SPUTUM PROCESSING WORKSHEET

ID NUMBER:								

FORM CODE: **SPW**
VERSION: 5.0 06/05/2018

Event_____

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

0c) Date Collected ☐☐/☐☐/☐☐☐☐

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

0e) Processing Ended ☐☐:☐☐ 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 (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 4 deg C in the refrigerator.

8) Processing Microbiology sample

Micro Sample	
Micro centrifuge tube (g)	8a)
Whole sputum (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. Record the weight of sputum and store in -80ºC. Ship sample on dry ice.

9) Processing %Solids/Osmotic Pressure Sample

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

*Weigh an empty micro centrifuge tube. Zero the balance. Pipette 200uL of sample. Transfer 50-200ul of sample to micro centrifuge tube. Record the weight of sample and store in -80ºC. Sample can be transferred to -20ºC prior to delivery.

10)Processing Whole Sample using EDTA-DPBS then EDTA-Sputolysin solution

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% sputolysin (ml)	10e1)
Volume of 0.2% sputolysin added to the sample (ml)	10f1)
Volume of DTT containing supernatant removed (ml)	10g1)
Volume of EDTA-DPBS (0.25 – 2 ml)	11g1 10h1)

*****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 deg 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 48-53µm nylon mesh into new 50mL tube. Spin the sample filtrate at 790g for 10 minutes. Remove and store the entire volume of DTT containing supernatant at -80 deg 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 – 1x10^6 cells/ml and make 4 cytospin slides.

11) EDTA-DPBS Supernatants for Nucleotides and Cytokines

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

*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 -80º C freezer.

11g) EDTA-Sputolysin Supernatants

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

12)Cell Counts

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

*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.

Initial Sample Concentration (cells/ml) = ([12e3 / 4] X 2 X 10^4)	12f)
TCC volume = (([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 0.1	$_{12h}$12i)

Final Adjusted Sample Concentration (cells/ml) *based on resuspension volume* (range = 0.5 – 1.0 x 10^6/mL)	12j)

13)Cytospins

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

*Slides are made using 60µL-90ul of cell suspension (at 0.5 - 1X10^6/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 Following air drying, fix all 4 slides in 95% ethanol – 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.

14)Cells for RNA

☐ **No0**

☐ **Yes1**

Zymo Research RNA/DNA cell pellet	1414a)

*The sample is spun down at 790g 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).

15)Comments