



KITASATO UNIVERSITY

Test Report (Transitional Report)

—In air-purifying devices based on feline coronavirus
Virus Removal Effectiveness Assessment Test—

The Educational Foundation of Kitasato Institute
Kitasato University Medical Center
Medical Environmental Science Center

General Information

Title	Test of Virus Removal Effectiveness of Air Purifier Devices on Feline Coronavirus
Test number	2005
Objective	Plabio Co., Ltd.'s air purifier's ability to remove cat corona virus is performed to determine its effectiveness.
Test Consignor	Title : PLABIO Co., Ltd. Location : 10F, 316, Yeongdong-daero, Gangnam-gu, Seoul, Korea 06177 Tel : +82-2-2088-2335 Fax: +82-2-2088-2334 Director : Jae Min, Park
Test Consignee	Name : The Educational Foundation of the Kitasato Institute. Kitasato University Medical Center Location : 6 Chome- 1 0 0 番地 Arai, Kitamoto, Saitama 364-8501, Japan Tel : +81-48-593-1212 Fax: +81-48-593-1262 Director : Kobayashi, Noritada
Testing Facility	Testing Facility Name : The Educational Foundation of the Kitasato Institute. Kitasato Medical Center Biomedical Building 1F Infection Lab Tel: +81-48-593-1212 Fax: +81-48-593-1262 Test Director: Kobayashi Noritada Yamazaki Oka Test Manager: Kobayashi Noritada Testing Period: July 1, 2020 ~ March 31, 2021 (Virus incubation period included)

Method and Procedure

1. Materials

1.1 Test equipment and materials

1.1.a Title: Air purifier

Notes: PLABIO's Plamedi Air Mini (small air purifier)

1.1.b Title: Air purifier

Notes: PLABIO's Plamedi Air Pro (large air purifier)

1.2 Main virus applied in test

Feline coronavirus

Title: Feline infectious peritonitis virus
(FIPV; Feline coronavirus)

Code : 79-1146

1.3 Cell applied in test

Title : fcwf4 cell

Code: fcwf4 p86

Notes: Epithelial cell derived from cat kidney

1.4 Medium

a. Cell growth medium for viral culture

10% FBS Embedded Eagle MEM(Gibco; Invitrogen Corp., CA, USA)
(NaHCO₃, Glutamine and Penicillin/Streptomycin embedded)

b. Virus maintenance medium

1% BSA Embedded Eagle MEM(Gibco)
(NaHCO₃, Glutamine, vitamin, biotin and Penicillin/Streptomycin embedded)

1.5 Test equipment

1.5.1 Title: Testing chamber (approx. 30cm³)

Notes: Acrylic test box with waterproof processing such as power supply (Toyo Seiki)

1.5.2 Title: Testing chamber (approx. 100cm³)

Notes: Acrylic test box with waterproof processing such as power supply (Toyo Seiki)

- 1.5.3 Title: Nebulizer
Notes: Special-order glass nebulizer
- 1.5.4 Title: Impinger
Code: 3008-62325
Manufacturer: GL Science Co., Ltd.
Notes: Used to collect air from the test box

2. Testing method

2.1 Preparation of the virus

Feline infectious peritonitis virus (FIPV; feline coronavirus: 79-1146), freeze-dried and preserved in a cryogenic freezer, was added to fcwf4 cells (cat kidney cells: fcwf4p86) in the Eagle MEM (Gibco) cell growth medium containing 10% FBS. The MOI (Multiplicity of infection) was infected at 0.01, and cultured for 72 hours under the conditions of 5% CO₂ at 37°C. (Generation 1). After 5 successive subcultures were incubated in large quantities, the virus solution was separated and purified by ultra-high-speed centrifugation at a silica density gradient, dispensed by 1 mL, and stored in a -80°C ultra-low temperature freezer until use. Part of the viral solution was tested for its cytopathic effect by a 10-fold step dilution to measure viral infectivity (TCID₅₀).

2.2 Experimental method

Performance test of PLABIO air purifier against pathogenic microorganisms.

a. Measurement conditions

Spraying time: 30 minutes

Number of tests: 10times/test model

Recovery capacity: 15ml/10minutes

Setting of air purifier

ON/OFF

Air volume: Model dependence (device setting value) fixed (small and large)

b. Method of measurement

① Prepared at a concentration of $10^{7.5\sim 8.5}$ TCID₅₀/mL, 50mL of Feline infectious peritonitis virus (FIPV; 79-1146) was added to the nebulizer and sprayed into the test box for 30 minutes.

② The air purifier operates under the ON/OFF binary, and air volume is relative to the model

of the device. According to the flow chart shown in Fig. 1, the air in the test box was collected for 10 minutes using an opening provided in the test box. A test box with a capacity of about 30cm³ was used for the performance test of the small air purifier, and a test box with a capacity of about 100cm³ was used for the performance test of the large air purifier.

- ③ The collected virus solution was diluted 10-fold, inoculated into fcwf4 cells on a 96-hole microplate, and infected via direct contact for 1 hour. After removing the virus solution, 0.1 mL of Eagle MEM (Gibco) containing 1% BSA was added to the virus growth medium. The medium was incubated for 96 hours under the conditions of 5% CO₂ at 37°C, and observed for its cytopathic effect (CPE) or metabolic hindrance every 24 hours in order to measure its virus infectivity (TCID₅₀). The performance of the air purifier was judged by comparing the amount of the virus recovered when plasma ions (hereafter referred to as “Object X”) were generated by the purifier versus when no plasma ions were generated.

Results

3.1 Virus removal effect of air purifier using Feline infectious peritonitis virus (FIPV; 79-1146) as an index

a: Small-sized air purifier: Plamedi Air Mini

The amount of FIPV in the test box when the air purifier was not operated was $>10^{6.5}$ TCID₅₀/mL after 0 minutes of spraying, after 1 minute $>10^{6.5}$ TCID₅₀/mL, after 5 minutes $>10^{6.5}$ TCID₅₀/mL, after 10 minutes $>10^{6.0}$ TCID₅₀/mL 、 After 30 minutes $>10^{5.5}$ TCID₅₀/mL, after 60 minutes $>10^{5.5}$ TCID₅₀/mL and after 90 minutes $>10^{4.9}$ TCID₅₀/mL (Figure 2 and Table 1).

The amount of FIPV in the test box when the air purifier was in operation was $>10^{6.5}$ TCID₅₀/mL after 0 minutes of spraying, after 1 minute $>10^{6.5}$ TCID₅₀/mL, after 5 minutes $>10^{6.5}$ TCID₅₀/mL, after 10 minutes $>10^{6.0}$ TCID₅₀/mL, After 30 minutes $>10^{4.4}$ TCID₅₀/mL, after 60 minutes $>10^{4.1}$ TCID₅₀/mL and after 90 minutes $>10^{3.4}$ TCID₅₀/mL (Figure 2 and Table 1).

b: Large-sized air purifier/sterilizer: Plamedi Air Pro

The amount of FIPV in the test box when the air purifier was not in operation was $>10^{7.8}$ TCID₅₀/mL after 0 minutes of spraying, after 1 minute $>10^{7.8}$ TCID₅₀/mL, after 5 minutes $>10^{7.8}$ TCID₅₀/mL, after 10 minutes $>10^{7.5}$ TCID₅₀/mL 、 After 30 minutes $>10^{7.3}$ TCID₅₀/mL, after 60 minutes $>10^{6.9}$ TCID₅₀/mL and after 90 minutes $>10^{5.9}$ TCID₅₀/mL (Figure 3 and Table 2).

The amount of FIPV in the test box when the air purifier was in operation was $>10^{8.0}$ TCID₅₀/mL after 0 minutes of spraying, $>10^{8.0}$ TCID₅₀/mL after 1 minute, $>10^{7.5}$ TCID₅₀/mL after 5 minutes, $>10^{7.0}$ TCID₅₀/mL after 10 minutes, after 30 minutes $>10^{6.3}$ TCID₅₀/mL, after 60 minutes $>10^{5.3}$ TCID₅₀/mL and $>10^{3.8}$ TCID₅₀/mL after 90 minutes (Figure 3 and Table 2).

Review

The virus removal ability test of PLABIO air purifier was evaluated using the Feline infectious peritonitis virus. In this test, after spraying the virus in the test box for 30 minutes and waiting for a set amount of time, the amount of the virus in the test box (as shown via infectious power value) was measured and examined. Labeling the plasma ions emitted by the air purifier as “Object X,” the measurement values were compared for when the virus came into contact with “Object X” versus when they did not come into contact with “Object X”.

When the small air purifier was not operated, the amount of the virus in the test box gradually decreased as time elapsed after spraying the virus solution. After the final measurement at 90 minutes, the value came to a figure of $10^{5.9}$ TCID₅₀/mL. On the other hand, it was observed that operating the air purifier for 90 minutes resulted in a decrease in the amount of the virus by approximately 99.9% (Table 1).

Similarly, for the large air purifier, when the air purifier was not operated, the virus in the test box gradually decreased as time elapsed after spraying the virus solution. After the final measurement at 90 minutes, the value came to a figure of $10^{5.9}$ TCID₅₀/mL. It was observed that operating the air purifier for 90-minutes resulted in a decrease in the amount of the virus by approximately 99.99% (Table 2). Regarding the trend in virus reduction, the large air purifier appeared to be more effective than the small air purifier.

As a result of the above, it is suggested that the air purifier used in this test has sufficient virus removal ability. It is also suggested that the removal ability is affected by the concentration of “Object X” in the test box, and the length of contact time between “Object X” and the virus.

Summary

1. In the performance test of the air purifier using Feline infectious peritonitis virus as an index, it is suggested that the air purifier used in this test has sufficient virus removal ability, depending on the concentration of “Object X”.
2. It is also suggested that the virus removal ability by the air purifier used in this test is affected by the length of contact time between “Object X” and the virus.

This test is currently in progress. This report was prepared based on the results obtained on November 16, 2020 (Director: Noritada Kobayashi). Please handle this report with utmost caution

Figure 1.

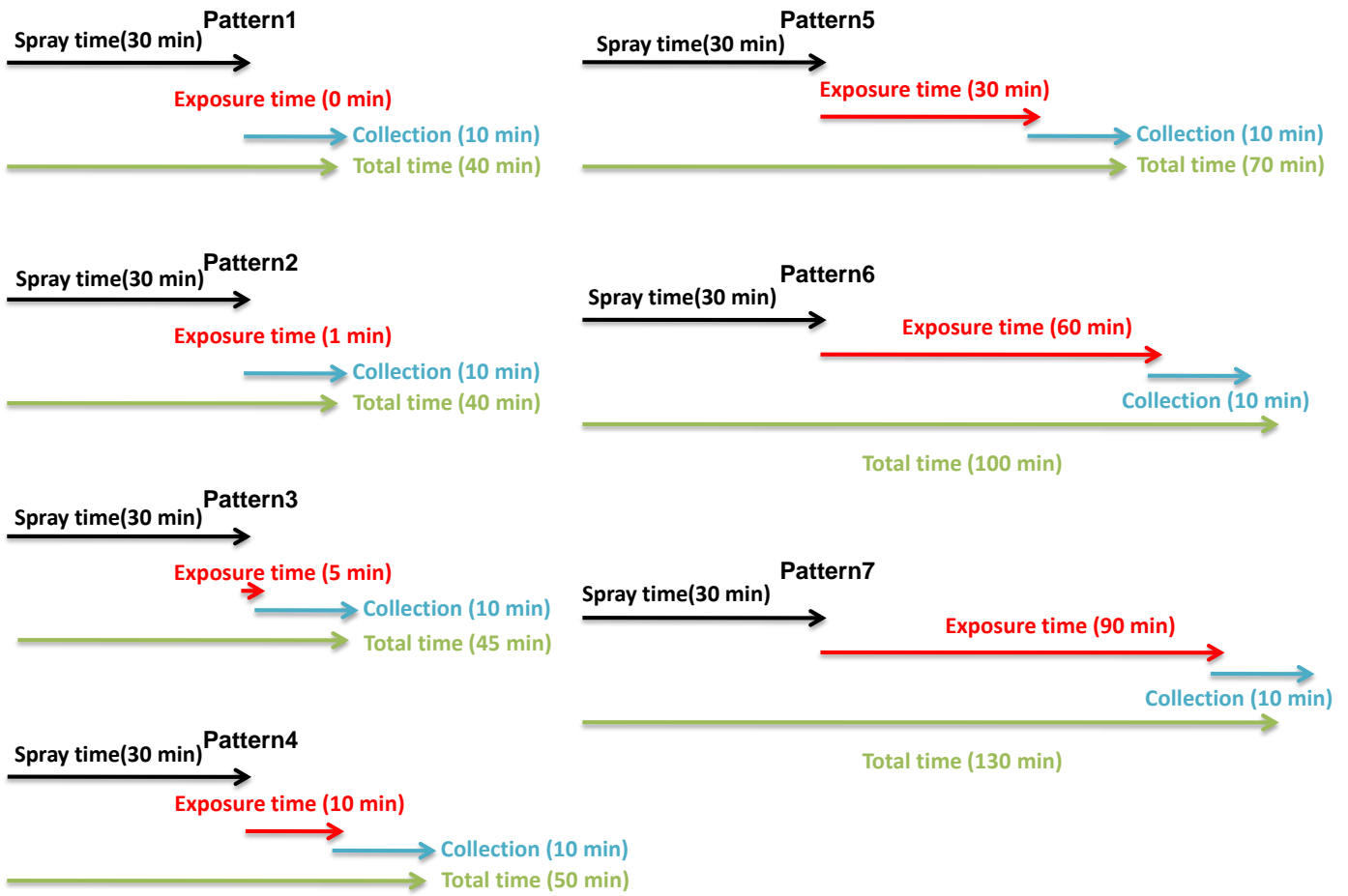


Figure 2. Results of small air purifier test

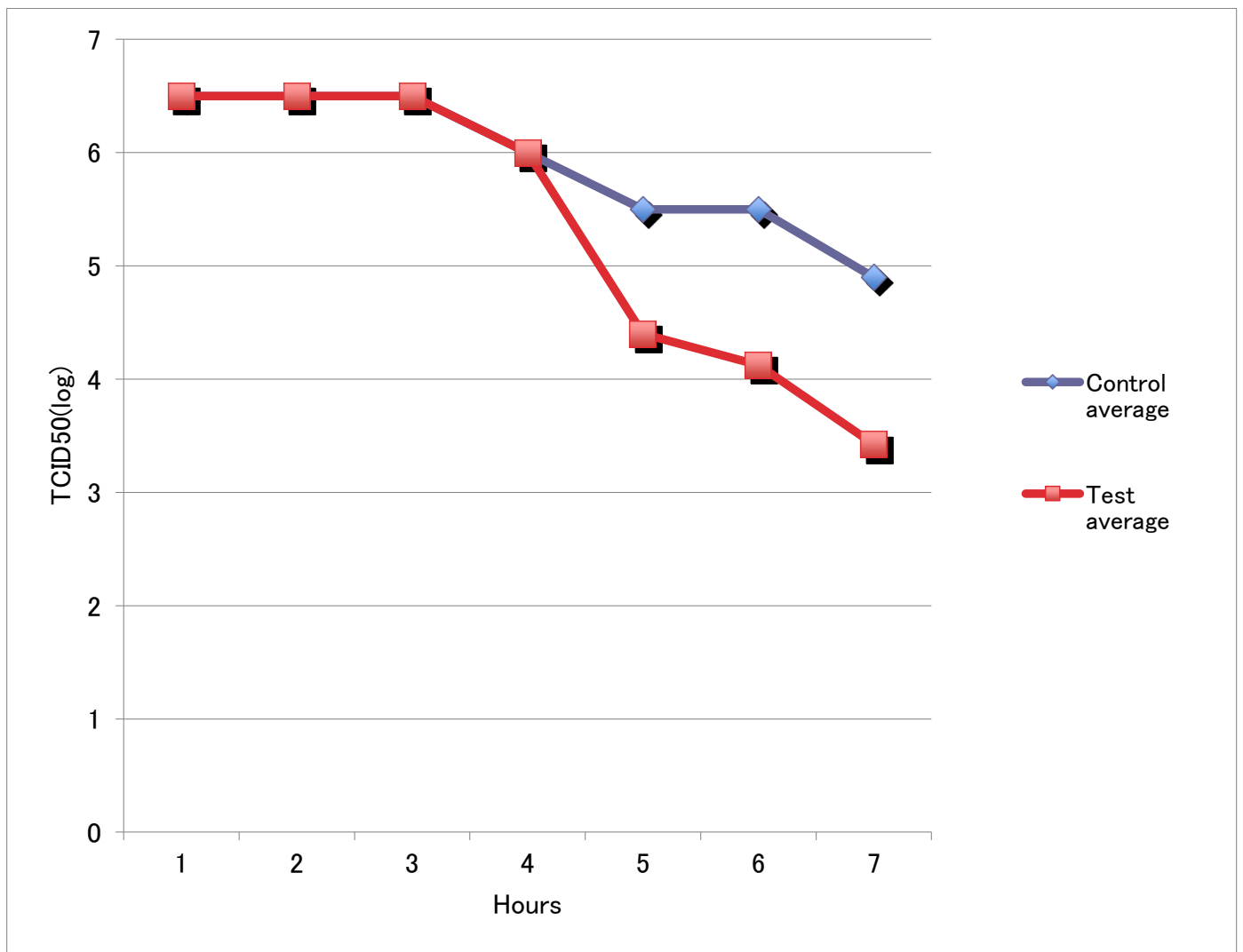


Figure 3. Results of large air purifier test

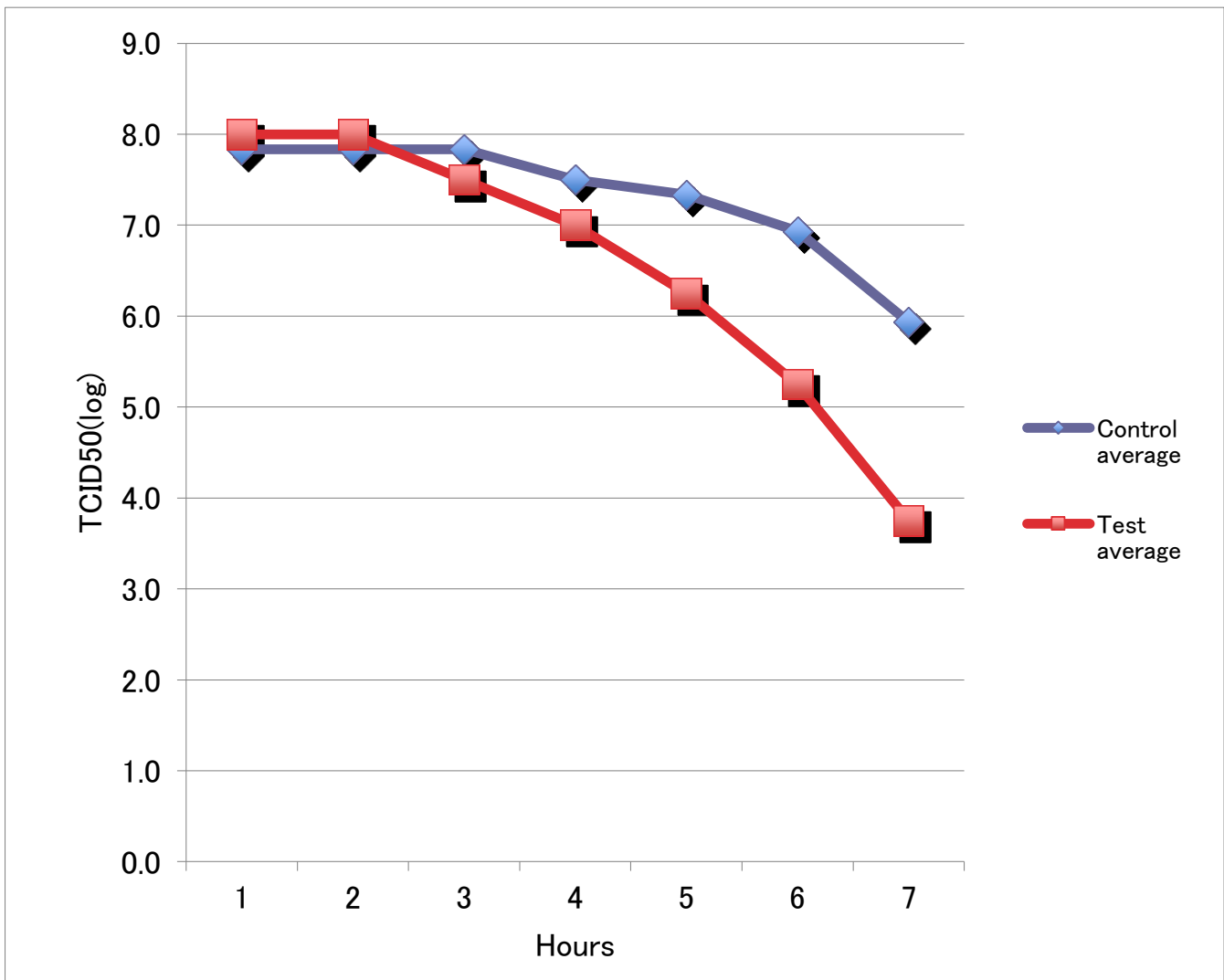


Table 1. Results of small air purifier test

Test number	2003
Standard test	Not applicable
Virus	FCV
Virus strain	HKV
Number of viruses	7.5TCID ₅₀
Number of recovered viruses	6.5TCID ₅₀
Medium	LV/MEM
Test subject	Plamedi Air Mini
Sterilization method	Electon / ion
Spray time	30 minutes
Test box pressure (before spraying):	0.04MPa
Test box pressure (during spraying):	0.2MPa
Air recovery amount in test box:	15mL/10 minutes (<-0.04MPa)

Control

Exposure time (minutes)	0	1	5	10	30	60	90
CID50(log) after 72 hrs of culture	6.5	6.5	6.5	6	5.5	5.5	5
CID50(log) after 72 hrs of culture	6.5	6.5	6.5	6	5.5	5.5	5
CID50(log) after 72 hrs of culture	6.5	6.5	6.5	6	5.5	5.5	4.8
CID50(log) after 72 hrs of culture	6.5	6.5	6.5	6	5.5	5.5	4.8
CID50(log) after 72 hrs of culture	6.5	6.5	6.5	6	5.5	5.5	4.8
CID50(log) after 72 hrs of culture	6.5	6.5	6.5	6	5.5	5.5	5
Control average	6.5	6.5	6.5	6	5.5	5.5	4.9

Operating

Exposure time (minutes)	0	1	5	10	30	60	90
CID50(log) after 72 hrs of culture	6.5	6.5	6.5	6	4.3	4	3
CID50(log) after 72 hrs of culture	6.5	6.5	6.5	6	4.8	4.5	3.5
CID50(log) after 72 hrs of culture	6.5	6.5	6.5	6	4.5	4	3
CID50(log) after 72 hrs of culture	6.5	6.5	6.5	6	4	4	4.2
CID50(log) after 72 hrs of culture	6.5	6.5	6.5	6	4.2	3.5	3
Test average	6.5	6.5	6.5	6	4.4	4.1	3.4

Table 2. Results of large air purifier test

Test number	2003
Standard test	Not applicable
Virus	FCV
Virus strain	HKV
Number of viruses	8. 5TCID ₅₀
Number of recovered viruses	7. 5TCID ₅₀
Medium	LV/MEM
Test subject	Plamedi Air Pro
Sterilization method	Electron / ion
Spray time	30 minutes
Test box pressure (before spraying):	0.04MPa
Test box pressure (during spraying):	0.2MPa
Air recovery amount in test box:	15mL/10 minutes (<-0.04MPa)

Control

Exposure time (minutes)	0	1	5	10	30	60	90
TCID ₅₀ (log) after 72 hrs of culture	8	8	8	7.5	7.5	7	6
TCID ₅₀ (log) after 72 hrs of culture	8	8	8	7.5	7.5	7	6
TCID ₅₀ (log) after 72 hrs of culture	7.5	7.5	7.5	7.5	7	6.8	5.8
TCID ₅₀ (log) after 72 hrs of culture	-	-	-	-	-	-	-
TCID ₅₀ (log) after 72 hrs of culture	-	-	-	-	-	-	-
TCID ₅₀ (log) after 72 hrs of culture	-	-	-	-	-	-	-
Control average	7.8	7.8	7.8	7.5	7.3	6.9	5.9

Operating

Exposure time (minutes)	0	1	5	10	30	60	90
TCID ₅₀ (log) after 72 hrs of culture	8	8	7.5	7	6.5	5.5	4
TCID ₅₀ (log) after 72 hrs of culture	8	8	7.5	7	6	5	3.5
TCID ₅₀ (log) after 72 hrs of culture	-	-	-	-	-	-	-
TCID ₅₀ (log) after 72 hrs of culture	-	-	-	-	-	-	-
TCID ₅₀ (log) after 72 hrs of culture	-	-	-	-	-	-	-
Test average	8.0	8.0	7.5	7.0	6.3	5.3	3.8