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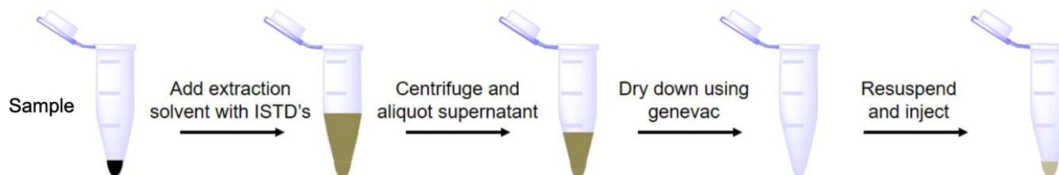
Experiment Details

Indole/Tryptophan Panel

a. Sample processing overview



Tryptophan Panel Sample Processing. Depiction of standard tryptophan panel sample processing workflow for liquid or solid samples.

b. Instrumentation used for analyses
   i. Agilent 1290 Infinity II / 6470 Triple Quadrupole (QQQ) LC-MS

c. Files reported and analysis details
   i. Quantitative files: Quantitative values are calculated after raw peak normalization with paired internal standards (Ex. Raw peak area for tryptophan/raw peak area of d_8-tryptophan). The 10-point line of the metabolite specific calibration curve is then used to calculate the endogenous concentration for each compound. Values are reported as **µM** concentration.
      1. Excel file of quantitative values
      2. Bar plot of quantitative values (**µM** y-axis)

d. Qualitative files: Qualitative values are calculated from the raw peak area of the endogenous compound normalized to the median raw peak area of two internal standards.
      1. Excel file of qualitative values
      2. Heatmap (coloration is the log2fold change of the median-normalized peak area for each compound. Grey indicates samples with raw peak areas below standard detection level).

HMMF Quality Control

Quality control samples:

- Control biological samples: These samples are used to evaluate all HMMF processes. Specifically, metabolite extraction efficiency and instrument performance. The samples are extracted with solvent containing known internal standard (IS) concentrations. These samples are processed and analyzed alongside project runs. Recovery, retention time and %CV are calculated for ISs.
- Method blanks (Labeled "MB" in datasets): These samples do not contain any metabolites and are included in datasets to indicate instrument noise for the m/z at the retention time extracted.
- PooledQC: A small aliquot of submitter samples are combined to create a PooledQC sample that is run to determine if the matrix submitted impacts processes.

Quality control ions:

- All submitted samples are extracted with solvent that includes deuterated or heavy atom standards with known concentrations to evaluate metabolite extraction efficiency and instrument performance.
- Quality control ions are tracked in all control samples and the submitted samples.

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Methods

Metabolite Extraction from Fecal/Cecal Material

Extraction solvent (80% methanol spiked with internal standards and stored at -80 ^{o}C) was added to pre-weighed fecal/cecal samples at a ratio of 100 mg of material/mL of extraction solvent in beadruptor tubes (Fisherbrand; 15-340-154). Samples were homogenized at 4 ^{o}C on a Bead Mill 24 Homogenizer (Fisher; 15-340-163), set at 1.6 m/s with 6 thirty-second cycles, 5 seconds off per cycle. Samples were then centrifuged at -10 ^{o}C, 20,000 x *g* for 15 min and the supernatant was used for subsequent metabolomic analysis.

Metabolite Extraction from Plasma/Serum/Culture Supernatant

Samples were incubated at -80 ^{o}C for at least one hour, or up to overnight. Extraction solvent (4 volumes of 100% methanol spiked with internal standards and stored at -80 ^{o}C) was added to the liquid sample (1 volume) in a microcentrifuge tube. Tubes were then centrifuged at -10 ^{o}C, 20,000 x *g* for 15 min and supernatant was used for subsequent metabolomic analysis.

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Indole and B vitamin Analysis

Indole-containing metabolites, B-vitamins and other targeted metabolites were analyzed by LC-MS/MS. The metabolite extract (400 µL) was added to pre-labeled microcentrifuge tubes. Samples were dried down completely using a Genevac EZ-2 Elite. Samples were resuspended in 100 µL of 50:50 water:methanol and added to an Eppendorf thermomixer® C at 4 ºC, 1000 rpm for 15 min to resuspend analytes. Samples were then centrifuged at 4 ºC, 20,000 x *g* for 15 min to remove insoluble debris. The supernatant (80 µL) was transferred to a fresh, prelabeled MS vial with inserts or 96 deep-well plate (Agilent 5065-4402). Samples were analyzed on an Agilent 1290 infinity II liquid chromatography system coupled to an Agilent 6470 triple quadrupole mass spectrometer, operating in positive mode, equipped with an Agilent Jet Stream Electrospray Ionization source. Each sample (2 µL) was injected into a Acquity UPLC HSS PFP column, 1.8 µm, 2.1 x 100 mm (Waters; 186005967) equipped with a Acquity UPLC HSS PFP VanGuard Pre-column, 100Å, 1.8 µm, 2.1 mm X 5 mm (Waters; 186005974) at 45 ºC. Mobile phase A was 0.35% formic acid in Water and mobile phase B was 0.35% formic acid in 95:5 acetonitrile:water. The flow rate was set to 0.5 mL/min starting at 0% B held constant for 3 min, then linearly increased to 50% over 5 min, then linearly increased to 95% B over 1 min, and held at 100% B for the next 3 min. Mobile phase B was then brought back down to 0% over 0.5 min and held at 0% for re-equilibration for 2.5 min. The QQQ electrospray conditions were set with capillary voltage at 4 kV, nozzle voltage at 500 V, and Dynamic MRM was used with cycle time of 500 ms. Transitions were monitored in positive mode for 46 analytes. An 11-point calibration curve (ranging from 0.88 nM to 909 µM) was prepared for tryptophan, tyrosine, phenylalanine, serotonin, 5-HIAA, melatonin, tryptamine, kynurenine, kynurenic acid, anthranilic acid, and niacin. Data analysis was performed using MassHunter Quant software (version B.10, Agilent Technologies) and confirmed by comparison with authentic standards. Normalized peak areas were calculated by dividing raw peak areas of targeted analytes by averaged raw peak areas of internal standards.