FILARIASIS RESEARCH REAGENT RESOURCE CENTER:

University of Wisconsin Oshkosh

 STANDARD OPERATING PROCEDURE

TITLE: Collection of *A. viteae* adults from Infected Hamsters

SOP NUMBER: A. vit 1

1. PURPOSE

The purpose of this document is to describe the procedure used when necropsying hamsters for the collection of *A. viteae* adults

2. MATERIALS

 2.1 CO2 euthanasia chamber and CO2

2.2 Medium sized sterile plastic Petri dishes

2.3 2 containers large enough to fit the carcass of the hamster plus dissection medium of choice. Styrofoam coffee cups work well.

2.4 Dissection medium: Hanks' Balanced Salt Solution (HBSS) or RPMI 1640 supplemented with penicillin (final concentration 100ug/ml) and streptomycin (final concentration 100ug/ml) (P/S). Prepare the medium under sterile conditions and warm to 37º C prior to use.

2.5 Sterile surgical scissors

2.6 2 pairs sterile fine tipped forceps, blunt dissection probes, and/or handmade dissection tools made from a 27 ga. hypodermic needle bent at a 45° angle and fixed to the end of a cotton tipped applicator.

2.7 Small hair clippers

2.8 Gloves

2.9 Dissection microscope

3. PROCEDURES

3.1 Prior to necropsy, euthanize the hamster(s) by exposure to CO2 per your approved IACUC protocol. Anesthetizing the hamster with a gas such as isoflurane prior to euthanasia is acceptable. Alternate: Use of other approved euthanasia method that will not negatively affect the worms.

3.2 Shave the entire body of the hamster(s).

3.3 Wearing gloves, carefully skin the euthanized animal with your fingers or using blunt dissection with scissors. Using sharp instruments can damage or destroy a large number of worms. Make incisions with scissors as close to the all four feet as possible and then use fingers to separate the skin from the carcass.  Most of the worms will be found on the inside of the skin, but some will be found in the connective tissues of the superficial muscles of the carcass after it is skinned. Do not allow the skin or carcass to dry as you skin the hamster or the worms could die; frequently moisten both with your dissection medium.

**Note:** If you use a razor blade or scissors to remove the skin from the entire body you will cut many of the worms.

3.4 After skinning, place the carcass in a container of your dissection medium so that the whole carcass is submerged.  Place the skin in a separate container of warm dissection medium.

Some worms will migrate out of the skin and carcass as they soak in the dissection medium.

**Note:** Using a large container to soak the skin and carcass will make recovery of these worms more difficult. A standard Styrofoam cup works well for this soaking.

3.5 Examine the skin and the carcass at least three times using a dissection scope, soaking both in dissection medium between examinations.  After examination of the skin and carcass, use the tips of forceps to disrupt fat and connective tissue. This will allow any worms that are in deeper tissue to migrate to more superficial tissue and be found during the next examination.

3.6 When a worm is found, carefully remove it. This can be done using the forceps, dissection probe, or handmade dissection tools. The worms are often under the fascia of the tissue and care must be used when removing them, especially if they are wanted alive (i.e., for culturing). The worms can be placed in a sterile Petri dish of HBSS or RPMI + P/S after removal.

3.7 Time to Completion: 3-5 hours per hamster