



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.**

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

Tested by : Nguyen Le Khanh Hang . MS

Vuong Duc Cuong MS

Nguyen Co Thach BcS

Tested in : Viral Special Pathogen Laboratory

Department of Virology

National Institute of Hygiene and Epidemiology

No1- Yersin street - Hanoi-Vietnam

Tel : 84-4-39726857/216

email : lom9nihe@yahoo.com

Supervised by : Dr Le thi Quynh Mai

Hang
Sept, 14th, 2009



National Institute of Hygiene and Epidemiology

No1- Yersin Street - Hanoi-Vietnam

1. Objective

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

2. Materials and Method

2.1. Materials

- Chamber (be provided 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 μ l virus + 9.9 ml *diluent*).
 3. Add 100 μ l of *diluent* (with or without TPCK-trypsin, 2 μ g/ml*) to all wells, except column 1, of a 96-well tissue culture plate. (Perform titration of virus in quadruplicate cultures).
 4. Add 146 μ l virus of 1/100 working stock to column 1. Perform 1/2 \log_{10} dilutions of virus



National Institute of Hygiene and Epidemiology

No1- Yersin Street - Hanoi-Vietnam

5. Transfer 46 μ l serially from column 1 \rightarrow 2 \rightarrow 3 \rightarrow ... 11. Dilutions will be $10^2, 10^{-2.5}, 10^{-3}, \dots, 10^{-7}$. Incubate virus at 37°C in 5% CO₂ for 1 hr.

6. Results :

- Virus strains HN 31868: 10.000 TCID₅₀/ ml

2.2.2. Experimental performance

a. Setting up air –purifier system.

- 4 ml of virus solution with concentration from 10.000 TCID₅₀/ ml to be added in Petri dish.
- Remove the ceiling board from the chamber
- Make sure the air purifier be turn off
- Remove the ceiling board of air purifier
- To set up 4 petri dishes of virus in to chamber.
- Return the ceiling board of air purifier
- To cover the chamber by ceiling 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.



National Institute of Hygiene and Epidemiology

No1- Yersin Street - Hanoi-Vietnam

- 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 different time of experiments into a MDCK flat.
- Allow inoculate to adsorb for 60 minutes at 37⁰C.
- 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 observation of individual 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



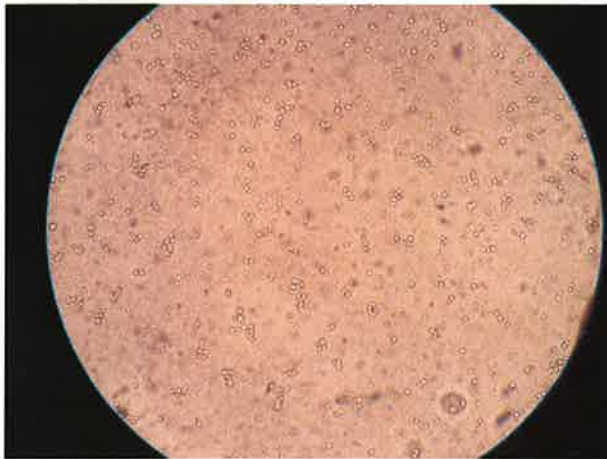
National Institute of Hygiene and Epidemiology

No1- Yersin Street - Hanoi-Vietnam

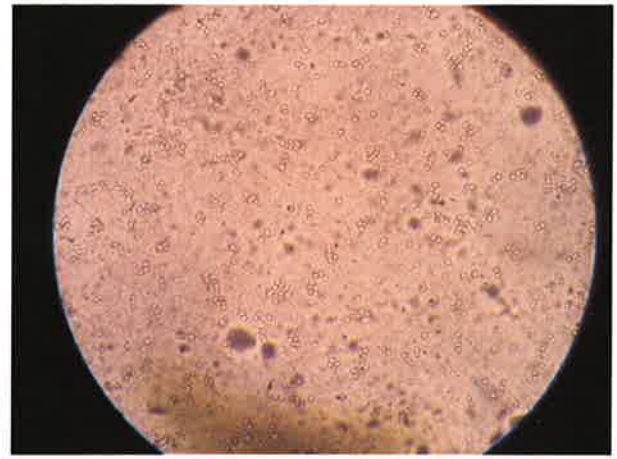
3. Results

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

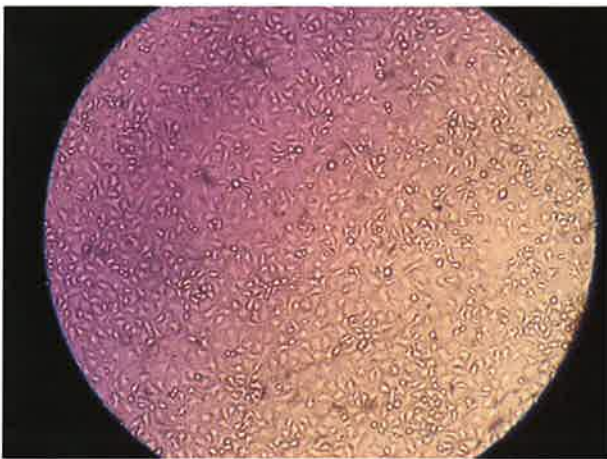
7th day: HN 31868-0 hour



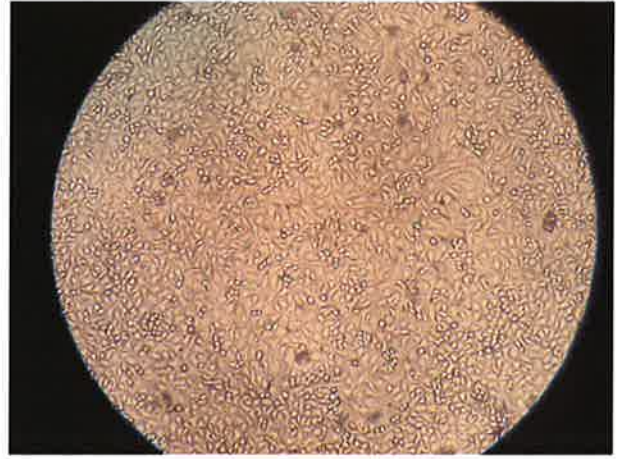
7th day: HN 31868-1 hour



7th day: HN 31868-4 hour



7th day: HN 31868-Control





National Institute of Hygiene and Epidemiology

No1- Yersin Street - Hanoi-Vietnam

3. Conclusion

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