Drosophila infection protocol using septic injury

We use *Pseudomonas aeruginosa* ***PA14***, which is a virulent natural isolate requiring BSL-2.

Make sure you clean all tools and work area with 70% ethanol before and after working.
(A dedicated workspace for BSL-2 fly work is best.)

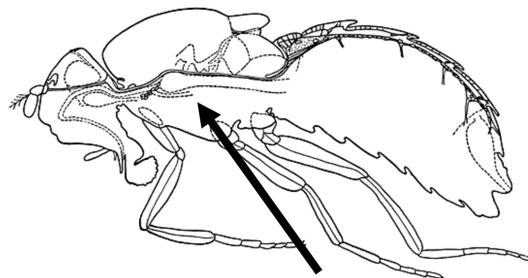
1. Grow a 3 ml overnight culture in Nutrient Broth. Shake at 37°C.

2. Use an inoculum with an A_{600}= 0.005 (measured in 200 µl on our 96 well plate reader- note that this absorbance will differ depending on the reader). This corresponds to ~ 3.0×10^7 CFU/ml and can be made by diluting the overnight culture 6 or 7 µl : 1500 µl in PBS.

3. Tighten a sharpened tungsten needle (Fine Science Tools cat #10130-05 = 0.125mm diameter) in a pin holder and be careful not to bend it or ram it into anything. If you do, sharpen the needle or get a new one.

4. Gas adult flies with CO_2 for inoculation. 20 flies/vial (or biological replicate) is a good number. Control for age, diet, genotype, and sex. Only gas the number of flies you can stab in 10 minutes or so.

5. Dip the needle into the inoculum and drag up against the tube rim to prevent a large droplet. Pierce the thorax of the fly just underneath the wing on either flank (arrow). Move fly to front of pad to separate from uninoculated flies.



Miller (1950)

- Just prick the animal; don't impale it. You won't be able see the wound, but should feel the needle breach the cuticle.
- A good control is to dip a clean needle into PBS without *PA14*. This should not kill any flies.

6. Count surviving flies daily for one week. Expect some flies to die within 2-3 days after injury in all genotypes. Flies should be counted around the same time each day.

7. Use Kaplan-Meier estimation curve and a Mantel-Cox or log-rank test to quantify survival.