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Laboratory Evaluation of the disinfectant Virocid® as a virucidal agent against High Pathogenic Avian Influenza Virus, H5N1 subtype

Client: CID LINES

Test Product: Virocid® diluted in sterile phosphate buffer saline (PBS) at a concentration of 0.25%

Test virus: Highly Pathogenic Avian Influenza Virus H5N1 subtype, strain A/duck/Vietnam/12/05

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1. TEST PROCEDURE

The following method was the "Use-dilution test" used by the Association of Official Agricultural Chemists (Washington, USA) to evaluate the efficacy of disinfectants against bacteria, duly modified for Newcastle disease virus and for avian influenza ones (2, 3).

The procedure includes:

1) disinfectant toxicity test on SPF chicken embryos at the recommended dilution (0.25%);

2) evaluation of the disinfectants activity at the recommended-dilution.

The H5N1 AI viral isolate was obtained from a National Centre for Veterinary Diagnostics, in Ha Noi (Vietnam). The virus was then titrated by a standardised virological technique (4).

The titred viral suspension was used to produce 60 ml of H5N1n infected allantoic fluid. Sterile stainless steel rings were used as carriers (8mm x 10mm x 2mm).

TOXICITY TEST

• Two carrier rings were placed in 30 ml of uninfected SPF (Specific Pathogen Free) eggs allantoic fluid for 15 minutes.

• They were removed from the allantoic fluid and placed vertically in Petri dishes lined with filter paper.

• The carrier rings were incubated for 20 min at 37° C.

• Each ring was transferred in a Petri dish containing 10 ml of disinfectant at the dilution of 1% with phosphate buffer saline (PBS) and incubated for 10 minutes at room temperature.

• Each ring was placed in 1.5 ml of cell culture medium (Eagle Minimum Essential Medium containing 5% triptose broth, 5% calf foetal serum, 1% L-glutamine, 5% antibiotic solution) and then incubated for 10 minutes at room temperature.

• 0.15 ml of the broth were inoculated by the allantoic cavity route into 9 day old SPF chicken embryos. The eggs were incubated at 37 C and candled daily for 10 days.

DISINFECTANT EFFICACY

• Two rings were placed in 30 ml of infected allantoic fluid for 15 min. The infectious allantoic fluid was harvested from eggs inoculated with 100 µl of a viral suspension containing $10^{6.5}$ Egg Infectious Dose 50% (EID_{50}).

• They were removed from the allantoic fluid and placed vertically in Petri dishes lined with filter paper.

• Each carrier was incubated for 20 min at 37° C.

• Two rings were transferred in a Petri dish containing 10 ml of the disinfectant at a dilution of 0.25%

• After 10 minutes each carrier was placed in 1.5 ml of cell culture medium and incubated for 10 min at room temperature.

• 0.15 ml of the medium was inoculated by the allantoic cavity route into 9 day old SPF chicken embryos. The eggs were incubated at 37 C and candled daily. Two blind passages of seven days each were carried out. Viral isolation was performed following the guidelines indicated in directive 92/40/EEC (1).

TEST TO EVALUATE THE VIRUS INFECTIVITY CARRIED BY RINGS

• Two rings were placed in 30 ml of infected allantoic fluid for 15 min. The amount of the infectious allantoic fluid was harvested from eggs inoculated with 100 µl of a viral suspension containing $10^{6.5}$ EID_{50}.

• They were removed from the allantoic fluid and placed vertically in Petri dishes lined with filter paper.

• Each carrier was incubated for 20 min at 37° C.

• Each ring was placed in 10 ml of sterile PBS.

• After 10 minutes each ring was placed in 1.5 ml of cell culture medium and incubated for 10 min.

• 0,15 ml of the medium was inoculated by the allantoic cavity route into 9 day old SPF chicken embryos. The eggs were incubated at 37° C and candled daily. Two blind passages of seven days each were carried out. The viral isolation was performed following the procedure reported in directive 92/40/EEC (1).

RESULTS

The results are shown in the table n. 1.

1) The product has had no significant toxic effect on embryos that preserved their vitality until the end of the observation period (10 days).

2) The eggs inoculated to evaluate the efficacy of the disinfectant survived until the end of the observation period which included two blind passages of seven days each. The allantoic fluid obtained from eggs at the end of the second passage showed no haemagglutinanting activity, which implies the absence of virus replication. On the contrary, the eggs used to evaluate the virus infectivity died within two days post-inoculation. The death of the entirety of these eggs gives evidence of the preserved virus infectivity and the high virus concentration in the medium in which the carrier was placed.

Table 1: test results

	Embryonated eggs										Results
	1	2	3	4	5	6	7	8	9	10	
Toxicity test	-	-	-	-	-	-	-	-	-	-	All alive after 10 days
Infectivity test	+	+	+	+	+	+	+	+	+	+	All dead after 48 hours from inoculation
Disinfectant efficacy	-	-	-	-	-	-	-	-	-	-	All alive after two blind passages

CONCLUSION

The Virocid® at the recommended concentration (0.25%) is not toxic for developing chicken embryos and is able to fully inactivate a viral suspension of High Pathogenic Avian Influenza virus, strain A/duck/Vietnam/12/05 containing $10^{7.5}$ EID_{50}/ml.

REFERENCES

1) CEC (1992). Council Directive 92/40/EEC of 19 May 1992 introducing Community measures for the control of avian influenza. *Official Journal of the European Commission*, L 167, 1-15.

2) Davison S., Benson C. E., Ziegler A. F. & Eckroade R. J. (1999). Evaluation of disinfectants with the additions of antifreezing compounds against nonpathogenic H7N2 Avian Influenza virus. *Avian Diseases* 43: 533-537

3) Lorenz D. E. & Jann G. J. (1964). Use-dilution test and Newcastle disease virus. *Applied Microbiology* **12**, 1: 24-26

4) PDP VIR 05 "Isolamento e tipizzazione preliminare dei virus influenzali aviari"