U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service

Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30329-4027

Processing of Sputum Specimens for Nucleic Acid Extraction

PURPOSE

Sputum specimens collected from patients for molecular diagnostic testing frequently contain mucoid or mucopurulent material that make the specimen too viscous for efficient processing for downstream nucleic acid extraction.

This procedure provides guidance for liquification of sputum specimens prior to downstream nucleic acid extraction and molecular testing.

MATERIALS (Disclaimer: Names of vendors or manufacturers are provided as examples of suitable product sources. Inclusion does not imply endorsement by the Centers for Disease Control and Prevention.)

- Nuclease-free PCR grade water
- Thermo Scientific™ Pierce™ DTT (Dithiothreitol), No-Weigh™ Format (Fisher Scientific catalog A39255)
- Sterile, 0.01 M Phosphate Buffered Saline (PBS), pH 7.2
- Micropipettes
- Aerosol barrier pipette tips
- 1.5 mL or 2.0 mL microcentrifuge tubes (DNase/RNase free)
- 10 mL or 15mL, sterile screw cap tubes (polypropylene)

PRECAUTIONS

- Handling and processing specimens should be performed in accordance with national biological safety guidelines. Refer to the following: https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF
- Handle specimens carefully to avoid cross-contamination, including changing gloves between samples. All specimens should be kept cold during processing.

PREPARING SPUTUM SAMPLES BEFORE EXTRACTION

- Rehydrate Thermo Scientific™ Pierce™ DTT (Dithiothreitol) by adding 100 µL of nuclease-free water to one microtube containing DTT and gently mix with pipette tip to completely dissolve (500 mM final concentration).
- Add the entire 100 µL of freshly prepared DTT to 5mL of cold sterile 0.01 M PBS (pH 7.2) and mix briefly.
- In a microcentrifuge tube, add diluted DTT solution to an equal volume of specimen. (e.g. 500 µL of sputum + 500 µL of diluted DTT). **Note:** Use DTT immediately. Discard any unused reconstituted DTT.
- Incubate sample at room temperature with intermittent mixing until the sample is liquified (up to 30 minutes).
- Use liquified sample for downstream nucleic acid extraction following the extraction system manufacturer's guidelines. Retain any residual liquified sample at -70°C.

QUESTIONS

Please send questions or comments by email to respvirus@cdc.gov.