

SPUTUM PROCESSING WORKSHEET

ID NUMBER:	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
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FORM CODE: **SPW**
VERSION: 1.0 10/17/2024

Event: _____

0a) Date of Collection: / /

0b) Staff Code:

0c) Processing Started: : AM₁ / PM₂

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*):

- 2a) Minimal
- 2b) Mild
- 2c) Moderate
- 2d) Excessive

3) Consistency (*check all that apply*):

- 3a) Watery
- 3b) Mucoïd
- 3c) Purulent (puss)

4) Mucus "plugs" (material that is colored and/or more opaque than clear surrounding saliva)

(*check all that apply*):

- 4a) Numerous
- 4b) Moderate number
- 4c) Sparse
- 4d) Large
- 4e) Small
- 4f) Dense/flocculent
- 4g) Diffuse opacity
- 4h) None

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

- 5a) Clear
- 5b) White
- 5c) Yellow/Tan
- 5d) Brown
- 5e) Green

6) General Notes/Comments:

6a) Sputum processing condition:

- Condition 1: Induced initial sample Wt. is > 1 g₁ → **Perform Steps 7-12**
- Condition 2: Induced initial sample Wt. is 0.5 g-1 g₂ → **Perform Steps 7-12**
- Condition 3: Induced initial sample Wt. is < 0.5 g₃ → **Perform Steps 7-8**
- Condition 4: Induced initial sample Wt. is < 0.5 g and a spontaneous sputum sample was acquired prior to induction₄ → **Perform Steps 7-8 with induced sample; then 9-12 with spontaneous sample**
- Condition 5: No induced sputum sample was produced; but a spontaneous sample was acquired₅

6a1) Sputum processing for Condition 5:

- Condition A: Spontaneous sample Wt. is > 1 g₁ → **Perform Steps 7-12**
- Condition B: Spontaneous sample Wt. is 0.5 g-1 g₂ → **Perform Steps 7-12**
- Condition C: Spontaneous sample Wt. is < 0.5 g₃ → **Perform Steps 7-8**

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 0.100-0.250 g. Add 0.5 mL of guanidine reduction buffer (6M) to the sample and store at 2-8°C in the refrigerator.*

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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 ~0.500 g of whole sputum sample and record the weight (where there is < 0.500 g available, don't use less than 0.250 g). Add an equal volume (~500 μL) of Zymo Research RNA/DNA Shield to the sample and mix. Divide the sample into two equal aliquots and store at -80°C . Ship sample on dry ice.*

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

Weight of Centrifuge tube (g)	9a)
Weight of Sputum (g)	9b)
Volume of EDTA-DPBS added to sample (mL)	9c)
Volume of supernatant removed (mL) (see section 10 for process)	9d)
Volume of EDTA added to stock sputolysin to make 0.2% DTT (mL)	9e)
Volume of 0.2% DTT added to the sample (mL)	9f)
Volume of DTT containing supernatant removed (mL)	9g)
Resuspension volume of EDTA-DPBS (0.25 – 2 mL)	9h)

**Weigh a 50 mL conical test tube. Zero the balance. After separating any clear runny saliva from the more viscous "main" sample, add remaining "main" sample to 50 mL conical test tube and record weight in grams. Add 8x sample weight of cold EDTA-DPBS buffer, homogenize for 15 minutes on a rotating tumbler, then spin at 790 g at 10 minutes. Remove half the volume of supernatant (leaving behind the "master" sample) and spin this at 1500 g for 10 minutes. Collect the supernatant and store at -80°C (see text below section 10). 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 minutes on a rotating tumbler - then filter the sample through a pre-wetted 48-53 μm 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 -80°C (see EDTA-DTT Supernatant Table in 10 below). Resuspend the cell pellet in 0.25 – 2 mL of cold EDTA-DPBS depending on thickness of pellet ("thin" pellets require less volume; "thick" pellets require more volume).*

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10) EDTA-DPBS Supernatants for Nucleotides and Cytokines

Supernatants	Number of aliquots	Volume stored per aliquot (µL)
Nucleotides	10a1)	10b1)
Cytokines	10a2)	10b2)
Cytokine Zymo Research RNA/DNA	10a3)	10b3)

**If the supernatant volume is greater than 8.6 mL, obtain four 1000 µL aliquots for nucleotides and four 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 one nucleotide sample between 200-500 µL and one cytokine sample at 200 µL. Mix one of the next cytokine aliquots 1:1 with 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 -80°C freezer.

EDTA-DTT Supernatants

Supernatants	Volume stored (mL)
Sputolysin Sup 01	10c1)
Sputolysin Sup 02	10c2)

11) Cell Counts

Cell Counts:	# Dead	# Live	Total	Squamous Epithelial
Quadrant 1	11a1)	11a2)	11a3)	11a4)
Quadrant 2	11b1)	11b2)	11b3)	11b4)
Quadrant 3	11c1)	11c2)	11c3)	11c4)
Quadrant 4	11d1)	11d2)	11d3)	11d4)
Totals	11e1)	11e2)	11e3)	11e4)

**Count live (clear) and dead (cell interior is blue) cells in each of the four corner quadrants. Include bronchial epithelial cells (BECs) but exclude RBCs. 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 – 1x10⁶ cells/mL and make four cytospin slides.*

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Sample Concentration (cells/mL) = $([11e3 / 4] \times 2 \times 10^4)$	11f)
Total Cell Count = $(([11e3 / 4] \times 2 \times 10^4) \times 9h)$ (ref range: 1/2 million – 1 million)	^{12f} 11g)
Number of cells/weight = (11g) / weight of selected sample (9b)	^{12g} 11h)
Viability = $(11e2 \text{ live cells} / 11e3 \text{ total cells}) \times 100$	^{12h} 11i)
Final Adjusted Sample Concentration (cells/mL) based on resuspension volume (range = 0.5 – 1.0 x 10 ⁶ /mL)	11j)

12) Cytospins

	# of slides stored	Amount of cell suspension used (µL)
Hema 3 stained slides	¹³ 12a)	12b)

**Slides are made using 60-90 µL of cell suspension (at 0.5 - 1X10⁶/mL). Make one slide using 60 µL, 2nd slide using 70 µL, 3rd slide using 80 µL, and a 4th slide using 90 µL. Spin six minutes at 450 rpm. 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 four slides in 95% ethanol – DO NOT COVER SLIP THE SLIDES. Ship all slides to the sputum reading center. Store all slides in SOURCE box at room temperature.*

13) Comments: _____

^{0e}14) Processing Ended: : AM₁ / PM₂

END OF FORM