

**The in Vitro Inactivation Effect of Norwex Antibacterial Agent
on Filter Upon Bird Influenza Virus (H5N1)**

Commissioned by:

XXXXXXXXXXXXXXXXXX

(Testing Facility information removed for compliance with #3, as below.)

Tested by:

**Harbin Veterinary Research Institute of China Agricultural Scientific
Academy & Animal Flu Key-point Open Lab & National Bird Flu
Reference Lab of the Ministry of Agriculture**

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Interpretation

1. This Test Report holds true only for the result of the tested contents of the samples delivered for the test.
2. This Test Report would be invalidated if alteration, addition and deletion is found, as well as free from signature and official stamp.
3. Both of this Test Report and the name of this test and verification organization are disallowed to utilize as product label and for advertisement, commercial propaganda and appraisal of high-quality product etc.
4. This Test Report is in quadruplication, two copies are archived by each of the test organization and sample delivery organization respectively.

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Harbin Veterinary Research Institute of China Agricultural Scientific Academy & Animal Flu Key-point Open Lab & National Bird Flu Reference Lab of the Ministry of Agriculture

**The in vitro inactivation effect
Of NORWEX ANTIBACTERIAL AGENT ON Filter upon bird influenza virus
(H5N1)**

This laboratory has conducted the test of the NORWEX ANTIBACTERIAL AGENT ON Filter delivered by XXXXXXXXXXXXX Science & Technology Co., Ltd. for in vitro disinfections and deactivation of influenza virus. The Test Report for the tested result is as follows:

1. Stuff and means for the test

1.1 The product for the test

NORWEX ANTIBACTERIAL AGENT ON Filter (The sample is filtered membrane that has been treated with medicament, the negative control is non-medicament filtered membrane, and size of both the sample and the control is $5 \times 5 \text{cm}^2$). The samples were provided by XXXXXXXXXXXXX Science & Technology Co., Ltd., and free from lot number.

1.2 Viral strain

The viral stain is H5N1 bird influenza virus in sub-type high pathogenicity, which is preserved by relying on Harbin Veterinary Research Institute of China Agricultural Scientific Academy & Animal Flu Key-point Open Lab & National Bird Flu Reference Lab of the Ministry of Agriculture.

1.3 Chick embryo

The SPF chick embryo of the age in 10 days was provided by the Experimental Animal Center of Harbin Veterinary Research Institute of China Agricultural Scientific Academy.

1.4 Preparative test

1.4.1 Determination of virus EID₅₀

Inoculate the H5 sub-type bird influenza that is diluted by 10 times series into the SPF chick embryo of the age in 10 days. Each degree of dilution is for 5 chick embryos (0.1ml/embryo), and then to determine the viral half infective dose (EID₅₀).

1.4.2 Preparation for the tested product — NORWEX ANTIBACTERIAL AGENT ON Filter

Make the NORWEX ANTIBACTERIAL AGENT ON Filter into the size of $5 \times 5 \text{cm}^2$, and get it for use after sterilization through high pressure.

1.4.3 The toxicity test for the chick embryo of the NORWEX ANTIBACTERIAL AGENT ON Filter product for the test.

Soak the NORWEX ANTIBACTERIAL AGENT Filter sized $5 \times 5 \text{cm}^2$ in 5ml sterilizing normal

saline for 4h, 3h, 2h, 1h and 0.5h respectively. After that, take out each of the 5 kinds of the soak solution respectively and inoculate each kind of the soak solution to five SPF chick embryos of 10 days old with the dose of 0.2ml/embryo. Place the inoculated chick embryos in the 37°C incubator to cultivate them for 96h. Remove the chick embryo that has died within 24h, and write down the death information of the chick embryo, so as to determine the toxicity of the makings of the tested subject.

1.5 Test for the killing and inactivating rate of the tested product NORWEX ANTIBACTERIAL AGENT Filter against the virus

Klein-Defors suspension method is to be adopted to conduct the killing and inactivating test for the high pathogenic bird influenza virus; quantitative EID₅₀ virus suspended material is applied to call the NORWEX ANTIBACTERIAL AGENT Filter sized 5×5cm² into play in different time, and then dilute the tested product with sterilizing normal saline by 10 times in series. After that, check the condition of the infected chick embryo.

1.5.1 Suspension killing and inactivating test of Klein-Defors

Soak the NORWEX ANTIBACTERIAL AGENT Filter sample sized 5×5cm² into the suspension of 5ml 10^{7.5}EID₅₀ H5 sub-type bird influenza virus for 4h, 3h, 2h, 1h and 0.5h respectively under the condition of 20±1 , and then have the soak solution diluted with sterilizing normal saline by gradually increasing 10 times. As for the negative Control Group No.1, negative filtered membrane shall be substituted for the NORWEX ANTIBACTERIAL AGENT Filter sample sized 5×5cm², and for the negative Control Group No.2, 5ml 10^{7.5}EID₅₀ H5 sub-type bird influenza virus suspension is to be put in use and treated with the same method. The diluents shall be inoculated the SPF chick embryo of 10 days old; each degree of dilution shall be used for the inoculation of 5 chick embryo (0.1ml/embryo). Place the inoculated chick embryos in the 37°C incubator for cultivation, and write down the death information of the chick embryo. Remove the chick embryo that died within 24h, take out that died after 24h timely, and take off the entire ones from the incubator till 96h. Dislodge allantoic fluid from the dead embryo to perform hemagglutination (HA) test. It should be diagnosed infestation of chick embryo if the hemagglutination test assumes positive.

Calculate the infectious positive rate, content of EID₅₀ in the samples and virus-killing and inactivating rate of the chick embryos in both of the tested group and the control groups according to infectious result of the chick embryo by using the following equation:

The positive rate = the chick embryo quantity that assumes positive in hemagglutination/the quantity of inoculated chick embryos; the logarithm for the content of EID₅₀ in the samples = $L - d(S - 0.5)$

(L is the logarithm of the minimum dilution multiple; d is the logarithmic difference among the degrees of dilution; S is the sum of the positive rate of various dilution series).

The disinfected and inactivated rate of the virus = (the content of EID50 in the control sample - the content of EID50 in the tested sample) / the content of EID50 in the control sample ×100%.

2 Result

2.1 Toxicity test for the chick embryo applied with the tested product - NORWEX ANTIBACTERIAL AGENT Filter

It's observed from Table 1 that after the NORWEX ANTIBACTERIAL AGENT Filter sized 5×5cm² is soaked in 5ml sterilizing normal saline for 4h, 3h, 2h, 1h and 0.5h respectively. After that, take out each of the 5 kinds of the soak solution respectively and inoculate each kind of the soak solution to five SPF chick embryos of 10 days old, the test result shows that with regard to the chick embryos (with injection of 0.2ml liquid), it's free of visual pathological change after the soak solution of NORWEX ANTIBACTERIAL AGENT Filter sized 5×5cm² is inoculated.

Table 1: The maximum non-poisonous dosage applied to the chick embryo with the tested product

	The soaking duration of the NORWEX ANTIBACTERIAL AGENT Filter sized 5×5cm ² in 5ml sterilizing normal saline				
	4h 时	3h	2h	1h	0.5h
Death information of the chick embryos	0/5	0/5	0/5	0/5	0/5

Note: The numerator is the quantity of the died embryo, and the denominator is the quantity of inoculated embryo.

2.2 The killing and inactivating effect of the tested product upon the bird influenza virus

When the NORWEX ANTIBACTERIAL AGENT Filter sample sized 5×5cm² is soaked in the suspension of 5ml 10^{7.5}EID₅₀ H5 sub-type bird influenza virus for 4h, 3h, 2h, 1h and 0.5h respectively under the condition of 20±1 , the killed and inactivated rate of the H5 sub-type bird influenza virus by applying the NORWEX ANTIBACTERIAL AGENT Filter sample sized 5×5cm² is 100%、100%、100%、100%、99% respectively. For the result, refer to Table 2.

Table 2: The killing and inactivating effect of the NORWEX ANTIBACTERIAL AGENT Filter sample sized 5×5cm² upon the H5N1 sub-type bird influenza virus

	The soaking duration of the NORWEX ANTIBACTERIAL AGENT Filter sized 5×5cm ² in the 5ml10 ^{7.5} EID ₅₀ H5 sub-type bird influenza virus suspension				
	4h	3h	2h	1h	0.5h

The NORWEX ANTIBACTERIAL AGENT Filter sized 5×5cm ²	100%	100%	100%	100%	99%
Negative Control Group 1	0	0	0	0	0
Negative Control Group 2	0	0	0	0	0

3 Conclusion

Through study on that the NORWEX ANTIBACTERIAL AGENT Filter sample sized 5×5cm² is soaked in the suspension of 5ml 10^{7.5}EID₅₀ H5 sub-type bird influenza virus for 4h, 3h, 2h, 1h and 0.5h respectively by adopting the test method of klein-defors suspension to kill and inactivate virus and infection, therewith test the effect of the tested product to kill and inactivate in vitro bird influenza virus. The test result indicates that after the NORWEX ANTIBACTERIAL AGENT Filter sized 5×5cm² acts on bird influenza virus for 1h, the killed and inactivated rate of the virus could reach 100%, within the range of which the effect to kill virus is conspicuous; After it acts on bird influenza virus for 30min, the killed and inactivated rate of the virus could reach 99%, within the range of which there is certain effect to kill virus.

Harbin Veterinary Research Institute of China Agricultural Scientific Academy

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