

Standard Operating Procedure for Animal Tissue Homogenization

1. Purpose

This Standard Operating Procedure (SOP) will provide information necessary for the uniform completion of tissue homogenization of organs harvested from laboratory animals infected with experimental pulmonary aspergillosis and will provide sufficient information to allow determination of colony forming units of *Aspergillus* within these tissues.

2. Scope

This SOP will encompass homogenization of organs from mice and guinea pigs and will provide two functionally equivalent methods for homogenization of the tissues derived from these model animals.

3. Definitions.

“Homogenization” means to prepare uniform dilution of a known quantity of tissue suspended within a known quantity of a suitable diluent and uniformly macerate or crush the tissue in such a manner as to disperse minute fragments of tissue evenly throughout the mixture.

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

• **Method A. Tissue Grinder**

- Weigh paper
- Petri dish
- Wheaton® Potter-Elvehjem Tissue Grinders [Grinding Chambers (#358039 10ml and #358054 30 ml) with Teflon® pestles ¼” diameter stainless steel rod] (Millville, NJ)
- Amphyl (Revco) [Acceptable equivalent: Vesphene (Steris) or Decon (Decon Labs)]
- Tissue Grinder: RW16 Basic S1 Overhead stirrer, 15 V 50/60 Hz (IKA® works Inc., Wilmington NC)
- Snap top tubes (5ml, 12 x 75 mm) (BD Falcon #352054)
- Petri dish
- Homogenization buffer (sterile saline, gentamicin [0.8 µg/ml] chloramphenicol [0.05mg/ml] (Sigma-Aldrich).
- Parafilm
- Potato Dextrose Agar plates (PDA)

• **Materials and Reagents: Method B. WhirlBag**

- Whirl Pak Bags® (Fisher Scientific, Pittsburgh, PA)
- Sabouraud dextrose plates (SAB)
- PDA plates
- Sabouraud dextrose agar

- Petri dish
 - Sterile saline
 - chloramphenicol (Sigma-Aldrich)
 - Sharpie style pen
6. Procedure
- ***NOTE: Due to the nature of working with infected animal tissues, all procedures described in this SOP should be performed in a biohazard laminar flow hood with proper caution. Hood bottom should be lined with absorbent pads to soak up any animal tissue spillage or aerosol induced by any of the procedures described (i.e. Whirlbag seepage or breakage). Disinfectant (70% alcohol or Amphyl®) should be accessible inside the hood at all times.***
 - **Protocol A. Tissue Grinder homogenization**
 - Using sterile technique, freshly harvested organs (placed on Petri dishes) are individually weighed, weights recorded and processed as described:
 - **Guinea pig:** One gram of each organ is extracted (spleen will be less than one gram) and placed in a 30ml Wheaton® glass homogenization tube containing 9ml of Homogenization buffer.
 - *Note: Due to the difficulty of homogenizing the lung tissue, roughly chop the tissue before placing into the tube with the buffer prior to homogenization.*
 - **Mice:** Entire organ is placed in a 10ml Wheaton® glass homogenization tube and bring up entire volume (tissue + buffer) to a total of 2ml (add 2ml of buffer to an empty 10ml Wheaton tube and use this volume as your guide) with Homogenization buffer.
 - Any tissue left over is sterilely placed in a Whirl Pak bag® labeled with study number, animal identification, date of extraction, and name of organ. Bags are flash frozen in liquid nitrogen and stored at -70°C.
 - An appropriate sized pestle is placed in the homogenization tube containing the organ and buffer. Organ is homogenized using the tissue grinder: RW16 Basic S1 Overhead stirrer, (IKA® works Inc., Wilmington NC)] set at a speed of approximately 1300 rpm or a setting of 8 on the instrument scale. Homogenize the tissue by going up and down the tube with the pestle twice only so as to keep the state of homogenization of each organ uniform from sample to sample.
 - Based on the volume, pour the homogenate into a 5ml snap top tube for mouse homogenates, or a 15ml conical tube for guinea pig homogenates.
 - Label PDA plates and snap top tubes with animal number, organ, and dilution (i.e. GP 1, lung, 0 or -1 or -2).
 - Make appropriate dilutions (1:10, 1:100, 1:1000) in 900µl of homogenization buffer and plate in duplicate (100µl of the same dilution on each ½ of a single PDA plate).

- Place parafilm sealed plates at 37°C for 24-36 hours and count colonies from the different dilutions. Calculate CFU/gram of organ.
- **Method B. Whirlbag technique**
 - Murine Lungs
 - In sterile fashion, dissect lungs (do not include heart) and weigh them in Petri dish. Place lungs in Whirlbag and add 5ml of sterile saline.
 - Close bag expressing as much air out as possible (leaving approximately 4cm of length from the point at which the bag is secured shut to the tissue to allow for “crushing” of the tissue.
 - Utilizing a large sharpie pen, roll it toward the bottom of the bag over the lung tissue being careful not to rupture the bag. Crush the tissue in this fashion with approximately 25 crush strokes. Attempt to take the parenchymal tissue off of the connective tissue as you crush the specimen.
 - Pour homogenates into snap top tubes and make dilutions.
 - Plate 0.1 ml of concentrated specimen in duplicate on PDA plates
 - ***NOTE: If the bag should rupture, then transfer the contents to a new bag rinsing the original with an extra 5ml of saline. Plating should be done with usual volume 1ml but calculations revised to reflect the change in volume.***
 - Murine Brain
 - In sterile fashion, dissect brain and weigh it in Petri dish. Place brain in Whirlbag and add 2ml of sterile saline.
 - Close bag expressing as much air out as possible (leaving approximately 4cm of length from the point at which the bag is secured shut to the tissue to allow for “crushing” of the tissue.
 - Utilizing a large sharpie pen, roll it toward the bottom of the bag over the brain tissue being careful not to rupture the bag. Crush the tissue in this fashion with approximately 10 crush strokes.
 - Pour into 5ml snap top tube, make serial dilutions and plate 0.100 ml in duplicate.
 - Plate the rest of the sample (1.8ml) on one plate. Rinse empty bag with an additional 2ml sterile saline and plate the additional 2ml onto a new plate. The total colony forming units will be the total amount of colonies from both plates.
- **Alternative Plating Method: Overlay Technique**
 - Dilute specimen 1:10 (1 ml specimen to 9 ml of Sterile saline) and plate 1ml of this dilution on a Sabouraud dextrose plate.
 - Overlay plate with SAB agar supplemented with 0.05mg/ml chloramphenicol and swirl 10-12x clockwise and 10-12x counter clockwise. Allow agar to solidify.
 - Incubate overnight at 37°C and count colonies early in the morning (due to possible overgrowth of fungus on the plates).

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- After counting, leave the plates an additional 24 hours at room temperature to finalize counts.
- Calculate the CFU per mouse/per gram of tissue.

7. Attachments

N/A

8. Deliverables

Two types of deliverables are possible. First, an accounting of colony forming units per gram of tissue examined is expected. Second, aliquots of these homogenates should be prepared and frozen (-20°C) for reference / experimental purposes.

9. References

Graybill JR, Kaster SR. Experimental murine aspergillosis. Comparison of amphotericin B and a new polyene antifungal drug, SCH 28191. *Am Rev Respir Dis.* 1984 Feb;129(2):292-5.

Patterson TF, George D, Ingersoll R, Minitier P, Andriole VT. Efficacy of SCH 39304 in treatment of experimental invasive aspergillosis. *Antimicrob Agents Chemother.* 1991 Oct;35(10):1985-8

10. History

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11. Examples of Deliverables

N/A