S-1 SOIL FIELD SAMPLING V.1

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Protocol status: Working
We use this protocol and it's working

DISCLAIMER

This work is supported by the US Army Medical Research and Development Command under Contract No.W81XWH-21-C-0001, W81XWH-22-C-0093 and HT9425-23-C-0059. The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army or Navy position, policy or decision unless so designated by other documentation.

ABSTRACT

This protocol describes soil field sampling.

GUIDELINES

OBJECTIVE

To outline steps for properly collecting sediment samples from waterholes to evaluate the risk of zoonotic disease transmission by the detection of pathogens from environmental DNA (eDNA).

SUMMARY/SCOPE

The overarching aim of the REDI-NET is to develop a collaborative laboratory network between domestic and international partnering institutions to address disease surveillance needs in order to effectively detect, predict and contain potentially emergent zoonosis. This SOP provides guidance on the protocol for the correct sediment sampling at CONUS/OCONUS sites to evaluate and predict the risk of zoonotic disease transmission.

RESPONSIBLE PERSON

Principal Investigator, Study Coordinator, Entomology Component Lead, Managers

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Note

NOTE: All study procedures must be conducted in compliance with national and local policies for prevention and control of COVID-19 infection.

MAINTENANCE OF EQUIPMENT

BEFORE EACH COLLECTION

1. Clean corer and scoops
2. Freeze and clean ice packs
3. Clean cool-boxes
4. Fully charge all equipment (e.g., GPS unit, tablets/phone). Make sure the tablet has enough free-space for field sampling pictures.

AFTER EACH COLLECTION

1. Clean all equipment thoroughly between sampling sites, including boots, cooler box (inside and outside), etc.
2. Store sterile equipment separate from used equipment and samples.

QUALITY CONTROL

This SOP is reviewed by the applicable supervisor annually or as required in order to maintain its relevance.

Note

NOTE: Because ‘the edge’ of the water body may change due to precipitation changes, the edge should be defined at each month's sampling. If the water body has a "drop off" (embankment of more than 20cm), the soil and water samples should be taken where the water "meets" the soil. Aim to collect at locations where finer sediment is present, if possible.

MATERIALS

EQUIPMENT AND MATERIALS

Note

NOTE: If product number is listed, please ensure use of this or equivalent product.

	A	B	C
	Equipment / Material	**Description**	**Mfg / Product #**
	Rubber boots	Waterproof Boots	Locally Sourced
	Dipper Head or Corer	Clean, Stainless steel or plastic device to collect sediment	Locally Sourced
	Spoon or scoop	Clean, Stainless steel or plastic device to move sediment to tubes	Locally Sourced
	Sterile Falcon® tubes (50ml)	Sterile, tubes for holding samples	Midwest Scientific
	Writing utensils	Pen / pencils, marker pens	Locally Sourced
	Plastic bags	Black (for used material)	Locally Sourced
	Camera (if no Tablet available)	For taking picture of sampling sites	Locally Sourced
	Paper towels	For sample drying collection container after sampling	Locally Sourced
	Depth finder	Example: wooden dowel, broom handle, or similar	Locally Sourced
	Cooler boxes	For cooling soil samples during transport	Locally Sourced
	Sealable plastic bags	For sample transportation	Zip-lock® slider
	GPS unit	WGS 84 and precision of 5 decimal degrees	Locally Sourced
	Sediment Field Sampling Form	REDI-NET DCS S-1	REDI-NET Data Portal
	Tablet	For data entry and picture of sampling site	Locally sourced

SAFETY WARNINGS

RISKS AND PERSONAL PROTECTION

1. Know the risk associated with the study site and sampling location and take appropriate personal safety precautions and/or wear appropriate equipment.
2. Wear light-colored long pants and a long sleeve shirt. Wear rubber boots or hiking shoes and white socks, and tuck pant legs into socks. Be sure that clothing is sturdy and can withstand long thorns.
3. To avoid contamination, never open a sample container until a sample is taken.

SAMPLING TEAMS

1

Note

NOTE: Suggested sampling frequency at each site is one time per month or, when not logistically possible, at minimum two times per unique season representative of the laboratory setting (e.g., early wet/late wet; early dry/late dry; rainy/dry season).

Field sampling of eDNA sediment samples involves two people. One person serves as the ‘sampler’ and the other person serves as a ‘helper’. The helper can look up details in these instructions when needed, keep track of samples, handle objects that are contamination risks, serve as a second set of eyes for potential contamination, and ensure safety of the sampler in potentially hazardous field conditions.

SAMPLING SITE SELECTION

2 Find two suitable sampling locations within the water body representing different ecological systems, if possible.

3 At each sampling location, soil samples should be taken from two points, one at the edge of the waterhole and the second one meter in from the edge. Sediment collection at each sampling location should be done in triplicate.

4 Because ‘the edge’ of the water body may change due to precipitation changes, the edge should be defined at each month's sampling.

5 If the water body has a "drop off" (embankment of more than 20 cm), the soil and water samples should be taken where the water "meets" the soil. Aim to collect at locations where finer sediment is present, if possible.

SAMPLE COLLECTION

6

Note

NOTES:

- Water samples collection should be performed before sediment samples collection at each sampling location point.
- Sampling from the edge should begin before sampling at the one meter location. The edge of the waterbody may change between collection times (depending on the rain/drought).

At each sampling location, the helper opens the bag containing the vials to have one open and ready for the sampler. The helper will open and close the bags containing the collection materials. This is to prevent potential contamination of samples.

7 Collect sediment using a suitable and appropriately labeled sampling device (such as a clean dipper head or corer). The Sampler takes the appropriately labeled sampling device (for example, SL1-edge would be the device for sampling location 1 at the edge).

7.1 If using a dipper head, scrape the surface of the bottom of the water body. Gently pour off any water within the dipper head over bare ground (not over the water body) taking care to minimize loss of fine surface sediments.

7.2 If using a corer, sample from the first 1-3 cm depth of the bottom of the water body. Cut the exposed sediment with a stainless steel or plastic (HDPE or PTFE) cutter.

8 The Sampler takes the appropriately labeled spoon or scoop (for example, SL1-edge would be the spoon or scoop for sampling location 1 at the edge) from the Helper and fills an appropriately labeled 50 mL falcon® tube with 10 mL of sediment. Do NOT place rocks in the tube. Cover sediment with water from the collection site leaving no airspace.

Note

NOTE: A single sampling device, as well as, spoon or scoop can be used for all triplicate sediment samples from the same sampling location (for example, SL1-edge replicate 1, 2, and 3).

9 Close the Falcon® tube and dry it using paper towels.

10 Label the Falcon® tube.

11 Place the Falcon® tube into the cooler box filled with ice for storage.

12 Repeat the sediment sampling 3 times per sampling location point (for example, three times at the edge and three times at the 1 m point).

13 Record sediment quality characteristics and information relating to samples collected.

14 Repeat steps 7 to 13, 1 m away from the edge of the waterhole.

15 Clean boots thoroughly between sampling location sites.

16 Repeat steps 7 to 15 at the second sampling location until all the samples are collected.

SOIL STORAGE

17 Keep freshly collected sediment in labeled, sealed vials at 4 °C cold chain (cooler box) until return to laboratory. Soil samples are not recommended to be stored for long periods of time at 4 °C.

18 Once at the laboratory, samples can be stored at -20 °C for up to 2 weeks or -80 °C for longer periods of time.