BEST PRACTICES FOR ENVIRONMENTAL DETECTION AND MONITORING OF AIRBORNE VIRUSES WITH CORIOLIS® AIR SAMPLERS

The appearance of coronavirus SARS-CoV-2 and the subsequent pandemic of COVID-19 has shown biosafety to be one of the biggest challenges of this century. While scientists are still deciphering the transmission behavior of the virus, public health authorities struggle to elaborate strategies to ensure the safety of their citizens post confinement. As with most respiratory viruses, the environment is an important potential source of contamination. A recent study has found that SARS-CoV-2 can remain viable in aerosols for at least 3 hours, and up to days on surfaces. Reliable environmental air monitoring solutions become critical to safely reopen public areas such as schools or restaurants. Such solutions will also help understand and prevent the infection of healthcare workers in hospitals.

The detection of viruses in air samples presents many challenges: compared to other microorganisms, viruses are present in the air at a very diluted ratio which translates in the necessity of sampling a relatively large amount of air to have reliable analyses results – a few m^3. The integrity of the virus also has to be maintained through each step of the workflow to have reliable virus viability estimates. Traditional air sampling devices are often impedimented by low airflow rates, which translates into a time-consuming sampling process for viruses. Bertin Technologies has developed air samplers that can be used for the detection and monitoring of airborne viruses in a wide range of environments, from hospitals to office buildings.

In this White Paper, we present the best practices for virus monitoring in air samples and describe how experts have used Bertin's Coriolis® air samplers in a wide range of environments: hospitals, farms, and industrial settings, for the detection of different airborne viruses: RSV (Respiratory Syncytial Virus) rotaviruses, influenza A and bacteriophages.

OPTIMIZE ENVIRONMENTAL MONITORING WORKFLOWS USING THE CORIOLIS® µ

SUMMARY

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BEST PRACTICES FOR VIRUS-LADEN PARTICLES DETECTION WITH CORIOLIS® MICRO AIR SAMPLER

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 manage the current outbreak and design the appropriate prevention and control measures. This can only be accomplished through the use of reliable and efficient solutions for the sampling of virus-laden aerosols. Among the conventionally used sampling methods, cyclone-based air sampling is considered to be one of the simplest and most effective. The Coriolis® µ cyclonic air sampler has been tried and approved for many years by renowned aerovirologists. In this document, we have gathered the best practices for virus monitoring in air samples, with the end of helping Coriolis® users optimize their environmental monitoring workflow.

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..)	**Use** appropriate parameters for airflow rate and sampling cycles duration	**Store** and transport samples in appropriate conditions to avoid RNA degradation	**Optional** reconcentration step	**Get** reliable results in hours with rapid microbiology techniques	**Decontaminate** the Coriolis® after each experiment

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BEST PRACTICES FOR VIRUS-LADEN PARTICLES DETECTION WITH CORIOLIS® MICRO AIR SAMPLER

/ SAMPLING STRATEGY

- Airflow rate: samplers with a flow rate higher than 200L/min may tend to degrade RNA virus during collection [1]. For this reason, we recommend using an airflow rate of 200L/min or below.
- Sampling duration: as viruses are most often present in the air at a very diluted concentration [2], most studies experimental design involves the collection of at least 1m^3 of air during each experiment, which corresponds to a sampling duration of a minimum of 5 min at a speed of 200L/min. When possible, a higher sampling duration should be preferred to collect the maximum volume of air possible, ideally 20 to 30 min at 200L/min. We recommend collecting at least 3m^3 of air for all experiments dedicated to virus detection. The Coriolis® µ can collect air for up 1h on battery, and up to 6 hours with the Long Time Monitoring option on the main power supply. The Coriolis® compact can function for up to 8 hours at 50L/min on battery.
- Device position: there are many sampling strategies possible for the positioning of the Coriolis®, depending on the room layout and the virus 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 [3], 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.
- Sampling liquid: surfactants such as Triton may affect the integrity of most viruses membranes. Therefore, we recommend against adding any surfactant to the collection liquid. The optimal collection liquid for virus-laden aerosols sampling is Phosphate Buffer Saline buffer. A culture medium such as DMEM or MEM is also a suitable alternative. On the other hand, most RNA shields should be avoided due to high evaporation speeds. 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.

/ SAMPLE STORAGE

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

To obtain a final solution at a suitable concentration for analysis, most protocols ([5],[6]) require a reconcentration step with a tangential flow filtration device, such as the Amicon 100 kDa Amicon Ultra-15 (Millipore). However, in environments where the virus concentration step is sufficiently high, this step can be omitted by choosing a small starting volume of collection liquid (such as 5mL), then taking a small aliquot of around 150 µL for DNA or RNA extraction. We recommend concentrating your samples by a factor of at least 50.

BEST PRACTICES FOR VIRUS-LADEN PARTICLES DETECTION WITH CORIOLIS® MICRO AIR SAMPLER

/ SAMPLE ANALYSIS

As samples obtained with the Corolis® µ are in liquid form, they are compatible with all rapid microbiology techniques, such as qPCR, RT-qPCR, microarrays. Samples can also be analyzed with virus viability assays [7]. It has been shown that liquid air samplers are less likely to damage viruses during sampling than dry air samplers [8], which implies that they are more adapted to virus viability studies. Regarding the DNA/RNA extraction step, we recommend having a positive control to validate the extraction protocol.

/ DECONTAMINATION

The Coriolis® µ device should be decontaminated after each experiment. The cane, the air intake, and the sampling cones can be autoclaved. An alternative solution is to soak these parts in a commercial bleach solution. As most commercial bleach concentrations vary between 5.25 and 8.25%, a dilution of 1 part commercial bleach for 49 parts of water will make for a suitable disinfectant solution. Both the cane and the air intake may also be cleaned with ethanol 70% between each sampling. These solutions can be used to clean the external parts of Coriolis® air sampler applied with a wet sponge or a rag
The main unit of the Coriolis® µ air sampler can be decontaminated using Hydrogen peroxide vapour (H202) and running the device in decontamination mode.
Alternatively, for virus sampling, the main unit can be decontaminated by being run them with an ethanol-filled cone for 15min at 300L/min in a ventilated room. This will generate an aerosol of Ethanol that will disinfect the Coriolis® main engine. Indeed, ethanol at 70% is a well-known disinfectant that has been shown to be effective on enveloped virus (hands or surfaces) even at lower concentrations. It is to be noted that, while this operation will destroy all viruses, spores won't be eliminated.
All chemicals handling and decontamination procedures should also follow the user's laboratory safety guidelines (regarding protective equipment..)

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1. Brisebois, Evelyne, et al. "Human viral pathogens are pervasive in wastewater treatment center aerosols." *Journal of Environmental Sciences* 67 (2018): 45-53.
2. Hammond, G. W., R. L. Raddatz, and D. E. Gelskey. "Impact of atmospheric dispersion and transport of viral aerosols on the epidemiology of influenza." Reviews of infectious diseases 11.3 (1989): 494-497.
3. Mubareka, Samira, et al. "Influenza virus emitted by naturally-infected hosts in a healthcare setting." *Journal of Clinical Virology* 73 (2015): 105-107.
4. Scoizec, Axelle, et al. "Détection du génome de virus influenza aviaire A de sous type H5 dans des échantillons d'air.«
5. Brisebois, Evelyne, et al. "Human viral pathogens are pervasive in wastewater treatment center aerosols." *Journal of Environmental Sciences* 67 (2018): 45-53
6. Mubareka, Samira, et al. "Influenza virus emitted by naturally-infected hosts in a healthcare setting." *Journal of Clinical Virology* 73 (2015): 105-107.
7. Toulouse, Marie-Josée. "Validation de la neuraminidase à titre de marqueur enzymatique pour la détection de virus dans l'air." (2019).
8. Blachere, Francoise M., et al. "Detection of an avian lineage influenza A (H7N2) virus in air and surface samples at a New York City feline quarantine facility." *Influenza and other respiratory viruses* 12.5 (2018): 613-622.

EVIDENCE OF AIRBORNE TRANSMISSION OF SWINE INFLUENZA A VIRUS IN EXPERIMENTAL CONDITIONS

S. Hervé, Cador C., N. Barbier, S. Gorin, F. Paboeuf, N. Rose and G. Simon
ANSES, Ploufragan-Plouzané Laboratory, Swine Virology Immunology Unit, France

/ CONTEXT

Swine Influenza A virus (swIAV) may transmit through aerosols. The virus has been detected in air samples collected in affected pig farms [1] and a relationship between airborne swIAV detection and the number of infected pigs was shown [2]. Here, we report swIAV detection in air samples collected in experimental rooms housing specific-pathogen-free (SPF) pigs without or with maternally-derived antibodies (MDA). Thirty-three MDA- piglets were assigned to 3 independent rooms (rooms 1 to 3) and 33 MDA+ piglets to 3 others (rooms 4 to 6) [3]. In each room there were 2 seeder pigs (intra-tracheally inoculated with A/Sw/France/Cotes d'Armor/0388/09 (H1N1) (10^6 EID_{50} in 5 mL) and 4 pen-mates in direct-contact, as well as 5 indirect-contact pigs in a neighboring pen, 30 cm apart. (Figure 1).

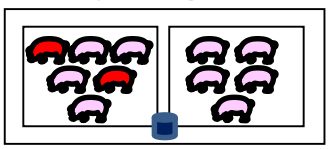

Figure 1. An experimental room was composed of two pens. The air sampler collector was placed in-between. The inoculated pigs are colored in red.

/ MATERIALS

- Coriolis® µ, sterile cones, 15mL of collection liquid (Bertin Technologies)
- Amicon® Ultra-15 Centrifugal Filter 30K Device (Merck Millipore Ltd)
- Nasal swabs Virocult® MW 951 sent (Medical Wire)
- RNeasy Mini Kit© (Qiagen GmbH)
- LSI VetMAX™ Swine Influenza A kit (Life Technologies)

/ PROTOCOL

The Coriolis® µ was put down in the room between the 2 pens, 70 cm away from the ground, at the height of piglets but without direct contact with them (Figure 1). Air samples were taken 3 times a week for 25 days post-infection (DPI). At each collection time, the collector ran during 10 min to collect 3000 L of aerosols in 15 mL of 0.005% Triton solution. The air samples were then concentrated thanks to an ultrafiltration step using Amicon® Filter Device and centrifugation for 30 min at 3900 × g. Viral RNA was purified from 150 µL eluate and 5 µL RNA extract were tested by real-time M gene RT-PCR to detect the swIAV genome. Nasal swabs were taken on a daily basis until DPI 14, then every 2 days. RNA was extracted from 200 µL supernatants and 5 µL submitted to RT-PCR.

/ RESULTS

The swIAV genome was detected in aerosols from all rooms, from DPI 2 or DPI 4, until the end of the experiment (Figure 2). In each room the viral genome load peaked at DPI 9.

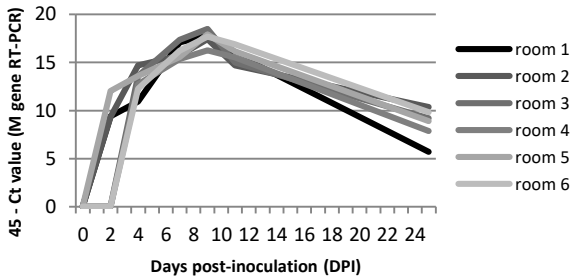

Figure 2. SwIAV genome load in air samples deduced from RT-PCR analyses (45-Ct value)

All indirect-contact (IC) pigs were shown to have been infected. They shed the virus from DPI 4 or DPI 6 depending on their serological status, and until DPI 14 for the last one (Table 1).

	Days post-inoculation																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	17	18	21	23	25
IC_room 1																					
IC_room 2																					
IC_room 3																					
IC_room 4																					
IC_room 5																					
IC_room 6																					

Table 1. SwIAV genome detection in nasal secretions of IC pigs. The room was considered positive (in red) when at least 1 out the 5 IC pigs was found RT-PCR positive.

Thus, the swIAV genome was detected in the air 2 days before the first IC piglet shed the virus and still up to 15 days after the last one did (room 2).

[1] Corzo, C. A. Culhane, M. Dee, S. Morrison, R. B. Torremorell, M. (2013). Airborne detection and quantification of swine influenza a virus in air samples collected inside, outside and downwind from swine barns. PLoS One, 8, e71444.

[2] Corzo, C. A. Romagosa, A. Dee, S. A. Gramer, M. R. Morrison, R. B. Torremorell, M. (2013). Relationship between airborne detection of influenza A virus and the number of infected pigs. The Veterinary Journal, 196 (171-175).

[3] Cador, C. Hervé, S. Andraud, M. Gorin, S. Paboeuf, F. Barbier, N. Quéguiner, S. Deblanc, C. Simon, G. Rose, N. (2016). Maternally-derived antibodies do not prevent transmission of swine influenza A virus between pigs. Veterinary Research 47:86.

CORM-203-SL037

Thanks to the Coriolis® µ instrument, the genome of swIAV was detected in air samples collected in experimental rooms housing inoculated and contact SPF piglets, in addition to detection in nasal swabs taken from animals and estimation of indirect transmission rate that revealed that 1.41 piglets became infected per day via the air [3]. Altogether, these results demonstrate that aerosols are a key point in swIAV spread and persistence in pig herds. They confirm that detection of swIAV in air samples collected within commercial farms would give information on airborne transmission within confinement building environments. Such investigation would help monitor ventilation systems and airflows accurately, in order to reduce the infectious process.

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AIRBORNE VIRUS DETECTION IN HOSPITAL ENVIRONMENTS

Amiens Hospital (France)

/ CONTEXT

In winter time, two viral infections are the cause of 80% of the consultations in French hospitals: the bronchiolitis & the gastro-enteritis. The incriminated germs in this pathologies are respectively the RSV (Respiratory Synticial Virus) and the Rotavirus. The flow of patients, especially children, would improve the viral nosocomial infections apparition and dissemination.

In this study, the Coriolis® air sampler has been coupled with PCR detection method for RSV detection in children hospital rooms.

/ RESULTS

- 44 air samples, at most 2 days after the diagnostic.
- Positive results by RT-PCR RSV real time for 6 samples for 3 children.
- The earliest the air sampling is done and the closest from the patient it is, the most probable it is to detect air borne virus.
- The concentration of viruses in the air are very low and would necessitate new study to go further (under process on multiplex PCR and quantification PCR).

/ MATERIALS

- Coriolis® + sterile cones (Bertin technologies).
- Collection liquid: 15 ml of Hanks liquid.
- Specific filters for sample concentration.
- Extraction kits for RT-PCR / Kit RSV.

/ CUSTOMER

CHU Amiens Picardie

/ PROTOCOL

- Sampling step : 300 L/min - 10 min. Filtration and concentration of the sample.
- Automated RNA extraction.
- RT-PCR RSV.

CORM-203-SL008

The Coriolis® µ air sampler is thus capable to collect **airborne viruses detected by RT- PCR analysis**, in patient narrow environment.

This study is going to be completed but still shows that airborne viruses can be controlled whereas they are often responsible for **nosocomial infections** or epidemic dissemination.

These data open a new way for airborne viruses contamination control.

DETECTION OF AIRBORNE VIRULENT BACTERIOPHAGE OF DAIRY STARTER CULTURE IN A CHEESE FACTORY

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Daniel Verreault M.Sc, Caroline Duchaine PhD.

/ CONTEXT

Phage infection of Lactococcus lactis (commercially important bacterium used to make fermented dairy products) during fermentation of milk is a troublesome and persistent economic problem in factories where fermented dairy products are produced such as in cheese factories.

In this study, the concentration of the lactococcal 936-species bacteriophages was evaluated in aerosols collected in a cheese factory with different sampling techniques: either filters (polycarbonate and Teflon) or liquid sampling (Coriolis® and BioSampler).

/ MATERIALS

- Coriolis® µ, sterile cones.
- BioSampler (SKC).
- Liquid: Sterile water+ 0.01%Tween20.
- PC (Polycarbonate) filter on 37 mm cassette.
- PTFE (Teflon) filter on 37 mm cassette.
- Real time PCR.

/ PROTOCOL

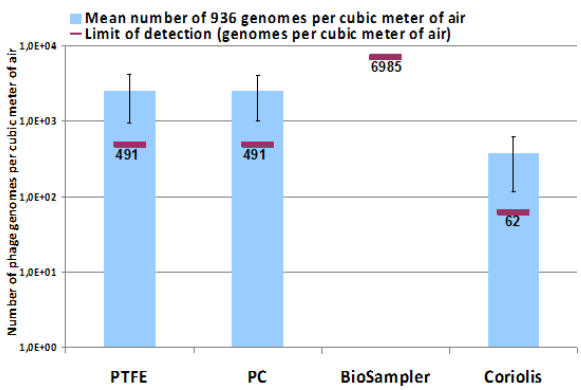
- Coriolis® µ (n=5): 3 x 10 minutes; 300 L/min.
- BioSampler (n=6): 20 min, 12.5 L/min.
- PC and PTFE filters (n=6): 12 hours; 2 L/min.
- Real time PCR (SYBR Green) : number of phage genomes per cubic meter of air.

/ RESULTS

Coriolis®µ: Conclusive result with a fast sampling (30 minutes) and a limit of detection 8 to 113 times better than other samplers.

PC et PTFE filters: Conclusive results (above the limit of detection); the time of sampling (12 hours) is still a restrictive step.

BioSampler: Inconclusive results (limit of detection)



/ CUSTOMER

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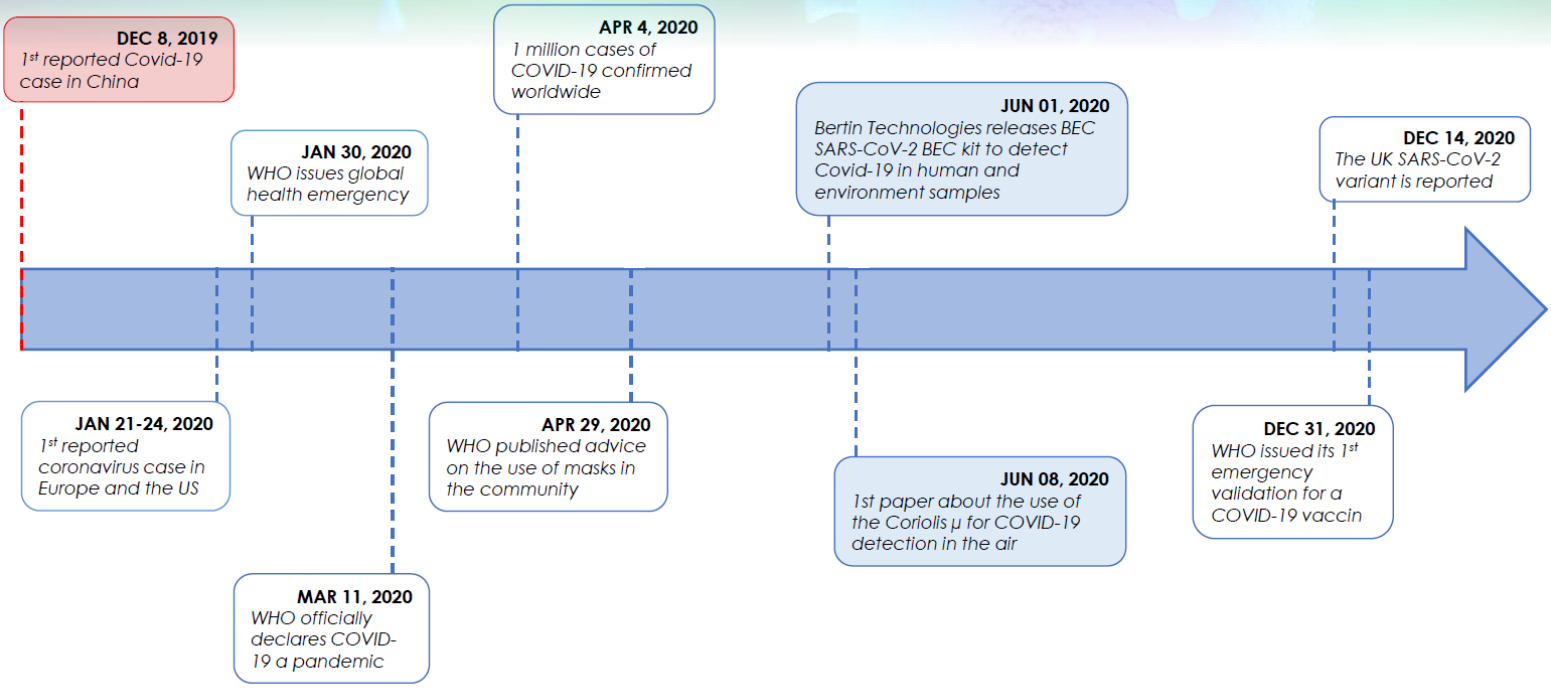
DE CARDIOLOGIE
ET DE PNEUMOLOGIE
DE QUÉBEC

CORM-203-SL008

The **Coriolis®** is an efficient air sampler to **detect low concentrations of airborne virulent bacteriophage** in the air. Furthermore thanks to its ergonomic design using Coriolis® is easy in industrial area such as cheese factories. In both **industrial and epidemiological context**, short-time sampling (high airflow rate), efficiency and an ergonomic design are important assets to detect airborne contaminants and to react as soon as possible.

LATEST PUBLICATIONS ON VIRUS DETECTION WITH THE CORIOLIS AIR SAMPLERS



/Transmission of SARS-CoV-2 by children attending school. Interim report on an observational, longitudinal sampling study of infected children, contacts, and the environment

Transmission of SARS-CoV-2 by children and young people in school settings has not been directly evaluated, nor the main mechanisms of transmission identified. The study set out to undertake sequential longitudinal sampling of infected children, their contacts, and the environment.

Cases of COVID-19 were identified through statutory notification and matched to schools reporting cases. Cases of COVID-19 and their contacts from school and home were longitudinally sampled and tested for SARS-CoV-2. Surfaces and air in the home and school environment were also subject to longitudinal sampling and testing.

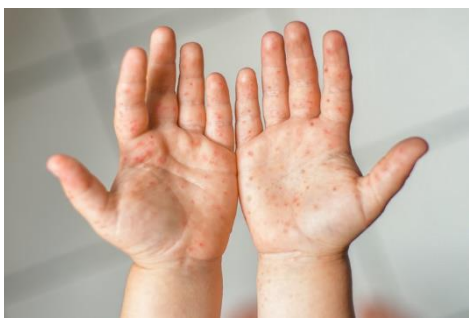
Air sampling was undertaken in the same three school rooms for periods of 10 minutes (300 liters/minute, Coriolis micro, Bertin Instruments, France), with an infected subject present in one of the rooms during sampling.

Cordery, Rebecca, et al. "Transmission of SARS-CoV-2 by children attending school. Interim report on an observational, longitudinal sampling study of infected children, contacts, and the environment." medRxiv (2021). [preprint]

LATEST PUBLICATIONS ON VIRUS DETECTION WITH THE CORIOLIS AIR SAMPLERS

/ Environmental and air sampling are efficient methods for the detection and quantification of foot-and-mouth disease virus

In this study, both environmental and aerosol sampling methods were evaluated. The recovery of FMDV (Foot-and-mouth disease virus) from a variety of different surfaces was assessed and data on the collection efficiency of the Coriolis micro air sampler was obtained over distances of up to 150 cm.



Brown, Emma, et al. "Environmental and air sampling are efficient methods for the detection and quantification of foot-and-mouth disease virus." Journal of virological methods 287 (2021): 113988.

/ Detection of influenza virus in air samples of patient rooms

During the 2017-2018 influenza season, patients with confirmed influenza or RSV infections were enrolled. Room air samples were collected close (0.30 m) to and distant (2.20 m) from patients' heads. Real-time polymerase chain reaction was used to detect and quantify viral particles in the air samples. The plaque assay was used to determine the infectiousness of the detected viruses.

Chamseddine, A., et al. "Detection of influenza virus in air samples of patient rooms." Journal of Hospital Infection 108 (2021): 33-42.

/ Virus transmission by ultrasonic scaler and its prevention by antiviral agent

The purpose of this research was to develop an experimental model for testing the spread of viruses during an ultrasonic scaler (USS) operation and to examine the prevention of spreading by replacing the coolant with an antiviral agent. In a virus transmission tunnel, USS operation with saline coolant and delivery of a viral suspension to the vicinity of USS tip generated droplets and aerosol containing Equine Arteritis Virus (EAV). Evaluation of droplet transmission was evaluated with adherent 48h cell culture monolayer RK13 cell lines in standard 48-well-plates positioned at a distance from 30 to 55 cm. The aerosol was collected by a cyclone aero-sampler flow of 100l/min. Antiviral activity of 0.25% sodium hypochlorite or electrolyzed water (EOW) was tested by suspension test. The two tested antiviral agents' transmission prevention ability was evaluated by repeating the same experiment as with saline coolant.

Fidler, Ales, and Andrej Steyer. "Virus transmission by ultrasonic scaler and its prevention by antiviral agent." bioRxiv (2021).

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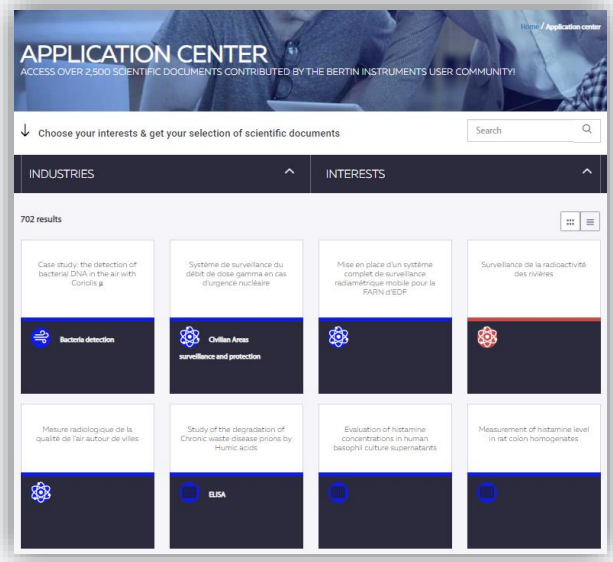

Coriolis® µ: microbial air sampler for air bio-contamination control:

- 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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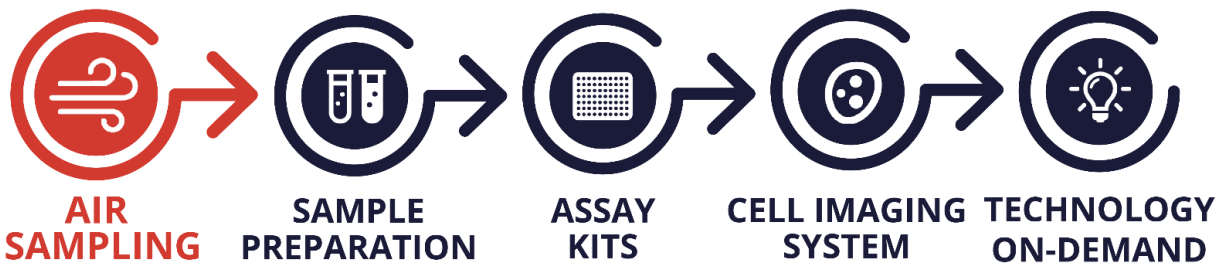

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