Test report of Dopair filtration system of ATA company: Influenza H1N1 and Adenovirus.

Summary

1. Scientific and technological background

VirNext is a technological platform of service and innovation with the aim to answer to scientific and technological needs of manufacturers in the fields of virology and microbiology. VirNext is specialized in the evaluation of physical, chemical and biological technologies for indoor air, surface and water decontamination.

ATA Company commissioned VirNext technological platform to evaluate the efficiency of the "Dopair/Room Dopair" indoor air purification system for the decontamination of viruses in confined space. This purification system is composed of a filter system.

Confined space indoor air favours exposition to chemical and biological harmful compounds; which can have a hard sanitary impact. Pollutants in confined space are known to be involved in respiratory deficiency, cardiovascular diseases, rhinitis, allergies and cancer. The nature of these pollutants depends on environmental confined spaces. For medical and paramedical sectors, the main biological pollutants are microorganisms, and notably viruses. Among the viral strains involved, a first category is the enveloped viruses, which possessed a lipidic membrane of cellular origin. These enveloped viruses are considered as poorly resistant to environmental conditions. Among them, we can point the *Coronaviridae*, the *Orthomyxoviridae* (influenza virus type A and B), the *Paramyxoviridae* (human respiratory Ssyncytial virus) (hRSV), human parainfluenza viruses (hPIV) and metapneumovirus) are rediscovered. Another category is non-enveloped viruses, which possess a viral proteic shell. These viruses demonstrate a moderate to high resistance to environmental factors. Among them, we can point the Adenoviruses, the *Picornaviridae* (Rhinovirus, Poliovirus and Enterovirus).
Altogether, these viruses are responsible of respiratory diseases such as flu, bronchiolitis, acute respiratory illness and nosocomial infections.

VirNext has developed experimental procedures in order to evaluate the efficiency of Room Dopair/Dopair filter system to decontaminate confined space. This confined space was contaminated with enveloped or non-enveloped viruses: influenza virus (H1N1) (sensitive to environmental parameters) and Adenovirus type 5 (Ad-5) (less sensitive to environmental parameters). The influenza H1N1 viruses have an important pleiomorphy, with spherical viruses (80 to 120 nm of diameter) and ovoid forms (150 to 600 nm of diameter). Adenoviruses have a typical icosaedric form (100 to 400 nm of diameter).

Caller:

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2. Methodology

The experimental strategy consists of the evaluation of the capacity of "Room Dopair/Dopair" system, developed by ATA-Medical Company, to decontaminate a confined space with microorganisms. This confined space was materialised by a nebulization chamber with a volume of 2.5m^3 where an artificial atmosphere containing microorganisms can be generated. These atmospheres were obtained by nebulization of concentrated solutions containing the microorganisms. Test samples were harvested by suction of total volume of chamber using cyclonic movement (Coriolis, Bertin Technologies). During this suction, the harvested microorganisms were resuspended in a collection buffer.

3. Evaluation of purifier efficiency

3.1 Experimental conditions

Date: 7/02/2014 (Influenza H1N1) and 11/02/2014 (Adenovirus type 5)

Temperature: 20°C

Flow of Room Dopair/Dopair filter system: 160m^3/h

Functioning time:

Functioning time of Room Dopair system has been defined in order to evaluate decontamination efficacy on confined space after passage of 5 chamber volumes (12.5m^3 in 5 minutes), 10 chamber volumes (25m^3 in 10 minutes), 20 chamber volumes (50m^3 in 20 minutes).

Number of samples: 14 for each microorganism

Concentration of microorganism solutions:

- Influenza virus H1N1 **:** 10^8 $TCID_{50}$/mL
- Adenovirus type 5*:* 10^8 $TCID_{50}$/mL

Collection parameters: 10 minutes (2.5 m^3) in 7 mL of collection medium (phosphate buffer)

Evaluation method:

- Influenza: the amount of infectious virus by limit-dilution on MDCK (Madin-Darby Canine Kidney) cells, incubation at 37°C, 5% of CO_2 during 96 hours.
- Adenovirus**:** the amount of infectious virus by limit-dilution on A549 (Carcinomic Human Alveolar Basal Epithelial) cells, incubation at 37°C, 5% of CO_2 during 7 days.

3.2 Results:

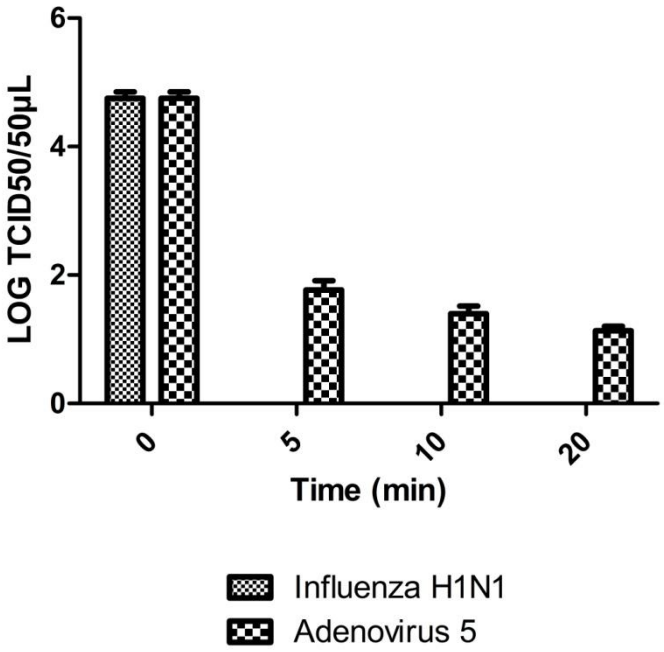

Figure 1: Evaluation of « Room Dopair » filter system on viruses: *Influenza H1N1* and *Adenovirus type 5*.

The collecting data allow to define the efficiency of the « Room Dopair » system on decontamination of confined space with viruses.

- Reduction Log $TCID_{50}$/mL Influenza H1N1 :
  - 4,1 ± 0,1 Log in 5 minutes
  - 4,1 ± 0,1 Log in 10 minutes
  - 4,1 ± 0,1 Log in 20 minutes

- Reduction Log $TCID_{50}$/mL Adenovirus type 5:
  - 3.0 ± 0,2 Log in 5 minutes
  - 3.3 ± 0,2 Log in 10 minutes
  - 3.6 ± 0,1 Log in 20 minutes

3.3 Conclusion

The « Room Dopair/Dopair » system developed by ATA-Medical Company allows the decontamination of a confined space of a volume of 2.5m^3 in 5 minutes with efficiencies of 99.9929 % and 99.905 %, for Influenza H1N1 and Adenovirus type *5* viruses, respectively.

Lyon the 5 mars 2014,

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APPENDIX 1

Name	Parametres		Results LOG TCID50/50µL	Readin of CPE- a) numbre of positive wells																	
	Condition system	Numbres of passages		10 -1		10 -2		10 -3		10 -4		10 -5		10 -6		10 -7		10 -8		10 -9	
14/F/A/HINI/Control -	n.a	n.a	0,00	0	0	0	0	0	0												
14/F/A/H1N1/1	OFF	0	5,00	4	4	4	4	4	4	4	4	3	1	0	0	0	0	0	0	0	0
14/F/A/H1N1/2	OFF	0	4,80	4	4	4	4	4	4	4	4	2	1	0	0	0	0	0	0	0	0
14/F/A/H1N1/3	OFF	0	4,50	4	4	4	4	4	4	4	3	1	0	0	0	0	0	0	0	0	0
14/F/A/H1N1/4	OFF	0	4,70	4	4	4	4	4	4	4	3	2	1	0	0	0	0	0	0	0	0
14/F/A/H1N1/5	ON	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/H1N1/6	ON	5	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/H1N1/7	ON	5	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/H1N1/8	ON	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/H1N1/9	ON	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/H1N1/10	ON	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/H1N1/11	ON	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/H1N1/12	ON	20	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/H1N1/13	ON	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 1: Quantification of influenza H1N1 viruses infectivity in $TCID_{50}$/50µL. a) 1 at 4virus, degree of CPE in 4 cellular culture units (microtitration plate), 0 : no virus, n.a : not applicable

Name	Parametres		Results LOG TCID50/50µL	Readin of CPE- a) numbre of positive wells																	
	Condition system	Numbres of passages		10 -1		10 -2		10 -3		10 -4		10 -5		10 -6		10 -7		10 -8		10 -9	
14/F/A/Ad5/Control -	n.a	n.a	0,00	0	0	0	0	0	0												
14/F/A/Ad5/1	OFF	0	5,00	4	4	4	4	4	4	4	4	3	1	0	0	0	0	0	0	0	0
14/F/A/Ad5/2	OFF	0	4,80	4	4	4	4	4	4	4	4	2	1	0	0	0	0	0	0	0	0
14/F/A/Ad5/3	OFF	0	4,50	4	4	4	4	4	4	4	3	1	0	0	0	0	0	0	0	0	0
14/F/A/Ad5/4	OFF	0	4,70	4	4	4	4	4	4	4	3	2	1	0	0	0	0	0	0	0	0
14/F/A/Ad5/5	ON	5	1,8	4	4	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/Ad5/6	ON	5	1,5	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/Ad5/7	ON	5	2	4	4	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/Ad5/8	ON	10	1,2	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/Ad5/9	ON	10	1,4	4	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/Ad5/10	ON	10	1,6	4	4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/Ad5/11	ON	20	1,2	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/Ad5/12	ON	20	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/F/A/Ad5/13	ON	20	1,2	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Tableau 2: Quantification of Adenovirus infectivity in $TCID_{50}$/50µL. a) 1 at 4virus, degree of CPE in 4 cellular culture units (microtitration plate), 0 : no virus, n.a : not applicable

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