SPUTUM PROCESSING WORKSHEET

ID NUMBER:									FORM CODE: **SPW** VERSION: 2.**0** 9/12/11	Visit Number		SEQ #			

0a) Form Date ..... ☐☐/☐☐/☐☐☐☐ 0b) Initials..... ☐☐☐

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:

- ☐ Minimal
- ☐ Mild
- ☐ Moderate
- ☐ Excessive

3) Consistency:

- ☐ Watery
- ☐ Mucoid
- ☐ Purulent (puss)

4) Mucus "plugs":

- ☐ Numerous
- ☐ Moderate number
- ☐ Sparse
- ☐ Large
- ☐ Small
- ☐ Dense/flocculent
- ☐ Diffuse opacity

5) Color of plugs:

- ☐ Clear
- ☐ White
- ☐ Yellow/Tan
- ☐ Brown
- ☐ Green

6) General Notes/Comments:

6a) Sputum processing method ..... ☐

Method 1 (remove three aliquots then process with EDTA)..... 1
Method 2 (Immediately process with EDTA)..... 2 → **Go to Item 10**

7) Processing Whole Sample using the Mucin Method (Complete only for method 1)

Mucin Sample	Weight (g)
Weighing tray	a)
Whole sputum	b)
Guanidine vol.	c)

*Sample size should be from 0.100-0.500g. If sample is 0.500g, 1mL of guanidine reduction buffer added. If less than 0.500g, 0.5mL guanidine reduction buffer added. Sample transferred from weighing tray to microcentrifuge tube. Sample should be stored in 4ºC refrigerator.

8) Processing Microbiology sample (Complete only for method 1)

Micro Sample	Weight (g)
Microcentrifuge tube	a)
Whole sputum	b)

*Weigh an empty microcentrifuge tube. Zero the balance. Measure 0.250g of whole sputum sample. Record the weight of sputum and store in -80ºC. Ship sample on dry ice.

9) Processing Viscoelastic Sample (Complete only for method 1)

Viscoelastic Sample	Weight (g)
Microcentrifuge tube	a)
Whole Sputum	b)

*Weigh an empty microcentrifuge tube. Zero the balance. Pipet 100uL of sample, drawing up whatever can be achieved. Transfer sample to microcentrifuge 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:

Weight of Centrifuge tube	a)
Weight of Sputum	b)
1% sputolysin volume	c)

Volume EDTA added to make 0.1% sputolysin	d)
Time of 15 minute tumble	e)
Volume EDTA added after 15 minute tumble	f)
Time of 5 minute tumble	g)

*****Weigh a 50mL centrifuge tube. Zero the balance. Add remaining sample to centrifuge tube and record weight in grams. Add 0.1% Sputolysin in mLs equal to 4X selected sample weight in grams (For example, 2g of sample would need 8mL of 0.1% sputolysin). Place sample on tumble for 15 minutes. Dilute sample with EDTA. Use the same volume that was added above. Continue to tumble for an additional 5 minutes. After 5 minute tumble, sample is filtered through 53µm nylon mesh into new 50mL tube. Cells are spun down at 500Xg for 10 minutes.

11)Supernatants for Nucleotides and Cytokines

Supernatants	Number of aliquots	Volume stored
Nucleotides	a)	b)
Cytokines	c)	d)

*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, 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 in -80ºC freezer.

Volume of Hanks added	e)

12)Cell Counts

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

*Count live (clear) and dead (blue) cells in each 4 corner grids. Count BEC's, but exclude RBC's and squamous.

TCC= (sum 4 grids/4 X 2 X 10^4 X vol. sample	f)
=TCC/weight of selected sample	g)
Viability = (live cells/total cells) X 100%	h)

13)Cytospins:

	# slides stored

Hema 3 stained slides	a)

*Slides are made using 60µL of cell suspension ($1X10^6$/mL). Spin 6 min. at 450rpm (program 6 on cytospin). If possible, fix and stain 2 slides in Hema 3 stain (10 dips in each) and fix 2 slides in 95% ethanol. After air drying, stained slides are fixed with cytoseal and a cover slip. All slides stored at room temperature.

14)Cells for RNA

Trizol cell pellet	

*Cells are spun down at 500Xg for 5 minutes, HBSS's is discarded and 1mL Trizol is added. Add 10uL of GGD. The number of cells left in the Trizol pellet will be equal to the TCC (12f) minus the total number of cells used to make slides in 13.