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Section: <b>Virology Manual</b>	Subject Title: <b>Appendix XXII - Sputolysin Procedure</b>	
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## **Appendix XXII**

### **SPUTOLYSIN PROCEDURE**

Sputolysin Reagent is a concentrate of dithiothreitol (DTT) in phosphate buffer used in the isolation of pathogens from mucoid sputum, BAL and other fluids.

#### **Materials**

- a. Sputolysin Reagent (Cat. No. 560000, CALBIOCHEM) - dilute a 10 mL vial to 100 mL with sterile dH<sub>2</sub>O. Stable for up to 48 hr. at 2 – 8°C.
- b. Microcentrifuge tubes
- c. Microcentrifuge
- d. Vortex mixer

#### **Procedures**

Sputum / mucoid BAL for PCP

- a. Adjust specimen s to approximately 0.5 McFarland standard with Hank's Buffered Saline Solution (HBSS) or maintenance medium.
- b. Suspend specimen (approximately 1 mL) with an equal volume of Sputolysin in a microcentrifuge tube by vortexing for 30 seconds.
- b. Incubate at room temperature for 15 minutes.
- c. Centrifuge at 5000 rpm for 5 minutes and remove supenate.
- d. Make a thick and a thin smear on the 2 wells of a PCP slide.

#### **Reference**

CALBIOCHEM Product Insert, revised 21 Jan 2000 (<http://www.calbiochem.com>)