EMORY UNIVERSITY SCHOOL OF MEDICINE Department of Medicine	**Clinical Biomarkers Laboratory** **Division of Pulmonary Allergy and Critical Care Medicine** **615 Michael St. Ste. 225, Atlanta GA, 30322**
Title: Preparation of tissue for high-resolution metabolomics	
SOP: HRM_SP_tissue_062016_01 Revision: 1	**Date effective:** 30 July 2016

Summary statement:
Samples are prepared for metabolomics analysis using established methods (Johnson et al. (2010). *Analyst*; Go et al. (2015). *Tox Sci*). Prior to analysis, lung samples were removed from storage at -80°C and thawed on ice. The 20-30 mg of each lung tissue sample was then mixed with ice cold LC-MS grade acetonitrile: water mixture (Sigma-Aldrich; 2:1 ratio; 15 µL per mg tissue) which contains 2.5 µL per 100 µL of internal standard solution with eight stable isotopic chemicals selected to cover a range of chemical properties. Samples are then sonicated on ice to ensure metabolite extraction as previously described (Chandler et al (2016) AJ Physiology; Hu et al. (2019) AJ Pathology). Following sonication, tissue lysates are then equilibrated for 30 min on ice, upon which precipitated proteins are removed by centrifuge (16.1 ×g at 4°C for 10 min). The resulting supernatant containing metabolite extract (100 µL) is removed, added to a low volume autosampler vial and maintained at 4°C until analysis (<22 h).

Chemicals Needed:

- 20000 µL LC-MS grade acetonitrile
- 500 µL stable isotope internal standard solution containing: [$^{13}C_6$]-D-glucose, [^{15}N,$^{13}C_5$]-L-methionine, [$^{13}C_5$]-L-glutamic acid, [^{15}N]-L-tyrosine, [3,3-$^{13}C_2$]-cystine, [trimethyl-$^{13}C_3$]-caffeine, [U-$^{13}C_5$, U-$^{15}N_2$]-L-glutamine, [^{15}N]-indole

Materials Needed

- 250 µL q3June2014
- 100 uL NIST SRM 1950
- 150 uL conditioning plasma
- 48 Study samples (≥20 mg of tissue sample required)
- Labeled 1.5mL microfuge tubes
- Calibrated P200 and P1000 Micropipettes with 200 µL and 1000 µL tips
- Refrigerated centrifuge at 4°C with speed ≥ 16,100 × *g*
- Vortexer
- Scale
- Sonicator
- Labeled, low-volume LC vials with snap caps

Instrumentation

- Centrifuge, Eppendorf 5430R, Room 225: Prior to starting sample preparation set speed to 16,100 × g and temperature to 4°C. Cool using "fast cool" option. When loading samples, makes sure samples are evenly distributed around the wheel. Sonicator, Q120AM Active Motif with CL-18 probe, Tissue samples are sonicated at 20% amplitude for 5s with 5s between each sonication, and is repeated 5x.

Sample preparation

Samples are to be prepared daily, and placed in the autosampler for analysis immediately upon completing sample preparation

1. Remove conditioning, QC and study samples from storage at -80°C and thaw on ice
2. Remove internal standard solution from storage at -80°C and thaw.
3. Label clean, microfuge tubes.
4. Add 125 µL of internal standard solution to 5000 µL acetonitrile, vortex and store on ice.
5. Carefully pipette 50 µL of thawed sample to appropriate microfuge tube. Ensure no air bubbles or clogs occur in pipette tip. Use a fresh tip for each sample
6. Carefully pipette 100 µL of acetonitrile/internal standard solution into each tube and close snap top.
7. Vortex each tube for 10 sec.
8. Place tube on ice and allow to equilibrate for 30 min.
9. For tissue samples, obtain 20-30mg of tissue and thaw on ice, calculate volume of 2:1 acetonitrile:water mixture is required for each sample (15 µL per mg of tissue) and add requisite amount to each sample.
10. Once added, samples are sonicated at 20% amplitude 5x at 5s intervals on ice.
11. Samples are allowed to equilibrate for 30 min.
12. Following equilibration period, centrifuge tubes at 4°C for 10 min at 16.1 × *g*.
13. Label clean, LC vials.
14. Carefully pipette 100 µL of supernatant into corresponding LC vial.
15. Cap.
16. Load into autosampler racks based on predetermined run order.

References

1. Johnson JM, Yu T, Strobel FH, Jones DP. A practical approach to detect unique metabolic patterns for personalized medicine. *The Analyst*. 2010;135:2864-2870.
2. Soltow QA, Strobel FH, Mansfield KG, Wachtman L, Park Y, Jones DP. High-performance metabolic profiling with dual chromatography-Fourier-transform mass spectrometry (DC-FTMS)

for study of the exposome. *Metabolomics*. 2013;9:S132-S143.

3. Go YM, Walker DI, Liang Y, Uppal K, Soltow QA, Tran V, et al. Reference Standardization for Mass Spectrometry and High-resolution Metabolomics Applications to Exposome Research. *Tox. Sci*. 2015;148:531-543.
4. Chandler JD, Hu X, Ko EJ, Park S, Lee YT, Orr M, Fernandes J, Uppal K, Kang SM, Jones DP, Go YM Metabolic pathways of lung inflammation revealed by high-resolution metabolomics (HRM) of H1N1 influenza virus infection in mice. *A. Journal of Physiology.* 2016;311(5); R906-R916.
5. Hu X, Kim KY, Lee Y, Fernandes J, Smith MR, Jung YJ, Orr M, Kang SM, Jones DP, and Go YM. Environmental Cadmium Enhances Lung Injury by Respiratory Syncytial Virus Infection. *A. Journal of Pathology*, 2019; 189(8); 1513-1525

SOP Details and Version Information

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