**Sputum FACS Protocol 9/25/19**

Materials:

* Sputum samples
* Flow cytometry analysis tubes with cell strainer top (35µm mesh size)
* 15mL conical tubes
* 2mL microcentrifuge tubes
* 1.5mL microcentrifuge tubes
* Phosphate buffered saline (Mg2+ and Ca2+ free)
* Disposable transfer pipettes

Steps:

*\*\*schedule time on the flow cytometer at the Flow Core with a prepared PO\*\**

**Preparing Sputum Cell Suspensions**

1. Turn centrifuge on and set to 4°C.
2. For each sputum sample to be analyzed, remove the plug and additional fluid necessary to bring the total volume to 2mL in 15mL conical tubes.
3. Add 2mL (1:1) of Sputasol (dithiothreitol) to each conical.
4. Incubate the mixture at 37°C for 15 minutes. The resulting mixture should be visibly homogenous.
5. *Make three separate dilutions in 2mL microcentrifuge tubes of each sample as follows:*
   1. *No dilution - 1mL of sample*
   2. *1:2 - 500µl of sample in 1mL PBS*
   3. *1:5 - 500uL of sample in 2.5mL PBS*
6. Remove 1mL of each into separate 1.5 microcentrifuge tubes and centrifuge each suspension at 10,000 x g for 2 minutes to pellet the cells.
7. Remove the supernatants. Re-suspend the pellets in 1mL of PBS. Briefly vortex until homogenous.
8. Aliquot 500µL of each sample into FACS tubes. Bring the total volume of each tube up to 1mL with PBS. Briefly vortex to homogenize.
9. Using a new disposable transfer pipette for each tube, draw up the suspension and expel it through the cell strainer top.

**Analyzing Cells via Flow Cytometry**

**NOTES:**