Assessing the airborne stability of influenza A virus

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1. Introduction

- Influenza A virus (IAV) is a **major respiratory virus** which in the last 105 years has caused four pandemics and is responsible for annual influenza epidemics in the U.K. [1].
- Evidence that **airborne transmission** is a prominent transmission route for IAV (**Figure 2**) [2].
- Seasonality of IAV infection potentially linked to seasonal fluctuations in climate [3].
- Reported levels of IAV inactivation under different atmospheric conditions are inconsistent between studies. [4]
- Inconsistencies hinder effective public health interventions for virus outbreaks.

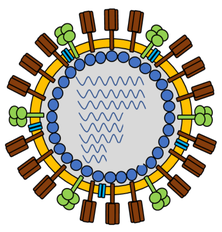

Figure 1: Diagram of the structure of IAV.

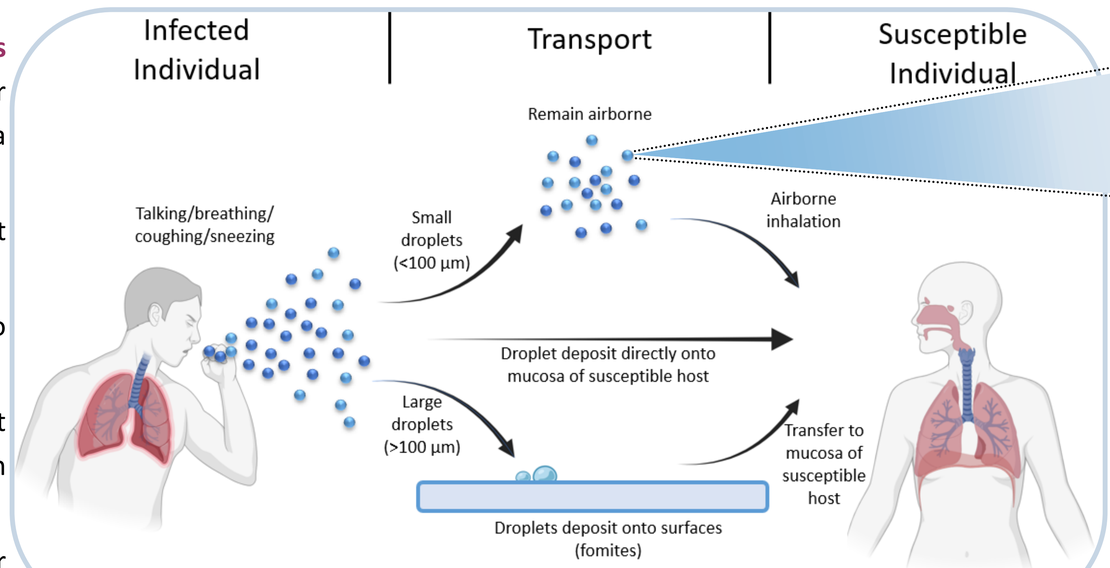

Figure 2: Possible transmission routes for IAV

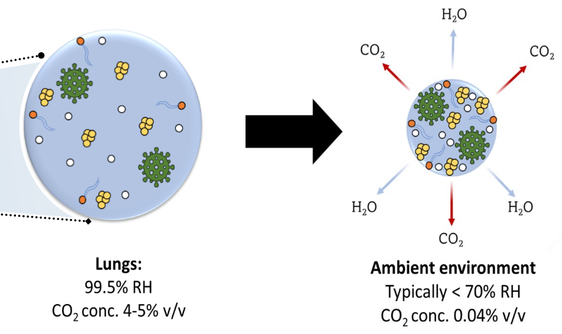

Figure 3: Equilibration of respiratory droplet with ambient environment. Depicts flux of CO_2 and water from respiratory droplet.

- Physicochemical changes in respired aerosol droplets may result in harmful microenvironments in which suspended pathogens must remain viable to transmit (**Figure 3**).
- Potential mechanisms of IAV inactivation include increased **solute concentrations** (e.g. salts or proteins), droplet **phase changes** and aerosol **pH changes** [5].

2. Objectives

1. To elucidate the influence of relative humidity (RH), temperature, and gas phase composition on IAV airborne viability
2. Identify the **mechanisms of IAV inactivation** in the aerosol phase

3. Methodology

Controlled Electrodynamic Levitation and Extraction of a Bioaerosol onto a Substrate (CELEBS)

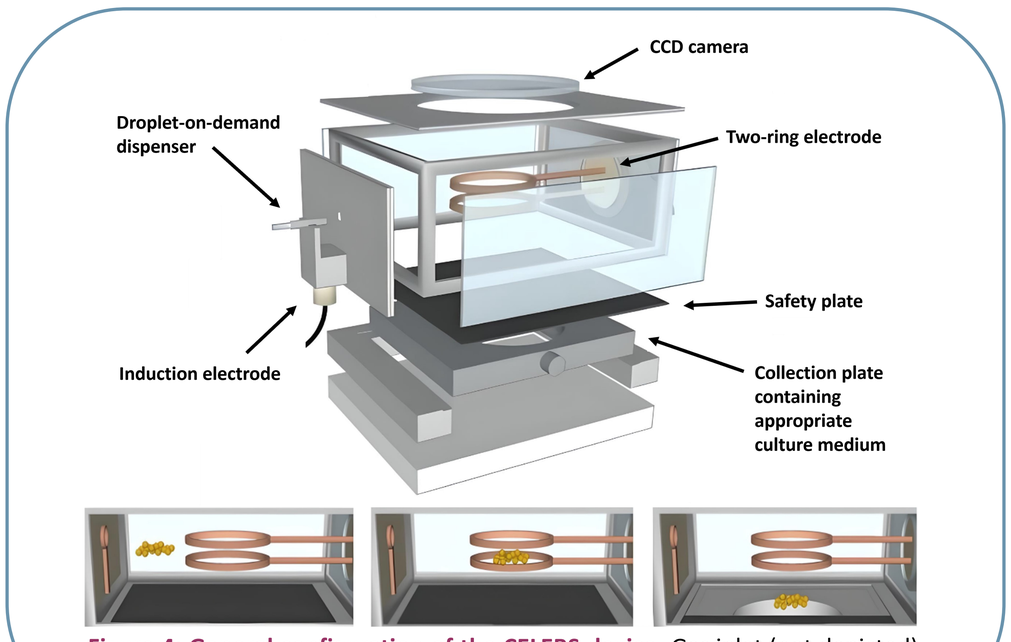

Figure 4: General configuration of the CELEBS device. Gas inlet (not depicted) allows control over environmental conditions experienced by trapped droplets.

- Allows the influence of environmental conditions (i.e. temperature, RH, and gas airflow composition) on virus viability to be investigated.
- Simulates the aerosol phase by levitating droplets in an electromagnetic field produced by two concentric ring electrodes (**Figure 4-5**)
- Atmospheric conditions experienced by the pathogen are controlled by a laminar airflow which is passed over levitated droplets
- After exposure to a desired atmospheric condition droplets are deposited into cell tissue growth media and the impact on virus viability is assessed using an infectivity assay.

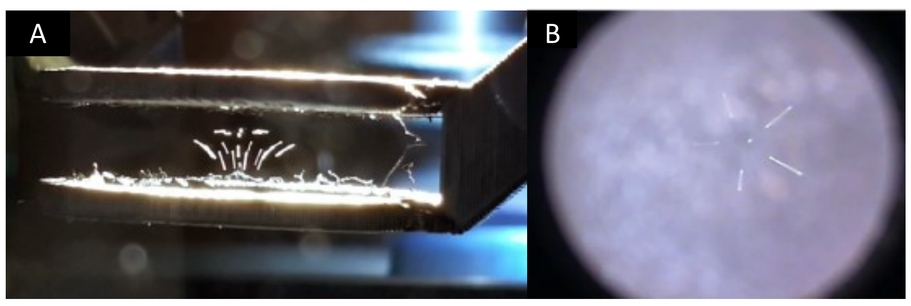

Figure 5: CELEBS generated droplets levitated in electromagnetic field. A) Side view of a population of droplets levitating in the CELEBS device. **B)** Overhead view of five droplets levitating in CELEBS device

Detection and quantification of single infectious particles following levitation

- Due to the small volume (around 100 pL) and low number of droplets produced by the CELEBS device the number of virions per levitation can be as low as 1 virion. Therefore, a **highly accurate and sensitive** method of quantifying infectious virions is required.
- Plaque assays are one of the most accurate methods for direct quantification of infectious virions
- Plaque assays use an overlay to localise virus spread, resulting in the formation of visible zones of cell death termed plaques (**Figure 6**) . Each plaque is assumed to be a result of a single virus infection.
- Here we use a **modified plaque assay** to quantify infectious virions post -levitation in the CELEBS device (**Figure 7**).

Figure 6: Example of plaques formed by IAV.

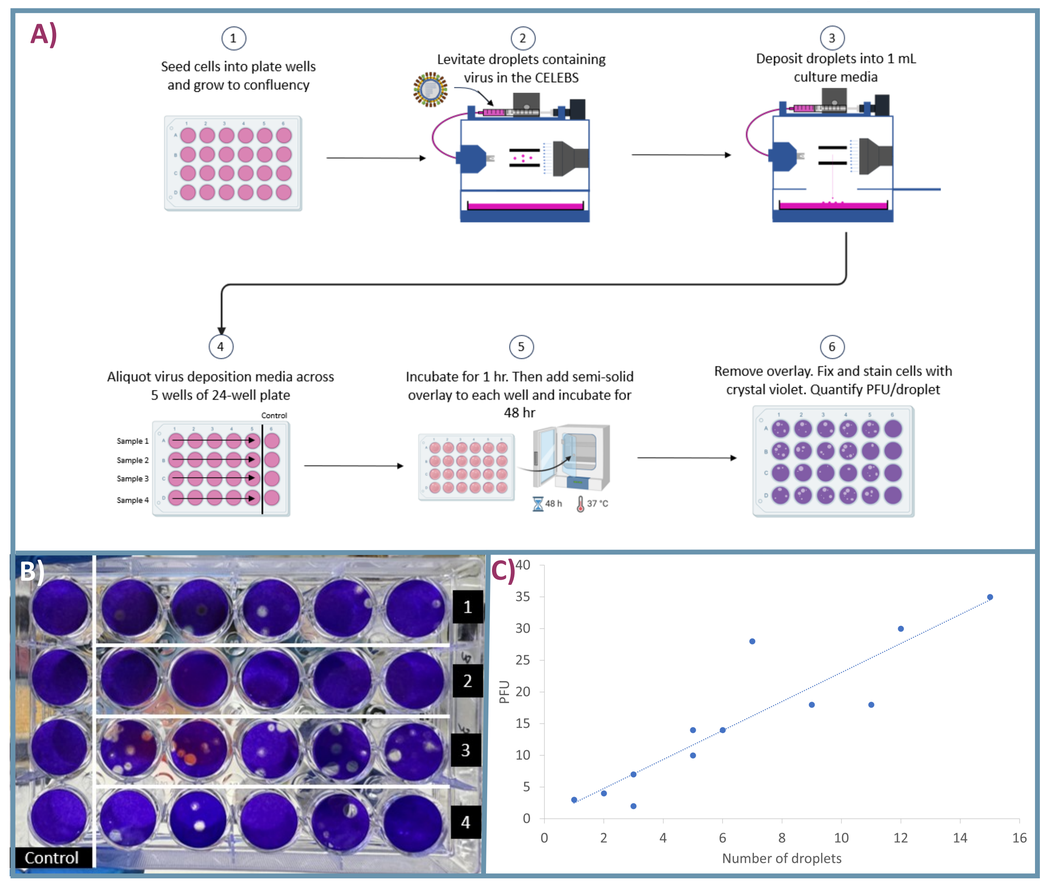

Figure 7: Plaque-based virus detection assay. A) Flow diagram of protocol to quantify infectious virions per levitated droplet after exposure to a desired environments condition. **B)** Plaques formed after 48 hrs by Influenza A strain WSN **C)** Correlation between the number of levitated droplets in the CELEBS and the counted PFUs.

4. Next Steps

- **Investigation of RH dependent decay profile for IAV.** Strains to be investigated include WSN, PR8, X31 and Udorn.
- **Investigate the effect of suspension medium composition on IAV viability.** Including altering salt and protein concentrations.
- **Effect of atmospheric CO_2 on the infectivity of IAV.** Previous research on SARS-CoV-2 demonstrates that atmospheric CO_2 concentrations play a significant role in controlling SARS-CoV-2 infectivity, possibly by altering aerosol pH (**Figure 8**) [7].
- **Identify the physicochemical changes occurring within aerosol droplets that lead to variations in IAV viability.**

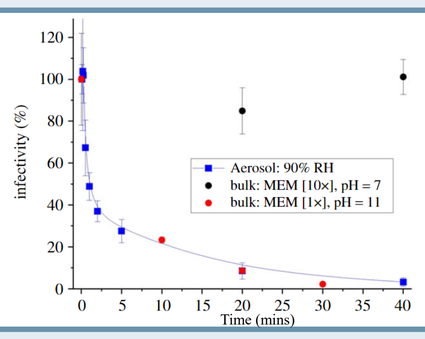

Figure 8: Comparison of the decay profile of SARS-CoV-2 in the aerosol phase (blue) with bulk solution measurements (High salt (black) and high pH (red)) [7]

8. References

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