Guidelines for the Hands-on Training on BD AccuriC6

1. **Ice bucket** with samples covered with aluminum foil
   Note: *All the samples (unless otherwise specified by the protocol) should remain at +4^{o}C protected from light*

2. Plenty of the **unstained cells**. Cell concentration to be 1-5 x 10^6/ml. 1 ml volume should be enough. Useful Tips to Improve Sample Quality could be found at http://www.rockefeller.edu/fcrc/tips/sampleprep
   Note: *These unstained cells should fully match the sample origin and preparation to be used in the future data acquisition on Accuri C6.*

3. **Cell samples stained with different concentrations of antibody** in order to find the proper titer for each antibody (if applicable):
   a. For the antibody previously not used in the lab – bring "wide" titration.
      For example: 3x; x; 1/3x; 1/9x; 1/27x; 1/81x (where "x" is the concentration suggested by the vendor)
   b. For the antibody previously used in the lab – bring "narrow" titrations.
      For example: 3y; y; 1/3y; 1/9y (where "y" is the concentration suggested by the Labmates)
   c. For Live Dead Fixable Dyes - bring "narrow" titration.
      For example: 2z; z; 1/2z (where "z" is the concentration suggested by the vendor). Make sure to follow the vendor's staining protocol (time, temperature, special buffer)
      Note: *Cell concentrations to be 1-5 x 10^6/ml. 500 ul volume should be enough.*

4. 15 ml tube with the **"FACS Buffer"** (http://www.rockefeller.edu/fcrc/tips/cellsorting) to use in case we need to dilute the samples or titer non-fixable Dead Cell Exclusion Dye (DCE).

5. 1.5 ml of the 1 ug/ml **stock of the non-fixable DCE** (for example 7AAD, PI, ToPro3, etc.) to prepare titration and stain samples.
   Note: *These dyes remain in the "FACS Buffer" with single cell suspension (no wash step).*

6. **Automatic pipets** (20, 200 and 1000 ul).
   Note: *FCRC doesn't lend pipets in the Analysis room, but offers tips.*

7. **Sharpie marker** (to label tubes).