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### **PRELIMINARY REPORT ON THE EFFICACY OF BLUOXY DISINFECTANT AGAINST SWINE INFLUENZA VIRUS (H1N1)**

With reference to the above matter, the results of the test are as show as below:

#### **The Efficacy of BluOxy Disinfectant Against Swine Influenza Virus (H1N1)**

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#### **Introduction**

BluOxy is a plant-based solution developed for purpose of cleansing, sanitizing and deodorizing room ambient. The product was also reported to have ability of killing a wide range of fungi, spores and bacteria that are harmful to human. This communication reports the preliminary efficacy test result of BluOxy against Swine Influenza Virus (H1N1).

#### **Materials and Methods**

##### *Preparation of virus pool*

The virus H1N1 strain was propagated in Chicken Embryonated Eggs (CEEs) and the virus titer was determined by Hemaaglunitination test (HA).

##### *Disinfectant*

The disinfectant namely BluOxy<sup>TM</sup> was used in this experiment. Two different dilutions of disinfectant were used in this study. The concentrated stock was diluted to concentration of 2% while the solution from the small personal size bottle which is formulated at 5% was used directly in the study.

#### *A 'carrier' dilution method*

A "carrier" is used according to standard protocols for evaluating the effect of surface disinfectant (Sattar et al., 2001). Two (2) pieces of sterile stainless steel penicylinders (type 304 stainless steel) as a carrier was immersed in 7 ml of H1N1 virus pool suspension for 15 minutes. Then, the carriers (rings) were then blot dried and left in incubator for 30 minutes at 37°C (Mermert, Western Germany). Each ring was immersed in 3 ml of disinfectants for 5 minutes. One control carrier was immersed in 1 ml PBS, pH 7.2. Then, 1 ml of diluents (PBS) was added to each ring to elute the virus. These elutes were further assayed for subsequent qualitative analysis by inoculating it into 5 of CEEs.

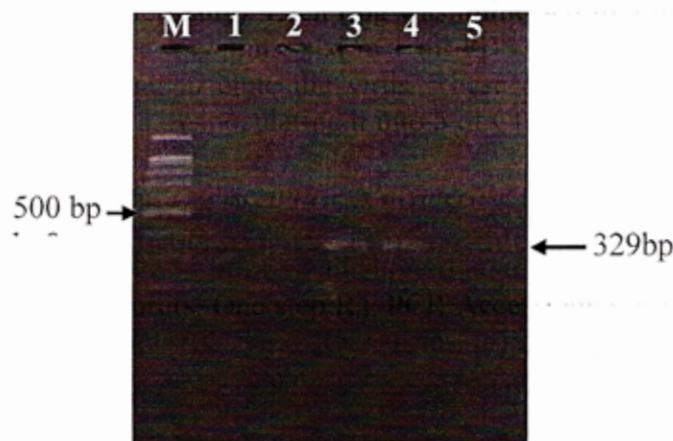
#### *Extraction of virus and Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR)*

The viral RNA was extracted from infected allantoic fluid using TRIzol reagent (Invitrogen) as described by the manufacturers. One step RT-PCR AccessQuick RT-PCR Kit (Promega, USA) was employed to amplify the viral RNA using primer NP1529R/ NP1200F (M.-S. Lee et al., 2001).

#### **Results**

No amplicon was detected from BluOxy<sup>TM</sup> treated (2% and 5% ) allantoic fluid. However, all the positive control shown amplicon sized at 329 bp. Negative control was also produced no amplicon. The results are illustrated in Figure 1.

Figure 1: RT- PCR product using Primer NP1529R/NP1200F (M.-S. Lee et al., 2001)



Legend: **M:** Marker, 100bp; **1:** BluOxy<sup>TM</sup> Treated at concentration of 2%; **2:** BluOxy<sup>TM</sup> Treated at 5% concentration; **3:** Positive control (Not Treated with BluOxy<sup>TM</sup>); **4:** Positive Control; **5:** Negative Control

#### **Discussion**

Absence of amplicon indicated that the Swine Influenza Virus H1N1 virus was completely destroyed by the BluOxy<sup>TM</sup>. This indicated that BluOxy<sup>TM</sup> posed virucidal property. However, according to the Environmental Protection Agency (EPA) and Canada General Standard Board (CGSB) guidelines for the evaluation of disinfectant, there is a requirement to conduct virus infectivity assay (Arshad et al., 2007). As such, further work needs to be conducted.

### **Conclusion**

In conclusion, the BluOxy™ disinfectant tested in this study can be considered as an effective agent against Swine Influenza Virus (H1N1), as reflected by the absence of amplicon in BluOxy™ treated allantoic fluid.

### **References**

1. Alexander, D.J (2001). Orthomyxoviridae. In: Jordan, F.T.W., Pattison, M. (Eds), Poultry Diseases, 5<sup>th</sup> ed. W.B. Saunders, London, pp.281-290.
2. Canadian General Standard Board (CGSB). Assessments of the efficacy of antimicrobial agents for use on environmental surfaces and medical devices. National Standard of Canada CAN/CGSB-2.161.97. Ottawa: Canada General Standards Board, 1997.
3. Chen, J.H.S. (1991). Methods of testing virucides. In: Disinfections, Sterilization and Preservation. Seymour S.Block. 4<sup>th</sup> ed., ed. Lea & Febiger, Philadelphia. pp. 1076-1093.
4. Sattar, S.S., and Springthorpe, V.S. (2001). Methods of testing the virucides activity of chemicals. In: Disinfections, Sterilization and Preservation. 5<sup>th</sup> ed. S.S. Block, ed. Lippincott Williams & Wilkins, Philadelphia. pp. 1391-1412.
5. Sattar, S.A, and Springthorpe, V.S. (1988). Test protocols for virucides: need for improvements. Presented at: The 4<sup>th</sup> International Conference on Progress in Chemical Disinfection. State University of New York, Binghamton, NY: April (1988): 369-386.
6. Arshad, S.S, Elmi Salina, A.M, Khairul Anuar,M., Tan, S.W, Amlizawaty,A., Mohd Kamarudin, A.I, Siti Khatijah, M., Omar, A.R (2007). Efficacy of Disinfectants Against Avian Influenza Virus. Presented at: The 19th Veterinary Association Malaysia Congress. 3-5 August 2007: 47-49.
7. M.-S Lee et al. Identification and Subtyping of Avian Influenza Viruses by reverse transcription-PCR. Journal of Virological Methods Volume 97, Issues 1-2, September 2001: 13-22.

Sincerely,



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