Standard Operating Procedure for Processing Animal Body Fluids Samples for PCR, Galactomannan, (1→3)-β-D-glucan and Storage

1. Purpose

   This Standard Operating Procedure (SOP) will provide information necessary for the uniform processing and storage of body fluids 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 initial processing and subsequent storage of body fluids from mice and guinea pigs and will provide uniform methods for labeling of the specimens derived from these model animals.

3. Definitions.

   "Storage" means to prepare samples of body fluids 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

- 1.8 ml or 3.6 ml cryovials (Nunc)
- 1.5 ml microcentrifuge tubes
- Equipment for Platelia Aspergillus EIA assay
  - Microplate washer
  - Microplate spectrophotometer
  - Microcentrifuge
  - Heat block (120°C)
  - Platelia *Aspergillus* EIA kit (BioRad, Redmond, WA)
- Equipment for Fungitell (1→3)-β-D-glucan assay
  - 250 and 1000 µl beta-glucan free pipet tips (Associates of Cape Cod)
  - Beta-glucan free borosilicate glass tubes (Associates of Cape Cod)
  - Microplate spectrophotometer with kinetic reading and plate incubation capability
  - Fungitell (1→3)-β-D-glucan kits (Associates of Cape Cod, East Falmouth, MA)

6. Procedure

- Initial sample preparation and storage:
  - Use sterile technique during processing.
  - Blood samples are spun at 6000 x g for 10 minutes and 0.5 ml serum or plasma aliquots are to be frozen in cryovials and stored at -20°C. Label the tubes with study number, animal identification number, date of harvest and sample type.
  - BAL aliquots of 0.5 ml per sample are frozen in cryovials and stored at -20°C. Label the tubes as done in the previous step.
  - Whole blood aliquots of 3 ml are frozen in cryovials and stored at -20°C. Label the tubes as done in the previous step.

- Galactomannan Sample Preparation of Sera or BAL:
  - A 300 µl aliquot of serum or BAL is tested for galactomannan quantification using Platelia Aspergillus Galactomannan EIA kits (BioRad, Edmonds, WA) according to manufacturer's directions.
    - The remainder of the sample is stored at -20°C.

- (1→3)-β-D-glucan Preparation of Sera or BAL.
  - 10 µl (5 µl in duplicate) of serum or BAL is tested for (1→3)-β-D-glucan concentration measurements using the commercially available kit (Fungitell, Associates of Cape Cod) according to manufacturer's directions.
    - The remainder of the sample is stored at -20°C.

- Quantitative PCR Preparation of Sera, Plasma, BAL, or whole blood:
  - An aliquot of 500 µl of serum, plasma or BAL 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.
  - For whole blood, a 3 ml aliquot 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 body fluid specimens should be prepared and frozen (as instructed herein) for reference / experimental purposes.

9. References

Bio-Rad Platellia *Aspergillus* EIA kit operation manual
Associates of Cape Cod Fungitell 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. 52(7):2593-2598.

Wiederhold NP, Najvar LK, Vallor AC, Kirkpatrick WR, Bocanegra R, Molina D, Olivo M, Graybill JR, Patterson TF. Assessment of serum (1->3)-beta-D-glucan concentration as a measure of disease burden in a murine model of invasive pulmonary aspergillosis. *Antimicrob Agents Chemother*. 2008. 52(3):1176-1178.

10. History

Version 1.00. Original

11. Examples of Deliverables

N/A