

BRONCHOSCOPY SPUTUM PROCESSING WORKSHEET

	ID NUMBER: FORM CODE: BSW Visit VERSION: 2.0 03/06/12 Number SEQ #										
0a	Instructions: Complete this form while processing the sputum sample. Carefully record all data in the space provided. When entering times mark them as hours, minutes, and circle AM or PM.										
1.	Time processing began:										
2.	h h:m m (Circle One) 2. Color and Description of Sample: a. Salivary Contamination: Minimal Mid Moderate Excessive b. Consistency: Watery Mucoid Purulent (puss) c. Mucus "plugs": Numerous Moderate number Sparse Large Small Dense/flocculent Diffuse opacity d. Color of plugs: Clear White Yellow/Tan Brown Green										

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3. General Notes/Comments:
4. Time immunophenotyping processing began:
5. Weight of the 50mL Centrifuge tube: grams
Remember to zero the balance.
6. Weight of the sputum: grams
Make up 1x Sputolysin: combine 1 ml of sputolysin with 9 mls of distilled water and swirl gently to dissolve crystals
7. Volume of Sputolysin to add (weight of sputum x2):
8. Time placed in 37°C water bath:
Leave in bath approximately 20 minutes or until the sputum appears viscous with vortexing every 5 minutes.
9. Number of times vortexed:
10. Time removed from water bath: AM/PM (circle 1)
11. Volume of PBS added: mL
Centrifuge at 300 x g for 5 mins, then decant supernatant. Resuspend cells in 1.0 ml Stain Buffer. Briefly centrifuge immunophenotyping sputum assay antibody tubes (supplied by immunophenotyping core) at 300 x g for 2 mins and uncap tubes
12. Time pre-made sputum assay antibody tubes placed in centrifuge: AM/PM (circle 1)
Add 100 μ l of sputum sample to each tube. Wrap rack with tinfoil to protect from light.
13. Number of pre-made sputum assay antibody tubes used:

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Incubate tubes for 25 minutes at room temperature, with shaking or rocking.													
14. Time incubation begin: AM/PM (circle													cle 1)
15. Time incubation end: AM/PM (circle													cle 1)
Add 2 mls of Stain Buffer to each tube. Centrifuge at 300 x g for 5 mins. Decant supernatant. Prepare 2% formaldehyde solution: Combine 0.2 mls 20% formaldehyde with 1.8 mls Stain Buffer. Add 200 μ l of 2% formaldehyde to each tube. Replace caps on tubes; they should snap firmly into place. Wrap rack in a new sheet of tinfoil. Store at 4°C until ready to be shipped													
16. Time placed in 4°C storage: AM/PM (circle 1)													
17. Problems processing:													