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Version 2

## REDI-NET S-1 SOIL FIELD SAMPLING V.2

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**We use this protocol and it's working**

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## Disclaimer

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## 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

#### 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

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### 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 a suitable sampling location within the water body representative of the ecological system, if possible.
- 3 Four sediment samples should be taken from the edge of the waterhole at the sampling location (same sample location of water sampling).
- 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  $\pm 20$  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

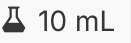
### Note

#### NOTES:

- Water samples collection should be performed before sediment samples collection (REDI-NET SOP S-1 Soil Collection).



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).
  - 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  $\pm$  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, Edge-1 would be the spoon or scoop for the first sediment sample) 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 sediment samples from the same sampling location).

- 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 4 times per sampling location point.
- 13 Record sediment quality characteristics and information relating to samples collected.





## SOIL STORAGE

- 14 Keep freshly collected sediment in labeled, sealed vials at  $4\text{ }^{\circ}\text{C}$  cold chain (cooler box) until return to laboratory. Soil samples are not recommended to be stored for long periods of time at  $4\text{ }^{\circ}\text{C}$ .
- 15 Once at the laboratory, samples can be stored at  $-20\text{ }^{\circ}\text{C}$  for up to 2 weeks or  $-80\text{ }^{\circ}\text{C}$  for longer periods of time.

## Protocol references

### REFERENCES

[REDI-NET Overview Summary](#)