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Environmental Conditioning and Aerosol Infection of Mice

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[Abstract] Influenza infection models in mice are widely used to study flu-mediated immune responses and pathology. However, most laboratory mice are housed at 20 °C and 50% relative humidity (RH). To better recapitulate influenza epidemics and immune responses during winter seasons, mice were housed at 20 °C under different humidity conditions, 10-20% or 50% RH. Here, we describe a protocol for using aerosolized droplets to infect mice with influenza under different environmental conditions. Using this method enables influenza infection studies performed under more physiologically relevant conditions which better mimics human viral exposure.

Keywords: Influenza, Humidity, Environment, Mouse, Infection, Aerosol challenge

[Background] Influenza A viruses (IAVs) are one of the major causes of seasonal respiratory infections in the world, resulting in half million deaths annually (Johnson *et al.*, 2014). IAV outbreaks occur during the winter season in temperate regions, peaking between November and March in the Northern Hemisphere and between May and September in the Southern Hemisphere (Tamerius *et al.*, 2013; Alonso *et al.*, 2015). Virological research in guinea pigs shows that low temperature and humidity enables better aerosol transmission of influenza virus (Lowen *et al.*, 2007). In addition, epidemiological studies demonstrate that a drop in absolute humidity correlates closely with the rise in influenza-related deaths in humans (Shaman *et al.*, 2010). It is worth noting that in tropical and subtropical climate regions, which are wet and warm, the virus can thrive on surfaces of objects and cause fomite transmission (Shek and Lee, 2003, Moura *et al.*, 2009).

Laboratory mice are generally maintained at macroenvironmental temperature and relative humidity ranges of 64 to 79 °F (17.8 to 26.1 °C) and 30% to 70%, respectively (Clark *et al.*, 1997). However, these environmental conditions do not reflect our home, office or school in the winter season. Moreover, intranasal inoculation of virus using pipette is the most standard route used to study influenza infection in mice, which significantly affects the effective humidity in the upper respiratory system, as a liquid solution is being delivered. On the contrary, influenza infections between humans occur by either aerosol droplets or through contaminated surface contact (Lakdawala and Subbarao, 2012). Therefore, to better mimic human infection, we utilized a protocol to infect mice with influenza virus through aerosol exposure after housing the animals in dry air conditions, similar to house found in the winter months. In this protocol, we describe the use of an environmental chamber to mimic indoor conditions found during the winter seasons, namely, 20 °C and 10-20% RH, along with the use of a nebulization system.

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Materials and Reagents

1. 15 ml conical tube (BD Falcon, catalog number: 352096)
2. Pipette tips (Fisherbrand, catalog numbers: 02-681-165, 02-681-147 or equivalent)
3. C57BL/6 mice carrying a functional *Mx1* allele (Horisberger *et al.*, 1983)
   Note: Most laboratory mouse strains are highly susceptible to influenza infection due to a defective Mx1 gene which is an important interferon stimulated gene to combat influenza (Iwasaki, 2016). Therefore, we prefer to use Mx1 congenic B6 mice to study host responses to influenza viruses.
4. Highly virulent A/PR/8/34 (H1N1; hvPR8) (Grimm *et al.*, 2007)
   Previously, the hvPR8 strain was generated by serial lung passages in Mx1 mice (Grimm *et al.*, 2007).
5. 1x PBS (Sigma, catalog number: D8537)

Equipment

1. Environmental chamber (Caron, model: 7000-10)
2. Nebulizer (Allied Healthcare Products, Schuco, model: S5000)
3. Mouse pie cage (Braintree Scientific, model: MPC-3 AERO)
4. Weight scaler (OHAUS, Scout Pro, model: SP202)
5. Pipettes (DENVILLE, P1000, P200)

Software

1. GraphPad Prism 7.0 (GraphPad Software; https://www.graphpad.com/)

Procedure

A. Pre-conditioning of mice in environmental chambers
   1. Transfer the mice to biosafety level 2 (BSL2) adaptive feeding for 3-7 days. Divide the mice randomly for housing under different humidity conditions at 20 °C, 5 mice for each group.
   2. House the mice in cages placed in chambers set to either 20% or 50% RH for 5-7 days.

B. Influenza infection via aerosol exposure (to be carried out under BSL2 condition)
   1. Dilute hvPR8 2 x 10^5 pfu/ml in PBS and prepare 4 ml of diluted virus solution.
      Note: This virus concentration is the lethal dose 50 (LD_{50}) for this study. If you change influenza virus or mouse strain, you should determine LD_{50} for your conditions before the experiment following this protocol. General protocol of measurement of LD_{50} is reported in Zhao et al. (2018).
   2. Put mice (up to 12 mice simultaneously) in the pie cage.

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3. Set nebulizer and pour 4 ml of diluted virus solution (3 ml is exact volume that would be will be used during 15 min of aerosol delivery).
4. Turn on the switch for the nebulizer and expose for 15 min (Figure 1 and Video 1).

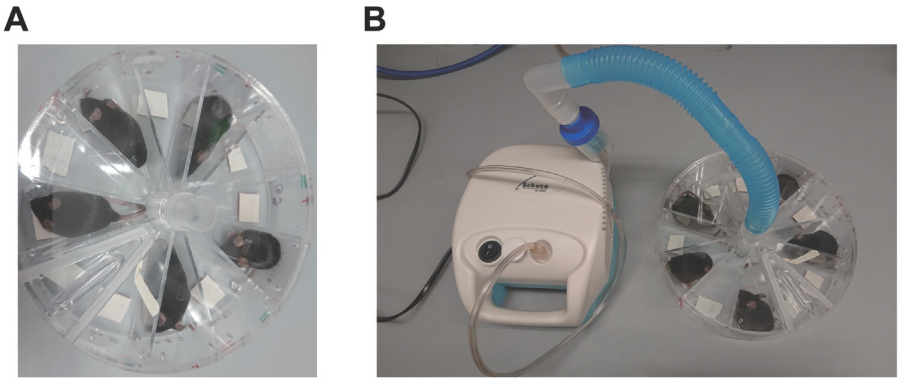

Figure 1. Representation of how to infect influenza virus in mice using a nebulizer. A. Mice are diagonally put into the pie cage if the number of mice is less than 12. B. Image of connected pie cage and nebulizer.

Video 1. Infection via aerosol administration

5. Put mice back in the cage and house them at respective environmental conditions.
6. Monitor the weight and death of the mice for 2 weeks (Figure 2 and Video 2). Euthanize the animals according to the guidelines of the institutional animal care and use committee.

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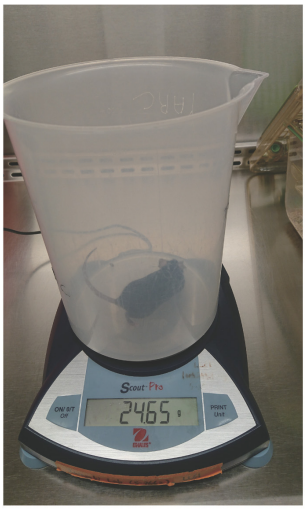
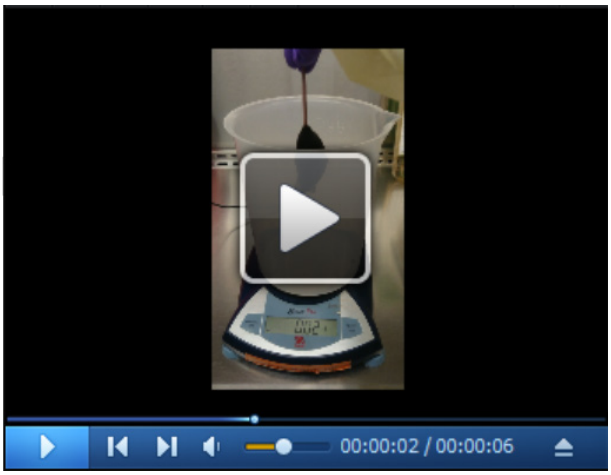


Figure 2. Measuring of body weight Mouse put on the weight scaler to measure the body weight



Video 2. Measure the body weight

Data analysis

Survival curve and weight loss are analyzed using GraphPad Prism (Figure 3).

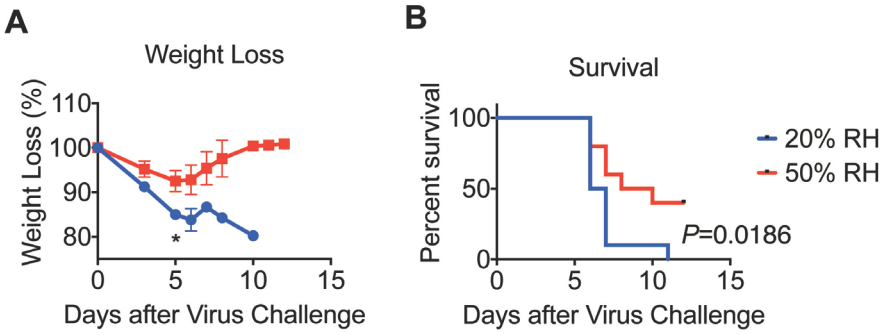


Figure 3. Low relative humidity leads to more severe disease. (A) Weight and (B) survival were

monitored for 11 days. Data are representative of five experiments and means ± SEM *$P < 0.05$; one-way ANOVA; log-rank (Mantel-Cox) (Adapted from Kudo *et al.*, 2019).

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Competing interests

The authors declare no competing interests.

Ethics

All procedures used in this study complied with federal and institutional policies of the Yale Animal Care and Use Committee (protocol #10365).

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