FEB 02, 2024

DOI:
dx.doi.org/10.17504/protocols.io.eq2lyjjkrlx9/v1

Protocol Citation: REDI-NET Consortium 2024. SW-2 SWAB PROCESSING. **protocols.io** https://dx.doi.org/10.17504/protocols.io.eq2lyjjkrlx9/v1

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

Created: Aug 24, 2023

SW-2 SWAB PROCESSING

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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 details procedures for total nucleic acid extraction from swab samples.

Last Modified: Feb 02, 2024

PROTOCOL integer ID: 86923

Keywords: Swab processing, total nucleic acid extraction, SAMPLE LYSIS

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

GUIDELINES

OBJECTIVE

To outline procedures for total nucleic acid extraction from swab samples.

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 procedures for total nucleic acid extraction from swab samples to provide materials for downstream library preparation and sequencing for pathogen detection.

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

Caution on RNA handling

1. RNases are very stable and difficult to inactivate, and only minute amounts are sufficient to destroy RNA.
2. Care should be taken to avoid inadvertently introducing RNases into the samples during or after the purification procedure.
3. Sample handling and extraction should be performed under an extraction hood and respecting Good Laboratory Practices.
4. Use filter tips all the time.

Storage of the buffers from IndiMag Pathogen Kit

1. Proteinase K is stable for at least 1 year after delivery when stored at Room temperature (15-25°C). To store for more than 1 year or if ambient

temperature often exceeds 25°C, storage at 2-8 °C is recommended. Do not add Proteinase K directly to the Buffer VXL mixture! This can cause clogs or precipitates.

2. Precipitation may form after storage at low temperature or prolonged storage. To dissolve precipitate, incubate Buffer VXL or ACB for 00:30:00 at 37 °C, with occasional shaking.
3. Reconstituted Buffer AW1 can be stored at Room temperature (15-25°C) for up to 1 year. Mix well after adding Ethanol.
4. Buffer AVE is RNase-free upon delivery. It contains sodium azide, an antimicrobial agent that prevents growth of RNase-producing organisms. However, as this buffer does not contain any RNase degrading chemicals, it will not actively inhibit RNases introduced by inappropriate handling. When handling Buffer AVE, take extreme care to avoid contamination with RNases. Follow general precautions for working with RNA, such as frequent change of gloves and keeping tubes closed whenever possible.

QUALITY CONTROL

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

APPENDICES

APPENDIX 1. MEASURING SPOON FOR 0.1 MM BEATING BEADS

The spoon (Next Advance, MSP01-RNA) is used for 0.1 mm beating beads measurement. One scoop equals to 100 uL.

APPENDIX 3. Expected Outcomes

	Sample	Amount	Sample condition	Elution volume	DNA conc. (ng/ul)	RNA conc. (ng/ul)
	Swab	1 swab	Frozen/Fresh	75	0-20	0-15

MATERIALS

EQUIPMENT AND MATERIALS

Note

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

	A	B
	Equipment	**Mfg / Product #**
	KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head or KingFisher™ Duo Prime Magnetic Particle Processor	ThermoFisher, 5400630 or ThermoFisher, 5400110
	Bullet Blender 24 Gold	Next Advance, BB24-AU
	Qubit 4 Fluorometer	ThermoFisher, Q33238
	Adjustable micropipettes	Locally sourced
	Multi-channel micropipettes	Locally sourced
	Vortex	Locally sourced
	Tube centrifuge	Locally sourced
	Plate centrifuge	Locally sourced
	Thermo Heater Mixer	Locally sourced

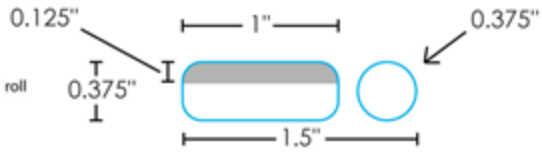
	A	B	C
	Material	**Description**	**Mfg / Product #**
	IndiMag Pathogen Kit	w/o plastics, 384 reactions	Indical Bioscience, SP947257
	Buffer ATL	200 mL, Tissue Lysis Buffer	Qiagen, 19076
	Reagent DX	1 mL, Antifoaming Reagent	Qiagen, 19088
	Measuring Spoon 100 µL	RNase Free, pack of 10. reusable	Next Advance, MSP01-RNA
	KingFisher™ Deepwell 96 Plate	KingFisher	ThermoFisher, 95040450
	KingFisher™ 96 KF microplate	KingFisher Flex *ONLY*	ThermoFisher, 97002540
	KingFisher™ 96 tip comb for DW magnets	KingFisher Flex *ONLY*	ThermoFisher, 97002534

	A	B	C
	KingFisher™ Duo Prime 12-tip comb	KingFisher Duo Prime *ONLY*	ThermoFisher, 97003500
	Elution Strip	KingFisher Duo Prime *ONLY*	ThermoFisher, 97003520
	KingFisher™ Duo Cap for Elution Strip	KingFisher Duo Prime *ONLY*	ThermoFisher, 97003540
	MicroAmp™ Clear Adhesive Film	KingFisher	ThermoFisher, 4306311
	RNase-Free Microfuge Tubes	Nonstick, 1.5 mL	ThermoFisher, AM12450
	Thermo Scientific Screw Cap Micro Tubes	1.5 mL Screw Cap Tube, NonKnurl, NonSkirted,Natural, E-Beam Sterile tube w/ attached cap	Fisher Scientific, 14-755-208
	Zirconium oxide beads	0.1 mm, 400 g	Fisher Scientific, 50-154-2950
	Qubit™ 1X dsDNA HS Assay Kit	(*consumable)*	ThermoFisher, Q33230
	Qubit™ RNA HS Assay Kit	(*consumable)*	ThermoFisher, Q32852
	Qubit Assay Tubes	For Qubit DNA/RNA measurement (*consumable*)	Thermo Fisher, Q32856
	RNaseZap™ RNase Decontamination Solution	To remove RNase from working area	ThermoFisher, AM9780
	ZymoBIOMICS Microbial Community Standard Material	For positive controls	Zymo Research, D6300
	Zika virus (ZIKV) positive control	For TNA extraction positive control	NMRC made
	Human gammaherpesvirus (EBV) positive control	For TNA extraction positive control	NMRC made
	Forceps	For use with samples	Locally sourced
	Ethanol	100% (molecular biology grade)	Locally sourced
	Isopropanol	100% (molecular biology grade)	Locally sourced
	Nuclease-free Water	To elute total nucleic acids	Locally sourced
	Dry ice	To maintain cold chain during sample handling	Locally sourced
	Ice bucket	To contain the dry ice	Locally sourced
	Kimwipes	To dry material	Locally sourced
	Falcon tubes	15 mL and 50 mL	Locally sourced
	Data sheets	REDI-NET DCS SP-1 Sample Processing Form	REDI-NET Data Portal

APPENDIX 4. SET-UP INSTRUCTIONS FOR BARCODE PRINTING

Material and Equipment

	A	B	C
	Equipment / Material	**Description**	**Mfg / Product #**
	Thermal Printer	Zebra ZD421T Desktop Dual Barcode Printer - 203 dpi	Uline, H-9581
	Thermal Transfer Ribbon	For use with Zebra thermal printer; Desktop thermal transfer ribbons - wax/resin, 4.33” x 244 (12/case)	Uline, S-18466
	Cryo-labels	667 1.00" x 0.38" Cap & Wrap CryoLabel® w/0.375" Cap, Blanks, 1" Core Color bar breakdown: Grey - 31,24,25,0 Orange - 0,80,95,0 Blue - 85,50,0,0 Brown - 35,60,80,25 Yellow - 0,0,100,0	Electronic Imaging Materials, #335774-COLOR
	Handheld scanner	To scan barcode	Zebra, LS2208-SR20001R-NA
	123Scan Software	To scan barcodes	123Scan software
	Laptop or desktop computer with Google Chrome and access to the REDI-NET data portal	To connect with the handheld scanner, the thermal printer and the REDI-NET Data Portal	Locally sourced



Cryo-labels

Equipment

NAME	TYPE	BRAND	SKU	LINK
KingFisher™ Flex Purification System, KingFisher with 96 Deep-well Head	Flex Purification System	Thermo Scientific™	5400630	https://www.thermofisher.com/order/catalog/product/5400630

Equipment

NAME	TYPE	BRAND	SKU	LINK
Bullet Blender 24 Gold (1.5 mL snap and screw cap tubes, 4°C cooling)	Homogenizer	Next Advance	BB24-AU	https://www.nextadvance.com/product/bullet-blender-24-gold/

Equipment

Qubit Fluorometer	NAME
Fluorometer	TYPE
Invitrogen	BRAND
Q33238	SKU
https://www.thermofisher.com/order/catalog/product/Q33238#/Q33238	LINK

IndiMag Pathogen Kit w/o plastics (384 reactions) **INDICAL BIOSCIENCE Catalog #SP947257**

Buffer AL, Lysis buffer **Qiagen Catalog #19076**

Reagent DX **Qiagen Catalog #19088**

Measuring Spoon 100 uL RNase Free pack of 10 **Next Advance Catalog #MSP01-RNA**

KingFisher™ Flex™ Systems Consumables, KingFisher Flex Microtiter Deepwell 96 plate, V-bottom **Thermo Fisher Catalog #95040450**

KingFisher™ Flex™ Systems Consumables, KingFisher 96 KF microplate (200µL) **Thermo Fisher Catalog #97002540**

KingFisher™ Flex™ Systems Consumables, KingFisher 96 tip comb for DW magnets **Thermo Fisher Catalog #97002534**

KingFisher™ Duo and KingFisher™ Duo Prime Consumables, 12-tip comb, for Microtiter 96 Deepwell plate **Thermo Fisher Catalog #97003500**

KingFisher™ Duo and KingFisher™ Duo Prime Consumables, Elution strip **Thermo Fisher Catalog #97003520**

KingFisher™ Duo and KingFisher™ Duo Prime Consumables, KingFisher Duo Cap for elution strip **Thermo Fisher Catalog #97003540**

MicroAmp™ Clear Adhesive Film **Thermo Fisher Catalog #4306311**

Nonstick, RNase-free Microfuge Tubes, 1.5 mL **Thermo Fisher Catalog #AM12450**

Thermo Scientific™ Screw Cap Micro Tubes **Fisher Scientific Catalog #14-755-208**

Bertin Corp 0.1mm Zirconium oxide beads (450g) (qty 500) **Fisher Scientific Catalog #50-154-2950**

Qubit 1X dsDNA High Sensitivity Assay Kit **Thermo Fisher Scientific Catalog #Q33230**

Qubit RNA HS (High Sensitivity) assay **Thermo Fisher Scientific Catalog #Q32852**

Qubit™ Assay Tubes **Invitrogen - Thermo Fisher Catalog #Q32856**

RNaseZap™ RNase Decontamination Solution **Thermo Fisher Scientific Catalog #AM9780**

ZymoBIOMICS Microbial Community Standard **Zymo Research Catalog #D6300**

SAFETY WARNINGS

RISK AND PERSONAL PROTECTION

1. Caution should be taken while processing samples as some chemicals may be harmful. Please use a fume-hood when required to avoid inhaling harmful chemicals.
2. Gloves should be worn all the time when handling samples.
3. Decontaminants such as DNA/RNaZap could irritate the skin, avoid contact with skin while preparing the workbench for nucleic acid extractions.

BEFORE START INSTRUCTIONS

BEFORE START

1. Pre-cool the Bullet Blender by adding dry ice into the cooling compartment and running the cooling program.
2. Clean the work surfaces with RNaseZap, then wipe the surfaces with 70% molecular biology grade ethanol to remove additional contaminants.
3. Pre-heat the heater mixer at 56 °C.
4. Transfer 0.1 mm zirconium oxide beads (2 spoons, Appendix 1) to Thermo Scientific Screw Cap Micro 1.5ml Tubes.
5. Buffer ATL may form precipitates upon storage. If necessary, warm to 56 °C until the precipitates have fully dissolved. Prepare buffer ATL-DX: add 100 µL Reagent DX to 15 mL Buffer ATL. If smaller amounts are needed, transfer 1.5 mL of Buffer ATL into a sterile 2ml vial and add 10 µL Reagent DX. Mix well, after addition of Reagent DX. After preparation, the mixture is stable for 6 months at Room temperature (15-25°C).
6. For the first time use of IndiMag pathogen kit, add 100% ethanol to Buffer AW1 and AW2, and add 100% isopropanol to ACB as indicated on the bottles.
7. MagAttract Suspension G from IndiMag pathogen kit needs to be vortexed thoroughly for 00:03:00 (before first use) or 1 minute (before subsequent uses) to ensure that the magnetic silica particles are fully resuspended.
8. Aliquot nuclease-free water in big bottle into a few 15ml tubes for preparing TNA elution in KingFisher Flex or KingFisher Duo Prime to avoid cross-contamination.

1. SAMPLE LYSIS

1

Note

NOTE: *To prevent contamination samples nucleic acid extraction and amplification (PCR) should be performed in separate rooms.*

Add 500 µL of ATL-DX buffer and 20 µL Proteinase K to the bead tubes prepared in Step 4 of Before Start section under the Guidelines & Warning tab.

2 Place the sample swab in the bead tube with ATL buffer and Proteinase K. Use sterile scissors to carefully cut off the swab heads or transfer the pre-cut swab head from the original container to the bead tube with

forceps.

3 Include a positive control for each batch of samples: transfer 37.5 µL ZymoBIOMICS Microbial Community Standard Material, 100 µL EBV, and 100 µL ZIKV standard into a tube from Step 3 of Before Start section. Add 250 µL ATL-DX buffer and 20 µL proteinase K.

4 Include a negative control for each batch of samples: a bead tube from Step 3 of Before Start section with 500 µL ATL-DX buffer and 20 µL proteinase K.

5 Incubate samples in a thermomixer at 1400 rpm, 56°C, 00:10:00.

6 Refill the dry ice compartment of the Bullet Blender if necessary. After incubation, load all samples into the Bullet Blender.

7 Set the speed at 12 and the time at 3. Press Start.

8 Let the samples settle for 00:01:00 in the Bullet Blender and then repeat Step 7. 1m

Note

STOPPING POINT: lysed samples can be stored at 4 °C Overnight.

2. INSTRUMENT SET UP

9

Note

NOTE: KingFisher Flex only, if using KingFisher Duo Prime, go to section 3

Confirm 96 deep-well magnetic heads and 96 well deep-well heat blocks are being used.

10 Ensure the program **IndiMag_Pathogen_KF_Flex_4wash** or the program has been downloaded and loaded onto the KingFisher Flex instrument.

2.1 SET UP THE PROCESSING PLATES

11 Set up the Tip Comb, Wash, and Elution Plates outside the instrument according to the following table.

Note

NOTE: *DO NOT use the elution buffer provided by the kit for TNA elution. The ingredients in the elution buffer inhibit the downstream DNA sequencing efficiency.*

	A	B	C	D	E
	Plate ID	**Plate position**	**Plate type**	**Reagent**	**Volume per well**
	Tip comb	7	Place a 96 Deep-well Tip comb in a deep-well plate		
	Elution	6	Deep-Well	Nuclease-free water	75 µL
	Wash 4	5	Deep-Well	100% ethanol	750 µL
	Wash 3	4	Deep-Well	80% ethanol	750 µL
	Wash 2	3	Deep-Well	Buffer AW2	700 µL
	Wash 1	2	Deep-Well	Buffer AW1	700 µL
	Sample	1	Sample Lysate	Lysate and lysis buffer	985 µL

2.2 EXTRACTION

12 Centrifuge the bead tubes with lysate from Step 8 for 12000 x g, 00:05:00. 5m

13 Transfer 290 µL supernatant without any particle carryover to the wells of the Deep-well plate. This plate becomes the Sample Plate.

14 Add 135 µL Buffer VXL, 540 µL Buffer ACB, and 20 µL MagAttract Suspension G to each sample in the sample plate. For multiple samples, make a master mix with 10% overage. Invert slowly to mix the master mix; avoid foaming (it can be mixed on a Hula mixer for 2 min). Add 695 µL mixture to each sample. Each well including sample lysate should be 985 µL.

15 Select the program IndiMag_Pathogen_KF_Flex_4wash on the instrument.

16 Start the run, then load the prepared plates into position when prompted by the instrument.

2.3 QUANTIFICATION AND STORAGE

17 After the running protocol is completed (~35 minutes), immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to the final tube or plate of choice for final storage.

18 In a 0.6mL microcentrifuge tube, use 3 µL total nucleic acid for DNA and RNA concentration measurement using Qubit 4 Fluorometer following manufacturer instructions (Kits needed: Qubit 1X dsDNA HS Assay Kit and Qubit RNA HS Assay Kit) (see Appendix 2 and Appendix 3).

19 Proceed with sample testing following the REDI-NET SOP SW-4 Swab Testing or store at -20 °C for less than 2 weeks (for long-term storage the sample needs to be stored at -80 °C following the REDI-NET SOP SW-3 Swab Storage).

3. INSTRUMENT SET UP

20

Note

NOTE: KingFisher Duo Prime only, if using KingFisher Flex, go to 2.

Confirm 12-tip magnetic heads and 12 well deep-well heat blocks are being used.

21 Ensure the program **IndiMag_Pathogen_KF_Duo_4wash** has been downloaded and loaded onto the KingFisher Duo Prime instrument.

3.1 SET UP THE SAMPLE PLATE AND ELUTION STRIP

22 Set up the Sample Plate according to the table below:

A	B	C	D
Row ID	**Plate Row**	**Reagent**	**Volume per well**
Sample row	A	Lysate and lysis buffer	985 µL
Wash 1	B	Buffer AW1	700 µL
Wash 2	C	Buffer AW2	700 µL
Wash 3	D	80% ethanol	750 µL
Wash 4	E	100% ethanol	750 µL
Tip Comb	F	Tip comb	
	G	Empty	
	H		

23 Set up the Elution Strip according to the table below:

Note

NOTE: *DO NOT use the elution buffer provided by the kit for TNA elution. The ingredients in the elution buffer inhibit the downstream DNA sequencing efficiency.*

	A	B	C	D
	Strip ID	**Row**	**Reagent**	**Volume per well**
	Elution	A	Nuclease-free water	75 µL

3.2 EXTRACTION

24 Centrifuge the bead tubes with lysate from Step 8 for 12000 x g, 00:05:00. 5m

25 Transfer 290 µL supernatant without any particle carryover to the wells of the Deep-well Row A. This row becomes the Sample Row.

26 Add 135 µL Buffer VXL, 540 µL Buffer ACB, and 20 µL MagAttract Suspension G to each sample in the Sample Plate. For multiple samples, make a master mix with 10% overage. Invert slowly to mix the master mix and avoid foaming. Add 695 µL mixture to each sample. Each well including sample lysate should be 985 µL.

27 Select the program IndiMag_Pathogen_KF_Duo_4wash on the instrument.

28 Start the run, then load the prepared plate and Elution Strip into position when prompted by the instrument.

Note

Keep the door open while extraction is in process. The chamber of the KingFisher Duo Prime is small. Closing the door makes the ethanol vapor restrained inside the chamber and increases the ethanol contamination.

3.3 QUANTIFICATION AND STORAGE

29 After the running protocol is completed (~35 minutes), immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to the final tube or plate of choice for final storage.

30 In a 0.6mL microcentrifuge tube, use 3 µL total nucleic acid for DNA and RNA concentration measurement using Qubit 4 Fluorometer following manufacturer instructions (Kits needed: Qubit 1X dsDNA HS Assay Kit and Qubit RNA HS Assay Kit) (see Appendix 2 and Appendix 3).

31 Proceed with sample testing following the REDI-NET SOP SW-4 Swab Testing or store at -20 °C for less than 2 weeks (for long-term storage the sample needs to be stored at -80 °C following the REDI-NET SOP SW-3 Swab Storage).

APPENDIX 2. DNA and RNA Measurement using QUBIT FLUOROMETER 4.0

4m

32 DNA quantification:

32.1 According to the volume of sample used, add the 1x HS dsDNA Qubit Assay for a final volume o 2m 200 µL (i.e., if using 3 µL of sample, add 197 µL of 1x HS dsDNA Qubit Assay). Vortex for 5 - 10 seconds, then incubate for 00:02:00 at Room temperature

33 RNA Quantification:

33.1 In a new microcentrifuge tube/falcon tube (depending on the number of samples processed), prepare a working solution of the Qubit HS RNA Assay:

	A	B	C
	Reagents	**Volume/sample**	**Volume for n+1 sample**
	Qubit RNA HS Assay buffer	199 µL	…. µL
	Qubit RNA HS Assay Dye	1 µL	…. µL

33.2 In a new 0.6ml tube, mix 197 µL of Qubit HS RNA Assay working solution and 3 µL of the sample. Vortex for 5 - 10 seconds, then incubate for 00:02:00 at Room temperature before reading. 2m