Laboratory Operating Procedure

RNA extraction from swabs and tissue samples

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LOP-Version: 1

Attachments:

Introduction

RNA is extracted using a Qiagen QIAamp viral RNA mini kit according to the manufacturers' instructions except that carrier RNA is omitted from the AVL lysis buffer. The extraction process involves lysis of virus under highly denaturing conditions using AVL lysis buffer containing guanidine-HCL. Following addition of 100% ethanol any virus present and any RNases introduced with the sample are inactivated. Nucleic acid present in the sample is then bound to a silica fibre-containing filter. Contaminating material (that may be inhibitory to the final PCR) is removed by washing the silica fibre filter with two preparatory wash buffers (AW1, AW2) prior to elution of the purified nucleic acids (RNA and DNA) in nuclease free water.

Sample Material

Swabs are transported to the laboratory in a dry state at ambient temperature. The swabs are introduced into 1ml of brain heart infusion broth (BHIB) or PBS containing antibiotics (1000IU penicillinG;10µg/ml amphotericin B; 1mg/ml gentamicin). The swab tip is cut off, placed in the BHIB, agitated briefly in order to release material into the medium. The swab suspension in BHIB is the starting material for the extraction.

Tissues or carcasses are transported to the laboratory at ambient temperature. A tissue homogenate is obtained by finely cutting the tissue and incubating in1ml of PBS containing antibiotics (1000IU penicillinG;10µg/ml amphotericin B; 1mg/ml gentamicin) for 30–60 min at ambient temperature (+/-22°C). The suspension is clarified by centrifugation for 5 minutes at 2000 rpm and removing the supernatant.

Chorio- allantoic fluid is collected from infected eggs and stored at -80°C or used directly for RNA extraction, as required.

Equipment and Reagents

(general remarks and list of equipment and material needed, bullet points)

Equipment

Microfuge with rotor for 2ml microfuge tubes and centrifugation up to 20000g
1.5 - 2ml microfuge tubes
Filter pipette tips
Pipettor

Reagents

- *Qiagen QIAamp viral RNA mini kit*
- 96-100% ethanol

General remarks

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Literature

- *Handbook supplied with kit*

Before getting started

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Procedure

- Equilibrate all reagents to room temperature prior to use and if buffers contain precipitate, warm in an 80°C incubator.

- In an appropriate class of MSC, add 140µl of sample suspension to 560µl AVL buffer without carrier RNA and mix thoroughly. Incubate at room temperature for 10 minutes.

- Add 560µl ethanol to the sample, mix by gentle aspiration for 15 seconds. At this point infectious influenza virus in the sample will be inactivated and the tube can be removed from the MSC using specified surface decontamination procedures.

- Briefly centrifuge the tube to remove drops from the inside of the lid. Add 630µl of the solution to a spin column in a 2ml collection tube and centrifuge 6000 x g (8000rpm) for 1 minute. Place the spin column in a clean collection tube and discard the tube containing the filtrate. Repeat this step until all the sample has been passed through the column

- Add 500µl AW1 buffer to spin column and centrifuge 6000 x g (8000rpm) for 1 minute. Repeat this step

- Add 500µl AW2 buffer to spin column and centrifuge 20,000 x g (13,000rpm) for 3 minutes. Discard the flow-through

- Repeat the centrifugation at 20,000g (13000rpm) for 1 minute to remove residual AW2 and dry the filter.

- Place spin column in a clean 1.5ml microcentrifuge tube and discard the tube containing the filtrate. Add 50µl of room temperature RNase free water. Incubate at room temperature for 1 minute. Centrifuge at 6000 x g (8000rpm) for 1 minute.

- Store the eluted RNA as appropriate.

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