SPUTUM PROCESSING WORKSHEET

ID NUMBER:								

FORM CODE: **SPW**
VERSION: 6.0 04/22/2019

Event________________________

0a) Form Date ☐☐ / ☐☐ / ☐☐☐☐ 0b) Staff Code ☐☐☐

0c) Date Collected ☐☐/☐☐/☐☐☐☐

0d) Processing Started ☐☐:☐☐ AM/PM (circle one)

Instructions: *Complete this form while processing the sputum sample. Carefully record all data in the space provided.*

1) Weight of Entire Sample ☐☐☐.☐☐ grams

Color and Description of Sample:

2) Salivary Contamination (check all that apply):
   a) ☐ Minimal
   b) ☐ Mild
   c) ☐ Moderate
   d) ☐ Excessive

3) Consistency (check all that apply):
   a) ☐ Watery
   b) ☐ Mucoid
   c) ☐ Purulent (puss)

4) Mucus "plugs" (material that is colored and/or more opaque than clear surrounding saliva) (check all that apply):
   a) ☐ Numerous
   b) ☐ Moderate number
   c) ☐ Sparse
   d) ☐ Large
   e) ☐ Small
   f) ☐ Dense/flocculent
   g) ☐ Diffuse opacity
   h) ☐ None

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

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

6) General Notes/Comments:

6a) Sputum processing condition

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

6a1) Sputum processing for Condition 5

☐ Condition A: Spontaneous sample Wt. is > 1g[1] → **Perform Steps 7-14 below**
☐ Condition B: Spontaneous sample Wt. is 0.5g-1g[2] → **Perform Step 7; then 10-14 below**
☐ Condition C: Spontaneous sample Wt. is <0.5g[3] → **Perform Steps 10-14 below**

7) Processing Whole Sample using the Mucin Method

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

*Sample size should be from 0.100-0.250g. Add 0.5ml of guanidine reduction buffer (6M) to the sample and store at 2-8 ^{0} C in the refrigerator.

8) Processing Microbiology sample

Micro Sample	
Micro centrifuge tube (g)	8a)
Whole sputum weight (g)	8b)
Vol. of Zymo added (µl)	8c)
Vol. per aliquot (8b+8c)/2	8d)

*Weigh an empty micro centrifuge tube. Zero the balance. Measure ~0.200g of whole sputum sample and record the weight. Add an equal volume (~200µl) of Zymo Research RNA/DNA Shield to the sample and mix. Divide the sample into 2 equal aliquots and store at -80ºC. Ship sample on dry ice.

9) Processing %Solids/Osmotic Pressure Sample

Osmo Sample	
Micro centrifuge tube (g)	9a)
Whole sputum weight (g)	9b)

*Weigh an empty micro centrifuge tube. Zero the balance. Use a 200µl air displacement pipette and draw up whatever can be achieved. Transfer entire volume (50-200µl) of sample to micro centrifuge tube. Record the weight (g) of the sample and store at -80ºC. Sample can be transferred to -20ºC prior to delivery.

10) Processing Total Remaining Whole Sample for Cytokines, Nucleotides, Cell Counts, Cytospins and RNA

Weight of Centrifuge tube (g)	10a1)
Weight of Sputum (g)	10b1)
Volume of EDTA-DPBS added to sample (ml)	10c1)
Volume of supernatant removed (ml) (see section 11 below for process)	10d1)
Volume of EDTA added to stock sputolysin to make 0.2% DTT (ml)	10e1)
Volume of 0.2% DTT added to the sample (ml)	10f1)
Volume of DTT containing supernatant removed (ml)	10g1)
Resuspension Volume of EDTA-DPBS (0.25 – 2 ml)	10h1)

***Weigh a 50ml conical test tube. Zero the balance. Add remaining sample to 50 ml conical test 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 1500 g for 10 min. Collect the supernatant and store at -80ºC (see text below section 11). To the "master" sample, add a volume of 0.2% sputolysin (diluted with 1mM EDTA) that is equal to the same volume you previously removed from the master sample; i.e., half the volume of DPBS you added to initial weight of sample. For example, if initial volume of DPBS added to the sample = 8 ml, you need to add 4 ml of 0.2% sputolysin as follows: 0.8 ml DTT from stock vial (1%) + 3.2 ml of cold DPBS = 4 ml of 0.2% sputolysin). Pipet the sample up and down with P1000 pipette to break up any clumps (this is especially necessary for very viscous/thick samples), then homogenize the sample for 15 min. on a rotating tumbler - then filter the sample through a pre-wetted 48-53µm nylon mesh into new 50 ml tube. Spin the sample filtrate at 790 g for 10 minutes. Remove and store the entire volume of DTT containing supernatant at -80ºC (see EDTA-DTT Supernatant Table in 11 below). Resuspend the cell pellet in cold EDTA-DPBS (0.25 – 2ml depending on thickness of pellet).

11) EDTA-DPBS Supernatants for Nucleotides and Cytokines

Supernatants	Number of aliquots	Volume stored per aliquot (µl)
Nucleotides	a1)	b1)
Cytokines	c1)	d1)
Cytokine Zymo Research RNA/DNA	e1)	f1)

*If the supernatant volume is greater than 8.6 ml, obtain 4 1000 µl aliquots for nucleotides and, 4 1000 µl aliquots for cytokines. Of the remaining sample, take 600 µl and mix it 1:1 with Zymo Research RNA/DNA shield. Apply the label called "SPU_DPBS_Zymo" to the aliquot containing the Zymo Research RNA/DNA shield.
If the sample volume is less than 8.6 ml start by getting 1 nucleotide sample between 200-500 µl and 1 cytokine sample at 200 µl. Mix one of the cytokine aliquots1:1with Zymo Research RNA/DNA shield and apply the label called "SPU_DPBS_Zymo" to this aliquot. If there is sample leftover after that, then continue alternating between nucleotide and cytokine aliquots (i.e., 200-500 µl for nucleotides, 200 µl for cytokines) until finished.
All supernatant samples are immediately stored in a -80º C freezer.

EDTA-DTT Supernatants

Supernatants	Volume stored (ml)
Sputolysin Sup 01	g1)
Sputolysin Sup 02	g2)

12) Cell Counts

Cell Counts:	# Dead	# Live	Total	Squamous Epithelial
a) Quadrant 1	1)	2)	3)	4)
b) Quadrant 2	1)	2)	3)	4)
c) Quadrant 3	1)	2)	3)	4)
d) Quadrant 4	1)	2)	3)	4)
e) Totals:	1)	2)	3)	4)

*Count live (clear) and dead (cell interior is blue) cells in each of the 4 corner quadrants. Include bronchial epithelial cells (BEC's), but exclude RBC's. Count squamous epithelial cells but do not include them in the total live/dead cell count. Perform total cell count and cell viability. Adjust the cell concentration of the sample to 0.5 – $1x10^6$ cells/ml and make 4 cytospin slides.

Sample Concentration (cells/ml) = ([12e3 / 4] X 2 X 10^4)	12f)
Total Cell Count = (([12e3 / 4] x 2 x 10^4) x 10h1) (ref range: 1/2 million – 1 million)	12f 12g)
Number of cells/weight = (12g) / weight of selected sample (10b1)	12g 12h)
Viability = (12e2 live cells / 12e3 total cells) X 100	12h 12i)
Final Adjusted Sample Concentration (cells/ml) *based on resuspension volume* (range = 0.5 – 1.0 x 10^6/ml)	12j)

13) Cytospins

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

*Slides are made using 60 µL-90 µl of cell suspension (at 0.5 - 1X10^6/ml). Make 1 slide using 60 µl, 2nd slide using 70 µl, 3rd slide using 80 µl and a 4th slide using 90 µl. Spin 6 min. at 450rpm. Note: these volumes are based on the Shandon Cytospin IV model. If you are using a different cytospin instrument, refer to the manufacturer's instructions on appropriate sample concentrations and volumes to use. Following air drying, fix all 4 slides in 95% ethanol – DO NOT COVER SLIP THE SLIDES. Ship all slides to the slide reading center. Store all slides in SPIROMICS box at room temperature.

14) Cells for RNA

☐ No 0

☐ Yes 1

Zymo Research RNA/DNA cell pellet	14 14a)

*The sample is spun down at 790g for 10 minutes. Discard the supernatant and resuspend the pellet in 500 µl of DPBS. Add 500 µl of Zymo Research RNA/DNA shield to the sample. Then divide this into two equal 500 µl aliquots for storage Store in -80°C.The number of cells left in the Zymo Research RNA/DNA pellet will be equal to the TCC (12g) minus the total number of cells used to make slides in (13b (0.0 – 0.3) ml X 12j).

15) Comments

0e 16) Processing Ended ☐☐:☐☐ AM/PM (circle one)

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