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## Intranasal Infection of Mice with H1N1 Virus

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**Protocol status:** Working

**We use this protocol and it's working.**

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## Abstract

**Objective:** *This SOP addresses the infection of adult mice with **mouse-adapted influenza H1N1 A/FM/1/1947 strain influenza** via the intranasal route*

Adult mice (as young as 3 weeks of age; typical age for our studies is 4-6 weeks) are inoculated on their nose pad with 2000 plaque forming units, PFUs of virus. Upon inhalation, the virus reaches alveolar space, leading to fulminant pneumonia associated with macrophage and neutrophil recruitment into the interstitium and alveolar sacs associated with cytokine release. At LD50, 50% of wild-type mice (CD1; black6 strains) succumb to the infection within 7-10 days. The other 50% recover. See dosing note for sex-differences observed in C57Bl/6J below.

## Intranasal Infection with H1N1: Survival and Tissue Collection

- 1 Thaw one aliquot of H1N1 (stored at -80 deg). Keep on ice.
- 2 In a CL2 biosafety cabinet dilute aliquoted stock virus in phosphate buffered saline (PBS) accordingly and vortex (approximately 5 sec) after each dilution. Keep virus dilution on ice and discard stock aliquot after use.
- 3 Prior to infection, anesthetize mice with isoflurane (3%) while maintaining oxygen at a flow rate of 1 L/min.
- 4
  1. Once anesthetized, remove mouse and scruff with one hand, tilting the head of the animal back slightly. Administer 50 µl of diluted virus between both nostrils of the animal using a P100/200 micropipette.
  - a. If infecting both female and male mice, infect all male mice first.
  - b. Note: dosing of wild-type C57BL/6J mice (> 4 weeks) sees a strong sex effect: 2000 PFU. Wild-type male mice have a higher survival rate than wild-type female mice at this dose.
- 5 If taking weight measurements, place the animal on the scale before returning it to its home cage to recover from the anesthetic.
- 6
  1. Mice should be monitored once daily until 5 days post-infection (dpi), and at least twice daily thereafter (once in the early morning and once in the late afternoon). Mice show weight decline by approx. 4 dpi, and visible signs of sickness approx. 5 dpi. Mice will succumb to illness typically between 6-12 dpi.
  - a. Humane endpoint: moribund state and/or respiratory distress with 25% body weight loss or loss of 30% bodyweight (irrespective of respiratory function).
  - b. If the readout is survival and if a mouse is found dead during a morning check, mark the time-to-death as the day prior.

Table 1: Animal disease severity scores over course of infection

A	B
Disease Score	Disease Phenotype
1	Percolated fur but no detectable behavioral differences compared to untreated mice
2	Percolated fur and a huddle reflex but are still active



A	B
3	Less active and are relatively passive when handled
4	Inactive mice that exhibit only limited response when handled
5	Moribund

- c. The survival experiment is stopped after 14 dpi. Survivors may be aged to monitor post-infection outcomes dependent on study goals.
- d. Weight can also be used as a readout – weights are taken at inoculation, and then daily from 4dpi until 14dpi when the experiment is finished.