DETECTION OF AIRBORNE MICROORGANISMS WITHIN HEALTHCARE SETTINGS WITH THE CORIOLIS AIR SAMPLERS

The emergence of new respiratory viruses such as the SARS-CoV-2 virus or avian influenza has highlighted the importance of **understanding the transmission of airborne pathogens within healthcare settings.** Identifying the key parameters of transmission is becoming a critical step to **control the spread** of potentially infectious bioaerosols and limit the risks to healthcare staff and patients.

Thanks to a large body of recent studies, **public health guidelines have been updated to recommend the use of personal protection equipment (PPE) for all hospital staff,** including workers that are not in direct contact with patients. However, due to the challenges presented by bioaerosol sampling, several outstanding questions remain on the risks of exposure to pathogens within healthcare settings. Indeed, traditional culture methods are time-consuming and unable to detect a large number of microorganisms present in the air.

Bertin Instruments has developed air samplers that can be used for the rapid detection and monitoring of airborne pathogens within healthcare facilities, the Coriolis air samplers.

In this White Paper, we present the best practices for bioaerosol monitoring in healthcare settings. We also describe how experts have used Bertin's Coriolis air samplers to detect many types of airborne pathogens in hospitals, including bacteria, fungi, and viruses and perform viability studies.

MONITOR AIRBORNE PATHOGEN TRANSMISSION IN HOSPITALS WITH THE CORIOLIS AIR SAMPLERS

SUMMARY

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TIPS AND TRICKS FOR THE DETECTION OF AIRBORNE MICROORGANISMS WITHIN HEALTHCARE SETTINGS WITH THE CORIOLIS AIR SAMPLERS

SAMPLING STRATEGY | SAMPLE COLLECTION | SAMPLE STORAGE | SAMPLE PROCESSING | SAMPLING ANALYSIS | DECONTAMINATION PROCEDURES

Choose a sampling strategy adapted to the environment (sampling room size, air flow patterns..)

- **Airflow:**
  In general, it is better to use the highest airflow possible on the Coriolis to capture the maximum amount of air. For the Coriolis µ, this corresponds to 300L/min and for the Coriolis Compact, 50L/min. There is one exception to this rule: for viruses, it is recommended to sample at an airflow equal to or inferior to 200L/min in order to avoid damaging viral particles during the sampling process.
- **Duration:**
  Similarly, it is preferable to sample for the longest time possible to allow for the collection of a large number of particles. In the case of viability studies, it is better to sample at least 3 m3 of air (corresponding to 10min with the Coriolis µ at 300L/min, 30min with the Coriolis µ at 100L/min and 1h with the Coriolis Compact).
- **Device position:**
  There are many sampling strategies possible for the positioning of the Coriolis, depending on the room layout and the microorganism concentration in the air. For healthcare settings, the optimal positioning of the device is at an approximate distance of 1 meter from the patient's bed, at approximately the same height as the patient's head. If air samples are collected in several points of a patient's room, it is preferable to start from the further distance away from the patient and then progress towards closer positions. For optimal particle collection, the device should always be placed on the trajectory of the airflow in the room. To be noted, the presence of animals or humans in a room can alter the airflow of the room and should be accounted for when designing the sampling strategy.
- **Sampling liquid with Coriolis µ:**
  For most applications, the optimal sampling solution is a saline buffer (such as PBS: Phosphate Saline Buffer) with a small percentage of surfactant around 0.005%, for example, Triton or Tween. A culture medium such as DMEM or MEM is also a suitable alternative. Collection liquid vials can also be purchased from Bertin Technologies and our distributors worldwide. On the other hand, most RNA shields should be avoided due to high evaporation speeds.
  In the case of viruses, the same sampling solution can be used except for the addition of surfactants: surfactants such as Triton may affect the integrity of most viruses membranes. Therefore, we recommend against adding any surfactant to the collection liquid. The recommended starting sampling volume liquid range is between 5 and 15mL. When using the Long Time Monitoring option, it is preferable to inject sterile water (or a mix of sterile water and culture medium), rather than PBS, in order to avoid increasing the salt concentration in the cone.
- **Resuspension step for the Coriolis Compact:**
  In case users are interested in the DNA/RNA present in the sample (PCR and RT-PCR applications), we highly recommend immediately resuspending the sample in your chosen extraction kit's buffer, using a swab to scratch the walls of the cone, and following the instructions listed in the user manual. In the case of resuspension in Trizol, Quiazol (Qiagen), or RNA later, these solutions will protect the nucleic acids in the sample and allow for storage at room temperature. Importantly, if Trizol, Qiazol, or RNAlater are used, it will be necessary to transfer the sample to a tube compatible with these solutions as quickly as possible.
  In case users are interested in viabilities studies, a saline buffer such as Phosphate Buffer Saline (PBS) or cell culture medium can be used.

TIPS AND TRICKS FOR THE DETECTION OF AIRBORNE MICROORGANISMS WITHIN HEALTHCARE SETTINGS WITH THE CORIOLIS AIR SAMPLERS

SAMPLING STRATEGY | SAMPLE COLLECTION | SAMPLE STORAGE | SAMPLE PROCESSING | SAMPLING ANALYSIS | DECONTAMINATION PROCEDURES

Store and transport samples in appropriate conditions to avoid RNA degradation

- Before transportation, samples should be transferred from the cones into appropriate storage tubes. Samples can be stored for up to 24h at 4°C. For long-term storage, they can be frozen in cryotubes at -20°C or -80°C.

Sample processing: Reconcentration step

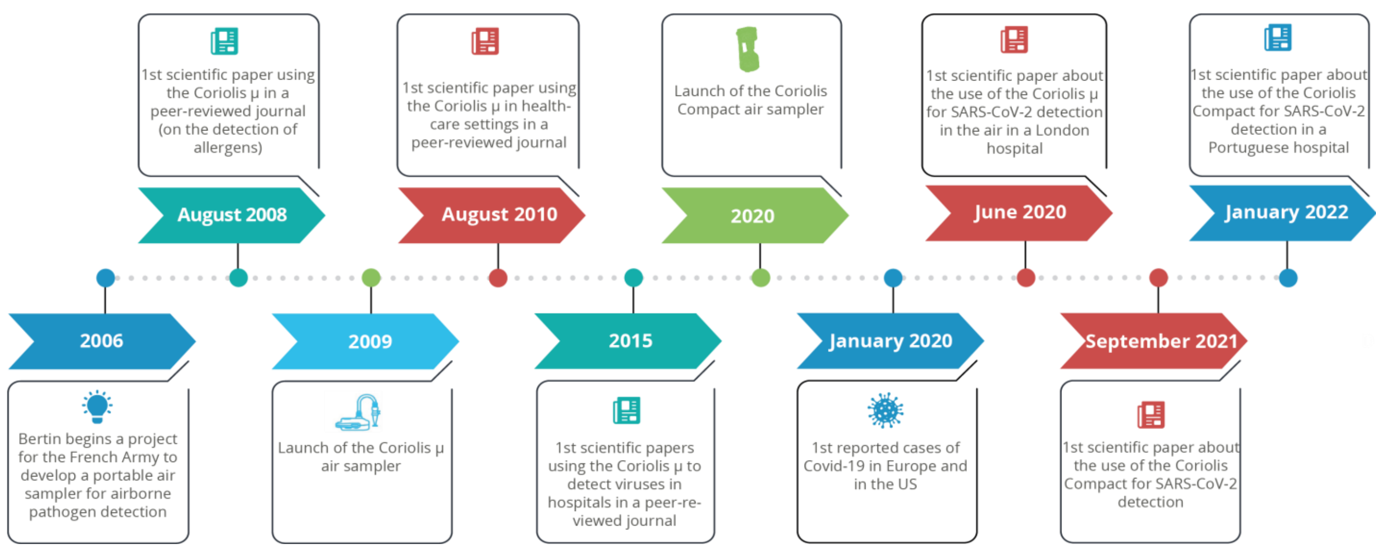
- For most samples, users can proceed directly with their chosen analysis technique sample preparation steps. In the case of viruses, for Coriolis µ samples, it is sometimes recommended to perform a reconcentration step with a tangential flow filtration device, such as the Amicon 100 kDa Amicon Ultra-15 (Millipore).

Sampling analysis: Get reliable results in hours with rapid microbiology techniques

- Coriolis samples are compatible with all rapid microbiology techniques, including qPCR, RT-qPCR, and microarrays, and with viability studies.
- For the DNA/RNA extraction step, we recommend having a positive control in order to validate the extraction protocol.

Decontaminate the Coriolis after each experiment

- Both Coriolis air samplers should be decontaminated after each experiment. We recommend following the Coriolis user manual guideline for decontamination procedures.



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EVALUATION OF SARS-COV-2 AIR CONTAMINATION IN HOSPITALS WITH CORIOLIS MICRO AIR SAMPLER

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/ CONTEXT

The current pandemic of Covid-19 has shown the vulnerability of our healthcare systems when faced with viral infections without a known treatment. Understanding the transmission behavior of SARS-CoV-2 in the air will be a crucial step to managing the current outbreak and design the appropriate prevention and control measures. In this document, we present how researchers in Imperial College have been evaluating SARS-CoV-2 surface and air contamination in a hospital during the peak of the COVID-19 pandemic in London, using surface swabs and the Coriolis µ air sampler coupled with RT-qPCR and viral culture.

/ PROTOCOL

Sampling design: Surface and air samples were collected in 8 different sites including 7 clinical areas and 1 public area at a North Western London hospital, during the peak of the Covid-19 pandemic (from April 2nd to April 20th, 2020). The list of sites can be found in **Figure 1**. All inpatient wards were fully occupied by patients with Covid-19 at the time of sampling, apart from the Emergency Department .

In each of these clinical areas, 4 air samples were collected (5 air samples were collected in the Emergency Department, and 3 in public areas of the hospital). Surface samples were collected by swabbing approximately 25 cm2 of items in the immediate vicinity of each air sample.

Collection: Sampling was carried out with the **Coriolis µ air sampler** (Bertin Technologies, France) at 100L/min for 10 min (corresponding to 1m³ of air), with 5 mL DMEM.

RT-qPCR Analysis: The RNA extraction step was realized on 140µL of sample using Qiagen viral RNA mini kit.

This was followed by absolute quantitative Real-time PCR targeting the envelop (E) gene of SARS-CoV-2 with AgPath One-step RT-PCR (Life Technologies).

Viral culture: Vero E6 (African Green monkey kidney) and Caco2 (human colon carcinoma) cells were used to culture virus from air and environmental samples.

/ CUSTOMER

Imperial College London

[1] Zhou, J. (2020). Investigating SARS-CoV-2 surface and air contamination in an acute healthcare setting during the peak of the Covid-19 pandemic in London. *Medrxiv*.

/ RESULTS

		AIR SAMPLES		
		Result	Concentration (copies/m³)	Notes
Cohort ward A	Staff room	Negative		
	Nurse station	Negative		
	Toilet B (outside the patients' bay)	Negative		
	Cohort bay B	Positive	7048	
Cohort ward B	Staff room	Negative		
	Patients' toilet (in the ward)	Suspect	464	
	Male bay	Suspect	1335	
	Male bay (side room)	Suspect	163	
Adult acute admission unit	Ward managers office	Negative		
	Nurse station	Positive	404	
	Patient bay 2	Negative		
	Patient bay 1	Negative		
Adult emergency department	'Green' majors	Negative		
	Nurse station	Negative		
	Ambulatory waiting	Negative		
	Patient assessment cubicles			
	Male toilet (next to the nurse station)			
	Resus bay (last patient > 2 hours)	Suspect	35	
Hospital public areas	QEQM main entrance	Suspect	1574	
	Male toilet at QEQM main entrance	Suspect	1545	
	Lift area QEQM ground floor	Negative		
Temporary CPAP ward	Nurse station	Suspect	1922	
	CPAP unit	Suspect	31	< 1m from 2 patients
		Negative		> 2 m from patients
	PPE doffing area	Negative		
Adult ICU	Staff room	Suspect	249	
	Nurse station inside ICU	Negative		
	Bay area	Suspect	164	
	Side room bay area	Suspect	307	
Operating theatres	Operating theatres	Negative		Before tracheostomy
		Negative		During tracheostomy
		Suspect	1163	During tracheostomy
		Negative		During tracheostomy
	Total	2/31 (6.4%) positive; 12/31 (38.7%) suspect		

Figure 1: PCR results from air samples. Samples where both of the PCRs performed from an air or surface sample detected SARS-CoV-2 RNA were defined as positive, and samples where one of the two PCRs performed from an air or surface sample detected SARS-CoV-2 RNA were defined as suspected. Adapted from [1].

SARS-CoV-2 RNA was detected on 114/218 (52.3%) of the surface samples and 14/31 (38.7%) of the air samples collected with the Coriolis µ air sampler. The concentrations detected indicated a viral load that would not be culturable, which was confirmed by viral culture assays. Viral RNA was detected on surfaces and in the air in public areas of the hospital but was more likely to be found in areas immediately. These results show that the Coriolis µ air sampler can be used successfully to evaluate SARS-CoV-2 air contamination, and also set guidelines for the use of PPE, social distancing, and hygiene in acute healthcare settings.

CORM-026-039-B

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RAPID QUANTIFICATION OF VIABLE FUNGI IN HOSPITAL ENVIRONMENTS USING SOLID-PHASE CYTOMETRY

Université Rennes 1, France

/ CONTEXT

Indoor fungal contamination is a major source of nosocomial diseases in hospitals. The monitoring of environmental fungal contamination is strongly recommended, especially for outbreak investigation in epidemic situations and during building construction and renovation work. Moreover, air assessment is also useful to validate the efficiency of air treatment in wards with high risk patients such as hematology.

Conventional culture methods for enumerating environmental fungi are time-consuming and underestimate the number of micro-organisms. Viability of a microorganism is an important criterion in hospital environment as it determines its infectivity.

In this study [1] we evaluated a combination of two recent technologies for sampling (Coriolis air sampler) and rapid detection of viable airborne fungi with a solid-phase cytometry (SPC) system.

/ MATERIALS

- Coriolis µ, sterile cones (Bertin Technologies), 15mL of collection liquid (AES Chemunex).
- Solid-phase cytometry (SPC) system: ChemScan RDI system (AES Chemunex).

/ PROTOCOL

- Sites selected for air sampling with different amounts of airborne fungi: an office (High), a conventional room (Medium), a corridor in haematology unit (Low), and a room with laminar air flow (Very low).
- Coriolis air sampling: 300L/min, 10 min, middle of the room at a height of 1 m above the floor.
- Samples from all areas were processed by SPC and by culture on malt extract agar (MEA).

/ RESULTS

Significantly more viable fungi (1.5-fold higher) were detected when the Coriolis samples were examined with SPC by comparison with results of cultures (Figure 1): in the office (220 ± 45 viable fungi/m3 vs 133 ± 31 cfu/m3), in the conventional room (154 ± 30 viable fungi/m3 vs 98 ± 16 cfu/m3) and in the haematology corridor (31 ± 9 viable fungi/m3 vs 23 ± 10 cfu/m3).

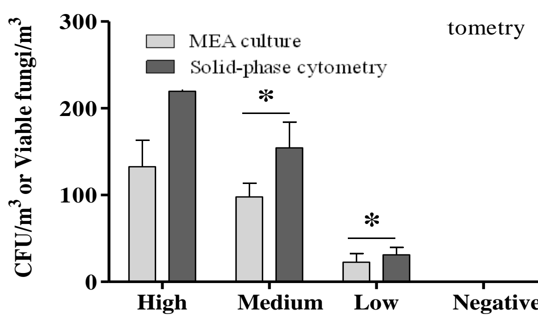

Fig.1: Fungi concentrations obtained by Coriolis sampling; mean of 10 sample ± 1 standard deviation
** Significative difference using ANOVA (p <0.05)*

One of the prominent advantages of SPC associated with Coriolis air sampling was its rapidity in comparison with the culture-based method (5 h versus 5 days).

[1] Méheust D, Le Cann P, Gangneux JP. **Rapid quantification of viable fungi in hospital environments: analysis of air and surface samples using solid-phase cytometry**. Journal of Hospital Infection, 2013 Feb;83(2):122-6. doi: 10.1016/j.jhin.2012.10.004. Epub 2013 Jan 11.

In this study, we showed that the combo **Solid-phase cytometry (SCP) with Coriolis air sampling** allows for rapid monitoring of **viable fungi in hospital environments.**

Coriolis and SPC can thus be used to provide an early warning and a rapid implementation of corrective measures. Viable fungi detection may be an important tool to assess infectious risk in wards with immunosuppressed patients.

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VIABLE AIRBORNE BACTERIA COUNT WITH CORIOLIS COMPACT AIR SAMPLER: EVALUATION OF THE PERFORMANCE OF THE GENANO AIR PURIFIER IN HOSPITALS

coriolis
Microbial Air Sampler

/ CONTEXT

Airborne microorganisms are an increasingly important source of concern in healthcare settings. Indeed, nosocomial infections are a major cause of mortality in hospitalized patients. Several studies have shown that environmental contamination has a strong impact on the nosocomial transmission of bacteria such as S. aureus and M. tuberculosis (1, 2). For this reason, it is crucial for healthcare facilities to implement decontamination protocols that allow for the control of the level of microorganisms in the air.

Air purifying solutions can help decrease the level of airborne microorganisms in the air and reduce the risk of healthcare-associated infections. In this study, researchers have used the Coriolis Compact air sampler (Bertin) to assess the performance of the GENANO 5250A (Genano, Espoo Finland) in hospitals room to decrease the number of airborne microorganisms in the air. Airborne viable bacteria count and particle count were measured with the Bactester Fluorescence Microbial Imaging Machine (Bactester, Fukuoka, Japan).

/ MATERIALS

- Coriolis Compact air sampler & cones
- The Genano 5250A (Genano, Espoo, Finland) air purifier

/ PROTOCOL

- The Genano 5250A (Genano, Espoo, Finland) air purifier was used to purify the air in a CT scanner room & in an elevator room in a Japanese hospital. The CT scanner room & the elevator room are both ISO CL9 environments. The CT room has a mechanical ventilation system with an airflow rate of 500m3/h.
- The Coriolis Compact air sampler (Bertin Technologies, Montigny-le-Bretonneux, France) was used to collect air samples in the CT scanner room & in the elevator. CT Room: 3 consecutive cycles of 10min, the collection cone was changed at the end of each cycle (the cone changing took approximately 10s). Elevator: 3 consecutive cycles of 5 min, the collection cone was changed at the end of each cycle (the cone changing took approximately 10s).
- The air samples were analyzed with the Bactester Fluorescence Microbial Imaging Machine (Bactester, Fukuoka, Japan) which allows for viable bacterial counting without culture. Particles were also counted with the RION KC-22B particle detector (Rion, Tokyo, Japan). Results are shown in Figure 5 and Figure 6.

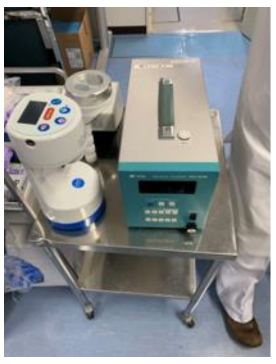

Figure 1: Coriolis Compact air sampler and particle air counter

Figure 2: Genano 5250A air purifier in CT room

Figure 3: Sample collection in CT Room

Figure 4: Sample collection in Elevator

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VIABLE AIRBORNE BACTERIA COUNT WITH CORIOLIS COMPACT AIR SAMPLER: EVALUATION OF THE PERFORMANCE OF THE GENANO AIR PURIFIER IN HOSPITALS

/ RESULTS

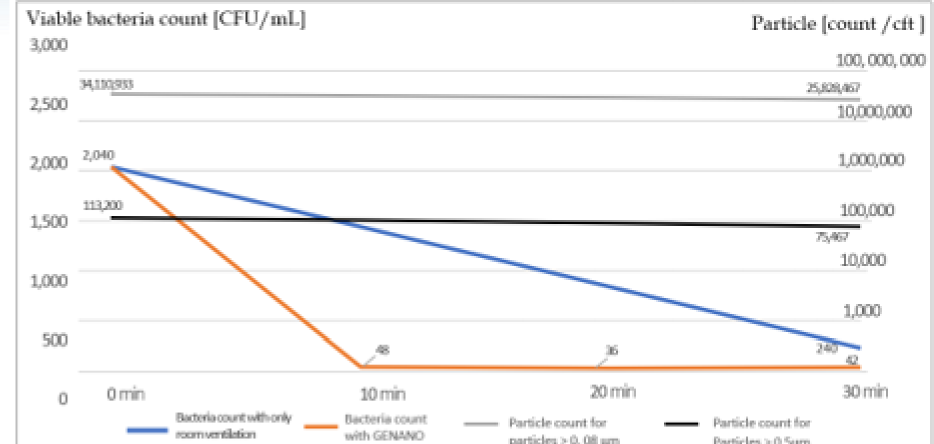

Figure 5: Airborne viable bacteria count and particle count in air samples collected in the CT scanner room.
Light and dark grey: particle count using normal room ventilation (without Genano)
The initial particle count was around 110,000 CFU/mL for 0.5um particles (ISO CL9 environment). The initial viable bacteria count value was around 2,040 CFU/mL . After 30min with only the room ventilation (and the doors closed), the number of particles with a diameter higher than 0.5 µm was 75,467/cft , and the viable bacteria count was 240 CFU/mL. After 30min with Genano, the viable bacteria was down to 42 CFU/mL. The number of viable bacteria after Genano is 6 times lower than without Genano.

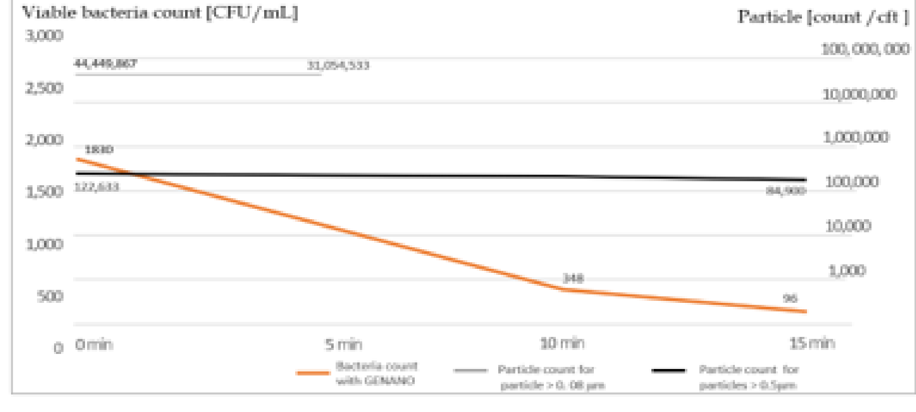

Figure 6: Airborne viable bacteria count and particle count in air samples collected in the elevator
The initial particle count was around 120,000 CFU/mL for 0.5um particles (ISO CL9 environment). The initial viable bacteria count value was around 1,830 CFU/mL. After 30min with Genano, the viable bacteria was down to 96 CFU/mL.

Using the Coriolis Compact air sampler**, a reduction of airborne viable bacteria by 198 CFU/mL** has been observed after using the Genano air purifier for 30min in the hospital CT scan room. This corresponds to **a reduction of 82,5%** compared to a situation where only the normal room ventilation is used. The findings of this study **confirm the cleaning efficiency of the Genano purifier** in healthcare environments and indicate that the Genano could be used to safely increase the number of patients in CT scan rooms. These results also show that **the Coriolis Compact air sampler can be used successfully to assess bacterial air contamination,** which can inform hygiene and social distancing rules within hospitals.

CORC-026-SL002-A

DETECTION OF SARS-COV-2 AEROSOL DURING RESPIRATORY EQUIPMENT REPROCESSING

- **Context:**
  Understanding of aerosolization during the decontamination process of potentially contaminated material in a Brazilian hospital
- **Sampling strategy:**
  The Coriolis µ air sampler (Bertin Technologies, France) was set at 300 L/min for 10 min. The air sampler was placed at 50 cm from the respiratory equipment being cleaned or assembled, or 50 cm away from the patient's face. A sterile cone was used with 15 mL of 0.9% saline.
- **Analysis:**
  RNA extraction with QIAmp viral RNA mini kit (QIAGEN, Germany), PCR RT-PCR was carried out using RealStar® SARS-CoV-2 RTPCR Kit 1.0 (Altona Diagnostics, Germany) followed by DNA amplification (Roche LightCycler 96 System; Roche Diagnostics, Switzerland). Viral culture was carried out on Vero cells (ATCC CCL-81™; ATCC, USA).
- **Results:**
  SARS-CoV-2 RNA was detected in only 1 air sample, before the beginning of the decontamination process, showing a **Ct value of 36.88. Cell cultures were negative**
- **Conclusion:**
  routine sterilizations of contaminated respiratory therapy equipment are not considered aerosol-generating procedures (AGP).

SAMPLING OF P. JIROVECII IN THE SURROUNDING AIR OF PATIENTS WITH PNEUMOCYSTIS PNEUMONIA

- **Context:**
  The fungus *Pneumocystis jirovecii* is responsible for a disease affecting immunocompromised patients: Pneumocystis pneumonia. The objective of the study was to study the burdens of Pneumocystis jirovecii in exhaled air from infected patients.
- **Sampling strategy:**
  Air samples were collected in each patient's room with the Coriolis µ air sampler (Bertin Technologies, France). Collection of 1 air sample of 1.5 m 3 (5 min) at a distance of 1 m from patient's head + air samples inside and outside the room: at the door entrance and in the corridor (3m, 5m, and 8m from the patient)
- **Analysis:**
  DNA extraction using the QIAamp DNA Mini Kit (Qiagen), followed by RT-qPCR targeting the mitochondrial large subunit (mtLSU) rRNA gene of *P. jirovecii*
- **Results:**
  33/56 positive air samples : the lower the distance from the patient, the higher the quantity of *P.jirovecii* DNA detected at: 1m - 15/19 samples (78.9%), 3m - 9/13 (69.2%), 5m - 5/12 (41.7%), 8m - 4/12 (33.3%). This study provides the first quantitative data on the spread of P. jirovecii in exhaled air from infected patients

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Coriolis®: microbial air sampler for air bio-contamination control:

- Airborne particles concentration in a liquid sample
- Technology adapted to virus, bacteria, molds, pollens, spores...
- Compatible with culture and molecular biology standard methods

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