Inactivation Effects of Virkon on Avian Influenza Virus and Newcastle Disease Virus

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Commissioned by Nedtex Company (Taiwan), we used the disinfectant submitted for test, "Virkon" to conduct an inactivation test on Avian influenza virus and Newcastle disease virus. The results were reported as follows:

1 **Materials**

1.1 **Disinfectant** Broad-spectrum viricidal disinfectant "Virkon" was provided by Nedtex Company (Taiwan), the principal components of which were Potassium monopersulphate /Potassium hydrogen sulphate /Potassium sulphate triple salt.

1.2 **Virus** The H9-subtype international reference low virulent strain of Avian influenza virus A/Turkey/Wisconsia/1/66 (H9N2) was kindly presented by Central Veterinary Laboratory (U.K.). The H5 subtype virulent strain of Avian influenza virus A/Goose/Guangdong/1/96 (H5N1) was isolated, identified and preserved by Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences. The virulent strain F48E9 of Newcastle disease virus was preserved by Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences.

1.3 **SPF chick embryo** 9-10 days old, provided by Experimental Animal Center of Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences.

2 **Methods**

2.1 **Virus inactivation test** Suspension test was adopted for inactivation test. A proportion dilution method was used to remove disinfectant.

2.2 **Preparation of virus suspension** Avian influenza virus H9N2, H5N1 and Newcastle disease virus were diluted appropriately, and a dose of 0.2mL/embryo was inoculated into each SPF chick embryo of 10 days old, and the 50% infective dose (EID_{50}) of virus for chick embryo was determined.

2.2 **Preparation of Virkon** 1000mg Virkon was weighed and dissolved in 2.0ml, 2.8ml, 4.0 ml, 8.0 ml, 10.0 ml, 15.0 ml and 20.0 ml sterile normal saline to obtain 1:2, 1:2.8, 1:4, 1:8, 1:10, 1:15 and 1:20 Virkon solutions. They were diluted to the concentrations required when the test was performed.

2.3 **Maximal innoxious dose of Virkon solution to chick embryo** 1:20, 1:40, 1:80, 1:100 and 1:150 Virkon solutions were inoculated into 10-day-old chick embryo allantoic cavity. 4 chick embryos were inoculated for each dilution (0.2 ml/embryo). The inoculated chick embryos were cultured in a 37 incubator for 96 hours. The death of chick embryos was observed, and the dead and living chick embryos after 96 hours were anatomized to observe if there were lesions.

2.4 **Suspension test** Avian influenza virus 10^4EID_{50} H5 suspension, 10^5EID_{50} H9 suspension and

10^5EID_{50} Newcastle disease virus suspension were mixed with 1:200, 1:280, 1:400, 1:800, 1:1000, 1:1500 and 1:2000 disinfectant solutions respectively (1:10), and they were kept at 20±1 for 5 and 10 minutes, and then a 1:10 serial dilution was conducted with normal saline. The control group was treated using the same method with disinfectant solution replaced by sterile normal saline. The solutions were inoculated into 10-day-old chick embryo allantoic cavity, and 4 chick embryos were inoculated for each dilution (0.2 ml/embryo). The inoculated chick embryos were cultured in a 37 incubator. The chick embryos dying within 24 hours were removed. After 24 hours, the dead embryos were taken out in time, and the embryo liquid was taken from each dead embryo for hemagglutination test, until 96 hours (Avian influenza virus) and 144 hours (Newcastle disease virus) after incubation. If the result of hemagglutination test was positive, the chick embryo was infected, indicating that there was surviving Avian influenza virus or Newcastle disease virus.

According to the results of chick embryo infection, the positive rates of chick embryo infection, EID_{50}, and the inactivation rates of influenza virus and Newcastle disease virus in the test group and the control group were calculated using the following formula.

Positive rate=number of infected chick embryos/number of inoculated chick embryos

Log ELD_{50}=L-d(S-0.5)

(L is the logarithm of minimum dilution; d is the logarithm of the proportion of dilution; S is the sum of the positive rates of each diluted line.)

Virus inactivation rate = (ELD_{50} of control sample-ELD_{50} of test sample)/ELD_{50} of control sample×100%

3 **Results**

3.1 After 1:200, 1:280, 1:400, **1:800**, 1:1000, 1:1500 and 1:2000 Virkon solutions were interacted with AIV **H9** for **5** minutes, the inactivation rates were 100%, 100%, 100%, 100%, **100%,** 99.995% and 99.97% respectively. After 1:200, 1:280, 1:400, 1:800, **1:1000**, 1:1500 and 1:2000 Virkon solutions were interacted with AIV **H9** for **10** minutes, the inactivation rates were 100%, 100%, 100%, 100%, 100%, **100%** and 99.99% respectively.

3.2 After 1:200, 1:280, 1:400, **1:800**, 1:1000, 1:1500 and 1:2000 Virkon solutions were interacted with AIV **H5** for **5** minutes, the inactivation rates were 100%, 100%, 100%, **100%,** 99.99%, 95% and 85% respectively. After 1:200, 1:280, 1:400, **1:800**, 1:1000, 1:1500 and 1:2000 Virkon solutions were interacted with AIV **H5** for **10** minutes, the inactivation rates were 100%, 100%, 100%, **100%,** 99.99%, 99.97% and 87% respectively.

3.3 After 1:200, 1:280, 1:400, 1:800, 1:1000, **1:1500** and 1:2000 Virkon solutions were interacted with **NDV** for **5** minutes, the inactivation rates were 100%, 100%, 100%, 100%, 100%, **100%** and 99.999% respectively. After 1:200, 1:280, 1:400, 1:800, 1:1000, 1:1500 and **1:2000** Virkon solutions were interacted with **NDV** for **10** minutes, the inactivation rates were 100%, 100%, 100%, 100%, 100%, 100% and **100%** respectively.

3.4 The maximal innoxious dose of Virkon solution to chick embryo was 1:40.

4 **Conclusion**

1:200, 1:280, 1:400, 1:800, 1:1000, 1:1500 and 1:2000 Virkon solutions are effective for both AIVH9 and NDV. The complete inactivation concentrations are 1:200, 1:280, 1:400, 1:800 and 1:1000, and the suggested concentration for AIV H9 in clinical use is 1:1000. However, the concentration can be increased according to the conditions. The suggested concentration for NDV in clinical use is 1:1500. However, the concentration can be increased according to the conditions.1:200, 1:280, 1:400, 1:800 and 1:1000 Virkon solutions are effective for AIVH5, the complete inactivation concentrations are 1:200, 1:280, 1:400 and 1:800, and the suggested concentration for AIV H5 in clinical use is 1:800.

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