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:
  o Use sterile technique during processing.
  o 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.
  o 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.
  o 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:
  o 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.
  o 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:
  o 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.
  o 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 Platelia Aspergillus EIA kit operation manual
   Associates of Cape Cod Fungitell kit operation manual


10. History
   Version 1.00. Original

11. Examples of Deliverables
   N/A