

## BRONCHOSCOPY SUB-STUDY SPECIMEN PROCESSING WORKSHEET

ID NUMBER:

FORM CODE: BPW  
VERSION: 3.0 07/17/2023

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:

μL

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:

μL

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:

μL

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5d) Time aliquots placed in freezer:

		:			AM <sub>1</sub> / PM <sub>2</sub>
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6) for biomarkers [1 x 8.5 mL red top (for serum) tube]

6a) Time processed:

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

6b) Number of aliquots:

--	--

6c) Volume in last aliquot:

				µL
--	--	--	--	----

6d) Time aliquots placed in freezer:

		:			AM <sub>1</sub> / PM <sub>2</sub>
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**NASAL SWAB**

7) Specimen source:

7a) Number of swabs processed right naris:

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7b) Number of swabs processed left naris:

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8) Problems processing?

No<sub>0</sub> → **Go to 9**

Yes<sub>1</sub>

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

8a)  Blood in the sample

8b)  Other

8b1) If Other, please specify: \_\_\_\_\_

9) Time processed:

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

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

**ORAL SPECIMEN**

11) Time processed:

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

12) Number of 15 mL freezer jars:

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13) Time 15 mL freezer jars placed in refrigerator:

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

14) Date 15 mL freezer jars moved to freezer:

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

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>

77) Number of aliquots:

*NOTE: ONLY count the number of basal cell stock aliquots that will be shipped to Dr. Crystal's lab. DO NOT include the one aliquot used to confirm cell viability in the number reported in item 77.*

**END OF FORM**