

Standard Operating Procedure for *Aspergillus spp.* DNA Extraction for Quantitative Real-time Polymerase Chain Reaction

1. Purpose

This Standard Operating Procedure (SOP) will provide information necessary for extraction of *Aspergillus* DNA from tissue homogenates from organs harvested from laboratory animals infected with experimental pulmonary aspergillosis. Additional information is provided to encompass additional processing as needed for further experimentation or investigation.

2. Scope

This SOP will encompass generation of secondary homogenates from initial homogenates obtained from mice and guinea pigs and subsequent extraction of *Aspergillus* DNA, utilizing a commercially available kit, to determine fungal tissue burden utilizing quantitative real-time PCR.

3. Definitions.

“2°homogenate” means to prepare a quantity of initial tissue homogenate for use in extraction of DNA.

4. Responsibilities

This SOP shall be utilized by employees of Research assistant status or higher without additional training. Research technicians may perform this work upon receipt of training.

5. Equipment

- 1.5 ml Eppendorf tubes
- Buffered pipet tips
- 100% Ethanol
- Sterile saline
- High speed table top centrifuge (Eppendorf Centrifuge 5415 D)
- Sterile 2ml screw-cap centrifuge tubes (Sarstedt, Newton N.C.)
- 0.5-mm-diameter glass beads (Biospec, Bartlesville, OK)
- Biospec Bead Beater homogenizer (Biospec)
- Ribonuclease A (50mg/ml) (Sigma,)
- QIAamp DNA Mini Kit (Qiagen®, Valencia CA)
- Two water-baths set at 50°C and 70°C

6. Procedure

- Preparation of 2° Organ Homogenate for quantitative PCR preparation:
 - Primary tissue homogenate is prepared as stated in *Standard Operating Procedure Animal Tissue Homogenization*.
 - In sterile 2ml screw-cap Sarstedt centrifuge tubes, add 0.5-mm-diameter glass beads (approximately 0.7ml) and 300 µl of sterile saline.
 - An aliquot of 500µl of fresh homogenate is added to the tube (labeled with animal number, organ, study number or date), and the sample is bead beaten on the homogenization setting (3200 rpm) for 1 minute 30 seconds on a

Bead beater homogenizer (Biospec) according to manufacturer's instructions. (Secondary [2°] homogenate)

- 100 µl of the 2° homogenate is then processed for DNA extraction [see SOP Determination of Tissue Fungal Burden Utilizing Quantitative Real Time Polymerase Chain Reaction (qPCR)]. The remainder of the sample is stored at -20°C.
- DNA extraction:
 - One hundred microliters of the 2° tissue homogenate is aliquoted into a 1.5ml eppendorf tube. Fungal DNA extractions are done utilizing the Qiagen® DNA Mini-kit. 100 µl of buffer ATL and 20 ml of Proteinase K solution (provided in the kit) is added to the sample.
 - Samples are incubated at 55°C overnight.
 - The next day, samples are processed according to manufacturer's instructions and applied to DNA mini-kit columns; and DNA is recovered in 150 µl of elution buffer and stored at -20°C if not screened immediately.
- Note for maximal DNA recovery:
 - Warm elution buffer in a 50°C water bath prior to use.
 - Let the elution buffer sit on the column at least 2 min. before spinning for 2 min.
 - After first elution through the column, pass the eluate once again through the column.
 - Average amount of fungal DNA recovered from GP lungs at the 1hr sacrifice should result in 1.5 µg of fungal DNA/gram tissue (As quantified by quantitative RT-PCR results).

7. Attachments

N/A

8. Deliverables

Aliquots of extracted DNA from each sample should be stored at -20C for reference / experimental purposes.

9. References

QIAamp® DNA Mini Kit and QIAamp® DNA Blood Mini Kit Handbook (2/2003) (Qiagen®, Valencia CA)

10. History

Version 1.00.

11. Examples of Deliverables

N/A