

SPUTUM PROCESSING WORKSHEET

| | | | | | | | | | |
|------------|---|---|---|---|---|---|---|---|---|
| ID NUMBER: | □ | □ | □ | □ | □ | □ | □ | □ | □ |
|------------|---|---|---|---|---|---|---|---|---|

FORM CODE: **SPW**
 VERSION: 1.0 03/03/2025

Event: _____

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

0b) Staff Code: □ □ □

0c) Processing Started: □ □ : □ □ AM₁ / PM₂

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:

6a) Sputum processing condition:

- Condition 1: Induced initial sample Wt. is > 1 g₁ → **Perform Steps 7-13**
- Condition 2: Induced initial sample Wt. is 0.5 g-1 g₂ → **Perform Steps 7 and 9-13**
- Condition 3: Induced initial sample Wt. is < 0.5 g₃ → **Perform Steps 9-13**
- Condition 4: Induced initial sample Wt. is < 0.5 g and a spontaneous sputum sample was acquired prior to induction₄ → **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 acquired₅

6a1) Sputum processing for Condition 5:

- Condition A: Spontaneous sample Wt. is > 1 g₁ → **Perform Steps 7-13**
- Condition B: Spontaneous sample Wt. is 0.5 g-1 g₂ → **Perform Steps 7-13**
- Condition C: Spontaneous sample Wt. is < 0.5 g₃ → **Perform Steps 7-8**

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 %Solids/Osmotic Pressure Sample

| 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 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 = $([11e3 / 4] \times 2 \times 10^4) \times 9h$ (ref range: 1/2 million – 1 million) | ^{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

No₀

Yes₁

| | |
|--|------|
| Micro centrifuge tube (g) | 13a) |
| Whole sputum weight (g) | 13b) |
| Vol. of Zymo added (µL) | 13c) |
| Vol. per aliquot $(13b+13c)/2$ (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: _____

13/14) Comments: _____

0e/14) Processing Ended: : AM₁ / PM₂

END OF FORM