FILARIASIS RESEARCH REAGENT RESOURCE CENTER:

University of Wisconsin Oshkosh

STANDARD OPERATING PROCEDURE

TITLE: Culture of Infective Larvae or Adults of *Acanthocheilonema viteae*

SOP NUMBER: A. vit. 2

1. PURPOSE

This document describes the procedures used in culturing adult or infective larval *Acanthocheilonema viteae*.

1. MATERIALS
   1. Third-stage larvae (L3) or adult *A. viteae*
   2. Hanks' Balanced Salt Solution (HBSS) or RPMI 1640 supplemented with penicillin (final concentration 100 units/mL) and streptomycin (final concentration 100ug/mL) (P/S). Prepare the media under sterile conditions and warm to 37º C prior to use.
   3. NCTC-135 medium powder
   4. Sterile dH2O
   5. Isocove's Modified Dulbecco's Medium (IMDM)
   6. Non-heat treated fetal bovine serum
   7. Two sterile handmade dissection tools (27 gauge needles bent at a 45º angle and placed on the end of a cotton tip applicator). These ‘worm hooks’ can be used to move adult worms from container to container.
   8. p200 micropipette with sterile tips for moving L3 from container to container
   9. Container for culturing (i.e., tissue culture flask)
   10. Dissection microscope
   11. Tissue culture incubator capable of maintaining 37ºC with 5% CO2
2. PROCEDURES
   1. Prepare worm culture medium: In a sterile 500 or 1000 mL screw top bottle, aseptically combine the following under sterile conditions:

150 mL sterile water

150 mL IMDM

60 mL fetal bovine serum (FBS) (non-heat activated)

3 mL Penicillin/Streptomycin (P/S)

1.4 g NCTC-135 powder

This will result in a culture medium of 1:1 (v/v) NCTC-135 and IMDM supplemented with 20% FBS, 100 units penicillin/mL, and 100µg of streptomycin/mL.

* 1. Isolate *A. viteae* L3 (SOP Number A. vit. 6: **Collection of infective larvae of *Acanthocheilonema viteae* from Infected Ticks (*Ornithodoros tartakowskyi*)**) or adults (SOP Number A. vit. 1: **Collection of *Acanthocheilonema viteae* adults from Hamsters**)
  2. Rinse worms three times in 37ºC HBSS or RPMI 1640 + P/S

**Rinsing L3:** Place the dish containing L3 on the dissection microscope at an angle so all worms are in the front 1/3 of the dish. Using the pipette, remove contaminants and as much of the medium as possible. Add fresh medium to the dish and wait for the worms to settle. Repeat at least three times as needed.

**Rinsing adults:** Use the worm hooks to gently transfer adults into a dish of fresh HBSS or RPMI + P/S. Allow worms to sit in dish ~1 min. Repeat at least three times. When using the worm hooks, be careful not to poke the worms with the sharp points, but rather use the hooks to scoop the worms.

* 1. Transfer worms to culture container containing 37ºC worm culture medium

**Note:** It is important to use warm medium, as cold or room temperature medium might shock the worms.

* 1. Culture worms in incubator at 37ºC with 5% CO2
  2. Culture medium must be removed and replaced with fresh medium if worms are cultured for longer than 12 to 24 hours. Adult females have been successfully cultured for extended periods of time in a ratio of one worm to 5 mL of culture medium, with medium changes occurring every two to three days (Maki, J. and Weinstein, P. P., 1989. *Dipetalonema viteae*: Survival of adult females and microfilarial release in both a chemically defined and serum-supplemented medium. J. Parasit., 75(6), 953-957).
  3. Time to Completion: 1-2 hours