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Research Article

Development of a Bioaerosol Sampling Method for Airborne Pathogen Detection with Focus on SARS-CoV-2

Sarah L. Paralovo,[1] **Koen Vanden Driessche**,[2] **Reinoud Cartuyvels**,[3] **Borislav Lazarov**,[1] **Erika Vlieghe**,[2] **Laura Vanstraelen**,[3] **Rita Smets**,[3] **Maarten Spruyt**,[1] **Sabine Kreps**,[4] **Nady Hufkens**,[4] **and Marianne Stranger**[1]

[1]*Indoor Air Quality Team, Flemish Institute for Technological Research (VITO), Mol, Belgium*
[2]*Department of General Internal Medicine, Infectious Diseases and Tropical Medicine, Antwerp University Hospital (UZA), Antwerp, Belgium*
[3]*Clinical Laboratory, Campus Virga Jesse, Jessa Ziekenhuis vzw, Hasselt, Belgium*
[4]*Reference-Laboratory Team, Flemish Institute for Technological Research (VITO), Mol, Belgium*

Correspondence should be addressed to Sarah L. Paralovo; sarah.limaparalovo@vito.be

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As worldwide evidence shows that the predominant transmission route of SARS-CoV-2 and other respiratory pathogens is airborne, the need for suitable methods for the sampling of bioparticles directly from the air is more urgent than ever. The present paper describes the development of a method for the collection of biological aerosols, using a preexisting cyclonic impinger, the Coriolis μ, combined with a lysis buffer and subsequent qPCR analysis of the generated samples in lab. Four phases of method development are described: exploratory, validation, blank tests, and application. The application phase consisted of a field experiment in which the method was simultaneously applied at two daycare facilities. The method achieved a good level of accuracy and reliability in detecting different types of infectious agents in the air, with a global uncertainty of 19.6%. Furthermore, our method allows the simultaneous detection of 26 different respiratory pathogens in air samples, it is relatively simple, and the equipment is easy to use. Additionally, the time to collect a representative sample is short compared to other methods. The method does not cause significant disturbance to those present in the sampled rooms, and it is safe for operators and flexible, meaning it can be used in virtually any environment regardless of use, size, or occupancy. Further research is being developed to allow quantitative analysis of the collected samples and to test the methods' ability to assess the viability of the microorganisms collected in the sample.

1. Introduction

The scientific community has been aware of the importance of monitoring indoor air quality for many decades, but since the COVID-19 pandemic hit the world in early 2020, this awareness has transformed into a commitment. After a long period of confusion and debate on the main routes of SARS-CoV-2 transmission, the dominant role of infectious aerosol particles is now being recognized following an ever-increasing amount of evidence [1, 2]. Practical methods for air sampling of viruses and microorganisms in indoor environments are therefore needed to study the implications of airborne detection and interventions to reduce the infectious risk.

While reference methods for assessing various air pollutants in indoor environments do exist, the monitoring of airborne biological agents in suspension is knowingly challenging [3]. Such monitoring is usually conducted via the collection of bioaerosols suspended in the indoor air, rather than via automatic sensors. Any method for separating particles from the rest of the air (e.g., sedimentation, filtration, inertial impaction, impingement in liquids, and thermal

and electrostatic precipitation) can in principle be applied to collect bioaerosols [4]. Suspended bioparticles can be collected using liquid media, filters, or impaction on a solid surface like a culture dish [5]. After collection, the microorganisms from the bioaerosol can be cultured, counted, and/or identified through an appropriate analytical method.

Both collection and analytical methods must be selected in accordance with the primary assessment goals. When the intention is to evaluate the viability of airborne biological agents (i.e., the ability to multiply and/or infect when provided with the appropriate conditions), the collection method must include an adequate medium to keep the target organisms in the bioaerosol viable, and then, the organisms must be cultured for an appropriate period under favorable nutrient conditions to allow for multiplication.

On the other hand, keeping the collected bioaerosols (viruses or microorganisms) viable may present a health risk depending on the biocontaminant of interest. In the case of SARS-CoV-2 monitoring, it is easy to understand why neutralizing the sample at the moment of collection may be preferable. In such situations, the analysis of the collected samples is not based on culture, but rather on detection, identification, and quantification (if possible). Analytical methods that do not require viability include microscopy (with or without fluorescent probes), flow cytometry, some bio- and immunological essays, and polymerase chain reaction (PCR) techniques [6–9].

Specifically for harmful viral agents, the sample collection procedure can be challenging. Virus-laden aerosols are usually present at very low concentrations in the air, and therefore, it may be necessary to sample a large volume of air to allow for detection. This often means a time-consuming process, requiring sampling periods of up to a week [10] or involving heavy and noisy high-volume pumps. Moreover, some sampling techniques, such as impaction and filtration, can dry out bioaerosol particles and damage the viral genetic material during collection, hindering the applicability of PCR analysis, for example [3].

Some published studies have compared the efficacy of different sampling techniques to collect airborne viruses, either experimentally or in the field. One study tested 4 different samplers for collecting an experimentally generated aerosol of H1N1 virus: an impinger incorporating cyclonic airflow (SKC BioSampler), Teflon filters, gelatin filters, and a cascade impactor [11]. The impinger/cyclone measured the highest concentrations of viral RNA, followed by the filters and then the impactor. Another large study compared the sampling efficiency of six different impinger/cyclone air samplers, a filter-based sampler, and a cascade impactor by collecting artificially generated aerosols of MS2 bacteriophages and swine/avian influenza viruses [12]. In that study, high flow rate samplers generally collected greater absolute quantities of virus, both viable (i.e., determined via titration and culture) and total (i.e., determined via qPCR), than low flow samplers, but low flow rate samplers generally measured higher, and likely more accurate, airborne concentrations of viable and total viral RNA (i.e., determined by dividing the absolute quantities determined via PCR by the total volume of sampled air). The study concludes that a complementary two-sampler approach may work best: a high flow sampler may provide low limits of detection to determine if any virus is present in the air, and if a virus is detected, a lower flow sampler may measure airborne virus concentrations more accurately.

Since 2020, researchers in the field have focused their attention into optimizing specifically the sampling of airborne SARS-CoV-2. Most of this research happened in hospital wards dedicated to COVID-19 patients and adjacent hospital/medical environments and applied many different sampling methods/devices and varying parameters for comparison. Many of these studies reported none or very few (weakly) positive samples, even when sampling in the presence of symptomatic COVID-19 patients [13–19].

One important study, which helped to demonstrate the predominant role of the airborne route for SARS-CoV-2 transmission, was able to collect 4 positive samples (out of 6) in a hospital in Florida using a novel type of air sampler, based on a water vapor condensation technique that retains the viability of the collected virus [20]. A more recent study from Truyols-Vives et al. [21] seemed even more effective in detecting SARS-CoV-2 in the air of hospital rooms in Spain, where almost half of all the 65 collected samples were positive. They attributed the good performance of their method to a combination of two main factors: the use of liquid impingement (SKC Biosampler®) to collect the virus (which helps to preserve the genetic material) and of digital droplet PCR (ddPCR) to analyze the samples (which is a newer technology that allows for lower detection limits compared to traditional qPCR). Later that same year, Truyols-Vives et al. [22] optimized their sampling protocol even further by substituting their liquid sampling media by a mineral oil, with a much lower evaporation rate in the same sampling time. With this change, they were able to detect SARS-CoV-2 in 80% of the 15 samples collected in the presence of a COVID-19 patient.

Similar studies in environments other than hospital/healthcare-related spaces are rarer. One study by Setti et al. [23] analyzed 34 ambient particulate matter (PM) samples from an industrial area in Italy and detected traces of SARS-CoV-2 in almost 60% of them. A disadvantage of their study design is the very long sampling time (24 h per sample). While long sampling times can help collecting a minimal detectable amount of genetic material, it simultaneously hinders the ability to make comparison studies on shorter term events (e.g., viral build-up during classes or the effect of air cleaning). Truyols-Vives et al. [21] further argue that some RNA degradation may occur during longer sampling periods. Hadei et al. [24] applied shorter sampling periods when collecting air samples in various public spaces in Iran. They were able to detect SARS-CoV-2 in 64% of the 28 samples collected on PTFE or glass fiber filters, using 40 or 3.5 l min^{-1} pumps (respectively) for 1 to 1.5 h. Despite the small sample size for each environment in their study, their results suggest that public spaces may carry an increased risk of airborne SARS-CoV-2 compared to hospital spaces, possibly due to significantly higher ventilation rates in the latter.

In this context, the work reported in this paper details the process of adapting a sampling and detection method

for airborne pathogens, including SARS-CoV-2. We hypothesize that, by combining adequate sampling parameters and strategies, we can reach a method that is equally well applicable to varied types of indoor environments, while maintaining a good level of simplicity, accuracy, and safety for operators. Field experiments performed during method development are also described.

2. Materials and Methods

2.1. Bioaerosol Collection. Liquid impingement was selected as the sampling method, following the example of Truyols-Vives et al. [21, 22], who were comparatively more efficient in collecting positive SARS-CoV-2 samples in the presence of COVID-19 patients than other researchers using different methods (e.g., Dumont-Leblond et al. [14] and Stern et al. [19]). In our study, airborne bioparticles were collected from the indoor air using a cyclonic impinger manufactured by Bertin Technologies (St-Berthely, France), Coriolis μ. This sampler is designed to collect airborne particles with diameters ranging from 0.5 to 20 μm by combining cyclonic impingement in any chosen liquid with a high airflow rate [25]. By generating samples directly in a liquid phase, the Coriolis μ is directly compatible with various microbiological analysis methods (e.g., PCR-type or culture) depending on the liquid media chosen. The highest collection efficiency of the device is for particles of ~5 μm diameter [25], matching the size fraction currently considered of the highest infectious potential [26].

The Coriolis μ allows a collection medium volume of up to 15 ml. The inlet airflow rate ranges from 100 to 300 l min^{-1} on increments of 50 l min^{-1}, which represents an advantage compared with the impinger used by Truyols-Vives et al. [21, 22], that operated at the significantly lower flow rate of 12.5 l min^{-1}. This means that the Coriolis μ can sample a much larger total volume of air by applying the same sampling period as applied by Truyols-Vives et al. [21, 22]. The sampling period of the stand-alone Coriolis μ device can be set from 1 to 10 min, in increments of 1 min. An add-on is available for long-term monitoring, which extends the sampling time to up to 6 hours, while an additional pump delivers extra liquid medium into the sampling cup at a specified rate, mitigating significant decreases in sample volume caused by evaporation [25].

In the exploratory phase of this research, a combination of different sampling airflow rates, sampling periods, and liquid medium volumes was tested. The liquid medium selected for bioaerosol collection was a lysis buffer, which is a solution that breaks open cells allowing for molecular biology analysis of cellular macromolecules (e.g., for DNA extraction) [27]. An important reason for using lysis buffer directly during sampling was the immediate deactivation of any pathological microorganism that may be collected in the sample, increasing safety during transportation and handling of samples. Moreover, the use of lysis buffer avoids extensive sample preparation in the lab, minimizing the introduction of potential analytical artefacts. Finally, following the example of Truyols-Vives et al. [22], using a liquid sampling medium of higher viscosity such as the lysis buffer allows for sampling periods longer than 10 minutes without significant evaporation (i.e., with minimal loss of liquid sample volume) even without the use of the add-on for long-term monitoring (which was not utilized in our research, sampling times longer than 10 min were enabled via bypassing the device's time-block mechanism).

The device operator was requested to wear an FFP2 respirator and nitrile gloves at all times when near the sampler and/or manipulating sampling cups or samples. The sampling spaces were only entered to start/stop and change the sampling cups on the device (which usually takes less than one minute).

2.2. Sampling Sites and Setups. Bioaerosol sampling was conducted in four distinct phases during this research. Initially, an exploratory phase was carried out to refine the methodology. Subsequently, a validation phase employed the optimized method configuration in both positive control and negative control environments. Following that, a blank test phase involved the application of the adapted method in areas devoid of potential pathogenic bioaerosol sources to collect blank samples, enabling the assessment of potential contamination by various infectious agents. Finally, the new method was applied in field experiments.

The exploratory phase occurred in an employee cafeteria in Mol, Belgium. A total of 24 samples were taken in the cafeteria over 3 consecutive days in October 2021 during lunch breaks (8 samples per day). In these experiments, two Coriolis μ devices were used simultaneously side-by-side to compare the efficiency of different sampling configurations. The sampling configurations comprised different combinations of sampling airflow rates (100 to 300 l min^{-1}), sampling periods (10 to 30 min), and liquid medium volume (V = 2 to 8 ml). Table 1 details these for each of the 24 samples taken in the cafeteria. All 24 samples were analyzed via qPCR exclusively for genetic markers of SARS-CoV-2 RNA.

Due to the composition of the lysis buffer, a thick foam is generated in the sampling cup during the sampling process, which may potentially impede the collection cup's vortex formation. Hence, varying quantities of an antifoam agent (IDEXX Laboratories, Inc.) were added to the buffer. The total volume of buffer used did not exceed 8 ml, as an extra measure to avoid that the generated foam would reach the air inlet of the Coriolis μ. Figure 1 shows the setup configuration of the 1st sampling day in the cafeteria.

Once the optimal sampling conditions were determined in the exploratory phase, validation experiments were performed, first in a presumed positive control environment. The positive control test involved the placement of Coriolis μ impingers in close proximity to an individual (one of the coauthors of this paper) who had tested positive for SARS-CoV-2, thereby creating an environment where the presence of viral bioaerosols was expected (however not confirmed by alternative tests, which ideally would be the case for a positive control test). The subject selected for the experiment was a 39-year-old male, residing in Brussels, with only mild symptoms of COVID-19 (i.e., headache), but with a "strongly positive" SARS-CoV-2 test result (PCR CT value < 16 from a nasal swab taken a couple of days after

Table 1: Description of samples taken during the exploratory phase experiments in a cafeteria in Mol, Belgium.

Day	Sample ID	Location	Sampling length (min)	Air sampling rate (l/min)	V air sample (m^3)	V collection media (ml)	Antifoaming agent (# droplets)
1	Cafe # 01	Center	30	100	3	8	3
	Cafe # 02		10	100	1	5	3
	Cafe # 03		10	100	1	5	3
	Cafe # 04		10	100	1	5	3
	Cafe # 05		30	100	3	8	2
	Cafe # 06		10	100	1	5	2
	Cafe # 07		10	100	1	5	2
	Cafe # 08		10	100	1	5	2
2	Cafe # 09		30	100	3	5	1
	Cafe # 10		10	100	1	5	1
	Cafe # 11		10	100	1	5	1
	Cafe # 12		10	100	1	5	1
	Cafe # 13		30	100	3	3	1
	Cafe # 14		10	100	1	3	1
	Cafe # 15		10	100	1	3	1
	Cafe # 16		10	100	1	3	1
	Cafe # 17		10	100	1	2	1
	Cafe # 18		10	100	1	2	0
3	Cafe # 19	Close to left wall	10	150	1,5	2	0
	Cafe # 20		10	150	1,5	3	0
	Cafe # 21		10	200	2	2	1
	Cafe # 22		10	200	2	2	0
	Cafe # 23		30	150	4,5	3	1
	Cafe # 24		30	150	4,5	2	1

Figure 1: Exploratory phase experiment in a cafeteria in Mol, Belgium.

symptom onset). The nasal swab and subsequent PCR test were conducted by the subject at a third-party lab, unrelated and independent from this study. Air samples were taken in the subject's apartment during two separate days in December 2021: the first (day 1) three days after the subject's positive PCR test result and the second (day 2) when he was released from the 10-day isolation period that was mandatory at that time in Belgium (i.e., on the 11^{th} day after the subject's positive PCR test result). The second sampling day was presumed to be a negative control test (i.e., in an environment where the presence of viral bioaerosols was not expected), since the subject had strictly followed the isolation measures for the officially required period at that time of the pandemic and therefore should not be emitting viral particles at significant levels anymore. However, it should be noted that confirmation through alternative testing methods was once again unavailable. A total of 10 samples were taken in the subject's residence, 5 on each sampling day. These 10 samples were analyzed with qPCR for genetic markers of SARS-CoV-2 RNA. Table 2 provides an overview of the sampling setups employed for each of the 10 samples collected in the presumed positive/negative control environment.

The third experimental phase described in this paper involved a series of tests that aimed to assess the contamination risk; thus, there is a risk of false-positive results generated during both sample collection and lab analysis. Firstly, a total of 60 field blanks were collected from an unoccupied and unfurnished office over 5 different days (12 samples per day, using two Coriolis μ devices simultaneously), utilizing sterilized device parts. Secondly, several field blanks were collected in outdoor locations (away from people), using nonsterilized device parts, after other samples had been taken in occupied indoor spaces, i.e., following sample collection in a cafeteria (total of 12 blanks), two schools (total of 5 blanks), and two daycares (total of 4 blanks). The sampling configuration for the Coriolis μ device was the same as in the exploratory phase of this research (and validated in

Table 2: Description of samples taken in the validation phase experiments in the residence of the subject who tested positive for COVID-19.

Day	Sample ID	Coriolis μ location	Subject's position/activity
1 (positive control)	House # 01	Office desk	Seated at 2 m distance from the Coriolis μ and engaged in continuous speech during sampling
	House # 02		
	House # 03	Office desk	Seated at 20 cm distance from the Coriolis μ and engaged in continuous speech during sampling
	House # 04		
	House # 05	Office desk	Same conditions as above (20 cm distance and talking) but sampling cup reused from previous sampling, after rinsing with sterile water + ethanol
2 (negative control)	House # 06	Bedroom (occupied overnight, unoccupied during sampling)	Subject absent
	House # 07	Living room (occupied by PCR-negative partner of subject)	Subject absent
	House # 08	Office desk	Seated at 2 m distance from the Coriolis μ and engaged in continuous speech during sampling
	House # 09	Office desk	Seated at 20 cm distance from the Coriolis μ and engage in continuous speech during sampling
	House # 10	Office desk	Same conditions as above (20 cm distance and talking) but sampling cup reused from previous sampling, after rinsing with sterile water + ethanol

the second phase described in the previous paragraph). The following genetic markers for infectious agents were included as targets for the PCR lab analysis in this phase (in addition to SARS-CoV-2 RNA): adenovirus DNA; bocavirus DNA; coronavirus 229E, NL63, OC43, and HKU1 RNA; enterovirus RNA; hMPV RNA; influenza A and B RNA; parainfluenza 1-4 RNA; rhinovirus RNA; RSV-A and B RNA; herpes simplex and varicella-zoster virus DNA; *Bordetella pertussis* DNA; *Bordetella parapertussis* DNA; *Bordetella holmesii* DNA; *Chlamydophila pneumoniae* DNA; *Legionella pneumophila* DNA; *Mycoplasma pneumoniae* DNA; and *streptococcus pneumoniae* DNA.

Lastly, the application phase of this study was carried out during normal working hours at two different daycare facilities in the province of Antwerp, Belgium. This application was part of a larger study performed for the Flemish Government looking into airborne pathogens in public spaces. The first facility (henceforth called C1) was a naturally ventilated ground floor space (average ventilation rate measured via tracer gas decay at approx. $1.1\,h^{-1}$ under normal cold weather conditions), which had formerly been a retail store, located in a residential area. A total of 13 babies and toddlers were cared for at the time of the experiment. The building featured large front windows of the unopenable shopping window type. Additionally, it had an exterior door that led to an enclosed (fenced) outdoor playground situated on the street side. Behind the indoor playroom of the toddlers (aged >18 months), there was a kitchen with an openable window. At the back of the daycare center, there was a bedroom for the children's nap time. Figure 2 shows a sketch of C1 along with a picture of the toddler's playroom.

The second daycare facility (henceforth called C2) consisted of a terraced building located in an urban environment and had a mechanical ventilation system (based on mechanical air extraction and natural air supply; ventilation rate measured at approx. $3.6\,h^{-1}$ under cold weather conditions with the front door and the door to the storage room open, the door to the play area slightly open, and the window in the storage room tilted). The facility provided care for 70 children in total at the time of the experiment, who were divided into groups according to their age. In this experiment, the toddlers' building was assessed. Two rooms in that building were selected for sampling, one on the ground floor and the other on the first floor. In both rooms, there was also a duplex-style sleeping area integrated within the space. Playing and sleeping areas could therefore not be closed off from each other. A sketch of the rooms is provided in Figure 3, along with a picture of the ground floor room (both rooms share identical dimensions and spatial arrangements).

The two daycares were assessed simultaneously during a 2-week period in March 2022, when the official daily COVID-19 incidence in Belgium was about 60/100,000 inhabitants [28]. During this period, bioaerosol sampling was carried out with the Coriolis μ at 4 different days. Each sampling day, a total of 4 to 6 air samples were collected with the Coriolis μ (same sampling configuration as described in the previous research phases), aiming at having 2 samples per day at each sampling space whenever possible. The genetic markers of infectious agents included in the samples' PCR analysis were the ones listed for the blank test phase above.

2.3. Sample Analysis. Environmental air samples, collected in cell lysis buffer (Promega Corp., Madison, U.S.), were transferred on-site from the collecting vessel of the Coriolis μ to 15 ml sterile centrifuge tubes immediately after collection. At the end of each sampling day, the sample batch was sent via taxi service straight to the Jessa Hospital microbiology laboratory.

All samples were tested with an in-house developed multiplex PCR test for the following respiratory pathogens: adenovirus; bocavirus; coronaviruses NL63, OC43, and HKU1;

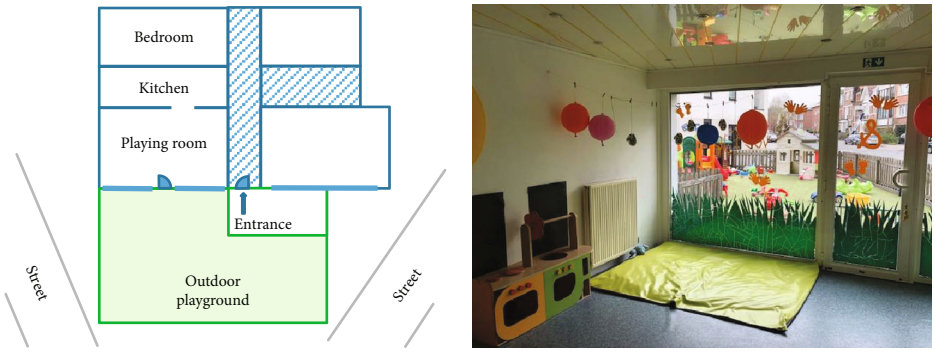

Figure 2: (a) Sketch of daycare 1 (C1) and (b) picture of the toddlers' playroom.

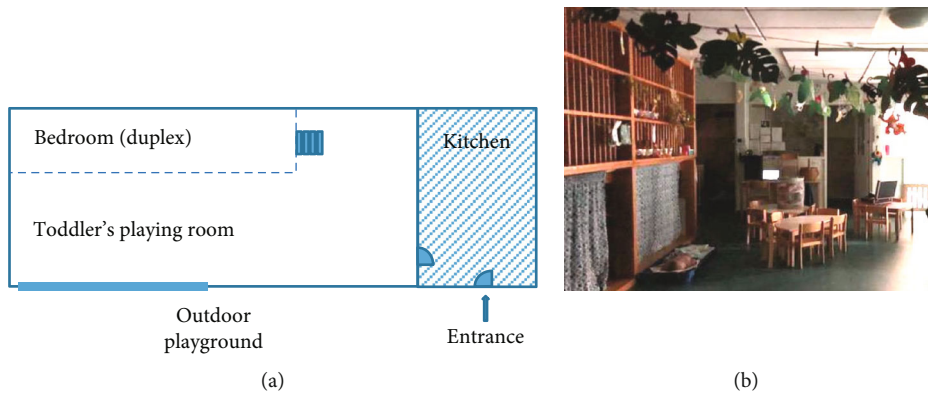

Figure 3: (a) Sketch of daycare 2 (C2) and (b) picture of the ground floor playing room for toddlers.

enterovirus, influenza viruses A and B; parainfluenza viruses 1-4; rhinovirus; respiratory syncytial virus (RSV) A and B; human metapneumovirus (hMPV); herpes simplex virus; varicella-zoster virus; *Bordetella pertussis*; *Bordetella parapertussis*; *Bordetella holmesii*; *Chlamydophila pneumoniae*; *Legionella pneumophila*; *Mycoplasma pneumoniae*; and *Streptococcus pneumoniae*.

DNA and RNA extraction was performed on a 1 ml sample using the DSP Virus/Pathogen Midi Kit on the automated sample preparation system QIAsymphony SP (Qiagen, Hilden, Germany) according to the manufacturers' instructions. Prior to extraction and amplification, RNA and DNA controls (porcine delta virus and Puumala hantavirus, Department of Viroscience, Erasmus Medical Centre Rotterdam) were added to each sample. Subsequently, 60 μl of the eluate was added to the TaqMan Fast Virus 1-Step Master Mix (Life Technologies, Carlsbad, U.S.) and pipetted utilizing the QIAgility instrument (Qiagen) into custom-made microwell strips. Each test strip contained 8 microwell cups filled with pathogen-specific primers and probes for a total of 28 targets (Eurogentec, Liège, Belgium). Amplification was performed on the QuantStudio7 PCR instrument (Thermo Fisher Scientific, Waltham, U.S.).

All samples were tested for SARS-CoV-2 RNA on the fully automated Alinity m System (Abbott, Illinois, U.S.) according to the manufacturers' instructions. This assay detects highly conserved and SARS-CoV-2-specific target regions in the RdRp and N genes of the SARS-CoV-2 genome. The literature shows that the Abbott Alinity m SARS-CoV-2 assay performs well [29, 30]. Samples with a Ct value (threshold cycle) of 34.5 or less were considered as positive (for SARS-CoV-2). Ct values higher than 34.5 were reported as limit value for this pathogen. This cut-off value was based on a nationwide calibration of different commercially available analysis methods with documented samples (virus culture) coming from the National Reference Laboratory (UZ Leuven) and corresponds to 25 virus particles per milliliter of sample, which was also demonstrated in our laboratory for the SARS-CoV-2 RNA kit on the Alinity m System. The highest Ct value in the Alinity m System is 45; i.e., once this value is reached, the analysis automatically stops and the sample is labeled as "negative." For all the other targeted pathogens besides SARS-CoV-2, samples with a Ct value under 35 were considered as positive. When the Ct value was 35 or higher, the PCR was repeated in singleplex and reported as "limit value" when the repeat Ct was again ≥35. The in-house method for the other respiratory pathogens was extensively validated in our lab and is under ISO 15189 accreditation for many years. The RNA/DNA extraction method is part of the integral analysis process that

has been extensively validated and accredited in its entirety. During the validation process, the extraction method was also optimized to achieve optimal sensitivity.

2.4. Uncertainty Analysis. Uncertainty measurement is typically applicable in quantitative analyses as a parameter that quantifies the dispersion of results. However, in qualitative analyses, such as the method currently under development in this study (where samples yield only binary outcomes of positive or negative), the expression of uncertainty cannot be rendered in a similar manner [31, 32]. In such instances, recognizing that uncertainty measurements are inherently probabilistic, the uncertainty can be conveyed as the probability of making an erroneous decision or of an outcome being inaccurate [32]. To assess the overall uncertainty of the proposed method, the individual sources of error must be mathematically combined using the summation in quadrature approach [31], as shown in

$$\text{Combined uncertainty} = \sqrt{a^2 + b^2 + c^2 + (\cdots)}, \quad (1)$$

where a, b, and c represent the individual errors related to each factor influencing the final result. For the Coriolis μ method, the two possible types of contamination (onsite and in lab) leading to false-positive results can be considered as the primary sources of uncertainty, and both are in this paper expressed in percentages of false-positive blanks.

3. Results and Discussion

3.1. Exploratory Tests. For the first day of sampling in the employee cafeteria, a greater volume of liquid media (8 ml) was used for extended sampling (30 min) to mitigate any potential issues arising from buffer evaporation. However, this larger volume resulted in the buffer foam rising high enough to touch the Coriolis μ air inlet, which could potentially lead to contamination of subsequent samples. The air inlets of both Coriolis μ devices were thoroughly disinfected with disinfecting wipes after sampling (cafe # 01 and # 05) with 8 ml of collection medium, and none of the results showed any contamination. Nevertheless, 5 ml was determined as the maximum collection medium volume.

Regarding the use of antifoaming agent, the addition of 2 and 3 droplets to the buffer did not reduce foaming visually, compared to the use of 1 droplet only. Without the addition of antifoaming agent, however, moderately more intense foam formation was observed on the 3rd sampling day. It was thus determined that 1 droplet of antifoaming agent per sampling cup was required. The highest sampling rate tested (200 l min^{-1}) resulted in excessive foaming, even with antifoaming agent. The intermediate flow rate (150 l min^{-1}) led to only moderately more foaming than the lowest flow rate (100 l min^{-1}), but the noise level was considerably higher for the former (according to the manufacturer, the highest flow rate generates a noise level of 70 dB) [25]. Considering that the sampling method is intended to be used also in environments where noise is a concern (e.g., schools, nurseries, and elderly care homes), the lowest sampling airflow rate (100 l min^{-1}) was selected.

The PCR analysis of the 24 cafeteria samples indicated one "limit value" sample for SARS-CoV-2 (i.e., non-negative, traces of SARS-CoV-2 RNA were detected in the sample with Ct value > 35): cafe # 13. This sample was collected during 30 min. Simultaneously, with a second Coriolis μ side-by-side, sample cafe # 14, # 15, and # 16 were collected successively, each during 10 minutes of sampling, and all three turned out completely negative in the qPCR analysis. This could mean that a longer sampling time of 30 min is more effective in collecting viral particles from the indoor air. Sample cafe # 13 was collected in 3 ml buffer fluid for 30 min of sampling at 100 l min^{-1} airflow rate. For subsequent field experiments, this sampling configuration of the Coriolis μ was therefore picked as the protocol, with 1 droplet of antifoaming agent as medium.

3.2. Validation. Table 3 shows the results obtained after qPCR analysis of the samples taken at the residence of the SARS-CoV-2-infected patient in Brussels. Traces of SARS-CoV-2 were detected in all five samples taken near the subject at peak infectiousness (on the first sampling day, the 3rd day after his positive nasal swab), while the 5 samples taken in the presence of the same subject on the second sampling day (the 11th day after his positive nasal swab, when the infective potential was assumed to be insignificant, i.e., after the mandatory 10-day isolation period) were negative.

It is of course important to consider that, despite air sampling taking place right next to a COVID-19 patient, this does not mean that air samples should necessarily be expected to result positive, because not all patients exhale SARS-CoV-2 particles. In fact, in many cases, the viral replication phase is ended when patients are admitted in a hospital. As seen many studies conducted in hospitals in which sampling took place close to a COVID-19 patient [13–19], most (if not all) samples resulted negative. In such studies, it is likely that many of those negative samples correspond to air samplings occurring beside a noninfective patient. On the contrary, in the present study, a COVID-19 patient expected to be in the viral replication phase was selected, as was the case in the Truyols-Vives et al. [22] study.

In this context, if the relying assumptions are properly acknowledged (i.e., assuming that every individual who tests positive for SARS-CoV-2 after a nasal swab PCR exhales viral bioaerosols at a sufficient quantity for accurate detection and that the same individuals stop exhaling viral bioaerosols at detectable levels after the adequate isolation period), the results in Table 3 can be considered as successful positive and negative controls, respectively, for the method validation. In fact, it could be argued that this method was more successful even than the protocol optimized by Truyols-Vives et al. [22], who collected several samples exclusively in the presence of COVID-19 patients, but only detected viral traces in about 80% of these samples. Despite the total number of samples collected in their study (15) being higher than the number collected in the present study (5), we had a 100% success rate of capturing viral traces in the presence of a COVID-19 positive individual.

Table 3: Results obtained after PCR analysis of 10 air samples collected at the apartment in Brussels, Belgium.

Sampling day	Sample ID	Presence of SARS-CoV-2	Ct value
1	House # 01	Non-negative (LV)*	35.01
	House # 02	Non-negative (LV)*†	39.77†
	House # 03	Non-negative (LV)*	37.69
	House # 04	Non-negative (LV)*	37.58
	House # 05	Non-negative (LV)*	38.20
2	House # 06	Negative	—
	House # 07	Negative	—
	House # 08	Negative	—
	House # 09	Negative	—
	House # 10	Negative	—

*For SARS-CoV-2, samples with Ct < 34.5 are reported as positive and with Ct > 34.5 as "limit value" (LV). For the other pathogens, the cut-off is Ct = 35. †For SARS-CoV-2, Ct = 39.3 was calculated as limit of blank (LoB). Although not strictly applicable as a cut-off, samples with Ct > 39.3 have increased probability of being false positives. LoBs were not derived for other pathogens.

Table 3 also presents the cycle threshold (Ct) values for each of the samples in which SARS-CoV-2 was detected. In a PCR analysis, any viral RNA contained in the sample is isolated and then amplified via multiple cycles to produce a detectable amount of RNA. The Ct value is the number of cycles necessary to detect the virus of interest, and when that happens, the PCR automatically stops running. If there is no positive signal after 45 cycles, the test result is considered negative. Therefore, the Ct value is inversely proportional to the amount of viral RNA in the sample. For example, a test which detects a positive result in the 12^{th} cycle (i.e., Ct value = 12) contains over 10^7 times more genetic material than a sample with Ct value = 35 [33]. Although the Ct value is not always a measure of infectivity, because the genetic target of both viable and nonviable microorganisms is measured without distinction, it could still be considered a useful proxy, especially when applied to samples taken straight from the air, as opposed to those taken from human body fluids (i.e., not yet spread to the environment and thus not immediately in risk of contact with other individuals).

Air samples will normally contain much smaller quantities of viral RNA compared to human samples, regardless of the concentration of viral-laden bioaerosols in the assessed environment, because the bioparticles emitted by infected person(s) disperse throughout the whole air volume contained in the room they are in, leading to high dilution factors. This means that the Ct value thresholds commonly considered to determine if a sample is positive, e.g., nasal swabs (usually Ct value < 30), might not be as adequate for samples taken with the Coriolis μ. Therefore, it seems necessary to determine new threshold Ct values specifically applicable to viral samples collected directly from the air, to better ascertain if an air sample is truly positive or negative. It is important to note that determining air samples as positive for SARS-CoV-2 provides only a quick yet superficial assessment of the potential infectivity of bioaerosols in a given space. The actually infectious dose of SARS-CoV-2 suspended in aerosol has not yet been determined (i.e., dose-response studies are lacking), mostly because such studies are much more complex to prepare and perform.

We should note that the validation tests were preliminary, involving only one patient. Given the varied response of individuals to SARS-CoV-2, further validation requires inclusion of more patients. During the measurements, constraints such as limited time, specialized personnel, and resources prevented the inclusion of additional test subjects. A comprehensive clinical trial is planned for the near future.

3.3. Blank Tests. For the third phase of the method development, blank samples from indoor and outdoor locations were analyzed. Traces of SARS-CoV-2 RNA were reported in 3 out of the 60 indoor blanks (5%), collected at the unoccupied office, with Ct values of 38.8 and higher. Table 4 shows the results obtained only for the 3 samples which registered non-negative for SARS-CoV-2 RNA during the PCR analysis (none of the other 57 blank samples presented any traces of either SARS-CoV-2 or any other of the 28 targeted genetic markers). Even in these 3 samples in which genetic markers were detected, the only pathogen picked up was SARS-CoV-2. Since the air samples were analyzed in a lab that routinely provides analysis of swab samples from human origin (which, when positive, tend to be much more highly concentrated when compared to air samples), it is possible that some carry-over from highly concentrated samples happened to the blank samples during laboratory analysis, resulting in false positives. There is also the possibility of actual presence of pathogen bioaerosols at the sampling site or blank contamination during sampling by the Coriolis μ parts. These three potential sources of contamination are however considerably unlikely.

The chance of contamination during the lab analysis is small because (at least for SARS-CoV-2) it is performed as a fully automatic method in a closed system, and negative controls are also analyzed at each run. The chance of actual presence of pathogen bioaerosols during sampling is also small, since the office was cleaned and the surface where the Coriolis μ was placed was disinfected with alcohol before the test, the room remained unoccupied during the test (as it had been for many consecutive days before then), and the researcher entered the room only for placing/removing sampling cups (<1 min each time) and was wearing an FFP2 mask and nitrile gloves for the entire test. Lastly, the chance of blank contamination during sampling is small because the Coriolis μ parts were thoroughly washed and autoclaved before collection (thus presumed to be free of contamination).

Regardless of the actual source of this potential contamination, their high Ct values do indicate a strong possibility of consisting of false-positive samples. From the results of these 3 most likely false positives, the lowest Ct value was 38.8. Therefore, samples in which the genetic marker is detected at Ct values around and above 38.8 must be considered with greater caution, due to its high uncertainty and thus increased chance of constituting a false positive.

Table 4: Results obtained after PCR analysis only for the 3 (out of 60) blank samples collected at the unoccupied office which were not completely clear of genetic material.

Infectious agent	Blank 1	Blank 2	Blank 3
Varicella-zoster virus DNA	Negative	Negative	Negative
Herpes simplex virus DNA	Negative	Negative	Negative
Bordetella pertussis DNA	Negative	Negative	Negative
Bordetella parapertussis DNA	Negative	Negative	Negative
Bordetella holmesii DNA	Negative	Negative	Negative
Chlamydophila pneumoniae DNA	Negative	Negative	Negative
Legionella pneumophila DNA	Negative	Negative	Negative
Mycoplasma pneumoniae DNA	Negative	Negative	Negative
Streptococcus pneumoniae DNA	Negative	Negative	Negative
Adenovirus DNA	Negative	Negative	Negative
Corona virus 229E RNA	Negative	Negative	Negative
Coronavirus HKU1 RNA	Negative	Negative	Negative
Coronavirus NL63 RNA	Negative	Negative	Negative
Coronavirus OC43 RNA	Negative	Negative	Negative
SARS-CoV-2 RNA (RT-PCR)	LV* (Ct = 38.8)	LV* (Ct = 39.1)	LV* (Ct = 39.2)
Human metapneumovirus RNA	Negative	Negative	Negative
Bocavirus DNA	Negative	Negative	Negative
Bocavirus DNA	Negative	Negative	Negative
Rhinovirus	Negative	Negative	Negative
Influenza A virus RNA	Negative	Negative	Negative
Influenza B virus RNA	Negative	Negative	Negative
Parainfluenza 1 virus RNA	Negative	Negative	Negative
Parainfluenza 2 virus RNA	Negative	Negative	Negative
Parainfluenza 3 virus RNA	Negative	Negative	Negative
Parainfluenza 4 virus RNA	Negative	Negative	Negative
Enterovirus RNA	Negative	Negative	Negative
RSV A RNA	Negative	Negative	Negative
RSV B RNA	Negative	Negative	Negative

*Limit value = genetic marker was detected in the sample, but at a high Ct value (closer to the detection limit).

This should, however, not be used as a strict rule or cut-off value, but rather as an indicator of an increased uncertainty of the PCR analysis result, since at these high Ct values, it is inherently difficult to distinguish true positives from false positives.

According to Armbruster and Pry [34], the limit of blank (LoB) is the "highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested" and is calculated as follows: $\mathrm{LoB} = \mathrm{mean}_{\mathrm{blank}} + 1.645(\mathrm{SD}_{\mathrm{blank}})$. In theory, calculating this value could help to determine whether Ct values within the 35-45 range can be considered as positives. In our method, the Ct values of the three indoor blanks (38.8/39.1/39.2) could technically be used to calculate an LoB using the expression above, resulting in Ct = 39.3. However, we are not convinced that such a calculation is applicable in this case, since a Ct value is not an analyte concentration. Although related, there is not enough data available yet to confidently relate Ct values to SARS-CoV-2 air concentrations.

Regarding the outdoor field blanks, in 5 out of 21 samples (24%), at least one of the targeted genetic markers was detected (4 times for SARS-CoV-2 and one time for rhinovirus). Table 5 shows the results obtained for these 5 samples. From the 5 field blanks in which traces of genetic markers were detected, 3 presented SARS-CoV-2 at a Ct value above 38.8, indicating a very small quantity of viral material and thus a higher probability of configuring as false positives resulting from carry-over contamination during lab analysis, and one had a Ct value below this value (37.7). For this latter sample and for one outdoor field blank taken at the school (which was negative for SARS-CoV-2, but positive at LV for rhinovirus), it is less clear; i.e., contamination could have happened in lab or upon collection in the field (by carry-over from non-negative samples to blanks onsite, since the outdoor blanks were always collected at the end of the sampling day, using the same Coriolis μ parts that were used for collecting indoor samples).

In these experiments, the uncertainty regarding potential contamination by SARS-CoV-2 carry-over in the group of

Table 5: Results obtained after PCR analysis only for the 5 (out of 21) field blanks collected at the cafeteria, schools, and daycare facilities which were not completely clean.

Infectious agent	Cafeteria blank 1	Cafeteria blank 2	Cafeteria blank 3	Daycare blank 1	School blank 1
Varicella-zoster virus DNA	Negative	Negative	Negative	Negative	Negative
Herpes simplex virus DNA	Negative	Negative	Negative	Negative	Negative
Bordetella pertussis DNA	Negative	Negative	Negative	Negative	Negative
Bordetella parapertussis DNA	Negative	Negative	Negative	Negative	Negative
Bordetella holmesii DNA	Negative	Negative	Negative	Negative	Negative
Chlamydophila pneumoniae DNA	Negative	Negative	Negative	Negative	Negative
Legionella pneumophila DNA	Negative	Negative	Negative	Negative	Negative
Mycoplasma pneumoniae DNA	Negative	Negative	Negative	Negative	Negative
Streptococcus pneumoniae DNA	Negative	Negative	Negative	Negative	Negative
Adenovirus DNA	Negative	Negative	Negative	Negative	Negative
Coronavirus 229E RNA	Negative	Negative	Negative	Negative	Negative
Coronavirus HKU1 RNA	Negative	Negative	Negative	Negative	Negative
Coronavirus NL63 RNA	Negative	Negative	Negative	Negative	Negative
Coronavirus OC43 RNA	Negative	Negative	Negative	Negative	Negative
SARS-CoV-2 RNA (RT-PCR)	LV* (Ct = 37.7)	LV* (Ct = 40.4)†	LV* (Ct = 40.7)†	LV* (Ct = 40.1)†	Negative
Human metapneumovirus RNA	Negative	Negative	Negative	Negative	Negative
Bocavirus DNA	Negative	Negative	Negative	Negative	Negative
Bocavirus DNA	Negative	Negative	Negative	Negative	Negative
Rhinovirus	Negative	Negative	Negative	Negative	LV* (Ct = 36.0)
Influenza A virus RNA	Negative	Negative	Negative	Negative	Negative
Influenza B virus RNA	Negative	Negative	Negative	Negative	Negative
Parainfluenza 1 virus RNA	Negative	Negative	Negative	Negative	Negative
Parainfluenza 2 virus RNA	Negative	Negative	Negative	Negative	Negative
Parainfluenza 3 virus RNA	Negative	Negative	Negative	Negative	Negative
Parainfluenza 4 virus RNA	Negative	Negative	Negative	Negative	Negative
Enterovirus RNA	Negative	Negative	Negative	Negative	Negative
RSV A RNA	Negative	Negative	Negative	Negative	Negative
RSV B RNA	Negative	Negative	Negative	Negative	Negative

*Limit value = genetic marker was detected in the sample, but at a high Ct value (close to the detection limit). †Ct > LoB: for SARS-CoV-2, Ct > 39.3 indicates samples with increased probability of being false positives.

indoor blanks was of 5% (i.e., 3 out of 60 indoor blanks), and in the group of outdoor blanks, it was of 19% (i.e., 4 out of 21 outdoor field blanks). Combining both via a summation in quadrature, according to Bell [31], it gives a global uncertainty of 19.6% for the Coriolis μ sampling method for SARS-CoV-2 described in this paper. A recommendation for reducing the risk of contamination onsite, and consequently reducing uncertainty, is substituting the Coriolis μ parts for autoclaved ones between consecutive samples, as was done for the indoor blanks.

3.4. Real-Life Application. Table 6 summarizes the results obtained after qPCR analysis of the air samples collected at the two daycare facilities. No medical tests were performed on the children or employees of the daycare facilities to attest if they were infected by any of the pathogens, because such efforts would require much more time and funds than available, besides needing healthcare specialists to get samples and proper consent forms for all subjects. Alternatively, the employees responsible for each of the assessed rooms were asked to register the number of children present each day of the experiment and the number of children absent due to illness. This information is shown in Table 7.

Overall, C1 had fewer cases of ill children during the experiment, while the first floor room in C2 had the most cases. It is however important to note that these numbers are not expected to directly correlate to the level of infective bioaerosols present in the rooms: first because the children confirmed ill were in fact absent during the samplings and second because asymptomatic individuals can also emit infective bioaerosols, even if at lower rates than symptomatic individuals. These numbers simply provide a general overview of the potential circulation of respiratory symptoms among the different groups.

Of the 22 samples collected at both daycare facilities during the 4 sampling days, 8 contained traces of SARS-CoV-2 according to the qPCR test, i.e., a ratio of 36% non-negative samples in total. Four of these eight samples contained traces of more than one pathogen simultaneously, demonstrating that the method seems capable of

Table 6: Results of qPCR analysis from the air samples collected with the Coriolis μ at the two daycare facilities (Ct values shown are for SARS-CoV-2; the presence of other pathogens is indicated by superscript numbering).

			Week 1		Week 2	
			Day 1	Day 2	Day 3	Day 4
C1	Playing room	M*	LV* (CT 36.55)[1]	Negative	LV (CT 36.99)	Positive (*vl** < 1000, CT 33.86)[5]
		A*	—	Negative	LV (CT 34.66)	Negative
	Bedroom	A	Negative[2]	—	LV (CT 35.33)[3]	—
C2	Ground floor room	M	Negative	Negative	LV (CT 36.16)	LV (CT 39.82)†
		A	—	Negative	Negative[4]	Negative
	1st floor room	M	Negative	Negative	Negative	LV (CT 36.82)[6]
		A	—	Negative	—	Negative

*LV = limit value. †Ct > LoB: increased possibility of false-pos; *vl* = viral load; M = sample collected in the morning; A = sample collected in the afternoon. [1]Streptococcus pneumoniae DNA positive (Ct = 34). [2]Streptococcus pneumoniae DNA positive (Ct = 33) + coronavirus OC43 RNA at LV (Ct = 38). [3]Streptococcus pneumoniae DNA positive (Ct = 33) + rhinovirus positive (Ct = 35). [4]Influenza A virus RNA at LV (Ct = 37 CDC/38 Schülze). [5]Streptococcus pneumoniae DNA positive (Ct = 33) + rhinovirus positive (Ct = 33) + influenza A virus RNA at LV (Ct = 38 CDC/41 Schülze) + enterovirus RNA at LV (Ct = 36). [6]Rhinovirus positive (Ct = 34).

Table 7: Number of children present and absent due to illness in each of the assessed rooms during the experiment in daycares C1 and C2.

		Week 1		Week 2	
		Day 1	Day 2	Day 3	Day 4
C1	Present	11	11	15	13
	Absent	0	1	0	1
C2 ground floor	Present	14	13	17	13
	Absent	2	2	0	1
C2 1st floor	Present	13	12	14	15
	Absent	2	3	1	1

detecting the infection potential for several respiratory illnesses at once (e.g., COVID-19, pneumonia, common cold, and influenza). Two other samples contained only other pathogens, but not SARS-CoV-2. One of the 8 non-negative samples even reached the level of a clear "positive" in terms of Ct values of nasal swab (<34.5) samples instead of only limit value, i.e., a level comparable to nasal swabs of infected humans. We were surprised to obtain such a concentrated sample from the air, particularly because the sampling site was not healthcare-related (i.e., the presence of highly infectious persons, and thus of strong "emission sources," was not expected). However, in line with our results, generally fewer and less-concentrated SARS-CoV-2 positive samples have been retrieved from hospital air [13–19] compared to public spaces [23, 24].

From Table 6, 80% of the samples were completely free of the targeted genetic markers in week one, while in week 2, this rate fell to only 33%, suggesting that the first week of sampling had a lower incidence of pathogenic (and potentially infective) aerosols present in the facilities. More importantly, however, comparing both facilities, only 21% of samples were non-negative in C2, while in C1, this rate was 67%, suggesting that the air in C2 had an overall lower infective potential than C1. It is possible that this discrepancy is due to the presence of more numerous infectious children in C1 than in C2, although the levels of respiratory symptoms (e.g., coughing and nasal mucus discharge) perceived by the research team among the children were seemingly similar between both facilities.

However, the difference in the rate of non-negative samples between the facilities was in fact expected, as a reflection of the better ventilation in C2 (which counts with a mechanical ventilation system). Even before the COVID-19 pandemic, Stockwell et al. [35] performed a systematic review on the concentrations and composition of indoor bioaerosols in different areas within hospitals and the effects of different ventilation systems. They observed that areas with natural ventilation had the highest total bioaerosol concentrations, compared with areas using conventional mechanical ventilation systems, while areas using sophisticated systems (e.g., increased ACHs, directional flow, and filtration systems) had the lowest total bioaerosol concentrations. This demonstrates that more efficient mechanical ventilation systems can improve IAQ within hospitals and thus assist in reducing the risk of airborne pathogen transmission. Although their review focused exclusively on hospitals, one could extrapolate their findings to other public spaces where infected individuals might be present [35].

In our study, another important indication of the beneficial role of increased ventilation is the fact that, in the two sampling days of the second week, it happened in 3 sampling spaces that the first collected sample contained traces of SARS-CoV-2 (excluding the sample with Ct value > 38.8, which has a higher likelihood of being a false positive) and

the subsequent sample collected in the same space, in the same day and with the same group of children, was negative. In all three cases, the first sample was collected earlier in the morning, when doors and windows were kept closed for thermal comfort, and the second later in the afternoon, after the children had their outdoor playing time, during which the doors remained open for airing, a common practice in both facilities when the weather is sunny. The two samples taken at C1 on day 4 are also illustrative: the sample with the most viral material present in the whole experiment (the only one which had a clear "positive" result for SARS-CoV-2, besides the presence of 4 other pathogens simultaneously) was collected in the morning, and then, the second sample collected later in the same day and in the presence of the same group of children, right after the half-hour airing, was completely negative. As such, these results are in line with the understanding that better ventilation, especially when combined with lengthier periods of airing, can be efficient in diminishing the presence of different airborne pathogens in daycare facilities and consequently in reducing the risk of airborne pathogen transmission in these spaces.

4. Conclusion

The present paper reports on the work performed to develop a suitable method for collecting biological pathogen particles directly from the air. This method makes use of a preexisting cyclonic impinger, the Coriolis μ, combined with a lysis buffer to immediately inactivate the collected biocontaminants upon collection and posterior qPCR analysis of the generated liquid samples in the lab. The sampling material (lysis buffer) and performant analysis methods are widely used to detect respiratory viruses, and the Abbott Alinity m SARS-CoV-2 assay is a reliable method for the detection of SARS-CoV-2 in nasopharyngeal swabs. Until now, there has been little to no experience with applying this method for the analysis of air samples. However, we expected this method to perform well since a significant matrix effect by the air in the lysis buffer is highly unlikely. Further optimalisation of the pre-PCR stage to obtain higher concentrations of viral genomes and thus a higher sensitivity should be examined in future research.

We described four phases of method development (exploratory, validation, blank tests, and application), showing that the method is very promising in detecting the presence of virus in the air of different types of indoor spaces (global uncertainty of 19.6%) with a reasonable level of accuracy and reliability (more elaborate tests to determine the actual levels of accuracy and reliability are planned in the near future). Further advantages of the proposed method are the feasibility of simultaneous detection of different types of infectious agents in the air, the practicality and ease-of-use offered by the equipment, and the relatively short sampling period needed to generate a significant sample, making it ideal to use for intervention evaluation and for follow-up assessments over time. Moreover, the method does not cause significant disturbance to occupants (e.g., noise-wise), presents more safety for operators (especially when combined with adequate PPE, e.g., FFP2 mask), and is mobile and flexible, allowing it to be used in virtually any indoor environment regardless of use, size, or occupancy. Such versatility offers an opportunity to greatly enhance the current general understanding of virus behavior in the air and also in combination with the potential effects of ventilation and/or air cleaning, as demonstrated in the field experiments performed at the two daycare facilities described in this paper.

As this research is still ongoing, the next steps in the method development include organizing a comprehensive clinical trial to consolidate the validation of this method with more test subjects serving as positive and negative controls. Further tests also target enabling quantitative analysis of the collected samples, i.e., to accurately quantify the concentration of the target organism in the collected bioaerosols, as well as the detection limits. The goal is to perform a series of controlled experiments in the lab, with bioaerosol generation at specified concentrations, in order to construct a calibration curve linking bioaerosol microorganisms' concentration in the air to qPCR results (possibly in connection to sample Ct values). It is also intended to optimize the use of different liquid solutions as collection media. For example, by using specific nutrient media to collect the bioaerosol, it is possible to assess the viability of microorganisms of interest. This is an important step to validate the general assumption that any suspended microorganism is viable. Finally, another important future goal is to investigate the distribution of bioaerosols across different indoor environments and how this is affected by air mixing.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Consent

This paper does not contain any identifiable patient information. Written informed consent for publication was not obtained.

Disclosure

The positive SARS-CoV-2 PCR test result, which was documented as part of the method validation in this paper, was obtained as per the mandatory protocol mandated for all Belgian citizens infected with COVID-19 during that phase of the pandemic. The individual with COVID-19, who also contributed as a coauthor to this study, proposed to collect the air samples himself, voluntarily.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

S.L. Paralovo, K.V. Driessche, R. Cartuyvels, and M. Stranger conceptualized the study. S.L. Paralovo, B. Lazarov, L. Vanstraelen, R. Smets, M. Spruyt, S. Kreps, and N. Hufkens

performed measurements, sample analysis, and data curation in the study. M. Stranger acquired the funding. S.L. Paralovo, K.V. Driessche, R. Cartuyvels, E. Vlieghe, and M. Stranger were responsible for formal analysis and investigations in the study. S.L. Paralovo, R. Cartuyvels, B. Lazarov, S. Kreps, and M. Stranger developed the methodology. M. Stranger coordinated the project. R. Cartuyvels, M. Spruyt, and M. Stranger acquired the resources. K.V. Driessche, R. Cartuyvels, and M. Stranger supervised the study. S.L. Paralovo, K.V. Driessche, R. Cartuyvels, B. Lazarov, and M. Stranger validated the study. S.L. Paralovo and M. Stranger wrote the original draft. All authors contributed to reviewing and editing the manuscript.

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