

## How to make cholesterol Andersen lab, winter 2015

*C. elegans* requires cholesterol to progress through the four larval stages. Previously, we found that some filters were removing cholesterol from K medium and S medium. Most worms arrested in larval development. For this reason, we add cholesterol after filtration or autoclaving media for NGMA plates.

### For NGMA plates:

Use the unfiltered but sterile 5 mg/mL cholesterol in ethanol.

To make this cholesterol,

1. Take an autoclaved sterile 250 mL bottle
2. Add 1 g of powdered cholesterol to the bottle
3. Add 200 mL of 100% ethanol to the bottle
4. Cap and shake to resuspend
5. Store at room temperature on the reagents shelf for up to six months

### For HTA plates:

Use the filtered sterile 5 mg/mL cholesterol in ethanol.

To make this cholesterol,

1. Take a 50 mL conical tube
2. Add 125 mg of powdered cholesterol to the tube
3. Add 25 mL of 100% ethanol to the tube
4. Cap and shake to resuspend
5. Aliquot using a 25 mL syringe fitted with a filter (Millipore Millex-LG cat # SLLG025SS)
6. Dispense 1 mL of 5 mg/mL cholesterol through the filter into autoclaved sterile 1.7 mL microfuge tubes
7. Cap and store at room temperature for up to six months
8. 125  $\mu$ L of 5 mg/mL cholesterol in ethanol should be added to 500 mL of K medium.