Preparation of mosquito diluent

**Materials:**

1. Biosafety cabinet

2. Water Bath (37 C)

3. Glass bottle (sterile), 1 liter

4. Graduated cylinder (sterile), 1 liter

5. Assorted pipette-aids, serological pipettes, and 1000 uL pipette tips

6. 70% ETOH

7. Pipette boat with small amount of water or non-bleach disinfectant

8. Tape and sharpie for labeling

**Reagents:**

1. Phosphate Buffered Saline (PBS) 1X - 779 mL

2. Fetal Bovine Serum (FBS) - 200 mL (stored in refrigerator short term or freezer long term) **Note:** **If using a new bottle, make sure to heat-inactivate the FBS. Note also that Equalfetal is preferred for this application (cheaper).**

3. Penicillin Streptomycin (P/S) 100X - 10 mL (stored frozen)

4. Fungizone (Amphotericin B), 250ug/mL - 10 mL (stored frozen)

5. Gentamicin, 50 mg/mL - 1 mL (stored in refrigerator)

**Precautions:**

Standard BSL-2 PPE. Prepare inside biosafety cabinet.

**Protocol:**

1. \_\_\_\_\_ Thaw P/S and Fungizone (and heat-inactivated FBS, if stored in freezer) in water bath.

2. \_\_\_\_\_ Set up biosafety cabinet with all reagents and required materials. Remember to wipe down each reagent and material with 70% ETOH before placing in hood.

3. \_\_\_\_\_ Measure 779 mL of PBS, 1X, into 1 liter glass bottle.

4. \_\_\_\_\_ Add 200 mL of FBS.

5. \_\_\_\_\_ Add 10 mL of Penicillin Streptomycin, 100X.

6. \_\_\_\_\_ Add 10 mL of Fungizone (Amphotericin B), 250ug/mL.

7. \_\_\_\_\_ Add 1 mL of Gentamicin, 50 mg/mL.

8. \_\_\_\_\_ Clean biosafety cabinet and store/dispose all reagents.

9. \_\_\_\_\_ Label bottle as “mosquito diluent” and include today’s date. Store in refrigerator (4 C).

**Purpose:** Mosquito diluent is used as an all-purpose solvent in which to store and/or homogenize mosquito tissue.

**Purchasing information:**

1. EqualFETAL (FBS): Atlas Biologicals, 500 mL (EF-0500-A) **We usually purchase this from PMF.**

2. Penicillin Streptomycin (P/S) 100X: Corning, 100 mL (30-002-CI)

3. Fungizone (Amphotericin B), 250ug/mL: GE Healthcare Life Sciences , 50 mL (SV30078.01)

4. Gentamycin Sulfate, 50 mg/mL: Lab Scientific, 5 g (2304)