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Infectivity Of Airborne Influenza A Virus: The Effect Of Matrix And Air Composition

Recurrent epidemics and pandemics of respiratory diseases have long had a strong impact on societies. Studying the effect of environmental factors on the conservation of airborne virus infectivity, in addition to helping to better understand the inactivation properties of viruses, could lead to easily implemented intervention strategies.

In this study, we experimentally investigate the infectivity of influenza A virus (IAV) contained in an aerosol by mimicking as closely as possible a host-to-host transmission scenario, using the novel LAPI BREATH facility described in Motos et al. (2024). The virus-containing aerosol is produced through bubble-bursting of a virus-containing medium simulating aerosol generation by normal breathing through reopening of small airways in the deep lungs. In order to respect the natural dynamics of the aerosol and its settling, particles are introduced and exposed to controlled conditions for up to 5 hours in a large polytetrafluoroethylene chamber (~1.6 m3) in which no fan or rotation is used to resuspend the particles. Virus sampling is based on particle condensational growth followed by liquid-to-liquid collection, similar to the deposition process occurring in our lungs upon inhalation.

We first study the effects of matrix composition on IAV infectivity, then explore that of air composition. We test the effect of adding protein (sucrose) to a saline matrix (phosphate-buffered saline, PBS), before moving to a synthetic lung fluid (SLF). We then expose IAV to various concentrations of CO2 and HNO3. We choose to express our results in terms of 99% inactivation time (*t99*), i.e., the time required for a 2-log inactivation of the initial airborne virus population.

We show that addition of sucrose to the PBS protects IAV at 55% relative humidity (RH; average *t99* ~40 min versus ~18 min in PBS) but not at 25% and 85%. RH. Interestingly, SLF protects IAV to the same degree at 55% RH but causes faster inactivation than plain PBS at low and high RH. An increase in CO2 concentration from ~440 ppm to ~4000 ppm has no effect on IAV infectivity at 25% and 85% RH, but causes an increase in *t99* at 55% RH (from 1h 23 min to 45 min). Exposure to HNO3 was shown to be highly effective in inactivating IAV. At 55% RH, concentrations as low as 5 and 30 ppb were sufficient to decrease *t99* from approximately 45 min to 25 min (1.8-fold) and 6 min (8-fold), respectively. At low RH, higher concentrations are required to achieve a similar level of inactivation, probably due to particle efflorescence or to the small amount of water in particles, mitigating the effect of pH.

In a dry indoor environment (~25% RH), the risk of IAV transmission expressed by *t99* is higher by factors of 3 to 5 than in more humid conditions (~55% RH), according to our chamber measurements. While there is a lack of medical knowledge about the health risks of exposure to acidic vapours at concentrations more than two orders of magnitude below occupational exposure limits, our results suggest that indoor acidification at such concentrations could be as effective as humidification in reducing the transmission of IAV. Importantly, the combination of humidification and acidification appears to be an extremely effective method. To the best of our knowledge, this is the first time that airborne IAV are exposed to artificially modified air composition, except for RH. Our results support previous modelling work highlighting aerosol acidity as an important but overlooked parameter governing airborne IAV inactivation (Luo et al., 2022).

Luo, B. et al.: Expiratory Aerosol pH: The Overlooked Driver of Airborne Virus Inactivation, Environ. Sci. Technol., 57, 486–497.

Motos, G. et al.: Dependence of aerosol-borne influenza A virus infectivity on relative humidity and aerosol composition, Front. Microbiol., 15.

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