Recovery of Spotted DNA Extracts from Whatman FTA Elute MicroCards

1 Procedure:

1.1 Obtain the spotted extract card(s) via the "Fbio_Spotted Extract Request" workflow in Qualtrax.

1.1.1 The spotted card for the original extraction negative should also be pulled and reconstituted with the spotted sample.

1.2 Ad-Hoc samples into LIMS.

1.3 Once all necessary samples are in LIMS, add them onto the Reconstitution of Evaporated Extract Worksheet for further processing.

1.4 The worksheet should contain 1 new reconstitution negative control for the recovery process and 1 extraction negative associated with the spotted samples being reconstituted, unless that original extraction negative was already previously reconstituted. Print the LIMS labels and label the appropriate number of 1.5 mL tubes.

1.5 Select the Extract Type for each sample in the worksheet.

1.6 Have a witness verify the samples, including the labels on the spotted extract cards against the list of samples LIMS.

1.7 Clean a 3mm hole puncher by punching a blank FTA Card 3 times, followed by wiping the puncher with a lint free wipe that has been prewet with 70% Ethanol.

1.8 Use the 3mm hole punch to punch out sample into a labeled 1.5 mL tube based on the chart below. The entire stain may be punched as needed, regardless of the concentration. The entire stain should be punched for spotted negative controls. All cards should be resealed in a kapak pouch and returned to storage, including those where the entire stain was punched.

Original Quantitation Value	*Recommended # of 3 mm punches for Recovery*
1 ng/uL and above	At least 8 punches
100 pg/uL to 999 pg/uL	At least 10 punches
Less than 100 pg/uL	Punch entire stain

1.8.1 A maximum of ten punches per 1.5mL tube is suggested for enhanced recovery. If more than ten punches are needed, separate the punches into multiple 1.5mL tubes.

1.8.2 If multiple tubes are being used for the reconstitution of one sample indicate in the sample comments how many additional tubes and the number of punches in each tube. Print additional LIMS labels for each additional tube.

1.8.3 The reconstitution extract from these samples can be combined during the microcon.

1.9 Heat the tubes with the punches at 80°C for 20 minutes to increase binding of the DNA to the card to prevent loss during washing.

1.10 Record the incubation temperature and the instrument in the 'Additional Information' section and the 'Instrument' box at the top of the worksheet.

1.11 Wash Step: Add 500 µL of sterile/ultrapure water to each tube, vortex for 5 seconds.

1.11.1 Note: Make sure the punches are moving around during the vortex step to ensure all punches are being washed. You may need to add more water if there are a large number of punches. Otherwise, there is the potential for PCR inhibitors to be left behind.

1.12 Briefly centrifuge the tubes, remove, and discard the supernatant.

1.13 Add sterile/ultrapure water to each tube, 50 uL per hole punch. Briefly centrifuge the tubes to ensure the punches are all immersed in water. Record this volume as the amount of water added. The initial volume should be marked as "0".

1.14 Place tubes in thermomixer at 95°C for 30 minutes, shaking at 500 rpm. At the end of the incubation step, remove the tubes from the heat block and vortex for 60 seconds.

1.15 Record the thermomixer temperature and the instrument in the 'Additional Information' section and the 'Instrument' box at the top of the worksheet.

1.16 Briefly centrifuge the tubes.

1.17 Collect the eluted DNA solution with a pipette and transfer to a 1.5 mL tube labeled with the LIMS output label. Discard the tube containing the discs/hole punches.

1.18 Fill out the 'Pass/Fail' response and complete the Reconstituted By review task and in the e-signature comment area , indicate how many tubes were created for each sample.

1.19 All reconstituted samples should then proceed to microcon. Create the MCON submission for the samples.

1.20 Verify the samples listed on the Mcon Submission Worksheet click [Save] and close the Submission worksheet.

1.20.1 On the Microcon Worksheet, enter the number of tubes present for each sample in the sample comments area. This should match the reconstitution sheet.

1.20.2 Samples should be microconned down to 25 uL. See Microcon DNA Fast Flow DNA Concentration and Purification.

1.20.3 Samples can be microconned to concentrate, however, for samples where color from the card is left behind (usually samples with a greater number of punches), microcon to clean and concentrate.

1.20.4 Filter a maximum of 400µL at one time. For samples with more than 400µL, transfer the microcon membrane to a new collection tube as needed.

1.20.4.1 If samples were separated into multiple tubes during the reconstitution extraction, combine the reconstituted extracts using the same microcon membrane filter and continue to add the remaining extract to the microcon filter (up to 400µL each time).

1.20.5 When filling out the 'Pass/Fail' response and complete the Extract By review task and in the e-signature comment area, indicate how many tubes were created for each sample.

1.20.6 Store the final microconned extracts at 4°C and update the storage location in LIMS. The empty reconstitution tubes from the reconstitution worksheet may be discarded.

1.20.7 After re-extraction, if sample testing will continue in Forensic Biology, a quantitation of the sample(s) is recommended.

1.20.8 If samples will be transferred outside of Forensic Biology, refer to QC710 - Locating and Processing of Retained and/or Spotted Extracts