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REDI-NET FV-2 FLYING VECTOR PROCESSING

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REDI-NET Consortium¹

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Remote Emerging Disea...



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We use this protocol and it's working

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Disclaimer

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Abstract

This protocol details flying vector processing for total nucleic acid extraction.

Guidelines

OBJECTIVE

To clearly document the correct process for effective standardized field collections of ticks, and recommended personal protection measures.

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 field tick collections in the vicinity of watering holes by dragging.

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 forceps with 70%-ethanol.
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. Tick drags should be sealed into trash bags for transport and cleaned *without detergents* (only use tap water and then air-dry) before the next collection.
3. Lint roller sheets should be disposed of in the waste bag after all ticks have been removed for testing.
4. Store sterile equipment separate from used equipment and samples.

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. The step is described on 6.6.4 the preparation before tick homogenization. One spoon equals to  100 µL .



APPENDIX 4 EXPECTED OUTCOMES

Sample	Amount	Sample condition	Elution volume	DNA conc. (ng/ul)	RNA conc. (ng/ul)
Tick	1 unfed adult or 10 nymphs or 60 larvae	Frozen/live	75	20 - 30	10 - 20

Materials

EQUIPMENT AND MATERIALS

Note

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

A	B
Equipment	Mfg / Product #
Dino-Lite 5MP Edge	AM7115MZT
Adjustable DinoScope Stand	MS35B
Magnetic light source (x2)	IMAGE Grill Lights Magnetic BBQ Grill Light
AAA batteries	EBL Pack of 8 AAA Batteries 1,100mAh AAA
Wifi or ethernet internet connexion	(WPA-2 security only. WPA-e not yet supported)
Laptop or desktop computer with Google Chrome	(Setup requires Ethernet or laptop with Bluetooth LE capabilities); PC preferred for DinoScope
Dino-Lite Driver Software	REDI-NET Program
BioQuip Portable Chill Table	BioQuip, 1429
Multiplex imaging system Tick e-ID device	Vectech
AC power converted to US standard	120 V and 60Hz AC
● KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head ● KingFisher™ Duo Prime Magnetic Particle Processor	ThermoFisher, 5400630 (Flex) or ThermoFisher, 5400110 (Duo Prime)
Bullet Blender 24 Gold	Next Advance, BB24-AU
Adjustable micropipettes	Locally sourced
Multi-channel micropipettes	Locally sourced
Vortex	Locally sourced
Tube centrifuge	Locally sourced
Plate centrifuge	Locally sourced
Qubit 4 Fluorometer	ThermoFisher, Q33238

A	B
Thermo Heater Mixer	Locally sourced

Equipment

Dino-Lite 5MP Edge AM7115MZTNAME

MicroscopeTYPE

Dino-LiteBRAND

AM7115MZTSKU

<https://www.dinolite.us/products/usb-microscopes/usb-edge/am7115mzt/>LINK

magnification range of this microscope is 20x to 220xSPECIFICATIONS

Equipment

Adjustable Vertical Mount standNAME

vertical standTYPE

Dino-LiteBRAND

MS35BSKU

<https://www.dinolite.us/products/accessories/stands/ms35b/>LINK

Equipment

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

Equipment

Qubit™ 4 Fluorometer, with WiFi		NAME
Fluorometer		TYPE
Invitrogen		BRAND
Q33238		SKU
https://www.thermofisher.com/order/catalog/product/Q33238#/Q33238		LINK

Equipment

KingFisher™ Duo Prime Purification System

NAME

Purification System

TYPE

Thermo Scientific™

BRAND

5400110

SKU

<https://www.thermofisher.com/order/catalog/product/5400110?SID=srch-srp-5400110>^{LINK}

A	B	C
Material	Description	Mfg / Product #
Posi-Click™ 5 mL Microcentrifuge Tubes	For storage of individual specimen	Thomas Scientific, 1149Y05
96 well Eppendorf tube racks	To hold specimen on chill plate during imaging	1164M62
Porcelain chill table stage or white paper note cards	To better visualize tick during imaging	Locally sourced
Petri dishes, disposable	To hold ticks under DinoScope	Bioquip, 4787
Color lab tape	For labeling and sealing boxes of ticks	Thomas Scientific, 1184X64
ZymoBIOMICS Microbial Community Standard Material	For TNA extraction positive control	Zymo Research, D6300
ATCC (DENV-1) positive control	For TNA extraction positive control; BSL-2	ATCC, VR-1856 or locally sourced
Human gammaherpesvirus (EBV) positive control	For TNA extraction positive control	Naval Medical Research Center
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



A	B	C
Measuring Spoon 100 µL	RNase Free, pack of 10, reusable	Next Advance, MSP01-RNA
Navy RINO RNA lysis kit	Bead lysis kits	Next Advance, NAVYR5-RNA
Clear RINO brand microcentrifuge tubes	1.5 mL, screw-cap	Next Advance, TUBE1R5-S
Zirconium oxidase beads	0.1 mm, 400 g	Fisher Scientific, 50-154-2950
KingFisher™ Deepwell 96 Plate	KingFisher	ThermoFisher, 95040450
KingFisher™ 96 tip comb for DW magnets	KingFisher Flex <i>ONLY</i>	ThermoFisher, 97002534
KingFisher™ Duo Prime 12-tip comb	KingFisher Duo Prime <i>ONLY</i>	ThermoFisher, 97003500
Elution Strip	KingFisher Duo Prime <i>ONLY</i>	ThermoFisher, 97003520
KingFisher™ Duo Cap for Elution Strip	KingFisher Duo Prime <i>ONLY</i>	ThermoFisher, 97003540
BRAND Self-adhesive Plate Sealing Film	Aluminum (<i>consumable</i>)	Fisher Scientific, 13-882-329 or equivalent
MicroAmp™ Clear Adhesive Film	KingFisher	ThermoFisher, 4306311
Nonstick, RNase-Free Microfuge Tubes	1.5 mL	ThermoFisher, AM12450
Nonstick, RNase-Free Microfuge Tubes	2.0 mL	ThermoFisher, AM12475
Qubit assays tubes	For Qubit™ DNA/RNA measuring (<i>consumable</i>)	Thermo Fisher, Q32856
RNaseZap™ RNase Decontamination Solution	To remove RNase from the working area	ThermoFisher, AM9780
Qubit™ 1X dsDNA HS Assay Kit	(<i>consumable</i>)	ThermoFisher, Q33230
Qubit™ RNA HS Assay Kit	(<i>consumable</i>)	ThermoFisher, Q32852
Ethanol	100% (molecular biology grade)	Locally sourced
Isopropanol	100% (molecular biology grade)	Locally sourced
Nuclease-free Water	For negative control	Locally sourced
Dry ice	To maintain cold chain during sample handling using Bullet Blender	Locally sourced
Ice bucket and ice	To keep sample cold	Locally sourced

	A	B	C
	Kimwipes	To dry material	Locally sourced
	Falcon tubes	15 mL and 50 mL	Locally sourced
	Forceps	Fine point, straight tip, stainless, sterile; For sample handling	BioQuip, 4731
	Sterile 1x PBS	To clean tick sample	Locally sourced
	Sterile petri dishes	To hold tick sample during cleaning	Locally sourced
	Data sheets	REDI-NET DCS T-2	REDI-NET Data Portal

⊗ Posi-Lock™ 5 mL Microcentrifuge Tubes **Thomas Scientific Catalog #1149Y05**

⊗ 96 Place Reversible Racks with Covers **Thomas Scientific Catalog #1164M62**

⊗ Color LAB-TAPE™ 0.94 **Thomas Scientific Catalog #1184X64**

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

⊗ 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**

⊗ Sterile Microcentrifuge Tube 1.5 mL (RINO®) 500/case **Next Advance Catalog #TUBE1R5-S**

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

⊗ KingFisher™ Plastics for 96 deep-well format **Thermo Fisher Scientific Catalog #95040450**

⊗ 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**



BRAND[™] Self-adhesive Plate Sealing Film **Fisher Scientific Catalog #13-882-329**



MicroAmp Clear Adhesive Film **Applied Biosystems (ThermoFisher Scientific) Catalog #4306311**



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



Navy RINO RNA Lysis Kit 250 pack (1.5 mL) **Next Advance Catalog #NAVYR5-RNA**



Nonstick, RNase-free Microfuge Tubes, 2.0 mL **Thermo Fisher Catalog #AM12475**



Qubit assay tubes **Thermo Fisher Scientific Catalog #Q32856**



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



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



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

APPENDIX 12: Tray Cleaning Materials

1. VECTECH IDX tray
2. Lens cleaner, glass cleaner or ethanol
3. Kimwipe or microfiber cloth
4. Phillips head screwdriver

APPENDIX 14. SET-UP INSTRUCTIONS FOR BARCODE PRINTING



Safety warnings

RISKS 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/RNaseZap could irritate the skin, please, try to avoid contact with skin while preparing workbench for nucleic acid extraction.

Before start

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. Transfer  0.1 mm zirconium oxide beads (2 spoons, Appendix 1) to Thermo Scientific Screw Cap Micro 1.5 ml Tubes.
4. 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.
5. MagAttract Suspension G from IndiMag pathogen kit needs to be vortexed thoroughly for  00:03:00 (before first use) or  00:01:00 (before subsequent uses) to ensure that the magnetic silica particles are fully resuspended.
6. Aliquot nuclease-free water in big bottle into a few  15 mL tubes for preparing TNA elution in KingFisher Flex or KingFisher Duo Prime to avoid cross-contamination.

1. SORT FLYING VECTOR SAMPLES

1

Note

NOTE: 1) The sections 6.1 to 6.5 in this SOP are for imaging adult female mosquitoes. The sections 6.6 to 6.16 in this SOP are for total nucleic extraction from samples previously imaged and identified.

Sort flying vectors by species, collection date, and collection location. You may also consider sorting specimens by trap type (if using multiple at the same location).

Note

NOTE: Only female flying vectors should be processed for testing.

- 2 Following sampling, flying vectors can be stored at  -20 °C for up to 2 months and at  -80 °C for long term storage.

2. PREPARING MOSQUITOES e-ID (IDX): Device setup using Bluetooth

3

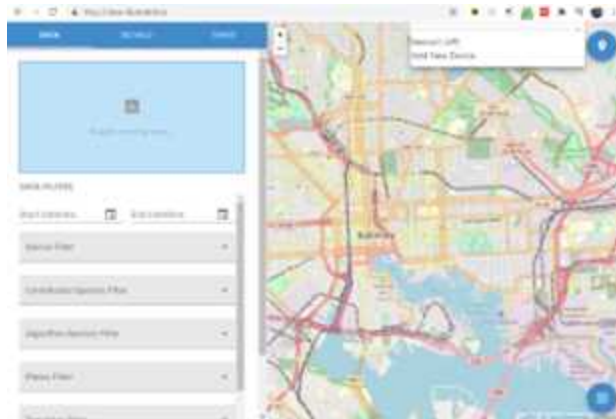
Note

NOTE: If the Tick e-ID is not recognizing the device, please, logout from google and your institution, and reboot the computer. Then login to the Tick e-ID platform using google account. Current device is not supporting larval ticks, which will only be imaged using DinoScope in section 5 and 6.

Plug in the device. On a phone or computer which has both Google Chrome and Bluetooth capabilities, visit <https://mos-id.vectech.io> (if on mobile, use the desktop site option) and log in. For first time use, click the **"Sign Up"** tab, enter in a username (email), and a password. We will approve your account, and then you can continue.

- 4 If set-up with ethernet is desired, skip to Section 3.
- 5 Click the drop-down arrow at the top-center of the page (see figure). Click **"Add New Device,"** click on the bluetooth icon button. When a device with a name **"Agamotto"**

appears, select the device and click **"Pair"**. When given the opportunity, select and enter your Wi-Fi credentials [user name, passcode] (the website may throw an error, but ignore this error). Return to the **"Data"** tab.



- 6 Click the drop-down arrow at the top-center of the page, and you should see a device with status **[booting]** or **[Idle]**. If [booting], wait for status to change to [idle] and stay. Then select **"Device."** Setup is complete. If your device soon changes to [Idle] under the device drop down menu, continue to 4. Imaging Protocol.

3. PREPARING MOSQUITOES e-ID (IDX): Device setup using Ethernet

- 7 If setting up with ethernet instead of Bluetooth, follow these steps. Select the user menu at the bottom right corner of the screen. Select **"Connect Device."**



- 8 Select **"Device."** If the device name is not known, contact Vectech for support. Then select your Wi-Fi name from the **"Wi-Fi SSD"** drop down menu. If you do not see your Wi-Fi in the drop down, verify the network is functioning on other devices. Then enter your Wi-Fi password. Select connect.

- 9 If a successful Wi-Fi connection is established, disconnect the device from power and ethernet, then reconnect power. If your device soon changes to [**Idle**] under the device drop down menu, continue to Section 4. Imaging Using Mosquitoes e-Id.
- 10 If you have trouble connecting to the device via Wi-Fi, try to connect an Ethernet cable directly from your device to your modem. You should soon see your device status change to [**Idle**] under the device drop down menu.



4. IMAGING USING MOSQUITOES E-ID

- 11 A single representative individual mosquito from a pool will suffice for documentation.
- 12 On <https://mos-id.vectech.io>, navigate to the “**Trays**” tab. Verify that the device is plugged in. The device should flash lights when booting up.
- 13 Organize the specimens to be multi-imaged in sample tubes laid out in tubes in a 96-well sample rack (8×12). **MAINTAIN THE COLD CHAIN.** Keep samples in dry ice or in polystyrene boxes filled with cool beans until ready to use. Place a glass tray on the chill table as you fill the rows before imaging and immediately return to storage tubes once imaged. Sequentially lay out the sequential sample across the row, with each row of 12 equaling one tray of multiplex photos.

Note

Tip: If you run samples in sequential order from top left to bottom right through the tray (A1-A4; A5- 8, A9-12), the system will auto-fill your sample numbers in the associated data fields.



Figure 1: Placement of samples in the tray for identification.

- 14 Mosquitoes will need to be collected, stored, and moved dry. Any liquid contact will degrade important identifying features.
- 15 Repeat step 12, until all 12 specimens are placed in the tray, dorsal side facing up.
- 16 Close the tray by placing the lid on the well-side of the tray until you hear the gentle click as the magnets connect to keep the tray closed.



Figure 2: Insertion of the tray.

- 17 Insert tray into device tray slot. Tray will “click” into place when fully inserted.
- 18 To capture the dorsal view, go to the **“Trays”** tab in the website, click, **“Capture New Tray.”**

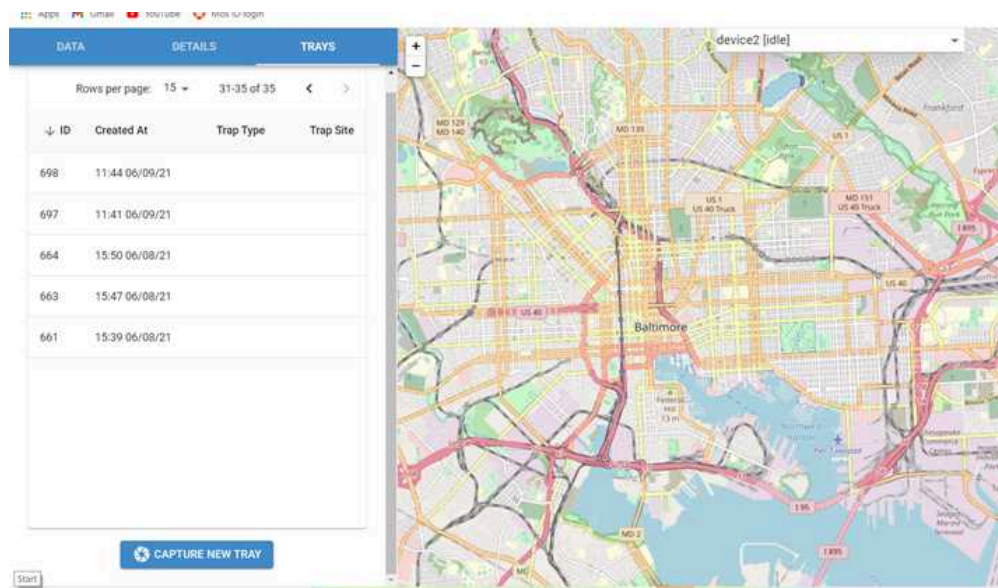


Figure 3: Screenshot of the “Capture New Tray” window.

19 MmosquitoesImages auto upload.

Note

NOTE:

- Writing the tray number and the well-ID on the side of the tube after the mosquitoes e-ID imaging process could be helpful for double-checking and labeling Dinoscope images when done several days apart and for TNA extraction information.

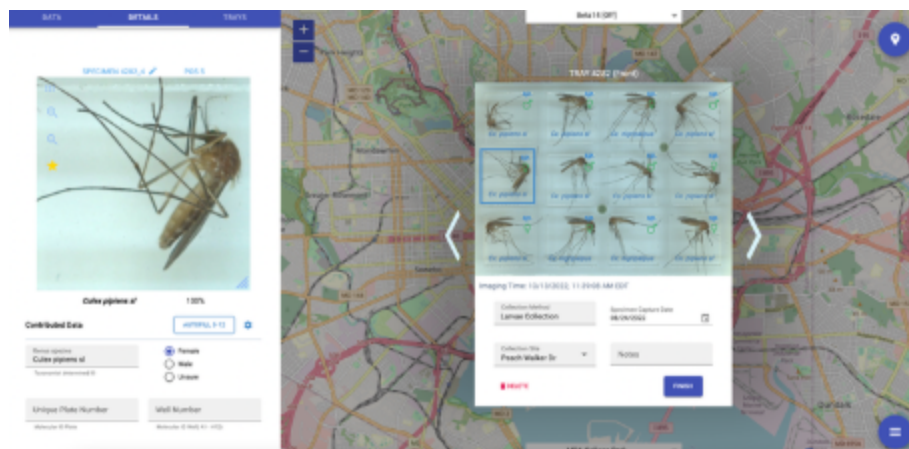


Figure 4: <https://www.vectech.io/idx-mosquito-control>.

20 Enter the following information:

20.1 Genus and species (if known).

Note

This field will autofill by clicking the arrow to view the next specimen. Autofill for this field is: previous specimen=current specimen. Once a field has data saved, autofill is no longer active.

20.2 Plate and well number.

Note

This field will autofill by clicking the arrow to view the next specimen. Autofill for this field is: for well, A1-H12, consecutive; for plate, previous specimen=current specimen. Once a field has data saved, autofill is no longer active.

21 Remove tray, flip upside down (i.e. dorsal to ventral), and insert tray again to capture the ventral image of the mosquito.

22 Click **"Capture Other Side"**.

23 Click **"Finish,"** and return specimens to original storage. Make a note of the tray number that corresponds to your sample row to facilitate data updates at a later date.

5. PREPARING DINOSCOPE

24 Place a portable chill table on the adjustable stand.

25 Place white porcelain stage directly on top of the chill table surface. If a porcelain stage is not available, cut a  5 cm x  square from a paper note card and use color lab tape to attach to the bottom of a petri dish to make a stage.

26 Once in place, secure the stage by taping either porcelain or petri dish stands to sides of the chill table.

27 Adjust the DinoScope holster on the stand so it is  4.5 cm above the surface of the stage.

Note

Note: make sure to secure the rear set screw just below the holster to secure placement on the vertical post. (See [Appendix 4](#) for more information).

- 28 Turn on the chill table (skip if not maintaining a cold chain) and wait until the surface temperature monitor reads $-20\text{ }^{\circ}\text{C}$.
- 29 Ensure all specimens slated for imaging are arranged in individual Eppendorf tubes and stored in 96-well racks labeled with a plate number.
- 30 Use a Kimwipe and 75% ethanol to wipe clean the stage surface. If using a petri dish stage, replace paper cards.

6. IMAGING USING DINOSCOPE

31

Note

Note: Flying vectors should be pooled to maximize sample processing efficiency (see pooling strategy QRG). A single, representative specimen from a pool of like specimens should be imaged (e.g., one female *Aedes aegypti* in a pool of twenty).

Remove a specimen from tube with forceps and place in the center of the stage with the dorsal side facing up.

- 32 Using the DinoScope interface and stand fine adjustment, bring the specimen into focus. Specimens should be centered in the field of view. Make sure all important characters are visible.

Note

NOTE: Additional adjustments may need to be made to the camera settings, possibly camera holster placement depending on size of specimen. (See [Appendix 1](#) for more information on DinoScope camera settings profile).

- 33 Annotate with the file naming convention: Sample ID-View (D = Dorsal, V = Ventral)
Example: RUSFV-00001D.

- 34 Ensure the correct magnification is entered into the Magnification field and that all metadata is included in the image output (magnification, magnification profile, scale, username and annotation) but does not overlap with specimen in the image.

Note

NOTE: Please do not use abbreviations in the annotations.

- 35 Just before taking an image readjust the white balance for **each individual** image to standardize the background color for all images.
- 36 Take a single image and review the photo to ensure quality (focus, frame, taxonomic characters). If the image is not satisfactory delete and repeat steps 31 to 35.
- 37 Use forceps to flip the specimen so that the ventral side is facing up.
- 38 Repeat steps 31 to 35 for ventral view.
- 39 Return specimen to tube and repeat steps 30 to 39 until a dorsal and ventral image are captured for all specimens.
- 40 Review both photos for quality and record mosquito characteristics.
- 41 When the imaging session is completed, copy photos from the dated folder and save.

7. BEFORE FLYING VECTOR HOMOGENIZATION

42

Note

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

Pre-cool the Bullet Blender by adding dry ice into the cooling compartment and running the cooling program.



- 43 Clean the work surfaces with RNaseZap, then wipe the surfaces with 70% molecular biology grade ethanol to remove additional contaminants.
- 44 Transfer $\pm$ 0.1 mm zirconium oxide beads (two spoons, Appendix 5) to Clear RINO brand 1.5 mL screw-cap microcentrifuge tubes.
- 45 For the 1st time use of the IndiMag pathogen kit, add 100% ethanol to Buffer AW1 and AW2, and add 100% Isopropanol to ACB as indicated on the bottles.
- 46 Buffer ATL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved. Prepare buffer ATL-DX: add $100\ \mu\text{L}$ Reagent DX to $15\ \text{mL}$ Buffer ATL. If smaller amounts are needed, transfer $1.5\ \text{mL}$ of Buffer ATL into a sterile 2 ml vial and add $10\ \mu\text{L}$ Reagent DX. Mix well, after addition of Reagent DX. After preparation, the mixture is stable for 6 months at Room temperature (15°C - 25°C).
- 47 MagAttract Suspension G from the IndiMag pathogen kit needs to be vortexed thoroughly for $00:03:00$ (before first use) or $00:01:00$ (before subsequent uses) to ensure that the magnetic silica particles are fully resuspended.
- 48 Prepare a few $15\ \text{mL}$ or $50\ \text{mL}$ conical centrifuge tubes with nuclease-free water for preparing TNA elution in KingFisher Flex or KingFisher Duo Prime to avoid cross-contamination.



4m

8. FLYING VECTOR HOMOGENIZATION

- 49 Clean forceps with 70% ethanol and Kimwipes before use and between samples.
- 50 Label navy RINO RNA lysis tubes on the cap.

Note

Avoid labeling on the side of tubes due to potential damage during beads beating process.



51 Transfer ≤ 20 individuals into the labeled RINO tube and place tubes on ice.



Note

NOTE: Pooling strategies will vary between labs. Consult a Gold Lab for feedback when designing a testing strategy.

52 Add  400 μL cold sterile 1xPBS into each navy RINO tube with flying vectors.

53 Ensure the Bullet Blender is fully cooled down. Add more dry ice into the cooling compartment of Bullet Blender, if necessary, then load the RINO tubes with the samples.

54 Set the controls for Speed 10 and Time 3. Press Start.

55 Centrifuge the suspension at  100 x g, 4°C, 00:01:00 to pellet debris.

1m



56 Without disturbing the tubes, carefully transfer the top  320 μL supernatant into  1.5 mL tubes.

9. MICROBE LYSIS

57



Note

NOTE: Check section 7 for the buffer preparation and storage details, before setting up microbe lysis and KingFisher instrument.

Add  80 μL of ATL-DX Lysis Buffer to the  1.5 mL tubes containing  0.1 mm beads from step 44 and label the sample or pool ID on the tubes. Also, prepare two tubes for positive and negative controls.

58 Transfer  320 μL homogenate (individual or pooled) to each of the pre-labeled bead tubes.



- 59 Include a positive control for each batch of samples: transfer  37.5 μL ZymoBIOMICS Microbial Community Standard Material into a tube from step 57. Add  282.5 μL 1x PBS to make the final control mix volume 320 μL .
- 60 Include a negative control for each batch of samples: a bead tube with  320 μL cold sterile 1xPBS only.
- 61 Add more dry ice into the cooling compartment of Bullet Blender, if necessary and then load the all bead tubes (samples and controls).
- 62 Set the speed at 12 and time at 3. Press Start.
- 63 Let the samples settle for  00:01:00 and then repeat step 62. 
- 64 Centrifuge the tube at  120 x g, 4°C, 00:05:00 . 
- 65 Carefully transfer the  350 μL supernatant from the RINO tube to a new snap-cap  1.5 mL microcentrifuge tube, avoiding bead carryover (slight bead contamination is tolerated).  

Note

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

10. INSTRUMENT SET UP

66

Note

NOTE: KingFisher Flex only, if using KingFisher Duo Prime, go to section 11

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

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

10.1 SET UP THE PROCESSING PLATES

68 Set up the Wash, Elution, and Tip Comb 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

10.2 EXTRACTION

69 Add  20 µL of Proteinase K into wells (based on number of samples) of a new Deep-well plate.



- 70 Transfer  270 μL supernatant of step 65 without any particle carryover to the wells of the Deep-well plate containing proteinase K. This plate becomes the Sample Plate.
- 71 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 (can be mixed on Hula mixer for 2 min). Add  695 μL mixture to each sample. 
- 72 Select the program **IndiMag_Pathogen_KF_Flex_4wash** on the instrument.
- 73 Start the run, then load the prepared plates into position when prompted by the instrument.

10.3. QUANTIFICATION AND STORAGE

- 74 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.
- 75 In a  0.6 mL microcentrifuge tube, use  1 μL total nucleic acid for DNA and RNA concentration measurement using Qubit 4 Fluorometer following manufacturer instructions.

Note

Kits needed: Qubit 1X dsDNA HS Assay Kit and Qubit RNA HS Assay Kit. (see Appendix 6)

- 76 Proceed with sample testing following the REDI-NET SOP FV-4 Flying vector Testing or store at  -20 $^{\circ}\text{C}$ for less than 2 weeks.

Note

For long-term storage the sample needs to be stored at  -80 $^{\circ}\text{C}$ following the REDI-NET SOP FV-3 Flying Vector Storage.

11. INSTRUMENT SET UP

77

Note

NOTE: KingFisher Duo Prime only, if using KingFisher Flex, go to section 10.

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

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

11.1 SET UP THE SAMPLE PLATE AND ELUTION STRIP

- 79 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	700 µL
		G	Empty	
		H		

- 80 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
	Row ID	Plate Row	Reagent	Volume per well
	Elution	A	Nuclease-free water	75 µL

11.2. EXTRACTION

81 Add  20 µL of Proteinase K into wells (based on number of samples) of a sample row. 

82 Transfer  270 µL supernatant from step 69 without any particle carryover to the wells of the sample row containing proteinase K.

83 Add  135 µL Buffer VXL,  540 µL Buffer ACB, and  20 µL MagAttract Suspension G to each sample in the sample row. For multiple samples, make a master mix with 10% overage. Invert slowly to mix the master mix, avoid foaming (can be mixed on Hula mixer for 2 min). Add  695 µL mixture to each sample. 

84 Select program **IndiMag_Pathogen_KF_Duo_4wash** on the instrument.

85 Start the run, then load the prepared plate/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.

11.3 QUANTIFICATION AND STORAGE

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

- 87 Use  1 μL total nucleic acid for DNA and RNA concentration measurement using Qubit 4 Fluorometer.

Note

Kits needed: Qubit 1X dsDNA HS Assay Kit and Qubit RNA HS Assay Kit. (see Appendix 6).

- 88 Proceed with sample testing following the REDI-NET SOP FV-4 Flying Vector Testing or store at  -20 °C for less than 2 weeks.

Note

For long-term storage the sample needs to be stored at  -80 °C following the REDI-NET SOP FV-3 Flying Vector Storage.

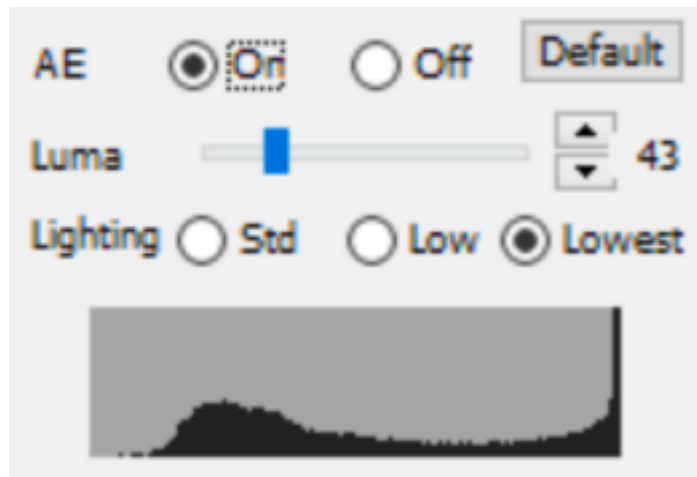
APPENDIX 1. DINOLITE DIGITAL MICROSCOPE CAMERA SETTINGS

89



CAMERA SETTINGS

90



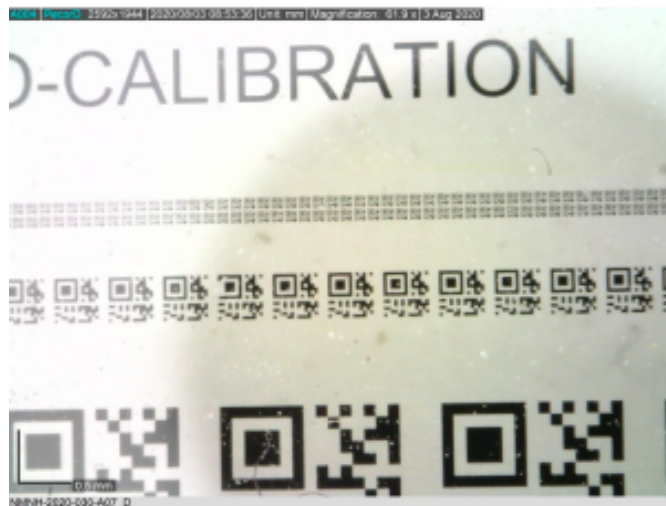
AUTO EXPOSURE SETTINGS

91



LED BRIGHTNESS

92



CALIBRATION

Note

NOTE: Use the automatic calibration target included with the DinoScope to calibrate the camera each time the camera position is changed. For more information on how to calibrate the DinoScope, view this video: <https://www.youtube.com/watch?v=mivZvOLtEBA>

APPENDIX 2. SAMPLE IMAGE

93



APPENDIX 3. DINO-LITE DIGITAL MICROSCOPE

94

**Dino-Lite**
Digital Microscope

Dino-Lite US | Dunwell Tech, Inc

sales@dunwell.com
888-668-2442
19803 Hamilton Ave
Torrance, CA 90502

AM7115MZT

\$995

Product Images



Description

The Dino-Lite 5MP Edge AM7115MZT delivers unsurpassed image quality with a magnification range from 20x to 220x and now with illumination flexibility options. Integrated with cutting-edge optics, advanced 5MP sensor, and low-loss MJPEG compression, the AM7115MZT provides incredibly crisp and fluent imaging with low power consumption. The innovative Flexible LED Control (FLC) enables partial illumination to enhance edge contrast and provides intensity adjustment for better adaptability. Together with built-on polarizer, interchangeable front caps, and rugged metal housing, the AM7115MZT is made to be the tool of choice for wide range of applications.

1<https://www.dinolite.us/en/am7115mzt>

Additional Information

Measurement	Yes
Flexible LED Control	Yes
Automatic Magnification Reading	No
Extended Depth of Field	No
Enhanced Dynamic Range	No
Wireless Ready	No
Magnification Lock	Yes
Body Material	Aluminum Alloy
Microtouch Sensor	Yes
LEDs	White (8)
Axial	No
Polarization	Yes
Excitation Wavelength (LED)	N/A
Emission Wavelength (Filter)	N/A
Magnification Range	10x - 220x
Working Distance	Standard
Lens Type	Glass
Megapixels	5 MP
Image Save Formats (Windows)	BMP, GIF, PNG, JPG, TIF, RAS, PNM, TGA, PCX, MNG, WBMP, JP2, JPC, PGX
Image Save Formats (Mac OS)	JPEG, PNG
Video Save Formats (Windows)	WMV, FLV, SWF
Operating System	Windows XP, Vista, 7, 8, 10, Mac OS 10.5+
Connection Type	USB 2.0
Included Software	DinoCapture 2.0 (Windows), DinoXcope (Mac OS)
Imaging Standards	UVC
Sensor Type	CMOS
Resolution	2592 × 1944 pixels

2

<https://www.dinonline.us/en/lan7115enut>

Frame Rate (max)	30 FPS
Dimensions	10.5cm (L) x 3.2cm (D) (4.13" x 1.26")
Weight	135g (4.76oz)
Cable Length	182cm (71.65 in)
Package Includes	Microscope, Carry Pouch, Software CD, Edge Calibration Target, Alternate end caps
Warranty Period	2 years
Manufactured in	Taiwan
Service & Support	U.S. (www.dinolite.us) English & Spanish
Regulatory Approval	CE, FCC



APPENDIX 4. MS35BE ELECTROSTATIC DISCHARGE PROTECTION MODEL



sales@dunwell.com
888-668-2442
19803 Hamilton Ave
Torrance, CA 90502



MS35BE

\$129

Product Images



Description

The MS35BE Electrostatic discharge protection model, is a versatile, lightweight and economical vertical stand with fine adjustment designed for use with the Dino-Lite hand held digital microscope cameras. It has a range of up to 10 inches of vertical working distance from object (up to 20 inches with the optional extension post MSA2P). The main body assembly with built in fine adjustment slides up or down into position on the vertical post and can be rotated 360 degrees. The Dino-Lite handheld microscope (not included) slides directly into a holster which is mounted onto a peg located on the main body. A ball and socket type peg allows for up to 45 degrees of adjustable lateral viewing angles while an optional, separate rigid vertical peg provides a dedicated straight-on view allowing for more accurate measurements. The precision machine crafted components provide smooth adjustment and positioning in a well balanced and stable ergonomic design. Manufactured from a combination of resilient stainless steel and lightweight recycled aluminum, the MS35BE can easily and conveniently be moved from one work area to another and will endure the harshest of work

1

<https://www.dinolite.us/en/ms35be>

environments

Additional Information

Warranty Period	1 year
Manufactured in	China
Service & Support	U.S. (www.dinolite.us) English & Spanish
Regulatory Approval	ROHS
Dimensions	12.5cm(W) x 20.5cm(L) x 30.5cm (H)/ 4.92" x 8.07" x 12"
Weight	1.1kg/ 2.42lbs.
Material	Stand: Metal, Holster: Plastic, Base: Metal
Flexibility	Multi Angle
Compatible with	All Dino-Lite Handheld Microscopes
Package Includes	Stand, Holster
Package Dimensions	30.5cm(L) x 15.2cm(W) x 6.7cm(H)/ 12" x 5.98" x 2.64"
Package Weight	1.2kg/ 2.65lbs.



2

<https://www.dinolite.us/en/m35be>

APPENDIX 5. MEASURING SPOON FOR 0.1 mm BEATING BEADS

- 96 The spoon (Next Advance, MSP01-RNA) is used for 0.1 mm beating beads measurement. The step is described on 6.6.4 the preparation before homogenization. One spoon

equals to 100 μL .



APPENDIX 6. DNA and RNA Measurement Using QUBIT FLUOROMETER 4.0

97 DNA quantification:

According to the volume of sample used, add the 1xHS dsDNA Qubit Assay for a final volume of 200 μL (i.e., if using 1 μL of sample, add 199 μL of 1x HS dsDNA Qubit Assay.

98 RNA Quantification:

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

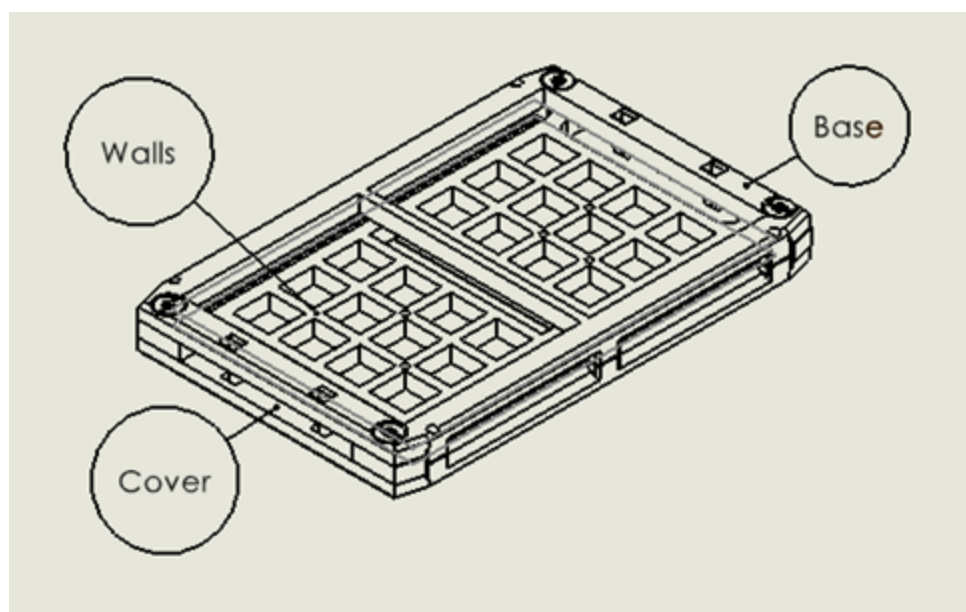
	A	B	C
	Reagents	Volume/sample	Volume for n+1 sample
	Qubit RNA HS Assay buffer	199 μL	 μL

	A	B	C
	Qubit RNA HS Assay Dye	1 μ L	 μ L

- 98.1 In a new 0.6 mL tube, mix 199 μ L of Qubit HS RNA Assay working solution and 1 μ L of the sample. Incubate for 1 minute at room temperature before reading.

APPENDIX 7. IDX TRAY STANDARD OPERATING PROCEDURES

99 Naming Conventions



100 Guidelines

- 100.1 Use gloves while handling the tray.
- 100.2 The tray should be cleaned prior to every imaging session according to the steps provided in this SOP.
- 100.3 Specimens should be as free of ethanol as possible before being placed in the tray.



100.4 The tray's cleanliness should be checked every 10 captures. If debris or smudges are observed, clean with lens cleaner and wipes, removing the tray walls to clean as necessary.

101 **Tray Cleaning: Materials**

101.1 Vectech IDX tray

101.2 Lens cleaner, glass cleaner or ethanol

101.3 Kimwipe or microfiber cloth

101.4 Phillips head screwdriver

102 **Tray Cleaning: Steps**

102.1 Remove the screws that secure the tray walls to the base of the tray and set the tray walls aside.

102.2 Apply a lens cleaner to the glass and wipe the area with a kimwipe or microfiber cloth. Rub the area lightly until the area is dry and free of debris. Repeat for both the top and bottom of the tray base and cover.

102.3 Hold up the tray to a light and look through the glass to check for remaining smudges or debris. Clean remaining areas as necessary.

102.4 Replace the tray walls onto the base of the tray (the half with the gold inserts). The indentations in the tray wall piece should be facing the gold inserts in the tray base. Replace the screws.



Protocol references

REFERENCES

REDI-NET Overview Summary

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