STANDARD OPERATING PROCEDURE

Urea Extraction for IRMS

1. METHOD OBJECTIVE: To prepare animal tissue for stable isotope analysis by extracting urea.

2. METHOD VARIATIONS: Due to the variety of sample types, variation in the conditions of analysis may require modifications to the method used for different samples. This procedure includes variations for skin/muscle tissue, whole blood and blood components (RBC, serum and plasma). If samples also need to be lipid-extracted, follow SOP CAIS-043-1.1 first, then proceed with this SOP. Some samples may dissolve completely during the deionized water extraction (e.g. blood serum). If this happens, refreeze and freeze-dry the sample and continue processing without DI extraction.

3. SAFETY PRECAUTIONS: Although proper training is required to conduct these preparations, one must remain mindful of the inherent hazards in some types of samples. Use gloves, googles, and lab coat when handling hot samples and handle in a fume hood.

4. EQUIPMENT, MATERIALS AND REAGENTS:

4.1. EQUIPMENT: heating sonicator, freeze-dryer, -80C freezer, 5 mL pipet and tips, 250mL Erlenmeyer flask, test tube rack, ball mill (for solid tissue only), centrifuge

4.2. MATERIALS: 7mL screw top test tubes and phenolic black caps (Wheaton W240822), deionized ultrapure water

5. PROCEDURE: Urea extraction from whole blood and blood components is described in section 5.1, from muscle and skin tissue in 5.2.

5.1. Blood Plasma, Serum and whole blood - If samples also need to be lipid-extracted, follow SOP CAIS-043-1.1 first, then skip to step 5 of this SOP.

5.1.1. Keep liquid samples refrigerated if components need to be separated; Store liquid samples frozen (at -20C) immediately after centrifuge separation Thaw sample and:

5.1.1.1 For plasma and serum samples, transfer 1000 μL of the sample to a 7-mL test tube using a fresh pipet tip with the end clipped off (~2mm) to prevent clogging

5.1.1.2 For whole blood samples, transfer 500 μL of the sample to a 7-mL test tube using a fresh pipet tip with the end clipped off (~2mm) to prevent clogging

5.1.2. Refreeze the subsample in the test tube then freeze-dry over night

5.1.3. Add 5 mL of ultrapure deionized water to each test tube and cap tightly with phenolic black caps

5.1.4. Sonicate samples with heat "on" under a fume hood for 15 minutes

5.1.5. Check samples for changes in extract volume; samples with little or no extract remaining after sonication may have a leaky cap or cracked test tube rim.

5.1.5.1. If the solids dissolve completely during the deionized water extraction (e.g. blood serum) refreeze and freeze-dry the sample and continue processing without DI extraction.

5.1.5.2. If the solids float or do not settle to the bottom of the test tube after each sonication, centrifuge for 5 minutes.

5.1.6. Decant the used solution into a waste container; Replace cap or transfer the extracted material to a clean test tube as necessary to avoid extract loss during further sonication.

5.1.7. Repeat steps 4-8 for a second and third time

5.1.8. Freeze and then freeze-dry extracted samples overnight

5.1.9. Homogenize the extracted tissue within the test tube using a clean glass rod

5.1.10. Weigh and encapsulate extracted sample according to SOP CAIS-040-1.2

5.1.11. Analyze encapsulated sample according to SOP CAIS-039-1.1

5.2. Muscle/Skin Tissue - If samples also need to be lipid-extracted, follow SOP CAIS-043-1.1 first, then skip to step 5 of this SOP.

5.2.1. Freeze (at -20C) samples immediately after collection

5.2.2. Freeze-dry whole sample in original containers for ~48 hrs

5.2.3. Grind whole sample with ball mill according to SOP CAIS-037-1.1

5.2.4. Transfer ~1 g (dry weight) of ground sample into 7 mL glass test tubes

5.2.5. Add 5 mL of ultrapure deionized water to each test tube and cap tightly with phenolic black caps

5.2.6. Sonicate with heat "on" samples under a fume hood for 15 minutes

5.2.7. Check samples for changes in extract volume; samples with little or no extract remaining after sonication may have a leaky cap or cracked test tube rim.

5.2.8. If the solids float or do not settle to the bottom of the test tube after each sonication, centrifuge for 5 minutes.

5.2.9. Decant the used solution into a waste container; Replace cap or transfer the extracted material to a clean test tube as necessary to avoid extract loss during further sonication.

5.2.10. Repeat steps 5.2.5-5.2.9 for a second and third rinse

5.2.11. Freeze-dry samples over night to remove any remaining extractant

5.2.12. Homogenize the extracted tissue within the test tube using a glass stir rod

5.2.13. Encapsulate the extracted sample according to SOP CAIS-040-1.2.

5.2.14. Analyze the extracted sample according to SOP CAIS-039-1.1.

6. QUALITY CONTROL: Calculate atomic C:N ratio from using the measured atom% of each element:

6.1 C:N = (atoms of C)/(atoms of N)

6.2 If C:N is greater than 3.0, it likely still contains lipids and another aliquot of sample should be extracted using additional petroleum ether rinses

6.3 Report mean C:N and standard errors for tissues subject to SIA

7. DATA MANAGEMENT: Extracted tissue samples are listed in the client file and results of analysis are archived on one computer and one backup external drive, and readily retrievable for data mining, review and reporting.