



## INSTRUCTIONS FOR BRONCHOSCOPY SUBSTUDY SPECIMEN PROCESSING WORKSHEET BPW, VERSION 4.0, QUESTION BY QUESTION (QxQ)

### I. GENERAL INSTRUCTIONS

The Bronchoscopy Substudy Specimen Processing Worksheet (BPW) is to be completed during the participant's Bronchoscopy Substudy Visit 2.

**Header Information:** The header information consists of key fields which uniquely identify each recorded instance of a form. For the Event field, record if this is happening at Visit 5 or another event.

0a. Date of Collection: Record the date the data was collected or abstracted. Select the date from the pop-up calendar in the data management system (DMS) or type the date in the space provided. Dates should be entered in the mm/dd/yyyy format.

0b. Staff Code: Record the SPIROMICS staff code of the person who collected or abstracted the data. This code is assigned to each person at each site by the GIC. If you do not have a staff code and are collecting SPIROMICS data, please contact the GIC in order to receive your own individual staff code.

### II. DETAILED INSTRUCTIONS FOR EACH ITEM

Please answer every question on this form.

#### BLOOD

Item 1. **For local lab CBC:** 1 x 4 mL purple top (EDTA) tube.

Item 1a. Record the time the 1 x 4 mL purple top (EDTA) tube was processed.

Item 2. **For immunophenotyping:** 3 x 10 mL purple top (EDTA) tubes.

Item 2a. Record the time the 3 x 10 mL purple top (EDTA) tubes were processed.

Item 2b. Record the time the aliquots were placed in the refrigerator.

Item 3. **For biomarkers:** 1 x 10 mL purple top (EDTA) tube.

Item 3a. Record the time the 1 x 10 mL purple top (EDTA) tube was processed.

Item 3b. Record the number of aliquots.

Item 3c. Record the volume in the last aliquot.

Item 3d. Record the time the aliquots were placed in the freezer.

Item 4. **For biomarkers:** 1 x 10 mL purple top (EDTA) tube.

Item 4a. Record the time the 1 x 10 mL purple top (EDTA) tube was processed.

Item 4b. Record the number of aliquots.

Item 4c. Record the volume in the last aliquot.

Item 4d. Record the time the aliquots were placed in the freezer.

Item 5. **For biomarkers:** 1 x 8.5 mL red top (for serum).

- Item 5a. Record the time the 1 x 8.5 mL red top (for serum) tube was processed.
- Item 5b. Record the number of aliquots.
- Item 5c. Record the volume in the last aliquot.
- Item 5d. Record the time the aliquots were placed in the freezer.

Item 6. **For biomarkers:** 1 x 8.5 mL red top (for serum).

- Item 6a. Record the time the 1 x 8.5 mL red top (for serum) tube was processed.
- Item 6b. Record the number of aliquots.
- Item 6c. Record the volume in the last aliquot.
- Item 6d. Record the time the aliquots were placed in the freezer.

## **NASAL EPITHELIAL SWABS**

Item 7. **Specimen source:**

- Item 7a. Record the number of brushes processed from the right nare.
- Item 7b. Record the number of brushes processed from the left nare.

Item 8. **Problems processing:** Select only one option among the two possible choices.

- Select No if there were no problems processing the nasal epithelial swabs. [Go to Q9]
- Select Yes if there were problems processing the nasal epithelial swabs.

If there were problems processing, specify all problems in 8a and 8b.

Item 9. **Time nasal swabs processed:** Record the time the nasal epithelial swabs were processed.

Item 10. **Time nasal swab samples placed in freezer:** Record the time the nasal epithelial swab samples were placed in the freezer.

## **ORAL SPECIMEN**

Item 11. **Time oral specimen processed:** Record the time the oral specimen was processed.

Item 12. **Number of freezer jars:** Record the number of 15mL freezer jars.

Item 13. **Time freezer jars placed in refrigerator:** Record the time the 15mL freezer jars were placed in the refrigerator.

Item 14. **Date freezer jars moved to freezer:** Record the date the 15mL freezer jars were moved to the freezer.

Item 15. **Time freezer jars placed in freezer:** Record the time the 15mL freezer jars were placed in the freezer.

## **SALINE SPECIMEN**

Item 16. **Time Scope Saline placed in freezer:** Record the time the Scope Saline was placed in the freezer.

## **PROTECTED BRUSH SPECIMEN**

Item 17. **Time protected brush specimen processed:** Record the time the protected brush specimen was processed.

- Item 18. **Problems processing:** Select only one option among the two possible choices.
- Select No if there were no problems processing the protected brush specimen. [Go to Q19]
  - Select Yes if there were problems processing the protected brush specimen.

If there were problems processing, specify all problems in 18a and 18b.

- Item 19. **Time protected brush specimen placed in refrigerator:** Record the time the protected brush specimen was placed in the refrigerator.
- Item 20. **Date protected brush specimen moved to freezer:** Record the time the protected brush specimen was moved to the freezer.
- Item 21. **Time protected brush specimen placed in freezer:** Record the time the protected brush specimen was placed in the freezer.

### INITIAL AIRWAY WASH

- Item 22. **Airway wash return** Select only one option among the two possible choices.
- Select No if a total of 8mL or less was returned from the airway washes. [Go to Q27]
  - Select Yes if a total of more than 8mL was returned from the airway washes.

### Microbiome sample in RNAlater:

- Item 23. **Time microbiome sample in RNAlater processed:** Record the time the microbiome sample in RNAlater was processed.
- Item 24. **Time microbiome sample in RNAlater placed in refrigerator:** Record the time the microbiome sample in RNAlater was placed in the refrigerator.
- Item 25. **Date microbiome sample in RNAlater moved to freezer:** Record the date the microbiome sample in RNAlater was moved to freezer.
- Item 26. **Time microbiome sample in RNAlater placed in freezer:** Record the time microbiome sample in RNAlater was placed in freezer.

### Cell count:

- Item 27. **Time cell count processed:** Record the time the cell count was processed.
- Item 28. **Total volume returned:** Record the total volume returned.
- Item 29. **Cell count:** Record the cell count (# cells/mL = (# cells in 4 squares/4) x 2 x 10<sup>4</sup>).
- Item 30. **Total cell count:** Record the total cell count (# cells/mL x wash returned).
- Item 31. **Cytospin suspension:** Record the cytospin suspension (total cells in 10 mL tube = # cells/mL x volume of wash in 10mL tube).
- Item 32. **Volume to resuspend pellet in with PBS:** Record the volume to resuspend pellet in with PBS (# cells in tube/0.7 x 10<sup>6</sup>).
- Item 33. **Number of cytospin slides created:** Record the number of cytospin slides created.

### Supernatant aliquots

- Item 34. **Time supernatant aliquots processed:** Record the time the supernatant aliquots were processed.
- Item 35. **Number of 500ul aliquots created:** Record the number of 500ul aliquots that were created.
- Item 36. **Time supernatant aliquots placed in freezer:** Record the time the supernatant aliquots were placed in the freezer.

### RNA prep of cell pellet

- Item 37. **Time RNA prep of cell pellet processed:** Record the time the RNA prep of cell pellet was processed.
- Item 38. **Time RNA prep of cell pellet placed in freezer:** Record the time the RNA prep of cell pellet was placed in the freezer.

### **BAL**

#### Microbiome sample with no reagent:

- Item 39. **Time microbiome sample with no reagent transferred to 15 mL conical:** Record the time the microbiome sample with no reagent was transferred to 15mL conical.
- Item 40. **Time microbiome sample with no reagent placed in freezer:** Record the time the microbiome sample with no reagent was placed in the freezer.

#### Microbiome sample with RNAlater:

- Item 41. **Time microbiome sample with RNAlater processed:** Record the time the microbiome sample with RNAlater was processed.
- Item 42. **Time microbiome sample with RNAlater place in refrigerator:** Record the time the microbiome sample with RNAlater was placed in the refrigerator.
- Item 43. **Date microbiome sample with RNAlater moved to freezer:** Record the date the microbiome sample with RNAlater was moved to the freezer.
- Item 44. **Time microbiome sample with RNAlater placed in freezer:** Record the time the microbiome sample with RNAlater was placed in the freezer.
- Item 45. **Total volume of mixed pooled BAL fluid prior to centrifugation:** Record the total volume of mixed pooled BAL fluid prior to centrifugation.  
**NOTE: this is the volume after microbiome samples are completed.**

#### Supernatant sample:

- Item 46. **Time supernatant sample processed:** Record the time the supernatant sample was processed.
- Item 47. **Number of 1 mL aliquots made:** Record the number of 1 mL aliquots that were made.

Item 48. **Number of 15mL aliquots made:** Record the number of 15mL aliquots that were made.

Item 49. **Time supernatant sample placed in freezer:** Record the time the supernatant sample aliquots were placed in the freezer.

Cell counts:

Item 50. **Time cell counts processed:** Record the time the cell counts were processed.

Item 51. **Total volume returned:** Record the total volume returned.

Item 52. **LIVE cell count:** Record the LIVE cell count (# of live cells/mL= (# live cells in 4 squares/4) x 2 x 10<sup>4</sup>).

Item 53. **DEAD cell count:** Record the DEAD cell count (# of dead cells/mL= (# dead cells in 4 squares/4) x 2 x 10<sup>4</sup>).

Item 54. **TOTAL cell count:** Record the TOTAL cell count (# live cells/mL x resuspension volume (10 ml)).

Item 55. **Cytospin suspension:** Record the cytospin suspension (total cells in 10 mL tube= # cells/mL x 10mL).

Item 56. **Volume to resuspend pellet in with PBS:** Record the volume to resuspend pellet in with PBS (# cells in tube/0.7 x 10<sup>6</sup>).

Cytospin slide sample:

Item 57. **Time cytospin slide sample processed:** Record the time the cytospin slide sample was processed.

Item 58. **Number of cytospin slides:** Record the number of cytospin slides.

Item 59. **Time cytospin slide sample fixed:** Record the time the cytospin slide samples were fixed.

Alveolar Macrophage Isolation

**NOTE: The Alveolar Macrophage Isolation MUST be processed EXACTLY 2 hours after collection**

Item 60. **Time alveolar macrophage isolation processed:** Record the time the alveolar macrophage isolation was processed.

Item 61. **Macrophage plating:** Select only one option among the two possible choices.

- Select No if the macrophage plating of 1x10<sup>6</sup> BAL cells was not completed.
- Select Yes if the macrophage plating of 1x10<sup>6</sup> BAL cells was completed.

Item 62. **RNA extraction:** Select only one option among the two possible choices.

- Select No if the RNA was not extracted with 600 ul of QIAzol buffer.
- Select Yes if the RNA was extracted with 600 ul of QIAzol buffer.

Item 63. **Processed less than 2 hours after collection:** Select only one option among the two possible choices.

- Select No if the time of processing was not less than 2 hours after collection. [Go to Q64]
- Select Yes if the time of processing was less than 2 hours after collection.

Item 63a. Record the number of minutes since collection.

Item 63b. Record the reason the processing occurred sooner than 2 hours after collection.

Item 64. **Processed more than 2 hours after collection:** Select only one option among the two possible choices.

- Select No if the time of processing was not more than 2 hours after collection. [Go to Q65]
- Select Yes if the time of processing was more than 2 hours after collection.

Item 64a. Record the number of minutes since collection.

Item 64b. Record the reason the processing occurred more than 2 hours after collection.

Item 65. **Time processing complete:** Record the time the alveolar macrophage isolation processing was completed.

Item 66. **Time placed in freezer:** Record the time the alveolar macrophage isolation was placed in the freezer.

#### Immunophenotyping BAL:

Item 67. **Time immunophenotyping BAL processed:** Record the time the immunophenotyping BAL was processed.

Item 68. **Time immunophenotyping BAL processing complete:** Record the time the immunophenotyping BAL processing was completed.

Item 69. **Time immunophenotyping BAL placed in refrigerator:** Record the time the immunophenotyping BAL was placed in the refrigerator.

#### **LARGE AIRWAY EPITHELIAL BRUSHES X 3 (RNA)**

Item 70. **Problems processing:** Select only one option among the two possible choices.

- Select No if there were no problems processing the large airway epithelial brushes x 3 (RNA). [Go to Q71]
- Select Yes if there were problems processing the large airway epithelial brushes x 3 (RNA).

If there were problems processing, specify all problems in 70a and 70b.

#### Cytospin Slides:

Item 71. **Time cytospin slides processed:** Record the time the cytospin slides were processed.

Item 72. **Cell count:** Record the cell count (# cells/mL = (# cells in 4 squares/4) x 2 x 10<sup>4</sup>).

Item 73. **Number of cytospin slides:** Record the number of cytospin slides.

#### Epithelial RNA Extraction:

Item 74. **Time epithelial RNA extraction processed:** Record the time the epithelial RNA extraction was processed.

Item 75. **Time epithelial RNA extraction placed in freezer:** Record the time the epithelial RNA extraction was placed in the freezer.

## LARGE AIRWAY EPITHELIAL BRUSHES X 2 (DNA)

Item 76. **Problems processing:** Select only one option among the two possible choices.

- Select No if there were no problems processing the large airway epithelial brushes x 2 (DNA). [Go to Q77]
- Select Yes if there were problems processing the large airway epithelial brushes x 2 (DNA).

If there were problems processing, specify all problems in 76a and 76b.

Item 77. **Time large airway epithelial brushes x 2 (DNA) processed:** Record the time the large airway epithelial brushes x 2 (DNA) were processed.

Item 78. **Time large airway epithelial brushes x 2 (DNA) placed in freezer:** Record the time the large airway epithelial brushes x 2 (DNA) were placed in the freezer.

## LARGE AIRWAY EPITHELIAL BRUSHES X 2 (MUCIN)

Item 79. **Problems processing:** Select only one option among the two possible choices.

- Select No if there were no problems processing the large airway epithelial brushes x 2 (MUCIN). [Go to Q80]
- Select Yes if there were problems processing the large airway epithelial brushes x 2 (MUCIN).

If there were problems processing, specify all problems in 79a and 79b.

Item 80. **Time large airway epithelial brushes x 2 (MUCIN) processed:** Record the time the large airway epithelial brushes x 2 (MUCIN) were processed.

Item 81. **Time PBS tube placed in freezer:** Record the time the PBS tube was placed in the freezer.

Item 82. **Time Urea tube placed in refrigerator:** Record the time the Urea tube was placed in the refrigerator.

## SMALL AIRWAY EPITHELIAL BRUSHES (OPTIONAL)

**If no small airway epithelial brushes were collected, go to END OF FORM.**

Item 83. **Problems processing:** Select only one option among the two possible choices.

- Select No if there were no problems processing the small airway epithelial brushes. [Go to Q84]
- Select Yes if there were problems processing the small airway epithelial brushes.

If there were problems processing, specify all problems in 83a and 83b.

### Cytospin Slides:

Item 84. **Time cytospin slides processed:** Record the time the cytospin slides were processed.

Item 85. **Cell count:** Record the cell count (# cells/mL = (# cells in 4 squares/4) x 2 x 10<sup>4</sup>).

Item 86. **Number of cytospin slides:** Record the number of cytospin slides.

Epithelial RNA Extraction:

Item 87. **Time epithelial RNA extraction processed:** Record the time the epithelial RNA extraction was processed.

Item 88. **Time RNA tube placed in freezer:** Record the time the RNA tube was placed in the freezer.

Item 89. **Time DNA tubes placed in freezer:** Record the time the epithelial DNA tubes were place in the freezer.

Save and close the form.