# Test Report of Virucidal effect on Avian Influenza Virus H7N9 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

I. This report is only responsible for the sample(s) tested.

II. Any alteration, addition and deletion made to the report without official seal (paging seal included) affixed will lead to invalidity of the test report. Any photocopies of this test report are invalid.

III. Any disagreement on the test results should be proposed to the test organization in fifteen (15) days upon receipt of the test report, overdue inadmissible.

IV. The test is only to evaluate the virucidal effect of the sample tested, all product promotions and marketing activities for commercial purposes should rigidly comply with the test report and related interpretation, otherwise corresponding responsibilities will be pursued by the test organization.

V. The test report is made in four copies, two for the clients and two for the test organization for archiving.

VI. The test report is valid for three years.

## Abstract

**Aim:** To evaluate the *in vitro* virucidal effect of "Germagic filters" for highly pathogenic avian influenza virus H7N9（A/Anhui/1/2013）.

**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 H7N9 strain 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 H7N9 under the defined lab conditions.


## Materials and Methods

### 1. Materials

1.1 Test sample: "Germagic filter", each $cm^2$ contains 0.155ml of "Germagic" coating, provided by Chiaphua Industries Limited.

1.2 Cell: Madin-Darby Canine Kidney (MDCK) cells, purchased from the Cell Bank of Typical Culture Collection of Chinese Academy of Sciences.

1.3 Virus strain: highly pathogenic avian influenza virus H7N9（A/Anhui/1/2013）was provided by BSL-3 laboratory in Guangdong Inspection and Quarantine Technology Center.

1.4 Neutralizing agent: 9g NaCl+ 2.2g Tween 80 + 20ml 1M $NaS_2O_3$, dissolved in 1L of deionized

water. The neutralizing agent was diluted with hard water in a ratio of 1:2.5.

1.5 All virological experiments about highly pathogenic avian influenza virus H7N9 were performed in BSL-3 laboratory in Guangdong Inspection and Quarantine Technology Center.

## 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 10minutes after mixing.

(2) Serial dilutions (dilution step: 1:10) were prepared in the culture medium and inoculated into monolayer MDCK cell at 34 ℃ 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℃. The plates were then blot dried on paper towels, followed by the addition of 100 ml 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 16cm$^2$ 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 ℃ for 10minutes, 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 of7.0 ml PBS and 7.0 ml neutralizing agent into group 1and 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℃,5% $CO_2$ 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℃ with 5%$CO_2$ for 2-4 days. Any microscopic changes in the cells were recorded when reading the tests for cytopathic effects (CPEs). Tissue Culture Infective Dose ($TCID_{50}$) 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 16cm$^2$ small pieces, respectively.

(2) "Germagic filter" and blank filter were added with 1.0ml H7N9 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 cell, 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℃, 5% $CO_2$ incubator for 2 hours, discard the supernatant.

Add 400IU/ml of double antibiotics culture medium, and incubate at 34℃with 5% $CO_2$ for 2-4 days. Any microscopic changes in the cells were recorded when reading the tests for cytopathic effects (CPEs).Tissue Culture Infective Dose ($TCID_{50}$) 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 cell, the "Germagic filter" and diluted neutralizing agent mixture showed almost no toxicity to MDCK cell.

### 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 Log($TCID_{50}$/ml) | Test 2 Log($TCID_{50}$/ml) | Test 3 Log($TCID_{50}$/ml) | Mean 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 highly pathogenic avian influenza virus H7N9 under the defined test condition. Results indicated that "Germagic filter" could effectively kill H7N9 at the specified time, See Table 2 and Table 3.

**Table 2. Virucidal effect of "Germagic filter" to H7N9 at Various Contact Time**

| Virus Strain | Time (minutes) | Test 1 Log($TCID_{50}$/ml) | Test 2 Log($TCID_{50}$/ml) | Test 3 Log($TCID_{50}$/ml) | Mean Log($TCID_{50}$/ml) | Mean of control group Log($TCID_{50}$/ml) |
|---|---|---|---|---|---|---|
| H7N9 | 10 | 3.50 | 3.67 | 3.50 | 3.56 | 6.25 |
| | 30 | 3.50 | 3.50 | 3.75 | 3.58 | 6.25 |
| | 60 | 2.75 | 3.50 | 3.75 | 3.33 | 6.50 |

**Table 3. The Negative Logarithm Means of Virucidal Effect of "Germagic filter" to H7N9**

| Virus Strain | Time (minutes) | Mean Virucidal Rate | Negative Logarithm Means of Virucidal Effect |
|---|---|---|---|
| H7N9 | 10 | 99.79% | 2.69 |
| | 30 | 99.78% | 2.67 |
| | 60 | 99.93% | 3.17 |

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