Examination Report

Evaluation test of cleaning of virus with air cleaning equipment, with Feline infectious peritonitis virus as indicator

KITASATO Research Center Kitasato Medical Center Hospital Kitasato Center of Medical Sciences Title:

Evaluation test of cleaning of virus with air cleaning equipment,

with Feline infectious peritonitis virus as indicator

Examination number: 00422

Objectives: Evaluate performance of ion generator developed by Samsung Electronics for removal of feline infectious peritonitis virus, to rate

performance of air cleaner with an internal ion generator.

Name of the party that requested the examination: Samsung Electronics Co. Ltd.

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Person in charge: Rae-Eun Park

Name of the party that carried out the examination:

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Person in charge: Tatsuo SUZUKI

Location of the examination:

Name:

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Person in charge of the experiment:

Noritada Kobayashi

Person in who conducted the experiment:

Noritada Kobayashi

Masataka Ootsuka

Person in charge of the examination:

Tatsuo Suzuki

Examination period (including culture period for virus): 4 January 2005 – 15 October 2005

Examination report drafted on: 24 October 2005 Examination report created on: 7 November 2005 Examination report approved on: 10 November 2005

Method

1: Materials 1.1: Subject

> Name: Air cleaner Code number:

Note: Samsung Electronics Air Cleaner with Ion Generator

1.2: Virus

Feline infectious peritonitis virus

Name: Feline infectious peritonitis virus (FIPV)

Root number: 79-1146

1.3: Cells

Name: fcwf4 cell Root name: fcwf4p86

Note: Feline liver epitheloid cells

1.4: Culture media

a: Cell breeding culture media for virus cultivation 10%FBS Eagle inclusion MEM (Glbco: Invitrogen Corp., CA, USA) (NaHCO3, Glutamine and Penicillin/Streptomycin)

b: Culture media for maintenance of virus 1% BSA Eagle inclusion MEM (Glbco) (NaHCO3, Glutamine, vitamin, biotin and Penicillin/Streptomycin)

2: Test method

2.1: Virus adjustment

FIPV (freeze-dried in the ultra low temperature freezer) is infected in culture media for virus cultivation (10%FBS Eagle inclusion MEM(Gibco)) with fcwf4 cell at M.O.I. 0.01, and cultured for 72 hours under 37C 5%CO2 (1 generation). After 5 consecutive generations it was cultivated in large volume, after which virus solution was separated and purified using density gradient centrifugation method. This was separated by 1ml, and was kept at -80C until experiment. Some of the virus solution was used for measurement of virus infection value (TCID50) by verifying cells cytopathic effect by ten-fold serial dilution.

2.2: Test method

Performance test of air cleaner with ion generator against microbes

a: Measurement condition

Spraying duration: 30 minutes

Number of tests: 3 times per testing condition

Collection volume: 30ml/10 minutes

Air cleaner setting

I: Wind power: Strong, Ion: OFF II: Wind power: Strong, Ion: ON

When Ion:ON, air cleaner was put in function 30 minutes prior to

spraying of virus solution.

b: measurement method

1: Put FIPV prepared at $10^{7.24-7.26}$ TCID₅₀/mL into the nebulizer, and sprayed into the test box for 30minutes.

- 2: Air cleaner was set at two settings stated above, and examination was carried out in that order. After spraying of virus solution, the air inside the test box was collected for 10 minutes via the impinger through air collecting hole made on the test box.
- 3: Collected air was ten-fold serial-diluted, then inoculated on fcwf4 cells applied on 96-hole microplate for 1 hour. After removing the virus solution, virus maintenance media of 1%BSA inclusion Eagle MEM (Gibco)0.1ml was added, then cultured for 96 hours under 37C 5% CO2. Every 24 hours, Cytopathic effect and metabolite inhibition was observed and $TCID_{50}$ was measured. Volume of virus collected was compared in order to evaluate performance of the air cleaner.

Results:

3.1 Virus cleaning effect of air cleaner based on feline infectious peritonitis virus FIPV 79-1146.

FIPV released into the test box remained 10 $^{7.24-7.26}$ TCID₅₀/mL throughout the test. Volume of FIPV of the test box when ion generator was off was 10 $^{6.30-6.50}$ TCID₅₀/mL after 0 minute of termination of spraying, then 10 $^{6.12-6.23}$ TCID₅₀/mL after 10 minutes, 10 $^{5.62-5.68}$ TCID₅₀/mL after 20 minutes, 10 $^{4.36-4.43}$ TCID₅₀/mL after 30 minutes, 10 $^{3.40-3.50}$ TCID₅₀/mL after 40 minutes, 10 $^{2.18-2.23}$ TCID₅₀/mL after 50 minutes (Chart 2 and Graph 1).

When Ion was On, FIPV volume was 10 $^{5.23\sim5.60}$ TCID $_{50}$ /mL after 0 minutes, 10 $^{4.50\sim4.60}$ TCID $_{50}$ /mL after 10 minutes, 10 $^{3.23\sim3.24}$ TCID $_{50}$ /mL after 20 minutes, <10 $^{2.29\sim2.36}$ TCID $_{50}$ /mL after 30 minutes, <10 $^{1.80\sim1.88}$ TCID $_{50}$ /mL after 40 minutes, and less than detection threshold after 50 minutes (Chart 2 and Graph 1).

Note: Virus cleaning performance was tested using Feline infectious peritonitis virus. In this test, we have referred to the results gained in preliminary exams measuring performance against MRSA and Influenza virus A, and we have set a set amount of leave period after spraying of virus for 30 minutes, then compared volume of virus inside the test box in two cases where lon generator was on and off.

When lon generator was turned off, as time elapsed after the spraying was finished the volume of virus within the test box decreased, and after 50 minutes it reached less than $^{102,23}TCID^{50}/mL$. On the other hand, when ion was generated, $1/10\sim1/100$ decrease of virus within the test box (compared with the case when ion was not generated) was observed. Especially after 20 minutes of spraying, volume of virus was approximately 1/100 of that when ion was not generated (Chart 2 and Graph 2).

When ion was not generated, virus volume inside the test box immediately after spraying (0 minute) was approximately 1/10 of virus inserted inside the box. Also, natural decrease of virus volume was observed as time elapsed after spraying. So, we have set volume of collected virus at each elapsed time after spraying when ion was not generated as a base figure rather than the amount of virus input, to calculate virus removal percentage when ion generator was in function. This was calculated as 89.285% at 0 minutes, 97.927% at 10 minutes, 99.623% at 20 minutes, 99.152% at 30 minutes, and more than 97.488% at 40 minutes. After more than 40 minutes has elapsed, removal rate decreased but this can be understood as a result of natural decrease in volume of virus compared. The result suggests that approximately 20 minutes of exposure period is required for removal function.

Following the above results, it was proved that the air cleaner used in this examination has enough performance ability of virus removal. It was also suggested that the performance level of virus removal depends on contact period between generated ion and virus.

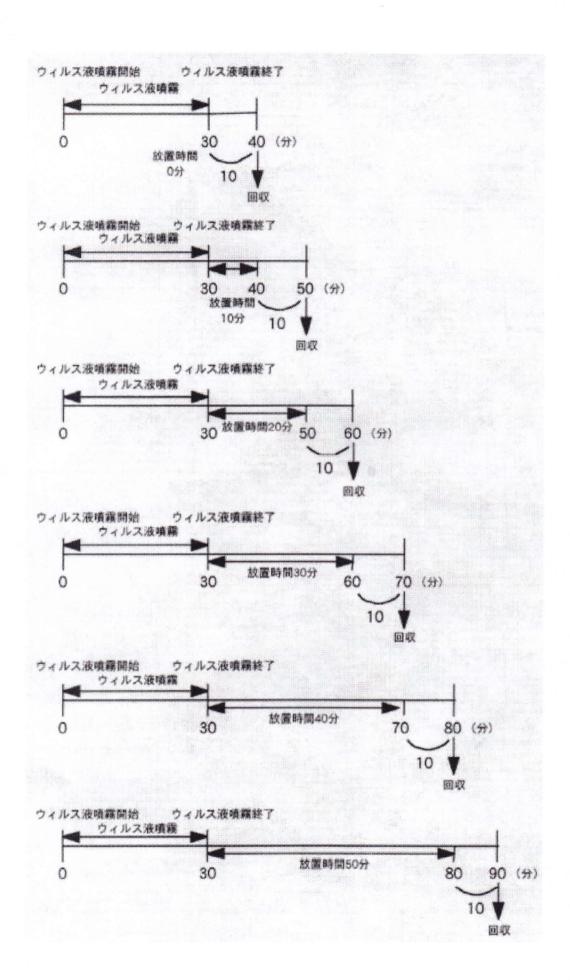
Summary 1: It was clarified that air cleaners used in this examination has virus removal function in relation to feline infectious peritonitis virus.

Summary 2: It was suggested that virus removal performance is affected by contact time duration between ion and virus.

Reference

Virus Examination, general chapter, 1973 Virus Examination, particular chapter, 1973 Virus Examination Protocol, 1995.

Examination report drafted on: 24 October 2005 Examination report drafted by: Noritada Kobayashi Examination report created on: 7 November 2005 Examination report created by: Noritada Kobayashi Examination report approved on: 10 November 2005 Person in charge of the examination: Tatsuo Suzuki Chart 1: Air Cleaner Exam flow chart

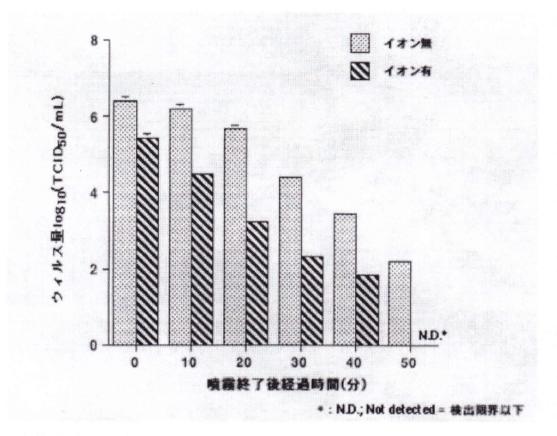


ウィルス液噴霧開始 Start of virus solution spraying ウィルス液噴霧 Spraying of virus solution ウィルス液噴霧終了 End of virus solution spraying

放置時間 Leave period 分 Minutes

回収 Collection

Chart 2: Air cleaner's virus removal performance against FIPV (Wind power: Strong)



イオン有 Ion ON イオン無 Ion OFF

ウィルス量 Virus volume 噴霧終了後経過時間(分) Minute elapsed after spraying 検出限界以下 not detected

Graph 1: Air cleaner (with internal lon generator) virus removal performance against FIPV

	排気側ウィルス量(TCID _® /mL)		
久置時間(分)	イオン未発生	イオン発生	
0	6.40	5.60	
10	6.20	4.60	
20	5.68	3.24	
30	4.40	2.36	
40	3.50	<1.80	
50	2.20	検出限界以下	

	排気側ウィルス量(TCID _{ss} /mL)		
放置時間(分)	イオン未発生	イオン発生	
0	6.30	5.23	
10	6.12	4.50	
20	5.62	3.23	
30	4.36	2.29	
40	3.40	<1.88	
50	2.18	検出限界以下	

	排気側ウィルス量(TCID _{in} /mL)		
放置時間(分)	イオン未発生	イオン発生	
0	6.50	5.46	
10	6.23	4.50	
20	5.67	3.23	
30	4.43	<2.35	
40	3.46	<1.88	
50	2.23	検出限界以下	

排気側ウィルス量	Virus volume on exhaust side
放置時間 (分)	Leave period (time)
イオン未発生	Ion non-generated
イオン発生	Ion generated
検出限界以下	Not detected

FIPV was sprayed at $10^{7.24-7.28}$ TCID₅₀/mL into the test box of 1m³ for 30 minutes. Then the air inside the box was collected from collection hole made on the exhaust side at 0, 10, 20, 30, 40 and 50 minutes after spraying. Collection was done in 10 minutes and virus infectivity titer was measured with TCID₅₀ method. At all examples, wind power of the air cleaner was set at Strong.