C. elegans Staph. aureus killing assay (SKA) Protocol

by Erik Andersen June 10, 2011

Assay timing (each step is described below)

Day 1: Streak out NCTC8325 from frozen stock onto tryptic soy agar (TSA) plates
Day 2: Chunk strains for pathogenesis assay
Day 3: Pick five L4s from chunked strains to new plates, pour *aureus* plates, inoculate NCTC8325 to brain heart infusion (BHI) broth (at 2 PM)
Day 4: Spot NCTC8325 to center of *aureus* plate and put plates at 37ºC (at 2 PM)
Day 5: Take plates out of 37ºC (at 2 PM)
Day 6: Setup 30-50 L4s to each *aureus* plate
Days 7-11: Score number of dead animals, and for the last time point, score the number alive too

Plate preparation

1. Prepare *aureus* plate medium and autoclave

	250 mL	500 mL	1 L
BHI agar (Difco)	13 g	26 g	52 g
BactoAgar	1.63 g	3.25 g	6.5 g
Sterile water	250 mL	500 mL	1000 mL

Autoclave, allow to cool to 55 ºC and add the following

Nalidixic acid (10 mg/mL)	250 μL	500 μL	1000 μL

2. Pour 4 mL per 35 X 10 mm plate (Falcon #351008). Flame tops of plates to remove bubbles, if needed.
3. Inoculate *Staph. aureus* (strain NCTC8325) from a freshly streaked TSA plate.
4. Grow at 37ºC overnight for exactly 24 hours.
5. Spot 10 μL of PA14 onto each plate and put the plates at 37ºC for 24 hours. Be careful not to scratch or puncture the tops of the plates.
6. Remove the plates from 37ºC and keep at room temp for exactly 24 hours. Use plates immediately.

Killing Assay

1. Put 30-40 L4 hermaphrodites onto each *aureus* plate.
2. Put the plates at 25ºC.
3. Starting 24 hours later, score the number of dead worms.
4. Pick off the dead worms. Dead worms do not move when prodded and sometimes appear clear.
5. Score every eight hours, until 96 hours. Then, score every 16 hours. For the 128-hour time point, score the number of dead worms and the number of surviving worms.
6. Plot the percent alive at each time point for each genotype.