Test Report of GERMAGIC Filter Membrane Killing MERS-CoV

Name of Sample: GERMAGIC filter membrane

Applicant: GERMAGIC Co., Ltd.

Test Type: Commissioned testing

Test Institute: Guangzhou Institute of Respiratory Health

Test Date: 19th Sep. 2016

Illustration

I. This inspection report is only responsible for the samples submitted for inspection.

II. The alteration, addition and deletion of this inspection report are invalid, the unsealed official seal is invalid, and the copy is invalid.

III. If you have any objection to this inspection report, you can file a review application within 15 days from the date you receive the report.

IV. This study is limited to investigating the virus killing effect of samples sent for test, any product promotion and market activities for commercial purposes must be strictly in accordance with this experimental result report and related interpretations, otherwise the testing unit has the right to pursue corresponding responsibilities.

V. 4 copies of this inspection report, 2 copies shall be submitted to the inspection unit, and 2 copies shall be filed by the inspection agency.

VI. This inspection report is valid for three years.

Summary

Objective: To evaluate the killing effect of GERMAGIC filter membrane on MERS-CoV.

Method: Refer to the 2.1.1.10 virus inactivation test in the "Disinfection Technical Regulation" and the test product manual to formulate this research plan, including cytotoxicity test, disinfectant neutralization test and virus killing test.

Results: The cytotoxicity test, disinfectant neutralization and virus killing test have been completed.
(1) The neutralizer GERMAGIC filter membrane and neutralization product are basically non-toxic or low toxicity to the experimental cells;
(2) GERMAGIC filter membrane neutralizer identification test showed that the neutralizer can completely neutralize the disinfection effect of the filter;
(3) Under the experimental conditions set in this test, GERMAGIC filter membrane reacted with virus suspension and can better kill virus MERS-CoV in 10 , 30 or 60 minutes.
Conclusion: The neutralizer passed the neutralizer identification test and complied with the neutralizer compliance standard in *Disinfection Technical Regulation 2.1.1.10.5*; under the experimental conditions set in this test, the GERMAGIC filter membrane can kill the MERS -CoV with a certain effect.

Materials and Methods

1. Test materials

1.1 Test sample: GERMAGIC filter membrane, the content of GERMAGIC haze-killing layer per square centimeter is 0.0155ml, provided by GERMAGIC Co., Ltd. In the meantime, a control filter membrane was provided.
1.2 Cells: Vero 81, provided by Stanley Perlman laboratory
1.3 Virus strain: MERS-CoV (EMC strain), provided by Stanley Perlman laboratory. Virus titer $\geqq 10^5$ $TCID_{50}$/ml.
1.4 Neutralizer: 9g NaCl + 2.2g Tween 80 + 20ml 1M NaS_2O_3, dissolved in 1L deionized water. Before using the neutralizer, mix the neutralizer and hard water in a ratio of 1:2.5
1.5 Test site: MERS-CoV related tests were conducted at the University of Iowa biosafety laboratory. Neutralizer identification experiment was conducted at Guangzhou Institute of Respiratory Health, Guangzhou city, Guangdong province, China.

2. Test methods

2.1 Cytotoxicity test (MTT method)
(1) The following 4 groups are mixed separately:
1. "Germagic" filter membrane + neutralizer 7.0ml

2. "Germagic" filter membrane + PBS 7.0ml
3. Neutralizer 7.0ml
4.PBS 7.0ml
After mixing, place it at room temperature for 10min.
(2) Each of the above groups was serially diluted 5 titers with a 10-fold gradient of PBS, and the dilutions of each group were added to a 96-well cell culture plate with cells prepared in advance, with 4 wells for each dilution and 100 μl per well.
(3) Place in a 37℃, 5% CO_2 incubator for 1 hour, discard the supernatant, and add cell maintenance medium. Put it in a 37℃, 5% CO_2 incubator for 48 hours and observe the cell growth status, and do MTT test to evaluate the toxicity to the cells。
(4) MTT test method was used to measure the inhibition rate, MTT solution (5mg/ml) 20μl was added to each well to establish a cell-free blank control group and set at 37℃ for 4 hours. Discard the supernatant, add 100μl of 2-methyl sulfoxide (DMSO) to each well, and shake at low speed for 10 minutes to fully dissolve the crystals. Select the 490nm wavelength and measure the absorbance of each well on the microplate reader.

Calculate the inhibition rate according to the following formula:

Inhibition rate = [(average OD value of PBS group-average OD value of blank control group)-(average OD value of test group-average OD value of blank control group)] / (average OD value of PBS group-average OD value of blank control group) x100 %.

2.2 Neutralizer identification test

The influenza A virus PR8 strain is used as the representative strain to evaluate the candidate neutralizers. For the experimental scheme and evaluation standard, refer to residual disinfectant chemical neutralization identification test in "Disinfection Technical Regulation" 2.1.1.10.5
(1) Cut the "Germagic " filter membrane into a $16cm^2$ square, clean and sterilize, and mix according to the following 5 groups:
1. "Germagic" filter membrane + virus suspension 1.0ml
2. "Germagic" filter membrane + virus suspension 1.0ml
3. "Germagic" filter membrane + neutralizer 7.0ml
4. Neutralizer 7.0ml + virus suspension 1.0ml
5. PBS 7.0ml + virus suspension 1.0ml
(2) After mixing, the 3rd group was placed at room temperature for 10 minutes, added 1.0ml of virus suspension and placed at room temperature for 1 hour, 4℃ for use. Group 1 and Group 2 were placed at room temperature for 1 hour, Group 1 was added with 7.0ml of PBS, Group 2 was added with 7.0ml of neutralizer, and placed at room temperature for 10 minutes, at 4℃ for use. Groups 4 and 5 were allowed to stand at room temperature for 1 hour at 4℃.
(3) Dilute the mixed solution of the above groups in 10 times, add the diluted solution to the 96-well cell culture plate containing cells grown to a single layer, set 4 wells per dilution, 100μl per well, and set the normal control group, add an equal amount of culture medium.

(4) Place in an incubator at 37℃, 5% CO_2 for 2 hours, discard the supernatant, add 400IU/ml culture solution (containing TPCK with a concentration of 1.5μg/ml), and place Incubate at 34℃, 5% CO_2 for 2 ~ 4 days. Observe the cell growth status every day, and record the occurrence of CPE when the cells appear atrophy, rounding, and shedding (CPE). According to the Reed-Muench formula, calculate half of the infection volume $TCID_{50}$.

2.3 Virus killing experiment

For the virus killing experiment plan and evaluation standard, please refer to 2.1.1.10 virus inactivation test in "Disinfection Technical Regulation".

(1) Cut the "Germagic" filter membrane and blank material into 16cm^2 squares, clean and sterilize them for later use.

(2) "Germagic" filter membrane and blank material were mixed with 1.0ml of virus suspension, and left at room temperature for 10, 30 or 60 minutes, adding neutralizing agent 7.0ml, left at room temperature for 10 minutes, 4℃ as standby and repeated the test for 3 times.

(3) Dilute the above-mentioned mixtures in 10 times, add the dilutions to the 96-well cell culture plate containing cells grown to a single layer, set 4 wells per dilution, 100μl/well, and set the normal control group, add an equal amount of culture medium.

(4) Place in an incubator at 37℃, 5% CO_2 and continue incubating for 2 hours, discard the supernatant and add 400IU/ml culture solution containing dual antibodies, place in an incubator at 34℃, 5% CO_2 for 2 ~4 days. MERS-CoV virus maintenance medium is DMEM medium containing 2% FBS. Observe the cell growth status every day, and record the occurrence of CPE when the cells appear to be round and detached (CPE). According to the Reed-Muench formula, calculate half of the infection volume $TCID_{50}$.

3. Result

3.1 Cytotoxicity test

MTT results show that the neutralizer is basically non-toxic or low-toxic to MDCK and Vero 81. The "Germagic" filter membrane and neutralization product are basically non-toxic or low-toxic to MDCK and Vero 81 after certain dilution.

3.2 Neutralizer identification test

The results of neutralization identification test basically conformed to the qualification standard of 2.1.1.10.5 neutralization in the Disinfection Technical Regulation. The 1st group and 2nd group can better kill the influenza A virus PR8 strain, the 3rd group of the influenza A virus PR8 strain titer is similar to the blank group of the 5th group. The results of Group 4 showed that the neutralizer is not toxic to the virus, see Table 1.

Table 1. "Germagic" filter membrane neutralizer identification test results

Group	1st test	2nd test	3rd test	Average Value
	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 Virus killing test

Under the experimental conditions of this test, the "Germagic" filter membrane reacted with the MERS-CoV virus suspension. The results show that, within the time point specified in this test, the "Germagic" filter membrane has a good killing effect on the MERS-CoV virus, see Table 2 and Table 3.

Table 2. Killing effect of "Germagic" filter membrane in contact with MERS-CoV at different times

Virus	Time (min)	1st test	2nd test	3rd test	Average Value	Average value of Control group
		Log($TCID_{50}$/ml)	Log($TCID_{50}$/ml)	Log($TCID_{50}$/ml)	Log($TCID_{50}$/ml)	Log($TCID_{50}$/ml)
MERS-CoV	10	4.60	4.70	4.48	4.59	5.25
	30	3.51	3.69	3.48	3.56	5.42
	60	3.26	3.1	3.28	3.21	5.13

Table 3. Negative logarithmic value of the average virus inactivation of MERS-CoV by the "Germagic" filter membrane

Virus	Time(min)	Average virus inactivation rate	Negative logarithm of average virus inactivation
MERS-CoV	10	78.1%	0.66
	30	98.6%	1.86
	60	98.8%	1.92

The following is blank