FILARIASIS RESEARCH REAGENT RESOURCE CENTER:

University of Wisconsin Oshkosh

STANDARD OPERATING PROCEDURE

TITLE: Tick Rearing Techniques using Commercially Obtained Blood as Food Source

SOP NUMBER: A. vit. 10

1. PURPOSE

The purpose of this document is to detail the methods used in rearing *Ornithodoros tartakowskyi* on commercially obtained blood.

1. MATERIALS
	1. Small plastic vials with snap top (i.e., 7 Dram)
	2. Parafilm® “M” laboratory film
	3. Small Petri dish (35 mm x 10 mm)
	4. 100 µm nylon or metal mesh
	5. Autoclaved sand, Plaster of Paris, filter paper, or other absorbent material
	6. Forceps
	7. Plastic shoebox
	8. Double-sided tape
	9. Camel hair paintbrush
	10. Commercially available aseptically collected blood (i.e., rabbit or cow)
	11. Heat block or other heat source capable of maintaining 37-39 C.
	12. Laboratory rocker
	13. Insectary or incubator capable of maintaining 26.7 ± 1°C and 80 ± 5% relative humidity
2. PROCEDURE
	1. Housing the ticks: Ticks are housed in small (7 Dram) plastic vials with snap tops. The snap top of the vial has a 13 mm diameter hole drilled through it for gas exchange. The hole is covered with 100 µm nylon screen secured to the top with E6000 multi-purpose adhesive (Eclectic Products, Pineville, LA). The bottom of the vial is covered with approximately 12-25 mm of autoclaved sand. Other substrates (i.e., filter paper) have been used. The snap top is secured onto the vial with Parafilm®, which also serves as a barrier for the escape of larvae.
	2. Monthly Feeding of the Ticks: Commercially purchased defibrinated rabbit and cow blood (Hemostat, Dixon, CA) have produced good results when membrane feeding soft ticks. Construction of the membrane feeder is as follows.

Enough blood (Hemostat; Dixon, CA) is dispensed into the lid of a small Petri dish to entirely fill it (approx. 4.5 mL). A piece of Parafilm® “M” is stretched as thinly as possible and placed over the blood-filled dish, ensuring no air bubbles are between the Parafilm® and the blood. The Parafilm® is then wrapped around the bottom of the dish, sealing the blood in the dish. This membrane feeder is warmed to 37ºC.

Ticks to be fed are transferred to an empty container that has a mouth smaller than the diameter of the membrane feeder. This container will sit on top of the blood-filled dish, so it must be of minimal weight to ensure it doesn’t tear through the Parafilm®. Another 7 Dram plastic vial works well for this application; the bottom of a small Petri dish can also be used. Depending on the size/life stage of the ticks, approximately 20-50 ticks can be fed on each membrane feeder.

A heat block set to 37-39ºC is placed onto a laboratory rocker. A Pyrex® dish approximately 5 cm deep is set onto the heat block, and its inner walls are lined with petroleum jelly or double-sided tape to act as a containment system should any ticks escape from the membrane feeders. The warmed membrane feeders are placed into the dish, and one plastic vial containing ticks is inverted onto each feeder. Direct light should be avoided during feeding. The rocker is turned on medium speed to continuously mix the blood. The ticks are allowed to fully engorge and are then carefully removed from the feeder with a camel hair paintbrush. They are then returned to their housing vials lined with sand or another absorbent material. This material serves to collect the coxal fluid released by the ticks after they feed. The top is immediately placed onto each vial and secured into place with Parafilm®. The vials are appropriately labeled and returned to the incubator.

1. CLIMATIC CONDITIONS OF INSECTARY OR INCUBATOR

4.1 The temperature in the insectary or incubator should be maintained at 26.7 ± 1°C, and the relative humidity should be maintained at 80 ± 5%. Incubators set at 29°C and 100% relative humidity have also been used.