

BRONCHOSCOPY SUB-STUDY SPECIMEN PROCESSING WORKSHEET

ID NUMBER:

FORM CODE: BPW
VERSION: 5.0 05/16/2024

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:

: AM₁ / PM₂

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

2a) Time processed:

: AM₁ / PM₂

2b) Time aliquots placed in refrigerator:

: AM₁ / PM₂

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

3a) Time processed:

: AM₁ / PM₂

3b) Number of aliquots:

3c) Volume in last aliquot:

μ L

3d) Time aliquots placed in freezer:

: AM₁ / PM₂

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

4a) Time processed:

: AM₁ / PM₂

4b) Number of aliquots:

4c) Volume in last aliquot:

μ L

4d) Time aliquots placed in freezer:

: AM₁ / PM₂

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

5a) Time processed:

: AM₁ / PM₂

5b) Number of aliquots:

5c) Volume in last aliquot:

μ L

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15) Time 15 mL freezer jars placed in freezer:

: AM₁ / PM₂

SALINE SPECIMEN

16) Time Scope Saline placed in freezer:

: AM₁ / PM₂

PROTECTED BRUSH SPECIMEN

17) Time processed:

: AM₁ / PM₂

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

20) Date moved to freezer:

/ /

21) Time placed in freezer:

: AM₁ / PM₂

BAL

Microbiome Sample with No Reagent:

22) Time transferred to 15 mL conical:

: AM₁ / PM₂

23) Time placed in freezer:

: AM₁ / PM₂

Microbiome Sample with RNA/ater™:

24) Time processed:

: AM₁ / PM₂

25) Time placed in refrigerator:

: AM₁ / PM₂

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26) Date moved to freezer: / /

27) Time placed in freezer: : AM₁ / PM₂

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: : AM₁ / PM₂

30) Number of 1 mL aliquots made:

31) Number of 15 mL aliquots made:

32) Time placed in freezer: : AM₁ / PM₂

Cell Counts:

33) Time processed: : AM₁ / PM₂

34) Total volume returned: . mL

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

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

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Cytospin Slide Sample:

41) Time processed:

: AM₁ / PM₂

42) Number of cytospin slides:

43) Time fixed:

: AM₁ / PM₂

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

44) Time processed:

: AM₁ / PM₂

45) Was the macrophage plating of 1×10^6 BAL cells completed?

No₀

Yes₁

46) Was the RNA extracted with 600 μ L of QIAzol buffer?

No₀

Yes₁

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

No₀ → **Go to 48**

Yes₁

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?

No₀ → **Go to 49**

Yes₁

If Yes,

48a) Minutes since collection:

minutes

48b) Reason processed more than 2 hours after: _____

49) Time processing complete:

: AM₁ / PM₂

50) Time placed in freezer:

: AM₁ / PM₂

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Immunophenotyping BAL:

51) Time processed:

: AM₁ / PM₂

52) Time processing complete:

: AM₁ / PM₂

53) Time placed in refrigerator:

: AM₁ / PM₂

CYTOLOGICAL BRUSHES FOR RNA (x 3)

54) Problems processing?

No₀ → **Go to 55**

Yes₁

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:

: AM₁ / PM₂

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

cells/mL

57) Number of cytospin slides:

Epithelial RNA Extraction:

58) Time processed:

: AM₁ / PM₂

59) Time placed in freezer:

: AM₁ / PM₂

CYTOLOGICAL BRUSHES FOR DNA (x 2)

60) Problems processing?

No₀ → **Go to 61**

Yes₁

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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:

: AM₁ / PM₂

62) Time placed in freezer:

: AM₁ / PM₂

MICROCYTOLOGICAL BRUSHES FOR MUCIN (x 2)

63) Problems processing?

- No₀ → **Go to 64**
- Yes₁

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:

: AM₁ / PM₂

65) Time PBS tube placed in freezer:

: AM₁ / PM₂

66) Time Urea tube placed in refrigerator:

: AM₁ / PM₂

SMALL AIRWAY EPITHELIAL BRUSHES

67) Problems processing?

- No₀ → **Go to 68**
- Yes₁

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:

: AM₁ / PM₂

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

cells/mL

70) Number of cytospin slides:

Epithelial RNA Extraction:

71) Time processed:

: AM₁ / PM₂

72) Time RNA tube placed in freezer:

: AM₁ / PM₂

73) Time DNA tubes placed in freezer:

: AM₁ / PM₂

Basal Cells for Culture:

74) Time put into culture:

: AM₁ / PM₂

74a) Date put into culture:

/ /

75) Time put into passage:

: AM₁ / PM₂

75a) Date put into passage:

/ /

76) Time frozen:

: AM₁ / PM₂

76a) Date frozen:

/ /

77) Number of aliquots:

END OF FORM