



INSTRUCTIONS FOR SPUTUM PROCESSING WORKSHEET FORM SPW, VERSION 5.0, QUESTION BY QUESTION (QxQ)

I. GENERAL INSTRUCTIONS

The Sputum Processing Worksheet Form is completed while processing the sputum sample collected at Clinic Visit 5.

Note: The completion of this form is necessary to run the following reports: “Visit 5 Shipping Manifest-Box 2” and “Visit 5 Shipping Contents Report”. Ensure that this form has been entered before any of the listed reports are run.

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. Form Date: Record the date form was completed. 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.

0c. 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.

0d. Time of Collection: Record the time the data was collected or abstracted in hours:minutes in 24 clock time.

II. DETAILED INSTRUCTIONS FOR EACH ITEM

Item 1. **Weight of Entire Sample** Record the weight of entire sample in grams in the space provided.

Color and Description of Sample

Item 2. **Salivary Contamination** Select all the options among the four possible choices that apply.

- Select Minimal if the salivary contamination is minimal.
- Select Mild if the salivary contamination is mild.
- Select Moderate if the salivary contamination is moderate.
- Select Excessive if the salivary contamination is excessive.

Item 3. **Consistency** Select all the options among the three possible choices that apply.

- Select Watery if the sample consistency is watery.
- Select Muroid (involving, resembling, or of the nature of mucus) if the sample consistency is muroid.
- Select Purulent (consisting of, containing, or discharging pus) if the sample consistency is purulent.

Item 4. **Mucus “plugs” (material that is colored and/or more opaque than clear surrounding saliva)**

Select all the options among the eight possible choices that apply.

- Select Numerous if the mucus “plugs” are numerous.
- Select Moderate number if the mucus “plugs” are moderate in number.
- Select Sparse if the mucus “plugs” are sparse in number.
- Select Large if the mucus “plugs” are large in number.
- Select Small if the mucus “plugs” are small in number.
- Select Dense/flocculent if the mucus “plugs” are dense/flocculent in number.
- Select Diffuse opacity if the mucus “plugs” are diffuse opacity.
- Select None if there are no mucus “plugs.”

Item 5. **Color of mucus “plugs”** Select all the options among the five possible choices that apply.

- Select Clear if the mucus “plugs” appear clear.
- Select White if the mucus “plugs” appear white in color.
- Select Yellow/tan if the mucus “plugs” appear yellow/tan in color.
- Select Brown if the mucus “plugs” appear brown in color.
- Select Green if the mucus “plugs” appear green in color.

General Notes/Comments

Item 6a. **Sputum processing condition** Select one of the options among the five possible choices that apply and follow the instructions for continuing.

- Select Condition 1: Induced initial sample Wt. is > 1g if the initial sample Wt. is > 1g. [Perform Steps 7-14]
- Select Condition 2: Induced initial sample Wt. is 0.5g-1g if the initial sample Wt. is 0.5g-1g. [Perform Step 7, then 10 -14]
- Select Condition 3: Induced initial sample Wt. is <0.5g if the initial sample Wt. is <0.5g. [Perform Steps 10-14]
- Select Condition 4: Induced initial sample Wt. is <0.5g and a spontaneous sputum sample was acquired prior to induction if the initial sample Wt. is <0.5g and a spontaneous sputum sample was acquired prior to induction. [Perform Steps 7-8 with induced sample; then 10-14 with spontaneous sample.]
- Select Condition 5: No induced sputum sample was produced; but a spontaneous sample was acquired if no induced sputum sample was produced; but a spontaneous sample was acquired.

Item 6a1. **Sputum processing for Condition 5** Select one of the options among the three possible choices that apply and follow the instructions for continuing.

- Select Condition A: Spontaneous sample Wt. is > 1g if the spontaneous sample Wt. is > 1g. [Perform Steps 7-14]
- Select Condition B: Spontaneous sample Wt. is 0.5g-1g if the spontaneous sample Wt. is 0.5g-1g. [Perform Step 7, then 10 -14]
- Select Condition C: Spontaneous sample Wt. is <0.5g if the spontaneous sample Wt. is <0.5g. [Perform Steps 10-14]

Items 7a-c. **Processing Whole Sample using the Mucin Method** Record the value each item in the space provided.

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

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

Item 7c. Record the weight of the guanidine volume in milliliters.

Note: Sample size should be from 0.100-0.250g. Add 0.5ml of guanidine reduction buffer (6M) to the sample and store at 4 degrees C in the refrigerator.

Items 8a-b. **Processing Microbiology Sample** Record the value each item in the space provided.

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

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

Item 8c. Record the volume of Zymo added in μl .

Item 8d. Record the volume per aliquot $(8b+8c)/2$.

Note: Weigh an empty micro centrifuge tube. Zero the balance. Measure 0.200g of whole sputum sample. Record the weight of sputum and store in -80°C . Ship sample on dry ice.

Items 9a-b. **Processing %Solids/Osmotic Pressure Sample** Record the value each item in the space provided.

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

Item 9b. Record the weight of the whole sputum in grams.

Note: Weigh an empty micro centrifuge tube. Zero the balance. Pipette 200 μL of sample.

Transfer 50-200 μl of sample to micro centrifuge tube. Record the weight of sample and store in -80°C . Sample can be transferred to -20°C prior to delivery.

Items 10a1-g1. **Processing Whole Sample using the Mucin Method** Record the value each item in the space provided.

Item 10a1. Record the weight of the centrifuge tube in grams.

Item 10b1. Record the weight of the whole sputum in grams.

Item 10c1. Record the volume of EDTA-DPBS added to sample in milliliters.

Item 10d1. Record the volume of supernatant removed in milliliters (see section 11 below for process).

Item 10e1. Record the volume of EDTA added to stock sputolysin to make 0.2% sputolysin in milliliters.

Item 10f1. Record the volume of 0.2% sputolysin added to the sample in milliliters.

Item 10g1. Record the volume of DTT containing supernatant removed in milliliters.

Item 10h1. Record the volume of EDTA-DPBS in milliliters (0.25-2 ml).

Note: Weigh a 50mL centrifuge tube. Zero the balance. Add remaining sample to centrifuge tube and record weight in grams. Add 8x sample weight of cold EDTA-DPBS buffer, homogenize for 15 min on a rotating tumbler, then spin at 790g at 10 min. Remove half the volume of supernatant (leaving behind the "master sample") and spin this at 1500g for 10 min. Collect the supernatant of this sample and store at -80 degrees C (* see below). To the "master" sample, add a volume of 0.2% sputolysin that is equal to the same volume you just removed from the master sample (note: stock sputolysin is at 1% - dilute this to 0.2% with 1mM EDTA as in following example: if you require 3ml of 0.2% sputolysin, add 0.6ml of stock sputolysin (1%) to 2.4ml of 1mM EDTA). Homogenize the sample for 15 min. on a rotating tumbler then filter the sample through a 48-53 μm nylon mesh into new 50mL tube. Spin the sample filtrate at 790Xg for 10 minutes. Remove and store the entire volume of DTT containing supernatant at -80 degrees C. Resuspend the cell pellet in cold EDTA-DPBS (0.25 – 2ml depending on thickness of pellet), perform hemocytometry for total cell count and cell viability. Adjust the cell concentration of the sample to 0.5 – 1×10^6 cells/ml and make 4 cytospin slides.

Items 11a1-f1. **EDTA-DPBS Supernatants for Nucleotides and Cytokines** Record the value each item in the space provided.

Item 11a1. Record the number of aliquots of Nucleotides.

Item 11b1. Record the volume of Nucleotides stored in milliliters.

Item 11c1. Record the number of aliquots of Cytokines.

Item 11d1. Record the volume of Cytokines stored in milliliters.

Item 11e1. Record the number of aliquots of Cytokine Zymo Research RNA/DNA.

Item 11f1. Record the volume of Cytokine Zymo Research RNA/DNA stored in milliliters.

Note: If the sample volume is greater than 8mL, obtain 4 1000 ul aliquots for nucleotides, 4 1000 ul aliquots for cytokines. For the remaining sample, take up to 600ul and mix it 1:1 with Zymo Research RNA/DNA shield. When there is a limited volume, start by getting a nucleotide sample between 200-500 ul and one cytokine sample at 200ul. If there is sample leftover after that, then continue alternating between nucleotide and cytokine aliquots (i.e., 200-500uL for nucleotides, 200uL for cytokines) until finished. **Ensure that one of the cytokine aliquots is mixed 1:1 with Zymo Research RNA/DNA shield.** All supernatant samples are immediately stored in a -80° C freezer.

Item 11g1. **EDTA-Sputolysin Supernatants** Record the volume stored of Sputolysin Sup 01 in milliliters.

Item 11g2. **EDTA-Sputolysin Supernatants** Record the volume stored of Sputolysin Sup 02 in milliliters.

Items 12a1-e3. **Cell Counts** Record the value each cell count in the space provided.

Item 12a1. Record the number of dead cells in Quadrant 1.

Item 12a2. Record the number of live cells in Quadrant 1.

Item 12a3. The number of total cells in Quadrant 1 will be calculated and autofill once you have entered the data for Q12a1 and 12a2. If the cell does not autofill, save the form and refresh.

Item 12b1. Record the number of dead cells in Quadrant 2.

Item 12b2. Record the number of live cells in Quadrant 2.

Item 12b3. The number of total cells in Quadrant 2 will be calculated and autofill once you have entered the data for Q12b1 and 12b2. If the cell does not autofill, save the form and refresh.

Item 12c1. Record the number of dead cells in Quadrant 3.

Item 12c2. Record the number of live cells in Quadrant 3.

Item 12c3. The number of total cells in Quadrant 3 will be calculated and autofill once you have entered the data for Q12c1 and 12c2. If the cell does not autofill, save the form and refresh.

Item 12d1. Record the number of dead cells in Quadrant 4.

Item 12d2. Record the number of live cells in Quadrant 4.

Item 12d3. The number of total cells in Quadrant 4 will be calculated and autofill once you have entered the data for Q12d1 and 12d2. If the cell does not autofill, save the form and refresh.

Item 12e1. The total number of dead cells will be calculated and autofill once you have entered the data for Q12a1, 12b1, 12c1, and 12d1. If the cell does not autofill, save the form and refresh.

Item 12e2. The total number of live cells will be calculated and autofill once you have entered the data for Q12a2, 12b2, 12c2, and 12d2. If the cell does not autofill, save the form and refresh.

Item 12e3. The number of total cells will be calculated and autofill once you have entered the all data for cell count chart. If the cell does not autofill, save the form and refresh.

Note: Count live (clear) and dead (cell interior is blue) cells in each of the 4 corner quadrants. Count BEC's, but exclude RBC's and squamous epithelial cells.

Item 12f. Record the Initial Sample Concentration (cells/ml) = $([12e3 / 4] \times 2 \times 10^4)$.

Item 12g. Record the TCC = $(([12e3 / 4] \times 2 \times 10^4) \times 10h1)$ (ref range: 1/2 million – 1 million).

Item 12h. Record the number of cells/weight = $(12g) / \text{weight of selected sample (10b1)}$.

Item 12i. Record the Viability = $(12e2 \text{ live cells} / 12e3 \text{ total cells}) \times 0.1$.

Item 12j. Record the Final Adjusted Sample Concentration (cells/ml) based on resuspension volume (range = 0.5 – 1.0 x 10⁶/mL).

Item 13. **Cytospins**

Note: Slides are made using 60µL-90ul of cell suspension (at 0.5 - 1X10⁶/mL). Make 1 slide using 60ul, 2nd slide using 70ul, 3rd slide using 80ul and a 4th slide using 90ul. Spin 6 min. at 450rpm. Fix all 4 slides in 95% ethanol followed by air drying. **DO NOT COVER SLIP THE SLIDES.** Select the 2 best quality slides after review under the microscope, and in a covered slide box ship to the central slide reading lab. Keep the other 2 fixed slides in a stored slide box for back up. All slides stored at room temperature.

Item 13a. Record the number of slides stored of Hema 3 stained slides in the space provided.

Item 13b. Record the amount of amount of cell suspension used (µl) in the Hema 3 stained slides in the space provided.

Item 14. **Zymo Research RNA/DNA cell pellet** Select only one option among the two possible choices that apply.

- Select No if cells for RNA were not collected.
- Select Yes if cells for RNA not collected.

Item 14a. Record the value for Zymo Research RNA/DNA cell pellet.

Note: The sample is spun down at 790xg for 10 minutes. Discard the supernatant and resuspend the pellet in 0.5ml of DPBS. Add 0.5 ml of Zymo Research RNA/DNA shield to the sample. Store in -80°C. The number of cells left in the Zymo Research RNA/DNA pellet will be equal to the TCC volume (12g) minus the total number of cells used to make slides in 13b (0.0 – 0.3 ml X 12f).

Item 15 **Comments** Record any additional comments in the space provided.

Save and close the form.