

## BRONCHOSCOPY SUB-STUDY SPECIMEN PROCESSING WORKSHEET

ID NUMBER:

FORM CODE: BPW  
VERSION: 2.0 05/09/2023

Event: \_\_\_\_\_

0a) Date of Collection:   /   /

0b) Staff Code:

**Instructions:** This form should be completed during the participant's Bronchoscopy Visit 2.

**BLOOD**

1) for local lab CBC [1 x 4 mL purple top (EDTA) tube]

1a) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

2) for immunophenotyping [3 x 10 mL purple top (EDTA) tubes]

2a) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

2b) Time aliquots placed in refrigerator:

:   AM<sub>1</sub> / PM<sub>2</sub>

3) for biomarkers [1 x 10 mL purple top (EDTA) tube]

3a) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

3b) Number of aliquots:

3c) Volume in last aliquot:

$\mu$ L

3d) Time aliquots placed in freezer:

:   AM<sub>1</sub> / PM<sub>2</sub>

4) for biomarkers [1 x 10 mL purple top (EDTA) tube]

4a) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

4b) Number of aliquots:

4c) Volume in last aliquot:

$\mu$ L

4d) Time aliquots placed in freezer:

:   AM<sub>1</sub> / PM<sub>2</sub>

5) for biomarkers [1 x 8.5 mL red top (for serum) tube]

5a) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

5b) Number of aliquots:

5c) Volume in last aliquot:

$\mu$ L



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15) Time 15 mL freezer jars placed in freezer:

:   AM<sub>1</sub> / PM<sub>2</sub>

**SALINE SPECIMEN**

16) Time Scope Saline placed in freezer:

:   AM<sub>1</sub> / PM<sub>2</sub>

**PROTECTED BRUSH SPECIMEN**

17) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

18) Problems processing?

No<sub>0</sub> → **Go to 19**

Yes<sub>1</sub>

If Yes, please specify the problem (*check all that apply*):

18a)  Blood in the sample

18b)  Other

18b1) If Other, please specify: \_\_\_\_\_

19) Time placed in refrigerator:

:   AM<sub>1</sub> / PM<sub>2</sub>

20) Date moved to freezer:

/   /

21) Time placed in freezer:

:   AM<sub>1</sub> / PM<sub>2</sub>

**BAL**

**Microbiome Sample with No Reagent:**

22) Time transferred to 15 mL conical:

:   AM<sub>1</sub> / PM<sub>2</sub>

23) Time placed in freezer:

:   AM<sub>1</sub> / PM<sub>2</sub>

**Microbiome Sample with RNA/ater™:**

24) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

25) Time placed in refrigerator:

:   AM<sub>1</sub> / PM<sub>2</sub>

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26) Date moved to freezer:   /   /

27) Time placed in freezer:   :   AM<sub>1</sub> / PM<sub>2</sub>

28) Total volume of mixed pooled BAL fluid prior to centrifugation:     .  mL  
*(This is the volume after both microbiome samples are completed)*

**Supernatant Sample:**

29) Time processed:   :   AM<sub>1</sub> / PM<sub>2</sub>

30) Number of 1 mL aliquots made:

31) Number of 15 mL aliquots made:

32) Time placed in freezer:   :   AM<sub>1</sub> / PM<sub>2</sub>

**Cell Counts:**

33) Time processed:   :   AM<sub>1</sub> / PM<sub>2</sub>

34) Total volume returned:     .  mL

35) LIVE cell count = # of live cells/mL = (# live cells in 4 squares/4) x 2 x 10<sup>4</sup>:  
           cells/mL

36) DEAD cell count = # of dead cells/mL = (# dead cells in 4 squares/4) x 2 x 10<sup>4</sup>:  
           cells/mL

37) TOTAL cell count = # live cells/mL x resuspension volume (10 mL):  
           cells

38) % cell viability = [# of live cells/mL / (# of live cells/mL + # of dead cells/mL)] x 100:  
   .   %

*NOTE: The % cell viability value will be automatically calculated in the DMS.*

39) Cytospin suspension = Total cells in 10 mL tube = # cells/mL x 10 mL:  
           cells in 10 mL tube

40) Volume to resuspend pellet in with PBS = # cells in tube/1 x 10<sup>6</sup>:     .  mL

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**Cytospin Slide Sample:**

41) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

42) Number of cytospin slides:

43) Time fixed:

:   AM<sub>1</sub> / PM<sub>2</sub>

**Alveolar Macrophage Isolation (must be processed exactly 2 hours after collection):**

44) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

45) Was the macrophage plating of  $1 \times 10^6$  BAL cells completed?

No<sub>0</sub>

Yes<sub>1</sub>

46) Was the RNA extracted with 600  $\mu$ L of QIAzol buffer?

No<sub>0</sub>

Yes<sub>1</sub>

47) Is the time processed less than 2 hours after collection?

No<sub>0</sub> → **Go to 48**

Yes<sub>1</sub>

If Yes,

47a) Minutes since collection:

minutes

47b) Reason processed sooner than 2 hours after: \_\_\_\_\_

48) Is the time processed more than 2 hours after collection?

No<sub>0</sub> → **Go to 49**

Yes<sub>1</sub>

If Yes,

48a) Minutes since collection:

minutes

48b) Reason processed more than 2 hours after: \_\_\_\_\_

49) Time processing complete:

:   AM<sub>1</sub> / PM<sub>2</sub>

50) Time placed in freezer:

:   AM<sub>1</sub> / PM<sub>2</sub>

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**Immunophenotyping BAL:**

51) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

52) Time processing complete:

:   AM<sub>1</sub> / PM<sub>2</sub>

53) Time placed in refrigerator:

:   AM<sub>1</sub> / PM<sub>2</sub>

**CYTOLOGICAL BRUSHES FOR RNA (x 3)**

54) Problems processing?

No<sub>0</sub> → **Go to 55**

Yes<sub>1</sub>

If Yes, please specify the problem (*check all that apply*):

54a)  Blood in the sample

54b)  Other

54b1) If Other, please specify: \_\_\_\_\_

**Cytospin Slides:**

55) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

56) Cell count = # cells/mL = (# cells in 4 squares/4) x 2 x 10<sup>4</sup>:

cells/mL

57) Number of cytospin slides:

**Epithelial RNA Extraction:**

58) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

59) Time placed in freezer:

:   AM<sub>1</sub> / PM<sub>2</sub>

**CYTOLOGICAL BRUSHES FOR DNA (x 2)**

60) Problems processing?

No<sub>0</sub> → **Go to 61**

Yes<sub>1</sub>

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If Yes, please specify the problem (*check all that apply*):

- 60a)  Blood in the sample
- 60b)  Other

60b1) If Other, please specify: \_\_\_\_\_

61) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

62) Time placed in freezer:

:   AM<sub>1</sub> / PM<sub>2</sub>

**MICROCYTOLOGICAL BRUSHES FOR MUCIN (x 2)**

63) Problems processing?

- No<sub>0</sub> → **Go to 64**
- Yes<sub>1</sub>

If Yes, please specify the problem (*check all that apply*):

- 63a)  Blood in the sample
- 63b)  Other

63b1) If Other, please specify: \_\_\_\_\_

64) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

65) Time PBS tube placed in freezer:

:   AM<sub>1</sub> / PM<sub>2</sub>

66) Time Urea tube placed in refrigerator:

:   AM<sub>1</sub> / PM<sub>2</sub>

**SMALL AIRWAY EPITHELIAL BRUSHES**

67) Problems processing?

- No<sub>0</sub> → **Go to 68**
- Yes<sub>1</sub>

If Yes, please specify the problem (*check all that apply*):

- 67a)  Blood in the sample
- 67b)  Other

67b1) If Other, please specify: \_\_\_\_\_

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**Cytospin Slides:**

68) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

69) Cell count = # cells/mL = (# cells in 4 squares/4) x 2 x 10<sup>4</sup>:

cells/mL

70) Number of cytospin slides:

**Epithelial RNA Extraction:**

71) Time processed:

:   AM<sub>1</sub> / PM<sub>2</sub>

72) Time RNA tube placed in freezer:

:   AM<sub>1</sub> / PM<sub>2</sub>

73) Time DNA tubes placed in freezer:

:   AM<sub>1</sub> / PM<sub>2</sub>

**Basal Cells for Culture:**

74) Time put into culture:

:   AM<sub>1</sub> / PM<sub>2</sub>

75) Time put into passage:

:   AM<sub>1</sub> / PM<sub>2</sub>

76) Time frozen:

:   AM<sub>1</sub> / PM<sub>2</sub>

**END OF FORM**