

Dec 16, 2024

Version 2

## REDI-NET W-1 WATER FIELD SAMPLING V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.q26g7p8m9gwz/v2>



REDI-NET Consortium<sup>1</sup>

<sup>1</sup>REDI-NET Consortium

Remote Emerging Disea...



REDI-NET Consortium

University of Notre Dame, Naval Medical Research Center, Wal...

### Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.q26g7p8m9gwz/v2>

**Protocol Citation:** REDI-NET Consortium 2024. REDI-NET W-1 WATER FIELD SAMPLING. [protocols.io](https://dx.doi.org/10.17504/protocols.io.q26g7p8m9gwz/v2)  
<https://dx.doi.org/10.17504/protocols.io.q26g7p8m9gwz/v2> Version created by [REDI-NET Consortium](#)

**License:** This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** Working

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

**Created:** September 25, 2024

**Last Modified:** December 16, 2024

**Protocol Integer ID:** 108367

**Keywords:** WATER FIELD SAMPLING, SAMPLE COLLECTION, WATER STORAGE, water field sampling, water

**Funders Acknowledgements:**

USAMRAA

Grant ID: W81XWH-21-C-0001,

USAMRAA

Grant ID: W81XWH-22-C-0093

USAMRAA

Grant ID: HT9425-23-C-0059

USAMRAA

Grant ID: HT9425-24-C-0072

## 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, HT9425-23-C-0059, and HT9425-24-C-0072. 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 water field sampling.

## Guidelines

### OBJECTIVE

To outline steps to properly collect water samples and 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 sampling of water at CONUS/OCNUS sites to evaluate and predict the risk of zoonotic diseases 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 water collection devices.
2. Freeze and clean ice-packs.
3. Clean cool-boxes.
4. Charge all equipment(e.g., GPS unit, tablets). 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. 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                   | Personal protection                                                                           | Locally Sourced                                                                                                                                                       |
| (4) Water dippers              | Water collection device, labeled                                                              | BioQuip Mosquito Dipper & Handles - Product Details                                                                                                                   |
| Whirl-pak bags 532 ml capacity | 532 ml capacity, write on-bags, sterile                                                       | Uline, S-19793 (500/ctn)                                                                                                                                              |
| Writing utensils               | Pen / pencils, marker pens                                                                    | Locally Sourced                                                                                                                                                       |
| Litmus paper                   | Litmus pH paper strips                                                                        | <a href="https://www.amazon.com/s?k=Litmus+Paper+Ph+Test+Strips&amp;ref=nb_sb_noss_2">https://www.amazon.com/s?k=Litmus+Paper+Ph+Test+Strips&amp;ref=nb_sb_noss_2</a> |
| Depth finder                   | Example: wooden dowel, broom handle, or similar                                               | Locally Sourced                                                                                                                                                       |
| Paper towels                   | For drying Whirl-pak bag after sampling                                                       | Locally Sourced                                                                                                                                                       |
| Plastic bags                   | Black (for used material)                                                                     | Locally Sourced                                                                                                                                                       |
| Cooler boxes                   | For cooling water 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                                                                                                                                                       |
| Water collection forms         | REDI-NET DCS W-1 Water Collection                                                             | REDI-NET Data Portal                                                                                                                                                  |
| Tablet                         | For data entry and picture of the sampling sites                                              | Locally Sourced                                                                                                                                                       |



Water dippers



Whirl-pak bags

## Safety warnings

### RISKS AND PERSONAL PROTECTION

1. Know the risk associated with the study site and sampling location and take appropriate 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. Never open a sample container until the instant you will take the sample.
4. Samples should be collected **WITHOUT ENTERING THE WATER**, i.e. the collector stands only on the waterhole bank or muddy pond edges. This prevents disturbance of the substrate and may limit cross-contamination.



## 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 water 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 systems, if possible.
- 3 Water samples should be taken from the edge of the waterhole at the same sample location of soil collection. Water collection should be done in triplicate (3x  250 mL ) if the water is clear but collect only one sample (  250 mL ) if the water is cloudy.
- 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 (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 The Sampler takes the appropriately labeled water dipper from the bag and opens the corresponding Whirl-Pak® Bag which can be placed in a bucket for easier manipulation.

**Note**

**NOTE:** A single dipper can be used for all triplicate water samples from the same sampling location (for example, edge replicate 1, 2, and 3).

- 8 Check the depth of the water. If the depth of the water is greater than 1 meter, the water column should be gently mixed using the water dipper without disturbing bottom sediment.

- 9 Once the water column is homogenized, collect water filling the dipper, and empty the sample into the Whirl-Pak® Bag until the  250 mL sample is collected (3/4 of a 532 mL Whirl-Pak® Bag).

**Note**

**NOTE:** Remove debris from water samples as much as possible before pouring into Whirl-Pak® bags.

- 10 Once the water is collected, measure pH of the sample by emerging litmus paper.

- 11 Close the Whirl-Pak® Bag and dry it using paper towels.

- 12 Label the Whirl-Pak® Bag with a Sharpie or water-proof marker.



- 13 Place the Whirl-Pak® Bags into the cooler box filled with ice for storage.
- 14 Proceed with REDI-NET SOP S-1 Soil Collection at the same sampling location.
- 15 Repeat the water and soil sampling 3 times per sampling location point.
- 16 When all samples for a collection site are taken, bring the samples back to the laboratory facility for further processing according to REDI-NET SOP W-2 Water Processing (filtration should be done within  24:00:00 ).

1d

## WATER STORAGE

- 17 Keep freshly collected water in labeled whirl-pak bags at  4 °C cold chain (cooler box) until return to laboratory. Water samples can be stored at  4 °C for up to 1 week.
- 18 Once at the laboratory, it is optimal to filter the water samples and freeze the filter disk in separate, labeled ziplock bags as soon as possible. If water samples can not be processed on the same day of collection, whole sample bags should be frozen at  -80 °C . If  -80 °C storage is unavailable, water bags/ filter disks should be stored at  -20 °C until processing. Water samples can be stored at  -20 °C for up to 1 month and at  -80 °C for longer term storage.

## Protocol references

### REFERENCES

[REDI-NET Overview Summary](#)