



INTRANASAL COLONIZATION WITH *STREPTOCOCCUS PNEUMONIAE*

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BACKGROUND

- This protocol describes how to prepare the bacterium *Streptococcus pneumoniae* for intranasal inoculation of mice and how the mice should be monitored afterwards.
- We have performed these inoculations with the *S. pneumoniae* strains P1121 (serotype 23F), TIGR4 (serotype 4), and P1547 (serotype 6A).

NOTES

- Mice should be housed in a BSL Lvl 2 room and should have been allowed to rest in the room for one week previous to inoculation to remove the effects of moving stress
- Mice must weigh at least 20g to avoid unnecessary deaths
- The average C57Bl/6 mouse will clear the bacteria by 21 days post-inoculation. We usually perform time courses of 1, 3, 7, 14, and 21 days p.i.

EQUIPMENT AND MATERIALS

- Equipment
 - o Centrifuge for 1.5mL tubes
 - o Pipet gun and 5mL pipets
 - o 12mL polystyrene tubes
 - o Spectrophotometer
 - o Ear notcher or sharp scissors
 - o Mouse immobilizer (50mL conical tube with narrowed end cut off)
 - o Scale
 - o Petri dish w/ 15mL TSA + 5% defibrinated sheep's blood + 10µg/mL neomycin
- Solutions
 - o Glycerol stock of *S. pneumoniae* (85% bacteria in TSB, 15% Glycerol)
 - o 1X TSB, autoclaved
 - o 1X PBS, sterile

PROTOCOL

A. Preparing *S. pneumoniae* inoculum

1. In BSC, thaw *S. pneumoniae* stock as rapidly as possible and add whole stock to 5mL of TSB in 12mL polystyrene tube.

2. Incubate tube at 37°C, 5% CO₂ with the tube loosely capped (to allow CO₂ into tube) until the absorbance reading is between 0.45 and 0.55 (with reference recorded with pure TSB). This corresponds with the logarithmic growth phase of the culture. It usually takes between 2 and 3 hours for the bacteria to grow to this concentration.
3. Mix tube by inverting three times and then remove 1mL of the bacteria in TSB to a 1.5mL tube and centrifuge at 15000 rpm for 1min.
4. Remove TSB from pellet and resuspend in 100 µL PBS.
 - i. There should now be ~1x10⁸ CFUs of bacteria in the 100 µL. You will be inoculating each mouse with 10µL (or 1x10⁷ CFUs).
 - ii. To ensure that this is the case, create 1:10 serial dilutions from 10⁻¹ to 10⁻⁹ in PBS and plate dilutions 10⁻⁴ to 10⁻⁹ on the sheep's blood agar plate and incubate the plate upside down overnight. This plate will tell you the exact concentration in the inoculum so you can account for any differences between experiments.
5. Keep the inoculum on ice until performing the inoculation.

B. Inoculating mice

1. For each mouse:
 - i. Ear notch or clip the ears in a way that you will be able to tell all of the mice apart throughout the colonization.
 - ii. While still holding the mouse by the scruff of its neck, slip the immobilizer over its head and allow it to run upwards toward the narrowed end. The hole will be large enough for the mouse to stick its nose out, but not its mouth. Keep the mouse at the end of the tube by pushing its rear end with your thumb.
 - iii. Using a 10mL pipet, inoculate the mouse intranasally with 10µL of the inoculum. Attempt to get an equal amount into each nostril. You will notice bubbles coming from the nostrils. This is normal and is a good sign that the mouse is breathing the inoculum appropriately.
 - iv. Weigh the mouse to get a measurement of its starting weight.

C. Monitoring mice

1. Each mouse should be weighed every day during the colonization time course. If a mouse has lost 10% of its original weight it should be euthanized.
2. Also check the mice for signs of serious illness. Decreased body condition, lack of grooming, solitary positions in the cage, and trembling are common contraindications.

CLEAN-UP INSTRUCTIONS

1. Unused TSB growth medium and inoculum should be disposed of in 10% bleach for 30min before washing down sink.
2. Mice should be euthanized and discarded according to your institution's animal research ethics protocols.

REFERENCE:

Dorrington MG, Roche AM, Chauvin SE, Tu Z, Mossman KL, Weiser JN, Bowdish DM. MARCO is required for TLR2- and Nod2-mediated responses to *Streptococcus pneumoniae* and clearance of pneumococcal colonization in the murine nasopharynx. J Immunol. 2013 Jan 1;190(1):250-8. doi: 10.4049/jimmunol.1202113.

<http://www.jimmunol.org/content/190/1/250.long>