efficiency test report on the system Beewair BW60L and its ability to decontaminate confined spaces containing the human Coronavirus MERS

1. *Foreword*

VirNext is the technology research platform **VirPath laboratory,** hosting the **National Center French reference for influenza and respiratory viruses,** as well as the **National Virus Reference Center Influenzae (CNR) of the WHO.** VirNext specializes in the evaluation of physical, chemical and technological Biological intended to decontaminate indoor air, surfaces and water.

The company asked the Beewair **laboratory VirPath** and its platform **VirNext** evaluate the effectiveness an air purification system developed by Beewair to decontaminate areas containing Human Respiratory Syndrome coronavirus Middle East (**MERS-CoV).** This assessment was carried out in coordination with Pr. Sylvie van der Werf, Chief, Laboratory of Molecular Genetics RNA viruses (RIG) at the Pasteur Institute in Paris.

applicant:

Beewair

3 Allée de la Teppe

71850 Charnay Les Macon, France

Contact: Dr. Pierre-Alexandre Deveau

Laboratory tests:

Laboratory VirPath

platform VirNext

RTH Laennec Faculty of Medicine 2nd floor,

7-11 rue Guillaume Paradin, 69372 Lyon cedex 08, France

Contact: Dr. Vincent Moulès

2. *Methodology*

The experiment consists in evaluating the capabilities of the system developed by the company to decontaminate Beewair a confined space containing microorganisms. This confined space was materialized by a spray chamber of 1.7m 3 wherein an artificial atmosphere containing microorganisms could be generated with good reproducibility. These contaminated atmospheres are obtained by nebulization viral concentrate solutions.

Test samples were collected by aspiration of the total volume of the chamber using a cyclonic movement (Coriolis Be1tin Technologies). The clinical isolate MERS-CoV (ref 201327C2-2 / 12 / 13) was obtained from the Institut Pasteur (Prof. Sylvie van der Werf, GMVR) (1). All protocols used for this experiment for cell cultures, viral titrations and amplifications were established and referenced by the Institut Pasteur (2).

In the collection step, the collected viruses were resuspended in a buffer containing a collector cell culture medium. To obtain UV dose in accordance with the specifications of the air decontamination system testing, UV lamps were lit 10 minutes before each decontamination step in the absence of operation of integrated fans.

3. *Evaluation of the effectiveness of the system Beewair*

3. the Experimental

date: *7* July 2014

Temperature : 20 ° C

Feed Beewair system: 60 m 3 / h

The Beewair BW60L decontamination and air treatment system is comprised of a tangential reactor integrating the DBD-Lyse © modules including an overall irradiation power of 70 Watts (UVC 254 nm) and an average capacity of oxidation / digestion 80,000 TeraRad.

Running Time:

The operating time of the system was defined to evaluate the decontamination effectiveness of a confined space after passing 3 bedrooms volumes (5.1 m3, 306 seconds), 8 room volumes (13.6 m3, 816 seconds) and 12 B volume (20.4 m3 1224 seconds).

Step nebulization:

The artificial atmosphere containing MERS CoV was generated by 5 collision nebulizers (NSF 6 jet CN25, BGI USA) (Air pressure 1.7 bar, 24.6 psig) for 15 minutes, corresponding to the average number of viruses 2.6E7 $TCID_{50}$ by atmosphere. The MMD value (mass median diameter) Theoretical aerosol is 1.9 .mu.m.

Number of samples : 18

Concentration of the stock virus solution: *MERS CoV* $10^{7.3}$ $TCID_{50}$ / ml

The viral stock was generated after two viral amplifications artificial kidney cells (Vero E6, ATCC CRL 1586), glucose Dulbecco's Modified Eagle Medium with 4.5g / L (LONZA Ref BE 2-614F l) with addition of fetal calf serum 2%, L-glutamine 2 mM (Sigma-Aldrich), penicillin (225U / mL, Cambrex Biosciences) and streptomycin (225ug / ml, Cambrex Biosciences) as described previously (3). The Vero E6 cells were infected at multiplicity of infection (MOI) of 5 and the cell supernatants were harvested 48 hours after infection.

collection settings: viruses were collected during 7 minutes (200 L / min) in 7 mL of Dulbecco's Modified Eagle Medium with 4.5g / L (Ref LONZA the 2-614F BE) with fetal calf serum addition to 2%, 2mM L-glutamine (Sigma-Aldrich), penicillin (225U / mL, Cambrex Biosciences) and streptomycin (225ug / ml, Cambrex Biosciences).

Measurement Method:

Infectious virus titers were determined by dosage titration end point, according to the established protocol by Institut Pasteur (2). Briefly, 50 ul of 10 series for each sample dilutions were inoculated four wells of cells replicas Vero E6 high micro 96-well plates, incubated at 37 ° C 5% CO2. The presence of cytopathic effect was monitored 72 hours after infection. The infectious viral titers (TCID 50/50 ul) were then determined using the statistical method Reed & Munch (4).

Bibliography:

(1) Guery 8 Poissy J el Mansouf L, Séjourné C Ettahar N, Lemaire X, Vuotto F, Goffard A, it Behil the S, V Enouf, Caro V A mesh, Che D, Manuguerra JC, Mathieu D, Fontanet A, van der Werf S; MERS-CoV study group. Clinicat features and Viral diagnosis of two cases of infection with Middle East Respiratory Syndrome Coronavirus: a postponement of nosocomia the transmission. Lancet. J 2013 a 29; 381 (9885): 2265-72.

(2) SOPs GMVR unites Pasteur Institute: "Inoculation of coronavirus in Vero E6 cells" - version of 03.04.2014; "TCID 50 titration" - version *041061201 4*

(3) Wilde AH, VS Raj, Oudshoorn D Bestebroer TM, N van ieuwkoop S Limpens RW Posthuma CC, van der Meer Y Barcena million Haagmans BL, Snijder EJ, van den Hoogen BG. MERS-coronavirus replication induces severe in vitro Strongly cytopathology and is inhibited by cyclosporin A or interferon-a treatment. J Gen Virol. 2013 Aug; 94 (Pt 8): 1749-1760.

(4) Reed. LJ: M uench. H. A simpler method of estimating fifty percent endpoints per Am. J. Epidemio l. (1938) 27 (3): 493- 497

Hospitals of Lyon

3.2 Result s :

	viral concentration (TCIDS0 / 50µ1)					
Number of room volumes	title	Mean (Average)	SD (Standard Deviation)	Mean ± SD log title	inactivation factor	Efficiency (%)
3	1.00E + 05 1.00E + 05 3,16E + 04	7.72E + 04	3.95E + 04	4.83 ± 0.28	2.00 ± 0.00	99.99%
3	1.00E + 03 3,16E + 02 1.00E + 03	7.72E + 02	3.95E + 02	2.83 ± 0.28		
8	2,00E + 04 5,01E + 04 3,16E + 04	3.39E + 04	1.52E + 04	4.50 ± 0.20	3.40 ± 0.03	99.99%
8	1.00E + 01 1.00E + 0l 2,00E + 0l	1.33E + 0l	5.77E + 00	1.10 ± 0.17		
12	1.00E + 04 5,01E + 03 1.00E + 04	.03 8.34E	2.88E + 03	3.90 ± 0.17	> 3.90 ± 0.17	> 99.99%
12	1.00E + 00 1.00E + 00 1.00E + 00	<1.00E + 00	0.00E + 00	<0.00		

As indicated in the table above, the infectivity of MERS-CoV was significantly reduced following their passage through the Beewair system. Reduced viral titers expressed as log $TCID_{50}$ / 50µ1 as follows:

- 2.00 ± 0.00 Log for an operating time of 306 s (5.1m^3)
- 3.40 ± 0.03 Log for an operating time of 816 s (13.6 m^3)
- 3.90 ± 0.17 Log for an operating time of 1244 s (20.4 m^3)

4. *Conclusion*

The BW60L system developed by the company Beewair leads to effective inactivation of MERS-CoV human virus in confined spaces with an efficiency of 99.99% of the viral titer by a factor of 100 when passing 3 bedroom air volumes up almost 10^4 to 12 volumes of air.

Lyon 24 July 2014,

Dr. Vincent Moulès

Responsible for the technology platform VirNext Laboratory VirPath

Dr. Rosa-Calatrava Manual

Deputy Director of VirPath Laboratory, University Claude Bernard Lyon 1

Prof. Sylvie van der Werf

Head of Molecular Genetics Laboratory of RNA viruses (RIG), Institut Pasteur, Paris

Institut Pasteur
Unité de Génétique Moléculaire
des Virus à ARN
CNRS URA 3015
25-28, rue du Docteur Roux
75724 Paris Cedex 15