

Test Report of Virucidal effect on Influenza Viruses and Enteroviruses by Germagic Filters

Sample Name: Germagic Filters

Requested by: Chiaphua Industries Limited

Test Category: Entrusted Test

Tested by: Guangdong Inspection and Quarantine Technology Center

Guangzhou Institute of Respiratory Disease Medicine Co., Ltd

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Declaration

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Abstract

- Aim:** To evaluate the *in vitro* virucidal effect of “Germagic filters” for Influenza A virus (A/PR/8/34,H1N1) and Enterovirus clinical isolates (EV71).
- Method:** The protocol is developed according to Section 2.1.1.10 of “*Technical Standards for Disinfection*” 《消毒技术规范》, Test Protocol for Virucidal Tests and Package Insert of Test Products. Cytotoxicity test, neutralizing test for disinfectant and virucidal test are included in the Protocol.
- Results:** The cytotoxicity test, neutralizing test for disinfectant and *in vitro* virucidal test have been completed. (1) The neutralizing agent, “Germagic filter” and neutralized product have low toxicity to cells. (2) The neutralizing agent evaluation test for “Germagic filter” indicates the neutralizing agent can completely neutralizes the disinfectant. (3) The “Germagic filter” can effectively kill Influenza A virus (A/PR/8/34,H1N1) and Enterovirus clinical isolates(EV71) under defined lab conditions after reaching with virus suspensions for 10, 30 and 60 minutes, respectively
- Conclusion:** The neutralizing agent passed the neutralizing agent evaluation test. It complied with Section 2.1.1.10.5 Neutralizing agent eligibility criteria of “*Technical Standards for Disinfection*”. The “Germagic filter” could effectively kill Influenza virus (A/PR/8/34,H1N1) and Enterovirus clinical isolates (EV71) under the defined lab conditions.

Materials and Methods

1. Materials

- 1.1 Test sample: “Germagic filter”, each cm² contains 0.155ml of “Germagic” coating, provided by Chiaphua Industries Limited.
- 1.2 Cells: Madin-Darby Canine Kidney (MDCK) cells and Buffalo Green Monkey kidney (BGM) cells, purchased from the Cell Bank of Typical Culture Collection of Chinese Academy of Sciences.
- 1.3 Virus strains: Clinical isolates of Influenza A virus (A/PR/8/34,H1N1), and Enterovirus (EV71) were provided by Guangzhou Institute of Respiratory Disease Medicine Co., Ltd. Virus titer

$\geq 10^5$ TCID₅₀/ml.

1.4 Neutralizing agent: 9g NaCl+ 2.2g Tween 80 + 20ml 1M Na₂S₂O₃, dissolved in 1L of deionized water. The neutralizing agent was diluted with hard water in a ratio of 1:2.5.

2. Method

2.1 Cytotoxicity Test (MTT assay)

(1) To check for possible structural alteration of cells by the filter and neutralizing agent, which were divided into 4 groups,

- ①“Germagic filter”+ neutralizing agent 7.0 ml
- ②“Germagic filter”+ PBS 7.0 ml
- ③ Neutralizing agent 7.0 ml
- ④ PBS 7.0 ml

All groups were retained at room temperature for 10 minutes after mixing.

(2) Serial dilutions (dilution step: 1:10) were prepared in the culture medium and inoculated into monolayer MDCK and BGM cell at 34 °C for 48 hours. The viability was measured with the MTT assay. 20 μl of the MTT solution was added and incubated for 4 hours at 37°C. The plates were then blot dried on paper towels, followed by the addition of 100 μl of DMSO. Finally, absorbance was recorded at 490 nm using the mQuant enzyme-linked immunosorbent assay(ELISA) Reader, measured the growth inhibition ratio.

Inhibition ratio = [(mean OD of PBS – mean OD of blank control group) – (mean OD of test group – mean OD of blank control group)] / (mean OD of PBS group – mean OD of blank control group) × 100%。

2.2 Neutralizing Agent Evaluation Test

Influenza A virus(A/PR/8/34,H1N1) was chosen as model virus to evaluate the disinfectant-inactivating properties of the candidate neutralizing agent. Protocol and specification could refer to Section 2.1.1.10.5 Evaluation Test for Chemical Neutralization of Residual Disinfectant of “*Technical Standards for Disfection*”.

- (1) "Germagic filter" was cut into squares of 16cm² small pieces, following 5 groups,
 1. "Germagic filter"+ Influenza virus suspension 1.0 ml
 2. "Germagic filter"+ Influenza virus suspension 1.0 ml
 3. "Germagic filter"+ Neutralizing agent 7.0 ml
 4. Neutralizing agent 7.0 ml + Influenza virus suspension 1.0 ml
 5. PBS 7.0 ml + Influenza virus suspension 1.0 ml
- (2) The mixtures were maintained at 25 °C for 10 minutes, added 1.0 ml of influenza virus suspension, and kept at room temperature for 1 hour. Group 1 and group 2 were maintained at room temperature for 1 hour, followed by the addition of 7.0 ml PBS and 7.0 ml neutralizing agent into group 1 and group 2, respectively, and then keeping at room temperature for 10 minutes. Group 5 were retained at room temperature for 1 hour.
- (3) Serial dilutions (dilution step: 1:10) were prepared in the culture medium and inoculated into monolayer of MDCK cells at 37°C, 5% CO₂ incubator for 2 hours. Normal control group were added equal volume of culture medium. Discarding the supernatant, culture medium (containing 1.5µg/ml TPCK, 400IU/ml antibiotics) were added and incubated at 34°C with 5%CO₂ for 2-4 days. Any microscopic changes in the cells were recorded when reading the tests for cytopathic effects (CPEs). Tissue Culture Infective Dose (TCID₅₀) was calculated according to Reed-Muench analysis.

2.3 Virucidal Test

The protocol and evaluation criteria for Virucidal Test refer to Section 2.1.1.10 Virucidal test of "*Technical Standards for Disinfection*".

- (1) "Germagic filter" and blank filter were cut into squares of 16cm² small pieces, respectively.
- (2) "Germagic filter" and blank filter were added with 1.0ml influenza virus suspension or enterovirus suspension respectively, retained at room temperature for 10, 30 and 60 minutes. 7.0ml neutralizing agent was added into each groups, kept at room temperature for 10 minutes.
- (3) Serial dilutions (dilution step: 1:10) were prepared in the culture medium and inoculated into monolayer MDCK cells or BGM cells, 4 wells for each level of dilution, 100µl per well. Normal control group was using equal volume of culture medium.

- (4) The cells were incubated in 37°C, 5% CO₂ incubator for 2 hours, discard the supernatant. Add 400IU/ml of double antibiotics culture medium (containing 1.5µg/ml TPCK), and incubate at 34°C with 5% CO₂ for 2-4 days. Any microscopic changes in the cells were recorded when reading the tests for cytopathic effects (CPEs). Tissue Culture Infective Dose (TCID₅₀) was calculated according to Reed-Muench analysis.

3. Results

3.1 Cytotoxicity Test

The MTT assay indicated the neutralizing agents showed almost no toxicity to MDCK cells and BGM cells, the “Germagic filter” and diluted neutralizing agent mixture showed almost no toxicity to MDCK cells and BGM cells.

3.2 Neutralizing Agent Evaluation Test

The results of Neutralizing Agent Evaluation Test complied with Section 2.1.1.10.5 Neutralizing agent eligibility criteria of “Technical Standards for Disinfection”. Group 1 and group 2 could effectively kill influenza virus. The influenza virus titer in group 3 was similar with that in blank group (group 5). The results of group 4 indicated the neutralizing agent had no toxicity to virus.

Table 1. Results of Neutralizing Agent Evaluation Test for “Germagic filter”

Group	Test 1	Test 2	Test 3	Mean
	Log(TCID ₅₀ /ml)	Log(TCID ₅₀ /ml)	Log(TCID ₅₀ /ml)	Log(TCID ₅₀ /ml)
1	5.00	5.83	5.33	5.39
2	5.50	5.50	5.33	5.44
3	7.00	7.00	6.50	6.83
4	7.33	7.00	7.00	7.11
5	7.50	7.23	7.00	7.24

3.3 Virucidal Test

The “Germagic filter” reacted with Influenza virus and Enterovirus under the defined test condition. Results indicated that “Germagic filter” could effectively kill Influenza virus and Enterovirus at the specified time, See Table 2, Table 3 and Table 4.

Table 2. Virucidal effect of “Germagic filter” to Influenza virus at Various Contact Time

Virus Strain	Time (minutes)	Test 1	Test 2	Test 3	Mean	Mean of control group
		Log(TCID ₅₀ /ml)	Log(TCID ₅₀ /ml)	Log(TCID ₅₀ /ml)	Log(TCID ₅₀ /ml)	Log(TCID ₅₀ /ml)
PR8	10	5.50	5.00	5.00	5.17	6.32
	30	5.33	5.33	3.50	4.72	6.78
	60	0	0	2.33	0.78	6.72

Table 3. Virucidal effect of “Germagic filter” to Enterovirus at Various Contact Time

Virus	Time (minutes)	Test 1	Test 2	Test 3	Mean	Mean of control group
		Log(TCID ₅₀ /ml)	Log(TCID ₅₀ /ml)	Log(TCID ₅₀ /ml)	Log(TCID ₅₀ /ml)	Log(TCID ₅₀ /ml)
EV71	10	4.67	5.00	4.67	4.78	5.50
	30	4.67	5.50	5.50	5.22	7.33



Table 4. The Negative Logarithm Means of Virucidal Effect of “Germagic filter” to Influenza virus and Enterovirus

Virus Strain	Time (minutes)	Mean Virucidal Rate	Negative Logarithm Means of Virucidal Effect
	10	92.921%	1.15
PR8	30	99.129%	2.06
	60	>99.999%	5.94
	10	80.945%	0.72
EV71	30	99.224%	2.11

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