truXTRAC® DNA Kit for truCOLLECT™-plus - Column (10)

Adaptive Focused Acoustics® (AFA) -based
DNA extraction from the truCOLLECT-plus
using column-based purification

PN 520259

INTENDED USE

The truXTRAC® DNA Kit for truCOLLECT™-plus - Column is intended for use in life science applications, such as molecular biology. This kit is designed to be used in conjunction with the truCOLLECT-plus Stabilization and Transport kit (PN 520254) to extract and purify DNA from dry stabilized blood specimens.

This Research Use Only product is not intended for the diagnosis, prevention, or treatment of a disease.

INTRODUCTION

DNA extraction from blood is the method of choice for researchers, however, the logistics of collection, stabilization, and long-term storage, as well as DNA extraction from dried blood specimens remains difficult to adapt for use in downstream NGS-based analysis, due to inherently low DNA yields. DNA recovery from such dry-stabilized blood samples is performed using Covaris Adaptive Focused Acoustics (AFA®), which ensures rapid rehydration and detachment of blood cells from the truCOLLECT-plus swabs. Furthermore, optimized DNA extraction buffers in combination with AFA ensures the efficient isolation and subsequent column-based purification of high quality, molecular biology grade DNA. The yield of genomic DNA is dependent on white blood cell count and is generally in the range of 5 to 15 ng per microliter of blood.

If you require any assistance with this product please refer to Troubleshooting (Appendix B) in this protocol, check the FAQs found on our website, or contact Covaris Application Support at ApplicationSupport@covaris.com.

REVISION HISTORY

Part Number	Revision	Date	Description of change
010462	01	6/18	Kit Release of truXTRAC DNA Kit for truCOLLECT-plus – Column

KIT CONTENTS

○ AFA Conditioning Buffer	5 ml
○ Proteinase K (PK Solution)	2 x 300 µL
○ Buffer B3	4.5 ml
○ Buffer BW	6 ml
○ Buffer B5 (Concentrate)	2 ml
○ Buffer BE	3 ml
○ Purification Columns	10
○ Collection Tubes	30
○ microTUBE-500 AFA tube	10 tubes

SDS INFORMATION IS AVAILABLE AT http://covaris.com/resources/safety-data-sheets/

STORAGE

Upon arrival, store the Proteinase K solution at 2 to 8C.

Store all other kit components at room temperature.

LABORATORY EQUIPMENT, CHEMICALS, AND CONSUMABLES TO BE SUPPLIED BY USER

Required Laboratory Equipment and Accessories

- truCOLLECT-plus dry-stabilized sample (Covaris, PN 520254)
- Dry block heater with block to accommodate 1.5 ml tubes or temperature-controlled water bath able to accurately heat between 68-72C (70C)

Required Chemicals

- 200 proof Ethanol (e.g., AmericanBio, PN AB00515)

Required Consumables

- 1.5 ml Nuclease free Microfuge Tubes (2 per sample) (e.g., Eppendorf Safe-Lock Tubes, PN 022363212)
- Benchtop microcentrifuge (11,000 x g capability)
- Benchtop vortex

Covaris Focused-ultrasonicator Accessories and Plate Definitions

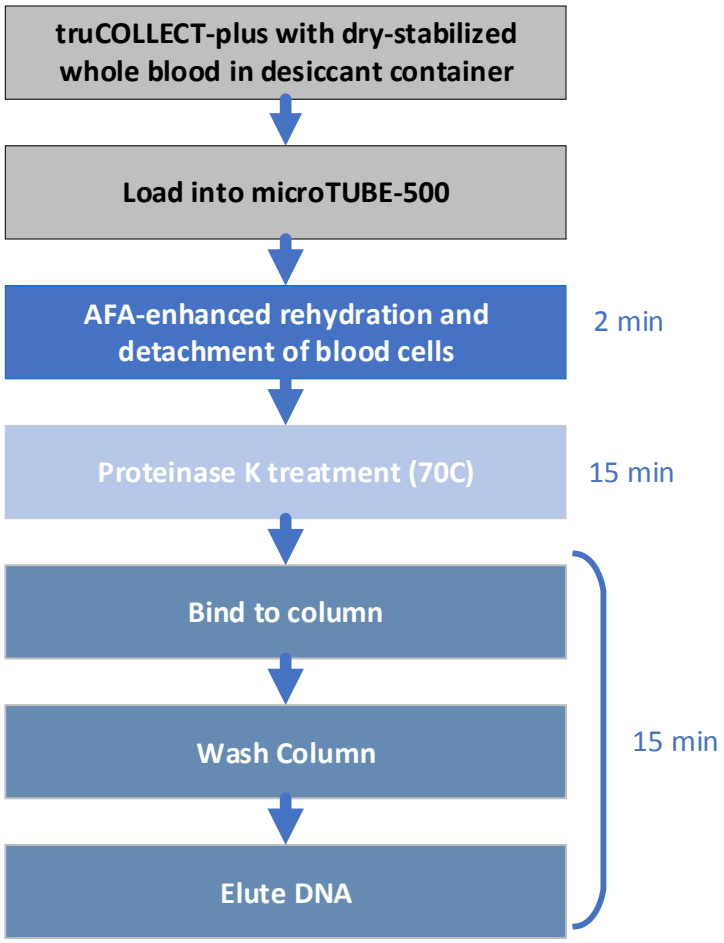
The table below contains the parts and plate definitions necessary to run the protocol.

Use the parts and plate definitions specific to your Covaris Focused-ultrasonicator.

Instrument	M220	ME220	E220	LE220
Holder/Rack Description (PN)	Holder XTU (500414)	Rack 4 Position microTUBE-500 (500525)	Rack 24 microTUBE-500 Screw-Cap (500452)	Rack 24 microTUBE-500 Screw-Cap (500452)
Plate definition file name	NA	<microTUBE-500 Screw-Cap>	<500452-24 microTUBE-500 Screw-Cap +6mm offset z enabled>	<500452-24 microTUBE-500 Screw-Cap +6mm offset z enabled>
Required Accessories (PN)	Insert XTU (500471)	ME220 Waveguide 4 Place (500534)	NA (Intensifier not required)	NA

truCOLLECT-plus Extraction And Purification Workflow

Using the Adaptive Focused Acoustics (AFA) process, dry-stabilized whole blood samples are rehydrated and detached from the swabs. Samples are treated with Proteinase K for 15 minutes at 70C. The genomic DNA is released and purified using columns.



1 – Preparation of Reagents

Follow these instructions before starting the DNA isolation process.

- **Buffer B5:** Before the 1st use of the kit, add 8 ml 100% ethanol to the Buffer B5, close bottle tightly and mix by inverting the bottle 5 times. Mark the bottle to indicate that the Isopropanol was added.

- Preheat the required amount of **BE Buffer** to 70°C in 1.5ml microcentrifuge tube(s). (Number of samples * 100 * 1.5 = total volume in µl to preheat)

2 – Preparation of Heat Block

1. Preheat dry block heater to 70C ± 2C. It is critical that this temperature is accurate to successfully execute the protocol.

2. Test the temperature of your heat block:
    a. Place a microcentrifuge tube (1.5 or 2 ml) filled with water into the heat block.
    b. Immerse a thermometer into the tube.
    c. Wait until the temperature has reached the plateau.
    d. Adjust the Set-temperature accordingly until the temperature inside the microcentrifuge tube has reached 70C ± 2C.

4 – FOCUSED-ULTRASONICATOR SETUP

For detailed instructions on how to prepare and use your instrument, please refer to the respective Covaris User Manual. If you do not see a Plate Definition on your system, please contact Covaris Technical Support (techsupport@covaris.com)

Refer to page 4 for Plate Definitions and required Focused-ultrasonicator accessories

1. **Create "truCOLLECT-plus" program in SonoLab™**
   Use the settings provided in the table below, specific to your Covaris instrument type, to create a program called "truCOLLECT-plus" using the Covaris SonoLab method editor. The truCOLLECT-plus protocols require the setup of dithering parameters. Save the program for later use. Refer to Appendix A for protocol creation.

Instrument	M220	ME220	E220	LE220
Peak Incident Power (PIP) (Watt)	50	75	75	350
Duty Factor (%)	25	25	25	25
Cycles Per Burst (CPB)	1000	1000	1000	1000
Treatment time (seconds)	120	120	120	120
Bath temperature (°C)	20	20	20	20
Water Level (run)	Fill to sample	Automatic	5	5
Dithering (See Appendix A)	NA	X Dither: 2.0mm Z Dither: 2.0mm Dither Speed: 10mm/sec	X Dither: 1.0mm Y Dither: 1.0mm X-Y Dither Speed: 10mm/sec Z Dither: 3.0mm Z Dither Speed: 10mm/sec	X Dither: 1.0mm X-Y Dither Speed: 10mm/sec Z Dither: 3.0mm Z Dither Speed: 10mm/sec
Plate definition	NA	<microTUBE-500 Screw-Cap>	<500452-24 microTUBE-500 Screw-Cap +6mm offset z enabled>	<500452-24 microTUBE-500 Screw-Cap +6mm offset z enabled>

1 – DNA PURIFICATION

1. Set up the dry-heat block as explained in Section-3 and verify the block temperature to be 70C. The heat block set to 70C is required for Proteinase K incubation (Step 13) and DNA elution after purification via column purification.

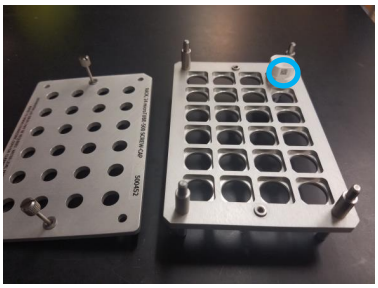
2. Add 400 µl of **AFA Conditioning Buffer** to a microTUBE-500 AFA processing tube. Prepare the appropriate number of microTUBE-500 tubes for the number of samples to be processed.

3. Open the truCOLLECT-plus desiccant storage container and remove the truCOLLECT-plus cap/swab.

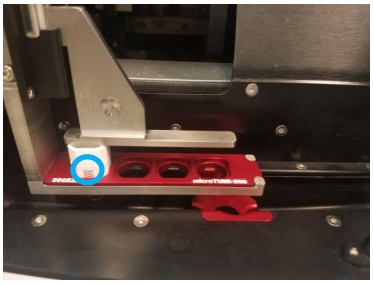
4. Carefully insert the swab into a microTUBE-500. Avoid spilling Conditioning Buffer. Seal by turning the cap.

5. Place the microTUBE-500s in the appropriate rack for your Focused-ultrasonicator. The rack must be positioned how it will fit into the instrument. The barcodes on the truCOLLECT cap/swab must be positioned facing you. See Appendix B.

E220 and LE220



ME220



6. Load the rack into the Focused-ultrasonicator for processing.

7. Process the sample using the "***truCOLLECT-plus***" program on your Covaris Focused-ultrasonicator.

8. Remove the microTUBE-500 rack from the Focused-ultrasonicator and remove the microTUBE-500s from the rack.

9. Carefully unscrew and remove the cap/swab. Slowly remove 350 µl of the sample and transfer to a 1.5 ml microcentrifuge tube. (See Appendix C - Troubleshooting for any issues.)

> CAUTION: Some swabs may break at the top of the handle due to the AFA-energetics. This will not impact DNA yields. Use caution when removing the cap/swabs.

10. Add 40 µl of Proteinase K.

11. Add 350 µl of Buffer B3.

12. Cap the tube and vortex for 5 seconds at maximum speed.

13. Place the 1.5 ml tube into a heat block pre-heated to 70C and incubate for 15 minutes at 70C.

14. Remove the tube from the heat block. Add 360 µl of **ethanol** (96-100%) and mix by vortexing 15 seconds.

15. Assemble a Purification Column on top of a Collection Tube.

16. Load 550 µl onto the Purification Column.

17. Centrifuge the Column/Tube assembly for 1 minute at 11,000 x g. Discard the flow-through.

18. Reassemble the Column/Tube assembly by placing the Column into the same Collection Tube.

19. Load the remaining sample volume onto the Purification Column.

20. Centrifuge the Column/Tube assembly for 1 minute at 11,000 x g. Discard the flow-through.

21. Place the Purification Column in a new Collection Tube.

22. **1st Wash:** Add **500 µl of BW Buffer**.

23. Centrifuge 1 minute at 11,000 x g. Discard the flow-through.

24. Place the Purification Column into a new Collection Tube.

25. **2nd Wash:** Add **600 µl of prepared B5 Buffer.**

26. Centrifuge 1 minute at 11,000 x g. Discard the flow-through.

27. Place the Purification Column back into the same Collection Tube and centrifuge 1 minute at 11,000 x g to remove residual ethanol.

28. Place the Purification Column into a new 1.5 ml microcentrifuge tube.

29. **Elute DNA:** Dispense 100 µl of **BE Buffer** (preheated to 70°C) directly into center of the Purification Column membrane.

30. Incubate at room temperature for 1 minute.

31. Centrifuge 1 minute at 11,000 x g to collect purified DNA sample.

32. Remove the Purification Column and cap the 1.5 ml tube.

Isolated DNA should be kept at 2-8C for short term storage (1 to 2 days) and -20C for long term storage.

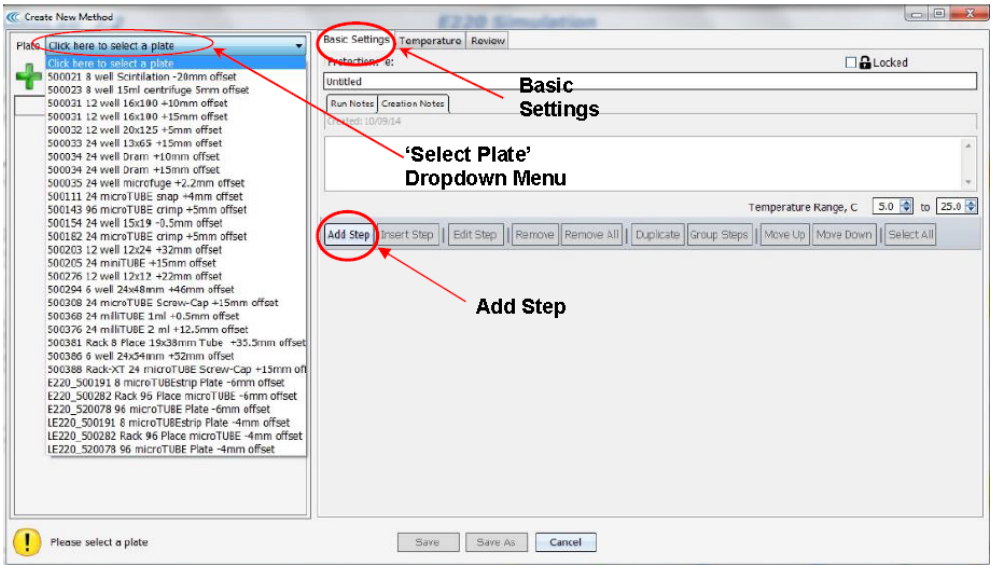
APPENDIX A – PROTOCOL CREATION IN SONOLAB

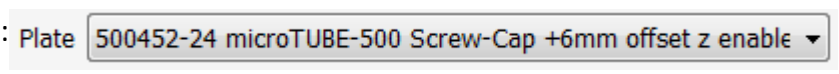
E220 and LE220 with SonoLab 7.3

1. Refer to your instrument user manual.
   a. https://covaris.com/wp-content/uploads/010277-E-and-LE-series-Manual_Rev-F.pdf
2. Refer to the protocols.

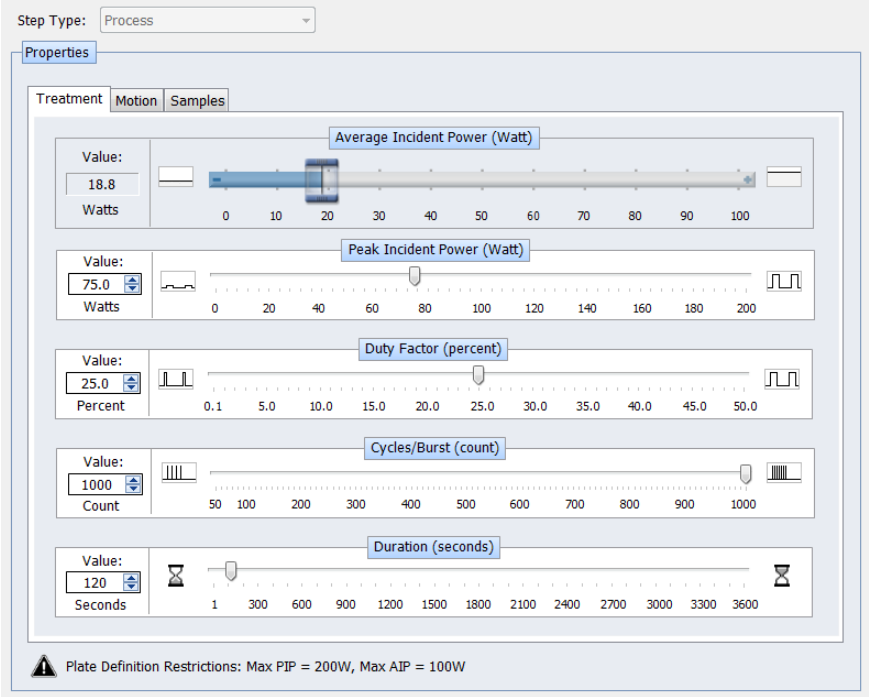
Instrument	E220	LE220
Peak Incident Power (PIP) (Watt)	75	350
Duty Factor (%)	25	25
Cycles Per Burst (CPB)	1000	1000
Treatment time (seconds)	120	120
Bath temperature (°C)	20	20
Water Level (run)	5	5
Dithering	X Dither: 1.0mm Y Dither: 1.0mm X-Y Dither Speed: 10mm/sec Z Dither: 3.0mm Z Dither Speed: 10mm/sec	X Dither: 1.0mm X-Y Dither Speed: 10mm/sec Z Dither: 3.0mm Z Dither Speed: 10mm/sec
Plate definition	<500452-24 microTUBE-500 Screw-Cap +6mm offset z enabled>	<500452-24 microTUBE-500 Screw-Cap +6mm offset z enabled>

3. On the Run screen, click the “New…” button in the Method window.
4. In the Basic Settings tab, first select the 500452 plate with z dither enabled.



a. Plate: 

5. Rename the “Untitled” protocol to “truCOLLECT-plus.”
6. Click the “Add Step” button. In the Treatment tab, input the settings for the truCOLLECT-plus. (The image below is for the E220.)



7. In the Motion tab, input the motion settings to enable x, y, and z dithering. (The image below is for the E220.)

8. Save the protocol.

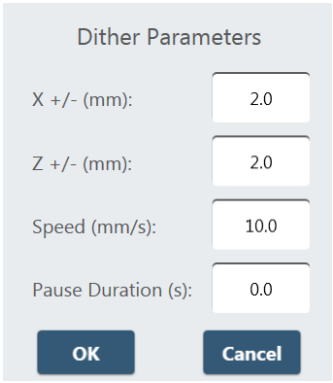
ME220 with SonoLab 8

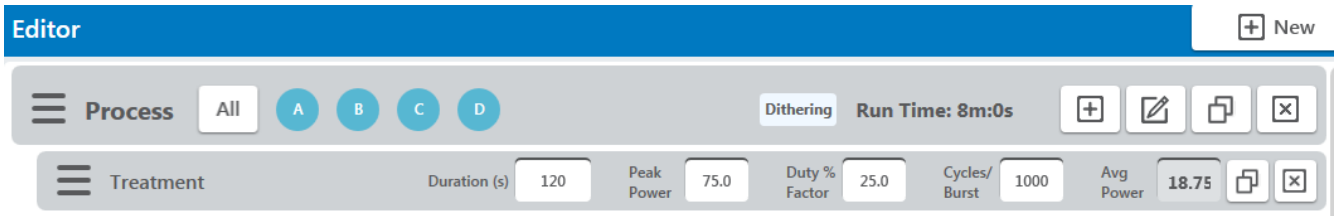
1. Refer to the ME220 User Manual https://covaris.com/wp-content/uploads/pn_010325.pdf
2. Select <microTUBE-500 Screw-Cap> from the drop-down menu.
3. Click “Edit.”
4. In the Editor box, select the number of samples to run in the Process bar.
5. Change the AFA settings in the Treatment bar.

Instrument	ME220
Peak Incident Power (PIP) (Watt)	75
Duty Factor (%)	25
Cycles Per Burst (CPB)	1000
Treatment time (seconds)	120
Bath temperature (°C)	20
Water Level (run)	Automatic
Dithering	X Dither: 2.0mm Z Dither: 2.0mm Dither Speed: 10mm/sec
Plate definition	<microTUBE-500 Screw-Cap>

6. Click the “Edit Process Settings” button.

7. Input the X- and Z-dithering values and click OK.



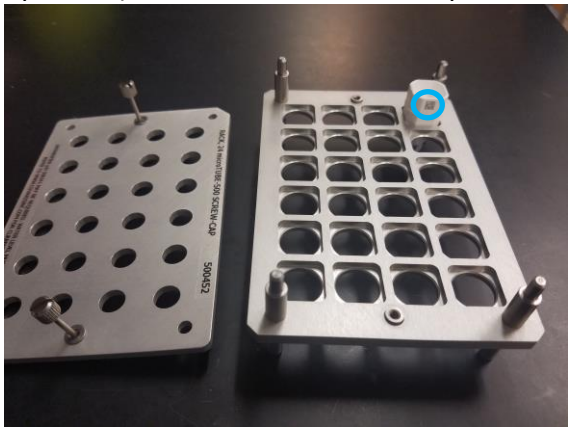


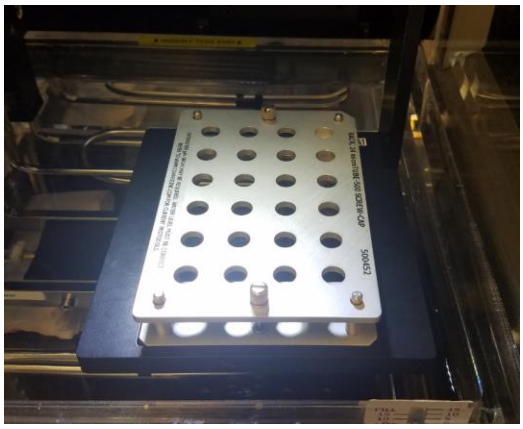
8. Rename the protocol as “truCOLLECT-plus.”
9. Click “Save.”

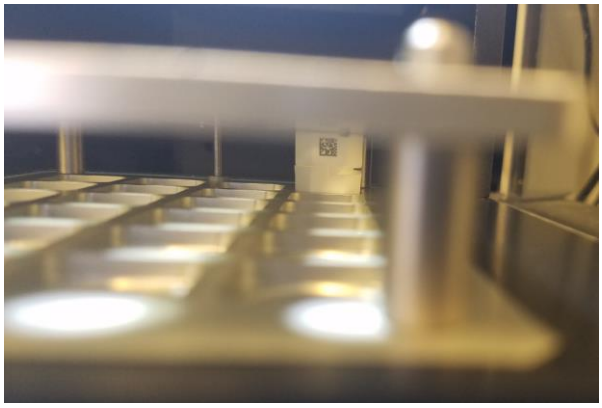
APPENDIX B – LOADING TRUCOLLECT-PLUS MICROTUBES IN THE FOCUSED-ULTRASONICATOR

E220 and LE220

1. The barcodes of the truCOLLECTs should be facing the bottom the rack 500452 (toward the operator) so the swabs are lined up in the same direction. Refer to the pictures below.

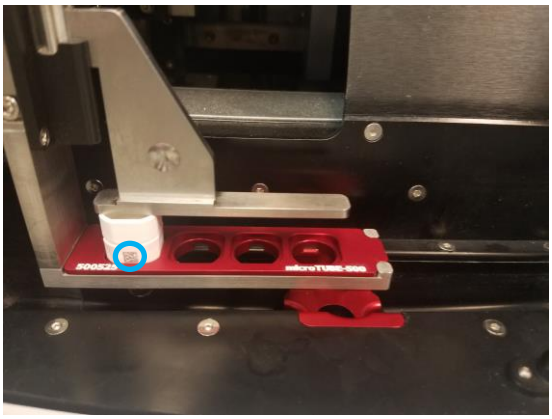






ME220

1. The barcodes of the truCOLLECTs should be facing the operator. Refer to the picture below.



APPENDIX C – TROUBLESHOOTING GUIDE

Issue	Cause	Solution	Comments / Suggestions
Swab handle breaks	AFA-energetics	Carefully remove the cap after AFA treatment. The swab may stay in the tube while removing the 350 µl for purification.	Sample loss will be negligible with remaining liquid.
Low concentration of DNA	Elution volume too high	Use lower elution volume in Step 29.	Only 100 µl has been tested.
Low sample volume recovery after AFA	Liquid absorbed by the swabs	Pipette liquid off the swab.	Due to the viscosity and absorbance capacity of the swab, the liquid can stick to the swab while removing it from the AFA tube.
Residual flow-through liquid when emptying a collection tube for reuse (Steps 17 and 27)	Pouring off flow-through results in liquid remaining around the rim of the collection tube.	Blot the top of the collection tube on a paper towel or kimwipe and dispose in biohazard waste.	Another option is to pipette the flow-through out of the collection tube.

ADDITIONAL NOTES

1. See following link: http://covaris.com/resources/protocols/ for updates to this document.
2. Patents pending