DETECTION OF POTENTIALLY INFECTIOUS VIRUSES AT WORKPLACES OF WASTEWATER TREATMENT PLANTS

IDENTIFYING GASTROINTESTINAL AND RESPIRATORY VIRUSES WITH THE CORIOLIS µ AIR SAMPLER

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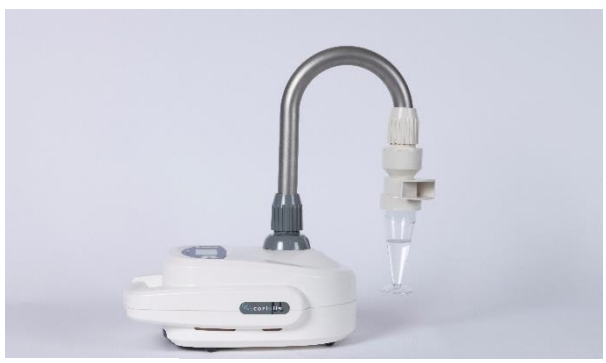
CONTEXT

Wastewater treatment generates aerosols of different sizes and all airborne biological agents can be subsequently deposited on surfaces. As a result, workers in wastewater treatment plants (WWTPs) may be exposed to viral agents during their occupational activities and, compared to general population, are more likely to develop a wide variety of work-related symptoms, including respiratory and gastrointestinal adverse outcomes.

Although several studies have already examined the presence of viruses in wastewater and WWTPs, the knowledge about potential infectivity of viruses in this occupational environment is still scarce. In this Application Note, we summarize the results of the first study to analyze, both qualitatively and quantitatively, the presence of the most common gastrointestinal and respiratory viruses in the occupational environment of WWTPs, using the Coriolis µ air sampler.

MATERIALS

- Coriolis µ (Bertin Technologies, France)
- Cone with 15 ml of 15 mL of universal viral transport medium (VTM)
- PMAxx Dye (Biotium, Inc., Hayward, USA)
- Kogene Power Prep Viral DNA/RNA Extraction Kit (Kogene Biotech, South Korea)
- CFX96 real-time PCR thermocycler (Bio-Rad, Hercules, USA)



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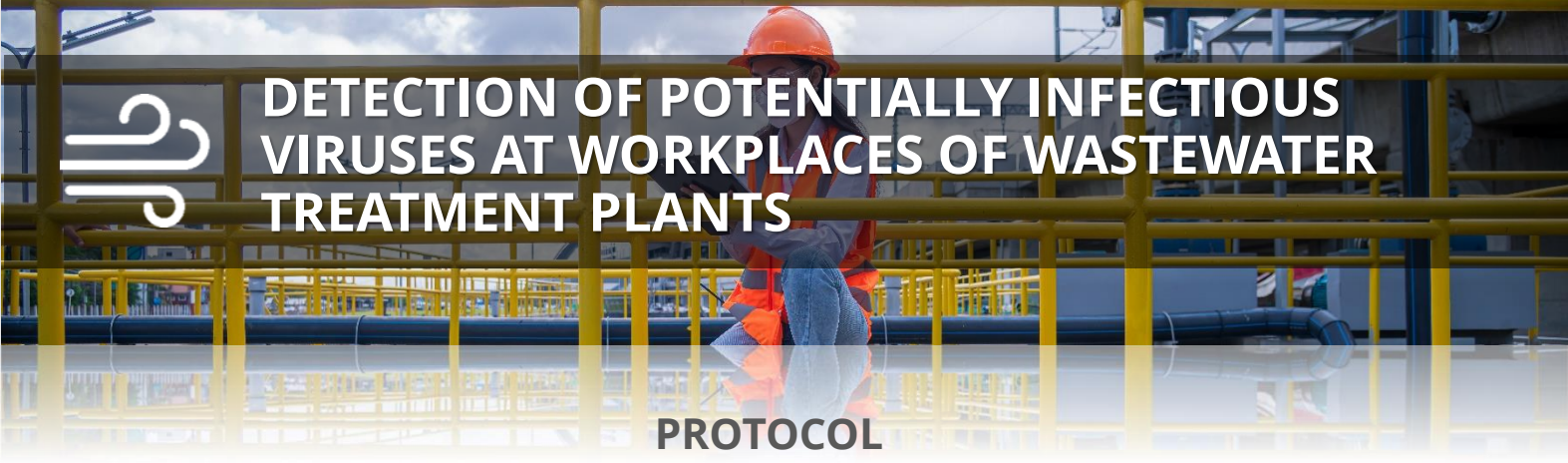

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PROTOCOL

- *Collection of air samples*

Bioaerosol and surface swab sampling was performed at workplaces in five different wastewater treatment plants. In total, 26 bioaerosol samples were collected with the Coriolis µ at the following sampling sites: wastewater pumping section, screens section, grit chamber, and dewatering and thickening sludge section.

- *Air sampling protocol*

The Coriolis µ was placed at a height of 1–1.5 m above the floor level to simulate aspiration from the human breathing zone. The air samples were collected for 10 min at a flow rate of 200 L/min, using sterile sampling cones filled with 15 mL of universal viral transport medium (VTM).

- *Detection of viruses*

All processed samples were divided into two equal aliquots (200 µL). The first one was intended for direct viral DNA/RNA isolation, the second one for PMA dye pretreatment allowing detection of potentially infectious viral particles. The treated samples were exposed to 40 W LED light with a wavelength of 460 nm for 15 min using a photo-activation system. Both qPCR/v-qPCR (for DNA viruses) and RT-qPCR/v-RT-qPCR (for RNA viruses) were performed using CFX96 real-time PCR thermocycler. The detection of AdVs, HBoV, RoVs, NoVs, IAV, and SARS-CoV-2 were carried out with Adenovirus, Bocavirus, Rotavirus, Norovirus (GI and GII), Influenza A, and SARS-CoV-2 VIASURE Real Time PCR Detection Kits.

RESULTS

This study revealed the presence of gastrointestinal and respiratory viral nucleic acids in the air, on surface as well as in influent and effluent wastewater samples.

Viruses		Number and percentage (%) of positive samples				
		Air C*)	Air M**)	Surface swabs	Influent wastewater	Effluent wastewater
qPCR/RT-qPCR	AdVs	12/26 (46.2)	8/26 (30.8)	23/54 (42.6)	15/15 (100)	6/15 (40)
	HBoV	2/26 (7.7)	ND	16/54 (29.6)	9/15 (60)	3/15 (20)
	NoV GI	4/26 (15.4)	ND	17/54 (31.5)	12/15 (80)	4/15 (26.7)
	NoV GII	6/26 (23.1)	ND	30/54 (55.6)	15/15 (100)	7/15 (46.7)
	RoVs	9/26 (34.6)	5/26 (19.2)	34/54 (63)	11/15 (73.3)	5/15 (33.3)
	IAV	ND	ND	ND	1/15 (6.7)	ND
	SARS-CoV-2	ND	ND	3/54 (5.6)	5/15 (33.3)	ND
	SARS-CoV-2/P	8/26 (11.5)	5/26 (19.2)	14/54 (25.9)	7/15 (46.7)	2/15 (13.3)
v-qPCR/v-RT-qPCR	AdVs	9/12 (75)	7/8 (87.5)	22/23 (95.7)	15/15 (100)	5/6 (83.3)
	HBoV	1/2 (50)	ND	13/16 (81.3)	9/9 (100)	3/3 (100)
	NoV GI	3/4 (75)	ND	12/17 (70.6)	11/12 (91.7)	3/4 (75)
	NoV GII	4/6 (66.7)	ND	28/30 (93.3)	15/15 (100)	6/7 (85.7)
	RoVs	6/9 (66.7)	4/5 (80)	32/34 (94.1)	11/11 (100)	4/5 (80)
	IAV	ND	ND	ND	1/1 (100)	ND
	SARS-CoV-2	ND	ND	1/3 (33.3)	5/5 (100)	0/15 (0)
	SARS-CoV-2/P	3/8 (37.5)	3/5 (60)	9/14 (64.3)	6/7 (85.7)	1/2 (50)

Table 1. - Number and percentage of virus-positive and potentially infectious virus-positive air, surface, influent and effluent wastewater samples as identified by qPCR/RT-qPCR in total studied samples and as identified by v-qPCR/v-RT-qPCR among all positive samples. C *) air samples collected with Coriolis µ impinger, M**) air samples collected with MAS-100NT impactor, *AdVs* adenoviruses, *HBoV* human bocavirus, *RoVs* rotaviruses, *NoV GI* Norwalk virus genogroup I, *NoV GII* Norwalk virus genogroup II, *IAV* influenza A virus, *SARS-CoV-2* severe acute respiratory syndrome coronavirus 2, *SARS-CoV-2/P* presumptive SARS-CoV-2 positive/other coronaviruses positive, *ND* not detected.

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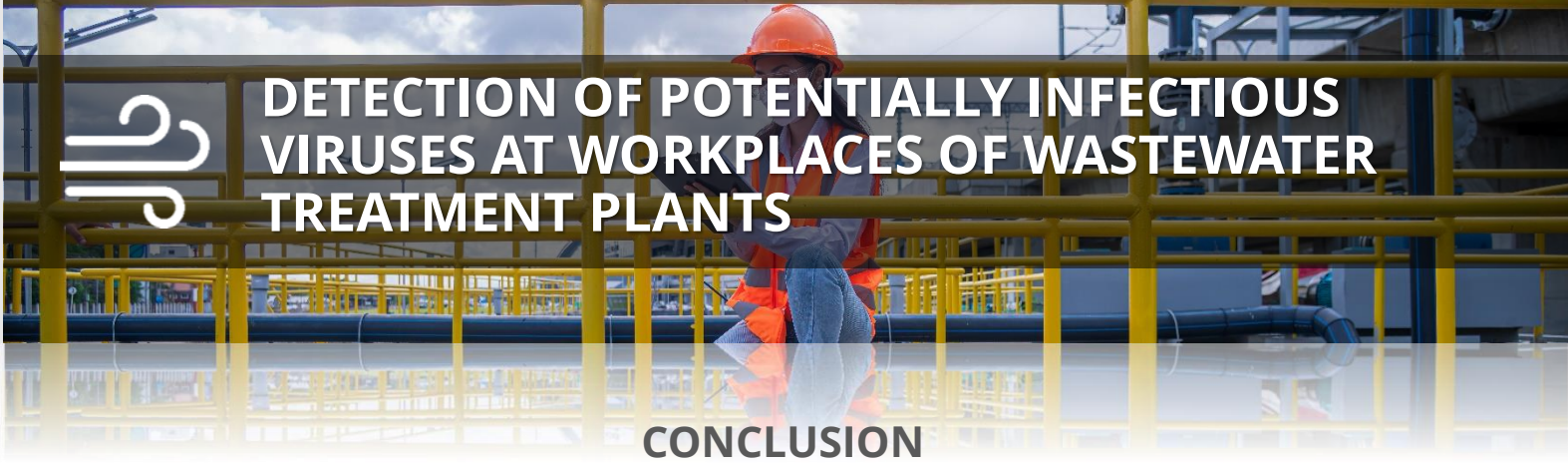

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CONCLUSION

- The use of Coriolis µ impinger allowed to detect two types of DNA (AdVs, HBoV) and four types of RNA viruses (NoV GI, NoV GII, RoVs, and presumptive SARS-CoV-2 or other coronaviruses).
- Although, the most contaminated area was in general the wastewater pumping section, the potentially infectious viruses occurred within all workplaces involved in wastewater treatment processes.
- The authors highlight that the identification and quantification of potentially infectious viruses in WWTPs and other occupational environments with high abundance of microbial contaminants are an important part of safety work management and proper health risk assessment.

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