

ID NUMBER:									
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FORM CODE: BSW
VERSION: 2.0 03/06/12

Visit Number		
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SEQ #			
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3. General Notes/Comments:

4. Time immunophenotyping processing began:

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 AM/PM (circle 1)

5. Weight of the 50mL Centrifuge tube:

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 grams

Remember to zero the balance.

6. Weight of the sputum:

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 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):

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 mL

8. Time placed in 37°C water bath:

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 AM/PM (circle 1)

Leave in bath approximately 20 minutes or until the sputum appears viscous with vortexing every 5 minutes.

9. Number of times vortexed:

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10. Time removed from water bath:

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 AM/PM (circle 1)

11. Volume of PBS added:

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 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:

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 AM/PM (circle 1)

Add 100 µl 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:

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 AM/PM (circle 1)

15. Time incubation end:

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 AM/PM (circle 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 µl 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:

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 AM/PM (circle 1)

17. Problems processing:
