

SPUTUM PROCESSING WORKSHEET, SPW QUESTION BY QUESTION (QxQ), VERSION 1.0

I. GENERAL INSTRUCTIONS

The Sputum Processing Worksheet (SPW) is to be completed while processing the sputum sample collected at the clinic visit.

NOTE: The completion of this form is necessary to run the following reports: “Visit Shipping Manifest - Box 2”, “Visit Shipping Manifest - Mucin”, and “Visit Shipping Manifest - Sputolysin”. Ensure that this form has been entered before any of the listed reports are run.

Please answer every question on this form.

NOTE: All response options in the paper form may not appear in CDART (e.g., ‘Don’t know’, ‘Declines to answer’, etc.). Beside each item input is a small double bracket icon which looks like this: >>. Clicking this icon displays a field dialogue box in which the “Field Status” selection menu allows you to choose from the following options: ‘Refused’, ‘No response’, ‘Doesn’t know’, ‘Not applicable’, ‘Maximum value’, ‘Minimum value’, and ‘Missing’. See MOP 6 – Section 3.2 for additional instructions on how to select a Field Status option.

Whenever numerical responses are required, enter the number so that the last digit appears in the rightmost box. Enter leading zeros where necessary to fill all boxes (e.g., enter 0.25 rather than .25).

II. INSTRUCTIONS FOR INDIVIDUAL ITEMS

Header Information: Consists of key fields which uniquely identify each subject and recorded occurrence of a form. For the “ID NUMBER”, record the 2 or 3-character, 6-digit number assigned to the specific participant. For the “Event”, record if this is happening at the E1 visit or another event.

- Item 0a.** Record the date the data was collected or abstracted in the MM/DD/YYYY format either by selecting the pop-up calendar in CDART or entering the date in the space provided.
- Item 0b.** Record the SPIROMICS III 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 III data, please contact the GIC in order to receive your own individual staff code.
- Item 0c.** Record the start time (i.e., hour/minute) of the sputum processing in the HH:MM AM/PM format in the space provided.
- Item 1.** Record the weight of the entire sputum sample in grams in the space provided.
- Item 2.** Select the check boxes to answer among the four possible choices all that apply to the salivary contamination.
- Item 3.** Select the check boxes to answer among the three possible choices all that apply to the sputum sample consistency.

- Item 4.** Select the check boxes to answer among the eight possible choices all that apply to the mucus “plugs”.
- Item 5.** Select the check boxes to answer among the five possible choices all that apply to the color of the mucus “plugs”.
- Item 6.** Record any other general notes and/or comments about the sputum sample in the space provided.

Item 6a. Select only one option among the five possible choices and follow the instructions for continuing as follows:

- If **Condition 1** selected, perform steps 7-13.
- If **Condition 2** selected, perform steps 7 and 9-13.
- If **Condition 3** selected, perform steps 9-13.
- If **Condition 4** selected, perform steps 7-8 with induced sample; then Items 9-13 with spontaneous sample.
- If **Condition 5** selected, got to Item 6a1.

Item 6a1. Select only one option among the three possible choices and follow the instructions for continuing as follows:

- If **Condition A** selected, perform steps 7-13.
- If **Condition B** selected, perform steps 7 and 9-13.
- If **Condition C** selected, perform steps 9-13.

Item 7.

Item 7a. Record the weight of the weighing tray in grams in the space provided.

Item 7b. Record the weight of the whole sputum sample in grams in the space provided.

Item 7c. Record the guanidine volume in milliliters in the space provided.

NOTE: According to SPIROMICS III MOP 5, Section 7.1.2, store sample IMMEDIATELY in a 2-8°C refrigerator.

Item 8.

Item 8a. Record the weight of the micro centrifuge tube in grams in the space provided.

Item 8b. Record the weight of the whole sputum sample in grams in the space provided.

NOTE: According to the SPIROMICS III MOP 5, Section 7.1.3, store sample IMMEDIATELY in a -80°C freezer.

Item 9.

Item 9a. Record the weight of the centrifuge tube in grams in the space provided.

Item 9b. Record the weight of the sputum sample in grams in the space provided.

Item 9c. Record the volume of EDTA-DPBS added to the sample in milliliters in the space provided.

- Item 9d.** Record the volume of supernatant removed in milliliters in the space provided. *NOTE: See section 10 for the process.*
- Item 9e.** Record the volume of EDTA-DPBS added to stock sputolysin to make 0.2% DTT in milliliters in the space provided.
- Item 9f.** Record the volume of 0.2% DDT added to the sample in milliliters in the space provided.
- Item 9g.** Record the volume of DTT containing supernatant removed in milliliters in the space provided.
- Item 9h.** Record the resuspension volume of EDTA-DPBS in milliliters in the space provided. *NOTE: Should be 0.25 – 2 mL.*

NOTE: According to the SPIROMICS III MOP 5, Section 7.1.4, store sample IMMEDIATELY in a -80°C freezer.

Item 10.

- Items 10a1-10a3.** Record the number of Nucleotides, Cytokines, and Cytokine Zymo Research RNA/DNA aliquots in the spaces provided, respectively.
- Items 10b1-10b3.** Record the volume per aliquot of Nucleotides, Cytokines, and Cytokine Zymo Research RNA/DNA stored in microliters in the spaces provided, respectively.

NOTE: According to the SPIROMICS III MOP 5, Section 7.1.4, store sample IMMEDIATELY in a -80°C freezer.

- Items 10c1-10c2.** Record the volume stored of Sputolysin Sup 01 and 02 in milliliters in the spaces provided, respectively.


NOTE: According to the SPIROMICS III MOP 5, Section 7.1.4, store sample IMMEDIATELY in a -80°C freezer.

Item 11.

- Items 11a1-11a2.** Record the number of Dead and Live cells in Quadrant 1 in the spaces provided, respectively.
- Item 11a3.** Select **Save and Reload** in CDART to automatically calculate the Total number of cells in Quadrant 1 based on the values entered in Items 11a1 and 11a2. *NOTE: If Item 11a1 and/or 11a2 is missing or not entered appropriately, Item 11a3 will not calculate.*
- Item 11a4.** Record the number of Squamous Epithelial cells in Quadrant 1 in the space provided. *NOTE: This cell count is not included in the Dead/Live cell count.*
- Items 11b1-11b4.** For Quadrant 2, follow the instructions as in Items 11a1-11a4 as the question pattern is identical.

Items 11c1-11c4. For Quadrant 3, follow the instructions as in Items 11a1-11a4 as the question pattern is identical.

Items 11d1-11d4. For Quadrant 4, follow the instructions as in Items 11a1-11a4 as the question pattern is identical.

Items 11e1-11e4. Select **Save and Reload** in CDART to automatically calculate the total numbers for Dead, Live, Total, and Squamous Epithelial cells based on the values entered in Items 11a1-11a4, 11b1-11b4, 11c1-11c4, and 11d1-11d4, respectively. Select the manual refresh icon (i.e., ) in CDART to automatically calculate the total number for Total cells based on the values entered in Items 11c1-11c4. *NOTE: If one or more of Items 11a1-11a4, 11b1-11b4, 11c1-11c4, and/or 11d1-11d4 is missing or not entered appropriately, Items 11e1, 11e2, 11e3, and/or 11e4 will not calculate, respectively.*

NOTE: See SPIROMICS III MOP 5 Section 7.1.5 for thorough instructions.

Item 11f. Select **Save and Reload** in CDART to automatically calculate the sample concentration in cells per milliliter based on the value calculated in Item 11e3 [i.e., $(11e3 / 4) \times 2 \times 10^4$]. *NOTE: If Item 11e3 is missing or not calculated appropriately, Item 11f will not calculate.*

Item 11g. Select **Save and Reload** in CDART to automatically calculate the total cell count (reference range: $\frac{1}{2}$ - 1 million) based on the values entered and/or calculated in Items 9h and 11e3 [i.e., $((11e3 / 4) \times 2 \times 10^4) \times 9h$]. *NOTE: If Items 9h and/or 11e3 is missing or not entered and/or calculated appropriately, Item 11g will not calculate.*

Item 11h. Select **Save and Reload** in CDART to automatically calculate the number of cells/weight based on the values entered and/or calculated in Items 9b and 11g [i.e., $11g / 9b$]. *NOTE: If Items 9b and/or 11g is missing or not entered and/or calculated appropriately, Item 11h will not calculate.*

Item 11i. Select **Save and Reload** in CDART to automatically calculate the viability based on the values entered and/or calculated in Items 11e2 and 11e3 [i.e., $(11e2 / 11e3) \times 100$]. *NOTE: If Items 11e2 and/or 11e3 is missing or not entered and/or calculated appropriately, Item 11i will not calculate.*

Item 11j. Record the final adjusted sample concentration in cells per milliliter based on the resuspension volume (range = $0.5 - 1.0 \times 10^6/\text{mL}$) in the space provided.

Item 12.

Item 12a. Record the number of Hema 3 stained slides stored in the space provided.

Item 12b. Record the amount of cell suspension used in the Hema 3 stained slides in microliters in the space provided.

NOTE: See SPIROMICS III MOP 5 Section 7.1.6 for thorough instructions. DO NOT COVER SLIP THE SLIDES. Store all slides in a box at room temperature.

Item 13. Select the check boxes to answer among the two possible choices all that apply to RNA Transcriptomics and Microbiome samples.

Item 13a. Record the weight of the micro centrifuge tube in grams in the space provided.

Item 13b. Record the whole sputum weight in grams in the space provided.

Item 13c. Record the volume of Zymo added in microliters in the space provided.

Item 13d. Select **Save and Reload** in CDART to automatically calculate the volume per aliquot in microliters (one for RNA Trans and one for Micro) based on the values calculated in Item 13b and Item 13c [i.e., $(1000 \cdot 13b + 13c) / 2$]. *NOTE: If either Item 13b or Item 13c are missing or not calculated appropriately, Item 13d will not calculate.*

NOTE: See SPIROMICS III MOP 5 Section 7.1.7 for thorough instructions. Store IMMEDIATELY in a -80°C freezer.

Item 14. Record any additional comments in the space provided.

Item 15. Record the time (i.e., hour/minute) sputum processing ended in the HH:MM AM/PM format in the space provided.

Select **Save and Close** at the bottom of the page/screen.