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HITRT/Microbiology/12

Report on *In-Vitro* modified quantitative surface test for the evaluation of virucidal efficacy of BactiBarrier Surface Protectant against Influenza H1N1 virus

PRODUCT NAME BACTIBARRIER SURFACE PROTECTANT

COMPOSITIONS 3-(Trihydroxysilyl) Propyldimethyloctadecyl ammonium chloride

TEST PROCEDURE

Sample evaluation conditions

A concentration of 0.5% of BactiBarrier surface protectant was directly sprayed for 7 seconds on a black cloth and kept for overnight drying. This dried cloth was then incubated with the pH1N1 pdm09 Influenza virus for a contact time of 10 minutes.

Testing of samples against Influenza H1N1 virus

The testing of the sample was carried out in accordance with the EN14476:2013 standard for disinfectant testing (modified). The BactiBarrier surface protectant was directly sprayed through an aerosol jet spray for 7 seconds on a polyester black cloth on four petri-plates. Each petri plates were designated for total number of days (15, 30, 45, 60 days) the BactiBarrier surface protectant was incubated. After the completion of incubation period, the BactiBarrier sprayed cloth were exposed to pH1N1 pdm09 Influenza virus stock for a contact time of 10 minutes. Post-infection (1, 15, 30, 45) the supernatant was aspirated and overlaid on susceptible cell line. The BactiBarrier exposed virus supernatant were overlaid on susceptible cell line BHK21 cells (Baby Hamster Kidney) and then incubated at 37°C. These

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cells were maintained in Minimum Essential Medium (MEM) with 10% Fetal Bovine Serum (FBS). They were maintained at 5% CO_2 at 37°C for a period of 4-6 days and observed for the presence of cytopathic effect (CPE).

The BactiBarrier exposed virus supernatent infection was carried out in a 24 well plate, keeping 3 replicates of the each subject. Prior to infection, BHK21 cell monolayers were washed with serum-free medium. For virus control well, pH1N1 pdm09 Influenza virus strain was adsorbed to cells for 60 minutes at 37 °C, the inoculum was removed and the cells were grown in MEM with FBS in suitable virus growth medium. They were maintained at 5% CO2 at 37°C for a period of 4-6 days and observed for the presence of cytopathic effect (CPE). Uninfected cells and virus infected cells were maintained as controls.
On day 6 (as per presence of CPE), the medium was removed, cells were fixed with 1 % glutaraldehyde and stained with amido black reagent. The virus titer was calculated using the Spearman-Karber formula and compared with the control virus titer. The evaluation was carried out using EN 14776:2013 (modified) protocol.

TEST SPECIFICATIONS FOR DAY 15

1. Method used - *In-vitro* modified quantitative surface test using Spearman Karber analysis
2. Cell line used for virucidal analysis – BHK21 (Baby Hamster Kidney cells)
3. Test organism - pH1N1 pdm09 Influenza virus strain
4. Media used – MEM (Minimum Essential Media)
5. Contact Time - 10 minutes
6. Start Date of experiment - 06.07.2020
7. End Date of experiment - 14.07.2020

TEST SPECIFICATIONS FOR DAY 30

1. Method used - *In-vitro* modified quantitative surface test using Spearman Karber analysis
2. Cell line used for virucidal analysis – BHK21 (Baby Hamster Kidney cells)
3. Test organism - pH1N1 pdm09 Influenza virus strain
4. Media used – MEM (Minimum Essential Media)
5. Contact Time - 10 minutes
6. Start Date of experiment - 21.07.2020
7. End Date of experiment - 29.07.2020

TEST SPECIFICATIONS FOR DAY 45

1. Method used - *In-vitro* modified quantitative surface test using Spearman Karber analysis
2. Cell line used for virucidal analysis – BHK21 (Baby Hamster Kidney cells)
3. Test organism - pH1N1 pdm09 Influenza virus strain
4. Media used – MEM (Minimum Essential Media)
5. Contact Time - 10 minutes
6. Start Date of experiment - 05.08.2020
7. End Date of experiment - 13.08.2020

TEST SPECIFICATIONS FOR DAY 60

1. Method used - *In-vitro* modified quantitative surface test using Spearman Karber analysis
2. Cell line used for virucidal analysis – BHK21 (Baby Hamster Kidney cells)
3. Test organism - pH1N1 pdm09 Influenza virus strain
4. Media used – MEM (Minimum Essential Media)
5. Contact Time - 10 minutes
6. Start Date of experiment - 20.08.2020
7. End Date of experiment - 28.08.2020

RESULTS

Virucidal efficacy of BactiBarrier Surface protectant against pH1N1 pdm09 Influenza virus

Test Organism	Testing Day	Initial Virus Log Titre	Final Virus Log Titre	Log reduction in viral count (TCID50)	Remarks
Influenza pH1N1 pdm09 virus strain	15	5.5	2.5	3	99.9% virucidal activity observed
	30	5.5	2.5	3	99.9% virucidal activity observed
	45	5.5	4.5	1	90% virucidal activity observed
	60	5.5	4.5	1	90% virucidal activity observed

CONCLUSION

The 'Bactibarrier Surface Protectant' sample submitted for testing the virucidal efficacy over a period of 60 days showed a reduction in cytopathic effect of Influenza pH1N1 pdm 09 virus in a range of 90.0% to 99.9% after exposing the virus to BactiBarrier for 10 minutes contact time.

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