L-1 LEECH FIELD SAMPLING V.1

REDI-NET Consortium[1]

[1]REDI-NET Consortium

REDI-NET Consortium
University of Notre Dame, Naval Medical Research Center, Wal...

VERSION 1

JAN 12, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.kxygx3peog8j/v1

Protocol Citation: REDI-NET Consortium 2024. L-1 LEECH FIELD SAMPLING. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygx3peog8j/v1Version created by REDI-NET Consortium

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 leech field sampling.

GUIDELINES

OBJECTIVE

To document the field processes for collecting hematophagous leeches (Family *Hirudinea*).

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 collection of blood-sucking leeches, to be used as sentinels for xenosurveillance of pathogens in wild and domestic animals frequenting the watering holes.

RESPONSIBLE PERSON

Principal Investigator, Study Coordinator, Field Team Lead, Managers

Created: Aug 09, 2023

Last Modified: Jan 12, 2024

PROTOCOL integer ID: 86276

Keywords: LEECH TRAPPING, LEECH STORAGE, DATA MANAGEMENT

Funders Acknowledgement:
USAMRAA
Grant ID: W81XWH-21-C-0001
USAMRAA
Grant ID: W81XWH-22-C-0093
USAMRAA
Grant ID: HT9425-23-C-0059

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 forceps with 70%-ethanol.
2. Clean and freeze ice-packs
3. Clean cool-boxes
4. Save 1cm squared of the bait liver in -80*C - labeled with sites and dates in a cryovial.
5. Fully charge all equipment (e.g., GPS unit, tablet).
6. 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.

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 #**

	A	B	C
	Leech traps (4 options)	▪ Pie dishes ▪ Bottle trap ▪ PVC Pipe trap ▪ Minnow trap see Appendix 2 for detailed description for making and baiting the leech traps	See Appendix 2
	Liver	Beef or Pork; for leech baiting 8oz per trap	Locally sourced
	Tight sealing tupperware box	For liver transportation	Locally Sourced
	Writing utensils	Pen / pencils, marker pens	Locally Sourced
	Container	Large volume (500 ml) jar or container for collecting samples; one per sampling location, pre-labeled Example: pickle jar	Locally Sourced
	Bright colored tape	For flagging	Uline; S-6089FP
	Flagged stakes	For trap retrieval	Locally Sourced
	Fine-tipped forceps	For leech removal	Carolina.com; Item # 624734
	Cooler and ice packs	For cooling leeches during transport	Locally Sourced
	GPS unit	WGS 84 and precision of 5 decimal degrees	Locally Sourced
	Data sheets	REDI-NET DCS L-1 Leech collection form	REDI-NET Data Portal
	Tablet	For data entry and picture of sampling site	Locally sourced

APPENDIX 2. LEECH TRAP CONSTRUCTION

Trap Option 1: Pie Pan Construction

	A	B	C
	Equipment / Material	**Description**	**Mfg / Product #**
	Pie pan (8”to 9” size)	Aluminum; 8”to 9” size	Locally sourced
	Mesh wire	To secure liver bait in the traps	Locally sourced
	Paper punch/ Metal skewer	To punch hole in the pie pan	Locally sourced
	Needle	To sew the mesh wire around the traps	Locally sourced

	A	B	C
	Scissors	To cut the mesh wire, the thread, and the twine	Locally sourced
	Twist tie	To seal the traps	
	Thread	To sew the mesh wire around the traps	Locally sourced
	Beef	liver To bait the leeches, 8 oz per trap	Locally sourced
	Twine/Fishing line	To attach and retrieve the traps (c.5 m per trap)	Locally sourced

Trap Option 2: Bottle Trap

	A	B	C
	Equipment / Material	**Description**	**Mfg / Product #**
	Plastic soda bottle (1.5L)	Clean, plastic; 1.5 L size	Locally sourced
	Stapler and staples	Stainless steel staples; to close the trap	Locally sourced
	Paper punch	To puncture the bottle	Locally sourced
	Icepick/Metal skewer	To punch hole in the bottle	Locally sourced
	Scissors	To cut the bottle	Locally sourced
	Beef liver	To bait the leeches, 8 oz per trap	Locally sourced
	Medium size rock	To weight down the traps	Locally sourced
	Fishing line	To attach and retrieve the traps (c. 5m per trap)	Locally sourced

Trap Option 3: PVC Pipe Trap

	A	B	C
	Equipment / Material	**Description**	**Mfg / Product #**
	Sewer drain plug	4 inch	Home Depot, 052063400112
	Sewer drain adaptor	4 inch	Home Depot, 052063444116
	Drain pipe drop-in grate PVC	4 inch, round, qty: 2	Home Depot, 052063950112
	Sewer drain Tee	4 inch	Home Depot, 052063404752
	Rope	50 feet	Home Depot, 030699706525
	Eyebolt	3/8 inch by 7 inch	Home Depot, 887480036046

	A	B	C
	Hex nuts	One for each eyebolt	Home Depot, 887480022445
	Drain pipe	4 inch by 10 feet (this will make 10-20 traps)	Home Depot, 0000-189-781

Trap Option 4: Bioquip Gee Minnow Trap

	A	B	C
	Equipment / Material	**Description**	**Mfg / Product #**
	Gee Minnow Trap	For trapping leeches, made of mesh galvanized wire and steel	BioQuip Products/ 2824
	Rope	50 feet	Home Depot, 030699706525
	Medium size rock	To weight down the traps	Locally sourced

SAFETY WARNINGS

RISKS AND PERSONAL PROTECTION

1. Know the risks 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.

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 iDNA (leech) 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, leech traps should be placed in triplicate at least 10-20 meters apart from the water and sediment sample collection point, and each trap should be distant by 5 meters from each other.

LEECH TRAPPING

4

Note

NOTE: Leech traps should be constructed and prepared ahead of time, and baited with fresh liver on site.

After identifying sample sites location in the water body, deploy the leech traps. Place a stake in the water body or flag a tree to indicate the sampling site location.

Note

Leech trapping should be conducted prior to water, sediment, and tick sampling.

5 Insert a piece of liver into the prepared traps, as instructed in Appendix 1. Leech Traps Construction (see Figures 1 and 2). Attach fishing line to trap and tie the remaining end around the flagged tree or stake to enable trap recovery.

6 Use nearby rocks to weigh the leech trap when placing it in water to ensure full submersion.

7 Repeat steps 4 - 6 to place traps at the second sampling location in the water body.

8 Check traps after 2–3 hours (after completing tick/water and soil collections).

9 Recover traps and carefully open to expose the liver bait. Using forceps, check each side of the liver for leeches.

Note

NOTE: All leeches should be collected from each trap and should be placed into one large container with water from their original environment by sampling location (see step 10).

10 Leeches can gently be removed from the liver bait with tweezers and placed directly into the large (example size 500 mL) pre-labeled field transport container with water collected from their original environment. Leeches can be aggregated from the three traps at the same sampling location (1 OR 2) and stored in a single container with water collected from their original environment.

Note

DO NOT mix leeches from different sampling locations.

NOTE: Leeches often regurgitate under stress so leeches should not be stored together.

11 Place all labeled storage containers into a cooler with ice until arriving back to the lab to prevent active swimming during transport; which promotes the consumption of the blood-meal.

12 After collecting all leeches from the first sampling location, advance to the second sampling location and repeat steps 9-11.

LEECH STORAGE

13 Keep freshly collected leeches alive in glass jars which allow air passage (net-lidded or lids with multiple

holes) and two-thirds filled with source water and tightly packed (so they will not spill) in a 4 °C cold chain (cooler box), ensuring air movement until return to laboratory. Storage at 4 °C is not recommended for long periods of time.

14 Once at the laboratory, if leech samples can not be processed on the same day of collection, remove from water and freeze individually at -80 °C. Leeches can be stored at -80 °C for up to 1 month.

Note

NOTE: If leech regurgitates during handling, soak up any fluid expelled using filter paper and store with leech. If -80 °C storage is unavailable, leeches should be stored individually at -20 °C and processed within 2 weeks.

APPENDIX 1.1 LEECH TRAP CONSTRUCTION -- Trap Option 1: P...

15 Gently fold the pie pan in half until the edges meet **(Figure 1A**).

Note

NOTE: Do NOT fold the center line.

Figure 1A: Leech traps construction.

16 Push the metal skewer through the edges to create 4 equally spaced holes to thread the ties through (Figure A1B, A1C).

Figure A1: Leech traps construction**.**

17 Measure and cut a piece of mesh wire that fits around the folded pie pan.

18 Carefully sew the mesh wire around the pie pan ensuring to leave openings for liver placement and to allow leeches into the trap (Figure A2).

Figure A2: Leech trap, pie pan design.

19 Cut a length of twine 3-4 m long. Tie the twine through the hole on one of the end holes. Thread through and twist tie two other holes shut, leaving one hole open (Figure A1D, A1E, and A1F).

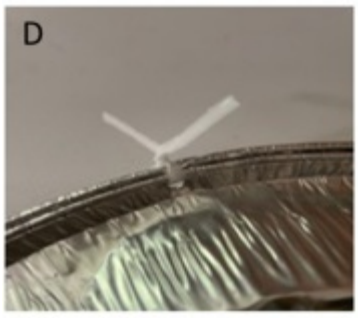

Figure A1: Leech traps construction**.**

Figure A1: Leech traps construction**.**

Note

NOTE: Steps 1–5 can be done in advance of field trips to save time.

20 Using rubber gloves, slip approximately 8 oz of liver (add the blood drippings too) into the cavity of the pre-prepared trap and seal the trap by threading and fixing the twist tie in the final hole.

21 Adjust the spaces between the tied parts of the open edge to ensure some space that allows the leeches to enter the trap (Figure A1G and A1H).

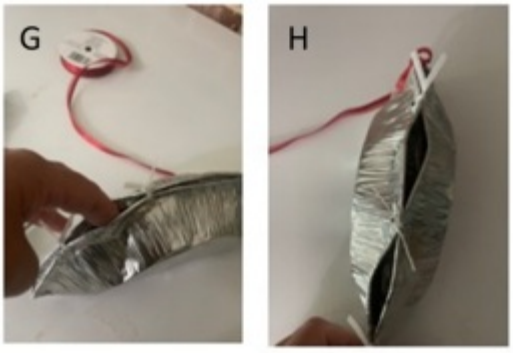

Figure A1: Leech traps construction**.**

Figure A3: Completed pie pan trap.

22 Move slightly away from the highest animal traffic area (so as not to damage the trap) and hold the trap in one hand and the end of twine in another, toss the leech trap into the water body, keeping hold of the cord.

Note

NOTE: Where carnivores are present it may be necessary to protect the leech trap by placing it inside a wire frame.

23 Wait until it sinks and secure the cord of the leech trap to sturdy vegetation, or fashion ground stakes to secure.

APPENDIX 1.2 LEECH TRAP CONSTRUCTION -- Trap Option 2: B...

24 Remove the soda bottle cap as this will not be needed for the trap (Figure A4).

Figure A4: Plastic soda bottle.

25 Cut the bottle top just above the labeled line as shown in Figure A5.

Figure A5: Cut bottle.

26 Make holes on the bottom portion and around the bottle using an icepick or scissors as shown in Figure A6.

Figure A6: Holes being made with icepick.

27 Place a piece of beef liver, approximately 8 oz, into the bottom of the bottle along with a medium-size rock. The rock will help sink the trap.

28 Invert the top of the soda bottle so it looks like a funnel and insert it into the bottom of the soda bottle as shown in Figure A7.

Figure A7: Top of bottle being inserted in bottom part.

29 Using a stapler, staple the funnel into position.

30 Punch two holes, one at the top and one at the bottom portion of the bottle, to tie the fishing line for throwing and retrieving the trap.

Figure A8: Tying of bottle with fishing line.

Figure A9: Completed bottle trap.

APPENDIX 1.3 LEECH TRAP CONSTRUCTION -- Trap Option 3: P...

31

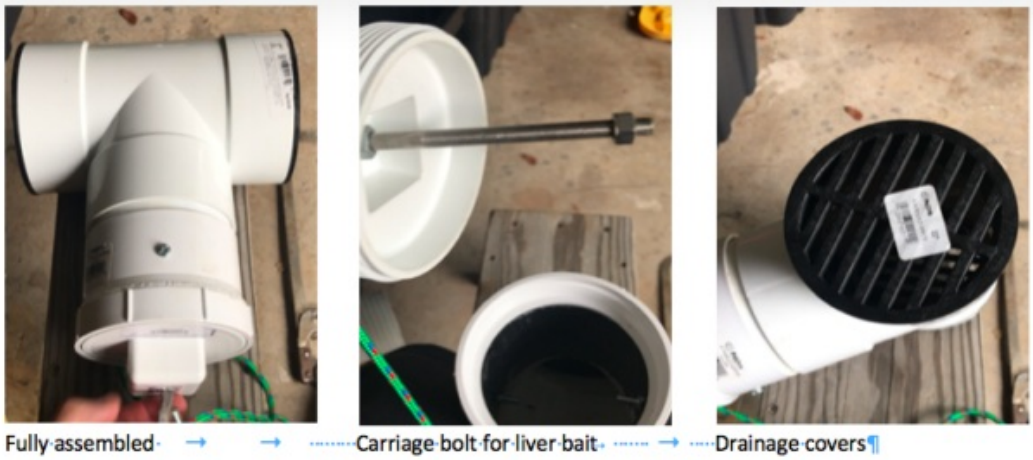

Figure A10: PVC pipe trap

Place a sewer grate onto both sides of the 'T' shaped drain.

32 Cut 6 in of the 10 ft x 4in PVC pipe using a hacksaw.

33 Place the 6 inch cut into the lower part of the T shaped drain. Then attach the 4 in sewer drain adapter to the other side.

34 Secure both the adapter and the Tee drain to the 6 in PVC section using nuts and bolts for both sides. (the image below only shows the top attached, the bottom segment will need to be attached with a bolt and nut as well).

35 Drill a hole using a 3/8 in drill bit through the top of the drain plug.

36 Push the 3/8 x 7 in eyebolt through the drain plug, then spin a 3/8 in hex bolt all the way to the top and add a second to the bottom.

37 Bait will be placed between these two hex bolts.

38 The 3/8 hex bolt might not go all the way to the top of the eyebolt and that is ok.

39 Attach rope to eyebolt loop.

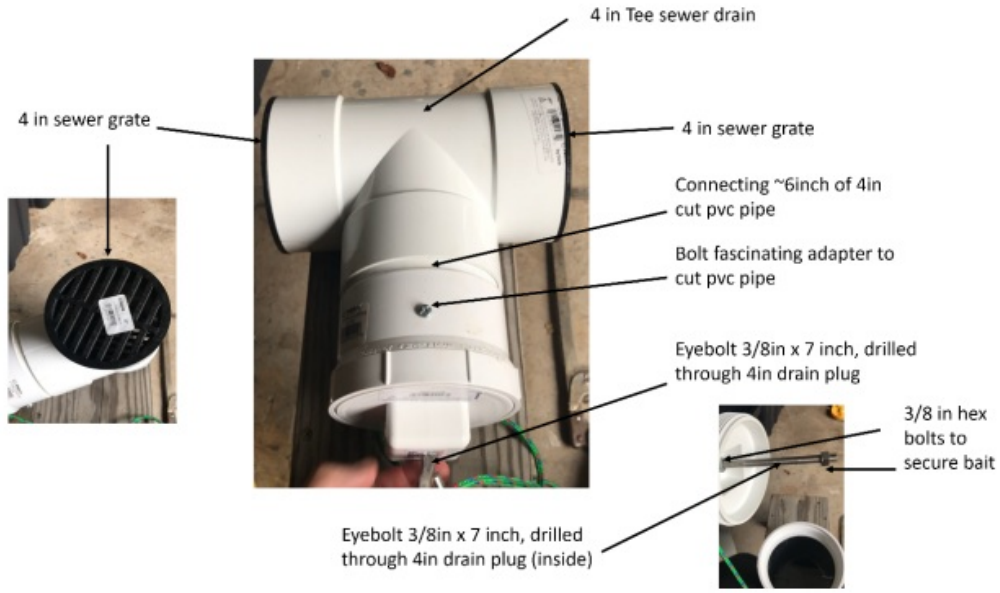

Figure A11: PVC pipe trap construction.

APPENDIX 1.4 LEECH TRAP CONSTRUCTION -- Trap Option 4: B...

40 Place a piece of beef liver, approximately 8 oz, into the bottom of the trap along with a medium-size rock. The rock will help sink the trap.

41 Close the trap using the latch.

42 Attach the rope to the trap for throwing and retrieving the trap.

> **Note**
>
> **NOTE**: There is no cap on the number of leeches during sampling - all leeches should be collected.

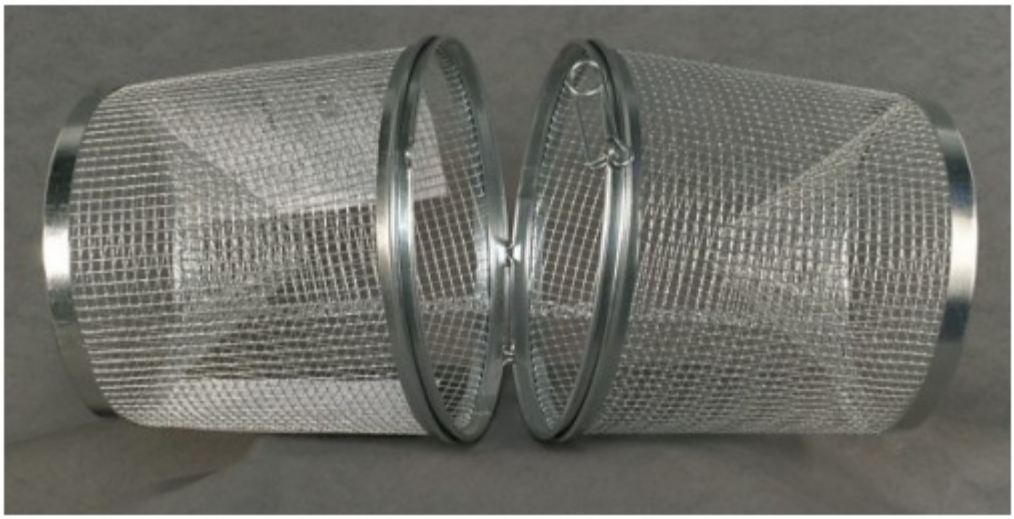

Figure A12: Minnow trap.