Transmission of airborne respiratory infections with and without air cleaners in a school in Switzerland: A modeling study of epidemiological, environmental, and molecular data

Statistical analysis plan (SAP)

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Contents

1 Introduction

This document describes the proposed presentation and analysis for the main paper(s) reporting results from a study estimating and comparing the transmission of airborne respiratory infections with and without air cleaners in schools. To study the effectiveness of air cleaners as an infection control measure, we installed air cleaners in the classrooms of two classes in a secondary school in the Canton of Solothurn, Switzerland (**Figure 1**). Over the study period between January 16 and March 11, 2023, we collected the following data (**Table 1**):

1. **Molecular data**: Bioaerosol and human saliva samples.
2. **Environmental data**: CO_2, aerosol and particle concentrations.
3. **Epidemiological data**: Daily absences from school.

Laboratory and molecular analysis will detect which respiratory infections (e.g. SARS-CoV-2 or Influenza virus) were spreading over the study. Molecular (positive samples), environmental (particle concentrations), and epidemiological data (absences related to respiratory infections) will then be compared between study conditions with and without air cleaners. The aim of the comparison is to assess whether air cleaners reduced the risk of airborne infection.

Any deviations from the statistical analysis plan will be described and justified in the final report. The analysis will be carried out by identified, appropriately qualified, and experienced statisticians, who will ensure the integrity of the data during their processing.

Figure 1: Study setting. Schematic study setup of classrooms where molecular and environmental data were collected. One air cleaner was placed in the front and the other in the back of the classrooms. All devices were placed at the head level of students when they were seated. Both classrooms were not equipped with an active HVAC (Heating, Ventilation, Air conditioning) system, but were ventilated using passive window ventilation.

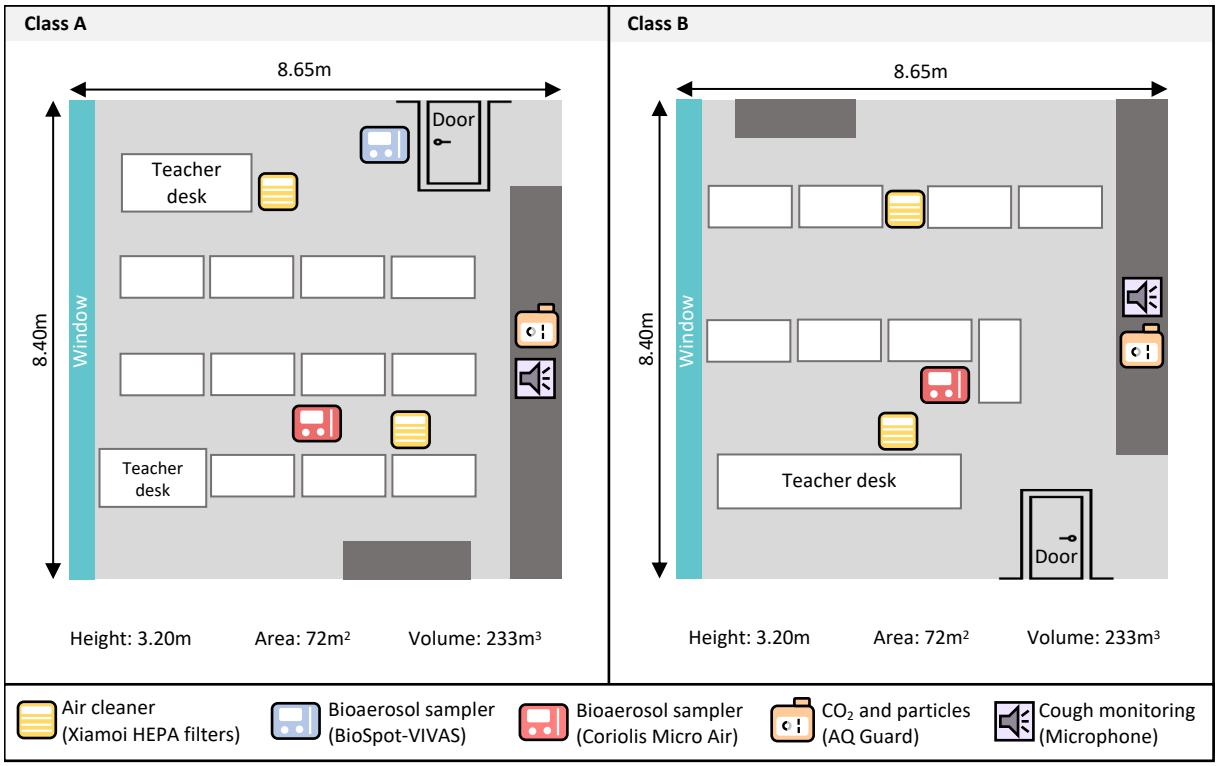

Table 1: Data. Type of data collected, method/device, and frequency in each class.

Type of data	Method / device	Class A	Class B	Frequency
Molecular data				
Bioaerosol sampling	Bioaerosol sampling devices: BioSpot-VIVAS (Aerosol Devices Inc., Ft. Collins, Colorado, United States) and Coriolis Micro Air (Bertin Instruments Montigny-le/Bretonneux, France)	x	(x) only Coriolis	daily
Saliva samples	Saliva samples	x	x	twice per week
Swabs from air cleaners	Swab samples from Xiaomi HEPA filters (Xiaomi Mi Air Pro 70m2, Shenzhen, China)	x	x	after each intervention phase (both classes) and before the vacation (only class B)
Environmental data				
CO_2, aerosol/particle concentrations, humidity, temperature	Air quality device: AQ Guard (Palas GmbH, Karlsruhe, Germany)	x	x	daily by minute
Coughs	Sounds collected via microphones and coughs detected with a Machine Learning model [1]	x	x	daily by seconds
Epidemiological data				
Absences	Survey	x	x	daily
Student characteristics	Survey	x	x	at study start

2 Background

In a previous study, we estimated and compared the transmission of airborne infections with and without air cleaners and mask mandates in five classes of two secondary schools in the Canton of Solothurn, Switzerland, between January and March 2022 [2]. Air cleaners (commercially available portable HEPA-filtration devices) were found to reduce particle concentrations, but no association was found with transmission. In the previous study, air cleaners were introduced towards the end of the study when most students were already infected with SARS-CoV-2, thus limiting their potential to reduce transmission. By contrast, in this study, we only assess the effectiveness of air cleaners and used a more efficient cross-over design (**Table 2**) to assess whether air cleaners are associated with reduced risk of airborne infection in schools.

Table 2: Study design. Seven-week study period from January 16 to March 11, 2023, excluding a week of vacation between February 6 and 11. Cross-over design where two portable air cleaners were installed during different time periods in each class (Class A: weeks 1, 2, 6, and 7; Class B: weeks 3, 4, and 5).

	Week 1 Jan 16-21	**Week 2** Jan 23-28	**Week 3** Jan 30-Feb 3	Vacation Feb 6-11	**Week 4** Feb 13-18	**Week 5** Feb 20-25	**Week 6** Feb 27-March 3	**Week 7** March 6-11
Class A	Air cleaner	Air cleaner	None		None	None	Air cleaner	Air cleaner
Class B	None	None	Air cleaner		Air cleaner	Air cleaner	None	None

2.1 Objectives of the study

The primary objective of the study is to estimate and compare the risk of airborne infection in schools. To this end, the following comparisons between study conditions (with and without air cleaners) are of interest:

1. **Molecular data**: Comparison of positive bioaerosol/saliva samples and viral load concentration.
2. **Environmental data**: Comparison of aerosol/particle concentrations.

3. **Epidemiological data**: Comparison of the number of absences related to respiratory infections.

2.2 Definitions

2.2.1 Case of respiratory infection

Based on epidemiological data, we will define a case of respiratory infection as an absence where the student reports (a) a positive lab test result confirming the respiratory infection, or (b) a sickness with at least one of the following symptoms: fever, coughing, tiredness, loss of test or smell, sore throat, headache, aches and pains, diarrhea, difficulty breathing or shortness of breath, stomach.

Students were asked to report the first date when they experienced symptoms. For example, a student absent on Monday may report Saturday or Sunday as the symptom onset date. We will denote cases by their symptom onset date. Note that, during the week, the symptom onset date will usually correspond to the absence date, unless the student went to school despite experiencing symptoms.

2.3 Primary outcome

The number of respiratory cases (absences related to respiratory infections by date of symptom onset).

2.4 Secondary outcomes

1. The number of positive bioaerosol and saliva samples.
2. Viral load concentration in bioaerosol samples.
3. The aerosol/particle number, and matter mass concentrations.
4. The number of coughs.

2.5 Variables

1. Molecular data
   (a) Number and proportion (%) of positive saliva and bioaerosol samples
   (b) Viral load concentration (viral genome load in copies per liter)
   (c) Spectrum of viral pathogens detected in bioaerosol and saliva samples, and on the HEPA filters of the air cleaners
2. Environmental data
   (a) CO_2 (parts per million [ppm])
   (b) Relative humidity (%) and temperature (°C)
   (c) Particle number (in $1/cm^3$, particle diameter between 175nm to 20μm) and matter mass concentrations (PM in μgm^{-3}; PM_1, $PM_{2.5}$, PM_4, PM_{10}, i.e. particles of sizes <1 to <10μm)
   (d) Number of detected coughs
3. Epidemiological data
   (a) Aggregated (baseline) data per class
      i. Number of students per class

ii. Vaccination and recovery status regarding COVID-19 of both students and teachers

iii. Age and gender

(b) Daily reporting per class

i. Number of absent students and teachers by sex

ii. Reason for absence: sickness, career days, or other

iii. Symptoms: fever, coughing, tiredness, loss of test or smell, sore throat, headache, aches and pains, diarrhea, difficulty breathing or shortness of breath, stomach, or other

iv. Date of symptom onset

v. Laboratory test performed for respiratory viruses: yes, no, or unknown

vi. Date when the laboratory test was taken

vii. Viruses tested: COVID-19, Influenza A and B, RSV, or other

viii. Laboratory test result: positive or negative

ix. Date when the absent student came back to school

4. Additional data (collected daily at 10min intervals)

(a) Working atmosphere: silent working, soft speaking, or loud speaking

(b) Ventilation: times of window or door opening

(c) Occupancy: breaks, lessons outside the classroom, lessons partly outside the classroom, or school-free hours

5. Secondary data

(a) Weekly number of cases and deaths, laboratory tests and share of positive tests for COVID-19 in the Canton of Solothurn [3]

(b) Weekly number of consultations per 100,000 people due to influenza-like illnesses (ILI) [4]

2.6 Hypothesis framework

The null hypothesis will be that there is no true difference in the outcomes between study conditions. We will use a Bayesian framework to assess the null hypothesis and use the posterior distribution of the parameter modeling the difference in the outcome by study condition to assess the direction and magnitude of the effects of air cleaners.

3 Study populations

The target population includes all students from two classes in a secondary school in the Canton of Solothurn, Switzerland. In total, there were 38 students in both classes (Class A: 20, Class B: 18).

4 Statistical analyses

4.1 Comparative analysis

We will employ descriptive statistics to summarize epidemiological, environmental, and molecular data. Categorical variables will be presented as the number and percentage of students, while continuous variables will be reported with the median and interquartile range (IQR). We will create summary tables to describe student characteristics (age and gender), vaccination status, recovery status, absences, number and share of positive samples, and viral load concentration. We will create figures (box-, bar- and line plots) to compare the share of positive samples, viral load concentration, and particle concentration.

4.2 Primary analysis

Linking observed cases to unobserved infections

The number of respiratory cases (absences related to respiratory infections by date of symptom onset) will be modeled with a Bayesian latent hierarchical regression model, which is similar to the model used in our previous study [2]. The model links the unobserved number of new infections to the observed number of new cases. The number of new cases in class j at time t is modeled with a Negative Binomial distribution

$$C_{jt} \sim \text{Negative-Binomial}(\mu_{jt}, \phi),$$

where μ_{jt} is the expected number of new cases and ϕ is the parameter modeling over-dispersion. The expected number of new cases is the weighted sum of the number of new infections I_{jt} in the previous days

$$\mu_{jt} = \sum_{s<t} I_{js} \cdot p_{\text{IN}}(t-s),$$

where $p_{\text{IN}}(t-s)$ denotes the probability distribution of the incubation period. The number of new infections is related to the presence of air cleaners using a log-link

$$\log I_{jt} = \log N_{jt} + \beta_0 + \beta_1 \cdot \text{AirCleaner}_{jt} + \beta_2 \cdot \text{Class}_t + \beta_3 \cdot \text{Students}_{jt} + \beta_4 \cdot \text{Ventilation}_{jt} + \beta_5 \cdot \text{CoV}_t + \beta_6 \cdot \text{ILI}_t,$$

where $N_{jt} = \sum_{s<t} I_{js}$ is the cumulative number of infections (model offset), β_0 is the rate of new infections without air cleaners (model intercept), and β_1 is the effect of air cleaners. The effect of air cleaners will be adjusted for class-specific effects, the number of students in school, the ventilation rate (air changes per hour computed from indoor CO_2 levels using the transient mass balance method [5]), the proportion of positive tests for SARS-CoV-2 in the community (from secondary data), and the number of consultations for influenza-like illnesses in the community (from secondary data).

Specifying the distribution of the incubation period

The pathogen of each respiratory infection cannot be identified from the epidemiological data unless students obtained a laboratory test result. As a consequence, different incubation periods will be considered in p_{IN}, reflecting a combination of the pathogen-specific incubation periods. The combination will be determined based on the proportion of positive bioaerosol/saliva samples for each pathogen found in the molecular analysis. The prior distributions for pathogen-specific incubation periods will be based on estimates published in the literature.

Adjusting for under-reporting of cases on weekends

Despite recording cases by date of symptom onset, our previous study recorded a higher proportion of cases on Mondays than on weekends, suggesting recall bias and under-reporting of cases on weekends [2]. To consider weekday effects in the reporting of cases, we will re-weight the expected number of cases each week as follows. Let $k \in$(1: Saturday, 2: Sunday, 3: Monday, ..., 7: Friday) denote the weekday with the week starting on Saturday. The re-weighted expected number of cases $\tilde{\mu}$ (class and day indexes omitted) are computed as

$$\begin{aligned} \tilde{\mu}_k &= \mu_k \cdot \nu_k \cdot \left(\frac{\sum_k \mu_k}{\sum_k \nu_k \cdot \mu_k} \right) \\ \sum_k \nu_k &= 1, \end{aligned}$$

where ν_k is the weight for weekday k. These weights will be modeled with a Dirichlet prior

$$\begin{aligned} \nu &\sim \text{Dirichlet}(c) \\ c_k &= \sum_j \sum_t C_{jt} \mathbb{I}_{\text{Weekday}(t)=k} \end{aligned}$$

where c_k is the total number of cases reported for weekday k and $\mathbb{I}$ is a binary indicator.

Modeling school-free days

Infections may have occurred during the week of vacation that falls into the study period. The expected number of infections and cases will be computed during vacation, but vacation days are not modeled (i.e. not incorporated into the model likelihood). In addition, we will assume lower transmission of respiratory infections on days without school (weekends and vacations). We incorporate our prior belief into the model intercept β_0

$$\beta_0 = \alpha + \omega \cdot \text{NoSchool},$$

where α is the rate of new infections on school days and $\alpha + \omega$ is the rate on days without school. We will model ω with an informative prior for a 10% decrease in new infections on school-free days

$$\omega \sim \text{Normal}(\log 1.1, 0.05) \ .$$

Seeding infections before study start

Cases in the first week of the study could indicate infections before the study commenced. We will therefore seed our model $2 \cdot \text{m}$ days before the study start, where m is the average incubation period. The number of infections before the study start will be modeled with an exponential prior

$$I_{jt} \sim \text{Exponential}(2 \cdot \text{m}) \quad t = -2 \cdot \text{m} + 1, \ldots, 0 \ .$$

Priors for modeling parameters

Unless stated otherwise, we will use weakly informative priors for all modeling parameters following recommendations on the choice of priors [6, 7, 8, 9, 10]. The continuous adjustment variables will be standardized to have zero

mean and a standard deviation of 0.5.

$$\begin{aligned}
\alpha &\sim \text{Student-t}_5(0, 10) \\
\beta_2, \ldots, \beta_6 &\sim \text{Student-t}_5(0, 2.5) \\
\frac{1}{\sqrt{\phi}} &\sim \text{Half-Normal}(0, 1),
\end{aligned}$$

where Student-t_5 is a Student-t distribution with 5 degrees of freedom. All priors represent our default but not definitive choices, and changes in the context of the data will be applied if necessary.

4.3 Secondary analyses

4.3.1 Number of positive bioaerosol and saliva samples

Molecular analysis will determine whether a bioaerosol or saliva sample is positive for a respiratory pathogen. If a sample is positive for multiple pathogens, we will denote this sample as positive for the pathogen with the highest viral load concentration. Let $p = 0, 1, \ldots, P$ denote the pathogen, where 0 refers to negative samples and P is the number of different pathogens detected over the study. The number of positive bioaerosol and saliva samples y_p in samples of sizes n will be analyzed with a Multinomial logistic regression model

$$\begin{aligned}
y_0, y_1, \cdots, y_P | n, \beta_0, \beta_1, \beta_2, \beta_3 &\sim \text{Multinomial-Logit}(\theta_0, \theta_1, \ldots \theta_P) \\
\theta &= \text{softmax}(\mu) \\
\mu &= \begin{cases} 0, & \text{if } p = 0, \\ \beta_{0[p]} + \beta_{1[p]} \cdot \text{AirCleaner} + \beta_2 \cdot \text{Class} + \beta_3 \cdot \text{Susceptibles}_p, & \text{if } p > 0. \end{cases} \\
\beta_0 &\sim \text{Normal}(\kappa_0, \tau_0) \\
\beta_1 &\sim \text{Normal}(\kappa_1, \tau_1) \\
\kappa_0 &\sim \text{Student-t}_5(0, 2.5) \\
\kappa_1, \beta_2, \beta_3 &\sim \text{Student-t}_5\left(0, \frac{2.5}{s_x}\right) \\
\tau_0, \tau_1 &\sim \text{Half-Student-t}_5(0, 1),
\end{aligned}$$

where $\text{softmax}(\mu) = \exp(\mu)/\sum \exp(\mu)$ and s_x is the empirical standard deviation of each input variable. Negative tests will be set as the reference category. Overall variation in positive samples by pathogen will be modeled with β_0 and the effect of air cleaners will be modeled with β_1. Both β_0 and β_1 will be modeled with a hierarchical prior, where κ is the average estimate across pathogens and τ is the variation of the estimate between pathogens. The effect of air cleaners will be adjusted for class-specific effects and the decreasing number of susceptibles over time (for each pathogen computed as the number of students minus the total number of positive samples).

4.3.2 Viral load concentration in bioaerosol samples

Molecular analysis will determine the viral load concentration in bioaerosol samples, which will be analyzed with a hierarchical linear regression model

$$
\begin{aligned}
y|\beta_0, \beta_1 &\sim \text{Normal}(\mu, s_y) \\
\mu &= \beta_{0[p]} + \beta_{1[p]} \cdot \text{AirCleaner} \\
\beta_0 &\sim \text{Normal}(\kappa_0, \tau_0) \\
\beta_1 &\sim \text{Normal}(\kappa_1, \tau_1) \\
\kappa_0 &\sim \text{Student-t}_5(\mu_y, 2.5 s_y) \\
\kappa_1 &\sim \text{Student-t}_5\left(0, 2.5\frac{s_y}{s_x}\right) \\
\tau_0, \tau_1 &\sim \text{Half-Student-t}_5(0, 1) \\
\sigma &\sim \text{Exponential}\left(\frac{1}{s_y}\right),
\end{aligned}
$$

where μ_y and s_y are the empirical mean and standard deviation of the outcome.

4.3.3 Aerosol/particle concentration

We will only analyze particle concentration data of the time periods during which students were in the classroom. Particle concentrations will be summarized daily with the mean across these time periods and a log transform will be applied. Each log-transformed average particle concentration will be analyzed separately with a linear regression model

$$
\begin{aligned}
\log y|\beta_0, \beta_1, \ldots, \beta_6 &\sim \text{Normal}(\mu, \sigma) \\
\mu &= \beta_0 + \beta_1 \cdot \text{AirCleaner} + \beta_2 \cdot \text{Class} + \beta_3 \cdot \text{Weekday} + \beta_4 \cdot \text{Students} + \beta_5 \cdot \text{Ventilation} + \beta_6 \cdot \text{Cases} \\
\beta_0 &\sim \text{Student-t}_5(\mu_y, 2.5 s_y) \\
\beta_1, \ldots, \beta_6 &\sim \text{Student-t}_5\left(0, 2.5\frac{s_y}{s_x}\right) \\
\sigma &\sim \text{Exponential}\left(\frac{1}{s_y}\right).
\end{aligned}
$$

The effect of air cleaners will be adjusted for class- and weekday-specific effects, the number of students in school, the ventilation rate (air changes per hour), and the cumulative number of cases related to respiratory infections. A log transform will be applied to all continuous input variables.

4.3.4 Number of coughs

The daily number of coughs will be analyzed with a Negative Binomial regression model

$$
\begin{aligned}
y|\beta_0, \beta_1, \ldots, \beta_6 &\sim \text{Negative-Binomial}(\mu, \phi) \\
\log \mu &= \log T + \beta_0 + \beta_1 \cdot \text{AirCleaner} + \beta_2 \cdot \text{Class} + \beta_3 \cdot \text{Weekday} + \beta_4 \cdot \text{Students} + \beta_5 \cdot \text{Ventilation} + \beta_6 \cdot \text{Cases} \\
\beta_0 &\sim \text{Student-t}_5(0, 2.5) \\
\beta_1, \ldots, \beta_6 &\sim \text{Student-t}_5\left(0, \frac{2.5}{s_x}\right) \\
\frac{1}{\sqrt{\phi}} &\sim \text{Half-Normal}(0, 1),
\end{aligned}
$$

where T is the daily duration that students were in the classroom and ϕ is the parameter modeling over-dispersion in count data.

5 Supplementary analyses

5.1 Modeling transmission risks

We will model transmission risks using the Wells-Riley equation [11] as modified by Rudnick and Milton [12]

$$P = \frac{D}{S} = 1 - \exp\left(-\frac{fIqt}{n}\right),$$

where P is the risk of infection, D is the number of disease cases, S is the number of susceptibles, f is the fraction of rebreathed air, I, is the number of infectious individuals in space, n is the total number of individuals in space, q is the generation rate of infectious quanta (doses of infectious particles), and t is the duration of exposure. Parameters D, S, and I will be computed based on epidemiological (or molecular) data. The fraction of rebreathed air will be computed from CO_2 levels. The number of individuals in space will be determined based on the number of absences. Exposure times will be determined based on the students' timetables.

We will first use the Wells-Riley equation to estimate the unknown parameter q. The risk of infection will then be modeled using the estimated q for exposure times representing a typical school day or week. These risks will be estimated separately for different pathogens detected over the study.

5.2 Targeted and deep sequencing

For positive samples (bioaerosol and saliva samples, swabs from HEPA filters), we will perform targeted sequencing and deep sequencing of viral RNA to compare genetic relatedness between viral strains detected in the air and human samples [13].

6 Missing data

We obtained informed consent from all but one student. As the reason for any absence(s) from this student and the symptoms will be unclear, we will consider any absence(s) from this student as unrelated to a respiratory infection. Particle concentrations will be summarized with the daily mean of the minutely values in the time periods when students were in the classroom. Missing values during these time periods will be linearly imputed. Missing values in adjustment variables will be imputed with a fully Bayesian approach where missing values are treated as parameters and a prior is assigned.

7 Statistical software employed

The statistical software R (version 4.2.1 or later) with RStudio will be used for all statistical analyses. Data preprocessing and comparative analyses will be performed in the `tidyverse` package (version 1.3.2). Bayesian analyses will be performed with the probabilistic programming language Stan (version 2.21.0), using the R interfaces provided by the `rstan` package (version 2.21.7), the `rstanarm` package (version 2.21.3), or the `brms` package (version 2.18.0).

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