National Institute of Hygiene and Epidemiology

No1- Yersin Street - Hanoi-Vietnam

Report for evaluation the inactivation performance of Daikin's streamer technology to pandemic influenza A/H1N1.

Perfomance period : Sept,6th to Sept, 14th 2009

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1. Objective

The goal of study to provide information of effective availble of Daikin's streamer technology to again pandemic influenza H1N1 viruses (Swine influenza)

2. Materials and Method

2.1.Materials

- Chamber (be provied by Daikin)
- Petri dish (50mm diameter)
- Timer.
- plastic consumables...
- Virus

2.2.Methods:

2.2.1 Virus titration

- **Stock Virus :**
  - Virus strains - HN 31868
- **Virus Titration**

1. Thaw an ampule of virus.Microneut uses only virus that has been freeze-thawed once.
2. Dilute virus 1/100 in *diluent* (100 µl virus + 9.9 ml *diluent*).
3. Add 100 µl of *diluent* (with or without TPCK-trypsin, 2 µlg/ml*) to all wells, except column 1, of a 96-well tissue culture plate. (Perform titration of virus in quadruplicate cultures).
4. Add 146 µl virus of 1/100 working stock to column 1. Perform 1/2 $\log_{10}$ dilutions of virus

5. Transfer 46 µl serially from column 1→2→3→…11. Dilutions will be 10^{-2},$10^{-2.5}$,10^{-3}…10^{-7}. Incubate virus at 37°C in 5% CO_2 for 1 hr.

6. Results :

   - Virus strains HN 31868: 10.000 $TCID_{50}$/ ml

2.2.2. Experimental performance

a. Seting up air –purifier system.

- 4 ml of virus solution with concentration from 10.000 $TCID_{50}$/ ml to be added in Petri dish.
- Remover the ceilling board from the chamber
- Make sure the air purifier be turn off
- Remove the ceilling board of air purifier
- To set up 4 petri dishs of virus in to chamber.
- Return the ceilling board of air purifier
- To cover the chamber by ceilling board of the chamber.
- Turn on the air purifier.

Take out petri dish at 1 hour different of incubation (1,2,3 4 hours).

b. Evaluation the efficient of air-purifier system by checking appearance of viruses

- **Preparation of cell culture flats**
  - Check the MDCK cells with microscope at 40X magnification.

- Decant growth medium into a beaker and wash two times with 5ml PBS (-) and a time with (D-MEM) containing 2 µg/ml of TPCKtrypsin.

➢ **Inoculation of cell culture flats**

- - Inoculate 250 µl of each virus collected from diffirent time of experiments into a MDCK flat.
- Allow inoculate to adsorb for 60 minutes at 37^0C.
- Add 5ml of complete media (D-MEM) containing 2 µg/ml of TPCKtrypsin with bovine serum albumine.
- Observe daily for cytopathogenic effect (CPE) among 7 days
- If CPE does not appearance , the test will be repeated 2 more time

2.3. **Data analysis**

- CPE obsevation of invidual Petri dish to be collected and repeat 2 more time in case CPE negative due to make sure the virus be inactivated after treatment by air –purifier.

Due to the unknown pathogenic potential of avian/human viruses, all experiments involving live virus will be carried in Biosafety level3 laboratories at High-tech center of National Institute of Hygiene and Epidemiology

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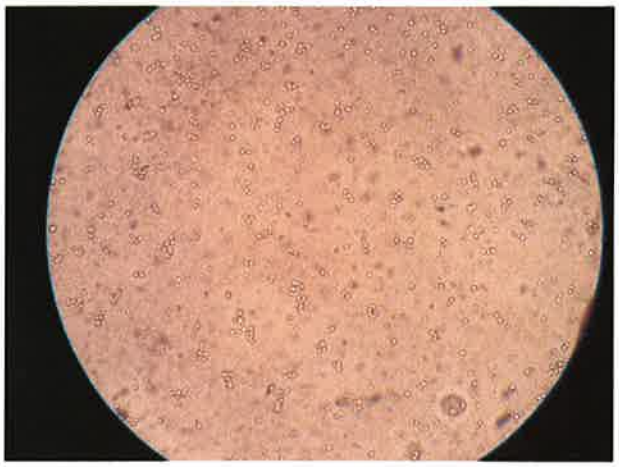
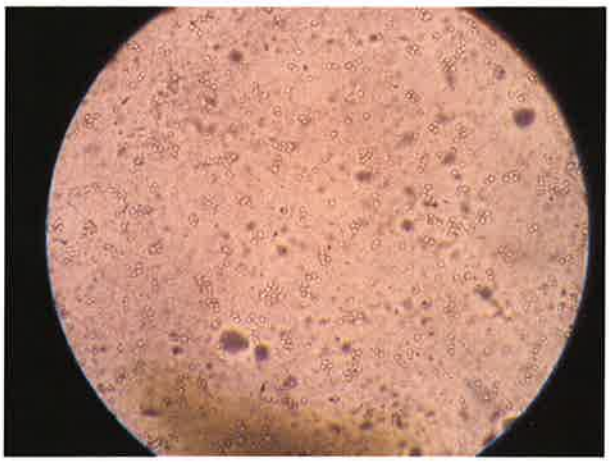
3. Results

3.1. Evaluation effective of DAIKIN's air purifier by cyto patho effect (CPE) on MDCK cells :

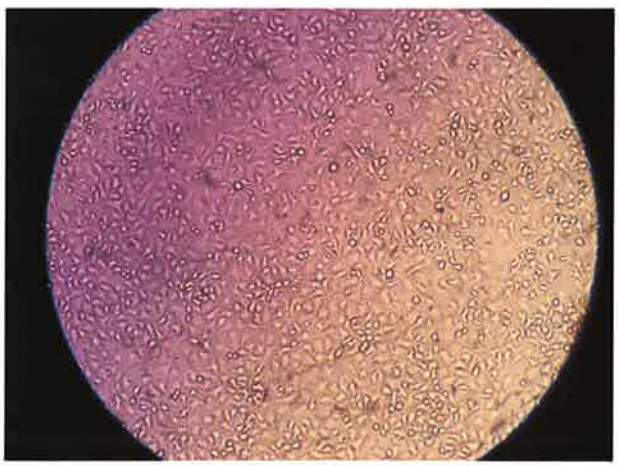
7th day: HN 31868-0 hour



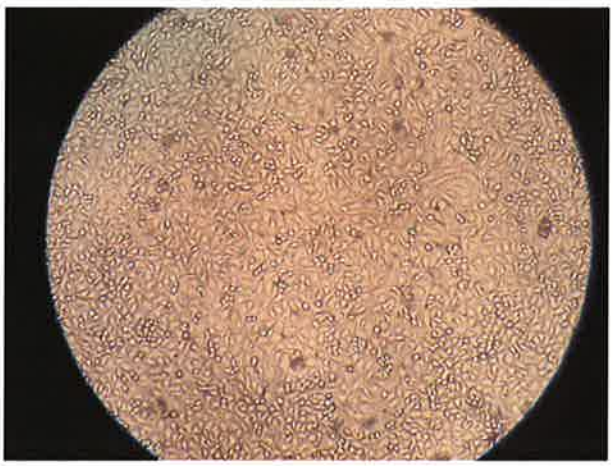
7th day: HN 31868-1 hour



7th day: HN 31868-4 hour



7th day: HN 31868-Control



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3. Conclusion

- DAIKIN's streamer technology has completely destroyed (100%) pandemic influenza H1N1 viruses (Swine influenza) after 4 hours of incubation.