







Excreta Quantification Protocol

1. Preparation of Vial Plugs for Quantification
 - a. Set 20 flies in vial containing blue food
 - b. Push plug to one inch from top of food.
 - i. ensure that the surface of the plug is flat allowing for equal access to all parts of the plug.
 - c. Allow flies to excrete for 48 hours (2 days)
 - d. After the 48 hours, remove plugs from the vials.
 - e. After removing the plugs, cut each plug 1 cm from the bottom to allow for clear imaging in ChemiDoc.
2. Imaging the Excreta.
 - a. Turn on the ChemiDoc Touch Imaging System
 - b. Insert the Chemi/UV/Stain-Free Tray
 - c. Place the cut portion of the plug containing the excreta centered on the tray.
 - d. Settings for the ChemiDoc
 - i. Image size: Small
 - ii. Width: 9.5cm
 - iii. Length: 7.6 cm
 - iv. Open Application  Select Blots  Colorimetric
 - v. Exposure: Intense Bands
 - e. Image the plug using the Auto-exposure setting on the ChemiDoc
 - f. Save the images captured from the gallery onto a USB drive.
3. Exporting the photos from .scn to .tif
 - a. Open ImageLab on computer
 - b. Click Open Photo and select the photo to export.
 - c. Once the image is open, Click File  Export  Export for Publication
 - d. In the pop-up window, select Entire Image and 600 dpi, then Click Export.
 - e. Click Yes to the pop-up after exporting
4. Analyzing/Quantifying Excreta
 - a. Open ImageJ on computer
 - b. Click File  New Image and select the image to be quantified
 - c. Under the Image menu, select Type, then 16-bit. Image should become black and white.
 - d. Now Image – Adjust - then Threshold. The threshold should be set between 140-155 on the sliding bar. (Use judgment to set threshold in order to obtain the most accurate representation of the wick.)
 - e. Then click Apply
 - f. To Quantify Excreta, click on the Analyze Menu  Analyze Particles
 - g. In the window that appears,
 - i. Size: 0-infinity
 - ii. Circularity: 0.2-1.00
 - iii. Show: Outlines
 - h. There will be a third window, “Drawing” which outlines and counts your droplets. **Check this image to make sure it looks reasonable to you, then close*

*it** Under the Image menu, you can scale the drawing to zoom in and look at each outlined droplet if you want. You don't need to select all excreta; it's more important to make sure that what's being selected is representative in terms of size and shape.

- i. A results window will pop-up. Select the results and paste into Excel.
- j. Keep columns 1 and 2 (Particle and Area) as these are the necessary parts of the data. (Columns 3,4,5 can be deleted)
- k. Make a separate Worksheet for each Male and Female and infected and not infected.
- l. Transfer the average area and number of particles for each into graph pad