



BRONCHOSCOPY SUBSTUDY SPECIMEN PROCESSING WORKSHEET

ID NUMBER:

FORM CODE: BPW
VERSION: 4.0 12/12/2019

Event: _____

0a) Date of Collection / /

0b) Staff Code

Instructions: This form should be completed during the participant's Bronchoscopy substudy visit #2.

BLOOD

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

1a) Time processed: : AM₁ / PM₂ (circle one)

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

2a) Time processed: : AM₁ / PM₂ (circle one)

2b) Time aliquots placed in refrigerator: : AM / PM (circle one)

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

3a) Time processed: : AM₁ / PM₂ (circle one)

3b) Number of aliquots:

3c) Volume in last aliquot: μL

3d) Time aliquots placed in freezer: : AM₁ / PM₂ (circle one)

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

4a) Time processed: : AM₁ / PM₂ (circle one)

4b) Number of aliquots:

4c) Volume in last aliquot: μL

4d) Time aliquots placed in freezer: : AM₁ / PM₂ (circle one)

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

5a) Time processed: : AM₁ / PM₂ (circle one)

5b) Number of aliquots:

5c) Volume in last aliquot: μL

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5d) Time aliquots placed in freezer: : AM₁ / PM₂ (circle one)

6) for biomarkers 1 x 8.5 mL red top (for serum) tube

6a) Time processed: : AM₁ / PM₂ (circle one)

6b) Number of aliquots:

6c) Volume in last aliquot: μL

6d) Time aliquots placed in freezer: : AM₁ / PM₂ (circle one)

NASAL EPITHELIAL SWABS:

7) Specimen source:

7a) Number of brushes processed right nare:

7b) Number of brushes processed left nare:

8) Problems Processing?

No₀ → **Go to 9**

Yes₁

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: : AM₁ / PM₂ (circle one)

10) Time placed in freezer: : AM₁ / PM₂ (circle one)

ORAL SPECIMEN

11) Time processed: : AM₁ / PM₂ (circle one)

12) Number of 15mL freezer jars:

13) Time 15mL freezer jars placed in refrigerator: : AM₁ / PM₂ (circle one)

14) Date 15mL freezer jars moved to freezer: / /

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15) Time 15mL freezer jars placed in freezer: : AM₁ / PM₂ (circle one)

SALINE SPECIMEN

16) Time Scope Saline placed in freezer: : AM₁ / PM₂ (circle one)

PROTECTED BRUSH SPECIMEN

17) Time processed: : AM₁ / PM₂ (circle one)

18) Problems processing?

No₀ → **Go to 19**

Yes₁

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: : AM₁ / PM₂ (circle one)

20) Date moved to freezer: / /

21) Time placed in freezer: : AM₁ / PM₂ (circle one)

INITIAL AIRWAY WASH

22) Was more than a total of 8 mL returned from the airway washes?

No₀ → **Go to 27**

Yes₁

Microbiome sample in RNA Later:

23) Time processed: : AM₁ / PM₂ (circle one)

24) Time placed in refrigerator: : AM₁ / PM₂ (circle one)

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25) Date moved to freezer: / /

26) Time placed in freezer: : AM₁ / PM₂ (circle one)

Cell count:

27) Time processed: : AM₁ / PM₂ (circle one)

28) Total volume returned: . mL

29) Cell count= # cells/mL= (# cells in 4 squares/4) x 2 x 10⁴ =
 cells/mL

30) Total cell count= # cells/mL x Wash returned = cells

31) Cytospin suspension= Total cells in 10 mL tube= # cells/mL x volume of wash in 10mL tube=
 cells in 10 mL tube

32) Volume to resuspend pellet in with PBS= # cells in tube/0.7 x 10⁶ = . mL

33) Number of cytospin slides created:

Supernatant aliquots

34) Time processed: : AM₁ / PM₂ (circle one)

35) Number of 500 µl aliquots created:

36) Time placed in freezer: : AM₁ / PM₂ (circle one)

RNA prep of cell pellet

37) Time processed: : AM₁ / PM₂ (circle one)

38) Time placed in freezer: : AM₁ / PM₂ (circle one)

BAL

Microbiome sample with no reagent:

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39) Time transferred to 15 mL conical: : AM₁ / PM₂ (circle one)

40) Time placed in freezer: : AM₁ / PM₂ (circle one)

Microbiome sample with RNA Later:

41) Time processed: : AM₁ / PM₂ (circle one)

42) Time placed in refrigerator: : AM₁ / PM₂ (circle one)

43) Date moved to freezer: / /

44) Time placed in freezer: : AM₁ / PM₂ (circle one)

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

Supernatant sample:

46) Time processed: : AM₁ / PM₂ (circle one)

47) Number of 1 mL aliquots made:

48) Number of 15 mL aliquots made:

49) Time placed in freezer: : AM₁ / PM₂ (circle one)

Cell counts:

50) Time processed: : AM₁ / PM₂ (circle one)

51) Total volume returned: . mL

52) LIVE cell count = # of live cells/mL = (# live cells in 4 squares/4) x 2 x 10⁴:
 cells/mL

53) DEAD cell count = # of dead cells/mL = (# dead cells in 4 squares/4) x 2 x 10⁴:
 cells/mL

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

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cells

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

cells in 10 mL tube

56) Volume to resuspend pellet in with PBS = # cells in tube/0.7 x 10⁶: . mL

Cytospin slide sample:

57) Time processed: : AM₁ / PM₂ (circle one)

58) Number of cytospin slides:

59) Time fixed: : AM₁ / PM₂ (circle one)

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

60) Time processed: : AM₁ / PM₂ (circle one)

61) Was the macrophage plating of 1x10⁶ BAL cells completed?

- No₀
 Yes₁

62) Was the RNA extracted with 600 µl of QIAzol buffer?

- No₀
 Yes₁

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

- No₀ → **Go to 64**
 Yes₁

If Yes,

63a) Minutes since collection: minutes

63b) Reason processed sooner than 2 hours after: _____

64) Time processed **more** than 2 hours after collection:

- No₀ → **Go to 65**
 Yes₁

If yes,

64a) Minutes since collection: minutes

64b) Reason processed more than 2 hours after: _____

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65) Time processing complete: : AM₁ / PM₂ (circle one)

66) Time placed in freezer: : AM₁ / PM₂ (circle one)

Immunophenotyping BAL:

67) Time processed: : AM₁ / PM₂ (circle one)

68) Time processing complete: : AM₁ / PM₂ (circle one)

69) Time placed in refrigerator: : AM₁ / PM₂ (circle one)

Large Airway Epithelial Brushes x 3 (RNA):

70) Problems Processing?

No₀ → **Go to 71**

Yes₁

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

70a) Blood in the sample

70b) Other

70b1) If Other, please specify: _____

Cytospin Slides:

71) Time processed: : AM₁ / PM₂ (circle one)

72) Cell count= # cells/mL= (# cells in 4 squares/4) x 2 x 10⁴:

cells/mL

73) Number of cytospin slides:

Epithelial RNA Extraction:

74) Time processed: : AM₁ / PM₂ (circle one)

75) Time placed in freezer: : AM₁ / PM₂ (circle one)

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Large Airway Epithelial Brushes x 2 (DNA):

76) Problems Processing?

No₀ → **Go to 77**

Yes₁

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

76a) Blood in the sample

76b) Other

76b1) If Other, please specify: _____

77) Time processed: : AM₁ / PM₂ (circle one)

78) Time placed in freezer: : AM₁ / PM₂ (circle one)

Large Airway Epithelial Brushes x 2 (Mucin):

79) Problems Processing?

No₀ → **Go to 80**

Yes₁

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

79a) Blood in the sample

79b) Other

79b1) If Other, please specify: _____

80) Time processed: : AM₁ / PM₂ (circle one)

81) Time PBS tube placed in freezer: : AM₁ / PM₂ (circle one)

82) Time Urea tube placed in refrigerator: : AM₁ / PM₂ (circle one)

Small Airway Epithelial Brushes (optional):

83) Problems Processing?

No₀ → **Go to 84**

Yes₁

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

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- 83a) Blood in the sample
83b) Other

83b1) If Other, please specify: _____

Cytospin Slides:

84) Time processed: : AM₁ / PM₂ (circle one)

85) Cell count= # cells/mL= (# cells in 4 squares/4) x 2 x 10⁴:

cells/mL

86) Number of cytospin slides:

Epithelial RNA Extraction:

87) Time processed: : AM₁ / PM₂ (circle one)

88) Time RNA tube placed in freezer: : AM₁ / PM₂ (circle one)

89) Time DNA tubes placed in freezer: : AM₁ / PM₂ (circle one)

END OF FORM