GC Assay for Cellular Cholesterol Mass

1. Must use cells cultured in 12-well dishes or larger.

2. Make sure all solutions are fresh and made under the cleanest standards and that glassware is new our cleaned with hexane and chloroform (not just choloform alone)—small contaminations will ruin the experiment.

3. Lipid extract your cells as usual with HIP except add β-sitosterol (Sigma 97% minimum purity) to the stock HIP so that each well gets a final amount of 5 µg β-sitosterol. Can make a concentrated stock of β-sitosterol in HIP (store under argon in freezer).

4. For FC: Take 1/3 of HIP, dry down in new borosilicate tube, bring up in 50 µl hexane, and transfer to GC injector vials. Inge Hanson in Richard Deckelbaum's lab (BB-4th floor, x53961) will then inject these into the GC for analysis.

5. For TC:
   a. Dry down the other 2/3 in hexane-washed tubes with sintered glass tops—do not use tubes with pastic tops and Teflon liners.
   b. Add 200 µl 50% KOH (50g KOH/100 ml ddH_2O) and 3 ml MeOH, cap with argon, and **vortex**.
   c. Heat 1 hour at 80^oC in heating block with oil.
   d. Allow to cool to room temp., and then add 3 ml H_2O and 5 ml HPLC grade hexane. **Vortex**.
   e. Spin 1000 rpm for 5 min and harvest top (hexane) phase.
   f. Dry down and bring up in hexane as in #3 above.

6. Calculations:
   a. (area under cholesterol peak ÷ area under β-sitosterol peak) x 5 = µg cholesterol
   b. CE = TC – FC (multiply by 1.7 if data is in mass instead of moles)