**Overview**

Thank you for your interest in the Microbial Culture & Metabolomics Core. We offer targeted metabolomic analysis (absolute quantification) of amino, bile, and short chain fatty acids in fecal, plasma, and microbial culture samples. Please refer to our website for a complete list of metabolites quantified in our assays. If you have not already done so, please fill out an [intake form](https://pennchopmicrobiome.chop.edu/microbial-culture-and-metabolomics-core-contact).

We will deliver a spreadsheet with absolute metabolite concentrations (per mass for stool samples or per volume for plasma/culture supernatant samples). Turn-around time for samples ranges from 4-8 weeks but depends on the sample queue at the time of submission and the number of samples submitted.



The core issues invoices on a quarterly basis. If you are within the University of Pennsylvania, invoices can be paid by providing a 26-digit budget code. If you are outside of the University of Pennsylvania, invoices can be paid by check made out to “The Trustees of the University of Pennsylvania”.

**Services Offered**

* Amino Acid Analysis: Plasma (Human, Mouse), Feces/Cecal Contents (Human, Mouse), Microbial Culture Supernatant
* Bile Acid Analysis: Plasma (Human, Mouse), Feces/Cecal Contents (Human, Mouse), Microbial Culture Supernatant
* Short Chain Fatty Acid Analysis: Feces (Human), Microbial Culture Supernatant

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| --- | --- | --- | --- |
| **Matrix** | **Amino Acids** | **Bile Acids** | **SCFAs** |
| **Plasma – Human** | X | X |  |
| **Plasma – Mouse** | X | X |  |
| **Feces – Human** | X | X | X |
| **Feces – Mouse** | X | X |  |
| **Microbial Culture Supernatant** | X | X | X |

**Analytes**

* Amino Acids: Alanine, Arginine, Asparagine/Aspartic Acid, Cysteine, Glutamine/Glutamic Acid, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Theronine, Tyrosine, Valine
* Bile Acids: Cholic Acid, Glycocholic Acid, Taurocholic Acid, Chenodeoxycholic Acid, Glycochenodeoxycholic Acid, Taurochenodeoxycholic Acid, Deoxycholic Acid, Glycodeoxycholic Acid, Taurodeoxycholic Acid, Lithocholic Acid, Glycolithocholic Acid, Taurolithocholic Acid, Muricholic Acids (α-, β-, γ-, σ-; mouse samples only)
* Short Chain Fatty Acids: Acetic Acid, Propionic Acid, Isobutyric Acid, Butyric Acid, Isovaleric Acid, Valeric Acid, Isocaproic Acid, Caproic Acid, Heptanoic Acid

**Sample Requirements**

1. ***Human Stool*** *–* One Eppendorf vial for bile and/or amino acid analysis with100-150 mg stool frozen neat. One Eppendorf vial for SCFA analysis with 100-150 mg stool frozen neat. *Exact weight for each sample must be included in the sample manifest.*
2. ***Mouse Stool*** – One Eppendorf vial for bile and/or amino acid analysis with25-100 mg stool frozen neat. *Exact weight for each sample must be included in the sample manifest.*
3. ***Plasma (Mouse or Human)*** – One vial with at least: 50 uL plasma for SCFA analysis, 25 uL for amino acid analysis, 25 uL for bile acid analysis, or 100 uL for a complete panel (AA, BA, SCFA analysis). *Note: Plasma SCFA levels in both mice and humans are typically below the detection limit for our SCFA assay using uPLC-UV technology. We do not recommend analyzing plasma samples for SCFAs.*
4. ***Bile (Mouse or Human)*** – One vial with at least 25 uL for bile acid analysis.
5. ***Bacterial Culture Supernatant*** – One vial for bile and/or amino acids with at least 50 uL of supernatant. One vial for SCFA analysis with at least 100 uL supernatant.

**Submission Requirements**

1. Please submit an electronic sample manifest containing a unique sample identifier for each aliquot, the sample type (e.g., stool, plasma), the sample weight (mg) or volume (uL), and what analysis you would like performed (i.e., AA, AA/BA, BA, SCFA).

|  |  |  |  |
| --- | --- | --- | --- |
| **Unique Sample ID** | **Sample Type** | **Sample Weight (mg)/Volume (uL)** | **Analysis** |
| Subject1-SCFA-Stool | Stool | 104 | SCFA |
| Subject1-AA/BA-Stool | Stool | 92 | AA/BA |
| Subject1-AA/BA-Plasma | Plasma | 50 | AA/BA |
| Subject2-SCFA-Stool | Stool | 110 | SCFA |

1. Deliver samples on dry ice to Microbial Culture and Metabolomics core. To drop off samples in person, please email Elliot (elliotf@pennmedicine.upenn.edu) to arrange a time. To ship samples, please ship overnight on dry ice. Do not ship samples on Friday. Please forward tracking information to Elliot (elliotf@pennmedicine.upenn.edu). Shipping address:

PennCHOP Microbial Culture & Metabolomics Core

c/o Elliot Friedman

936 BRB II/III

421 Curie Blvd.

Philadelphia, PA 19104

**Sample Collection Protocols**

Stool – Human (AA/BA/SCFA Analysis)

1. Tare scale.
2. For each sample weigh and record the weight two (2) empty vials (Eppendorf #
3. Aliquot sample into each vial (100-150 mg for each vial). Weigh and record the weight of each vial + sample.
4. Store aliquots at -80oC until shipment to Microbial Culture and Metabolomics Core. Send electronic record of sample IDs, vial weights, and vial+sample weights.

Stool/Cecal Contents – Mouse (AA/BA Analysis)

1. Tare scale.
2. For each sample weigh and record the weight of one empty vial (Eppendorf #
3. Aliquot sample into the vial (25-150 mg). Weigh and record the weight of each vial + sample.
4. Store aliquots at -80oC until shipment to Microbial Culture and Metabolomics Core. Send electronic record of sample IDs, vial weights, and vial+sample weights.

Plasma – Mouse and Human (AA/BA/SCFA Analysis)

1. Collect whole blood into appropriate anticoagulant-treated tubes:
	1. For human plasma: BD Vacutainer K2 EDTA 7.2 mg (#367844)
	2. For mouse plasma: BD Microtainer tube w/ Lithium Heparin additive (#365965)
2. Immediately after collection, incubate samples on wet ice for 10-40 minutes.
3. Centrifuge at 3000 rpm for 15 minutes at 4oC. The resulting supernatant is designated as plasma.
4. Keeping all tubes and vials on wet ice while handling, aliquot supernatant into:
5. For human plasma: cryovials (SARSTEDT #72.694.006)
6. For mouse plasma: safe-lock vials (Eppendorf #0030121589)
7. Store plasma aliquots at -80oC until shipment to Microbial Culture and Metabolomics Core.

**Tips for *human* blood collection**:

* If multiple tubes of blood are collected for the *same subject*: pool plasma in to a 15 mL conical tube before aliquoting.
* One full BD Vacutainer will usually yield 50% of blood volume as plasma.

Microbial Culture Supernatant (AA/BA/SCFA Analysis)

1. Collect culture broth and spin for 5 min at 13,000 g. Aliquot supernatant into new vial(s). Store at -80oC. Minimum sample requirements: One vial for bile and/or amino acids with at least 50 uL of supernatant. One vial for SCFA analysis with at least 100 uL supernatant.

**Publications and Abstracts**

Amino Acids

* Osmon, A., N.L. Mervosh, A.N. Strat, K.R. Meckel, T.J. Euston, G.D. Zipursky, J.D. Buxbaum, M.S. Breen, D.D. Kiraly. (2020). Effects of gene by microbiome interactions on behavioral and neurobiological phenotypes in a mouse model for autism spectrum disorder. *bioRxiv*.
* Ramsteijn, A. S., Jašarević, E., Houwing, D. J., Bale, T. L., & Olivier, J. D. (2020). Antidepressant treatment with fluoxetine during pregnancy and lactation modulates the gut microbiome and metabolome in a rat model relevant to depression. *Gut Microbes*, 1-19.
* Najem, C.E., J. Lee, C.E. Tanes, W.C. Strange, E.S. Friedman, A.G. Sreih, R.L. Rhee, A. Geara, H. Li, K. Bittinger, J.D. Lewis, P.A. Merkel. (2019). Characterizing the gut and plasma metabolome in patients with ANCA-associated vasculitis. European Congress of Rheumatology, June 12-15, Madrid, Spain.
* Najem, C.E., J. Lee, C.E. Tanes, W.C. Strange, E.S. Friedman, A.G. Sreih, R.L. Rhee, A. Geara, H. Li, K. Bittinger, J.D. Lewis, G.D. Wu, P.A. Merkel. (2018). Characterizing the gut and plasma metabolome in patients with ANCA-associated vasculitis. American College of Rheumatology Annual Meeting, October 20-24, Chicago, Illinois, USA.
* Ni, J., T.D. Shen, E.Z. Chen, A. Bailey, M. Roggiani, A. Sirota-Madi, E.S. Friedman, L. Wang, A. Lin, I. Nissim, L.A. Albenberg, R.N. Baldassano, J. Baun, R.J. Xaiver, C.B. Clish, M. Yudkoff, H. Li, M. Goulian, F.D. Bushman, J.D. Lewis, G.D. Wu. A role for bacterial urease in Crohn’s disease and gut dysbiosis. *Science Translational Medicine*. 2017: 9(416).

Bile Acids:

* Ramsteijn, A. S., E. Jašarević, D.J. Houwing, T.L. Bale, & J.D. Olivier. (2020). Antidepressant treatment with fluoxetine during pregnancy and lactation modulates the gut microbiome and metabolome in a rat model relevant to depression. *Gut Microbes*, 1-19.
* Grau, K.R., S. Zhu, S.T. Peterson, E.W. Helm, D. Philip, M. Phillips, A. Hernandez, H. Turula, P. Frasse, V.R. Graziano, C.B. Wilen, M.T. Baldridge, & S.M. Karst. 2020. The intestinal regionalization of acute norovirus infection is regulated by the microbiota via bile acid-mediated priming of type III interferon. *Nature Microbiology*, *5*(1), pp.84-92.
* Wang, S., R. Martins, M.C. Sullivan, E.S. Friedman, A.M. Misic, A. E-Fahmawi, E.C.P. De Martinis, K. O’Brien, Y. Chen, C. Bradley, G. Zhang, C.A. Hunter, R.N. Baldassano, M.P. Rondeau, D.P. Beiting. Diet-induced changes in microbial metabolism mediate colonization resistance in enteritis. *Microbiome.* 2019: 7 (1), 1-20.
* Najem, C.E., J. Lee, C.E. Tanes, W.C. Strange, E.S. Friedman, A.G. Sreih, R.L. Rhee, A. Geara, H. Li, K. Bittinger, J.D. Lewis, P.A. Merkel. (2019). Characterizing the gut and plasma metabolome in patients with ANCA-associated vasculitis. European Congress of Rheumatology, June 12-15, Madrid, Spain.
* Friedman, E.S., T.D. Shen, Y. Li, J. Jiang, L. Chau, L. Adorini, F. Babakhani, J. Edwards, D. Shapiro, C. Zhao, R.M. Carr, K. Bittinger, H. Li, G.D. Wu. FXR-Dependent Modification of the Human Small Intestinal Microbiome. *Gastroenterology*. 2018: 155, 1741–1752.
* Najem, C.E., J. Lee, C.E. Tanes, W.C. Strange, E.S. Friedman, A.G. Sreih, R.L. Rhee, A. Geara, H. Li, K. Bittinger, J.D. Lewis, G.D. Wu, P.A. Merkel. (2018). Characterizing the gut and plasma metabolome in patients with ANCA-associated vasculitis. American College of Rheumatology Annual Meeting, October 20-24, Chicago, Illinois, USA.

Short Chain Fatty Acids

* Osmon, A., N.L. Mervosh, A.N. Strat, K.R. Meckel, T.J. Euston, G.D. Zipursky, J.D. Buxbaum, M.S. Breen, D.D. Kiraly. (2020). Effects of gene by microbiome interactions on behavioral and neurobiological phenotypes in a mouse model for autism spectrum disorder. *bioRxiv*.
* Ramsteijn, A. S., Jašarević, E., Houwing, D. J., Bale, T. L., & Olivier, J. D. (2020). Antidepressant treatment with fluoxetine during pregnancy and lactation modulates the gut microbiome and metabolome in a rat model relevant to depression. *Gut Microbes*, 1-19.
* Uribe-Herranz, M., Rafail, S., Beghi, S., Gil-de-Gómez, L., Verginadis, I., Bittinger, K., Pustylnikov, S., Pierini, S., Perales-Linares, R., Blair, I.A., Mesaros, C.A., Snyder, N.W., Bushman, F., Koumenis, C., Facciabene, A.J. (2019). Gut microbiota modulate dendritic cell antigen presentation and radiotherapy-induced antitumor immune response. *The Journal of Clinical Investigation*, 130(1).
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* Najem, C.E., J. Lee, C.E. Tanes, W.C. Strange, E.S. Friedman, A.G. Sreih, R.L. Rhee, A. Geara, H. Li, K. Bittinger, J.D. Lewis, G.D. Wu, P.A. Merkel. (2018). Characterizing the gut and plasma metabolome in patients with ANCA-associated vasculitis. American College of Rheumatology Annual Meeting, October 20-24, Chicago, Illinois, USA.