

## SPUTUM PROCESSING WORKSHEET

ID NUMBER:	□	□	□	□	□	□	□	□	□
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FORM CODE: SPW  
 VERSION: 2.0 05/30/2025

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

0a) Date of Collection: □ □ / □ □ / □ □ □ □

0b) Staff Code: □ □ □

0c) Processing Started: □ □ : □ □ AM<sub>1</sub> / PM<sub>2</sub>

**Instructions:** Complete this form while processing the sputum sample. Carefully record all data in the space provided as you move through each of the sample processing steps using MOP 5 as the guide.

1) Weight of Entire Sample: □ □ □ . □ □ grams

**Color and Description of Sample:**

2) Salivary Contamination (*check all that apply*):

- 2a)  Minimal
- 2b)  Mild
- 2c)  Moderate
- 2d)  Excessive

3) Consistency (*check all that apply*):

- 3a)  Watery
- 3b)  Mucoïd (thick, sticky, slightly frothy)
- 3c)  Purulent (thick, colored, contains pus)

4) Mucus “plugs” (material that is colored and/or more opaque than clear surrounding saliva)  
*(check all that apply)*:

- 4a)  Numerous
- 4b)  Moderate number
- 4c)  Sparse
- 4d)  Large
- 4e)  Small
- 4f)  Dense/flocculent
- 4g)  Diffuse opacity
- 4h)  None

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5) Color of plugs (check all that apply):

- 5a)  Clear
- 5b)  White
- 5c)  Yellow/Tan
- 5d)  Brown
- 5e)  Green

6) General Notes/Comments:

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6a) Sputum processing condition:

- Condition 1: Induced initial sample Wt. is > 1 g<sub>1</sub> → Perform Steps 7-13
- Condition 2: Induced initial sample Wt. is 0.5 g-1 g<sub>2</sub> → Perform Steps 7 and 9-13
- Condition 3: Induced initial sample Wt. is < 0.5 g<sub>3</sub> → Perform Steps 9-13
- Condition 4: Induced initial sample Wt. is < 0.5 g and a spontaneous sputum sample was acquired prior to induction<sub>4</sub> → Perform Steps 7-8 with induced sample; then 9-13 with spontaneous sample
- Condition 5: No induced sputum sample was produced; but a spontaneous sample was acquired<sub>5</sub>

6a1) Sputum processing for Condition 5:

- Condition A: Spontaneous sample Wt. is > 1 g<sub>1</sub> → Perform Steps 7-13
- Condition B: Spontaneous sample Wt. is 0.5 g-1 g<sub>2</sub> → Perform Steps 7 and 9-13
- Condition C: Spontaneous sample Wt. is < 0.5 g<sub>3</sub> → Perform Steps 9-13

7) Processing Whole Sample using the Mucin Method

Mucin Sample	
Weighing tray (g)	7a)
Whole sputum weight (g)	7b)
Guanidine vol. (mL)	7c)

\*SPIROMICS III MOP 5, Section 7.1.2. Store IMMEDIATELY in a **2-8°C refrigerator**.

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8) Processing the Mucin Interactome

Osmo Sample	
Micro centrifuge tube (g)	8a)
Whole sputum weight (g)	8b)

*\*SPIROMICS III MOP 5, Section 7.1.3. Store IMMEDIATELY in a **-80°C freezer.***

9) Processing Total Remaining Whole Sample for Nucleotides, Cytokines, Cell Counts, Cytospins, RNA transcriptomics, and Microbiome

Weight of Centrifuge tube (g)	9a)
Weight of Sputum (g)	9b)
Volume of EDTA-DPBS added to sample (mL)	9c)
Volume of supernatant removed (mL) (see section 10 for process)	9d)
Volume of EDTA-DPBS added to stock sputolysin to make 0.2% DTT (mL)	9e)
Volume of 0.2% DTT added to the sample (mL)	9f)
Volume of DTT containing supernatant removed (mL)	9g)
Resuspension volume of EDTA-DPBS (0.25 – 2 mL)	9h)

*\*SPIROMICS III MOP 5, Section 7.1.4. Store IMMEDIATELY in a **-80°C freezer.***

10) EDTA-DPBS Supernatants for Nucleotides and Cytokines

Supernatants	Number of aliquots	Volume stored per aliquot (µL)
Nucleotides	10a1)	10b1)
Cytokines	10a2)	10b2)
Cytokine Zymo Research RNA/DNA	10a3)	10b3)

*\*SPIROMICS III MOP 5, Section 7.1.4. Store IMMEDIATELY in a **-80°C freezer.***

EDTA-DTT Supernatants

Supernatants	Volume stored (mL)
Sputolysin Sup 01	10c1)
Sputolysin Sup 02	10c2)

*\*SPIROMICS III MOP 5, Section 7.1.4. Store IMMEDIATELY in a **-80°C freezer.***

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### 11) Cell Counts

Cell Counts:	# Dead	# Live	Total	Squamous Epithelial
Quadrant 1	11a1)	11a2)	11a3)	11a4)
Quadrant 2	11b1)	11b2)	11b3)	11b4)
Quadrant 3	11c1)	11c2)	11c3)	11c4)
Quadrant 4	11d1)	11d2)	11d3)	11d4)
Totals	11e1)	11e2)	11e3)	11e4)

\*SPIROMICS III MOP 5, Section 7.1.5.

Sample Concentration (cells/mL) = $([11e3 / 4] \times 2 \times 10^4)$	11f)
Total Cell Count = $([11e3 / 4] \times 2 \times 10^4) \times 9h)$	12f)11g)
Number of cells/weight = (11g) / weight of selected sample (9b)	12g)11h)
Viability = $(11e2 \text{ live cells} / 11e3 \text{ total cells}) \times 100$	12h)11i)
Final Adjusted Sample Concentration (cells/mL) based on resuspension volume (range = $0.5 - 1.0 \times 10^6/\text{mL}$ )	11j)

\*SPIROMICS III MOP 5, Section 7.1.5.

### 12) Cytospins

	# of slides stored	Amount of cell suspension used (µL)
Hema 3 stained slides	13)12a)	12b)

\*SPIROMICS III MOP 5, Section 7.1.6. DO NOT COVER SLIP THE SLIDES. Store all slides in a box at **room temperature**.

### 13) RNA Transcriptomics and Microbiome samples

- No<sub>0</sub>  
 Yes<sub>1</sub>

Weight of centrifuge tube (g) (from item 9a above)	13a)
Whole sputum weight (g)	13b)
Vol. of Zymo added (µL)	13c)
Vol. per aliquot $(1000 \times 13b + 13c) / 2$ (one for RNA Trans and one for Micro)	13d)

\* SPIROMICS III MOP 5, Section 7.1.7. Store IMMEDIATELY at **-80°C freezer**.

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

1314) Comments: \_\_\_\_\_  
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0e/1415) Processing Ended:   :   AM<sub>1</sub> / PM<sub>2</sub>

**END OF FORM**