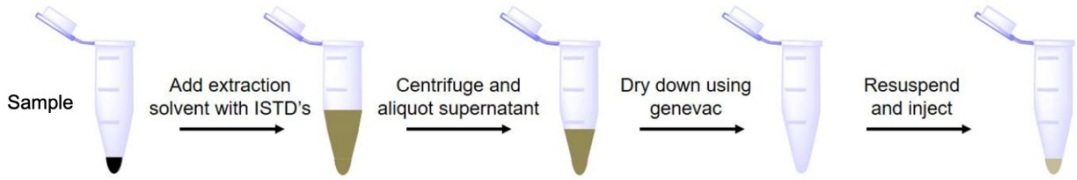
Experiment Details

Bile Acid Panel

a. Sample processing overview



Bile Acid Panel Sample Processing. Depiction of standard bile acid panel sample processing workflow for liquid or solid samples.

b. Instrumentation used for analyses
    i. Agilent 1290 Infinity II / 6546 Quadrupole Time of Flight (Q-TOF) LC/MS system
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 cholic acid/raw peak area of d_5-cholic acid). 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 **µg/mL** concentration.
        1. Excel file of quantitative values
    ii. 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

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

Methods

Metabolite Extraction from Fecal/Cecal Material

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

Bile Acid Analysis

Bile acids were analyzed using LCMS. The metabolite extract (75 µL) was added to prelabeled mass spectrometry autosampler vials (Microliter; 09-1200) and dried down completely under a nitrogen stream at 30 L/min (top) 1 L/min (bottom) at 30 ºC (Biotage SPE Dry 96 Dual; 3579M). Samples were resuspended in 50:50 Water:Methanol (750 µL). Vials were added to a thermomixer C (Eppendorf) to resuspend analytes at 4 ºC, 1000 rpm for 15 min with an infinite hold at 4 ºC. Samples were then transferred to prelabeled microcentrifuge tubes and centrifuged at 4 ºC, 20,000 x *g* for 15 min to remove insoluble debris. The supernatant (700 µL) was transferred to a fresh, prelabeled mass spectrometry autosampler vial. Samples were analyzed on a liquid chromatography system (Agilent 1290 infinity II) coupled to a quadrupole time-of-flight (QTOF) mass spectrometer (Agilent 6546), operating in negative mode, equipped with an Agilent Jet Stream Electrospray Ionization source. The sample (5 µL) was injected onto an XBridge© BEH C18 Column (3.5 µm, 2.1 x 100 mm; Waters Corporation, PN) fitted with an XBridge© BEH C18 guard (Waters Corporation, PN) at 45 ºC. Elution started with 72% A (Water, 0.1% formic acid) and 28% B (Acetone, 0.1% formic acid) with a flow rate of 0.4 mL/min for 1 min and linearly increased to 33% B over 5 min, then linearly increased to 65% B over 14 min. Then the flow rate was increased to 0.6 mL/min and B was increased to 98% over 0.5 min and these conditions were held constant for 3.5 min. Finally, re-equilibration at a flow rate of 0.4 mL/min of 28% B was performed for 3 min. The electrospray ionization conditions were set with the capillary voltage at 3.5 kV, nozzle voltage at 2 kV, and detection window set to 100-1700 *m/z* with continuous infusion of a reference mass (Agilent ESI TOF Biopolymer Analysis Reference Mix) for mass calibration. A ten-point calibration curve was used for quantitation. Data analysis was performed using MassHunter Profinder Analysis 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.