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 and Materials

- 1.8ml cryovials (Nunc)
- 1.5 ml microcentrifuge tubes
- Whirl Pak Bags® (Fisher Scientific, Pittsburgh, PA)
- Equipment for Platelia Aspergillus EIA assay
  - Microplate washer
  - Microplate spectrophotometer
  - Microcentrifuge
  - Heat block (120°C)
  - Platelia *Aspergillus* EIA kit (BioRad, Redmond, WA)

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).

- 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. Store at -70°C.
- For mice, the entire harvested organ is weighed and homogenized (see SOP for Animal Tissue Homogenization).
- Immediately aliquot the 1° organ homogenate into sterile cryovial tubes labeled with study number, animal identification number and name of organ (approx. 1.0 ml/tube). Store at -70°C.

- Galactomannan EIA Preparation of 1° Organ Homogenate.
  - For organs, vortex homogenate and aliquot 400 µl of fresh homogenate into a 1.5 ml microcentrifuge 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 *Aspergillus* Galactomannan EIA kits (BioRad, Edmonds, WA) according to manufacturer's directions.
    - The remainder of the supernatant and pellet is stored at -20°C.

- Quantitative PCR Preparation of 1° Organ Homogenate:
  - An aliquot of 500 µl of 1° tissue homogenate is processed for DNA extraction [see SOP for *Aspergillus* spp. DNA Extraction for Quantitative Real-time Polymerase Chain Reaction]. 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.

9. References

Bio-Rad Platelia 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*. 2001. 45(12): 3474–3481

Vallor AC, Kirkpatrick WR, Najvar LK, Bocanegra RC, Kinney MC, Fothergill AW, Herrera ML, Wickes BL, Graybill JR, Patterson TF. Assessment of Pulmonary *Aspergillus fumigatus* Tissue Burden by Quantitative PCR, Galactomannan EIA and Quantitative Culture in Guinea Pigs. *Antimicrob Agents Chemother.* 2008 Jul;52(7):2593-8.

10. History

Version 1.00. Original

Version 1.1 Revisions made to text for purposes of clarification and uniformity.

11. Examples of Deliverables

N/A