# INSTRUCTIONS FOR SPUTUM PROCESSING WORKSHEET
# SPW, VERSION 1.0 (QxQ)

## I. GENERAL INSTRUCTIONS

The Sputum Processing Worksheet is filled out by the lab technician processing the sputum collected during sputum induction.

Processing should begin within 30 minutes of sputum collection.

**Header Information:** The header information consists of key fields which uniquely identify each recorded instance of a form.

FORM DATE: Record date this is being completed. Select the date from the pop up calendar or type in the date in the space provided. Dates should be entered in the mm/dd/yyyy format.

INITIALS: Record the staff code of the person entering the data on this form. 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

Item1-6. Weight of Entire Sample and Color Description of Sample

***Prior to weighing the sample, pour the entire sample into a Petri dish and record Items 2-5.***

Item1a. ***After recording Items 2-5,*** weigh an empty 50mL tube with lid and record the weight on it.

Item1b. Weight of Entire Sample. Zero the balance on the scale. Pour the sample into the tube and measure the total weight in grams and record. Put sample on ice.

Item 2. Salivary Contamination: check whether *minimal, mild, moderate or excessive*

Item 3. Consistency: check whether *watery, mucoid or purulent (puss)*

Item 4. Mucus "plugs" consist of an exudate of serum proteins and cell debris. Check all that apply: Number of plugs: *numerous, moderate number or sparse;* size: *large or small;* quality: *dense/flocculent (resembling shreds or tufts)* ***or*** *diffuse (spreading or scattered) opacity.*

Item 5. Color of plugs, check all that apply: *clear, white, yellow/tan, brown,green*

Item 6. General Notes, Comments: This text field is optional and, if used, should be brief and concise. It allows for 254 text characters. If exceeded, the DMS will prompt you to truncate the text in the field. A common use of this field is to describe unusual color or consistency of the sputum.

Item 7. Processing Whole Sample using the Mucin Method

Item 7a. Weigh an empty microcentrifuge tube and record the weight.

Item 7b. Zero the balance. Measure 0.500g of whole sputum sample from Item 1. If there is only a small amount of sputum, you may measure as little 0.100g. Record weight of sputum.

Item 7c. Record the volume of guanidine reduction buffer: Add 1.0 mL of guanidine reduction buffer if sample is 0.500g. Add 0.5mL of guanidine reduction buffer if sample is less than 0.500g. Sample should then be stored 4◦C in a box labeled SPIROMICS Mucin.

Item 8. Processing Microbiology Sample:

Item 8a. Weigh an empty microcentrifuge tube and record the weight.

Item 8b. Zero the balance. From the remaining whole sputum sample from Item 1, measure a minimum of 0.300g. Record the weight of the whole sputum. Store at -80 ◦ C until shipment.

Item 9. Processing Viscoelastic Sample:

Item 9a. Weigh an empty microcentrifuge tube and record. Zero the balance.

From the remaining whole sputum sample from Item 1, obtain 25 microliters of sample, using a 100 ul pipette and drawing up whatever can be achieved. ***Exact volume is not important, but using a 100 ul pipette will ensure the 25 ul minimum has been achieved.***

Item 9b. Record the weight of the whole sputum sample. Sample should be delivered into an eppendorf tube stored immediately at -80◦. Sample may be transferred to -20◦ prior to shipment.

Item10. Processing Whole Sample using EDTA: Zero the balance.

Item10a. Record the weight of the empty 50mL centrifuge tube w/lid from Item 1a.

Item10b. Weight of sputum: Add the remaining whole sample to the centrifuge tube and record the weight in grams.

***Add 0.1% sputolysin (DTT) in mLs equal to 4 x the remaining sample weight.***

Item10b. 1% sputolysin (DTT) volume: Record the volume of 1% sputolysin used to make the 0.1% sputolysin solution. ***(DTT comes as a 1% solution in 10ml vials. Make the 0.1% solution by diluting 1:10 with EDTA)***

***(****For example: 2g of sample would need 8mL of 0.1% sputolysin. Mix 1mL 1.0% DTT + 10mL of EDTA for a total of 11mL of 0.1% DTT. You would draw off 8mL to add to the sputum sample, and discard the remaining 3mL).*

Item10c. Record the amount of EDTA added to make 0.1% sputolysin solution (DTT).

*Vortex the sample for 15 seconds and pipet up and down with a P1000 pipettor to break up any clumps. This is necessary for very viscous/thick samples.*

Item10d. Place sample on tumbler for 15 minutes and record the actual time in minutes that the specimen is on the tumbler.

Item10e. Dilute the sample with EDTA and record the volume added after the 15 minutes on the tumbler. ***Use the same volume, as the volume of the 0.1% sputolysin added: if 8mL of sputolysin was added, add 8mL of EDTA.***

Item10f. Record the actual time in minutes of the 5 minute tumble.

***After 5 minute tumble, filter the sample into a new 50mL tube through 53µm nylon mesh filter lining a funnel (pre-wet filter with buffer for better adhesion to the funnel).***

***Spin down cells at 500Xg for 10 minutes.***

Item 11. Supernatants for Nucleotides and Cytokines

***Save aliquots in 1.5mL tubes.***

***If sample volume is greater than 8ml obtain 4 1 mL aliquots for nucleotides, 4 1 mL aliquots for cytokines. When there is a limited volume start by getting a nucleotide sample between 200-500uL, 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.***

***Nucleotide and cytokine samples are stored at -80◦.***

Item 11a-b. Nucleotides: a. Record the number of aliquots obtained. b. Record the volume of aliquots.

Item 11c-d. Cytokines: a. Record the number of aliquots obtained. b. Record the volume of aliquots.

***Nucleotide and cytokine samples are stored in -80 freezer***

Item 11e. Volume of Hanks Balanced Salt Solution (HBSS) added to re-suspend cells in sputum pellet. *Add 1 or 2mL of HBSS.*

Item 12. Cell Counts

***Combine 10µL of re-suspended sample with 10µL of trypan blue stain.***

***Place 10µL of mixture on the hemacytometer.***

***Count live (clear) cells and dead (blue) cells in each of the 4 corner grids. Count basal epithelial cells, but exclude RBCs and squamous epithelial cells.*** *Over 40% squamous epithelial cells indicates a poor quality sample and will require returning to the filtration step above, and then commencing from that point forward.*

Item12a. Square 1: 1) Record number of dead (blue) cells 2) Record number of live (clear) cells. 3) Record the total of dead and live cells in Square 1.

Item12b. Square 2: 1) Record number of dead (blue) cells 2) Record number of live (clear) cells. 3) Record the total of dead and live cells in Square 2.

Item12c. Square 3: 1) Record number of dead (blue) cells 2) Record number of live (clear) cells. 3) Record the total of dead and live cells in Square 3.

Item12d. Square 4: 1) Record number of dead (blue) cells 2) Record number of live (clear) cells. 3) Record the total of dead and live cells in Square 4.

Item12e. Totals: 1) Record the total of the 4 corner grids of dead (blue) cells. 2) Record the total of the 4 corner grids of live (clear) cells. 3) Record the total sum of the 4 corner grids of dead and live cells.

Item12f. TCC Total Cell Count = [{sum 4 grids/4} x 2 x $10^4$] x volume of sample

Item12g. Cells/mg Determine the number of cells/mg of remaining sample = (TCC/weight of remaining sample). In other words: *(TCC-Response to Item 12f/weight of sample in Item 10a.)*

Item 12h. Viability. Viability = (live cells/total cells) x 100%. In other words: (Item 12e2./Item 12e3) x 100%

Item 13. Cytospins

***Spin down cell pellet and remove or add HBSS to give final concentration of 1x$10^6$cells/mL; mix to re-suspend. Slides are made using 60µL of cell suspension (single cytofunnel recommended). Make 4 slides if possible. You must make at least one slide.***

***Spin for 6 minutes at 450 rpm (program 6 on cytospin).***

***Fix and stain 2 slides in Hema 3 stain (10 dips in each) and rinse in distilled water. After air drying, stained slides are fixed with cytoseal and a coverslip. Store slides at room temperature.***

Item13a. Hema3. Record the number of slides stored

Item 14. Trizol Cell Pellet

***From remaining cell pellet above, spin down cells at 500xg for 5 minutes and discard HBSS. Add 1mL of Trizol reagent to cell pellet. Add 10uL of GGD. Pipet up and down to break up cells.***

Item 14. Record the number of cells.

***Store sample at -80◦C for later extraction of total RNA***