

# User-Developed Protocol

## Protocol for homogenisation of Cystic Fibrosis (CF) sputum samples in preparation for *Aspergillus* fungal DNA extraction

### Procedure for the homogenization of CF sputum samples prior to DNA extraction:

- 1) Collect or transfer the sputum sample into a sterile 50 mL plastic centrifuge tube.
- 2) Add an equal volume of Sputasol (dithiothreitol 1.4%) (Oxoid Ltd) to the sputum sample and shake the mixture well.
- 3) Incubate at 37°C for 30 minutes with periodic shaking.
- 4) Sonicate for 120 seconds at an amplitude of 295 µm.
  - a. Place the plastic centrifuge tube on ice in a polystyrene box (to prevent heat transfer during sonication).
  - b. Use plastic tubes only (glass may break during sonication).
  - c. In order to achieve a sonication amplitude of 295 µm, the volume of sample to be sonicated should first be determined and the correct sonication microtip, recommended by the manufacturer, should be used. The output is then adjusted to achieve the amplitude.
  - d. Any sonicator can be used but for this procedure a Sonics® VC505 ultrasonic processor was used in a sound abating enclosure. 3 mm stepped microtips were used with a four element coupler to process up to four samples simultaneously. This size of microtip will process samples from 0.5 to 10 mL in volume. A 40% output applied to a 3 mm microtip, in a four element coupler, will produce an amplitude of 295 µm. Sonication is performed continuously for 120 seconds.
- 5) Following sonication heat the sample at 80°C for 20 minutes (ensures mycobacterial decontamination) then allow to cool [This step only necessary if clinical suspicion of tuberculosis].

UDP 7: Protocol for homogenization of cystic fibrosis (CF) sputum samples in preparation for *Aspergillus* fungal DNA extraction

This protocol has been generated independently of Myconostica and is supplied for information only. The use of the protocol has not been validated by Myconostica, and users are responsible for their use. Myconostica has not established the diagnostic validity of our products when used in conjunction with this protocol.

## User-Developed Protocol

- 6) Perform MycXtra Fungal DNA extraction protocol, as per manufacturer's instructions - Procedure for clear free-flowing BAL samples.

### NB

**This protocol homogenizes the sputum sample causing breakdown of sputum mucins and biofilms without disrupting the *Aspergillus* fungal cell wall or fungal DNA integrity.**

**This procedure is not recommended for organisms other than *Aspergillus* as cell wall integrity may vary in different genera.**

### References

This UDP was kindly supplied by Caroline Baxter, University Hospital of South Manchester

Real Time PCR in the Identification and Management of *Aspergillus* in CF

<sup>1</sup>C G Baxter, <sup>2</sup>A M Jones, <sup>2</sup>A K Webb, <sup>1</sup>D W Denning. <sup>1</sup>*University Hospital of South Manchester, Education and Research Department, Manchester, UK;* <sup>2</sup>*Manchester Adult CF Unit, Manchester, UK* Doi:10.1136/thx.2010.150912.19

Sputasol (dithiothreitol 1.4%) (Oxoid Ltd, Basingstoke, UK). Product code - SR0233A

Sonics® VC505 ultrasonic processor (Sonics and Materials Inc, Newtown, CT, USA)

UDP 7: Protocol for homogenization of cystic fibrosis (CF) sputum samples in preparation for *Aspergillus* fungal DNA extraction

This protocol has been generated independently of Myconostica and is supplied for information only. The use of the protocol has not been validated by Myconostica, and users are responsible for their use. Myconostica has not established the diagnostic validity of our products when used in conjunction with this protocol.