Standard Operating Procedure for Processing Animal Tissue Samples for PCR, Galactomannan and Storage

1. Purpose

   This Standard Operating Procedure (SOP) will provide information necessary for the uniform storage of 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 storage of organs and homogenates from mice and guinea pigs and will provide uniform methods for labeling of the tissues and homogenates derived from these model animals.

3. Definitions.

   "Storage" means to prepare a quantity of tissue or tissue homogenate for long-term archival purposes.

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

- 1.8ml cryovials (Nunc)
- Liquid Nitrogen
- 1.5 ml Eppendorf tubes
- Whirl Pak Bags® (Fisher Scientific, Pittsburgh, PA)
- Sterile 2ml screw-cap centrifuge tubes (Sarstedt, Newton N.C.)
- 0.5-mm-diameter glass beads (Biospec, Bartlesville, OK)
- Biospec Bead Beater homogenizer (Biospec)
- Homogenization Buffer: sterile saline supplemented with gentamicin [0.8 µg/ml] and chloramphenicol [0.05mg/ml].
- High speed table top centrifuge (Eppendorf Centrifuge 5415 D)

6. Procedure

- Initial tissue preparation and storage:
  - Using sterile technique, freshly harvested organs are individually weighed and recorded.
    - One gram of each guinea pig (GP) tissue is extracted (if possible) for homogenization (see SOP for Animal Tissue Homogenization) in 9ml of homogenization buffer. (1° organ homogenate).
    - For mice, the entire harvested organ is weighed (record weight) and homogenized in 2ml of homogenization buffer.
    - The remainder of each organ is aseptically placed in a Whirl Pak bag® labeled with study number, animal identification number, date of

extraction, and name of organ. Bags are flash frozen in liquid nitrogen. Store at -70°C.
    - Immediately aliquot the 1° organ homogenate into sterile cryovial tubes labeled with study number, animal identification number and name of organ (approx. 1.5ml/tube) and flash freeze with liquid nitrogen. Store at -70°C.
  - Blood samples are spun at 6000 x g for 10 minutes and serum aliquots frozen in cryovials and stored at -20°C. Label the tubes as done in the previous step.
- Galactomannan EIA Preparation of 1° Organ Homogenate or Sera:
  - For organs, vortex homogenate and aliquot 400µl of fresh homogenate into a 1.5 ml eppendorf tube (labeled with animal number, organ and study name). Centrifuge at 2300 x g for 5 min. to pellet large fragments.
    - Extract 300 µl of supernatant into a clean tube (labeled with animal number, organ and study name) for galactomannan quantification using Platelia Galactomannan EIA kits (BioRad, Edmonds, WA) according to manufacturer's directions.
    - The remainder of the supernatant and pellet is stored at -20°C.
  - For serum samples, a 300 µl aliquot of serum is tested for galactomannan quantification, according to manufacturer's instructions, as noted in step 2.
- Quantitative PCR Preparation of 1° Organ Homogenate:
  - 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.

7. Attachments

   N/A

8. Deliverables

   Aliquots of these homogenates and the corresponding bulk tissues should be prepared and frozen (as instructed herein) for reference / experimental purposes. Additional aliquots of fresh (not frozen) samples may also be required for immediate testing or for further processing.

9. References

Bio-Rad Platellia kit operation manual
Qiagen kit operation manual

Bowman JC, Abruzzo GK, Anderson JW, Flattery AM, Gill CJ, Pikounis VB, Schmatz DM, Liberator PA, Douglas CM. (2001) Quantitative PCR Assay To Measure *Aspergillus fumigatus* Burden in a Murine Model of Disseminated Aspergillosis: Demonstration of Efficacy of Caspofungin Acetate. *Antimicrob Agents Chemother*. 45(12): 3474–3481

10. History

    Version 1.00.

11. Examples of Deliverables

    N/A