waterborne Outbreak Investigation Toolkit](#).

- **Biological contamination characterization and clearance** – Sampling to characterize contamination of a water body occurs after the outbreak initial response and a pathogen has been identified. From this point forward, targeted sampling will be done to determine the extent of contamination, monitor decontamination progress, and identifying residual contamination in efforts to determine when an area is eligible for resuming normal activities. Sampling programs, like [EPA’s ESAM](#), are tools that can assist individuals with developing sampling and analysis plans for microbial contamination during remediation activities. Additional information on sampling strategies for water contamination characterization and clearance can be found in the [EPA Choosing a Sampling Design for Environmental Data Collection](#).

4.3 Quality Control and Quality Assurance

Quality Control and Quality Assurance (QA/QC) practices during water sample collection methods are essential for maintaining accurate representations of the sampling site. The selection of QA/QC procedures and frequency will vary for each sampling scenario and available capacity. QA/QC practices during sample collection include field duplicates, field blanks, sample negative controls, and recording of supplies and reagent lot numbers and expiration dates. Further details regarding QA/QC procedures can be found in [EPA’s Guidance on Choosing a Sampling Design for Environmental Data Collection for use in Developing a Quality Assurance Project Plan and Sampling, Laboratory and Data Considerations for Microbial Data Collected in the Field](#).

Proper handling techniques are essential for preventing cross-contamination of samples and providing accurate results. Ways to prevent cross-contamination include using a new pair of gloves for each sample, avoid touching the inside of the sampling bottles or placing the tops of sampling containers on potentially contaminated surfaces, and sampling from the likely least suspected contaminated area to the most suspected contaminated area.

4.4 Water Quality Measurements

Water quality measurements provide indicators of general water quality and can help identify indicators of potential contamination. Many of these measurements can be performed in the field during sampling and are available in kits that provide rapid results. Useful water quality parameters to measure during sampling include temperature, pH, turbidity, disinfectant residual (free and total chlorine), conductivity, and dissolved oxygen. All field measurements should be properly documented on field notes and sent to the receiving laboratory.

4.5 Decontamination, Disposal, and Waste Management

All sampling equipment, PPE, and reusable supplies should be decontaminated thoroughly in an area free of contamination. The sampling plan should indicate the type of decontamination required after each sampling event.

All waste generated from sampling should be placed in a garbage, autoclave, or biohazard bag and secured until proper disposal. For high hazard sampling events, local regulatory agencies should be contacted to determine requirements for waste treatment and disposal.

5.0 Field Sampling Preparation

This section provides an overview of the sample documentation and supplies needed for field collection.

5.1 Sample Identification Numbers

A sample identification number, or sample ID, should be assigned to every sample collected. Sample identification numbers are used to uniquely identify each sample and usually contain information describing the sample type, matrix, location, and date/time of collection. The sample identification number should be created by the sample collector, the receiving laboratory, or a project manager prior to sampling. It is important to use this unique number consistently across the container label, field notes, and chain of custody forms. All information should be written legibly in waterproof ink.

5.2 Field Notes

Field notes help document sample collection operations and additional field activities that are not listed on the chain of custody form. All notes should be written legibly in waterproof/permanent ink. Any deviation from the sampling protocol or sampling plan should be documented. A photograph log that includes identifying landmarks is recommended when permitted.

Useful information to record in field notes are:

- Description of sampling location by GPS coordinates and site-specific markers
- Sampler(s) name
- Date, time, weather, and environmental conditions
- Field water quality measurements
- Start and stop times during sampling
- Individuals and agencies involved with sample collection and their contact information
- Field sampling methods and equipment used
- Level of PPE worn for sampling
- Photograph log

5.3 Chain of Custody Forms

The primary purpose of a Chain of Custody (COC) is to create an accurate written record that documents samples from collection through analysis at the receiving laboratory. This chronological record documents each individual in possession of the sample. An example chain of custody form can be found in Appendix 8.1. Sharing and storage of records should be conducted according to the lead agency policies and response requirements.

At a minimum, the COC should contain the following information:

- Sample identification number
- Date, time, and location of sample collection
- Hazards associated with the sample
- Names and signatures of samplers and individuals who obtained custody of the sample
- Date, time, and location of sample receipt facility/lab
- Any sample preservation methods

5.4 Custody Seals

Custody seals are used to ensure that samples have not been tampered with after collection. If required by the sample collection agency, custody seals can be placed across the seal of a

secondary leakproof container containing the sample (e.g., the seal of clear resealable plastic bag containing a 1 L sample or ultrafilter) and across the hinges on the shipping container, so that the container cannot be opened without the custody seals breaking.

5.5 General Field Equipment and Supplies

The equipment and supplies needed for sampling biological contaminants can vary based on the location, type of water source, and type of contaminant. All equipment used for sampling should be clean, and in working condition, and calibrated according to manufacturer's instructions (if required). Prior to sampling in a high containment zone, all equipment and materials should be identified and assembled with gloves for improved sampling ease when wearing high hazard PPE.

Table 2 provides a general list for routine sampling or for sampling in response to an outbreak. Other equipment or supplies may be needed based on the scenario. Table 3 provides a list of supplies for the specific sampling methods. The detailed steps of these methods are provided in the following section (Section 6).

Table 2. General Supplies for Water Sampling in the Field

Item
Stopwatch
Waterproof markers
Latex/nitrile gloves
Labeling tape
Insulated Cooler
Sodium thiosulfate or equivalent disinfectant neutralizing agent
GPS unit, area map, etc.
Record keeping documents (chain of custody, field logs, etc.)
Communication device (cellphone, two-way radio, etc.)
Paper towels
Alcohol, ethanol, and bleach wipes
Trash bags and clear resealable plastic bags
Water quality meters and test kits (pH, free and total chlorine, temperature, turbidimeter, etc.)
Camera (if allowed on site)
Shipping supplies
Custody tape (optional)
Graduated cylinder (1 L)

Table 3. Water Sampling Method Specific Supplies

Method	Item	Catalog #	Manufacturer
Grab	1-L wide-mouthed, sterile, with screw cap, polypropylene bottle(s)*	N3111000	Thermo Scientific
	Cubitainer, sterile (5-L or 20-L)*	EW-35204-88	Cole-Parmer
DEUF: <i>all water sources</i>	Hemodialyzer (hollow fiber ultrafilter with 30 kDa molecular weight cut off [20 nm pore size])	REXEED-25A	Asahi Kasei
	L/S 36 tubing (0.375 ID × ~0.56" OD) *	EW-96410-36	Cole-Parmer
	DIN adapters (3/8" hose barb x female DIN)	MPC-855NS.375PP	Molded Products
	SNP-8 tubing clamps	EW-06832-08	Cole-Parmer
	Blood port kidney storage end cap	MPC-40	Molded Products
	Dialysate port kidney storage side cap	MPC-60D	Molded Products
	Flow totalizing meter fitted with 2 straight barbed to male NPT threaded adapters, 3/8" x 3/4" NPT*. Use plumbers' tape on threading to prevent leakage if needed.	FTB691A-NPT	Omega
	2, hose barb x male NPT adapters, 3/8" x 3/4" NPT *	EW-30904-53	Cole-Parmer
	Distilled or deionized (DI) water		
	Disposable check valve, with 3/8" hose barbs (optional)	Model # 306306PS-0050S000-1402	Smart Products
	Long nose pliers		
	Scissors		
	500-mL wide mouth polypropylene bottle and cap*	70039	US Plastic
	Sodium thiosulfate, anhydrous*	S446-500	Fisher
	Alcohol, bleach, and ethanol wipes		
60-mL sterile syringe, luer lock or non-luer lock w/o needle*	309653	BD	
DEUF: <i>Pressurized from Standard US hose bib</i>	1/2" ID hose x swivel FGHT nylon swivel female insert*	63003	US Plastics
	SNP-12 tubing clamp *	EW-06832-12	Cole-Parmer
DEUF: <i>Pressurized from non-standard water faucet</i>	I/P 89 silicone tubing (0.375" ID × ~0.88" OD)*	EW-96510-89	Cole-Parmer
	Reducing connector 5/8" x 3/8"	EW-30622-00	Cole-Parmer
	SNP-28 tubing clamp*	EW-06832-28	Cole-Parmer
	SNP-24 tubing clamp*	EW-06832-24	Cole-Parmer
	SNP-19 tubing clamp*	EW-06832-20	Cole-Parmer
DEUF: <i>Non-pressurized</i>	Geopump peristaltic pump with pump head plus two Geotech modular batteries, 12-18V DC @ 70 watts batteries, or 90-260V AC @ 47-65 Hz batteries with terminals for alligator clips <i>or</i> hardwired AC power cord*	91352123 <i>or</i> 91351003	Geotech
	Masterflex Easy-Load II pump head for High Performance Precision Tubing*	EW-77200-52	Cole-Parmer
	4.8 oz. SS Tube Weight, 1/2" with Clamp*	87050024	GeoTech

*Indicates that equivalent products can be used. Names of vendors or manufacturers are provided as examples of suitable products and sources. Inclusion does not imply endorsement by CDC or EPA.

6.0 Water Sampling and Preservation Methods

6.1 Small Volume Grab Sampling (1 liter)

NOTE: *Under low hazard conditions, larger grab volumes may be collected in the sterile cubitainers (up to 20 L) for concentration and/or testing in a laboratory if field concentration is not possible. Of note, shipping of Category B water samples (suspected infectious materials) has a 1 L max volume.*

- 6.1.1** Ensure the containers are intact and sterile.
- 6.1.2** Label the container with sample identifier (ID) number associated with sample metadata (location, time, collector, volume, water quality measurements) that is also referenced on chain of custody.
- 6.1.3** Remove and put the cap aside, keeping it free from contamination during sampling.
 - **Sampling from a tap** - Wipe the outside and inside of tap with a bleach wipe, followed by an ethanol wipe and allow to air dry. If purging, allow tap to purge for 2 minutes prior to sample collection. Place container under tap and fill with water, leaving some headspace.

NOTE: *Sampler may remove aerator/screen from tap prior to collection if clogged or externally contaminated, being careful to not contaminate tap during removal.*
 - **Sampling from surface water** - Immerse the container opening first into the water, facing the opposite direction of water flow (if any) and allow water to run slowly into the bottle until it has minimal headspace.
- 6.1.4** If free chlorine is present or suspected in the water source during field testing, add 1 g sodium thiosulfate per 1 L water sample.
- 6.1.5** Replace the cap tightly.
- 6.1.6** Wipe any remaining liquid from outside of container with an alcohol wipe or paper towel.
- 6.1.7** Prepare the samples for any additional preservation requirements (Section 6.4).

6.2 Large Volume Dead-end Ultrafiltration Sampling: Pressurized Source

NOTE: *If unable to directly connect to pressurized system, collect pressurized sample in a large, sterile container (e.g., 20 L cubitainer) and follow the steps for collecting samples from an unpressured source using large volume dead-end ultrafiltration (Section 6.3). If the flow totalizer does not work due to low filtration rate, collect the filtrate in a graduated container to measure volume filtered. A disposable check valve is recommended for use if concerned about back flow and/or if required by local water utility.*

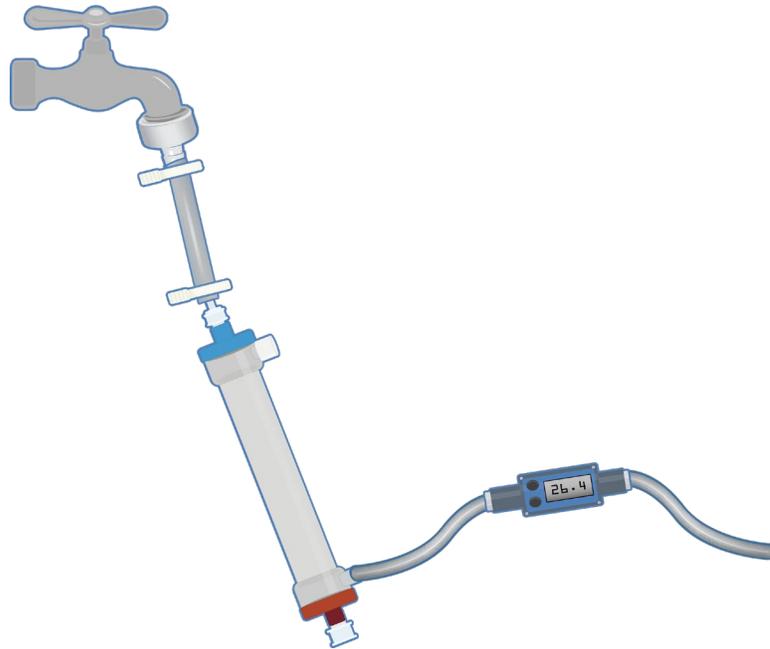


Figure 2. DEUF Pressurized Source

- 6.2.1** If free chlorine is present or suspected in the water source during field testing, prepare the sodium thiosulfate solution (1% w/v).
NOTE: *Sodium thiosulfate solution can be prepared ahead of time. Store at room temperature for up to 7-days and at 4°C for up to 6 months.*
- Add 500 mL of sterile, deionized water and 5 g sodium thiosulfate to a 1 L sterile bottle.
 - Shake to dissolve the solution and save for step 6.2.11.
- 6.2.2** Wipe the outside and inside of the tap with a bleach wipe followed by an ethanol wipe and allow to air dry.
- For pathogen detection, including high hazard pathogens, purging is not recommended. Contaminants can concentrate in stagnant pipe areas near sample points and are therefore ideal material for evaluating potential contamination.
 - For sampling scenarios where purging occurs (e.g., regulatory monitoring), allow the tap to purge for ≥ 2 minutes prior to sample collection.
- NOTE:** *Sampler may remove aerator/screen from tap prior to collection if clogged or externally contaminated, being careful to not contaminate tap during removal.*
- 6.2.3** Assemble the ultrafiltration set-up (Figure 3 and Figure 4).
NOTE: *There is no directionality to the ultrafilter. The end ports are color-coded for hemodialysis, but there is no difference in functionality for water sampling. Properly document Ultrafilter with Sample ID prior to sampling. Preparation of ultrafilter assembly (i.e., cutting and assembly of tubing) should be done prior to field entry.*



Figure 3. Ultrafilter before sampling

- Remove and dispose the end port cap from Port 4 of the ultrafilter and screw in the DIN adapter, firmly but no more than hand tight. This port is the influent port.
- Confirm the closure of Port 3 by screwing or pushing the cap provided on to the filter until it clicks.
- Remove and dispose the end port cap from Port 1 and screw in a blood port end cap.
- Cut a length of influent L/S 36 tubing to the length required to span the distance between the water source and the ultrafilter.
- Push the influent L/S 36 tubing on to the DIN adapter on Port 4 and secure with SNP-8 tubing clamp. Use pliers to tighten clamp.
- Remove and dispose the end port cap from Port 2. This port is the effluent port.
- Push the effluent L/S 36 tubing onto Port 2 (no clamp is needed).

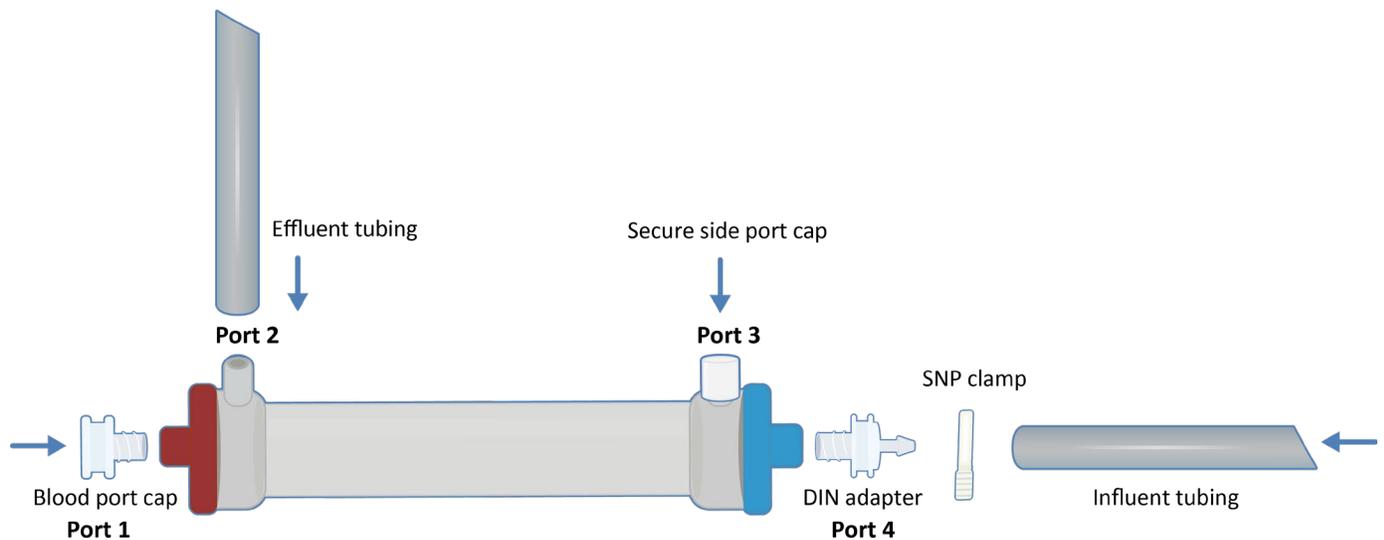


Figure 4. Ultrafiltration set-up assembly

6.2.4 Attach the flow totalizer.

- Attach straight barbed to male NPT threaded adapters to either end of the flow totalizer to allow for connection to tubing.
- Connect the ultrafilter effluent tubing to the end of flow totalizer side that ensures the arrow on the totalizer is pointing in the direction of the water flow (away from filter).
- Connect a final piece of tubing to the other end of the totalizer long enough to allow ultrafiltered water drainage into appropriate waste receptacle, or into a 20 L cubitainer/5 gallon bucket for transport to nearby drainage site.

- 6.2.5** Connect the influent tubing to the faucet or valve.
- If sampling from a standard garden faucet (hose bib), screw on the nylon swivel female adaptor onto the hose bib. Push influent tubing onto the male end of the hose bib adaptor and secure with a SNP-12 tubing clamp.
 - If a faucet is not standard (e.g., various types of kitchen and bathroom faucets and non-threaded outdoor faucets), push I/P 89 tubing over the faucet head and secure with a SNP-19, SNP-24, or SNP-28 tubing clamp. Use a reducing connector (5/8" to 3/8") to connect the I/P 89 and L/S 36 influent tubing, secure both sides with SNP-19 and SNP-8 tubing clamps, respectively.
NOTE: *If the connector is unable to secure to faucet, collect water in a sterile container using the method in Section 6.3.*
- 6.2.6** For ease of measurement during filtration, set the meter to liters, reset the flow totalizer to zero (if possible), and start the meter to read the flow rate. Record the initial flow totalizer meter reading on the COC (Appendix 8.1).
- 6.2.7** Turn the faucet on and gradually increase the flow until the desired flow rate is achieved (up to 4 L/min). Record the start time of filtration.
- 6.2.8** Calculate flow totalizer meter end reading by adding the desired water volume to the initial totalized flow reading; if using a meter that reads gallons, use the following conversions: 100 L=26.4 gal; 50 L=13.2 gal); continue filtration until that reading has been reached.
- If a flow totalizer is not available, measure the effluent flow rate and record the time and flow rate to estimate the total volume of water filtered by multiplying the cumulative filtration time and flow rate measurements.
 - Collect the filtered water in a 1 L graduated cylinder for 30 seconds.
 - Measure the volume of water in the cylinder and multiply by 2 to determine the flow rate per minute, and record on COC/data sheet (e.g., 900 mL X 2 = 1800 mL/min= 1.8 L/min).
 - Calculate the number of minutes required to filter desired volume of water (e.g., 100 L/1.8 L = 55.5 minutes).
 - Repeat this calculation every 5 minutes to accurately gauge how many minutes are required to filter desired volume of water.
- 6.2.9** During filtration, visually inspect the flow rate from the effluent tubing. Dramatic decreases in flow rate will indicate filter clogging, which can be due to water quality or entrapment of an object in the influent tubing or filter.
NOTE: *If clogging occurs (indicated by a dramatic decrease or stop in effluent flow), stop filtration and record flow totalizer reading.*
- 6.2.10** Stop filtration after desired water volume is filtered, record the final flow totalizer meter reading. A volume of 50 L is recommended for surface water and 100 L for drinking water.
- 6.2.11** Add sodium thiosulfate solution to the filter if needed (see step 6.2.1).
- Open the bottle of pre-made sodium thiosulfate.
 - Position the filter so that Port 4 (inlet) is facing up.
 - Remove the influent tubing from the faucet and cut the tubing so only 2-3 inches remains attached to the filter.
 - Remove the plunger from the 60 mL syringe. Insert the tip of the syringe into the remaining influent tubing securely.
NOTE: *An SNP-8 clamp can be used to secure tubing.*
 - Pour 60 mL of the 1% sodium thiosulfate solution into the syringe and gently push through the ultrafilter with the syringe plunger.

- Remove the syringe from the tubing before pulling out the plunger. Pulling out the plunger before detaching the syringe will create negative pressure.
- Repeat this process one time for a total of 120 mL 1% sodium thiosulfate pushed into the ultrafilter.

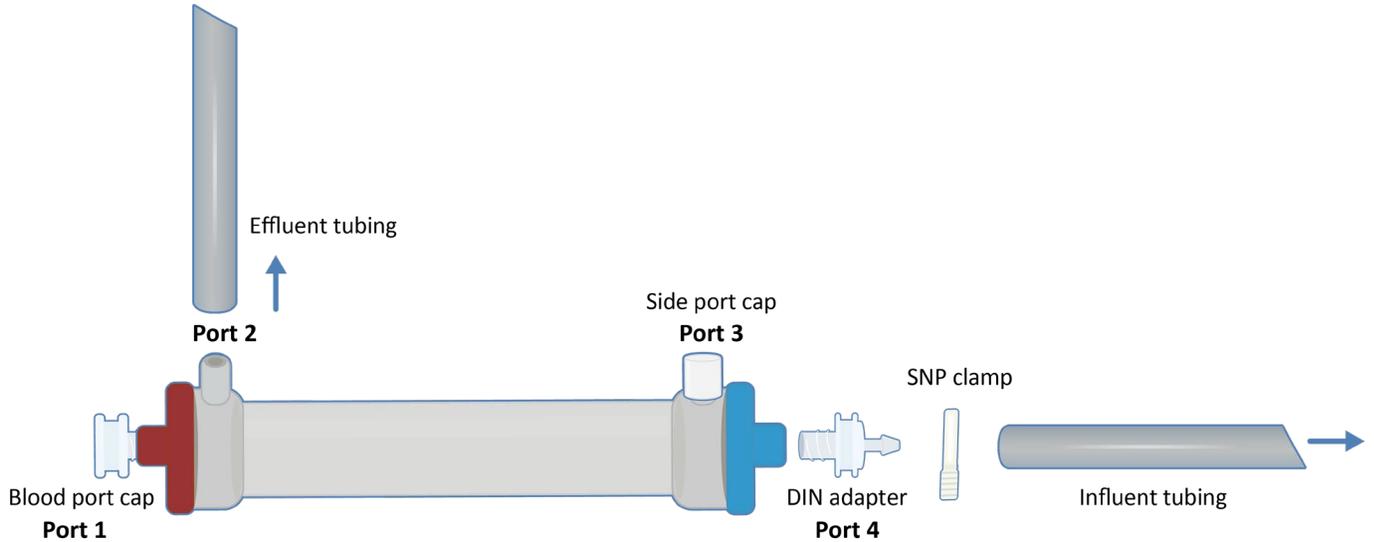


Figure 5. Disassemble the ultrafilter

- 6.2.12** Remove and discard all tubing from the ultrafilter (Figure 5). Screw a new kidney storage side cap on to Port 2 and place a new blood port storage end cap on Port 4 (Figure 6). Ensure caps are firmly tightened.

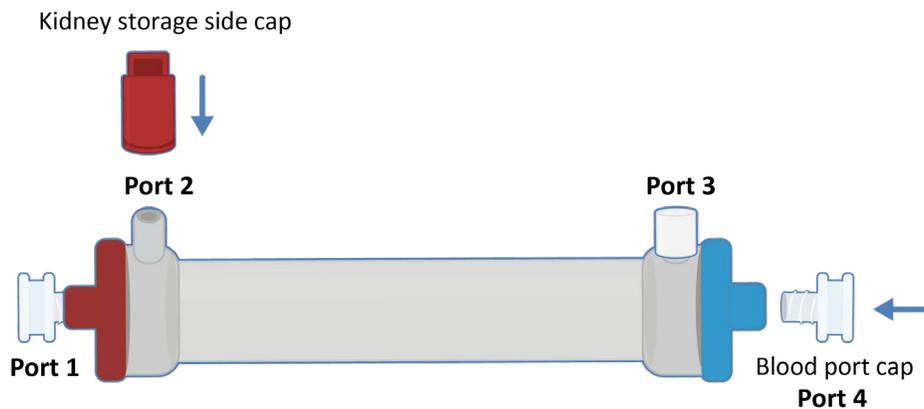


Figure 6. Prepare ultrafilter for storage and shipping

- 6.2.13** Prepare the samples for any additional preservation requirements (Section 6.4).

6.3 Large Volume Dead-end Ultrafiltration Sampling: Non-pressurized Source

NOTE: If the tubing length required to reach a non-pressurized source is sufficiently long that the pump does not produce enough suction to pull the water to the filter, then the tubing may need to be primed by starting the pump prior to attaching the tubing to the ultrafilter. Once the water begins to move through the tubing, attach the tubing to filter without opening the pump head. If priming the tubing still does not produce enough pressure to pull water through the filter, the non-pressure source may need to be pumped into a container and then ultrafiltered from the container using a shorter length of tubing. Overall, the volume of air pushed into the filter should be minimized, and the tubing length attached to the filter should be as short as possible to reach the unpressured water source for maximum field pump performance.

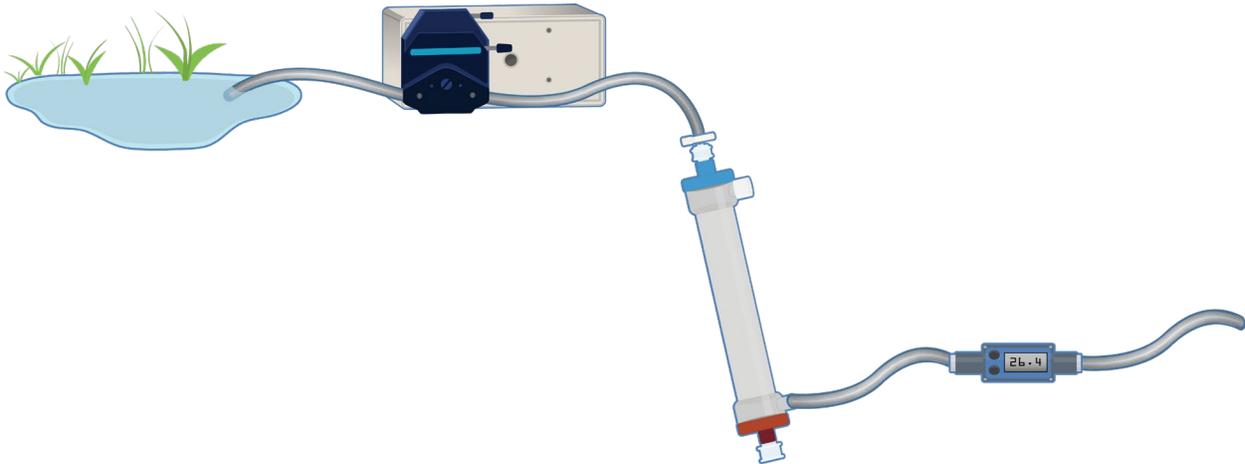


Figure 7. DEUF Non-Pressurized Source

- 6.3.1** If free chlorine is present or suspected in the water source during field testing, prepare the sodium thiosulfate solution (1% w/v).
- Add 500 mL of deionized water with 5 g sodium thiosulfate in a 1 L bottle.
 - Shake to dissolve the solution and save for step 6.3.14.
- 6.3.2** Assemble the ultrafiltration set-up (Figure 3 and Figure 4).
- NOTE:** There is no directionality to the ultrafilter. The end ports are color-coded for hemodialysis, but there is no difference in functionality for water sampling. Properly document Ultrafilter with Sample ID prior to sampling. Preparation of ultrafilter assembly (i.e., cutting and assembly of tubing) should be done prior to field entry.
- Remove and dispose the end port cap from Port 4 of the ultrafilter and screw in the DIN adapter, firmly but no more than hand tight. This port is the influent port.
 - Confirm the closure of Port 3 by screwing or pushing the cap provided onto the filter until it clicks.
 - Remove and dispose the end cap from Port 1 and screw in a blood port end cap.
 - Cut a length of influent L/S 36 tubing to the length required to span the distance between the water source and the ultrafilter.
 - Push influent L/S 36 tubing onto the DIN adapter on Port 4 and secure with SNP-8 tubing clamp. Use pliers to tighten clamp.
 - Remove and dispose the cap from Port 2. This port is the effluent port.
 - Push the effluent L/S 36 tubing onto Port 2 (no clamp is needed).
- 6.3.3** Attach the flow totalizer.

- Attach straight barbed to male NPT threaded adapters to either end of the flow totalizer to allow for connection to tubing.
 - Connect the ultrafilter effluent tubing to the end of the flow totalizer side that ensures the arrow on the totalizer is pointing in the direction of the water flow (away from filter).
 - Connect a final piece of tubing to the other end of the totalizer long enough to allow ultrafiltered water drainage into appropriate waste receptacle, or into a 20 L cubitainer/5 gallon bucket for transport to nearby drainage site.
- 6.3.4** For ease of measurement during filtration, set the meter to liters, reset the flow totalizer to zero (if possible), and start the meter to read the flow rate. Record the initial flow totalizer meter reading on the COC (Appendix 8.1).
- 6.3.5** Place the influent tubing into the body of water (or water that has been collected in a clean container). Weigh the tubing to ensure the end of the tubing will stay below the surface of the water (e.g., using a tubing weight following manufacturer's instructions). It is important to keep the end of the tubing below the surface to avoid bubbles being trapped in the line.
- 6.3.6** Feed the influent tubing through the pump head and close the pump head using the lever.
- 6.3.7** Plug in the appropriate power cord into the outlet in the back of the pump and the other end of the power cord into the power source. The power source can be any external 12-18 V DC @ 70 watts or 90-260 V AC 47-65 Hz. Place the battery in a location where it will not get wet.
- 6.3.8** Determine the desired direction of flow and set the toggle switch on the pump for the flow direction. Ensure the speed dial is set to zero before starting the pump.
- 6.3.9** Turn the pump "ON" and record the start time of filtration.
- 6.3.10** Once pumping has begun, the speed dial can be adjusted to gradually increase the speed to the maximum setting.
- 6.3.11** Calculate flow totalizer meter end reading by adding the desired water volume to the initial totalized flow reading; if using a meter that reads gallons, use the following conversions: 100 L=26.4 gal; 50 L=13.2 gal); continue filtration until that reading has been reached.
- If a flow totalizer is not available, measure the effluent flow rate and record the time and flow rate to estimate the total volume of water filtered by multiplying the cumulative filtration time and flow rate measurements.
 - Collect the filtered water in a 1 L graduated cylinder for 30 seconds.
 - Measure the volume of water in the cylinder and multiply by 2 to determine the flow rate per minute, and record on COC/data sheet (e.g., 900 mL X 2 = 1800 mL/min= 1.8 L/min).
 - Calculate the number of minutes required to filter desired volume of water (e.g., 100 L/1.8 L = 55.5 minutes).
 - Repeat this calculation every 5 minutes to accurately gauge how many minutes are required to filter desired volume of water.
- 6.3.12** During filtration, visually inspect the flow rate from the effluent tubing. Dramatic decreases in flow rate will indicate filter clogging, which can be due to water quality or entrapment of an object in the influent tubing or filter.
NOTE: *If clogging occurs (indicated by a dramatic decrease or stop in effluent flow), record the flow totalizer reading and slowly release the pump head to relieve pressure.*
- 6.3.13** Stop filtration after desired water volume is filtered, (or if filter has become clogged as described in the previous step) and record the final flow totalizer meter reading. A minimum of 50 L is recommended for surface water and 100 L for drinking water.
- 6.3.14** Add sodium thiosulfate solution if needed (see step 6.3.1).
-

NOTE: *The syringe method for addition of sodium thiosulfate can be used at this step instead of the pumping protocol described. Refer to step 6.2.11 for syringe protocol.*

- Open the bottle of pre-made sodium thiosulfate solution.
- Place the influent tubing into the sodium thiosulfate solution.
- Turn on the pump to draw the sodium thiosulfate solution into the filter. Continue until the sodium thiosulfate solution has been drawn through most of the influent tubing, but do not allow air to be pumped into the ultrafilter.
- Turn off the pump and *slowly* release lever on the pump head.

NOTE: *If possible, raising the end of the tubing in the water source above filter before releasing pump head will minimize backflush of sample out of the filter.*

6.3.15 Remove and discard all tubing from the ultrafilter (Figure 5). Screw a new kidney storage side cap on to Port 2 and place a new blood port storage end cap on Port 4 (Figure 6). Ensure all caps are firmly tightened.

6.3.16 Prepare the samples for any additional preservation requirements (Section 6.4).

6.4 Sample Preservation

Samples requiring preservation should be preserved as soon as possible to prevent degradation and maintain sample integrity. Preservation requirements are dependent on contaminants, type of sample, and method used to analyze the sample. Individuals unsure of proper preservation requirements should contact the receiving laboratory for assistance, as improper sample preservation can jeopardize the integrity of the sample.

Additional information on preservation requirements for specific contaminants can be found on the EPA's Environmental Sampling & Analytical Methods (ESAM) website- <https://www.epa.gov/esam>

6.4.1 Disinfection Reducing Agent - Water samples that have been treated or have tested positive for chlorine during field testing should be treated with sodium thiosulfate immediately after collection to dechlorinate the sample. The target final concentration of sodium thiosulfate in the sample is 0.1%.

6.4.2 Additional Preservation - Samples should be stored in a rigid cooler with ice or icepacks immediately after collection and during transportation to prevent biological degradation. Samples should be kept above 10 degrees Celsius, taking extra precaution to not freeze samples during transportation.

6.4.3 Holding Times - Holding time is the time from sample collection until initial analysis at the receiving laboratory. To maintain sample integrity, all samples should be shipped to a laboratory for analysis as soon as possible. Contact the receiving laboratory for recommendations if holding time is expected to exceed 24 hours after collection.

7.0 Sample Packaging and Shipment

7.1 Packaging and Shipping

After samples are collected, they are to undergo packaging procedures allowing for safe shipment to the receiving laboratory. Both low and high hazard samples should be packaged outside the area of contamination to preserve sample integrity.

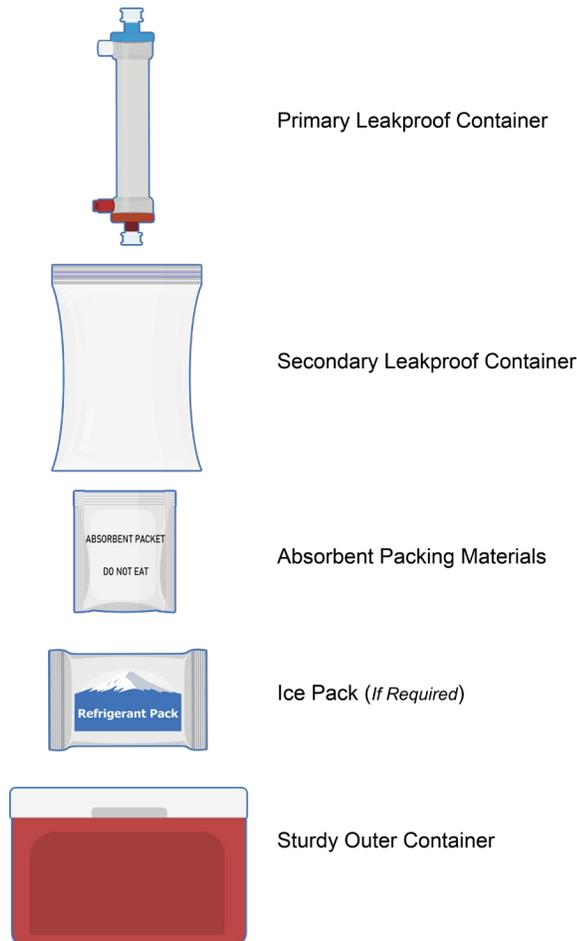


Figure 8. Shipping diagram

7.2 Low-Hazard Samples (Figure 8)

- 7.2.1 Ensure the shipping container is rigid enough to protect the sample.
- 7.2.2 Verify all samples are properly labeled, tightly sealed, and not leaking. Secure the tops of sample containers with clear tape or custody seals.
- 7.2.3 If using a cooler as a shipping container, ensure all the drain holes are properly sealed to prevent leakage.
- 7.2.4 Wipe the outside of the sample container with a bleach wipe before sealing in a clear resealable plastic bag.
- 7.2.5 Place ice packs or double bagged ice in container if sample requires preservation. If dry ice is used, ensure shipment is in accordance with 49CFR 173.217.
- 7.2.6 Add absorbent packing materials.

- 7.2.7 Place the chain of custody and any additional required paperwork in a clear resealable plastic bag and secure with tape to the underside of the shipping container lid.
- 7.2.8 Fill any extra space in cooler with protective wrap or packing material to prevent movement during transportation.
- 7.2.9 Secure the lid of the shipping container with tape.
- 7.2.10 Properly label the outside of the container with shipping information and handling instructions, (e.g., “This end up”).

7.3 High Hazard and Select Agent Samples

High hazard samples are to be triple packed, each container being leak-proof, contain absorbent material between each layer, and contained within a rigid final container. When sampling is conducted in high-hazard incidents, the sample packaging should be decontaminated in an area free of contamination if possible and packaged in a clean area. All samples identified as hazardous or potentially containing select agents should be packaged, labeled, and shipped by a trained and licensed HazMat technician or individual approved by the HHS Secretary or Animal and Plant Health Inspection Service (APHIS) Administrator, and should be in compliance with the Federal Select Agent Program and all applicable laws and regulations. Shipment of samples should be in accordance with the following domestic regulations: The Department of Transportation Hazardous Materials Regulations (49 CFR Parts 171-180) and United States Postal Service (USPS). 39 CFR Part 20.

8.0 Appendix

8.2 Federal Response Mandate

Safe water is a prerequisite for protection of public health, animal health, agriculture, food, and the environment. [Goal 2 of the National Biodefense Strategy of 2018](#) promotes measures to strengthen the resiliency of the water sector to prevent or contain water-borne disease outbreaks and improve the ability to detect biothreat agents in both finished and source waters. In addition to the responsibilities assigned to agencies, federal response and recovery activities are implemented under various legislations, regulations, and national policies. The Biological Incident Annex to the Response and Recovery Federal Interagency Operations Plans (2017) and the National Response Framework (NRF) provides details outlining the core capabilities and responsibilities of the CDC and EPA during a biological incident.

In addition to the response activities outlined by the NRF, EPA also has responsibilities relating to the safety and security of the water sector. Under the authorities of the Safe Drinking Water Act, and post-9/11 terrorist attacks Homeland Security Presidential Directive (HSPD)-7 (Critical Infrastructure Identification, Prioritization, and Protection), HSPD-10 (Biodefense for the 21st Century), Presidential Policy Directive (PPD)-21 (Critical Infrastructure Security and Resilience), and the latest National Biodefense Strategy of 2018, the EPA has been leading the water infrastructure protection mission in collaboration with the other federal partners. Under the [National Infrastructure Protection Plan \(NIPP\) Water and Wastewater Sector-Specific Plan for 2015](#), the Department of Health and Human Services (HHS) agencies including CDC, U.S. Food and Drug Administration (FDA), and Indian Health Service have been working closely with EPA. CDC, along with FDA have assisted EPA in defining CBR threats to drinking water. Additionally, since 2005 EPA-ORD's and CDC expert scientists and engineers have collaborated on research and development and both agencies participate in EPA ORD's Distribution System Research Consortium (DSRC).

8.3 Resources

Guidance for Building Field Capabilities to Respond to Drinking Water Contamination

(https://www.epa.gov/sites/production/files/2017-01/documents/field_capabilities_guidance_january2017.pdf)

Response Protocol Toolbox- Planning for and Responding to Drinking Water Contamination Threats and Incidents - Module 3: Site Characterization and Sampling Guide, EPA

(<https://www.epa.gov/waterutilityresponse/drinking-water-and-wastewater-utility-response-protocol-toolbox>)

Response Protocol Toolbox- Planning for and Responding to Drinking Water Contamination Threats and Incidents - Module 4: Analytical Guide, EPA

(<https://www.epa.gov/waterutilityresponse/drinking-water-and-wastewater-utility-response-protocol-toolbox>)

Sampling, Laboratory and Data Considerations for Microbial Data Collected in the Field, EPA, 2018

(https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=341832)

Validation of U.S. Environmental Protection Agency Environmental Sampling Techniques that Support the Detection and Recovery of Microorganisms, 2017

(https://www.epa.gov/sites/production/files/2015-01/documents/biosampling_validity_guidance.pdf)

Guidance on Choosing a Sampling Design for Environmental Data Collection, EPA, 2002

(<https://www.epa.gov/sites/production/files/2015-06/documents/g5s-final.pdf>)

Sample Collection Information Document for Pathogens--Companion to Selected Analytical Methods for Environmental Remediation and Recovery (SAM), EPA, 2017

(https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=339261)

Environmental Sampling and Analytical Methods (ESAM) Program 2017

(<https://www.epa.gov/esam>)

Technical Sampling Documents:

Drinking Water

Sampling Guidance for Unknown Contaminants in Drinking Water- EPA, 2017

(https://www.epa.gov/sites/production/files/2017-02/documents/sampling_guidance_for_unknown_contaminants_in_drinking_water_02152017_final.pdf)

Quick Guide To Drinking Water Sample Collection- EPA, 2016

(https://www.epa.gov/sites/production/files/2015-11/documents/drinking_water_sample_collection.pdf)

Potable Water Supply Sampling, EPA, 2013

(<https://www.epa.gov/quality/potable-water-supply-sampling>)

Surface Water

Surface Water Sampling, EPA, 2016

(<https://www.epa.gov/sites/production/files/2015-06/documents/Surfacewater-Sampling.pdf>)

Groundwater

Groundwater Sampling, EPA, 2013

(<https://www.epa.gov/sites/production/files/2015-06/documents/Groundwater-Sampling.pdf>)

Other Water Sampling

Industrial Stormwater Monitoring and Sampling Guide, EPA, 2009

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