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Coprostanol Extraction Protocol using Dionex ASE 100

DAILY SET-UP

- Refill solvent jar with methylene chloride (enough for day of samples ~70ml per sample, 10 to 12 samples per day)
- Turn on gas: open the tank valve, then adjust the regulator knob to 150 psi, then open the exit valve
- Make sure waste bottle on left is ~empty
- Install rinse/waste collection bottle on right; bottom end first, then top and toggle needle-housing to down position; check that housing is firmly seated against the collection bottle
- Turn on ASE with switch on back panel
- Install rinse (blue) cell in oven and press the "Rinse" button
- When rinse cycle is done (~5min), remove rinse collection bottle by toggling needle housing up
- Remove the rinse cell from oven

SAMPLE CELL LOADING

- Screw bottom onto cell by hand
- Place cellulose filter in cell at an angle and pack filter down to the bottom with tamping tool
- Weigh ~4g of freeze dried sample into the sample cell using custom funnel and record weight
- Remove funnel and add second cellulose filter on top of sample and pack down with tool
- Fill void space with clean sand ~47 g using custom funnel
- Dust off any debris from threads with kim wipe
- Screw on top cap by hand

EXTRACTION

- In the status screen, make sure method "12" is selected
- Install fresh, labeled collection bottle (bottom first), then lower needle housing
- Press "Start" and wait for preheat cycle to say "Oven ready"
- Install cell containing sample in oven (bottom first)
- Press "Start" again and when light stops flashing, wait ≈28 min
- Unload cell using insulated towel
- Put cell in freezer for ≈5 min to cool
- Toggle needle housing "Up," remove collection bottle and place in fume hood
- When cell is cool enough to handle, unscrew top and pour sand into "Used Sand" beaker
- Unscrew bottom cap and push filters and sediment into waste beaker
- Rinse cell and caps with acetone and dry thoroughly
- Store collection bottles in dark until ready for derivatization

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Cleaning Sand

- When enough "Used sand" is collected, add methylene chloride to beaker (enough to just cover the sand)
- Sonicate beaker with heat for ≈1 hour
- Pour off supernatant into waste container as much as possible
- Cover beaker loosely with foil and place in fume hood to evaporate for a few days until dry

Derivatization

- Transfer extract from collection bottle to evaporation tube
- Evaporate extract to dryness in water bath, ramping temperature from 40 to 50 C over 2 or 3 hours
- Resuspend extract using 2 successive 1-mL doses of methanol
- Use a new disposable pipette to transfer the extract to a 2-mL volumetric tube
- Bring up to 2mL using methanol
- Subsample 300 uL to GC vial
- Evaporate to dryness in oven at 60 C
- Add 20 uL piperidine using designated syringe
    - Cover with parafilm and store overnight in refrigerator if necessary
- Add 50 uL BSTFA and vortex
- Cap vial and incubate in 60 C oven for 30 min
- Add another 50 uL BSTFA and vortex
- Incubate in 60 C oven for another 30 min
- Uncap and bring up to 0.5 mL with hexane;
- Recap and vortex

Standards

- Pipet 200uL of 50ppm coprostanol stock solution into GC vial
- Evaporate to dryness at 60 C
- Add 20uL piperidine using designated syringe
- Add 50uL BSTFA and vortex
- Cap vial and incubate in 60 C oven for 30 min
- Add another 50 uL BSTFA and vortex
- Incubate in 60 C oven for another 30 min
- Uncap and bring up to 1 mL with hexane; i.e. add 880uL hexane to the 120uL of reagents
- Recap and vortex