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USEPA/USGS Sample Collection Protocol for Bacterial Pathogens in Surface Soil

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Version 1.0
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U.S. Environmental Protection Agency
Cincinnati, OH 45268

U.S. Geological Survey
St. Petersburg, FL

DISCLAIMER

The United States Environmental Protection Agency (Agency) through its Office of Research and Development funded and managed the research described here under a collaborative interagency agreement with United States Geological Survey (USGS) under IA# DW14957748. It has been subjected to Agency's administrative review and approved for publication. The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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ACRONYMS

ANSI	American National Standards Institute
CBRN	Chemical, biological, radiological and nuclear
CFR	Code of Federal Regulations
cm	Centimeter
COC	Chain of custody
FBI	Federal Bureau of Investigation
GPS	Geographic positioning system
HASP	Health and Safety Plan
HAZWOPER	Hazardous waste operation and emergency response
mL	Milliliter
OSHA	Occupational Safety and Health Administration
PPE	Personal protective equipment
QA	Quality assurance
QC	Quality control
SCP	Sample collection plan
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey

TRADEMARKS

Coleman®, Coleman Inc., Golden, CO, or
Excel®, Microsoft Corporation, Redmond, CA
FedEx®, Memphis, TN
Hype-Wipes®, Current Technologies, Crawfordsville, IN
Igloo®, Igloo Products Corp., Katy, TX
KimGuard® Kimberly-Clark, Irving, TX
Parafilm®, Pechiney Plastic Packaging Company, Chicago, IL
Pipetman® P200, Gilson, Inc., Middleton, WI
Pyrex®, Corning, Inc., Corning, NY
Tyvek®, Dupont, Wilmington, DE
UPS®, United Parcel Service, Atlanta, GA
Vegetronix™, Riverton, UT
Ziploc®, SC Johnson, Racine, WI

EXECUTIVE SUMMARY

This Sample Collection Procedure (SCP) describes the activities and considerations for the collection of bacterial pathogens from representative surface soil samples (0-5 cm). This sampling depth can be reached without the use of a drill rig, direct-push technology, or other mechanized equipment.

This procedure can be used in most soil types but is limited to sampling at or near the ground surface. This protocol has components for two different types of sampling applications: (1) typical sampling, when there is no suspicion of contamination (e.g., surveillance or background studies); and (2) in response to known or suspected accidental contamination (e.g., the presence of animal carcasses). This protocol does not cover sampling in response to a suspected bioterrorist or intentional release event.

Surface material is removed to the required depth (0-5 cm) and clean trowel or 50 ml sample tube is used to collect the sample. Sample containers are sealed, bagged, and shipped to the laboratory for analysis. Associated documentation, including a Field Data Log and Chain-of-Custody are also included in this document.

1.0 SCOPE AND APPLICATION

This protocol describes the procedures for collecting, handling, and shipping soil samples for the detection of naturally occurring bacterial microorganisms, specifically residing within the top 0-5 cm layer of soil. This top layer of soil is of interest since humans and grazing animals regularly come into contact with it and it may be a potential pathway for ingestion, inhalation, or dermal exposures.

This protocol has components for two different types of sampling applications: (1) typical sampling, when there is no suspicion of contamination (e.g., surveillance or background studies); and (2) in response to known or suspected accidental contamination (e.g., the presence of animal carcasses). Considerations specific to typical and suspected contamination sampling are noted throughout this document. This procedure does not cover sampling in response to a suspected bioterrorist or intentional release event, which require higher levels of biosafety and other considerations. See Figure 1 to determine which Level protocol to use for your purpose.

These are standard (i.e., typically applicable) operating procedures which may be varied or changed as required, dependent upon site conditions, equipment limitations or limitations imposed by the procedure. In all instances, the actual procedures used should be documented and described in an appropriate site report.

This document is not intended to be used as a substitute for a site-specific Quality Assurance Project Plan (QAPP) or a detailed sampling plan. This document is intended to be used as a reference for developing site-specific QAPPs and sampling plans.

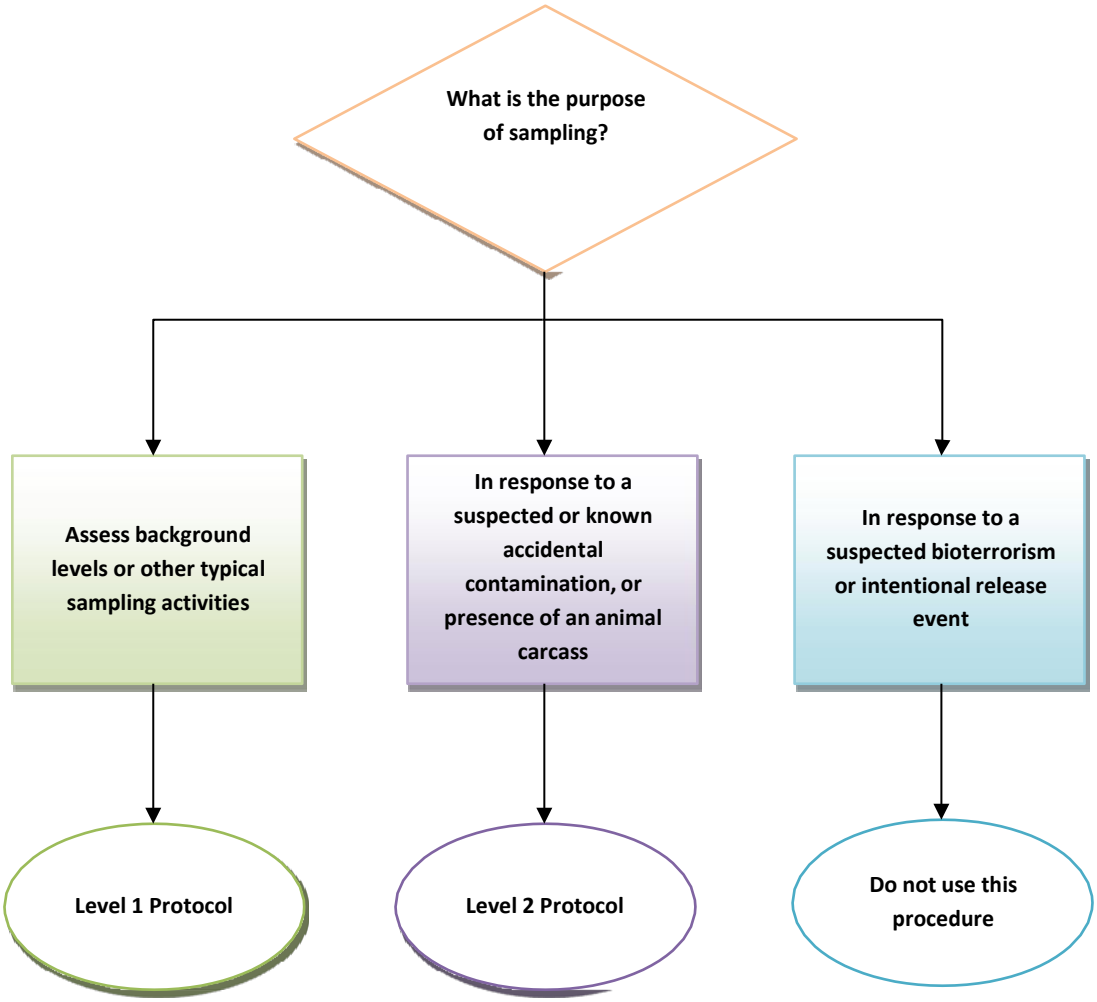

Figure 1. Flowchart for determining applicable soil collection protocol

2.0 PROCEDURE SUMMARY

This sampling protocol is based on the procedures used by U.S. Geological Survey (USGS) during its North American Geochemical Landscapes Pilot Studies (Smith, et al, 2009). Sterilized 50 mL tubes and aseptic technique are used for collection of 0-5 cm depth soils. This soil surface is the material with which humans and animals regularly come into contact during the course of a normal day's activity, and may be an exposure pathway to ingestion, inhalation, or dermal contact. The ***Level 1 Protocol*** is the sampling procedure for surveillance or background studies when there is no suspected intentional release and no animal carcasses present, or when the sampling purpose is not to investigate a suspected contaminated site. Level 1 Protocol can be performed with either one or two sampling personnel. The ***Level 2 Protocol*** is used when there is suspected or known accidental contamination, or the presence of animal carcasses, and refers to the additional factors (i.e., personal protective equipment) that must be utilized in conjunction with the standard protocol instructions. Level 2 Protocol sampling must be conducted using a two-person sampling team due to the PPE required. See Sections 6.0 and 10.0 for more information.

3.0 INTERFERENCES

When sampling soil, there are two primary potential issues to consider: cross contamination of samples and improper sample collection. Cross contamination issues can be eliminated or minimized through the use of dedicated sampling equipment. If this is not possible or practical, then decontamination of sampling equipment is necessary. Use of aseptic technique and appropriate personal protective equipment (PPE) such as disposable gloves and booties can help reduce contamination. Improper sample collection can involve disturbance of the matrix resulting in compaction of the sample, consequently producing variable, non-representative results.

Highly diverse substances in soil, which may include non-target microorganisms, organic matter, disinfection agents used at the site, and certain trace elements, can interfere with the analytical process. Communication with the analytical laboratory helps to identify and to avoid potential contaminants most likely to interfere with laboratory analysis.

Soil samples can contain rocks or sharp objects that can puncture containers, resulting in sample contamination from compromised integrity. These objects are removed or avoided during collection of these sample types. Soils and sediments are particularly rich in non-target microorganisms (e.g., bacteria and fungi) that can result in a sample with overgrowth from other microorganisms. The laboratory may need to use techniques to selectively isolate the target microorganism from the matrix material.

4.0 EQUIPMENT AND SUPPLIES

NOTE: Sampling kits containing the following supplies should be assembled prior to traveling to the field to collect samples. In addition, all sample vials should be labeled with sample ID stickers before sampling. *English units of measure are used for products and supplies described in those terms by suppliers.*

For **all levels** of sampling:

- ☐ Nitrile or vinyl gloves, non-powdered
- ☐ Surgical masks (i.e., N95 masks)[1]
- ☐ Safety goggles or safety glasses
- ☐ Disposable booties[1]
- ☐ Stainless steel trowels, one per sample site – thin bladed (will fit into the 50 ml tube) (Fisher Scientific, Waltham, MA, Part No. S02603; handle part no. #S02602; or equivalent)
- ☐ Alcohol wipes (70% ethanol)
- ☐ Bleach wipes (10% Hype-Wipes® or equivalent)

[1] Or as determined by the site/event-specific Health & Safety Plan or the sampling lead.

- ☐ Small zip-top bags (Ziploc® sandwich size ~6”x6” or equivalent)
- ☐ Benchtop bag holder (bag rack) – one per sample per site (Fisher Scientific, Part no. 01815-2 or equivalent) – (optional for one person collection)
- ☐ 50 mL pre-sterilized sample tubes with caps (Fisher Scientific, Part no. 06-443-19; or equivalent)**,** bagged in extra small zipper-seal bags (Ziploc snack size ~3”x5” or equivalent)
- ☐ GPS unit (standalone or smart phone app)
- ☐ Soil moisture meter (Vegetronix™, Part no. VG-Meter-200 basic or equivalent) Soil thermometer (Fisher Scientific, Part no. 14-648-46 or equivalent)
- ☐ Soil pH meter
- ☐ Field Data Sheets – one per sample site (Appendix A) Sample ID labels (pre-printed)
- ☐ Chain of custody forms (Appendix B)
- ☐ Custody seals (Fisher Scientific #05-719-337 or equivalent) Permanent markers
- ☐ Waterproof pens
- ☐ Precut Parafilm® strips (approx. 1.5” wide x 3” long)
- ☐ Shipping supplies (gel ice packs, paper or bubble shipping-wrap, packing tape) Cooler, durable plastic chest style (i.e., Coleman® or Igloo®)
- ☐ Cooler, small 6-pack size
- ☐ Paper towels
- ☐ Heavy duty garbage bags
- ☐ Large zip-top bags (Ziploc 1 gallon size ~11” x 11” or equivalent) KimGuard® Sterilization Wraps (cat# 19-135-120 or equivalent)
- ☐ Sterile Agvise Laboratories (Northwood, ND) soil for blanks or equivalent

Additional PPE for **Level 2 Sampling** (see Section 8.0, Health & Safety):

- ☐ Tyvek® suit
- ☐ Chemical, biological, radiological and nuclear (CBRN)-certified full face respirator [1]

5.0 SAMPLE DOCUMENTATION

Legibility and permanence are to be maintained for all documentation produced in collecting and processing samples. If errors are made, either the document error is struck out using a single line and initialed and dated, or it is rewritten, checked for accuracy, initialed and dated, and attached to the original for record keeping.

Pens and markers should be of black indelible ink capable of writing on damp labels and containers. Pens and markers taken to an area of known contamination should be discarded with waste or with used disposable PPE.

Logbooks, forms and reports should be assembled and maintained as permanent records. If taken to the sampling area, care should be taken to prevent contamination of the records. If

required to be taken into a known area of contamination, take only a blank copy of the form or page of the logbook. Once out of the contaminated area, they are to be rewritten into the original permanent records and verified as transcribed correctly once outside of the zone.

Written documents are generated and maintained as the primary records of the sampling event. However, information also may be entered into an electronic record during or, as soon as practical, following sample collection. See Appendix A for the Field Data Sheet to use with this protocol.

5.1 SAMPLE IDENTIFICATION AND LABELING

A unique sample ID is assigned to each sample and is maintained throughout the analysis process via an Excel® spreadsheet, sample ID labels and field data sheets. Prior to sample collection, label the tubes with the supplied labels and record date, time and sample ID number on the field sheets. If available to the involved laboratories, bar-coded labels may be used.

Field records/sample documentation are completed at the time each sample is collected (Appendix A), and should include the following information:

- Location of sample collection (i.e., general description)
- Unique sample identification number
- Date and time of sample collection
- Name of sample collector(s) Pertinent field data, if available (e.g., weather, soil type/condition, presence of carcasses**)**

5.2 CHAIN-OF-CUSTODY

When transferring samples, the persons transferring and receiving samples must sign and record the date and time on the chain of custody (COC) form. Custody transfers should account for each individual sample, although samples may be transferred as a group. Every person who takes custody must complete the appropriate section of the COC form. The sampler is responsible for properly packaging and dispatching samples to the analytical laboratory. This responsibility includes filling out, dating, and signing the appropriate portion of the COC form.

All packages sent to the sample management facility are accompanied by a COC form and other pertinent forms, such as field records/sample documentation forms. A copy of these forms should also be retained by the sampler (either carbon copy or photocopy). Forms are placed inside a waterproof, zip-top bag in the shipping container. If the container is being shipped by a courier service (e.g., FedEx®, UPS®), the courier does not have to sign the COC form. The COC form should also include the shipping label number(s).The chain is presumed to be maintained and evidenced by the courier shipping receipt. The shipping receipt is retained by sampling personnel for the permanent record.

5.3 CUSTODY SEALS

A Parafilm strip is placed over the seal of each sample container, and a custody seal is placed over the Parafilm to ensure the sample has not been opened or tampered with after collection and packaging. Two custody seals are also affixed to the shipping container/cooler, on opposite ends of the cooler, so that the cooler cannot be opened or hinges removed without breaking the seals.

6.0 ASEPTIC TECHNIQUE

Aseptic technique refers to procedures by which samples are collected without infecting the worker or contaminating the samples or the environment. Good aseptic technique is the first and most important step in ensuring consistent and accurate sampling results. Proper training in aseptic technique should be given to samplers.

The sampler(s) holds primary responsibility for conducting activities using aseptic technique. Level 1 Protocol activities may be performed with two-person sample teams or a single sampler, but Level 2 Protocol must be completed using a two-person team. For a two-person sampling team, aseptic sampling may use the designation of collector (sampler) and an assistant (assistant sampler or facilitator) who coordinate roles for sample collection, packaging, and documentation. The assistant sampler is responsible for providing the sampler with the appropriate tools and facilitating collection. Assistant duties may include, but are not limited to: opening and handing materials to the sampler as required, including sample collection containers, gloves, trowels, and packaging materials, performing any administrative functions including communication and photography (FBI Laboratory Publication, Handbook of Forensic Services 2007), as well as ensuring the Field Data Sheet is filled out. With a single person collecting samples, aseptic technique can be achieved with careful order of activities, such as filling out the data sheets prior to sampling and avoiding cross-contamination from the different supplies, documents, and equipment. Regardless of the number of personnel on the sampling team, the sampler should be the only person to come in contact with the environmental sample. The sampler is also responsible for signing the final chain-of-custody form.

A critical element of aseptic sampling is the use of a new pair of non-powdered, nitrile or vinyl examination gloves for each sample collected. This layer of gloves is in addition to the gloves that are part of standard PPE ensemble (that is, team members should have two or more layers of gloves on) for each sample collected. Given that the environment in which the samples are being collected is not sterile, the use of sterile gloves is not necessary. The gloves must always be changed between samples.

Care must be taken when removing gloves as well, and a careful procedure for removing gloves is necessary to avoid contamination of personnel hands, samples, or gloves. The following steps describe aseptic technique for doffing gloves between collection of samples and other activities, as described in Section 9.0:

1. Grasp one glove near the wrist with your thumb and forefinger.
2. Pull the glove toward your fingertips, allowing it to turn inside-out.

3. Pull the glove the rest of the way off your hand and continue to hold it in your other, still-gloved hand. Be careful not to touch anything with the freshly doffed hand.
4. Slide a finger of the now clean hand under the wrist cuff of the other glove.
5. Pull the glove toward your fingertips, allowing it to turn inside-out.
6. As the second glove is pulled off, it will encase the first glove.
7. Remove your hand completely and dispose of the gloves in a waste bag, touching only the uncontaminated side (the side that was originally touching your hand).

The following practices can be used as additional guidelines for collecting samples:

- Keep containers open a minimum amount of time, especially after the sample is collected.
- Hold containers at a 45 degree angle from vertical.
- Hold open containers away from sources of contamination (e.g., blowing air, other possibly contaminated objects).
- Do not touch the inside of sterile containers.
- Once a container is filled, do not touch the contents.
- Change gloves between samples or if your gloves become contaminated.
- Change booties in between sampling sites.
- Work as quickly as is consistent with careful technique.
- Keep the general work area as clean and uncluttered as possible.

7.0 QUALITY ASSURANCE/QUALITY CONTROL

Sample collectors should refer to the site-specific sample collection plan (SCP) to determine the kind and number of quality control (QC) samples that should be collected and procedures that should be performed. In some cases, additional samples or sample volume are needed to support laboratory QC sample analysis (e.g., matrix spikes). Because QC samples may be shipped to the laboratory as either known QC or blind samples, sample collectors should refer to the SCP to determine how QC samples are to be labeled for transport to the laboratory. The following sections cover different types of QC considerations that may be detailed in the site-specific sample collection plan, as deemed appropriate and applicable by the sample collection lead.

7.1 FIELD BLANKS

Field blanks are prepared in the laboratory before traveling to the site by filling a 50 ml sample tube with blank matrix material (i.e., standardized, sterile soil) and capping it. See Appendix C for the procedure for soil sterilization. One field blank is prepared for each sampling site.

Field blanks are used to monitor contamination that might be introduced into samples during sample collection, filtration, or preservation. Field blanks are typically used midway through collection at each sampling location. The field blank is submitted to the laboratory for analysis with the collected samples.

At each sampling site, typically halfway through the collection, open the cap to the field blank and allow the media to be exposed for a few seconds. Re-cap the tube, and seal with Parafilm. Ship the field blanks back to the laboratory with the samples.

7.2 Trip Blanks

Trip blanks are used to monitor contamination that might be introduced into samples during field handling and transport. Trip blanks are prepared in the laboratory, taken to the sampling site, and shipped back to the laboratory, unopened, with the samples. The media in trip blanks does not get exposed to the sampling procedures, unlike field blanks.

Trip blanks are prepared by filling a 50 ml sample tube with blank matrix material (i.e., standardized, sterile soil), capping the tube, and sealing with Parafilm. See Appendix C for the procedure for soil sterilization in the lab. One trip blank is prepared for each shipping cooler or container.

7.3 Field Replicates

Field replicates are samples collected in the same manner, location, and time as an initial sample. Sample collectors must ensure that sample replicates are as equivalent in content and mass or volume as possible. Variations can affect representative QC evaluations. In the case of a soil sample, the space of the initial sample should be enlarged to allow for at least twice the volume of sample to be taken. A field replicate is used to measure sample heterogeneity, sample collection methodology, and analytical procedures. The replicate sample is handled and documented in the same manner as the initial sample.

Number and frequency of replicates are determined by the site-specific plan. If multiple samples are taken from a single sampling point, they should be labeled as such and homogenized in the laboratory.

7.4 Background Samples

If samples are being taken from a suspected contaminated site, background samples should be collected from a known uncontaminated area to allow for the determination of natural or “background” biological concentrations for comparison.

7.5 Equipment

All equipment that is used to measure or analyze samples in the field requires calibration, routine maintenance, and at least annual standardization/verification or more frequently according to the manufacturer’s specifications. This equipment is calibrated following procedures included in the manufacturer’s product/equipment manual or performed in the laboratory. Equipment used to obtain volumetric sample measurements must be certified to appropriate volume specifications.

Analytical equipment used in the field, which includes pH meters, thermometers, soil moisture meters, and GPS units, requires calibration to ensure sample analyses are accurate. Calibration should be performed in the laboratory prior to sending equipment into the field, completed according to manufacturer's specifications, and calibrated as close to use as possible. Changes in elevation or large temperature variances since last calibration or standardization may require a new calibration or standardization.

7.6 SAMPLE CONTROL

Once samples have been collected, they must be maintained under controlled custody through shipment to an analytical laboratory. This control is required to ensure that samples are not compromised and that analytical data generated are representative of site conditions.

7.6.1 SAMPLE CUSTODY REQUIREMENTS

- Keep samples in an area where they can be observed by an authorized person or are under lock-and-key to prevent tampering
- Maintain samples in the same configuration or condition in which the samples arrived from the sampling site (e.g., containers sealed) until additional procedures are required

7.6.2 SAMPLE TRACKING

- As samples are transferred from collection through processing, packaging, and shipment, record sample progress.
- The person(s) performing each step is required to record their initials or signature on the label, sample tracking log, chain of custody form, and any other document associated with the sample to ensure the condition of the sample at that point of sample progression.

All sampling personnel are required to perform sample collection, processing, and packaging activities in a manner that does not compromise the integrity of the samples or the requirements associated with the sampling event.

- Follow documented procedures and adhere to requirements.
- Notify supervisor of problems or concerns.
- Adhere to all requirements regarding documentation of activities, conditions, observations, and measurements.

8.0 HEALTH AND SAFETY

Individuals collecting environmental samples place themselves at risk of exposure to soilborne pathogens. Sample collection personnel work within suspected contaminated environments and their sampling activities may mobilize and even cause re-aerosolization of soilborne pathogens. Therefore, precautions to protect investigators should be implemented prior to conducting an environmental sampling response. A health and safety plan (HASP) must be

developed that includes the following elements: emergency contact information (local hospital with maps), job hazard analysis, medical monitoring, training, decontamination, standard operating procedures, and appropriate selection and use of PPE. Elements of a comprehensive medical program include medical countermeasures, medical screening, monitoring, and follow-up care. These recommendations can be found in a number of separate guidance documents that are referenced below. These documents should be reviewed prior to developing and implementing a HASP. At a minimum, all sampling team members should be trained in Occupational Safety and Health Administration (OSHA) requirements for hazardous waste operations and emergency response (HAZWOPER) at 29 CFR 1910.120 or 29 CFR 1926.65 and have current medical screening. All sampling team members must also review and sign the HASP before engaging in any field activities.

HASPs will vary depending on the site, the sampling phase (e.g., site assessment for research purposes or for cleanup actions) and the responsible organization. The purpose of these plans is to ensure maximum protection to workers, the environment, and surrounding communities, in a way that is consistent with requirements needed to perform operational activities. At a minimum, HASPs should include instructions and guidelines regarding:

- Names, positions, and contact information of key personnel and of health and safety personnel
- Site- or event-specific risk assessment addressing sample collection activities and the potential pathogen exposures
- Training requirements
- Personal protective equipment (PPE) on site and usage requirements
- Medical screening requirements (maintain confidential documents properly and securely)
- Site or event control
- Emergency contact information, including location and phone numbers of local hospitals
- Emergency response plan, containing off-site emergency contact information such as local hazardous materials response teams or additional trained rescue personnel (29 CFR 1910.38)

Relevant safety and health guidance documents are:

- Protecting Investigators Performing Environmental Sampling for *Bacillus anthracis*: Personal Protective Equipment. (NIOSH website accessed Dec 2013) (http://www.cdc.gov/niosh/nas/rdrp/appendices/chapter6/a6-53.pdf)
- Centers for Disease Control, National Institute for Occupational Safety and Health. 2009. Recommendations for the Selection and Use of Respirators and Protective Clothing for Protection Against Biological Agents. (http://www.cdc.gov/niosh/docs/2009-132/default.html)

8.1 PPE FOR LEVEL 1 SAMPLING: DISPOSABLE RESPIRATOR

An N95 respirator (which filters 95% of airborne particulates) is a respiratory protective device designed to achieve a very close facial fit and very efficient filtration of airborne particles. Respirators that are at least as protective as an N95 respirator are recommended for protecting workers from exposure to particles or pathogens during activities such as sampling that may generate aerosolized particles or pathogens. Information on proper fit is available per the manufacturer's instructions.

N95 respirators are labeled as "single use", disposable devices. If your respirator is damaged or soiled, or if breathing becomes difficult, it should be removed, discarded properly, and replaced with a new respirator. At a minimum, new N95 respirators should be used at the start of each field day. All used N95 respirators are placed in sealed garbage bags for autoclaving and/or appropriate disposal (Section 11) immediately after leaving a potentially contaminated area.

8.2 PPE FOR LEVEL 1 SAMPLING: EYE PROTECTION

Safety goggles or safety glasses protect the eyes from debris, dust, aerosolized particles, and environmental hazards. Eye protection must be worn while working and sampling in the field. Consideration should be given to comfort and fit. Poorly fitting eye and face protection will not offer the necessary protection. Prescription safety spectacles should be fitted only by qualified optical personnel. Devices with adjustable features should be fitted on an individual basis to provide a comfortable fit that maintains the device in the proper position. Eye protection shall: provide adequate protection against the particular hazards for which they are designed; be of safe design and construction for the work to be performed; be reasonably comfortable when worn under the designated conditions; fit snugly and not unduly interfere with the movements of the wearer; be durable; and be capable of being disinfected and/or cleaned (OSHA).

Eye and face protection must comply with the American National Standards Institute, ANSI Z87.1-1989 standard if purchased after July 5, 1994 or ANSI Z87.1-1968 if purchased before July 5, 1994.

8.3 PPE FOR LEVEL 2 SAMPLING: FULL FACE RESPIRATOR

When used correctly, a respirator can selectively reduce exposure from aerosolized particles. A full face respirator consists of a face piece held to the wearer's head with straps, a cloth harness, or some other method. The face piece of the respirator covers the entire face, and may be an air purifying respirator (sometimes referred to as a gas mask, and it works as the wearer breathes) or a powered air purifying respirator (uses a powered fan which forces air through the cartridges and supplies filtered air to the wearer), which may provide additional protection and ease the strain on the sampler. Typically one or two cartridges attach securely to either type of mask, which has built into it a corresponding number of valves for inhalation and one for exhalation. Proper

filtering cartridges must also be selected based on specific contaminants at the sampling site. At a minimum a P100 cartridge should be used. Additional requirements may be necessary based on contaminants of concern at the sampling site. Cartridges and other requirements must be specified in the site-specific sampling plan by your health and safety manager.

All respirators require training and fit testing prior to use in the field. Training is extremely important in regard to the storage, maintenance, use, and discarding of the respirator. This information is provided by the supplier of the respirator (i.e., seller, distributor, or manufacturer) and your organization's health and safety personnel.

8.4 PPE FOR LEVEL 2 SAMPLING: PROTECTIVE CLOTHING

Protective clothing such as disposable suits (Tyvek®) must be worn to protect the body and to eliminate transfer of contamination away from the sample site. Disposable shoe coverings (i.e., booties) must also be worn.

All disposable protective clothing is placed in sealed garbage bags for autoclaving and/or appropriate disposal immediately after leaving a potentially contaminated area.

Required protective clothing, as well as proper donning and doffing techniques are specified in the site-specific sampling plan and the HASP.

8.5 PPE FOR ALL LEVELS OF SAMPLING APPLICATIONS: DISPOSABLE GLOVES

Disposable gloves made of nitrile (or equivalent) to protect hands from contact with potentially contaminated soils are worn. It is recommended to wear two pairs, so the outer layer of gloves are changed between the collection of each sample to prevent cross-contamination. Outer gloves are also disposed of whenever they become visibly contaminated or the integrity of the gloves is compromised. Outer gloves should be worn when removing all protective clothing and then removed and discarded before removing the respirator with clean, inner gloves. After all work with potentially infectious materials is completed, gloves are removed and disposed of (Section 11), and hands washed using antibacterial gel.

9.0 SAMPLE PREPARATION, COLLECTION, AND SHIPPING

The following section provides instructions for both Level 1 Protocol (one person sampling; 9.1) and Level 2 Protocol (two person collection team; 9.2). The activities in the subsequent sections (9.1.1 – 9.1.4 or 9.2.1 –9.2.4) are to be conducted for each sampling location. The collection steps (9.1.2 or 9.2.2) should be repeated for each sample taken for the sampling location. Specifics of the number of samples, locations, team members, and other considerations are noted in the site-specific sample collection plan for the sampling incident or need.

9.1 One Person Sampling Team (Only for Level 1 Sampling)

When possible, drive the vehicle right up to the sampling location and leave the cooler, bag rack, and sampling supplies in the vehicle to avoid contamination. Work from the trunk or truck bed. If this is not feasible, carry the sample kit to the site and place supplies on the Kimguard wrap to protect from contamination.

NOTE: Given that the environment in which the samples are being collected is not sterile, the use of sterile gloves is not necessary. However, gloves must be changed between samples.

9.1.1 Soil Readings

1. Spread the Kimguard wrap on the ground, adjacent to the sample location
2. Fill out initial sections of the field data sheet (through item 6).
3. Record the GPS location of the sample site on the field data sheet.
4. Don gloves (base pair + one) and mask and safety glasses/goggles.
5. Place the soil moisture meter, thermometer, and pH meter into the soil by pushing each meter's sensor just below the soil surface (Figure 2). If the soil is compacted use the trowel to break it up to allow placement of the sensors. In this case,.lightly compact the loosened soil by pressing the soil over the sensors.
6. Place the moisture meter body on the Kimguard wrap (Figure 2) to keep it from making contact with the ground.
7. Leave the sensors in the ground while soil samples are collected.
8. Doff top pair of gloves and dispose in a garbage bag.

Figure 2. Soil meter sensor placement of pH meter (red unit) and moisture meter (gray unit). Both models can also take temperature readings.

9.1.2 COLLECTION

1. Don fresh gloves over base pair[2].
2. Prepare a sample bag on the bag rack as shown in Figure 3, placing the rack on the Kimguard wrap or in the bed or trunk of vehicle.
3. Open the zip-top bag containing a pre sterilized 50 mL sample tube and remove the tube.
4. Open the tube, placing the cap open side down on the Kimguard wrap or holding it carefully in your hand. Avoid touching the inside of the cap.

Figure 3. Bag rack preparation for one person sampling.

5. Use the 50 mL tube to scoop up the soil from 0-5 cm, and/or use a clean trowel and fill the tube to at least the 40ml mark on the tube (Figure 4). If large pebbles/rocks, roots, leaves, or twigs are present, scrape them aside using the trowel to minimize carry over into the sample tube. If soil is compacted, use the trowel to break it up.
6. Place the dirty trowel on the ground until decontamination steps.

Figure 4. Soil collection using tube (left) and trowel (right).

[2] If multiple samples are to be taken per site, don multiple gloves over base pair.

7. Cap sample tube with lid.
8. Seal the lid with a Parafilm strip (Figure 5). Place a custody seal over the Parafilm strip.
9. Wipe down tube with an alcohol wipe, taking care to avoid the label.
10. Place sample tube into the prepped bag on the rack.
11. Doff top pair of gloves and discard in the trash bag. Don fresh gloves over base pair.

Figure 5. Sealing the sample tube cap with Parafilm.

12. Remove the sample bag (containing the collected sample) from the rack and seal.
13. Leave the bagged, sealed sample on the Kimguard wrap until all samples are collected at the site.
14. Doff top pair of gloves and discard in trash bag. Don fresh gloves over base pair.
15. Repeat Steps 3-15 of this section for remaining samples at site.
16. Doff top pair of gloves and discard in the trash bag.
17. Place the bagged samples into the large zip-top bag and seal.
18. Record soil moisture, temperature, and pH readings from meters.
19. Ensure all remaining sections of the field data sheet are completed for the site, including sample ID numbers.
20. Wipe down the large zip-top bag with a bleach wipe
21. Place the zip-top bag into a second zip-top bag to carry samples back to the vehicle (if sampling a distance from the vehicle). Remove the secondary bag before placing the sample bag into the cooler.

9.1.3 EQUIPMENT DECONTAMINATION

1. Doff top pair of gloves and discard in the trash bag. Don fresh gloves over base pair.
2. Remove the soil meters from the ground and wipe the sensors with alcohol wipes to remove any soil particulates.
3. Wipe the trowel with alcohol wipes to remove any soil particulates. Place in a zip-top bag (the same bag is used at all sites to collect dirty trowels).
4. Wipe the sensors with a 10% bleach wipe, place in an unused large Ziplock bag, seal, and allow for at least 30 minutes of contact time with residual bleach during travel to next sampling location. If travel time is less than 30 minutes, wait for 30 minutes to elapse before collecting samples at the next site.
5. Doff top pair of gloves and discard in the trash bag. Don fresh gloves over base pair.
6. Transport the sensors in the sealed bag. Ensure that sensors are wiped down with a dry paper towel prior to sampling at the next site.

9.1.4 PACKAGING AND SHIPPING

1. Return to the vehicle with bagged samples, decontaminated supplies, and garbage.
2. A large, heavy-duty trash bag should be placed in the cooler. Place the large zip-top plastic bag inside the bag in the cooler. Use only self-contained gel ice packs. NOTE: ensure the samples are not placed directly on the ice packs to avoid freezing the samples. Use the paper or bubble shipping-wrap to stabilize the contents of the cooler. Tie the outer heavy duty trash bag before closing the cooler.
3. Develop chain of custody form, sign, and place in the cooler.
4. Close the cooler lid.
5. Doff top pair of gloves and discard in the trash bag
6. Place two custody seals on the cooler (in a manner so that the cooler cannot be opened without breaking the seal) and sign and date the seals.
7. Wipe down the cooler with a bleach wipe.
8. Doff base pair of gloves and discard in the trash bag

Move to the next sampling location and repeat Sections 9.1.1 – 9.1.4 for each sampling location, as determined by the sample collection plan. Be sure to wipe the sensors down prior to sampling at the next site. Dispose of wipe in garbage bag.

At the end of the sampling effort, seal the garbage bag; place it into a second garbage bag, and seal. See Section 10 for instructions on disposal of sampling waste in the garbage bag.

9.2 TWO PERSON COLLECTION TEAM (ONLY FOR LEVEL 2 COLLECTION)

When possible, drive the vehicle right up to the sampling location and leave the cooler, bag rack, and sampling supplies in the vehicle to avoid contamination. Work from the trunk or truck bed. If this is not feasible, carry the sample kit to the site and place supplies on the Kimguard wrap to protect from contamination.

The responsibilities of the "Collector" and "Assistant" are specified here. See Section 6.0 on Aseptic Technique for more information on Collector and Assistant.

Given that the environment in which the samples are being collected is not sterile, the use of sterile gloves is not necessary. However, gloves must be changed between samples.

Sampling personnel must don PPE (Tyvek suit, respirator, gloves, booties) at the vehicle before proceeding with the following sampling steps.

9.2.1 SOIL READINGS

1. Arrive at sampling location already wearing all PPE (Tyvek suit, respirator, gloves [base pair + one], booties).
2. Assistant: Fill out initial sections of field data sheet.
3. Collector: Spread the Kimguard wrap on the ground, adjacent to the sampling location.
4. Assistant: Place the thermometer, pH, and the soil moisture meters into the soil by pushing each meter's sensor just below the soil surface (Figure 2). If the soil is compacted use the trowel to break it up to allow placement of the sensors. In this case, lightly compact the loosened soil by pressing the soil over the sensors.
5. Assistant: Place the moisture meter body on the Kimguard wrap to keep it from making contact with the ground.
6. Leave the moisture meter, pH, and thermometer sensors in the ground while soil samples are collected.
7. All: Doff top pair of gloves and dispose in a garbage bag.

9.2.2 COLLECTION

1. All personnel don fresh gloves over base pair[3].

2. Assistant: Open the bag containing the 50 mL tube, being careful not to touch the tube. Push the tube up to the bag opening to present to the Collector to take (Figure 6).
3. Collector: Take the 50 mL tube from the Assistant, avoiding touching the bag.
4. Collector: Open the tube, placing the cap open side down on the Kimguard wrap or holding it carefully in your hand. Avoid touching the inside of the cap.

Figure 6. Assistant presenting the sample tube to the Collector to take.

5. Collector: Use the 50 mL tube to scoop up the soil from 0-5 cm, and/or use a clean trowel and fill the tube to at least the 40ml mark on the tube (Figure 4). If large pebbles/rocks, roots, leaves, or twigs are present, scrape them aside using the trowel to minimize carry over into the sample tube. If soil is compacted, use the trowel to break it up.
6. Collector: Cap sample tube with lid.
7. Collector: Seal the lid with a Parafilm strip (Figure 5).
8. Place a custody seal over the Parafilm.
9. Collector: Wipe down tube with an alcohol wipe, taking care to avoid the label.
10. Collector: Place sample tube into small zip-top bag, held open by Assistant, and Assistant seal the bag.
11. Assistant: Place the sample bag into the large zip-top bag.
12. All personnel doff top pair of gloves and discard in the garbage bag. Don fresh gloves over the base pair.
13. Repeat Steps 2-12 of this section for remaining samples at site.

[3] If multiple samples are to be taken per site, don multiple gloves over base pair.

14. Assistant: Seal the large zip-top bag.
15. Assistant: Wipe down the large zip-top bag with a bleach wipe
16. Assistant: Place the zip-top bag into a second zip-top bag to carry samples back to the vehicle (if sampling a distance from the vehicle). Remove the secondary bag before placing the sample bag into the cooler.
17. All personnel doff top pair of gloves and discard in the trash bag. Don fresh gloves over base pair.
18. Assistant: Record soil moisture, temperature, and pH readings from meters.
19. Assistant: Ensure all remaining sections of the field data sheet are completed for the site, including sample ID numbers.

9.2.3 EQUIPMENT DECONTAMINATION

1. Collector completes this equipment decontamination process while the Assistant completes the packaging and shipping section.
2. Doff top pair of gloves and discard in the trash bag. Don fresh gloves over base pair.
3. Remove the soil meters from the ground and wipe the sensors with alcohol wipes to remove any soil particulates.
4. Wipe the trowel with alcohol wipes to remove any soil particulates. Place in a zip-top bag (same bag is used at all sites to collect dirty trowels).
5. Wipe the sensors with a 10% bleach wipe, place in an unused large Ziplock bag, seal, and allow for at least 30 minutes of contact time with residual bleach during travel to next sampling location. If travel time is less than 30 minutes, wait for 30 minutes to elapse before collecting samples at the next site.
6. Doff top pair of gloves and discard in the trash bag. Don fresh gloves over base pair.
7. Transport the sensors in the sealed bag. Ensure that sensors are wiped down with a dry paper towel prior to sampling at the next site.

9.2.4 PACKAGING AND SHIPPING

1. Return to the vehicle with bagged samples, decontaminated supplies, and garbage.
2. A large, heavy-duty trash bag should be placed in the cooler. Place the large zip-top plastic bag inside the bag in the cooler. Use only self-contained gel ice packs. NOTE: ensure the samples are not placed directly on the ice packs to avoid freezing the samples. Use the paper of bubble shipping wrap to

stabilize the contents of the cooler. Tie the outer heavy duty trash bag before closing the cooler.

3. Develop chain of custody form, sign, and place in the cooler.
4. Close the cooler lid.
5. Doff top pair of gloves and discard in the trash bag
6. Place two custody seals on the cooler (in a manner so that the cooler cannot be opened without breaking the seal) and sign and date the seals.
7. Wipe down the cooler with a bleach wipe.
8. Doff base pair of gloves and discard in the garbage bag.

Move to the next sampling location and repeat Sections 9.2.1 – 9.2.4 for each sampling location, as determined by the sample collection plan. Ensure fresh PPE (Tyvek suit, gloves, and booties) is donned for each sampling location. Be sure to wipe the sensors down prior to sampling at the next site. Dispose of wipe in garbage bag.

At the end of the sampling effort, seal the garbage bag; place it into a second garbage bag, and seal. See Section 10 for instructions on disposal of sampling waste in the garbage bag.

10.0 WASTE MANAGEMENT

The solutions and reagents used in this protocol pose little threat to the environment when managed properly.

All items used in the collection of samples in the field must be treated as contaminated and should be disinfected with bleach wipes. Alternately, they may be disposed of after use. All disposable materials is then be placed in the garbage bag. The coolers can be reused for other shipments. Do not stockpile empty containers. Clean and disinfect sampling equipment and supplies as soon as possible.

All waste resulting from sampling should be double bagged as noted in the Packaging and Shipping sections, placed in a separate cooler from the samples (6-pack size), and shipped back to the lab for disposal/decontamination.

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APPENDIX A: FIELD DATA SHEET

NOTE: *While field data sheets may vary within agencies, this format is provided for when this sample collection protocol is used. Using this field data sheet ensures consistency of field data collected, and ensures that all characteristics and information of the sample and sample site are recorded. A field form will be needed for each sample site. Multiple samples from the same sampling site can be recorded on one sheet; ensure that all sample number IDs are recorded. Record additional sample numbers in the "additional comments" field if needed.*

Field Form for Collection of Bacterial Pathogens in Soil

Date: _____ Time:_____

Name of Person Completing this Form: _____

Sample Collection Team:_____

Sample Collection Purpose: _____

1. City/State: _____

2. Site Number ID: _____

3. **Sample Number ID(s) (separate with commas):_____**

4. Location Description:_____

5. Latitude (dd): _____. _____ Longitude (dd): _____. _____

6. Weather Conditions:_____

7. Soil Type: % Coarse sand (similar to rock salt)_____; % Medium sand (sugar like)_____;
% Fine sand (powder like)_____

8. Soil moisture (circle one): Dry (dry/dusty) Wet (clearly visible moisture)
Moist (damp) Saturated (pooling water upon collection)
Soil Moisture: _____**%**

9. Soil temperature: _____° F / C

10. Soil pH:_____

11. Natural Geologic Features (i.e., river bank, depression):_____

12. Land Use (circle all that apply):

Natural/Native	Agricultural	Developed	Industrial
Residential	Urban	Rural	

13. Vegetative Features: ____Healthy vegetation/grasses

____Water killed

____Dried, closely cropped grasses

14. Land Cover (select classification category from top row, then circle applicable feature(s) from the column below it. See Appendix B for descriptions):

Barren	Developed	Forested Upland	Shrubland	Non-Natural Woody	Herbaceous Upland	Planted or Cultivated
Bare rock/ sand/clay Quarries/ strip mines/ gravel pits Transitional	Low Intensity Residential High Intensity Residential Commercial / Industrial/ Transporta-tIon	Deciduous Forest Evergreen Forest Mixed Forest	Shrubland	Orchard/ Vineyard/ Tree farm	Grasslands /Herbaceous	Pasture/ hay Row crops Small grains Fallow Urban/ recreational grasses

15. Other Anthropogenic Features in area (i.e., freshly tilled soil):

_____

_____

165. Additional Comments/Sketch:

APPENDIX B: CHAIN OF CUSTODY FORM

Collection of Bacterial Pathogens in Soil
Chain of Custody Form

_____of _____Pages

Submitted By: _____Number of Samples:_____

Received By: _____Sample Media:_____

Date/Time Received: _____Job Number: _____

Sample Identification Numbers

Custodial Locations

Received by: (time/date)	Purpose	Location	Returned to: (time/date)

(continued on next page; include both pages with sample kit)

Laboratory Checklist

Equipment Calibration:

Equipment calibration completed (initial and time/date):_____

List equipment calibrated: _____

_____

_____

Sample Kit Supplies Included (check all that apply):

For **all levels** of sampling:

- ☐ Nitrile or vinyl gloves, non-powdered
- ☐ Surgical masks (i.e., N95 masks)[4]
- ☐ Safety goggles or safety glasses
- ☐ Disposable booties[1]
- ☐ Stainless steel trowels, one per sample site – thin bladed (will fit into the 50 ml tube) (Fisher Scientific, Waltham, MA, Part No. S02603; handle part no. #S02602; or equivalent)
- ☐ Alcohol wipes (70% ethanol)
- ☐ Bleach wipes (10% Hype-Wipes® or equivalent)
- ☐ Small zip-top bags (Ziploc® sandwich size ~6”x6” or equivalent)
- ☐ Benchtop bag holder (bag rack) – one per sample per site (Fisher Scientific, Part no. 01815-2 or equivalent) – (optional for one person collection)
- ☐ 50 mL pre-sterilized sample tubes with caps (Fisher Scientific, Part no. 06-443-19; or equivalent), bagged in extra small zipper-seal bags (Ziploc snack size ~3”x5” or equivalent)
- ☐ GPS unit (standalone or smart phone app)
- ☐ Soil moisture meter (Vegetronix™, Part no. VG-Meter-200 basic or equivalent) Soil thermometer (Fisher Scientific, Part no. 14-648-46 or equivalent)
- ☐ Soil pH meter
- ☐ Field Data Sheets – one per sample site (Appendix A) Sample ID labels (pre-printed)
- ☐ Chain of custody forms (Appendix B)
- ☐ Custody seals (Fisher Scientific #05-719-337 or equivalent) Permanent markers
- ☐ Waterproof pens
- ☐ Precut Parafilm® strips (approx. 1.5” wide x 3” long)
- ☐ Shipping supplies (gel ice packs, paper or bubble shipping-wrap, packing tape)

[4] Or as determined by the site/event-specific Health & Safety Plan or the sampling lead.

- ☐ Cooler, durable plastic chest style (i.e., Coleman® or Igloo®)
- ☐ Cooler, small 6-pack size
- ☐ Paper towels
- ☐ Heavy duty garbage bags
- ☐ Large zip-top bags (Ziploc 1 gallon size ~11" x 11" or equivalent)
- ☐ KimGuard® Sterilization Wraps (cat# 19-135-120 or equivalent)
- ☐ Sterile Agvise Laboratories (Northwood, ND) soil for blanks or equivalent

Additional PPE for **Level 2 Sampling** (see Section 8.0, Health & Safety):

- ☐ Tyvek® suit
- ☐ Chemical, biological, radiological and nuclear (CBRN)-certified full face respirator [1]

Sample Kit Assembler Initials and Date:_____

APPENDIX C: LAND COVER CLASSIFICATION DESCRIPTIONS

These definitions are based on the U.S. Geological Survey's (USGS) National Land Cover Institute's Land Cover Class Definitions (USGS 1992).

Developed - Areas characterized by a high percentage (30 percent or greater) of constructed materials (e.g., asphalt, concrete, buildings).

> **Low Intensity Residentia**l - Includes areas with a mixture of constructed materials and vegetation. Constructed materials account for 30-80 percent of the cover. Vegetation may account for 20 to 70 percent of the cover. These areas most commonly include single-family housing units. Population densities will be lower than in high intensity residential areas.
> **High Intensity Residential** - Includes highly developed areas where people reside in high numbers. Examples include apartment complexes and row houses. Vegetation accounts for less than 20 percent of the cover. Constructed materials account for 80-100 percent of the cover.
> **Commercial/Industrial/Transportation** - Includes infrastructure (e.g., roads, railroads) and all highly developed areas not classified as High Intensity Residential.

Barren - Areas characterized by bare rock, gravel, sand, silt, clay, or other earthen material, with little or no "green" vegetation present regardless of its inherent ability to support life.
Vegetation, if present, is more widely spaced and scrubby than that in the "green" vegetated categories; lichen cover may be extensive.

> **Bare Rock/Sand/Clay** - Perennially barren areas of bedrock, desert pavement, scarps, talus, slides, volcanic material, glacial debris, beaches, and other accumulations of earthen material.
> **Quarries/Strip Mines/Gravel Pits** - Areas of extractive mining activities with significant surface expression.
> **Transitional** - Areas of sparse vegetative cover (less than 25 percent of cover) that are dynamically changing from one land cover to another, often because of land use activities. Examples include forest clear-cut areas, a transition phase between forest and agricultural land, the temporary clearing of vegetation, and changes due to natural causes like fire or flood.

Forested Upland - Areas characterized by tree cover (natural or semi-natural woody vegetation, generally greater than 6 meters tall); tree canopy accounts for 25-100 percent of the cover.

> **Deciduous Forest** - Areas dominated by trees where 75 percent or more of the tree species shed foliage simultaneously in response to seasonal change.
> **Evergreen Forest** - Areas dominated by trees where 75 percent or more of the tree species` maintain their leaves all year. Canopy is never without green foliage.
> **Mixed Forest** - Areas dominated by trees where neither deciduous nor evergreen species represent more than 75 percent of the cover present.

Shrubland - Areas characterized by natural or semi-natural woody vegetation with aerial stems, generally less than 6 meters tall, with individuals or clumps not touching to interlocking. Both evergreen and deciduous species of true shrubs, young trees, and trees or shrubs that are small or stunted because of environmental conditions are included.

> **Shrubland** - Areas dominated by shrubs; shrub canopy accounts for 25-100 percent of the cover. Shrub cover is generally greater than 25 percent when tree cover is less than 25 percent. Shrub cover may be less than 25 percent in cases when the cover of other life forms (e.g., herbaceous or tree) is less than 25 percent and shrubs cover exceeds the cover of the other life forms.

Non-Natural Woody - Areas dominated by non-natural woody vegetation; non-natural woody vegetative canopy accounts for 25-100 percent of the cover. The non-natural woody classification is subject to the availability of sufficient ancillary data to differentiate non-natural woody vegetation from natural woody vegetation.

> **Orchards/Vineyards/Tree Farm** - Orchards, vineyards, and other areas planted or maintained for the production of fruits, nuts, berries, or ornamentals.

Herbaceous Upland - Upland areas characterized by natural or semi-natural herbaceous vegetation; herbaceous vegetation accounts for 75-100 percent of the cover.

> **Grasslands/Herbaceous** - Areas dominated by upland grasses and forbs. In rare cases, herbaceous cover is less than 25 percent, but exceeds the combined cover of the woody species present. These areas are not subject to intensive management, but they are often utilized for grazing.

Planted/Cultivated - Areas characterized by herbaceous vegetation that has been planted or is intensively managed for the production of food, feed, or fiber; or is maintained in developed settings for specific purposes. Herbaceous vegetation accounts for 75-100 percent of the cover.

> **Pasture/Hay** - Areas of grasses, legumes, or grass-legume mixtures planted for livestock grazing or the production of seed or hay crops.
> **Row Crops** - Areas used for the production of crops, such as corn, soybeans, vegetables, tobacco, and cotton.
> **Small Grains** - Areas used for the production of graminoid crops such as wheat, barley, oats, and rice.
> **Fallow** - Areas used for the production of crops that do not exhibit visible vegetation as a resultof being tilled in a management practice that incorporates prescribed alternation between cropping and tillage.
> **Urban/Recreational Grasses** - Vegetation (primarily grasses) planted in developed settings for recreation, erosion control, or aesthetic purposes. Examples include parks, lawns, golf courses, airport grasses, and industrial site grasses.

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References

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APPENDIX D: STERILIZATION OF SOIL BY AUTOCLAVING

Purpose

Trip blanks and field blanks are quality control samples that are employed to ensure collected samples are not contaminated by exposure to improper field activities, handling or shipping. This standard operation procedure adapted from the U.S. Environmental Protection Agency standard operating procedure (SOP) *Sterilization of Soil by Autoclaving* (NHSRC BS-103) details the process for sterilizing soil by autoclaving for the purpose of preparing trip and field blanks.

Description

Two media are used to test soil sterility; a low nutrient medium such as R2A and R2B (Reasoner´s 2A and 2B agar), and a high nutrient medium such as Trypticase Soy Agar (TSA) and Trypticase Soy Broth (TSB) or Nutrient Agar (NA) and Nutrient Broth (NB). This procedure is suitable for use with sandy soil, clay soil, and loamy soil (high organic matter content). Soil moisture content before and after autoclaving should also be determined for each soil type at least one time. Soils are autoclaved using a gravity cycle with a 10 minutes drying time, because spores are added in an aqueous solution and saturation of the soil samples is not desirable. The standard soil microbiology method involves incubating all plates and broths at room temperature for 7 to 10 days. Because it is important to ensure that no viable spores of bacteria or fungi, requiring a host for vegetative growth, are present in the soil after autoclaving, nutrient-rich media replicates are also incubated at 37 °C for 3 days.

Equipment and Supplies

Equipment

- Autoclave: (Gravity, minimum of 45 minutes, 121 °C, 17 psi, 10 minute drying time)
- 37 °C incubator
- Room temperature incubator
- Biological Safety Cabinet (BSC)
- Vortex mixer
- Microscope (optional)

Media and Reagents

- Phosphate buffered saline (PBS) dilution blanks, 9 ml
- R2A plates
- R2B, 9 ml in 15 ml tubes
- Trypticase Soy Agar (TSA) or Nutrient Agar (NA) plates
- Trypticase Soy Broth (TSB) or Nutrient Broth (NB), 9 ml in 15 ml tubes

Supplies

- Pyrex® (Corning, NY) glass autoclavable pans (9 x 11.5 in. or 10 x 15 in.)
- Aluminum foil
- Sterile spatulas, beakers or other tools for transferring soil
- Pipette (Pipetman® P200 [Gilson, Inc., Middleton, WI] or equivalent) and sterile tips for 100 µl volumes

- Pipette (Pipetman® P200 [Gilson, Inc., Middleton, WI] or equivalent) and sterile tips for 100 µl volumes
- Plate spreaders
- Glass microscope slides (optional)
- Streaking loops (optional)
- Sterilized Agvise Laboratories[5] soils:
    - RMN-LS (0-6"), high clay content soil, Agvise Lab # 68189
    - MT-CL new site # 3 PF, high sand content soil, Agvise Lab # 68187
    - DU-L-PF. High organic matter loam soil, Agvise # 68194

Sterilization of Soil by Autoclaving

1. Place approximately 550 g of soil in a 9 x 11.5 inch Pyrex pan to a depth of approximately 1-2 cm, and cover with aluminum foil. If desired, tare the Pyrex pan, aluminum foil cover and any tape applied to get an accurate soil weight by then zeroing the balance to record total weight of pan, soil and foil. If using the larger Pyrex pan, approximately 600 g of soil should be weighed in a 1 L glass beaker and then placed in the Pyrex pan. Soil should be evenly spread in the pan and larger aggregates should be broken up.
2. Autoclave the soil for at least 45 minutes (55 minutes for the larger pan) at 121°C, 17 psi, with a 10 minute dry cycle.
3. Incubate for 24 hours at room temperature.
4. Repeat the autoclaving for at least 45 minutes (55 minutes for the larger pan) at 121°C, 17 psi., with a 10 minute dry cycle.
5. Cool to room temperature and weigh.

Confirming soil sterility

Soil sterility is confirmed by the absence of growth on solid or in liquid media.

Growth on agar

1. In triplicate, add approximately 1 g of soil to 9 ml phosphate buffered saline and vortex for 30 seconds.
2. Spread plate 100 µl on triplicate R2A and six TSA plates.
3. Incubate the R2A plates and three of the TSA plates for 7-10 days at 22-27°C, check for growth daily. Plates should be bagged but bags should not be tightly sealed. A beaker of water can be placed in the incubator to increase humidity. Thicker plates are preferred to prevent excessive medium dehydration of the plates during extended incubations.

[5] Agvise Laboratories, 902 13 Street North, P.O. Box 187, Benson, MN 56215; P: 320-843-4109, F: 320-843-2074 or Northwood, ND 58267; P: 701-587-6010, F: 701-587-6013.

4. Incubate the remaining three TSA plates at 37°C for at least three days, checking for growth daily.

Inspect all plates for colonies or any indication of growth. Any sign of growth indicates the sterilization procedure failed. Soil particles on plates can have the appearance of bacterial growth. This material can be re-streaked onto fresh plates and incubated under the same conditions to confirm that the material is not bacterial biomass. A sample can also be transferred to a glass slide and viewed under a microscope to determine if bacteria are present.

Growth in broth

1. In triplicate, add approximately 1 g of soil to 9 ml R2B and six TSB tubes and vortex 30 seconds.
2. Incubate the R2B tubes and three TSB tubes for 7-10 days at 22 -27 °C in a static incubator.
3. Incubate remaining three TSB tubes at 37°C for at least three days.
4. Turbidity after soil settling may indicate that the sterilization procedure failed. Absence of turbidity indicates presumptive soil sterilization.
5. Vortex the broth tubes, remove a 100 µl sample from each tube and spread onto a single agar plate; plate R2B on R2A and TSB on TSA.
6. Incubate at least 3 to 7 days at the same temperature as the source broth tube; no visible colonies (or other signs of growth such as surface sheen due to motility) serve to confirm soil sterility.

If any of the above incubations result in growth from soil samples, the autoclaved soil is discarded and not used for experiments. Repeating the sterilization procedure with the same soil is discouraged due to changes in the soil if repeatedly autoclaved.

Preparation of Trip and Field Blanks

1. In a biological safety cabinet, aseptically transfer the sterile soil to a sterile 50 mL sample tube.
2. Cap the tube with the lid.
3. Seal the cap with a strip of Parafilm® approximately 1.5 in (3.81 cm) x 3 in (7.62 cm)

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