Mass Spectrometry Protocol

I. SAMPLE PREP
   a. Sample should have been appropriately "cleaned up." (Filtered/washed, purified by column, extractions, etc.)
   b. Free from salts
   c. DILUTIONS – this is the most vital step. Take the time to ensure your dilutions are at the appropriate levels.
      i. 1 mg of compound in 1 mL = solution A (this is not a dilution)
      ii. 1 uL of solution A in 1 mL = solution B (1000 fold dilution)
      iii. You should walk into the room with 3 separate dilutions: 1 uL (B) in 1 mL (1 million fold dilution), 10 uL (B) in 1 mL (100,000 fold dilution), 40 uL (B) in 1 mL (60,000 fold dilution). Start with the lowest dilution and work your way up to the most concentrated. If you are not seeing anything at the 40 uL dilution DO NOT increase further. ASK SOMEONE to help you optimize the instrument.
   d. SOLUBILITY – At the very minimum you should completely understand what your sample is soluble in. Water should always be used as a 5-50% co-solvent. Never inject 100% of any one solvent.
      i. APPROPRIATE SOLVENTS (Primary solvents to be injected)
         1. LS/MS Grade Methanol
         2. LS/MS Grade Water
         3. LS/MS Grade Acetonitrile
      ii. COSOLVENTS (One drop to increase solubility in primary)
         1. Chloroform
         2. Dichloromethane
      iii. ADDITIVES (To promote ionization)
         1. Formic Acid 0.1%
         2. Ammonium Hydroxide 0.1%
      iv. NEVER inject under any circumstances
         1. THF
         2. **ANY** Solvent that is not at least LC/MS Grade
         3. BUFFERS (with the exception of ammonium based buffers)
         4. NON-VOLATILE SALTS
         5. Millipore/Nanopur water
         6. TAP water
         7. Old solvents (> 12 months)
      v. APCI (atmospheric pressure chemical ionization) source is available for compounds that are not soluble in above listed solvents by appointment only.

II. DATA COLLECTION
   a. Enter your target mass under the tune tab.

b. Run a **blank** of 1:1 ACN:Water (0.1% formic acid). Collect data for 2 minutes, average entire data set (right click and drag). Print spectra Blank 1)
c. Run **sample** from low dilution (3 uL/min ONLY)
d. OPTIMIZE
    i. Make sure you've correctly entered your target mass under the tune tab.
    ii. Ensure that under the mode tab you are collecting in maximum resolution
    iii. If you have trouble seeing your data:
        1. try switching polarity and ensure you have used an additive.
        2. Nebulizer pressure/dry gas pressure/dry gas temperature. Start at the high end of all the posted ranges and work backward paying attention to the relative intensity. Nebulizer pressure of 12 seems to be a fairly good level.
        3. Solvent composition. Use different amounts of aqueous and organic sovlents
    iv. Increasing your concentration above the final dilution (60,000 fold dilution) is unnecessary and prohibited. When in doubt ask for help.
e. IDEAL SIGNAL STRENGTH SHOULD BE 10^5 OR 10^6; SIGNAL SHOULD NEVER BE ABOVE 10^7.
f. IF SIGNAL REACHES 10^8 OR 10^9 --- STOP IMMEDIATELY BY TURNING OFF SYRINGE PUMP
g. Following sample data collection:
h. Print sample data spectrum
i. Disconnect line, flush with clean solvent.
j. Reconnect line and flow solvent until background returns to pre sample run levels.
k. Collect data for 2 minutes, average data, print **blank 2**.
l. Return the instrument to stand-by, wipe out housing.
m. Place a copy of all three spectra (blank 1, sample, blank 2) along with the completed sample information form in the binder in the mass spec room.

III. SCHEDULING

a. The goal is to have everyone log in and use the instrument completely independently, however, for the immediate future there will only be one way to log in to the instrument. You must find Billy in his lab or office to log you into the machine.
b. Create a google username and email it to Billy at wmotel@rx.umaryland.edu. He will add you to a google calendar where you can schedule time to use the instrument. The current hours of operation will be from 9AM-5PM, Monday thru Friday.