Prepared for: MIC Co., Ltd.

TEST REPORT

Viral Inactivation Test against Influenza Virus using "PBM Deomist"

Report (KitaKanHatsu) No. 21_0142
January 6, 2010

Prepared by: **KITASATO RESEARCH CENTER OF ENVIRONMENTAL SCIENCES**
1-15-1 Kitasato, Sagamihara-shi, Kanagawa-ken 228-0829, Japan
Chairman: Toshihiro Ito

1. **Purpose of Study**

   The viral inactivation effectiveness study was performed on “PBM Deomist”, tendered by MIC Co., Ltd., by using Influenza A virus.

2. **Sponsor of Study**

   Name: MIC Co., Ltd.

   Address: 2-2-15 Higashi-Asahina, Kanazawa-ku, Yokohama-shi, Kanagawa-ken, Japan

3. **Testing Facility**

   Name: Kitasato Research Center of Environmental Sciences, a judicial foundation

   Address: 1-15-1 Kitasato, Sagamihara-shi, Kanagawa-ken, Japan

   Study performed by: Yasuhiro Nojima, Viruses Section, Department of Microorganisms

4. **Study Period**

   November 13, 2009 – November 25, 2009

5. **Challenge Virus**

   Influenza A virus (H1N1)

6. **Test Agent**

   Antimicrobial agent: PBM Deomist, tendered by MIC Co., Ltd.

7. **Contact Time**

   0, 30, and 60 minutes

8. **Study Method**

   The study was performed in accordance with the test specifications prepared following discussion with the responsible person of MIC Co., Ltd.

   1) Incubation and preparation methods of challenge virus

      The influenza virus was inoculated into chorioallantic membrane cavity of embryonated eggs, cultured in an incubator, to obtain the choriallantic fluid. The virus fluid, purified by using density-gradient centrifugation, was used as the challenge virus fluid.

   2) Testing procedure

      The inactivation test of the Influenza A virus was performed in the following manner:

The test agent (0.9 mL) and Influenza A virus fluid (0.1mL; infectivity $7.6x10^{9}TCID_{50}$/mL) were loaded in a test tube, and mixed well in a vortex mixer. After specified reaction time at room temperature, 0.1mL of the mixture was sampled and immediately diluted with 9.9mL phosphate-buffered saline (PBS) in order to avoid toxicity to the cells to be used for determining viral infectivity. This fluid was used as the sample stock solution for determining viral infectivity. Meanwhile, the sample used for 0-minute reaction time was the mixture of virus fluid and PBS, recovered immediately after mixing, instead of allowing the test sample to react.

3) Determination of viral infectivity

Following 10-fold serial dilution of the sample stock solution (for determining viral infectivity) with PBS, 50μL Madin-Darby canine kidney (MDCK) cells, suspended in Dulbecco's modified Eagle's Medium (DMEM) containing 50μL stock solution (or its diluents) and 5% fetal bovine serum (FBS), were inoculated into a 96-well plate. They were then incubated in a CO_2 incubator for four days. After incubation, the cytopathic effects (CPE) were microscopically observed to determine the viral infectivity ($TCID_{50}$/mL) using the Reed-Muench method.

9. **Test Results**

The inactivation efficacy of PBM Deomist, tendered by MIC Co., Ltd., against Influenza A virus is shown in Table 1 and Table 2.

In controls, the viral infectivity at 30 min and 60 min contact times were found to be $2.9x10^{5}TCID_{50}$/mL and $3.1x10^{5}TCID_{50}$/mL, respectively, showing virtually no change from the initial infectivity (at 0 min; $2.9x10^{5}TCID_{50}$/mL).

When contacting the virus of $2.9x10^{5}TCID_{50}$/mL infectivity to PBM Deomist, the viral infectivity was below the identification limit ($6.3x10^{1}TCID_{50}$/mL) at 30 min, demonstrating 3.7-log_{10} or more reduction.

10. **Comments**

In this test, the inactivation efficacy against Influenza A virus by PBM Deomist, tendered MIC Co., Ltd., was studied.

It is thought that the test sample is an antimicrobial agent and not something similar to disinfectants, but the U.S. EPA (Environmental Protection Agency) recommends in its report a logarithmic infectivity reduction of 4-log_{10} as an acceptance criterion for disinfection efficacy and, when cytotoxicity is present against the cells used for determining viral infectivity, a minimum of 3-log_{10} reduction.

The present test demonstrated the viral infectivity reduction of 3.7-$\log_{10}$ or more in 30 minutes of contact time. Therefore, we conclude that PBM Deomist, tendered by MIC Co., Ltd., has viral inactivation efficacy.

References:

1) Antimicrobaials Division U.S. EPA, Confirmatory Virucidal Effectiveness Test, Using Feline Calicivirus As Surrogate for Norovirus

Table 1. Viral inactivation efficacy of PBM Deomist against Influenza A virus

Test agent	Contact time (minute[s])		
	0	30	60
PBM Deomist	$2.9x10^5$	$<6.3x10^1$	$<6.3x10^1$
Control (PBS)		$2.9x10^5$	$3.1x10^5$

Identification limit: $6.3x10^1 TCID_{50}/mL$

N.T.: not tested

Table 2. Changes of logarithmic reduction of viral infectivity over time

Test agent	Contact time (minutes)	
	30	60
PBM Deomist	>3.7	>3.7
Control (PBS)	0.0	0.0

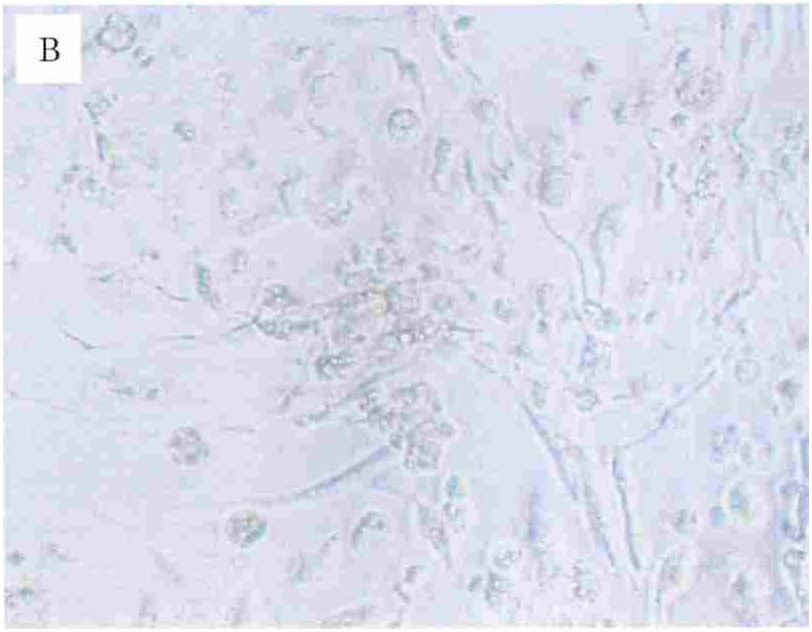
Calculation formula:

$\log_{10}$ (initial viral infectivity [$2.9x10^5 TCID_{50}/mL$] ÷ viral infectivity at respective contact time)

Reference data

Cytopathic effects produced by infecting Influenza virus

Non-infected MDCK cells



Infected MDCK cells (Day 4 of incubation)

The cells which are not infected with the virus showed growth in a sheet-like shape (photograph A), while the virus-infected cells showed morphological changes (cytopathic effects) by the growth of the virus (photograph B).