**DNA Extraction**

DNA extraction was performed at Ampath Laboratories. The BAL and sputum samples were collected in a 15 mL tube and transported on ice to the laboratory and stored at -80 ͦC upon arrival at the laboratory.

**1. Pre-processing DNA extraction:**

1.1 10-15 samples were extracted in a batch in a sterile and clean biohazard safety cabinet

1.2. Samples were thawed at room temperature ( ± 25 ͦC) for 15 minutes.

1.3 Each sample was vortexed at maximum speed with a vortex mixer (Labnet International, Edison, NJ, USA) for 10 seconds to ensure homogenization of the

Sample.

1.4 Five hundred microliter of the thawed sample was added to a sterile 2.0 mL

Eppendorf tube (Eppendorf, Hamburg, Germany).

1.5 Equal volume of the sample (500 µL) of prepared NALC and 1 X PBS was added to

the sample aliquot and vortexed at maximum speed (Labnet International, Edison, NJ,

USA).

1.6 To concentrate the sample, each sample aliquot was centrifuged in a microcentrifuge

(Thermo Fisher Scientific, Waltham, Massachusetss, USA) at 13 000 x g for 10

minutes.

1.7 The supernatant was carefully removed without disturbing the pellet.

**2. DNA extraction with the QIAamp DNA mini kit (Qiagen, Hilden, Germany)**

2.1 The Protocol for Bacteria, Isolation of bacterial DNA from biological fluids was used

and modified to accommodate the isolation of genomic DNA from Gram-positive

bacteria.

2.2 The pellet was resuspended in 180 µL of the separately prepared enzyme solution (20mg/mL lysozyme; 20 mM TRIS-HCL, pH 8.0; 2 mM EDTA; 1.2% Triton).

2.3 The suspension was incubated in a digital heat block (Labnet International, Edison,

NJ, USA) for 30 minutes at 37 ͦC.

2.4 Twenty microliter of the supplied proteinase K (Qiagen, Hilden, Germany) and 200µL of Buffer AL (Qiagen, Hilden, Germany) was added and vortexed at maximum speed with a vortex mixer (Labnet International, Edison, NJ, USA).

2.5 Another incubation step at 56 ͦC for 30 minutes was done followed by a further

incubation of 15 minutes at 95 ͦC.

2.6 200 µL of absolute ethanol (Sigma-Aldrich, Missouri, USA) was added to each

sample and pulse-vortexed for 15 seconds.

2.7. Washing steps was performed according to the manufacturer’s instructions.

2.8 The DNA was eluted in Buffer AE (Qiagen, Hilden, Germany) in two 25 µL elution

steps, resulting in a total elution volume of 50 µL.

2.9. Eluted DNA was stored at - 20 ͦC until further processing.