# **RAPPORT AIRIAS FASE 1**

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### Nederlandse samenvatting

Het onderzoeksproject Airias Fase 1 betreft een vooronderzoek naar de inzet van mobiele luchtreinigers in het onderwijs als bestrijdingsmiddel bij een nieuwe opleving of pandemie van een luchtwegvirus (zoals Corona). Hiervoor moeten mobiele luchtreinigers in staat zijn het aantal gevallen van overdracht van virusinfecties in scholen terug te dringen. Tot op heden bestaat er geen bewijs dat het aantal infecties in scholen met het gebruik van mobiele luchtreinigers afneemt, en hier is dringend behoefte aan om te bepalen of mobiele luchtreinigers als bestrijdingsmaatregel in de toekomst kunnen worden ingezet. Kwalitatief hoogwaardig bewijs hiervoor kan verkregen worden middels een epidemiologisch onderzoek in de vorm van een 'cluster randomized trial'. Hierbij wordt middels loting bepaald weke scholen uitgerust worden met mobiele luchtreinigers, en welke scholen niet. Een vergelijking tussen de twee groepen scholen levert vervolgens een directe meting op van het effect van luchtreinigers. Voor het bepalen van randvoorwaarden en ontwerp van een dergelijk grootschalig onderzoek binnen het onderwijs zijn enkele cruciale voorbereidende werkzaamheden in dit project uitgevoerd.

Allereerst is het nodig om te bepalen aan welke kenmerken mobiele luchtreinigers en hun opstelling (plaatsing in het lokaal) moeten voldoen om een effectieve reiniging van de lucht in klaslokalen te realiseren. Omdat er vele modellen van mobiele luchtreinigers op de markt zijn, vereist dit allereerst een zorgvuldige selectie van geschikte modellen en een veldtoets om de juist configuratie en werking in de praktijk vast te leggen. Deze selectie is deels gebaseerd op een eerder uitgevoerde studie, waarbij de werking, opstelling, en acceptatie van verschillende modellen zijn getest in een proefopstelling.

De overdracht van virussen is niet direct meetbaar en daarom moet het effect van een maatregel die deze overdracht moet remmen bepaald worden door te kijken naar een combinatie van andere, gerelateerde uitkomstmaten. Het meten van de mate van verontreiniging van lucht in klaslokalen met bacterie- en virusdeeltjes kan verder inzicht geven in de werking van luchtreinigers. Een eventueel verschil hierin tussen scholen met en zonder luchtreinigers geeft een onderbouwing voor de werking van luchtreinigers in het terugdringen van besmettingen. Of luchtreinigers de mate van luchtverontreiniging met bacterie- en virusdeeltjes inderdaad kan verminderen dient allereerst empirisch te worden vastgesteld. Bovendien dient een geschikte meetmethode te worden bepaald die binnen het grootschalige onderzoek toe te passen is in klaslokalen voor het bepalen van deze microbiële verontreiniging.

Binnen het grootschalige onderzoek naar mobiele luchtreinigers wordt voorgesteld om de metingen van verontreiniging van de lucht in klaslokalen te combineren met een analyse van ziekteverzuim data van scholen. Immers, ziekteverzuim onder leerlingen en personeel gedurende het winterseizoen is in veel gevallen veroorzaakt door een virusinfectie aan de luchtwegen. Hieruit volgt dat, wanneer het aantal gevallen van virusinfecties in de ene groep scholen afneemt door inzet van luchtreinigers, dit te meten moet zijn in een verschil in aantal ziekteverzuimdagen tussen scholen met, en zonder luchtreinigers. Een aanvullend voordeel van het gebruik van ziekteverzuimdata is dat ze relatief eenvoudig te verkrijgen en anoniem te analyseren zijn, waardoor actieve medewerking van leerlingen en personeel aan bijvoorbeeld grootschalig testen binnen het onderzoek wordt vermeden. Omdat onbekend is hoeveel ziekteverzuimdagen in het onderwijs worden veroorzaakt door luchtweginfecties (waar luchtreinigers mogelijk een effect op kunnen hebben), dient hierin nader inzicht verkregen te worden. Daarnaast is een reductie in ziekteverzuim een maatschappelijk relevante uitkomst, die gebruikt kan worden om het eventuele nut van luchtreinigers nader te kwantificeren.

Naast de effectiviteit van luchtreinigers is het uiterst belangrijk om te weten of de inzet ervan in de praktijk haalbaar en aanvaardbaar is voor kinderen en leerkrachten. Met name de invloed op comfort en welzijn is van belang. Een nader inzicht hierin is van groot belang om een succesvolle implementatie mogelijk te maken.

### Resultaten

Het project werd uitgevoerd door een consortium van onderzoekers van de Universiteit Utrecht, de Technische Universiteit Delft, de Universiteit van Amsterdam, de European Clinical Research Alliance in Infectious Diseases, onder leiding van het UMC Utrecht. De diverse vooronderzoeken leverde de volgende inzichten op:

De gemeten afname van fijnstofdeeltjes van PM<sub>2.5</sub> en PM<sub>10</sub> in de lucht van klaslokalen wanneer de luchtreinigers aanstonden, bevestigde het aanbevolen gebruiksprotocol en eigenschappen van de luchtreinigers d.w.z.: Het gebruik van twee mobiele luchtreinigers die aan tegenoverliggende zijden van het klaslokaal worden geplaatst, waarbij de luchtuitvoer naar boven gericht is met een minimale aanbevolen totale luchtreinigingscapaciteit van 800-1000 m<sup>3</sup>/h voor een klas met 30 personen.

Er werd een pilotstudie uitgevoerd naar het gebruik van mobiele luchtreinigers in vijf scholen van het primair onderwijs volgens het ontwerp van een cluster-gerandomiseerde, gekruiste trial. Bij gebruik van mobiele luchtreinigers in een opstelling conform bovenstaande aanbevelingen werd naast de afname in fijnstofdeeltjes van PM<sub>2.5</sub> en PM<sub>10</sub> ook een gedeeltelijke reductie waargenomen in de detectie van bacteriële en virale verontreiniging van humane oorsprong. Echter ook bij metingen in controle klaslokalen zonder luchtreinigers kon dit voorkomen. Dit suggereert een mogelijke afname in concentratie van aerosolen met deze verontreiniging in de lucht van klaslokalen, maar resultaten zijn niet eenduidig en vereisen een grootschalige steekproef, met meer herhaalde waarnemingen. Of deze bevindingen vervolgens ook geassocieerd zijn met een lager risico op infecties kan niet worden afgeleid uit deze onderzoeksresultaten en vereist een veel grotere trial met andere eindpunten.

Voor het meten van bacteriële en virale verontreiniging van stofdeeltjes in de lucht bleek gebruik van elektrostatische stofcollectoren bevestigd aan het plafond van het klaslokaal de meest geschikte methode.

Op basis van een analyse van ziekteverzuimgegevens van basisscholen, in combinatie met een vragenlijstonderzoek onder leerlingen die o.b.v. ziekte absent werden gemeld, werd een inschatting gemaakt van het aantal ziekteverzuimdagen dat gedurende een schooljaar is toe te schrijven aan luchtweginfecties. Ook werd gekeken welk deel hiervan mogelijk gerelateerd is aan school. De resultaten leveren een schatting op van ca. drie schooldagen per leerling per jaar, waarbij ziekteverzuim als gevolg van aan schoolbezoek gerelateerde luchtweginfecties aannemelijk is. Met deze schatting is, in inter-pandemische tijden, de mogelijke impact van infectiebestrijdingsmaatregelen in school op het totaal aantal ziekteverzuimdagen van leerlingen beperkt. Vanzelfsprekend kan de impact groter zijn naarmate er meer infecties rondgaan van bijvoorbeeld seizoensvirussen zoals RSV of bij een nieuw opkomende infectieziekte zoals bij corona, waarbij dan ook het ziekteverzuim hoger ligt. De pilotstudie in vijf scholen heeft ook belangrijke uitdagingen blootgelegd t.a.v. de praktische haalbaarheid en acceptatie van de inzet van mobiele luchtreinigers in het primair onderwijs. De grootte en vereiste positionering van de apparaten zorgde voor het moeizaam inpassen van de apparaten in de klas en resulteerde in een negatieve verandering in de ervaren klasomgeving door zowel leerkrachten als leerlingen. Het gebruik van luchtreinigers had een negatieve impact op verschillende indicatoren van omgevingscomfort (geluid, koude, frequentie van verstoringen en concentratievermogen) en dit resulteerde in een lage algehele tevredenheid over het gebruik van mobiele luchtreinigers, vooral onder leerkrachten. Als gevolg hiervan waren leerkrachten weinig geneigd om de mobiele luchtreinigers in de toekomst te gaan gebruiken.

Deze bevindingen hebben belangrijke implicaties voor het mogelijke succes van een implementatie van mobiele luchtreinigers in het primair onderwijs en vereisen een zorgvuldige overweging van hun nadelige effecten. Voor zoveel mogelijk behoud van comfort wordt aanbevolen een luchtreiniger te selecteren waarvan het maximale geluidsniveau van de luchtreinigers wanneer beide apparaten aanstaan onder de 35 dB(A) blijft en de luchtstroom snelheid in de gebruikszone van het klaslokaal <0.2 m/s. Door de inzet van mobiele luchtreinigers te beperken tot korte periodes, als tijdelijke bestrijdingsmaatregel voor pandemieën of epidemieën zou het ervaren discomfort verder kunnen worden beperkt, en daarmee mogelijk de acceptatie verhoogd. Dit moet verder onderzocht worden.

Ook bleek de deelnamebereidheid onder scholen voor het pilot onderzoek beperkt. Een belangrijke reden om af te zien van deelname was de extra inzet die gevraagd werden van de scholen voor handelingen in het kader van het onderzoek. Hiermee dient rekening gehouden te worden bij het plannen van een grootschalig onderzoek.

Samenvattend kunnen we concluderen dat mobiele luchtreinigers in de aanbevolen opstelling effectief lijken in het reduceren van stofdeeltjes. Voor microbiële verontreiniging van stofdeeltjes in de lucht in klaslokalen kan dit mogelijk ook gelden, maar zijn de resultaten niet eenduidig. Echter zijn er belangrijke barrières t.a.v. acceptatie en haalbaarheid voor het routinematig gebruik van mobiele luchtreinigers. Nader onderzoek is nodig om te bepalen of de afname in stofdeeltjes en mogelijke afname microbiële verontreiniging zich vertaalt in het voorkomen van via de lucht overdraagbare infecties en gerelateerd ziekteverzuim. De benodigde gegevens om een grootschalige cluster randomized trial hiernaar te ontwerpen zijn in dit project verzameld en een concept onderzoeksprotocol is opgesteld. In een inter-pandemische periode is deelname van 160 basisscholen nodig waarbij het onderzoek gedurende de wintermaanden wordt uitgevoerd, om een reductie van één ziekteverzuimdag per leerling per schooljaar als effect van mobiele luchtreinigers in klassen aan te tonen, dan wel uit te sluiten. Hierbij worden willekeurig 80 scholen uitgerust met mobiele luchtreinigers, en 80 scholen dienen als controle. Om een dergelijk onderzoek van de grond te krijgen is het belangrijk om voldoende scholen te motiveren hieraan deel te nemen, bijvoorbeeld door de extra werkzaamheden uit handen te nemen en/of een vorm van compensatie/beloning aan te bieden.

### Introduction

The main objectives of the Airias Fase 1 project was to conduct a feasibility and acceptability assessment of the use of mobile aircleaning devices (MACs) in classrooms in primary schools in the Netherlands, to test their technical performance in a real-world setting, and to obtain the required data to design a cluster randomized trial on the effect of MACs in classrooms on illness absenteeism in primary schools. De results of the Airias Fase 1 project are described in the following sections, referring to Activity A-F as specified in the approved grant application.

### Abbreviations

UMCU: University Medical Center Utrecht UU: Utrecht University TU Delft: Technical University Delft UvA: University of Amsterdam Ecraid: European Clinical Research Alliance on Infectious Diseases IRAS: Institute for Risk Assessment Sciences MAC: Mobile Aircleaning Device CI: Confidence Interval **TIG: The Implementation Group** GLMM: Generalized Linear Mixed-effects Model **IR: Incidence Rate** IQR: Interquartile range HEPA: High-efficiency particulate air **VOC: Volatile Organic Compounds TVOC: Total Volatile Organic Compounds** HVAC: Heating, ventilation, air conditioning **ES: electrostatics** AC: Activated Carbon  $PM_{1.0}$ : Airborne particles of diameter < 1  $\mu$ m  $PM_{2.5}$ : Airborne particles of diameter < 2.5  $\mu$ m  $PM_{10}$ : Airborne particles of diameter < 10  $\mu mn$ CADR: Clean Air Delivery Rate IAQ: Indoor Air Quality EDC: Electrostatic Dust fall Collector PCR: Polymerase Chain Reaction

Activity A: Trial protocol development

#### Introduction

One of the main objectives of this activity was to obtain the required data to design a cluster randomized trial on the effect of MACs in classrooms on illness absenteeism in primary schools. Activity A describes the research conducted to obtain estimates of the average number of absent days due to acute respiratory illness in students in primary schools that can be potentially reduced by infection control interventions in the school setting, such as MACs. Next, we describe how these results were used to design the optimal cluster randomized trial and the required number of schools that should participate in such a trial (sample size) to confirm or reject the hypothesis that MACs can be used in primary schools to reduce illness absenteeism. The concept trial protocol is included as attachment. Finally. We describe the results of the feasibility and acceptability assessment of the use of MACs in classrooms based on teacher and student interviews. The insights from this study can support development of communication and engagement strategies towards schools, teachers and students.

### School absenteeism data

### Number of absent days per student per year

School absenteeism data were obtained from the school administrative systems from the five schools participating in the pilot study. The data included for each absenteeism episode the start and end date, school and student group, but no personal information. Absenteeism data were collected for the entire 2022-2023 school year and the first half of the 2023-2024 school year, covering the period from summer to the Christmas holidays including the time during which the pilot study was conducted. Absenteeism data from the first grades were excluded due to the dynamic nature of group sizes throughout the school year and the availability of only final group sizes. Based on the remaining data, the average number of absent days per student due to illness for the 2022-2023 school year was estimated at 5.3 (95% CI: 3.9-7.2) days per school year (Figure A1A).

To confirm that the absenteeism rates of the five schools participating in the pilot study are representative for Dutch primary schools, we conducted a second analysis on school absenteeism data from Dutch primary schools obtained from The Implementation Group (TIG). This company hosts the school administrative system ParnasSys, used by more than 70 primary schools in the Netherlands. The anonymized data from this administrative system on school absenteeism for the school year 2022 - 2023 were provided to UMCU for research purposes. For the analyses, we selected data from 70 primary schools after a quality check on the data, where we checked the total days with school attendance above zero (as indicator of data entry) and correct assignment of registered holidays. The cumulative average number of absent days per student per school from these data is plotted in Figure A1B, with an average line indicating the median value across all schools. On average, students were absent due to illness for a total of 4.6 (IQR: 3.7-5.1) days during the 2022-2023 school year. These results suggest that the absenteeism rates in the five schools participating in the pilot study are representative.



**Figure A1** The cumulative average number of absent days per student during the 2022-2023 school year is plotted. A) Data from the five primary schools that participated in the pilot study is plotted in colors, in black the average of these five schools. B) Data from 70 Dutch primary schools during the 2022-2023 school year is plotted in color, in black the average of 70 schools. Estimates were obtained using a generalized linear mixed-effects model (GLMM) with a negative binomial distribution. The model treated the total number of absent days per class per year as a function of the total number of students per class, incorporating the logarithm of the number of students per class as an offset term and including school as a random effect. This allowed the exponentiated intercept to be directly interpreted as the average number of absent days per student per school year.

Next, we explored the seasonal pattern of absenteeism rates across the school year. We calculated the weekly incidence rate (IR) of absences, expressed as new absences per 100 student days. During the 2022-2023 school year, the average IR was 2.2 absenteeism episodes per 100 student days, The IR peaked between the Christmas and Spring break (3.7 episodes per 100 student days) and was lowest between May and Summer holiday (1.4 episodes per 100 student days), and between Summer break and Fall break (1.7 episodes per 100 student days, Figure A2). Substantial differences were observed among the schools in both the timing and magnitude of the absenteeism peaks.



**Figure A2** The weekly incidence rate (IR) is plotted as the number of new cases per 100 student days. The colored lines indicate individual school incidence, the black line indicates the average of all schools. Numbers below the graph indicate the IR per school period.

#### Proportion of absenteeism attributable to acute respiratory illness

In order to estimate the proportion of absenteeism attributable to acute respiratory infections, a survey was developed and distributed to parents of students on the day that the student was reported absent due to illness. An invitation to participate in the online, anonymous illness survey was sent by the school to parents/legal guardians. The survey included questions about the reason for absenteeism, the duration of absence, and the presence of symptoms in the student. During the study period, 371 surveys were distributed among parents of students reported absent due to illness, achieving an 81.7% completion rate. Survey results estimated that 73.8% of the missed days were attributable to acute respiratory illness, while 21.9% resulted from gastrointestinal illnesses and 4.7% were other diseases. From these proportions, it was estimated that per student 3.9 days of absenteeism per schoolyear are attributable to acute respiratory illness (73.8% \* 5.3 days). Parents were also queried about the presence of acute respiratory symptoms in other members of the household. In total 30.8% indicated that symptoms were previously present in the household, whereas 69.2% reported no symptoms or symptoms appearing later, suggesting the student likely contracted the infection outside the household setting, possibly at school. Considering these absences result from school-related infections and applying 100,000 bootstrap samples to the data, we estimate that 2.7 (95% CI: 1.8-3.7) days of absenteeism per student per school year are due to acute respiratory illness that could be targeted with school-related infection prevention interventions. We repeated this estimation using the data obtained from TIG as an alternative starting point (4.6 (IQR: 3.7-5.1) days of absenteeism due to illness per student per school year). This approach resulted in a slightly lower estimate of 2.3 (95% CI: 2.0-2.7) days of absenteeism per student per school year due to acute respiratory illness that could be targeted with school-related infection prevention interventions.

### Cluster randomized trial protocol

The effect of school-related infection prevention interventions on student absenteeism due to acute respiratory illness can be investigated in a cluster-randomized trial. In this study design, schools are randomly selected to receive the intervention or to serve as control school without the intervention. Thus, two groups of schools are created; one with and one without the intervention implemented. In this trial the intervention is the use of mobile air cleaning devices (MACs) in classrooms of primary schools during school hours. The primary outcome under study is the average illness absenteeism rates in schools with, compared to schools without the intervention over the study period. Thus, the effect of MACs can be measured by comparing the illness absenteeism rates between the two groups of schools. A relevant effect was defined upfront as a reduction in absenteeism rates due to illness by 20% or 25%. Based on an estimated average of 5.3 absent days per student per year, this corresponds to approximately one day less absenteeism due to illness per student per year. We determined the number of schools needed in a cluster-randomized trial (i.e. sample size) to be able to detect this difference of one day, in case the MACs are indeed effective in reducing absenteeism. The sample size calculation was based on an 80% statistical power using a simulation study based on two different algorithms (see Supplemetary files). As input for the simulation study, we used the results on illness absenteeism rates from the five schools participating in the pilot study as described above. We tested 3 different scenario's to run the trial:

1) Over the course of a full school year; or restricted to the period with highest absenteeism rates defined as 2) between Fall break and May holiday or 3) between Christmas holiday and Early April (around Easter break).

The results indicate that for an assumed effect 25% reduction of MACs on illness absenteeism a participation of 100 schools in the trial would yield results with acceptable statistical confidence (80% power). However, for an assumed effect of 20% reduction, the required number of schools participating in the trial would be at least 160 (Figure A3). Restricting the study period to the period with highest absenteeism rates 2) or 3) does not have a significant negative impact on the power. Both applied algorithms produce similar range results confirming robustness of the simulation results.

In conclusion, the simulation study suggests that the trial can be most efficiently conducted when the study period runs during the school weeks between Christmas and Easter break. For an assumed 25% reduction of the absenteeism rate, including and randomizing 100 primary schools yields a power that is estimated to be around 80% or higher. For an assumed 20% reduction, it is advised to randomize at least 160 schools. As only about ¾ of all illness absenteeism can be attributed to acute respiratory illness and could be potentially reduced by the effect of MACs, a reduction of 20% in illness absenteeism is the most realistic and conservative scenario. Therefore, we recommend a trial that includes and randomizes 160 schools.

For further details on the trial design, we refer to the concept trial protocol in appendix A.I



**Figure A3** The results of the different simulation scenarios are plotted. In grey the results assuming an effect size of 0.8 (20% reduction) are shown, in black the results assuming an effect size of 0.75 (75% reduction) are shown.

### Feasibility and acceptability of MACs; a qualitative exploration

This is a qualitative study conducted in five primary schools that participated in a pilot on the use of MACs between September – December 2023. The pilot is described in more details in Activity F. In brief, during the six-to-eight-week pilot, each school was equipped with MACs and measurements were conducted to explore the feasibility of these MACs in classrooms. During the study period, at least three consecutive weeks with and three without operating MACs were executed.

To maximize the scope of the pilot feasibility study, three different populations were interviewed: members of the school administration, teachers, and students.

#### Study methods

All interviews with members of the school administration and teachers were audio-recorded and conducted through Teams. All interviews with students were conducted face to face in schools. Participants received reimbursement for their time with a gift voucher ( $\leq$ 15).

Semi-structured in-depth interviews were conducted using an interview topic guide. The interviews explored feasibility of MACs in schools by exploring concepts of the Consolidated Framework of Intervention Research (CFIR) and Bowen framework (Bowen et al., 2009; Damschroder et al., 2022) among the members of the school administration, teachers, and students: acceptability, demand, implementation, practicality, integration, and perceived effectiveness (Bowen et al., 2009; Damschroder et al., 2009; Damschroder et al., 2022). The interviews were conducted by a researcher with expertise in qualitative research. Topics for the qualitative interviews included:

- Acceptability explored perceptions towards MACs of members of the school administration, teachers, and students and was operationalized by satisfaction and expectations towards the MACs.
- Demand is about the likelihood the MACs are used by members of the school administration, teachers, and students.
- Implementation is about the extent to which MACs are successfully implemented in schools. To investigate this parameter, barriers and facilitators were explored.
- Practicality includes the extent to which MACs can be used as intended by teachers.
- Integration reflects the level of change needed by teachers and students for the MAC to fit with their daily activities and practices.
- Perceived effectiveness was operationalized by beliefs about the effectiveness of the MACs in reducing diseases.

The interviews were conducted in cycles of six (Braun & Clarke, 2006; Guest et al., 2016). Data were systematically analyzed by the researchers according to the thematic analysis of Braun and Clarke (2006) to reflect a greater degree of data transformation [10]. Dutch quotes were translated into English by the research team using the forward-backward method.

### Results

### Participant characteristics

Six members of the school administration and 12 teachers scheduled an interview, of whom three members of the school administration and ten teachers participated in the pilot study. The reason for declining participating in the pilot study was lack of time. Twenty students scheduled an interview, of whom twenty participated in the pilot study of which, two were pilot test interviews. The results of these two test interviews were not included in further analysis. Experience ranged from one to fourteen years among members of the school administration and from one to twenty-three years among teachers. The age of students ranged from nine to thirteen years old.

### Acceptability

Acceptability was assessed through members of the school administration', teachers', and students' expectations of using MACs. Furthermore, positive and negative experiences were identified. Finally, the resulting satisfaction with MACs was explored.

### **Expectations**

When asked about expectations about air cleaners, the members of the school administration indicated that solutions were sought for struggles with the indoor climate. They explained that currently, the indoor climate is negatively affected due to temperature and CO<sub>2</sub>. The members of the school administration described struggles with temperature and CO<sub>2</sub> as the main reason to participate in the pilot study. Teachers expressed to experience similar issues with the indoor climate in their classrooms and expected the MACs to solve this. Students expressed expectations that MACs would make the air cleaner. This was also described as "it *does what it is supposed to do*".

### **Experiences**

The members of the school administration indicated that the experiences in winter versus summer could be very different due to the "cold" draft produced by the devices. The experience of

participating in a scientific study was described as very positive by the members of the school administration. With regards to the students, the members of the school administration underlined that the sound produced by the MACs may lead to a loss of concentration. The members of the school administration also indicated a negative experience with the size of the MACs.

Teachers described overwhelmingly negative experiences with the MACs. Although they were positive about conducting the study, many specific examples were given about their perceived negative experience:

- Teachers mentioned that MACs negatively affected the atmosphere in the classroom, learning ability, and concentration of children due to the sound produced.
- The airflow of the MACs was described as cold and compared to turning on the air conditioning or fan. Teachers chose to wear different or warmer clothing. The airflow also caused posters, drawings or other materials suspended to the wall to move due to the draft.
- Teachers indicated that they got used to the sound.
- The size of the MACs and the space they need in the classroom was added as a negative experience. Teachers explained to the interviewer that classrooms were small and that there was little to no space left to place the devices.
- Due to these negative experiences, teachers indicated that the MACs also have a negative effect on their work environment and work.

Students generally shared positive or neutral experiences regarding the MACs. Students indicated a lack of opinion about the MACs and got used to them in the classroom.

- Some students indicated that they were able to concentrate better during tests and independent work because of the constant and soft sound produced by the MACs.
- Students noticed that there is airflow coming from the MAC. This was compared to an air conditioning or a fan.
- Students noticed the sound of the MAC, but they believed the sound was described as not noticeable.

### **Satisfaction**

The members of the school administration indicated that they were satisfied with the MACs, but their satisfaction was reported about the use during the pilot. When the pilot would show that the MACs are effective in preventing diseases, they indicated that the effectiveness would outweigh the negative experiences and aspects of the MACs. Teachers indicated that this lack of conclusive information about the effect made it difficult for them to express their satisfaction. The students were satisfied with the MAC. They indicated that using the device was fine.

### Demand

Demand was assessed by exploring the needs and the intended use of the MACs in the future by members of the school administration, teachers, and students, and the likelihood that they would recommend the MACs to others.

### Needs of regarding air cleaners

The members of the school administration had a clear sense of need regarding the issues with the indoor climate. For this reason, they seized the opportunity for participating in this pilot with both hands. The needs described by members of the school administration centered around indoor climate, rather than 'air purification'. Teachers were aware that there is a need to improve the air

quality in the schools. There seemed to be some misunderstanding among them about what the MACs could improve about the air quality.

### Probability of using the device in the future

The members of the school administration indicated that they would like to work with the MACs again, provided they are effective in reducing diseases, and adjustments can be made in the way the devices are placed and used in the classrooms. Some teachers however, felt that despite potential effectiveness, it would not outweigh negative experiences. For others, effectiveness would outweigh negative experiences. The students explained they would like the MACs to remain in the classroom. They described the air as nice, and fresher compared to air without MACs.

### Likelihood of recommending the device to others

During the interviews, it was felt impossible for the members of the school administration to decide whether MACs would be recommended to others, as it is still unclear whether they are effective. This opinion was also shared by teachers. Students indicated that it would be good if other schools would also use the MACs.

### Implementation

The implementation of MACs in classrooms was explored by evaluating factors that facilitated and hindered implementation as perceived by members of the school administration, teachers and students.

### Barriers experienced

When asked about aspects of the MACs that could interfere with use, the members of the school administration and teachers indicated that the space the device takes up may hinder use. Practical aspects, such as a lack of power outlets in a classroom were also mentioned by both school administration and teachers. The members of the school administration observed that the size of the MACs and the space they took up in the classroom was as a cause of frustration among teachers. Indeed, teachers shared that the position of the MACs in the classroom was perceived as very negative. Due to the placement of the MACs, teachers and students shared that they had difficulty accessing the board. Some teachers had to stand in front of the board, rather than on the side of it as they were used to, when giving instructions. Teachers and students expressed difficulty reaching cabinets, or chairs and tables had to be moved.

### Facilitators experienced

When inquiring about aspects that may promote use, the ease of use of the MACs was described by teachers. No other suggestions were mentioned.

### Practicality

Practicality was explored by discussing the extent to which MACs can be used as intended by the primary users; i.e. teachers.

The convenience of the MACs was an aspect that all teachers were satisfied with. The operation was experienced as *"so easy, anyone can do it"*.

### Integration

Integration was explored by evaluating the ease of integration of operating the MACs into teachers' daily activities. Like the ease of operation, the ease of integrating the task of daily turning on the MACs was also experienced by teachers. This was explained by comparing the MACs with turning on the board or a computer to start the working day.

### Perceived effectiveness

Perceived effectiveness was operationalized by exploring beliefs about the effectiveness of the MACs in reducing diseases. Note that no data were generated during this pilot that determine whether the MACs are indeed effective in reducing diseases and this was not the objective of the pilot. Yet, perceptions on the effectiveness were discussed during the interviews.

Principals were unable to share observations about student absences. They are very curious about the results of the research. Principals were cautiously optimistic about the effectiveness of the MACs and shared anecdotes about experiences or observations that convinced them of the effectiveness of the MACs.

Although the airflow of the MACs was perceived as negative and cold, the airflow ensured that teachers were convinced that the MACs were actively working to improve air quality. Despite this belief, teachers did not express a sense of effectiveness about a decrease in diseases in schools. Both members of the administration and teachers indicated that they were very curious about results of effectiveness and hope that they are decisive and convincing.

Students were convinced of the effectiveness of the MACs. They described fresher, cleaner, and healthier air in the classroom.

#### Summary

In summary, the qualitative interviews have yielded important insights into the feasibility and acceptability of MACs in classrooms in primary schools. The need for non-pharmaceutical interventions that can help reduce transmission of respiratory viral pathogens, without compromising individual freedom in schools was acknowledged by members of the school administration and teachers. A general improvement in indoor climate and CO<sub>2</sub> is also desired, and high CO2 concentrations seemed to be the dominant problem experienced. As MACs do not reduce CO2 concentrations and cannot be considered an alternative to sufficient ventilation, it is crucial to ensure that expectations of schools willing to participate in future research match the objectives of the trial. It should be stressed that MACs do not solve the problem of high CO2 concentrations. Whether the MACs are effective in reducing disease would be an important determinant for willingness of future use or for recommending it to others. The most important barriers and facilitators identified for use of MACs in primary schools include:

#### Barriers

- Placement or position of the MACs in the classroom (see barriers).
- Expectation/needs regarding ventilation and CO<sub>2</sub>, both unrelated to the functionalities of the MACs.
- The experienced cold airflow of the MACs and loud sound.
- In the context of a study: lack of involvement of teachers in design of the study.

### Facilitators

- Support by the study research team (organization & communication) for implementation of the devices and to relief teachers from study related tasks.
- Ease of operating the MACs

### Recommendations

It is recommended to include at least 160 primary school in a cluster- randomized trial to evaluate the effect of MACs on reducing illness absenteeism, at an anticipated reduction of 1 illness day per student per year.

It is recommended to conduct such a trial during the season with highest incidence of illness absenteeism and should include at least the period between Christmas holidays and early April (as cut-off the Easter break could be selected). Limiting the duration of required use is expected to improve compliance and willingness to participate in the trial as it reduces the negative side effects experienced by teachers.

It is recommended to involve teacher representatives in the in-classroom protocol when designing the study (where to place the MACs, how to place the devices) and to explore the possibilities of allowing more flexibility in positioning of the devices.

It is recommended to involve representatives of primary schools in designing the communication plan for the trial.

There is a need for efforts to target misbeliefs about air cleaning/purification and their role in improving indoor climate among all stakeholders involved in the trial and potential future use of MACs in schools.

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### Appendix A.I

## Airias-trial Concept RESEARCH PROTOCOL (nWMO)

Version 1.0

PROTOCOL TITLE Clean-Air devices research in Schools; a cluster-randomized controlled trial

Short title (max 43 characters)	Airias-trial		
Version 1.0			
Date	10 Jun 2024		
Department Julius Centrum			
Coordinating investigator/project leader	Prof. dr. Patricia Bruijning-Verhagen		
Principal investigator (in Dutch: hoofdonderzoeker/uitvoerder)	Prof. dr. Patricia Bruijning-Verhagen		
Other investigator(s)			
Subsidising party	Ministerie van Onderwijs		

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### Introduction

Since the COVID19 pandemic, there is great interest in non-pharmaceutical interventions that can help reduce transmission of respiratory viral infections, without compromising individual freedom and daily life. Interventions that can help improve indoor air-quality and thereby potentially reduce airborne transmission have gained much interest. While sufficient ventilation is considered the cornerstone of this approach, air-cleaning devices may be of added value because they can remove virus laden particles from recirculated air. In addition, air-cleaning devices are mobile and can be installed in many different environments, making them ideal as temporary outbreak control or mitigation intervention.

Schools are known to be important drivers of transmission for many different respiratory viral infections and reducing transmission in schools has the potential to mitigate onward epidemic spread or contain outbreaks. For this reason, the potential for using air cleaning devices in schools to reduce transmission of respiratory viral infections is of great public health importance and needs to be further evaluated. A cluster randomized trial is the optimal design to assess the performance of air-cleaners in classrooms and the effect individual health of students and teachers.

Various types of air cleaning technologies are used as in-room air purifiers.<sup>1</sup> Most frequently used techniques are the high-efficiency particulate air (HEPA) filters and ultraviolet germicidal irradiation (UVGI). HEPA filters use mechanical filtration, meaning that they physically remove airborne particles from the air. These filters can be placed in HVAC (heating, ventilation, air conditioning) ducts or air purifiers. UVGI is a form of radiation that uses ultraviolet (UV) light to damage the deoxyribonucleic (DNA) of microorganisms, disrupting their ability to replicate and thus leaving them noninfectious. UVGI lamps can be placed within the ducts of the HVAC system, in the ceiling or upper wall of a room, or in air purifiers. To gain insight in commercially available mobile air cleaning devices (MACs) and their potential for practical use in schools, a study from Delft University of Technology (assigned by the Ministry of Education, Culture and Science, case no. 1325610) performed a comprehensive screening and assessment of 300 different types of MACs. Seven types of MACs were selected based on technical and practical characteristics for further technical assessments in the Experience room of the SenseLab at Delft University of Technology [5], which is of the half size of a classroom (70 m<sub>3</sub>), and with a typical classroom interior setting. Most of the MACs achieved a good level of clean air delivery rate (CADR) (900-1000 m3/h). Based on the results, three MACs were selected for conducting a pilot study in 45 classrooms at five primary schools. Indoor air quality (IAQ) parameters were monitored for a period of six weeks with three weeks the devices turned on and three weeks off. The results showed that the MACs proved to reduce the concentrations of PM<sub>2.5</sub> and PM<sub>10</sub> in the classrooms when turned on as compared to the off state, confirming the technical performance of the devices in a real-world classroom setting.

A trial to evaluate the effect of MACs in schools on transmission of respiratory pathogens is complicated because the occurrence of transmission cannot be directly observed. This is typically studied using indirect measures such as infection rates, determined by microbiological testing of exposed subjects, and sequence determination of detected pathogens to determine genetic relatedness. However, such outcomes require (repeated) biological sampling of subjects and detailed information of contact patterns. In the setting of students in schools, this approach is deemed unfeasible and undesirable and has the potential to introduce selection bias due to nonparticipation. Therefore, alternative outcomes that do not rely on active student participation and student biological sampling are needed. One such alternative outcome is the occurrence of schoolabsenteeism, as acute respiratory infections are one of the main reasons for missed school-days in children. We hypothesized that school-absenteeism can be used as a proxy for acute respiratory illness of infectious origin and that the level of temporal and spatial clustering of absenteeism in schools reflects school-related transmission of these infections. In preparation of designing this cluster randomized trial, we conducted a pilot study to determine the feasibility and acceptability of using MACs in primary schools. As a second objective of the pilot, we quantified school-absenteeism in students attending primary education. On average, student had 5.3 days of illness related absenteeism per school-year and in 74%, the primary reason for absenteeism was acute respiratory illness. In 69% of students reporting acute respiratory illness, no other household member had similar symptoms prior to the students symptoms suggesting that the infection may have been contracted outside the household setting including schools. In addition, we analyzed the spatiotemporal pattern of school-absenteeism and found a strong level of clustering in time and in student groups. Illness absenteeism rates followed as strong seasonal patterns with peak incidence between January and April. This pattern corresponds to the seasonality of main respiratory pathogens including RSV, influenza, and hMPV. We thus conclude that the majority of illness absenteeism in primary schools is caused by acute respiratory illness and that the spatiotemporal pattern of illness absenteeism in schools strongly suggests communicable disease. In the context of a controlled cluster randomized trial on transmission reducing interventions such as MACs, differences in illness-related absenteeism between clusters can thus be used to infer the effect of the intervention on transmission. More importantly, any intervention capable of reducing absenteeism has substantial societal relevance.

The primary objective of this trial in therefore to determine the effect of the use of MACs in classrooms on illness absenteeism rates in primary school pupils.

We focus on primary schools because of the higher incidence of acute respiratory infections in younger students and because clusters can be readily identified given that students are assigned to a fixed group and classroom for the duration of a schoolyear.

As MACs produce noise and draft, take up space, consume electricity, need to be operated by school personnel and require regular maintenance, the pilot study investigated the feasibility and acceptability of the use of MACs in school settings. Overall, it was concluded that is was feasible to use MACs, operated by teachers on a daily basis. Yet, teachers experienced substantial negative effects from using them on classroom space, noise, draft and concentration. Students experienced mostly no or non-substantial effects. It was recommended to restrict the use of MACs to periods of high disease burden due to acute respiratory infections, when their potential impact on absenteeism is most pronounced.

### Objectives

### Primary objective:

To determine the effect of using MACs in classrooms during the peak season of respiratory infections on illness absenteeism rates in pupils in grade 1-8 of primary education.

### Secondary objectives:

To determine the effect of using MACs in classrooms during the peak season of respiratory infections on illness absenteeism rates in pupils, stratified by grades 1-4 and 5-8.

To determine the effect of using MACs in classrooms during the peak season of respiratory infections on level of microbiological contamination in air dust samples from classrooms.

To determine the effect of using MACs in classrooms during the peak season of respiratory infections on the proportion of absenteeism due to acute respiratory illness.

To determine the effect of using MACs in classrooms during the peak season of respiratory infections on ventilation practices (e.g. opening doors and windows) as measured by classroom CO2 concentrations.

To determine the effect of MACs on indoor air quality measured using the following parameters in a subset of classrooms and schools: CO2, Temperature, PM1.0, PM2.5, PM10, Volatile organic compounds

To determine the level of adherence to recommended use of MACs by teachers during school-hours.

### Exploratory objectives

To assess the correlation between the level to microbiological contamination in air dust samples from classrooms and absenteeism rates among pupils in the classroom.

To determine factors influencing adherence to recommended use of MACs by teacher and school characteristics.

### Trial design

This is a cluster randomized trial. The unit of randomization is the school. Each school will be randomly assigned to the intervention arm or the control arm. Schools in the control arm will not receive the intervention. There will be no blinding of the intervention.

### Intervention

The intervention is the use of MACs in school classrooms, where MACs are operating during school hours. Schools in the intervention arm will be equipped with MACs for all classrooms that host a fixed group of students each day of the school week.

MAC setup per classroom:

- two MACs, one placed at the front and one at the back of the classroom, with an upward air supply towards the occupied zone that can together achieve a desired CADR of 800-1000 m3/h for 30 students per classroom.
- The two MACs both in operation should preferably not lead to a noise level above 35 dB(A) at the applied setting, and the induced air velocities should stay below the requirement (< 0.2 m/s).</li>

### Study period

It is recommended to run the trial during the period with highest incidence of acute respiratory infections and related absenteeism. Based on the pilot study, the selected period should at least cover the school-weeks between the Christmas holiday and the Spring break. A simulation study was used to determine the required number of schools for the trial and the optimal duration and season for the trial. Based on the results, a study period starting the first week after the Christmas holiday (January) and running until early April (around Easter) is most efficient.

### Study population

The population for study consists of primary schools in the Netherlands. Schools are eligible to participate based on the following criteria:

- Schools with at least 100 registered students.
- Willing to accommodate MACs according to the specified setup in the classrooms and able to operate them on a daily basis.
- Electronic system for absenteeism reporting and able to provide weekly numbers of absenteeism days due to illness per group.
- Willing to receive weekly visits by the study team during the study period to perform study related tasks including handling of equipment for air quality monitoring in classrooms, installing or removing environmental air samplers, checking MAC settings, and collection of (aggregated) absenteeism data.

### Sample size and power

The required sample size, i.e. the number of primary schools participating in the trial, was calculated based on simulation studies (Appendix 1). For an assumed 25% reduction of the rate of absenteeism days due to illness, including and randomizing 100 primary schools yields a power that is estimated to be around 80% or higher. For an assumed 20% reduction, it is advised to randomize at least 160 schools.

### Recruitment

For recruitment, it is proposed to collaborate with national or subnational school unions, counsels or other organizations for outreach activities, to organize information sessions for schools and promote participation during other events or school visits by these entities. In addition, we strongly recommend offering financial or in-kind incentives to the schools for participation. This strategy is proposed based on experiences from the pilot study where we experienced an overall low interest in participation. Buy-in form many different stakeholders will be essential to reach the target recruitment numbers.

### Measurements

### Absenteeism

Absenteeism data for each participating school will be collected as number of days and episodes of absenteeism due to illness aggregated per group and per school-day. No identifiable personal information will be collected. The difference in days of illness absenteeism between schools in the intervention and control arms will be used to estimate the effect of MACs on illness absenteeism.

### Air quality indicators

In a subset of classrooms, we will measure parameters of indoor air quality, including airborne particles,  $CO_2$  and volatile organic compounds (VOCs). The difference in parameters of indoor air quality between intervention and control schools will be used to estimate the effect of MACs on indoor air quality in primary schools.

Airborne particles i.e. aerosols, can be either liquid droplets or solid particles coming from a variety of sources. Indoor sources include 1) respiratory droplets generated by humans when breathing, speaking, coughing, or sneezing, which are the ones responsible for respiratory disease transmission, 2) dust (e.g., human dander and textile fibers) which can be aerosolized from surfaces via human activities (e.g. sweeping the floor of rubbing clothes), and 3) mold. Outdoor sources include pollution particles generated by car traffic and nearby industrial sites carried by the wind, which may enter the room via natural (window opening) or mechanical ventilation. CO<sub>2</sub> is often used as a proxy for the presence of humans since human breath is the only source of CO<sub>2</sub> which, when doors and windows are closed, accumulates in the air without escaping. Volatile organic compounds (VOCs) are chemical gases that may or may not be smelled such as benzene, ethylene glycol, formaldehyde, methylene chloride, tetrachloroethylene, toluene, xylene, and 1,3-butadiene. Indoor sources include perfume, flatulence as well as cleaning products. They may also be released from building materials such as paint, varnishes, caulks, adhesives, carpets and vinyl flooring.

The following parameters will be monitored:

- CO<sub>2</sub> (carbon dioxide) concentration in ppm (part per million)
- PM<sub>1</sub> (airborne particles of diameter < 1 μm) concentration in μg/m3 (micrograms per cubic meter)
- PM<sub>2.5</sub> (airborne particles of diameter < 2.5 μm) concentration in μg/m3 (micrograms per cubic meter)</li>
- $PM_{10}$  (airborne particles of diameter < 10  $\mu$ m) concentration in  $\mu$ g/m3 (micrograms per cubic meter)
- TVOC (total volatile organic compounds) concentration in ppb (part per billion)

 $CO_2$  concentration will be measured by an MH-Z19B sensor (range: 0-2000 ppm, accuracy: ±50 ppm). PM<sub>2.5</sub> and PM<sub>10</sub> concentrations will be measured by a SDS011 sensor (range: 0-999.9 µg/m<sup>3</sup>, accuracy: ±10%). Total VOC (TVOC) concentration will be measured by an SGP30 sensor (range: 0-60000 ppb, accuracy: 1, 6 and 32 ppb in ranges 0-2008 ppb, 2008-11110 ppb, and 11110-60000 ppb, respectively).

In the monitored classrooms, the sensors will be integrated on one panel (hereafter referred as the "IAQ sensor"). Each monitored classroom will be equipped with two IAQ sensors and these will be connected to a central unit from which data is saved on a SD card, with a logging interval of 5 minutes. The IAQ sensors are preferably placed one in the back of the room and one on the opposite of the windows, not nearby a person.

For determining the cleaning effect of the MACs on respiratory aerosols rather than all aerosols in the air, we will also monitor the outdoor PM concentrations at each school close to the school buildings, simultaneously with the indoor measurements.

### Environmental contamination

To passively collect microbe carrying dust during the trial, the Electrostatic Dust Cloth (EDC) and a smaller version adapted for this project (Mini-EDC). EDCs consist of two electrostatic cloths secured in a plastic frame. These types of cloths are commercially available for household usage and use electrostatic charge to hold onto dust. The mini-EDC is a modified version where a 6x6 cm section was cut from a dust cloth and then placed in a petri dish. The advantage of this would be that the smaller sampler takes up significantly less space in the classroom and is easier to handle and requires fewer materials in the lab.

During the pilot study, it was demonstrated that the use of MACs in classroom was associated with a decrease in both the total microbial load as measured by 16S and the other microbial and viral targets. To further quantify the reduction in specific viral targets (e.g. RSV, influenza, hMPV, rhinovirus), environmental sampling will be conducted using the previously tested sampling protocol: Placement of EDC samplers in each classroom at least 2 meters high and, on a surface, where they will not be disturbed such as the top of a bookcase. Ideally, they should also be placed in a central location away from strong air flows such as from an open window. To avoid taking up surface space in the classrooms it is proposed to hang the samplers from the ceiling. EDC replacement every 3 weeks should occur. This period provides sufficient time for the dust containing microbes to settle on the sample plates while allowing for different rounds of sampling throughout the study period to capture differences in circulating viruses over the course of the winter season.

### Illness absenteeism survey

When a student is reported absent to the school due to illness, the parents/legal guardians of the child will be invited by their school to participate in an online anonymous illness survey on the main reason for absenteeism in the child and presence of (respiratory) symptoms. Eligible subjects will be invited on the first day of absenteeism by the school. Students and staff can participate more than once if fulfilling the eligibility criteria again during the study period.

### Other parameters

Basic school characteristics will be collected at baseline, including building characteristic, student population and number of student groups per school grade. For schools in the intervention arm, location of MACs in each classroom will be mapped on school floor plans as well as location of air quality sensors and dust samples. MAC operation and ventilation practices per classroom will be checked and logged during weekly school visits.

### Data analyses

For the primary objective, we will compare the mean incidence rate of illness absenteeism in schools with and without MACs over the study period. The incidence rate will be calculated by dividing the number of absent days by the total number of student school days during the observation time. Student school days are counted summing the number of school days per student during the study period multiplied by the number of students. Incidence rates of illness absenteeism will be calculated per school and per cluster (intervention versus control) and stratified by age-group. The primary endpoint analysis will be based on a negative binomial mixed-effects regression model, with random intercept per school and class. The model will allow random effects per school and will be adjusted for seasonality. Secondary endpoint analysis will include stratified models per age-group.

The analysis will be repeated for other secondary endpoints including the level of microbiological contamination in air dust samples from classrooms; the proportion of absenteeism due to acute respiratory illness; classroom CO<sub>2</sub> concentrations; and other indoor air quality measures (temperature, PM<sub>1.0</sub>, PM<sub>2.5</sub>, PM<sub>10</sub>, Volatile organic compounds)

### Ethical considerations

The study will be conducted according to 'gedragscode gezondheidsonderzoek' and in accordance with the EU GDPR (General Data Protection Regulation) and in accordance with local laws and regulations.

### Administrative aspects

### Handling and storage of data and documents

All data will be entered and stored in the datacapture application Castor with authorized access only. Illness survey data will be collected without any personal identifiable information. School absenteeism data will first be aggregated to student group level before being shared with the study team and stored within Castor. Data storage is in the Netherlands.

### Amendments

Amendments are changes made to the research after a Confirmation Quality Check has been received. Any change that may cause the investigation to fall within the scope of the WMO is submitted to the ethical committee after a quality check by the research quality coordinator of the division. Other changes must undergo further review by the research quality coordinator of the division.

Activity B: execution plan air cleaners

### Introduction

This activity builds on the results of a previous project conducted by TU Delft with a grant from the Ministry of Education (Grant no 1325610). In this project, several MACs were tested under experimental conditions at the Senselab of the TU Delft for their performance and to define the optimal settings of use. Selected models were further tested in the pilot field study in schools. We here report the technical performance of the selected MACs when used in classroom settings at primary schools. We also provide recommendations for their future use in classrooms.

### Background

Airborne transmitted pathogen-laden respiratory particles, also called respiratory aerosols, is considered an important transmission route of respiratory infectious diseases such as COVID-19 [1]. Such aerosols are released when people breathe, speak, cough, or sneeze. School classrooms often have dense occupancy and long occupied hours per day, and thus are high exposure areas for human released aerosols of potential infectious origin [2]. To tackle this problem, MACs have been proposed to be adopted as a supplementary solution for school classrooms with limited ventilation [3].

Hence, in our prior study [4] (Grant no. 1325610), a comprehensive assessment was conducted on different types of MACs to provide a reference for practical usage. To do so, firstly, 152 products were pre-selected after screening more than 300 products found in the market. Categorization and comparison were then made based on the technical specifications of the products, considering the feasibility and affordability. Eventually, seven types of MACs (MAC1 to MAC7) were selected for further assessments, covering different combinations of air cleaning technologies, induced airflow patterns, fan capacities, and dimensions. Accordingly, they were tested for different settings (i.e., fan levels) and configurations (including location and number of devices), in the Experience room of the SenseLab at Delft University of Technology [5], which is of the half size of a classroom (70 m<sup>3</sup>), and with a typical classroom interior setting. The assessments included: 1) an aerosol decay test: the time evolution of aerosol concentration was monitored after filling the room with aerosols generated by a specific spraying technique, to calculate the aerosol removal rate and clean air delivery rate (CADR), and 2) a panel perception test: a panel of subjects was recruited to assess noise and air movement generated by the MACs, combined with measurements of sound pressure level and air velocity. Based on the results, the optimal condition of each type of MAC was determined, with sufficient clear air and an acceptable noise level.

As a follow-up of [4], this study aims to investigate the feasibility of using the selected MACs in real classrooms. Firstly, they were tested in a real classroom at the university, and from there, three were selected for a field study carried out in Dutch primary schools.

### Test in a prototype classroom

### Methods

An aerosol decay test was conducted in a classroom at the Faculty of Architecture and the Built Environment of Delft University of Technology, during July 2023, as shown in Figure B1. The classroom has a volume of 139 m<sup>3</sup>, with six openable windows and one door. The classroom is equipped with a mechanical ventilation system with air supplies on both sides and an air exhaust in the middle of the ceiling. The windows and door were closed during the test, while the mechanical ventilation was kept on. The procedure of the test was the same as performed in the lab study [4], with the same instruments and setup. An aerosol generator developed by [6] was used to continuously spray aerosols into the room during the build-up phase. Once the room was filled with aerosols, the aerosol generator was turned off, and the MACs were turned on to start the decay phase. The decay phase ended when the aerosol concentration decreased to a relatively low level (<  $5 \mu g/m^3$ ). The concentrations of PM<sub>2.5</sub> and PM<sub>10</sub> were monitored by six NOVA SDS011 PM sensors, which were evenly distributed in the room on six tables. One natural decay test was also performed without any MAC operating.



Figure B1. Set-up of the aerosol decay test in the classroom.

As previously mentioned, for each MAC, one condition was tested based on the results of the experimental study [4], as specified in Table 1. All the MACs were tested at the highest setting, except for MAC-7, which was tested at a low setting.

### Table B1. Conditions of the aerosol decay test of the MACs.

Device	Number of devices	Setting	Location
MAC1	2	10	Diagonally at 2 corners
MAC2	4	2	4 corners

MAC3	4	4	4 corners
MAC4	2	2	Diagonally at 2 corners
MAC5	1	5	Centre of front wall
MAC6	2	8	Diagonally at 2 corners
MAC7	2	4	Diagonally at 2 corners

The aerosol removal rate and CADR of the MACs were also calculated according to the methods used in [4]. The total decay and natural decay curves were described as equation (1):

$$C(t) = C_{\infty} + (C_0 - C_{\infty})e^{-kt}, \ k = k_{total} \text{ or } k_n$$
(1)

Where:

- *C* is the aerosol concentration [µg/m<sup>3</sup>]
- *t* is the time after the decay process starts [h]
- $C_0$  is the aerosol concentration when  $t = 0 [\mu g/m^3]$
- $C_{\infty}$  is the aerosol concentration when  $t >> k^{-1} [\mu g/m^3]$
- *k* is the decay coefficient [h<sup>-1</sup>]
- $k_{total}$  is the coefficient of the total decay, here also the total aerosol removal rate  $[h^{-1}]$
- *k<sub>n</sub>* is the coefficient of the natural decay [h<sup>-1</sup>]

The aerosol removal rate of the MACs  $k_{mac}$  [h<sup>-1</sup>] and CADR [m<sup>3</sup>/h] were then calculated using equation (2) and (3):

$$k_{mac} = k_{total} - k_n \tag{2}$$

$$CADR = k_{mac} \times room \ volume \tag{3}$$

### **Results and discussion**

The results of CADR of the tested MACs are presented in Figure 2, for both PM<sub>2.5</sub> and PM<sub>10</sub>. The minimum amount of ventilation ("clean" air) required by the Dutch Building Decree [7] in school classrooms is 8.5 l/s/p, while the recommended amount of ventilation for a good IAQ is 10 l/s/p [8]. Assuming a student occupancy of 30 persons, then the range of CADR is 918-1080 m<sup>3</sup>/h, as marked in the figures. For PM<sub>2.5</sub>, the CADR of MAC4 and MAC6 reached the minimum requirement, while MAC2 and MAC3 exceeded the recommended level. For PM<sub>10</sub>, the CADR of MAC5 passed the minimum requirement, while MAC2, MAC3, MAC4, and MAC6 were all above the recommended level. Among all the MACs, MAC1 always showed the lowest amount of CADR, which was possibly due to the horizontal design of the air supply, and thus, the clean air could not be effectively diffused throughout the room. Therefore, such MAC is not ideal for school classrooms. For MAC2 to MAC6, although similar results were found in the lab test [4], in the real classroom, the MACs tested

with four devices, i.e., MAC2 and MAC3, showed much higher CADR than the others, while the one tested with one device, i.e., MAC5, was much lower than the others, and the two tested with two devices, i.e., MAC4 and MAC6, were in between. This indicates the necessity of using multiple devices when the room size increases. Nevertheless, according to the results of the panel perception test conducted in [4], both MAC2 and MAC3 generated very loud noise which exceeded far beyond the limited level (35 dB(A) prescribed in [9]), and was considered to be not acceptable by the subjects, whereas MAC4 and MAC6 produced less noise and were more acceptable, and thus were more suitable to be used in practice. In addition, as MAC-7 was tested at the low setting, the noise level was well below the limit, yet a CADR of around 800 m<sup>3</sup>/h was still reached in the real classroom, which was thus suggested for use in practice as well.



**Figure B2**. CADR of PM<sub>2.5</sub> (left) and PM<sub>10</sub> (right) of the tested mobile air cleaners in the classroom. The minimum requirement and recommended value were calculated based on the assumption of a student occupancy of 30 persons.

Moreover, compared to the results of the lab test [4], in the real classroom, the CADR of all the MACs was found to be increased by 10% to 47% for both PM2.5 and PM10, except for MAC1. This could be due to the mechanical ventilation present in the classroom, which helped mixing the air during the decay phase, and most likely accelerated the aerosol removal, as  $k_n$  was found to have increased by 1.36 times compared to  $k_n$  in the lab. This indicates the potential of combining mechanical ventilation and MACs in school classrooms for a better aerosol removal.

### Field study

#### **Methods**

#### Selection of schools and classrooms

Following the studies conducted in the lab and the university classroom, a field study was carried out in November and December 2023. Five primary schools (denoted as School1 to School5) were enrolled in this study voluntarily. Among the five schools, School1 has eight classrooms, School3 has ten classrooms, and the rest have more than ten classrooms. Therefore, for School1 and School3, all classrooms were involved in this study, while in other schools nine classrooms were selected, which resulted in 45 classrooms in total. The selected classrooms cover all ages groups of students at the schools (5-12 years old) and were coded from 1 to n (n is the number of classrooms selected in each school). The classrooms are of a similar floor area of 40-50 m<sup>2</sup>, with a typical occupancy of 20-25 persons. All the classrooms have multiple openable windows and doors for natural ventilation, while only the classrooms in School1 are also equipped with a balanced mechanical ventilation system.
## Installation and operation of the mobile air cleaners

According to the results of the prior tests, three MACs, namely MAC4, MAC6, and MAC7, hereafter referred to as MAC-A, MAC-B, and MAC-C, respectively, were selected to be applied in the selected classrooms. The detailed information on the selected MACs is listed in Table B2. In School1, two classrooms were assigned with MAC-C, three were assigned with MAC-A, and three with MAC-B. In School3, four classrooms were assigned with MAC-C, three evenly assigned among the nine classrooms. The distribution of MACs among the classrooms was determined on a random basis, and each classroom had two devices, as determined in the prior tests.

Device <sup>a</sup>	Air cleaning technology <sup>be</sup>	Airflow pattern	Fan capacity (m³/h) <sup>b</sup>	Efficiency <sup>c</sup>	Noise level [dB(A)] <sup>c</sup>	Dimensions (cm) <sup>c</sup>	Number of devices	Price (including VAT)⁰ (€)
MAC-A	ES + AC		735	H13	27-55	34.0 × 34.0 × 85.5	2	1100
MAC-B	НЕРА		565	H13	18-51	33.2 x 33.6 x 60.6	2	500
MAC-C	НЕРА		750	H13	26-65	68.8 (Φ) x 25.4	2	1500

Table B2. Information on the selected mobile air cleaners.

<sup>a</sup> MAC: mobile air cleaner.

<sup>b</sup> ES: electrostatics; AC: activated carbon.

<sup>c</sup> As specified by the brand.

The schools were visited during the first week of November 2023, and the MACs were brought inside the classroom. Theoretically, the MACs should be placed in accordance with the locations mentioned in Table B1. However, it was observed that most of the classrooms were quite crowded and stuffed, leaving limited space for placing the MACs, and thus the locations needed to be adjusted from classroom to classroom. Still, it was ensured that in each classroom, one device was placed in the front and one in the back, with the air supply facing the occupied area. Furthermore, many of the classrooms were in short of power supplies/sockets, and thus extension cords and splitters were needed to properly plug all the electronic devices in the room.

For MAC-A and MAC-B, it is suggested to operate at the maximum setting, while for MAC-C it is suggested to use the low setting, as specified in Table B1. Instructions on how to switch on/off the

devices and how to set them to the suggested settings were provided on each device by the researchers.

Starting from the second week of November 2023, the field study lasted for six weeks, which consisted of two periods of three weeks with the MACs being turned ON and OFF, respectively. In School1, School4, and School5 the MACs started with the ON period, where the school directors and teachers were instructed to turn on/off the devices at the beginning/end of each school day during the first three weeks. Then the MACs were turned off for three weeks. For School2 and School3 it was performed in the opposite manner, with three weeks OFF followed by three weeks ON.

## Monitoring indoor air quality in the classrooms

The main indoor air quality (IAQ) parameters investigated in this study were aerosols, CO<sub>2</sub>, and volatile organic compounds (VOCs). Airborne particles, i.e. aerosols, can be either liquid droplets or solid particles coming from a variety of sources. Indoor sources include 1) respiratory droplets generated by humans when breathing, speaking, coughing, or sneezing, which are the ones responsible for respiratory disease transmission, 2) dust (e.g., human dander and textile fibres) which can be aerosolised from surfaces via human activities (e.g. sweeping the floor or rubbing clothes), and 3) mould. Outdoor sources include pollution particles generated by car traffic and nearby industrial sites carried by the wind, which may enter the room via natural (window opening) or mechanical ventilation. CO<sub>2</sub> is often used as a proxy for the presence of humans since human breath is the only source of CO<sub>2</sub> which, when doors and windows are closed, accumulates in the air without escaping. VOCs are chemical gases that may or may not be smelled such as benzene, ethylene glycol, formaldehyde, methylene chloride, tetrachloroethylene, toluene, xylene, and 1,3-butadiene. Indoor sources include perfume, flatulence as well as cleaning products. They may also be released from building materials such as paint, varnishes, caulks, adhesives, carpets and vinyl flooring.

In each school, IAQ was monitored in three classrooms equipped with different MACs (namely one with MAC-A, one with MAC-B, and one with MAC-C). The following parameters were monitored:

- CO2 (carbon dioxide) concentration in ppm (part per million)
- $PM_{2.5}$  (airborne particles of diameter < 2.5  $\mu m)$  concentration in  $\mu g/m^3$  (micrograms per cubic meter)
- $PM_{10}$  (airborne particles of diameter < 10  $\mu m)$  concentration in  $\mu g/m^3$  (micrograms per cubic meter)
- TVOC (total volatile organic compounds) concentration in ppb (part per billion)

CO<sub>2</sub> concentration was measured by an MH-Z19B sensor (range: 0-2000 ppm, accuracy: ±50 ppm). In schools 1, 2 and 4, PM<sub>2.5</sub> and PM<sub>10</sub> concentrations were measured by a SDS011sensor (range: 0-999.9  $\mu$ g/m<sup>3</sup>, accuracy: ±10%) [10]. In schools 3 and 5, PM<sub>2.5</sub> and PM<sub>10</sub> concentrations were measured by a PMS5003 sensor (range: 0-5000  $\mu$ g/m<sup>3</sup>, accuracy: ±10% in the range of PM<sub>2.5</sub> and PM<sub>10</sub> concentrations <100  $\mu$ g/m<sup>3</sup>) [11]. Total VOC (TVOC) concentration was measured by an SGP30 sensor (range: 0-60000 ppb, accuracy: 1, 6 and 32 ppb in ranges 0-2008 ppb, 2008-11110 ppb, and 11110-60000 ppb, respectively).

In the monitored classrooms, the sensors were integrated on one panel (hereafter referred as the "IAQ sensor") and were all connected to a central unit from which data is saved on a SD card, with a logging interval of 5 minutes. The IAQ sensors were mostly placed on the teachers' desk.

#### Data analysis

During the data cleaning process, it was found that although the IAQ sensors worked properly most of the time during the six-week period, some still showed no data for up to two days probably due to the teacher unplugging them by mistake and plugging them back on later. Furthermore, the IAQ sensor in the classroom of School5 equipped with MAC-A stopped working soon after being installed and could not be fixed, and thus no result can be reported. In addition, due to the days when the schools were off, e.g. Sinterklaas break on Wednesday December 6<sup>th</sup> 2023, as well as when the teachers had forgotten to turn the MACs on, in total 30 days out of 30×14 days (30 days for 14 classrooms) were excluded for data analysis, i.e. 7% of the expected amount of data.

The data was further trimmed based on the schedule of each classroom, namely the unoccupied hours were excluded. The concentrations of the IAQ parameters in each classroom were compared between the ON and OFF periods using Mann-Whitney tests, and the average concentrations of all classrooms were compared between the ON and OFF periods using Wilcoxon signed rank tests, all performed in IBM SPSS v28. The significance level was set at 0.05 (p < 0.05).

#### **Results and discussion**

The distributions of the daily average concentrations (excluding unoccupied hours) of the four IAQ parameters are shown in Figure B3 using violin plots. Each classroom features two violins labeled '1' and '2', corresponding to the 15 working days of the first and second period, respectively, with a color code indicating if the air cleaner was ON (green) or OFF (red) during that period. It is observed that the PM<sub>2.5</sub> and PM<sub>10</sub> concentrations were systematically lower during the ON period compared to the OFF period for all classrooms, while on the contrary CO<sub>2</sub> and TVOC concentrations could be lower during the ON period for some classrooms but higher for others. It is also noted that the range of daily average PM<sub>2.5</sub> and PM<sub>10</sub> concentrations is systematically narrower during the ON period than the OFF period, while no such conclusion can be drawn for the CO<sub>2</sub> and TVOC daily average concentrations which often span similar ranges for a given classroom.

It is also worth noting that for PM<sub>2.5</sub> and PM<sub>10</sub> concentrations most violins have a thick bottom part and a slender upper part. This indicates that most days feature a low average PM concentration, while a few days feature a significantly larger one. This could suggest a correlation with the outdoor air which might feature days of higher industrial pollution or higher traffic flow. However, for further investigation, measurements of outdoor air quality at each school need to be carried out.

The mean and standard deviation of the concentrations of the four IAQ parameters over the ON and OFF periods, as well as the results of the Mann-Whitney tests, are presented in Table B3 and B4, respectively. For PM<sub>2.5</sub>, the mean concentration at each classroom ranged from 0.87 to  $3.76 \ \mu g/m^3$  during the ON period and from 2.22 to  $10.36 \ \mu g/m^3$  during the OFF period, which in general increased by a factor of 2 from the ON period to the OFF period. For PM<sub>10</sub>, the mean concentration in each classroom ranged from  $3.48 \ to 16.20 \ \mu g/m^3$  during the OFF period, which also increased by a factor of 2 from the OFF period.

period. For CO<sub>2</sub>, the mean concentration at each classroom ranged from 777.2 to 1277.9 ppm during the ON period and from 786.6 to 1302.8 ppm during the OFF period, which are in general similar. For TVOC, the mean concentration in each classroom ranged from 544.5 to 7105.9 ppb during the ON period and from 537.5 to 5758.8 ppb during the OFF period, which is also similar between the ON and OFF periods.

The comparison for each classroom clearly showed that for both PM<sub>2.5</sub> and PM<sub>10</sub>, the concentrations when the MACs were turned on were significantly lower than when the MACs were turned off in all monitored classrooms (except for the classroom of school 2 equipped with MAC-A for PM<sub>2.5</sub>), regardless of the type of MAC or whether the classroom started with the ON period or the OFF period. Such results align with the findings of the previous tests conducted in the lab and the university classroom, which indicate the good capability of the selected MACs to remove aerosols in primary school classrooms. However, the differences in CO<sub>2</sub> and TVOC concentrations between the ON and OFF periods are also found to be significant in most of the classrooms. Yet unlike PM<sub>2.5</sub> and PM<sub>10</sub>, for CO<sub>2</sub> and TVOC, the concentrations can be both higher or lower during the ON period compared to the OFF period, varying among the classrooms.

Hence, to further determine that the lower concentration of PMs found in the ON period was mainly due to the operation of MACs instead of other factors (such as changes of situation in the monitored classrooms), a Wilcoxon signed rank test was performed for each IAQ parameter between the ON and OFF periods, using the two groups of classroom-averaged concentrations as the paired samples. The results are shown in Table B4. It is clearly seen that when grouping all the monitored classrooms, the differences between the ON and OFF periods are only significant for PM<sub>2.5</sub> and PM<sub>10</sub>, which confirms the previous statement. It is also indicated that such MACs had no effect on CO<sub>2</sub> concentrations, as expected, but also on TVOC, although MAC-A is claimed to also have an activated carbon filter.



Figure B3. Violin plots of the daily average concentration of (a) PM<sub>2.5</sub>, (b) PM<sub>10</sub>, (c) CO<sub>2</sub>, and (d) VOC in each classroom for the first ('1') and second ('2') period. The green and red violins correspond to the ON and OFF period, respectively. The mean value each period is indicated by the grey horizontal segments in each violin.

# Table B3. Results of the Mann-Whitney tests.

MAC	PM <sub>2.5</sub> (μg/m <sup>3</sup> )		PM <sub>10</sub> (μg/m <sup>3</sup> )		CO <sub>2</sub> (ppm)			TVOC (ppb)				
	ON*	OFF*	р	ON*	OFF*	р	ON*	OFF*	р	ON*	OFF*	р
MAC-B	0.6 (0.4-0.8)	1.8 (1.0-3.1)	< 0.001	4.5 (3.4-5.7)	7.1 (5.7-9.1)	< 0.001	1100 (940-1273)	1199 (1069-1379)	< 0.001	1047 (487-1976)	1241 (685-2293)	< 0.001
MAC-C	1.1 (0.9-1.6)	3.3 (2.1-4.7)	< 0.001	5.3 (4.0-6.9)	9.6 (7.3-13.1)	< 0.001	1033 (893-1241)	1102 (941-1243)	< 0.001	898 (558-1240)	1060 (595-1652)	< 0.001
MAC-A	1.2 (0.9-1.9)	2.9 (1.8-4.5)	< 0.001	4.7 (3.4-6.7)	8.6 (6.8-11.8)	< 0.001	890 (783-1073)	1040 (909-1235)	< 0.001	953 (469-1281)	1071 (634-1861)	< 0.001
MAC-B	2.0 (1.1-3.8)	2.5 (1.9-3.8)	< 0.001	6.0 (4.3-8.3)	10.7 (8.6-14.8)	< 0.001	839 (728-923)	776 (698-843)	< 0.001	659 (391-1534)	445 (255-676)	< 0.001
MAC-C	1.4 (0.9-2.2)	2.5 (2.0-3.2)	< 0.001	7.0 (5.5-9.1)	15.3 (12.1-18.9)	< 0.001	1242 (946-1525)	988 (830-1189)	< 0.001	2254 (1237-3705)	1519 (794-2646)	< 0.001
MAC-A	2.3 (1.1-4.0)	2.3 (1.7-3.3)	0.040	5.9 (3.5-10.0)	9.1 (6.9-11.6)	< 0.001	752 (686-823)	757 (680-837)	0.518	372 (244-557)	442 (257-745)	< 0.001
MAC-B	1.0 (0.0-3.0)	4.0 (2.0-7.0)	< 0.001	2.0 (0.0-4.0)	4.0 (2.0-8.0)	< 0.001	850 (735-955)	834 (733-923)	0.024	3630 (1563-7043)	2196 (1309-3369)	< 0.001
MAC-C	2.0 (1.0-4.0)	3.0 (2.0-5.0)	< 0.001	3.0 (1.0-5.0)	5.0 (3.0-7.0)	< 0.001	937 (830-1189)	896 (654-1072)	< 0.001	1792 (863-3327)	941 (346-2127)	< 0.001
MAC-A	1.3 (1.0-2.4)	3.2 (2.1-5.1)	< 0.001	5.5 (4.1-7.4)	9.8 (7.4-13.1)	< 0.001	1135 (961-1356)	1327 (1050-1548)	< 0.001	1812 (770-2717)	3123 (2096-6060)	< 0.001
MAC-B	1.2 (0.9-2.2)	2.5 (1.6-4.0)	< 0.001	7.6 (5.3-10.2)	8.6 (6.5-11.6)	< 0.001	873 (748-985)	896 (748-984)	0.460	807 (493-1513)	764 (354-1427)	0.002
MAC-C	0.9 (0.6-1.5)	1.8 (1.1-2.8)	< 0.001	3.5 (2.3-5.1)	5.3 (3.8-7.5)	< 0.001	1144 (903-1373)	1136 (937-1326)	0.660	1633 (800-2995)	2323 (1010-4417)	< 0.001
MAC-A	1.3 (1.0-2.4)	3.2 (2.1-5.1)	< 0.001	5.5 (4.1-7.4)	9.8 (7.4-13.1)	< 0.001	1135 (961-1356)	1327 (1050-1548)	< 0.001	1812 (770-2717)	3123 (2097-6060)	< 0.001
MAC-B	1.0 (0.0-3.0)	8.0 (3.0-16.0)	< 0.001	2.0 (1.0-4.0)	10.0 (5.0-17.0)	< 0.001	720 (569-954)	830 (648-1010)	< 0.001	923 (477-1584)	630 (287-1343)	0.648
MAC-C	1.0 (0.0-2.0)	6.0 (3.0-10.8)	< 0.001	1.0 (0.0-3.0)	6.0 (3.0-11.0)	< 0.001	1047 (909-1207)	1159 (967-1324)	< 0.001	547 (386-824)	683 (470-1044)	< 0.001
MAC-A	-	-	-	-	-	-	-	-	-	-	-	-
	MAC MAC-B MAC-C MAC-A MAC-B MAC-C MAC-A MAC-B MAC-C MAC-A MAC-B MAC-C MAC-A MAC-C MAC-A	MAC         ON*           MAC-B         0.6 (0.4-0.8)           MAC-C         1.1 (0.9-1.6)           MAC-A         1.2 (0.9-1.9)           MAC-B         2.0 (1.1-3.8)           MAC-B         2.0 (1.1-3.8)           MAC-A         2.3 (1.1-4.0)           MAC-B         1.0 (0.0-3.0)           MAC-C         2.0 (1.0-4.0)           MAC-B         1.2 (0.9-2.2)           MAC-B         1.2 (0.9-2.2)           MAC-B         1.2 (0.9-2.2)           MAC-C         0.9 (0.6-1.5)           MAC-A         1.3 (1.0-2.4)           MAC-B         1.0 (0.0-3.0)           MAC-B         1.0 (0.0-3.0)           MAC-A         1.3 (1.0-2.4)	MAC         PM2.5 (μg/m³)           ON*         OFF*           MAC-B         0.6 (0.4-0.8)         1.8 (1.0-3.1)           MAC-B         0.6 (0.4-0.8)         1.8 (1.0-3.1)           MAC-C         1.1 (0.9-1.6)         3.3 (2.1-4.7)           MAC-A         1.2 (0.9-1.9)         2.9 (1.8-4.5)           MAC-B         2.0 (1.1-3.8)         2.5 (1.9-3.8)           MAC-B         2.0 (1.1-3.8)         2.5 (1.9-3.8)           MAC-B         2.0 (1.1-4.0)         2.3 (1.7-3.3)           MAC-A         2.3 (1.1-4.0)         2.3 (1.7-3.3)           MAC-B         1.0 (0.0-3.0)         4.0 (2.0-7.0)           MAC-B         1.0 (0.0-3.0)         4.0 (2.0-7.0)           MAC-C         2.0 (1.0-4.0)         3.0 (2.0-5.0)           MAC-A         1.3 (1.0-2.4)         3.2 (2.1-5.1)           MAC-B         1.2 (0.9-2.2)         2.5 (1.6-4.0)           MAC-A         1.3 (1.0-2.4)         3.2 (2.1-5.1)           MAC-A         1.3 (1.0-2.4)         3.2 (2.1-5.1)           MAC-A         1.0 (0.0-3.0)         8.0 (3.0-16.0)           MAC-A         1.0 (0.0-2.0)         6.0 (3.0-10.8)           MAC-A         -         -	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	MAC $PM_{2.5} (\mu g/m^3)$ IMAC-B $ON^*$ $OFF^*$ p $ON^*$ MAC-B $0.6 (0.4 - 0.8)$ $1.8 (1.0 - 3.1)$ $< 0.001$ $4.5 (3.4 - 5.7)$ MAC-C $1.1 (0.9 - 1.6)$ $3.3 (2.1 - 4.7)$ $< 0.001$ $5.3 (4.0 - 6.9)$ MAC-A $1.2 (0.9 - 1.9)$ $2.9 (1.8 - 4.5)$ $< 0.001$ $4.7 (3.4 - 6.7)$ MAC-B $2.0 (1.1 - 3.8)$ $2.5 (1.9 - 3.8)$ $< 0.001$ $6.0 (4.3 - 8.3)$ MAC-B $2.0 (1.1 - 4.0)$ $2.3 (1.7 - 3.3)$ $0.040$ $5.9 (3.5 - 10.0)$ MAC-A $2.3 (1.1 - 4.0)$ $2.3 (1.7 - 3.3)$ $0.040$ $5.9 (3.5 - 10.0)$ MAC-B $1.0 (0.0 - 3.0)$ $4.0 (2.0 - 7.0)$ $< 0.001$ $2.0 (0.0 - 4.0)$ MAC-B $1.2 (0.9 - 2.2)$ $2.5 (1.6 - 4.0)$ $< 0.001$ $3.0 (1.0 - 5.0)$ MAC-B $1.2 (0.9 - 2.2)$ $2.5 (1.6 - 4.0)$ $< 0.001$ $5.5 (4.1 - 7.4)$ MAC-B $1.2 (0.9 - 2.2)$ $2.5 (1.6 - 4.0)$ $< 0.001$ $3.5 (2.3 - 5.1)$ MAC-B $1.0 (0.0 - 3.0)$ $8.0 (3.0 - 16.0)$ $< 0.001$ $5.5 (4.1 - 7.4)$ MAC-A $1.3 (1.0 - 2.4)$ $3.2 (2.1 - 5.1)$ $< 0.001$ $5.5 (4.1 - 7.4)$ MAC-B $1.0 (0.0 - 3.0)$ $8.0 (3.0 - 16.0)$ $< 0.001$ $1.0 (0.0 - 3.0)$ MAC-A $    -$	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	MAC         PM225 (µg/m³)         PM36 (µg/m³)         CC2 (ppm)         TVOC (ppb)           MAC         ON*         OFF*         p         ON*         OOF         C.0.01         1040 (909-1235)         C.0.01         1040 (909-1235)         C.0.01         526 (4525 (556-66)         TA*         <

Concentration: median (interquartile range).

Table B4. Results of the Wilcoxon signed rank tests.

IAQ parameter	ON*	OFF <sup>*</sup>	р	
PM <sub>2.5</sub> (μg/m³)	1.2 (1.0-1.55)	3.0 (2.5-3.5)	0.001	
PM <sub>10</sub> (μg/m <sup>3</sup> )	5.0 (2.8-5.9)	8.9 (5.8-9.9)	< 0.001	
CO <sub>2</sub> (ppm)	985 (847-1135)	1014 (833-1169)	0.158	
TVOC (ppb)	1000 (770-1812)	1066 (670-2228)	0.925	

\* Concentration: median (interquartile range).

Nonetheless, since no other filtration was adopted for the outdoor air coming into the monitored classrooms (via either natural ventilation or mechanical ventilation), as well as that the IAQ sensor used in this study was not able to differentiate the source of the aerosols detected, how good were the selected MACs at reducing respiratory aerosols cannot be determined. Still, a general conclusion can be drawn that the MACs effectively removed all kinds of aerosols in the classrooms.

The significant differences found in CO<sub>2</sub> and TVOC for individual classrooms might be due to the substantial variation of the concentrations throughout the day. Appendix B.I presents the examples of the time evolution of the IAQ parameters over two featured days (06:00-18:00): 1) Monday November 13<sup>th</sup>, 2023, representing the ON period for schools 1, 4 and 5, and the OFF period for schools 2 and 3; 2) Tuesday November 28<sup>th</sup>, 2023, representing the ON period for schools 2 and 3; and the OFF period for schools 1, 4, and 5.

## Conclusions

This field study was conducted in five primary schools, based on the findings of a previous experimental study, which determined the type of MACs adopted, as well as the settings and location of the MACs in the classroom. Forty-five classrooms were assigned with MACs, among which 15 were monitored for six weeks, with three weeks the MACs turned on and three weeks the MACs turned off.

The results showed that in spite of the limited space available in the classrooms, in many cases the MACs were not able to be placed exactly at the pre-determined locations by the experimental study [4], by keeping one primary rule, i.e. one device in the front and one in the back, with air supply towards the occupied zone, the MACs proved to work well. All MACs clearly reduced the concentrations of  $PM_{2.5}$  and  $PM_{10}$  in the classrooms when turned on as compared to the off state.

Although the measurements of the IAQ parameters at only one point in the classroom (nearby the teacher) is not representative of the whole classroom [12], for this pilot study it was enough to show the effect of the MACs. For future study on whether MACs can clean the air homogenously in the classroom, more sampling points should be included.

For determining the cleaning effect of the MACs on respiratory aerosols rather than all aerosols in the air, it is recommended to also monitor the outdoor PM concentrations at each school close to the school buildings, simultaneously with the indoor measurements. In addition, since respiratory

aerosols that linger in the air can be as small as 0.3-0.5  $\mu$ m, monitoring the concentration of PM<sub>1.0</sub> should be considered as well.

# Recommendations for future studies

Based on a lab study [13], a study in a classroom at the Faculty of Architecture and the Built Environment [14], and a field study in 15 classrooms, the following recommendations for future studies on the effect MACs on the health and comfort of children and teachers in classrooms can be made:

MACs:

- It is recommended to use two MACs, one placed at the front and one at the back of the classroom, with an upward air supply towards the occupied zone that can together achieve a desired CADR of 800-1000 m<sup>3</sup>/h for 30 students per classroom.
- The two MACs both in operation should preferably not lead to a noise level above 35 dB(A) at the applied setting, and the induced air velocities should stay below the requirement (< 0.2 m/s).</li>

Air quality measurements:

- It is recommended to monitor the IAQ parameters (CO<sub>2</sub>, PM<sub>2.5</sub>, PM<sub>10</sub>, VOC), as well as temperature (T) and relative humidity (RH), at two sampling points or more in the classroom: preferably in the back of the room and on the opposite of the windows, not nearby a person [15].
- Since respiratory aerosols that linger in the air can be as small as 0.3-0.5 μm, monitoring the concentration of PM<sub>1.0</sub> should be considered as well.
- Sampling points for monitoring outdoor CO<sub>2</sub>, T, RH, PM<sub>2.5</sub> and PM<sub>10</sub> should be included, the location and number of outdoor sampling points should depend on presence of fine particle sources in the area surrounding the school.

Checklist classroom:

- It is recommended to note the number of students in the classroom per day (if possible, per lesson/teaching hours during the time the classroom is occupied) during the monitoring period (with that information and the CO<sub>2</sub> measurements indoors and outdoors the ventilation rate can be determined).
- Additionally, the setting of the MACs and the setting of the mechanical ventilation system, if present, should be noted daily.
- Moreover, a simple method to monitor the opening and closing of windows could be added.

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# Appendix B.I

Examples of the time evolution of the IAQ parameters over two featured days (06:00-18:00): 1) Monday November 13<sup>th</sup>, 2023, representing the ON period for schools 1, 4 and 5, and the OFF period for schools 2 and 3; 2) Tuesday November 28<sup>th</sup>, 2023, representing the ON period for schools 2 and 3, and the OFF period for schools 1, 4, and 5.



**Figure Appendix B1**. Time evolution of PM2.5, PM10, CO2, and VOC concentrations in the classrooms monitored in School1, between 06:00 and 18:00 on 1) a day during the 1st period: Monday November 13th, 2023 (left) and 2) a





**Figure Appendix B2**. Time evolution of PM2.5, PM10, CO2, and VOC concentrations in the classrooms monitored in School2, between 06:00 and 18:00 on 1) a day during the 1st period: Monday November 13th, 2023 (left) and 2) a day during the 2nd period: Tuesday November 28th, 2023 (right). The background colour indicates whether the MAC is turned on (green) or off (red) on that day. Light data points correspond to unoccupied hours.



**Figure Appendix B3**. Time evolution of PM2.5, PM10, CO2, and VOC concentrations in the classrooms monitored in School3, between 06:00 and 18:00 on 1) a day during the 1st period: Monday November 13th, 2023 (left) and 2) a day during the 2nd period: Tuesday November 28th, 2023 (right). The background colour indicates whether the MAC is turned on (green) or off (red) on that day. Light data points correspond to unoccupied hours.



**Figure Appendix B4.** Time evolution of PM2.5, PM10, CO2, and VOC concentrations in the classrooms monitored in School4, between 06:00 and 18:00 on 1) a day during the 1st period: Monday November 13th, 2023 (left) and 2) a day during the 2nd period: Tuesday November 28th, 2023 (right). The background colour indicates whether the MAC is turned on (green) or off (red) on that day. Light data points correspond to unoccupied hours.



**Figure Appendix B5**. Time evolution of PM2.5, PM10, CO2, and VOC concentrations in the classrooms monitored in School5, between 06:00 and 18:00 on 1) a day during the 1st period: Monday November 13th, 2023 (left) and 2) a day during the 2nd period: Tuesday November 28th, 2023 (right). The background colour indicates whether the MAC is turned on (green) or off (red) on that day. Light data points correspond to unoccupied hours.

Activity C: Developing an environmental sampling plan

#### Introduction

The main objective of this activity was to develop an environmental sampling protocol to allow to study the effect of MACs in classrooms on microbial air levels in primary schools. Activity C describes the research conducted to develop the protocol and the application in a pilot study in actual classrooms. The insights from this study can support future research to study reduction effectiveness of MACs of microbiological contamination.

#### Background

Transmission of respiratory viruses and bacteria occurs from person to person by droplets of exhaled air from an infected person through breathing, talking, coughing and sneezing. Large exhaled droplets have a relatively small reach as gravity swiftly directs them towards the ground. Smaller 'aerosolized' droplets can linger in the air for longer time and therefore cross larger distances. Air currents can further increase the distance they can travel in indoor environments. Consequently, aerosolized droplets that contain infectious virus or bacteria can persist in air and may be able to infect persons that share the same indoor environment, even when they aren't near each other. It is hypothesized that a reduction of these aerosolized infected droplets in indoor environments can help reduce person-to-person transmission, which is the rationale for applying air purification techniques to remove these from the air. While the occurrence of person-to-person transmission through infectious aerosolized droplets cannot be directly observed, several techniques have been developed to quantify the presence of virus or bacteria containing aerosolized droplets in the air of indoor environments. By measuring the presence and amount of virus and bacteria (e.g. microbial contamination) present in air samples the potential for exposure in these environments can be inferred and compared between environments. Because these viruses and bacteria originate from infected persons, presence of specific viruses in air samples can also confirm circulation of the virus among the persons present in the room.

This activity aimed at establishing the optimal method to collect and test air samples from classrooms, to determine their level of microbial contamination in air for future large-scale application.

#### Investigating different air sampling techniques

To study microbiological contamination of classroom air in classrooms, we selected scalable and non-intrusive air sampling methods, that could easily be scaled up for a larger trial or for surveillance purposes. For these goals a simple, straight forward method, not needing expensive or noisy sampling equipment is advantageous. We first screened the literature on passive methods for collecting environmental microbial samples. The literature review was done previously and sought to collect the information available on the use of electrostatic dust cloths (EDCs) in the passive collection of airborne microbes. The review involved the systematic screening of 3,078 papers. In the process, other methods for passive microbial sampling were also found which helped inform our early method selection process. From these we selected three of the most practical and effective methods and organized pretesting to investigate practical implications. These three methods were the Electrostatic Dust Cloth (EDC), a smaller modified version of the EDC (mini-EDC), and the settle plate method. All three methods rely on the settlement of 'air-dust' on surfaces. By positioning the air-dust samplers at elevation (>2 meters) in the room, settlement of dust from other sources than air (e.g. floor, objects, persons) is limited.

EDCs consist of two electrostatic cloths secured in a plastic frame. These types of cloths are commercially available for household usage and use electrostatic charge to hold onto dust. The mini-EDC is a modified version where a 6x6cm section was cut from a dust cloth and then placed in a petri dish. The advantage of this would be that the smaller sampler takes up significantly less space in the classroom and are easier to handle and require fewer materials in the lab. Finally, the settle plate is simply an empty petri dish which is left open to collect dust and closed when sampling concludes. This method is also small and requires no preparation ahead of the sampling campaign so can be done quickly. These are all "low-tech" measurement methods which are not size or microbe specific and collect settling dust, and the microbes it contains, overtime. This ability to collect samples over an extended period is advantageous as it will be less impacted by local short-term variation and will be representative of average exposure during the sampling duration. As the bio-aerosol level in air is expected to be low, longer duration sampling is needed to have enough of the agent captured to detect its presence.

Prior to the school pilot, the sampling methods were compared in a field experiment in three different settings: a university library, a primary school, and a cow stable. The non-school settings were included to help evaluate the sensitivity range of these methods. The university library was assumed to be a low dust setting as the experiments were conducted during the summer break when the library was largely empty, whereas the cow shed is a high dust setting. Samplers were placed in six classrooms at the primary school, three locations in the university library and three locations at the farm. The methods were also tested for differing lengths of exposure time to determine if a shorter sampling time would yield enough collected dust to perform the quantitative polymerase chain reaction (qPCR) analysis.

#### Microbiological analysis

The qPCR analysis is a technique which selects and amplifies a specific target nucleotide sequence from genetic material, to determine if micro-organisms are present and at what concentration in the sample. The selected sequence for amplification is derived from specific regions of the bacterial or viral genome and determines which bacteria or viruses can be detected in the analysis. The qPCR technique can also be used to estimate total bacterial content, irrespective of the bacterial species present, by selecting a generic sequence for amplification that is present in the genome of all bacteria (16SrRNA). This 16SrRNA qPCR analysis was used to test the sensitivity of sampling methods in the different field experiment settings.

#### Results

In the high dust setting, the cow stables, the EDCs were overloaded with dust after being in place for three weeks making the qPCR analysis impossible without significant dilution due to inhibition problems. In the middle and low dust settings, the school and the library, 16SrRNA qPCR detected bacterial contamination at all time points with the mini-EDC technique. However, in the school setting, the EDC failed to collect a measurable amount of bacterial contamination after one week. In previous studies, also performed in indoor environments such as homes and schools, EDCs were used for a wide range of sampling periods varying from two to ten weeks (Spilak et al. 2015, Rocchi et al. 2015, Scherer et al., 2014). The results from the field experiments, support from the literature, and practical considerations lead us to select a sampling time of three weeks for the pilot study. A three-week sampling period allows multiple sampling in between school break periods. Both the EDC and mini-EDC were used in the pilot study as no clear distinction could be made based on the field experiments and the smaller size of the mini-EDC might prove useful.

Based on the results of the field experiments it was decided to exclude the settle plate method from use in the pilot project due to difficulties in processing these samples in the laboratory.

We found that finding a suitable location to place the samplers is often challenging in busy classrooms. The samplers should be placed at least two meters high and, on a surface, where they will not be disturbed such as the top of a bookcase. Ideally, they should also be placed in a central location away from strong air flows such as from an open window.

#### Application in the pilot study

To avoid taking up surface space in the classrooms it was decided that during the pilot study the samplers should be hang from the ceiling as can be seen in figure C1. This set-up prevented most accidental interference with samples but in rare instances extra material was found in the boxes ('pepernoten' thrown for Sinterklaas and some plant material from classroom decorations).

Originally, it was planned to hang the boxes in the center of the room, however in practice there was a concern that if the box moved due to air flow, they could trigger the motion activated security systems present in many of the ground floor classrooms. Because of this many of the boxes were hung close to the walls of the rooms. Lastly, a few classrooms had unusually high ceilings which could not be reached so the samplers were placed on top of shelves or closets.

Samplers were placed in all classrooms equipped with MACs. Samples were also placed in several classrooms without MACs, and 5 field blanks and 5 lab blanks were included (one per school).



Figure C1: Left; a sampler box (in red circle) hanging in a classroom Right; interior view of the cardboard box used for sampling containing an EDC (top) and a mini-EDC (bottom).

#### Sample analysis

The samples were analyzed by qPCR for the presence of four specific bacterial targets (*Staphylococcus aureus, Streptococcus salivarius, Staphylococcus epidermidis,* and *Moraxella catarrhalis*) that represent various niches (airways, skin, saliva) of the healthy human microbiome and were included as proxies for human presence. Three viral targets (Respiratory syncytial virus (RSV), Influenza A, and Influenza B) were selected for their role as common seasonal infections in school aged children. Additionally, the samples were analyzed for 16S rRNA, as estimate of total bacterial content of a sample. The amount of bacterial contamination present in each sample was calculated using reference curves and corrected for the differences in surface area between EDC and mini-EDC, extraction volume, and average field blank value.

For DNA/RNA extraction, samples were thawed and placed into tubes containing a mixture of LGC lysis buffer and Dulbecco's Phosphate Buffered Saline (PBS) in a flow cabinet. Next the tubes were agitated on a roller for 30 minutes, then placed on ice. The samples were then divided to enable two extraction procedures, 350ul was used with the LGC DNA extraction kit for total nucleic acid extraction to be used for analysis of the bacterial targets. The extractions proceeded according to steps published in *Fakunle et. al.* 2023. Another aliquot of 350ul was used with the Zymo extraction kit according to supplier instructions for the viral targets. All samples were analyzed in duplicate by qPCR using a BioRad CFX 384 instrument, with associated CFX Maestro software. Negative buffer-only controls were included in each run. The PCR reaction was allowed to run for 40 cycles as a low amount of DNA/RNA was expected. A standard curve of a dilution series of synthesized genetic fragments (gBlock) of the targeted region with known quantity was included in each run to allow the quantification of the amount of target DNA/RNA present in the samples. As output, we obtained Ct (cycle threshold) value, the number of cycles needed to replicate enough DNA/RNA to cross a threshold value to be detected, and the number of gene target copies in a sample based upon the Ct values of the sample and the standard curves.

#### Results

In total 70 EDC and 70 Mini EDC samples were collected in each of the two sampling periods from 68 sampled classrooms. Five samples were excluded from the analysis due to either field or lab processing errors. None of the samples were positive for either Influenza A or Influenza B, so these targets are excluded from the analysis. Field and lab blank samples tested negative for all targets, except 16SrRNA. For the comparison of the two sampling techniques, we compared the bacterial content as measured by 16SrRNA for different classrooms and periods (intervention versus control). EDCs yielded more 16SrRNA gene copies per square meter of surface area compared to the Mini-EDCs. Yield of mini-EDCs can be distinguished to a lesser extent from background (blank) signal. For both methods a trend is observed for lower yields with MAC on compared to MAC off and to control rooms (Figure C3).



*Figure C3: Boxplots comparing 16SrRNA yield from the EDCs compared to the mini-EDCs after adjusting for the differing surface areas and extraction volumes and correcting for background signal.* 

## Recommendations

When the results are adjusted to account for the differences in surface area and processing volume between the two sample types, the EDCs on average, yielded more 16srRNA compared to the Mini-EDC (Figure C3). This finding, along with the EDC method's established track record in other publications leads us to recommend the use of the EDC for future studies based on yield (Adams et. al. 2021, Jacobs et. al. 2014, Viegas et. al. 2020). Though both methods were able to detect a trend towards lower bacterial contamination when the MAC was switched on compared to when the MAC was switched off and the control period. This indicates that the mini-EDC method is still promising and could possibly be used if the practical concerns about available space in the classroom outweigh the issue of reduced yield.

We advise the use of a sampling period of three weeks as it provided sufficient time for the dust containing the microbes to settle on the samples, even if it was at relatively low concentrations. A longer sampling period is not recommended as this will reduce the opportunity to capture any seasonal differences. This may be important as there is strong variation over time in the circulation of different viruses, and other seasonal changes such as ambient temperature, humidity and ventilation practices may also impact levels of indoor air microbial contamination. This is also a practical decision, as it allows for two sampling periods in between school breaks within most school calendars.

During the school pilot study, the samplers were placed and picked up by the research team. This ensured proper application and handling. For application at scale, the procedures for appropriate

placing and handling of samples should be feasible to perform by non-research staff (i.e. members of the school administration or janitor). EDCs have been used in past research where study participants send the samples back to the laboratory by mail (Jacobs et al, 2013; Jonker et al. 2023). It should be acknowledged that for large-scale application considerable efforts are needed to make sure that school staff are well informed and regular contact and follow-up is needed to ensure timely return of the samples. Moreover, school staff having adequate time to place the samplers and collect the needed metadata is crucial. We consider both the EDCs and the mini-EDC method as described in this pilot suitable for large scale application with handling by non-research staff. Despite differences in yield, the practical advantage of the mini-EDCs small size could outweigh the EDC as the more feasible method in a large-scale study.

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#### School recruitment and selection

In April 2023, an email invitation was sent on behalf of the Ministry of Education to a list of primary schools that had previously indicated interest in participating in research on the use of MACs in schools, to inform them about the upcoming pilot. School interested to participate were requested to approach the Principal Investigator (UMCU) of the pilot. The aim was to enroll five or six primary schools in the 6-week pilot in the autumn of 2023.

The above actions resulted in a total of 3 individual schools and 2 school organizations, representing 38 schools five schools that approached the Principal investigator and expressed interest in participating in the pilot. In June 2023, the interested schools were contacted by email and phone and received further information on the pilot study and the activities involved in participation. Some schools remained unresponsive to these outreach activities. Of the remaining schools and school organizations, most declined participation. The main reason for declining was an already high workload and not being able to oversee the next school year yet when the pilot would take place (the recruitment process started just before the summer holidays). One school organization was interested to participate with five schools from the same organization. Given the low response rate it was decided to approach a second batch of schools that had participated before in a study performed in primary schools by TU Delft in 2017. Due to the unresponsiveness of this second batch and due to strict timelines for the pilot, it was decided in August 2023 to proceed with the school organization that could deliver 5 participating schools at once. Selecting this school organization had several advantages: Only one contract needed to be in place between the study sponsor (UMCU) and the school organization, instead of separate contracts with each school. Initial planning and operational procedures for the pilot could be negotiated with a single point of contact at the school organization, facilitating communication. The five school were close to each other in geographical location and therefore several school visits could be combined in one day improving efficiency of the study.

# Contract procedures

The school organization was selected in August 2023. Since the planning was to deliver the air cleaning devices end of October and start the pilot the 6<sup>th</sup> of November, about 2 months remained available to have both Sponsor and the school organization create, negotiate and sign the contract. A template collaboration agreement (in Dutch: samenwerkingsovereenkomst) from UMCU was used and adapted to accommodate and reflect the scope of the pilot. A separate data processing agreement was not deemed necessary as no personal identifiable and traceable data (in Dutch: herleidbaar) were collected during the study.

The contract was fully negotiated and finalized in the beginning of October and fully signed by the 26<sup>th</sup> of October 2023. It was not necessary for the school organization to have a wet-ink version of the contract, therefore only a scan with all signatures was shared between the signees, which greatly facilitated and expedited the signature process.

Delivery and ownership of the air cleaning devices was not part of the collaboration agreement. The MACs were donated to the school organization by the Ministry of Education.

The template contract is added in Appendix D.I.

#### Timelines

Below, an image to visualize the global timelines of the processes described above.



#### Recommendations for recruiting schools

For recruitment, it is proposed to collaborate with national or subnational school unions, counsels or other organizations for outreach activities, to organize information sessions for schools and promote participation during other events or school visits by these entities. In addition, we strongly recommend offering financial or in-kind incentives to the schools for participation. This strategy is proposed based on experiences from the pilot study where we experienced an overall low interest in participation. Buy-in from many different stakeholders will be essential to reach the target recruitment numbers. The expected additional workload was the main reason for non-participation of schools in the pilot project. It is recommended that schools are relieved from operational tasks related to participation in the trial as much as possible to enhance willingness to participate. This could be realized by transferring trial related tasks such as documentation, MAC maintenance and data collection to study staff.

Additional recommendations include:

- Collaboration with school organizations representing multiple schools, rather than single schools is advised for operational efficiency.
- When collaborating with school organizations, a single contract is sufficient to cover all schools from the organization that participate in the study.
- For contracts, enable digital signatures or scans (no wet-ink signatures)
- Ideally, participating schools are geographically clustered. This will enable visiting multiple schools in one day as part of the required study procedures.

• A separate agreement handling delivery, accountability and ownership of the air cleaning devices may be considered, as we experienced the schools had several questions about these topics.

# Appendix D.I Template contract

# SAMENWERKINGSOVEREENKOMST

#### **DE ONDERGETEKENDEN:**

<<u>Naam Sponsor></u>, een publiekrechtelijk rechtspersoon, gevestigd te <u><adres Sponsor></u>, te dezen rechtsgeldig vertegenwoordigd <u><naam invoeren></u>, hierna te noemen: '<u><naam</u> <u>Sponsor></u>,

en

 <Partij>, gevestigd te <adres partij>, te dezen rechtsgeldig vertegenwoordigd door <naam invoeren>, hierna te noemen: ''<Partij>"

hierna ook gezamenlijk te noemen de 'Partijen' en afzonderlijk de 'Partij'.

## OVERWEGENDE DAT:

- <<u>naam Sponsor></u> en <u><Partij></u> wensen met elkaar samen te werken in het kader van de studie Airias Fase 1 hierna te noemen de Studie;
- <naam Sponsor> wenst een aantal studiehandelingen te delegeren aan <Partij> als uiteengezet in Bijlage [1 tot en met 3] aangehecht aan- en daarom onderdeel van deze overeenkomst hierna de Overeenkomst;
- <Partij> en het <naam Sponsor> aan de Studie wil deelnemen volgens de voorwaarden van deze Overeenkomst;

## KOMEN ALS VOLGT OVEREEN:

## <u>Uitvoering van de Studie</u>

- <Partij> zal de Studie uitvoeren conform deze Overeenkomst, de Informatie Brief als uiteengezet in Bijlage 1, de Instructie als uiteengezet in Bijlage 2 en de geldende wet- en regelgeving. <a href="mailto:<a href="mailto:sal"><a href="mailto:<a href="mailto:sal">sal</a> een vergoeding betalen voor het uitvoeren van de Studie conform het Budget in Bijlage 3. In geval van vroegtijdige beëindiging van de Overeenkomst op basis van artikel 6b. zal de totaal door <a href="mailto:sal">sal</a> em Sponsor> onder deze Overeenkomst te betalen som worden berekend naar rato van de tot aan de datum van beëindiging volledig en correct ingevulde en door <naam Sponsor> ontvangen rapportageformulieren.
- 2. 2. Partij> voert handelingen uit van en voor de Studie als uiteengezet in Bijlage 1 en 2, draagt zorg voor en is verantwoordelijk voor de faciliteiten die noodzakelijk zijn voor het uitvoeren van de Studie, inclusief het gebruik van het Studie Apparaat.

- 3. <naam Sponsor> is verantwoordelijk voor het aanwezig zijn van alle voor de uitvoering van de Studie vereiste toestemmingen.
- 4. <Partij> zal medewerking verlenen aan regelmatige monitorbezoeken van <naam Sponsor> ter controle op de juiste uitoefening van de Studie op tijden die passend zijn voor alle betrokken Partijen en op aankondiging met een redelijke termijn. <Partij> zal tevens medewerking verlenen aan inspecties door overheidsfunctionarissen en <naam Sponsor> onverwijld informeren zodra zij op de hoogte zijn van een dergelijke inspectie.

#### Intellectuele eigendomsrechten

 Alle rechten op de resultaten van de Studie zullen uitsluitend toebehoren aan <a href="mailto:snaam"></a> Sponsor>.

#### <u>Financiën</u>

- a. <a href="https://www.en.style.com">studie uitvoeren conform het</a> Budget als uiteengezet in bijlage (2)
- b. In geval van vroegtijdige beëindiging van de overeenkomst op basis van artikel 7 a zal de totaal door <<u>naam Sponsor></u> onder deze overeenkomst te betalen som worden berekend naar rato van de tot aan de datum van beëindiging door bij <<u>naam Sponsor></u> aangemelde potentiële deelnemers.

#### Vertrouwelijkheid en bescherming persoonsgegevens

- a. <Partij> en <naam Sponsor> behandelt persoons- en onderzoeksgegevens waarover zij in het kader van deze Overeenkomst zullen beschikken, vertrouwelijk en handelen conform de toepasselijke wet- en regelgeving (waaronder de Algemene Verordening Gegevensbescherming ("AVG")) en de privacyregelingen in de gezondheidszorg. Deze verplichtingen gelden - met uitzondering van de persoonsgegevens - niet ten aanzien van informatie die bij het publiek bekend is anders dan door schending van geheimhouding.
- b. <<u>Partij</u> en <u>source</u> zullen zorgen dat zij voldoende technische en organisatorische maatregelen hebben genomen om aan de AVG te kunnen voldoen.
- c. Indien <<u>Partij</u>> zich kwalificeert als verwerker onder de AVG dan zullen Partijen een verwerkersovereenkomst sluiten.

## • Verzekering, vrijwaring en aansprakelijkheid

- a. Behoudens in geval van opzet of bewuste roekeloosheid, is de aansprakelijkheid van en jegens <naam Sponsor> beperkt tot € 500.000.
- b. Behoudens in geval van opzet of bewuste roekeloosheid, is de aansprakelijkheid van iedere Partij jegens de andere Partij uitgesloten voor indirecte en gevolgschade (waaronder winstderving, gemiste besparingen, schade door bedrijfsinterruptie en verlies aan goodwill).
- c. Het <naam Sponsor> is niet aansprakelijk voor de levering, het gebruik, de kwaliteit en schade van het Studie Apparaat. <Partij> vrijwaart het <naam Sponsor> voor aanspraken van derde ten aanzien van het Studie Apparaat.

#### Duur en beëindiging van de Overeenkomst

- a. Deze Overeenkomst treedt in werking na ondertekening door alle Partijen en eindigt van rechtswege op het eerdere moment van:
  - 1. voltooiing van de Studie, of
  - 2. voortijdige beëindiging in overeenstemming met artikel 6b.

- b. ledere Partij mag deze Overeenkomst door schriftelijk kennisgeving aan de andere Partijen met onmiddellijke ingang opzeggen (met uitsluiting van ontbinding en gedeelte ontbinding als uiteengezet in artikel 6:265 en 6:270 BW) wanneer:
  - 1. redelijkerwijs aannemelijk is dat de Studie dient te worden beëindigd in het belang van de gezondheid van de deelnemers;
  - 2. blijkt dat voortzetting van de Studie geen wetenschappelijk doel kan dienen;
  - 3. één van de Partijen failliet is verklaard, of als faillissement voor één van de Partijen is aangevraagd, of één van de Partijen als rechtspersoon is ontbonden;
  - 4. één van de Partijen de verplichtingen voortvloeiende uit de Overeenkomst niet nakomt en, voor zover nakoming niet blijvend onmogelijk is, deze nakoming is uitgebleven binnen dertig dagen na ontvangst door de tekortkomende Partij van een schriftelijk verzoek tot nakoming, tenzij de niet-nakoming niet in redelijke verhouding staat tot de voortijdige beëindiging van de Studie;
- c. Artikelen 2, 3, 4, 7 en 8 van deze Overeenkomst blijven na beëindiging daarvan van kracht.

# 8. <u>Overig</u>

- a. <Partij> zal zich inspannen om het Studie Apparaat in te zetten conform de Instructie. In het geval <Partij> om welke reden dan ook het Studie Apparaat niet te kan gebruiken conform de Instructie dan zal <Partij> hiervan schriftelijk melding maken naar het <naam Sponsor> binnen 5 werkdagen.
- b. Op deze Overeenkomst is de Nederlands wetgeving van toepassing en geschillen tussen Partijen naar aanleiding van deze Overeenkomst zullen uitsluitend worden berecht door de bevoegde rechter van de rechtbank Midden-Nederland, locatie Utrecht.
- c. De Partijen zullen niet elkaars naam of logo gebruiken in publicaties zonder voorafgaande schriftelijke toestemming van de betrokken Partij.
- d. De Partijen zullen hun rechten en verplichtingen uit deze Overeenkomst of de uitvoering daarvan niet overdragen aan een derde partij zonder voorafgaande schriftelijke toestemming van de andere Partijen.

Bijlage 1: Informatiebrief ouders, medewerkers, en kinderen

Bijlage 2: Afspraken met de scholen

Bijlage 3: Budget

Separate handtekeningen pagina volgt

# ALDUS OVEREENGEKOMEN EN ONDERTEKEND in tweevoud:

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Datum:			

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Functie:

Datum:

# Bijlage 1 - Informatiebrieven

# Bijlage 2: Afspraken met de scholen

- Toegang tot klaslokalen en openbare ruimtes is noodzakelijk voor het uitvoering van het project. Het project team heeft toestemming om deze ruimtes te betreden.
- Eén aangewezen persoon binnen de school is verantwoordelijk voor het aan en uitzetten van de apparaten, en zal regelmatig een check uitvoeren of de apparaten inderdaad nog aan/uit staan. De leraren zullen de apparaten zelf niet aan- en uit zetten.
- Docenten vullen dagelijks enkele gegevens in (in excel of op papier)
- De school neemt initiatief in het benaderen van kinderen die geschikt zijn voor deelname aan de interviews.
- De school informeert ouders die hun kind ziek melden over de mogelijkheid om deel te nemen aan een online vragenlijst

#### Bijlage 3: Budget studie, exclusief B.T.W.

Vergoeding Scholen: € 500,00 (ex BTW) per school

Betaling vindt plaats (tenzij er vroegtijdige opzegging van de overeenkomst plaatsvindt) na de onderzoeksperiode van 6 weken. <a href="mailto:</a> zal de instructies rondom facturatie verstrekken">mailto:</a>

Betalingen zullen gemaakt worden aan: <Partij> <rekeningnummer Partij> Activity E: Developing reporting forms and database for research results

## Data Forms

Before the start of the pilot, baseline information about the schools and classrooms was collected in an online report form (Castor database). This included information about the size of the classrooms, the usual number of students, the timetable of teaching-hours and other activities, etc. For the air cleaning devices and the air sensors, the device type/number and the location of the devices was recorded.

During the pilot, a separate reporting form for the control and intervention period was developed. In these forms it was recorded if there were any deviations in the class schedule. This information was needed to interpret the results from the ongoing air quality measurements. For instance, if students were not present in the classroom for a certain period during school hours. Also, on a daily basis teachers recorded information on the location and setting of the air cleaning devices and when the devices were turned on and off. On a weekly basis, during school visits, this was also checked by the study team.

Throughout the pilot, several anonymous surveys were conducted. During the control period students and teachers from group 6 to 8 were asked one time to complete an online survey on classroom comfort. During the intervention period this online survey was performed twice. An illness absenteeism survey was also created. Parents who reported their child absent due to illness were sent a link to an online survey to request more information on the nature of the illness.

All surveys were created in Castor and could be accessed through an online portal. Data entries were automatically captured in the secure online database.

For administrative data on illness absenteeism, an anonymous export from the school organization's IT department was received with the number of students reporting absent for illness or other reasons over the period of the 2022/2023 school year and between Aug and Dec 2023. An additional anonymous absenteeism dataset was made available from the implementation group a company that hosts school administrative software (Parnassys) that was used to determine if school absenteeism rates in the pilot schools were representative for the Netherlands.

Between the consortium partners information and documents were shared via a secured SURFdrive. All project-specific data and documents are being filed on the UMC Utrecht network drive that meets all legal requirements.

The reporting forms can be found in the supplementary documents.

Activity F: Pilot study
#### Introduction

Since the COVID19 pandemic, there is great interest in non-pharmaceutical interventions that can help reduce transmission of respiratory viral pathogens, without compromising individual freedom. Technical solutions that can help improve indoor air-quality and thereby potentially reduce airborne transmission have gained much interest. While sufficient ventilation is considered the cornerstone of this approach, air-cleaning devices may be of added value because they can remove virus laden particles from recirculated air. In addition, mobile versions of air-cleaning devices a can be installed in many different indoor environments, making them ideal as temporary outbreak control or mitigation intervention. Various types of air cleaning technologies are used to purify in-room air.<sup>1</sup> Most frequently used techniques are the high-efficiency particulate air (HEPA) filters and ultraviolet germicidal irradiation (UVGI). HEPA filters use mechanical filtration, meaning that they physically remove airborne particles from the air. These filters can be placed in HVAC (heating, ventilation, air conditioning) ducts or air purifiers. UVGI is a form of radiation that uses ultraviolet (UV) light to damage the DNA of microorganisms, disrupting their ability to replicate and thus leaving them noninfectious. UVGI lamps can be placed within the ducts of the HVAC system, in the ceiling or upper wall of a room, or in air purifiers.

Schools are known to be important drivers of transmission for many different respiratory viral pathogens. Reducing transmission in schools has the potential to mitigate onward epidemic spread or contain outbreaks. For this reason, the potential of mobile air cleaning devices (MACs) to reduce pathogen circulation in schools is of great public health importance and needs to be further evaluated. This study assessed the performance of MACs on improving air quality parameters in a real-world classroom setting and investigated whether MAC use was associated with a reduction in air derived microbiological contamination of the classroom.

MACs produce noise and draft, take up classroom space, consume electricity, need to be operated by school personnel and require regular maintenance. These characteristics demand a careful evaluation on the feasibility and acceptability of MACs in school settings before larger and randomized studies in schools to assess MACs efficacy in reducing respiratory infections can be considered. In this study we will explore this in primary schools as a first step towards further (efficacy) studies in these, and other educational settings. We focus on primary schools because of the continuity of classroom and group assignment allowing us to follow them over time during periods with and without MACs installed. Finally, we also used this study to obtain estimates of total absenteeism in schools due to acute respiratory illness that could be potentially targeted by interventions reducing school related transmission of respiratory pathogens.

#### Methods

#### Study design and school selection

This randomized cross-over pilot study on the performance and feasibility of MACs in primary schools was conducted between Oct and Dec 2023 in primary schools in the Netherlands. Each school had a three week intervention period with in-class MACs operating during school hours, and a three week control period without MACs in random order. Five or six schools at an estimated student group number of eight was considered for participation in the study given the number of MACs available.

Schools and school organizations that had previously indicated they were interested in participating in projects on the use of MACs were approached by mail and informed about the study. Schools were eligible if they: 1) accepted the positioning and operation of MACs in the classroom as per study manual during school hours; 2) were able to provide aggregated data on school absenteeism per student group; 3) willing to send out an anonymous (online) heath questionnaire to students reporting absent during the study period; 4) willing to cooperate with regular study visits to the schools conducted by the study team; 4) could facilitate anonymous surveys to be taken during school hours among students and teachers concerning the use of MACs. Each school was offered a compensation of 500 EUR for participation. Schools and school communities interested to participate were invited for an online meeting for additional information and Q&A. Schools that consented to participate following the meeting were contracted and included in the study. All schools were visited multiple times pre-, during and after the study period. At the pre- visit, schools received further details on the study set-up, the devices used, and instructions for data collection, survey invitations and device maintenance. For the duration of the study, school visits took place every week. During these visits the setting and location of the MACs were checked, the qualitative interviews and some of the surveys were performed. Post-study, a close-out visit was performed to evaluate the project with the school directors.

## Air-cleaning devices

Three different MAC models were selected for the study based on previous experimental studies described in [1, 2]. In brief, MAC models were selected based on their technical description and subsequently tested for their performance on CADR, the induced airflow pattern, noise level, and air velocity in previous tests in an experimental set-up of a classroom. Detailed information on the selected MACs (referred to as MAC-A, MAC-B and MAC-C) is listed in Table F1.

Deviceª	Air cleaning technology <sup>bc</sup>	Airflow pattern	Fan capacity (m <sup>3</sup> /h) <sup>c</sup>	Efficiency <sup>c</sup>	Noise level [dB(A)] <sup>c</sup>	Dimensions (cm) <sup>c</sup>	Number of devices
MAC-A	ES + AC		735	H13	27-55	34.0 × 34.0 × 85.5	2
MAC-B	НЕРА		565	H13	18-51	33.2 x 33.6 x 60.6	2
MAC-C	HEPA	()J	750	H13	26-65	68.8 (Φ) x 25.4	2

Table F1. Information on the selected MACs.

<sup>a</sup> MAC: mobile air cleaner.

<sup>b</sup> ES: electrostatics; AC: activated carbon.

<sup>c</sup> As specified by the brand.

## Position and operation mode of the air-cleaning devices in classrooms

Per school 8-10 classrooms were selected randomly, based on the previously received school floor plans. Next, the selected classrooms were divided into three groups, where each group was assigned with one type of MAC. The distribution of different MACs across the classrooms was determined on a random basis. Recommended positioning was based on the previous experiments with two MACs of the same model per classroom placed diagonally at 2 opposite corners with the air supply facing the occupied area. Schools were supported in positioning and setting-up the devices in each selected classroom on the first day of the study period, when a school visit took place. Teachers received written and verbal instructions on how to operate the MACs.

Teachers were also instructed to daily log the actual use of MACs during school hours on each day of the intervention period, and record any deviations from regular school timetables (e.g. extracurricular activities outside the classroom). All classrooms were equipped with openable windows and no specific instructions were given to teachers regarding the opening or closing of doors and windows, i.e. teachers were free to open them at any time as they did before MACs were installed.

## Microbiological contamination and air quality monitoring in classrooms

To study microbiological contamination of classroom air, we selected scalable and non-intrusive air sampling methods, that could easily be scaled up for a larger trial or for surveillance purposes. Based on a literature review and pre-testing of methods in the lab setting, we selected the Electrostatic Dust Cloth (EDC) and a smaller modified version of the EDC (mini-EDC).

The two samplers were placed side by side in a large, low-sided cardboard box adapted to be hung from the ceiling in the classrooms (Figure 1).



Figure F1: Left; a sampler box (in red circle) hanging in a classroom Right; interior view of a cardboard box used for sampling containing an EDC (top) and a mini-EDC (bottom).

Microbial sampling was done in each classroom equipped with MACs and additional classrooms were selected as control classrooms without MACs (if the school had remaining classrooms). Two schools had all classrooms equipped with MACs. In total 45 classrooms with, and 14 classrooms without MACs across the 5 participating schools were included for parallel sampling with EDC and mini-EDC. In addition, each school had one classroom with a blank sampler included alongside the EDC and mini-EDC sampler. The blanks remained taped shut during the sampling period to detect any sampling error or background signal from the sampler. At the start of period one, the samplers were placed in a box hung from the ceiling, at a height of at least 2 meters. Samplers stayed there

untouched for three weeks (period one) and were then collected and replaced. Samplers of period two were collected at completion of the study period three weeks later.

## Microbiological analysis of EDC samples

EDC samples were transported to the laboratory and stored in tubes at –80C until further processing. The samples were analyzed for the presence of four specific bacterial species (*Staphylococcus aureus, Staphylococcus salivarius, Staphylococcus epidermidis,* and *Moraxella catarrhalis*) that represent various niches (airways, skin, saliva) of the healthy human microbiome as proxies for human excreta. Three viral targets (Respiratory syncytial virus (RSV), Influenza A, and Influenza B) were selected for their role as common seasonal infections. Also, a 16S ribosomal RNA (16SrRNA) target was included as it can be used as a marker for a sample's total bacterial content. For further details we refer to Activity C.

## Monitoring of air quality parameters in classrooms

In each school, indoor air quality (IAQ) parameters were monitored in three classrooms: one classroom equipped with MAC-A, one equipped with MAC-B, and one equipped with MAC-C. The included IAQ parameters are:

- CO2 (carbon dioxide) concentration in ppm (part per million)
- $PM_{2.5}$  (airborne particles of diameter < 2.5  $\mu$ m) concentration in  $\mu$ g/m<sup>3</sup> (micrograms per cubic meter)
- $PM_{10}$  (airborne particles of diameter < 10  $\mu$ m) concentration in  $\mu$ g/m<sup>3</sup> (micrograms per cubic meter)
- TVOC (total volatile organic compounds) concentration in ppb (part per billion)

CO<sub>2</sub> concentration was measured by an MH-Z19B sensor (range: 0-2000 ppm, accuracy: ±50 ppm). In schools 1, 2 and 4, PM<sub>2.5</sub> and PM<sub>10</sub> concentrations were measured by an SDS011sensor (range: 0-999.9  $\mu$ g/m<sup>3</sup>, accuracy: ±10% [3]. In schools 3 and 5, PM<sub>2.5</sub> and PM<sub>10</sub> concentrations were measured by a PMS5003 sensor (range: 0-5000  $\mu$ g/m<sup>3</sup>, accuracy: ±10% in the range of PM<sub>2.5</sub> and PM<sub>10</sub> concentrations <100  $\mu$ g/m<sup>3</sup>) [4]. TVOC concentration was measured by an SGP30 sensor (range: 0-60000 ppb, accuracy: 1, 6 and 32 ppb in ranges 0-2008 ppb, 2008-11110 ppb, and 11110-60000 ppb, respectively). In the monitored classrooms, the sensors were integrated on one panel (hereafter referred to as the "IAQ sensor") and were all connected to a central unit from which data was saved on an SD card, with a logging interval of 5 minutes. The IAQ sensors were mostly placed on the teachers' desks.

## Feasibility surveys

To determine the feasibility of the use of MACs in elementary school, we assessed the following topics among teachers and students using the Bowen and CFIR framework [5, 6]: acceptability, demand, and implementation. The topic guide was pre-tested during two pilot interviews and subsequently revised:

- Acceptability explored expectations, satisfaction, and experiences of teachers and students with MACs.
- Demand investigated the likelihood of future use and recommending MACs among teachers and students.

- Implementation explored compliance of use of the MACS with the study protocol, barriers, and facilitators.
- Integration was operationalized as the ease of integration of use of MACS by teachers with their daily activities and practices.

For a detailed description of methods and surveys we refer to Activity A and the supplementary files. A 5-point Likert scale was used for each survey item. Two versions of the survey (i.e. one for teachers, one for students) was created in a web-based data capturing tool (Castor) to collect data anonymously from participants in schools. Teachers and students in grade six to eight (approximately nine to 11 years old) were invited to complete the survey once during the control period and twice during the period with MAC ON, at a two-week interval. Only teachers and students from classrooms equipped with MACs were invited to participate in the survey.

## Illness absenteeism survey

In order to estimate the proportion of absenteeism attributable to acute respiratory infections (ARI), a survey was developed and distributed to parents of students reported absent due to illness. An invitation to participate was sent by the school to parents/legal guardians on the first day of the student's absenteeism. The anonymous online survey included questions about the reason for absenteeism, the duration of absence, and the presence of symptoms in the student and other family members (supplementary files). Based on the survey results, the absenteeism was categorized as due to ARI, gastrointestinal illness or other/unknown illness. Based on the reported presence of symptoms in other family members, the ARI was categorized as likely school-related or not.

## School data collection

For each participating school, we collected the following information at baseline; number of students and staff, number of student groups per grade, school floorplan, school and group specific timetables, type of ventilation available for each classroom (natural ventilation only, mechanical inlet/outlet/combined inlet-outlet). Every day the teachers completed an Excel file with information on the number of students being present, the setting and location of the MACs and potential sampler disruption. School absenteeism data were obtained from the school administrative systems. The data were collected in the form of episodes per class, each characterized by a start and an end date, but without personal information. Absenteeism data were collected from the five schools for the entire 2022-2023 school year and the first half of the 2023-2024 school year, covering the period from summer to the Christmas holidays including the time during which the study was conducted.

## Statistical analysis

## The performance outcomes of this study include:

The difference in microbiological contamination in classroom air dust samples comparing the intervention period with MACs is ON, versus the control period. Upon analysis we found that there was overall a low level of DNA/RNA recovered from the samples. Many samples had Ct values which fell outside the range of the reference curves and therefore the number of cells could not accurately be calculated for any of the targets except for 16S rRNA. Instead, samples were marked as positive for a particular target if one or both duplicates had a Ct value, and negative if neither replicate had a Ct value. 16SrRNA measurements were adjusted for the difference in surface area between EDC and min-EDC, dilution volume and correct for background contamination of the samples as described in Activity C. We compared the percentage of samples positive for each microbiological target between

the control and intervention period using chi-square tests, and the bacterial load using the Wilcoxon Rank Sum test. In addition, we used the samples from the classrooms without MACs to detect any natural trends over time in microbiological contamination by comparing the 16SrRNA results for the first and second period.

The difference in  $PM_{2.5}$  and  $PM_{10}$  in classrooms comparing the period with MACs on, versus the control period. Data for analysis was restricted to the period of in-class presence of students based on the weekly timetable for each group. Measurements were summarized as median concentrations per period and per classroom and compared using Wilcoxon signed rank tests. All the tests were performed in IBM SPSS v28. The significance level was set at 0.05 (p < 0.05).

Feasibility was evaluated based on the survey results. These were analyzed using summary statistics for Likert-scores per survey item and grouped by aspect of feasibility: acceptability (i.e., expectations, satisfaction, and experiences), demand, implementation, and integration. Analyses were performed separately for teachers and students. We compared survey results with percentages of disagree, neutral and agree answers at the three time points and calculated the change in scores between intervention and control period separately for each experience indicator using unpaired t-tests and Mann-Whitney U-tests among teachers and students. A  $\geq$ 10% decline in score between the intervention period versus the control survey was used to define inferiority in experiences of teachers and students associated with the use of MACs. Statistical analyses were performed with SPSS, version 29 (IBM Corp., 2024).

The mean number of absent days per student due to acute respiratory illnesses (ARI) during a school year was estimated based on absenteeism data spanning one and a half school years were processed into weekly incidence rate (IR), expressed as new cases per 100 student days. Absenteeism data from the first grades were excluded due to the dynamic nature of group sizes throughout the school year. The average IR for the 2022-2023 school year was estimated using a multilevel generalized linear mixed-effects model (GLMM) with a negative binomial distribution and the logarithm of the number of student days as an offset term. School and week were included as random effects. We also calculated IR per school period - defined as intervals between holidays – to explore absenteeism patterns over the school year. IRs were then translated into cumulative number of absent days due to illness per student per school year and combined results on from the illness survey on the proportions of absences attributable to ARI and the fraction of those ARI cases considered school related. The mean and standard error of these parameters were used to generate 100,000 bootstrap samples, with each iteration calculating the product of the three parameters. Python version 3.11.5 was used for all data processing, visualization, and most statistical analyses. For the GLMM, R version 4.1.3 and the glmmTMB package (version 1.1.9) were used.

### Results

A total of 38 schools were approached and of these, five schools belonging to the same school community were willing to participate and included in the study. The remaining schools did not respond or declined, mainly for reasons of time-investment required for participation. The participating schools were located in the same district improving study efficiency. Together, the five primary schools had a student population of 1654. The number of classrooms per school varied between 8 and 23. The average student group-size was 24. A total of 45 classrooms, 8-10 per school were equipped with MACs (overview in table F2). In most classrooms space was limited for placing the MACs and adjustments were frequently needed in their positioning. Still, it was ensured that in

each classroom had one device placed in the front and one in the back, with the air supply facing the occupied area.

	Study period			Number of classrooms equipped with air-cleaning devices				
	Period 1	Period 2		MAC A	MAC B	MAC C	Total	
School 1	Intervention	Control		3	3	2	8	
School 2	Control	Intervention		3	3	3	9	
School 3	Control	Intervention		3	3	4	10	
School 4	Intervention	Control		3	3	3	9	
School 5	Intervention	Control		3	3	3	9	
Total	3 weeks	3 weeks		15	15	15	45	

Table F2; Study per	riod and dist	tribution of N	<b>MACs by schools</b>
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Period 1: 6 November – 24 November 2024; Period 2: 27 November- 15 December 2024

The study period lasted six weeks with a cross-over half-way. Period 1 lasted form 6 November – 24 November and period 2 from 27 November- 15 December 2023. School 1, 4 and 5 started with the intervention period (MACs ON), followed by the control period, while schools 2 and 3 started with the control period, followed by the intervention period. Schools were overall compliant with the assigned intervention during both periods. During the intervention period MACS were off 2.3% of school-hours translating to 0.35 day/school over the three weeks period, device settings were changed for 7.1% of the time and for 4.6% of the time the positioning of MACs deviated, meaning they were not on opposite sides of the classroom.

### Microbiological contamination and air quality in classrooms

In total 70 EDC and 70 Mini EDC samples were collected in each of the two sampling periods from 68 sampled classrooms. Five samples were excluded from the analysis due to either field or lab processing errors. None of the samples were positive for either Influenza A or Influenza B, so these targets are excluded from the analysis.

A summary of the microbiological results are presented in table F3. Here we compare the percentage of samples which were positive for the specific targets during the intervention period (MACs ON) versus the control period (MACs OFF), as well as the total bacterial load (16SRNA). In 8 out of 10 comparisons across the five microbial targets and two sampling methods the samples collected in the intervention period had lower detection rates than in the control period. In other words, with the MAC ON, it was less likely that microbial targets would be detected, suggesting a reduction in microbial load, although the magnitude could not be quantified. This is the case for all targets when measured by the mini-EDC and for all but *S.epidermidis* and *RSV* as measured by the EDCs. The large variability in detection across classrooms, targets and sample pairs (EDC and mini-EDC), sometimes with opposite results, limits our ability to detect statistically significant differences given the small scale of the study. Based on analysis of the samples from classrooms without MACs, no clear trend was observed in the microbial contamination over time (data not shown).

Table F3: Summary of the microbiological contamination in classrooms during control (MACs OFF) and intervention (MACs ON) periods.

	EDC			Mini EDC			
				_			
Bacterial/Viral	Control	Intervention	P-value (Chi	Control	Intervention	P-value (Chi	
Target	Period	Period	Squared)	Period	Period	Squared)	
S. salivarius	93,33%	73,81%	0.03 *	72,09%	50,00%	0.07	
S. epidermidis	75,56%	80,95%	0.73	79,07%	76,32%	0.98	
S.aureus	82,22%	64,29%	0.10	48,39%	42,86%	0.84	
M.catarrahlis	82,00%	69,23%	0.20	79,55%	60,00%	0.08	
RSV	48,89%	73,81%	0.03*	76,09%	54,55%	0.05*	
	EDC		Mini EDC				
			P-value			P-value	
			(Wilcoxon			(Wilcoxon	
	Control	Intervention	Rank Sum	Control	Intervention	Rank Sum	
16s rRNA	Period	Period	Test)	Period	Period	Test)	
	2.12 x 10 <sup>9</sup>	1.65 x 10 <sup>9</sup>	0.18	1.35 x 10 <sup>9</sup>	1.21 x 10 <sup>9</sup>	0.85	

The summary results on difference in  $CO_2$  measurement,  $PM_{2.5}$ ,  $PM_{10}$  and TVOF are presented in table F5. A significantly lower concentration of  $PM_{2.5}$ ,  $PM_{10}$  was measured during the period with MACs on, versus off, whereas differences for  $CO_2$  and TVOF were small and non-significant.

Table F5. Results of air quality measurements during the control (MACs OFF) and intervention MACs ON) period

IAQ parameter	ON <sup>*</sup>	OFF <sup>*</sup>	р	
PM2.5 (µg/m <sup>3</sup> )	1.2 (1.0-1.55)	3.0 (2.5-3.5)	0.001	
PM10 (µg/m <sup>3</sup> )	5.0 (2.8-5.9)	8.9 (5.8-9.9)	< 0.001	
CO <sub>2</sub> (ppm)	985 (847-1135)	1014 (833-1169)	0.158	
TVOC (ppb)	1000 (770-1812)	1066 (670-2228)	0.925	

\* Concentration: median (interquartile range), p-values based on Wilcoxon signed rank tests.

## Feasibility of MACs in classrooms

Of 695 eligible students, 274 (39.4%) completed the feasibility survey during the control period, and 213 (30.7%) and 136 students (19.6%) completed the first and second survey, respectively during the intervention period. Of the teachers, 17 completed the survey during the control period, while 16 and 14 completed the first and second survey during the intervention period. The complete survey results are summarized in Appendix F.I figures 1-XX.

## **Acceptability**

Among teachers, the proportion that indicated the MACs met their expectations declined from 40% to 8% between the first and second survey during the intervention period. Similarly, satisfaction about the MACs among teachers declined from 25% to 17% (appendix F.I Figure 1 and 2). Students were overall more satisfied about the MACs (38 and 34%, respectively, appendix F.I Figure 3).

## Experiences with MACs

Teachers experienced a more that 10% negative change in the experienced sound or noise, interruptions with routine activities and coldness in the classroom during the intervention period, compared to the control period (Appendix F.I figures 4-6). A similar, but less pronounced negative trend was observed among students for the experienced change in the classroom environment (>10% decline). Trends for ability to concentrate and the sound in the classroom among students were negative but did not reach the inferiority margin of -10% (Appendix F.I figures 7-9).

## Demand

During the intervention period, only 10% of teachers expressed they would recommend the MACs to others at the second survey and none at the first survey. Teachers also expressed a low likelihood of using the MACs themselves in the future at 8% during the first survey and 30% during the second survey (Appendix F.I figure 10).

## **Implementation**

Among teachers, only 10% reported any advantages during the second survey and none during the first survey of the intervention period, while 42% (first) and 60% (second) reported to perceive possible disadvantages (Appendix F.I figure 11).

The perceived ease of use was high at both intervention survey time-points (64% and 80% at first and second survey, respectively, Appendix F.I figure 12). However, the placement of the MACs was perceived as difficult by 58% and 44% of the teachers at first and second survey (Appendix F.I Figure 13).

### **Integration**

Most of the teachers reported that the MAC were easy to integrate in their daily activities with the current instructions (Appendix F.I figure 14).

### Illness absenteeism

Absenteeism data were obtained from the five schools for the entire 2022-2023 school year and the first half of the 2023-2024 school year. The incidence rate (IR) of absenteeism followed a seasonal pattern, peaking between the Christmas and Spring breaks (Figure 2A). During the 2022-2023 school year, the average IR was 2.2 absenteeism episodes per 100 student days, which increased to 3.7 episodes per 100 student days during the peak period. Substantial differences were observed among the schools in both the timing and magnitude of the absenteeism peaks (Figure 1A). Most absenteeism episodes lasted only one day (80.5%), with the average duration of an absenteeism episode being 1.23 days. On average, students were absent due to illness for a total of 5.3 (95% CI: 3.9-7.2) days during the 2022-2023 school year (Figure 2B).

During the study period, 371 surveys were distributed among parents of students reported absent due to illness, achieving an 81.7% completion rate. Survey results estimated that 73.8% of the missed days were attributable to acute respiratory illness, while 21.9% resulted from gastrointestinal illnesses and 4.7% were unclassified. From these proportions, it was estimated that per student 3.9 (95% CI: 2.7-5.2) days of absenteeism per schoolyear are attributable to acute respiratory illness (73.8% \* 5.3 days). Parents were also queried about the presence of acute respiratory symptoms in their households. In total 30.8% indicated that symptoms were previously present in other household members, whereas 69.2% reported no symptoms or symptoms appearing later, suggesting the student likely contracted the infection outside the household setting, possibly at school. Assuming these absences result from school-related infections, the acute respiratory illness related absenteeism that could be targeted by school-related infection prevention interventions is estimated at 2.7 (95% CI: 1.8-3.7) days per student per schoolyear.



**Figure 2** A) The weekly incidence rate (IR) is plotted as the number of new cases per 100 student days. In color the data from the individual schools is plotted, in black the data from the total population is plotted. Below the graph, the IR per school period is indicated. B) The cumulative average number of absent days per student during the 2022-2023 school year is plotted. In color the data from the individual schools is plotted, in black the data from the total population is plotted.

## Discussion

This cross-over trial on the use of MACs in primary schools provided important insights on their potential as infection control intervention in educational settings. With two devices per classroom with a minimal CADR capacity of 800-1000m<sup>3</sup>/hr, we observed generally lower detection rates of microbiological contamination of air dust samples and in concentrations of PM<sub>2.5</sub> and PM<sub>10</sub>, suggesting a decline in aerosolized particles resulting from the use of MACs. Whether these findings are subsequently associated with lower risk of infection cannot be inferred from our data and would require a much larger trial and different endpoints.

Our study also revealed major challenges regarding the practical feasibility and acceptability of implementing MACs in primary schools. The size and required positioning of the devices created difficulties in accommodating the devices and resulted in a negative change in perceived classroom environment by both teachers and students. The use of MACs had a negative impact on various indicators of environmental comfort (noise, cold, frequency of interruptions and ability to concentrate) and this resulted in overall low satisfaction with the use of MACs, in particular among teachers. Consequently, there was little appetite among teachers to use them in the future. These findings have important implications for potential success of implementing MACs in primary school settings and require careful consideration of their downside effects. Restricting the use of MACs to short periods of time as temporary mitigation measure for pandemic or epidemic control could limit the experienced nuisance and may improve their acceptance. This needs to be further investigated.

Based on the analysis of school absenteeism data together with data from the illness surveys we estimate that - during interpandemic times -, no more than three days of absenteeism per student in primary school are due to ARI during the school year and have a possible link with school-related infection. Therefore, we expect that the impact of school-based infection control interventions on overall absenteeism rates will most likely be limited. Naturally, the impact could be larger during times of highly elevated infection rate and associated absenteeism from seasonal pathogens (e.g. annual RSV or influenza peaks) or from newly emerging infections (e.g. COVID-19).

## Conclusions and recommendations

In summary, MACs when used in the configuration as described in this study appear to reduce aerosol concentration and microbiological contamination of air in classrooms of primary schools, but important barriers to acceptance of their use and feasibility of implementation were identified. Further studies are needed to determine whether the observed air purifying effects translate into reductions in infection rates of respiratory transmitted diseases among students and can help reduce school absenteeism. Based on an analysis of school absenteeism data, the overall impact of school-based infection control interventions on school absenteeism during interpandemic times is however expected to be small.

Based on the pilot study we conclude that a large-scale cluster randomized trial would be feasible, but buy-in and support from the educational community will be key to achieve the required sample size of 160 schools, and to gain acceptance for the use of MACs in classrooms in general.

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# Appendix F.I



Figure 1. Expectations of teachers about the MAC. Q: the use of the MAC meets my expectations.



Figure 2. Satisfaction of teachers with the MAC. Q: Overall, I am satisfied with the MAC.



Figure 3. Satisfaction of students with the MAC. Q: I think the MAC is (1) not nice to (5) very nice.



**Figure 4**. Experienced negative change in noise in the classroom by teachers. The doted lines represent a 10% inferiority margin. Opinions were asked on a scale from 1 (very annoying) to 5 (very nice). Teachers expressed to a negative change in sound or noise in the classroom during intervention I and intervention II period compared to control (> 10% inferiority margin). The experiences sound or noise did not differ between intervention I and intervention II period.



**Figure 5**. Experienced negative change in interruptions in the classes by teachers. The doted lines represent a 10% inferiority margin. Opinions were asked on a scale from 1 (very annoying) to 5 (very nice). Teachers expressed a negative change in perceived interruptions in the classroom in intervention period II compared to the control period and intervention I (> 10% inferiority margin). No differences were observed between the control and intervention I period.



**Figure 6**. Experienced negative change in perceived cold in the classroom by teachers. The doted lines represent a 10% inferiority margin. Opinions were asked on a scale from 1 (very annoying) to 5 (very nice). Teachers expressed fewer positive opinions about the experienced cold in the classroom in intervention period II compared to the control period (> 10% inferiority margin). No differences were observed between the control and intervention I period.



**Figure** 7. Experienced negative change in the opinions about the environment in the classroom by students. The dotes lines represent a 10% inferiority margin. Opinions were asked on a scale from 1 (very annoying) to 5 (very nice). Students expressed more positive opinions about the environment in the classroom during the control period compared to intervention measurement 1 and measurement 2 (> 10% inferiority margin).



**Figure 8**. Experienced negative change in the classroom environment by students. The lines represent a 10% inferiority margin. Perceived ability to concentrate was asked on a scale from 1 (very difficult) to 5 (very well). Students expressed a greater perceived ability to concentrate in the classroom during the control period. This was not significant as represented by the inferiority margin (mean scores of perceived ability to concentrate <10% inferiority margin).



**Figure 9**. Experienced negative change in annoyance of noise in the classroom by students. The lines represent a 10% inferiority margin. Opinions were asked on a scale from 1 (very annoying) to 5 (very nice). Students expressed more positive opinions about the sound in the classroom during the control period compared to intervention measurement 2 (> 10% inferiority margin).



*Figure 10.* Demand of teachers with the MAC. *Q* Recommend: How likely are you to recommend the MAC to others? *Q* probability: How likely is it that you would use the MAC after the pilot, if it remains present in the classroom?



*Figure 11. Implementation of MACs perceived by teachers. Q: Using the MAC provides me with: (1) disadvantages to (5) advantages?* 



*Figure 12. Implementation of MACs perceived by teachers. Q: The use of the MAC is: (1) difficult to (5) easy?* 



*Figure 13. Implementation of MACs perceived by teachers. Q: The MAC can easily be set up in my classroom. With (1) disagree to (5) agree.* 



*Figure 14. Ease of integration with current instructions. Q: I can operate and use the MAC well with the explanations and instructions I received. With (1) disagree to (5) agree.*