

## USER-DEVELOPED PROTOCOL

### Protocol for automated DNA extraction from BAL, bronchial aspirate and sputum samples using QIAcube Robotic workstation (Qiagen)

#### Procedure for the isolation of DNA using QIAcube Robotic workstation (Qiagen)

**NB: Ensure all reagents are free from fungal contamination.**

1) a) Non-viscous BAL samples do not require any pretreatment.

b) Viscous BAL/bronchial aspirate/sputum samples are treated with a brief freezing/thawing cycle at -20°C or treated like a Kock bacillus sample (neutralization reaction with HCl and NaOH). Please see UDP7 for homogenization of sputum samples from Cystic Fibrosis patients.

2) Take 200 µL of sample from the collector tube (if more sample volume is available, centrifuge at 3000 rpm for 5 minutes and concentrate to a final volume of 200 µL).

3) Using the QIAamp DNA Blood Mini kit (Qiagen) follow instructions according to manufacturer's protocol in the ProtocolSheet\_DNA\_QIAampDNABloodMini\_BloodOrBodyFluid\_ElutionVolume100.

4) DNA elution volume = 100 µL.

Use 10 µL DNA in the MycAssay™ according to the Instruction for Use.

#### References

This UDP was kindly supplied by Mrs. Gabriella Grillo, San Martino University-Hospital, Genoa, Italy (Personal Communication).

QIAamp® DNA Blood Mini Kit (50) plus QIAamp DNA Blood Mini Accessory Set A, cat. no. 1043368  
or

QIAamp® DNA Blood Mini Kit (250) plus QIAamp DNA Blood Mini Accessory Set B, cat. no. 1043369

UDP 8: Protocol for automated DNA extraction from BAL, bronchial aspirate and sputum samples using QIAcube Robotic workstation (Qiagen)

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