# GenTegraRNA-NEO™
# for Microsampling Tips
## Mitra® Device & 96-Autorack
## User Guide

**Adding Active Chemical Protection™**
**of RNA to the VAMS® tips on Mitra® Devices**

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For Research Use Only

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# 1 Overview

GenTegraRNA-NEO is a novel room temperature stabilization technology developed specifically for use with the Neoteryx Mitra devices with VAMS tips. This user guide provides the instructions for using the GenTegraRNA-NEO to treat the VAMS tips in preparation to collecting samples for the purpose of extracting stabilized RNA.

When needed, simply rehydrate the GenTegraRNA-NEO in preparation for treating the needed tips. When the matrix in the tube is rehydrated there is sufficient volume to treat 32 VAMS tips or eight Mitra devices that have four microsamplers per device.

## 1.1 Product Information

| | GenTegraRNA-NEO |
|---|---|
| **Catalog Number** | **GTRNEO-32** |
| **Product form** | **Dried material in 2ml vial** |
| **Shelf life as the dry matrix** | **3-years at room temperature; 6-months as a liquid stored at 4°C** |
| **Rehydration volume** | **1,100µL of molecular biology grade water** |
| **Solution Volume per tip** | **30µl, dried on the tip before use** |
| **Sample Amount** | **30µL of blood or other liquid sample** |
| **RNA concentration** | **Any** |
| **RNA Recovery** | **Equivalent to Sample volume** |
| **Drying Method** | **Air dry in biosafety hood** |

Upon arrival, GenTegraRNA-NEO is a dry rust colored material in the bottom of the tube. In the dry form GenTegraRNA-NEO has a shelf life estimated to be at least three years. When re-hydrated the solution may be stored at 4°C for up to 6-months.

If the pellet is not at the bottom of the tube, briefly centrifuge the tube before opening to ensure all the matrix is at the bottom of the tube before adding water.


**Figure 1** - Tube of GTRNEO-32, manufactured by GenTegra, in a 2mL tube with white cap. Each tube is sealed in a metal coated Mylar zip lock envelope.

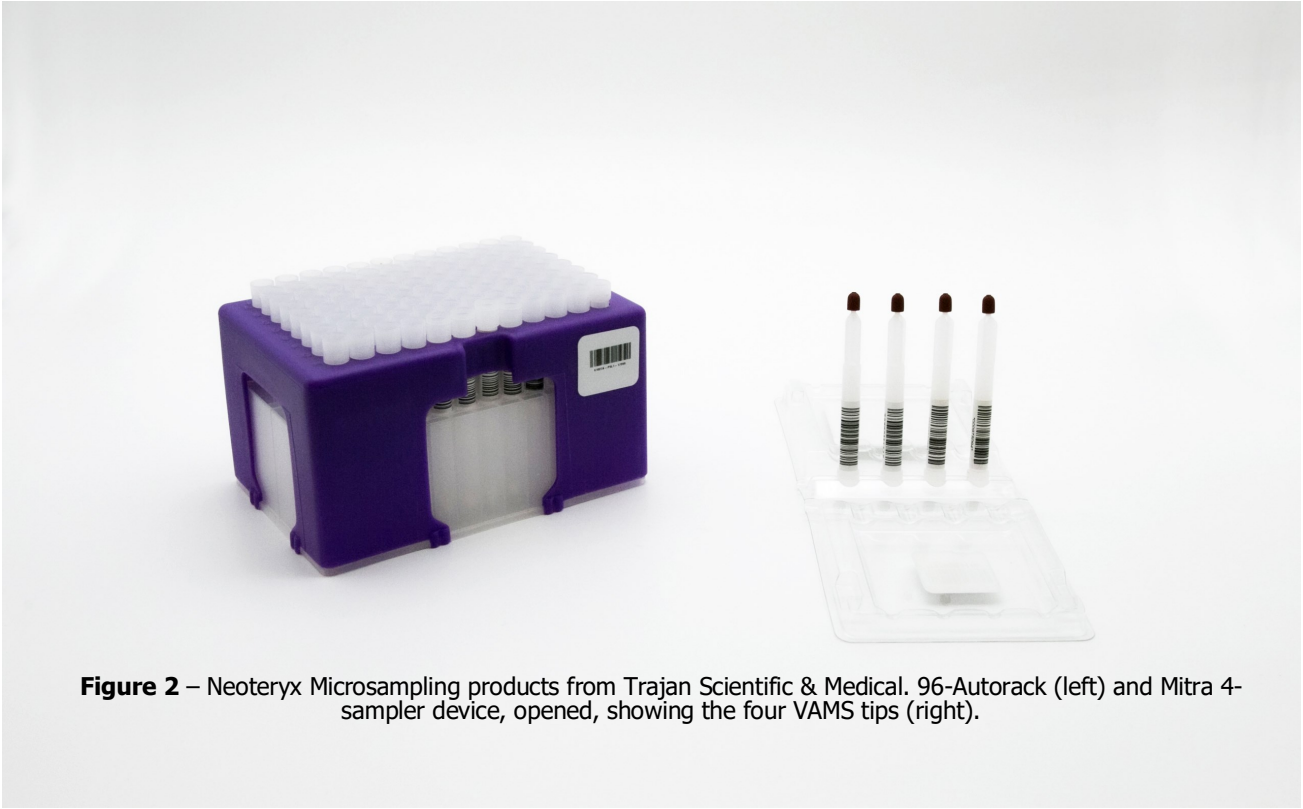


**Figure 2** – Neoteryx Microsampling products from Trajan Scientific & Medical. 96-Autorack (left) and Mitra 4-sampler device, opened, showing the four VAMS tips (right).

## 2 GenTegraRNA-NEO Preparation Protocols

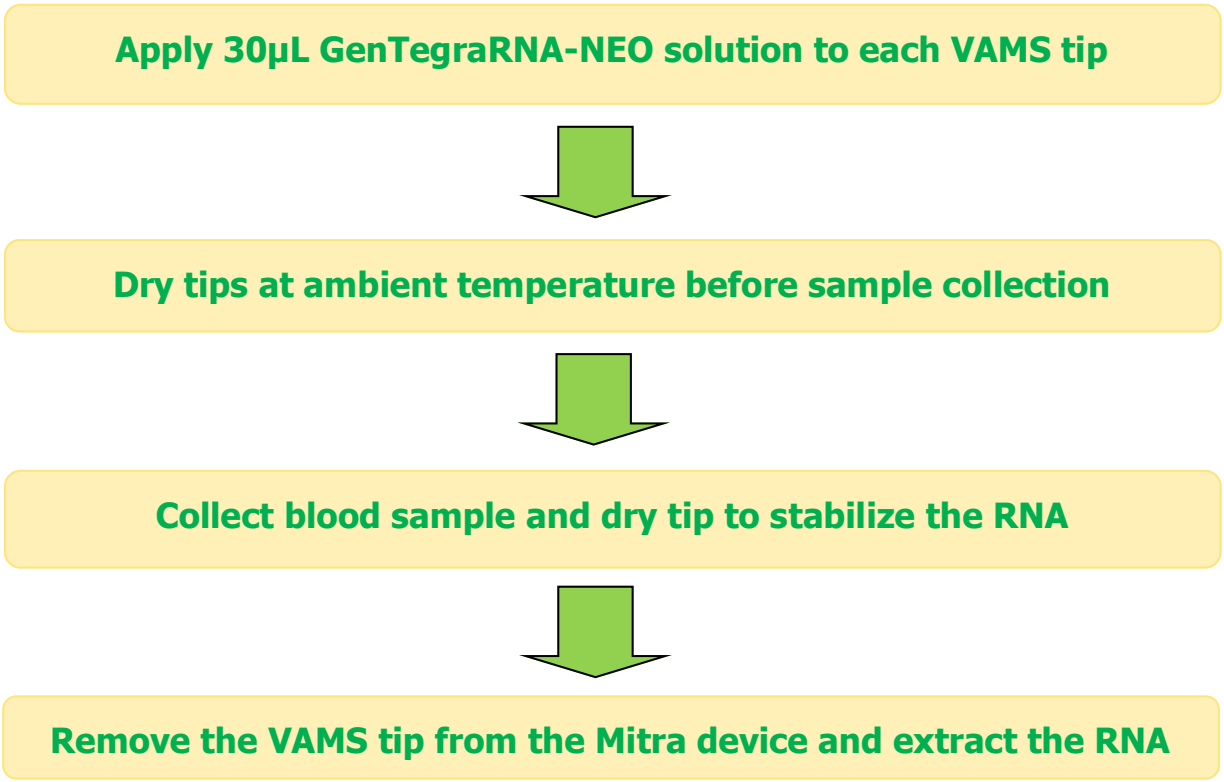



**Figure 3** - Simplified Workflow of GenTegraRNA-NEO Preparation

## 2.1 Rehydrating GenTegraRNA-NEO

The dry matrix must be rehydrated before it can be applied to the VAMS tips of the Mitra device.

1. Remove the cap from the tube and add 1100µL of molecular biology grade water to the tube and replace the cap.
2. Vortex the tube for 30 seconds then place the tube in a lab centrifuge and briefly spin at maximum speed to ensure all the solution is at the bottom of the tube. The solution is now ready for treating the Mitra devices.

## 2.2 Storing rehydrated GenTegraRNA-NEO solution

If you are treating fewer than 32 tips at a time and have extra solution left, the GTR-NEO rehydrated solution can be stored at 4°C for up to 6 months.

## 2.3 Treating VAMS tips on Mitra devices with GenTegraRNA-NEO solution

The solution prepared as above is ready to treat up to 32 of the Mitra devices with 30µL VAMS tips.

**Figure 4**

**Figure 5**

**Figure 6**

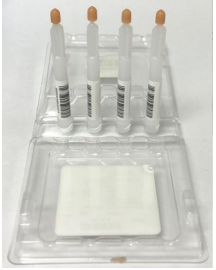


**Figure 7**

1. Place the number of Mitra devices (in this example we are using one 4-sampler Clamshell device but this will work with other device housings) you intend to treat within convenient reach (Figure 4)
2. Remove one Mitra sampler body with the attached VAMS® tip and place the tip into the reagent tube so about 20-50% of the tip is submerged in the solution. The solution will rapidly wick up into the tip. When color reaches the top of the tip, wait 5-seconds and then remove the sampler body with the treated tip from the solution (Figure 5)
3. Place the Mitra sampler body back into the device housing (Figure 6).
4. Repeat steps 2-3 for the remaining 3 sampler bodies so that all 4 VAMS tips are identically treated with reagent (Figure 7)
5. Repeat steps 2 through 4 for the remaining Mitra devices to be treated. The solution in the tube will treat 32 VAMS tips or 8 Mitra 4-sampler devices.
6. Place the treated Mitra devices in a clean area with good air flow for dying. A molecular biology hood is recommended and drying should be complete in 12-24 hours. See section 2.5 for more details on different methods for drying the treated tips.

If the VAMS tips do not dry overnight, then additional effort should be taken to reduce humidity and drying time to less than 24 hours. Do not use heat in excess of 37°C to accelerate the drying process. After the samples are dry, they are stable and can be stored at up to 30°C through the expiration date printed on the Mitra device. The GenTegraRNA-NEO treatment of the tips has no impact on the original VAMS tip warranty.

**NOTE**: If utilizing Mitra VAMS in specimen bags for a study, use scissors to cut the specimen bags at the top. After treating the device with the GenTegraRNA-NEO solution and drying the VAMS tips, return to the specimen bag and reseal the zip closure. Adding a gel desiccant packet would also be advised.

## 2.4 Treating the Mitra 96-Autorack tips with GenTegraRNA-NEO solution

If you are treating the 96 VAMS tips of the 96-Autorack with GenTegraRNA-NEO solution you will need an additional standard height 96-well V-bottom microplate and four tubes of GenTegraRNA-NEO, part number GTRNEO-32, and the 96-Autorack.

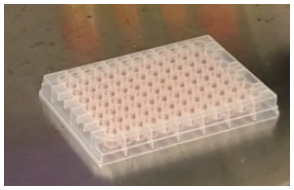


**Figure 8** V-bottom standard height microplate with 40µL of GenTegraRNA-NEO in each well. Notice slight orange color.

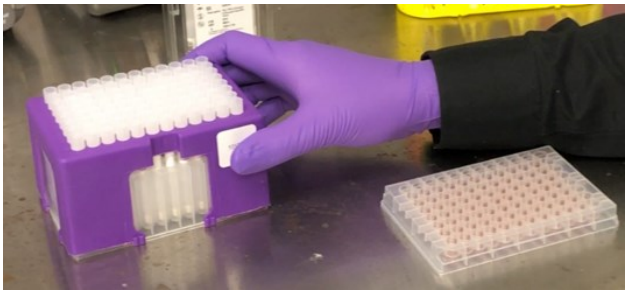


**Figure 9** Place the 96-Autorack near the microplate.

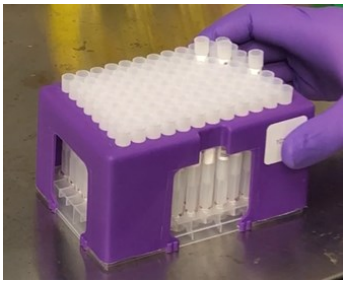


**Figure 10** Lift the rack of 96 Mitra devices off its protective high wall microplate and plate the rack of devices over the microplate. Some devices may not immediately drop into the well. Touch these gently and they will drop into place, then push the rack of device so it is flush with the bench surface.

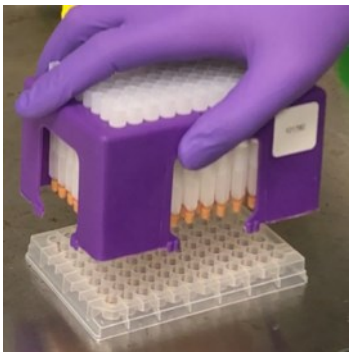


**Figure 11** After ten seconds with the tips in the solution, lift the rack off the microplate. You may need to hold the microplate down.

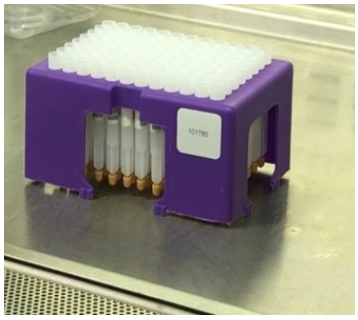


**Figure 12** Place the treated rack into a molecular biology hood to dry.

1. Pipette 40µL into each well of a standard height V-bottom microplate. Each tube of GenTegraRNA-NEO2 will provide enough solution to fill 26 wells with some excess remaining.
2. Place the rack holding the 96 Mitra samplers over the microplate and press down so rack is flush and bottomed on the surface where the microplate is sitting
3. Wait 10 seconds for solution to fill the tips
4. While holding the microplate down, carefully remove the rack holding the 96 tips from the microplate
5. Place the rack of tips in clean safe space to dry, a molecular biology hood is recommended, for about 12-24 hours at room temperature

If you plan to treat more than one 96-Autorack of Mitra samplers, you will need an additional three GTRNEO-32 tubes for each additional 96-Autorack. Rehydrate each GTRNEO-32 tube as outlined above. Add 30µL of GTR-NEO solution to each well of the microplate and repeat steps 2. through 5.

### 2.4.1 Storing GenTegraRNA-NEO treated Mitra devices

The dried GenTegraRNA-NEO formulation is extremely stable and has a shelf life of >3-years at room temperature. Mitra devices with treated VAMS tips should be stored according to the recommendations for the untreated Mitra devices. Store the Mitra devices with treated VAMS tips at up to 30°C through the expiration date printed on the Mitra device.

## 2.5 Drying methods for GenTegraRNA-NEO treated Mitra devices

### 2.5.1 Using a molecular biology hood

1. Place the treated Mitra devices in the hood where they will not be disturbed.
2. Turn the hood air flow on if necessary.
3. Dry tips for approximately 12-24 hours.
4. After drying the tips, they are stable and can be stored at up to 30°C through the expiration date printed on the Mitra device.

### 2.5.2 Using a vacuum chamber

A Vacuum may be used to dry treated tips faster. Drying time in a vacuum is approximately 3-4-hours.

1. Place the treated and closed Mitra devices in the vacuum chamber, such as a SpeedVac without heat or cooling.
2. Ensure that the temperature setting does not exceed 37°C.
3. Dry tips for approximately 3-4 hours.
4. After drying the tips, they may be stored at 30°C through the expiration date printed on the Mitra device. The GenTegraRNA-NEO treatment of the tips has no impact on the original VAMS tip shelf life.

### 2.5.3 Using a DIY Vacuum Desiccator

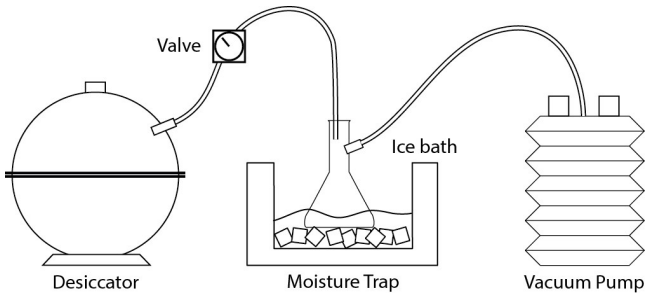



**Figure 8**—Schematic of a DIY Vacuum Desiccator

A Vacuum Desiccator may be used to dry the GenTegraRNA-NEO treated tips. Drying time is approximate and may need to be modified based on the system. The system consists of a vacuum desiccator, vacuum pump, a vapor trap, assorted tubing and a small ice bath. After the first use, ensure that tips are completely dry by visually inspecting .

1. Place the treated and closed Mitra devices conveniently in the desiccator. Close desiccator and turn on vacuum pump.
2. Dry the tips for approximately 3-4 hours.
3. Following drying, treated tips can be stored at 30°C through the expiration date printed on the Mitra device. The GenTegraRNA-NEO treatment has no impact on the original Mitra device shelf life.

# 3 Using GenTegraRNA-NEO treated Mitra devices

## 3.1 Blood sampling using the GenTegraRNA-NEO treated Mitra devices

Sample blood on the treated VAMS tips in the Mitra devices according to the instructions provided by Trajan.

## 3.2 RNA extraction protocol for Neoteryx Mitra devices with 30µL VAMS

The following outlines steps for sample extraction, lysis and RNA recovery prior to purification.

1. Add 2 tips to 2mL microcentrifuge flip top tube. See following for recommend tip removal methods. The capacity of your centrifuge rotor will determine the largest number of samples you can process at the same time.
2. Add 1mL Ambion TRIzol Reagent (Ref: Invitrogen Part#15596018) to each 2mL tube and vortex for ~20 seconds.
3. Incubate samples on a nutating mixer for 5 minutes at RT.
4. Remove tubes from the mixer and centrifuge tubes at max speed for 2 minutes.
5. Prepare a matching number of dolphin tubes with spin baskets (Ref: GVSPIN-200) inserted.
6. Transfer the liquid to the 2mL dolphin tube with spin basket. Carefully transfer tips into spin basket*.
7. Centrifuge the dolphin tube at max speed for 2 minutes.
8. Transfer all liquid from 2mL dolphin tube to 1.5mL microcentrifuge flip top tube.
9. Centrifuge the dolphin tube with spin basket (containing tips) at max speed for 5 minutes.
10. Transfer remaining liquid from the dolphin tube to the 1.5mL microcentrifuge tube. Discard dolphin tube, spin basket and tips.
11. Add 200µL Phase Separation Reagent, BCP (1-bromo-3-cholorpropane), to the 1.5mL microcentrifuge tube, close the top and vortex for ~20 seconds.
12. Place sample tubes on nutating mixer for 5 minutes at RT.
13. Centrifuge sample tubes at 12,000xg for 15 minutes at 4°C.
14. Remove 400µL of upper aqueous layer from samples and transfer to a new 2mL flip top microcentrifuge tube. The 2mL flip top tube may be replaced with a GenTegra RNAssure tube to provide enhanced RNA protection at room temperature.

**NOTE**: To use this protocol for extraction of liquid whole blood simply eliminate steps 4 through 10.

* Spin baskets are available from GenTegra as part number GVSPIN-200, which is a package of 200 tubes and baskets.

## 3.3 VAMS tip removal methods

### 3.3.1 Method 1: Using tweezers

Using a pair of tweezer carefully pull the VAMS tip off the sampler body.

Place the tip into a flip-top microcentrifuge tube. Repeat as needed.

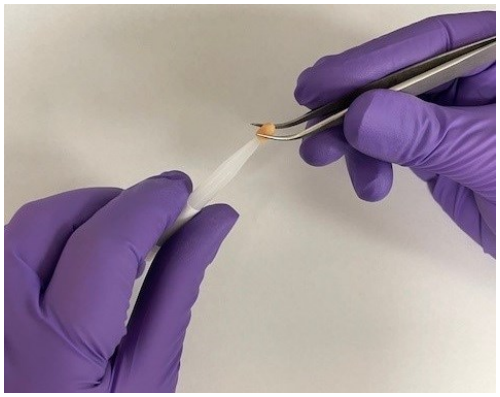


**Figure 9 a**—Holding the plastic carrier gentle pull the VAMS tip off the sampler body.

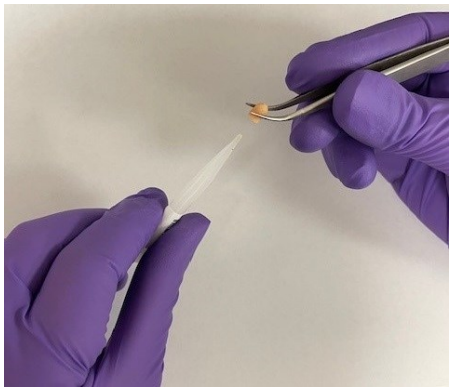


**Figure 9 b**—After removal place the VAMS tip in a microcentrifuge tube

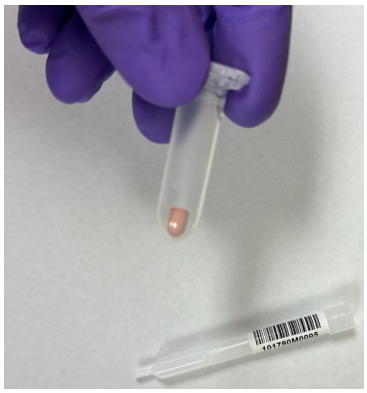


**Figure 9 c**—VAMS tip in the microcentrifuge tube.

### 3.3.2 Method 2: Using a micro-centrifuge tube

Use the cap of the flip-top centrifuge tube to gently pull the VAMS tip off the Metra device.

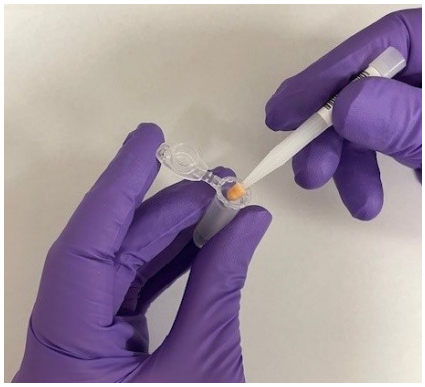


**Figure 10 a**—Holding the Mitra sampler body, place the VAMS tip inside the microcentrifuge tube.

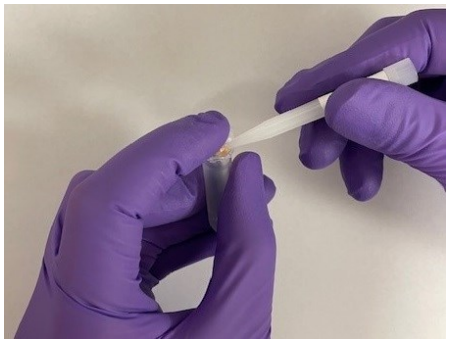


**Figure 10 b**—Close the flip top to hold the VAMS tip inside the microcentrifuge tube.

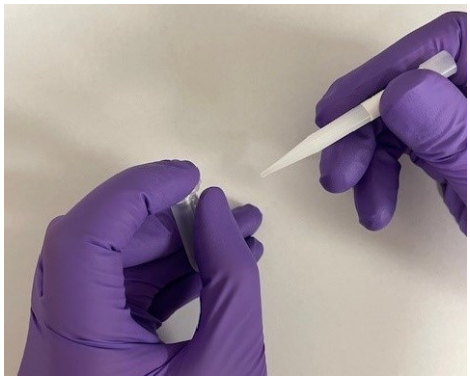


**Figure 10 c**—Gently pull the Mitra sampler body to dislodge the VAMS tip into the microcentrifuge tube.

Repeat as needed.

## 4 RNA Column Purification

The procedure outlined below is based on the Thermo Fisher Purelink RNA kit Cat# 12183020 (10 preps); 12183018A (50 preps); 12183025 (250 preps)

1. Create Lysis mix: Add 500µL PureLink Lysis buffer + 500µL 100% Ethanol per sample. Add 10µL BME, (2-mercaptoethanol) for every 1mL of Lysis buffer used.

   Example: For 10 samples, add 5mL Lysis buffer + 5mL 100% Ethanol + 50µL BME.

2. Add 800µL Lysis mix to 400µL of aqueous phase from step 14. Close the lid and vortex for ~20 seconds then centrifuge sample tubes for ~10 seconds at low speed to ensure there is no liquid on the tube lids.
3. Transfer 700µL of lysate into PureLink spin-column and spin at 12,000xg for 30 seconds. Discard flow-through in collection tube and reinsert spin-column into the same collection tube.
4. Repeat steps 1 to 3 with the remaining volume of lysate.
5. Add 700µL PureLink Wash Buffer 1 to spin-column and centrifuge at 12,000xg for 30 seconds. Discard collection tube and liquid. Replace it with a clean collection tube.
8. Add 500µL Wash Buffer 2 to spin-column and centrifuge at 12,000xg for 30 seconds. Discard flow-through in collection tube and reinsert spin-column into the same collection tube. Spin empty column and collection tube at 12,000xg for 2 minutes to ensure the spin-column is fully dry.
9. Remove spin-column and add it to a clean 1.5mL microcentrifuge tube.

**Note:** For better protection of the RNA the final collection tube can be replaced with a GenTegra RNAssure tube, catalog number GTR50-LQ.

10. Add 100µL of PureLink RNase free water to elute the RNA. Wait 2-5 minute and centrifuge at 12,00xg for 1 minute.
11. Discard spin-column and immediately store samples at 4°C for short term storage or freeze at -80°C for long term storage. Immediate refrigeration or freezing is not required if an RNAssure tube is used in step 9.

**Note:** Purified RNA may also be transferred to a GenTegraRNA tube, catalog number GTR5100-S, and dried for long term room temperature storage and for protection when shipping the RNA sample at ambient temperature to a sequencing service laboratory for RNAseq.

# 5…Technical Information

## 5.1 Expected Results

- Quantitative recovery of RNA
- Quality is comparable to input RNA in the original sample

## 5.2 Storage and Transport of RNA samples on GenTegraRNA-NEO treated tips

Transport conditions: 15°C to 30°C

## 5.3 Tested Applications Compatible with GenTegraRNA-NEO

- tRNA extraction from whole blood
- vRNA from blood

# 6 Application Information

## 6.1 RNA recovery from GenTegraRNA-NEO treated Mitra devices

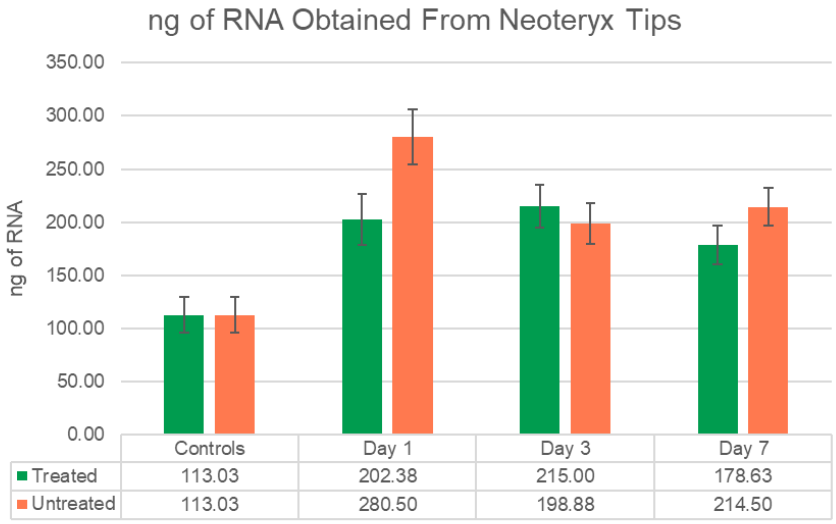



Figure 1: ng of RNA obtained from RNA extraction of 1 Mitra tip (30uL of dried whole blood) and eluted in 50uL of molecular grade water. Samples were extracted on days 1, 3 and 7, controls are WB samples that were extracted using the same method immediately after blood collection (GenTegra Data).

## 6.2 RNA Integrity (RINe) for GenTegraRNA-NEO treated Mitra Devices

### Protection is demonstrated at room temperature

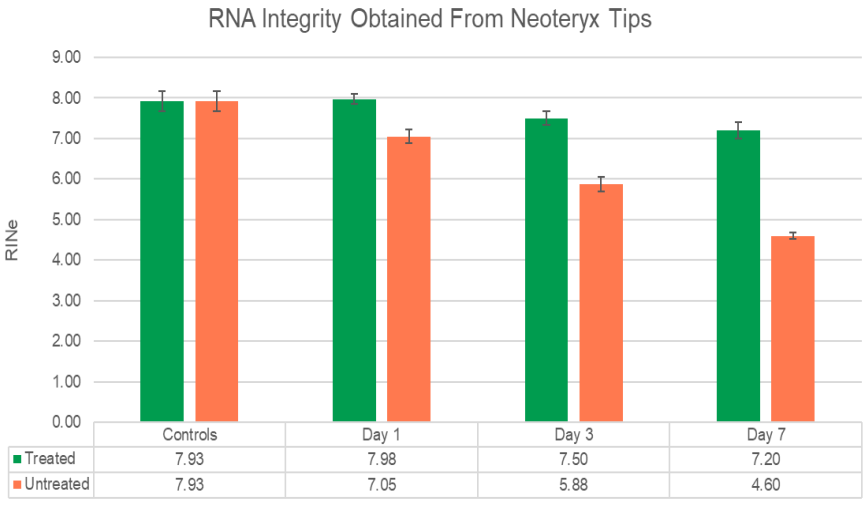
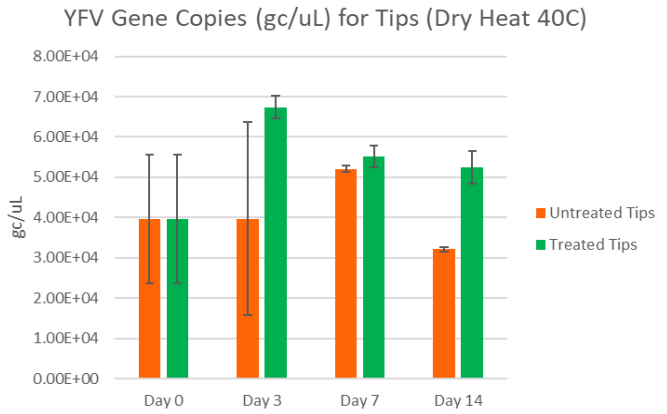
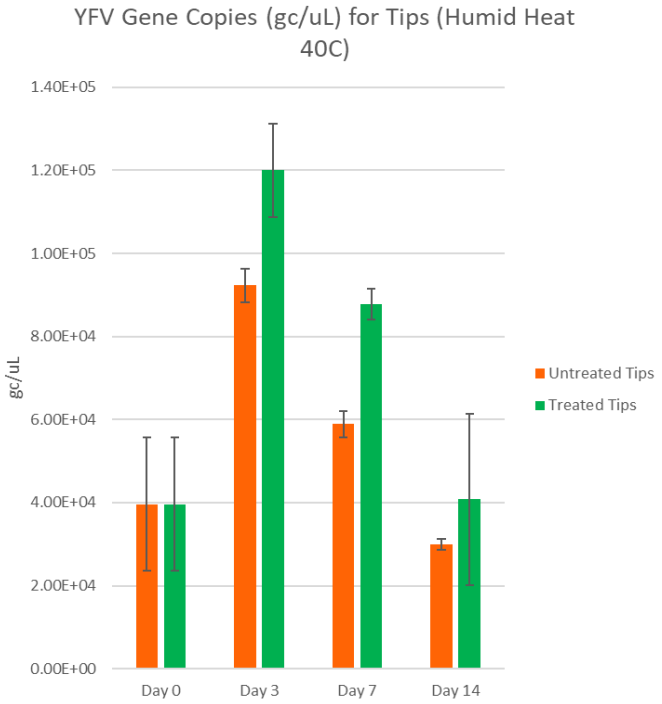



Figure 2: RINe results obtained from Tape Station 4200 after analyzing RNA extracted from 30uL of blood from 1 Mitra tip per sample, samples were extracted on days 1, 3 and 7, Controls are liquid WB samples extracted with the same method as the tips, but immediately after the blood was collected. (GenTegra Data).

## 6.3 Stabilization of RNA from Yellow Fever Virus Samples

### Mitra devices treated with GenTegraRNA-NEO supports samples collected in remote environments

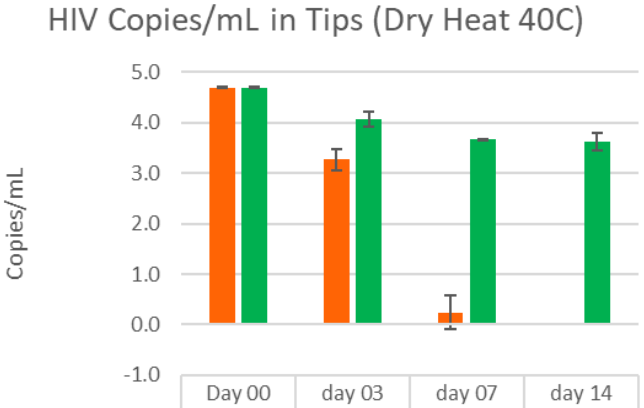


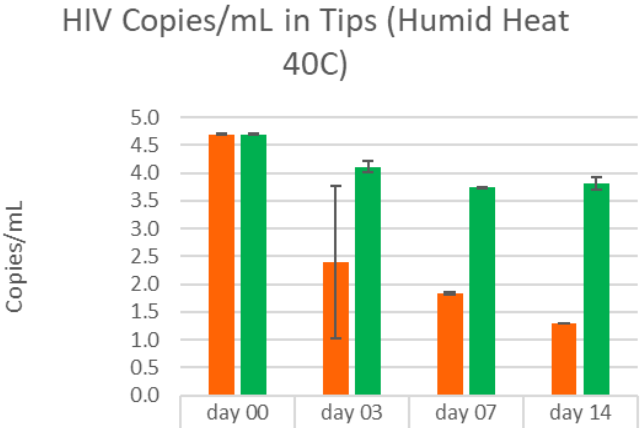

Number of tips extracted two per data point each data point two replicates

## 6.4 Stabilization of RNA from HIV Samples

### Mitra devices treated with GenTegraRNA-NEO prior to use




| | Day 00 | day 03 | day 07 | day 14 |
|---|---|---|---|---|
| Untreated Tips | 4.7 | 3.3 | 0.2 | 0.0 |
| Treated Tips | 4.7 | 4.1 | 3.7 | 3.6 |




| | day 00 | day 03 | day 07 | day 14 |
|---|---|---|---|---|
| Untreated Tips | 4.7 | 2.4 | 1.8 | 1.3 |
| Treated Tips | 4.7 | 4.1 | 3.7 | 3.8 |

RT-qPCR data shows that viral load is detectable in higher quantities in VAMS tips on Mitra devices protected with GenTegra chemistry compared to unprotected VAMS tips, showing the stabilizing effect of GenTegraRNA-NEO on the VAMS tips. Number of tips extracted one per data point each data point two replicates.

HIV samples were run at Stanford University in the lab of Dr Benjamin Pinsky.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