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Attn: MIC. Co., Ltd.

Test Report

Antiviral Testing of PBM DeoSpray

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1. Purpose of the Test

The purpose of this test is to evaluate the efficacy of viral inactivation by PBM Deo Spray, using feline calicivirus (norovirus alternative).

2. Testing Virus

Feline calicivirus F-9 strain, norovirus alternative)

3. Test Method

1) Preparation of Feline calicivirus F-9 strain

Crandell-Reese feline kidney (CRFK) was infected with Feline calicivirus (FCV), and then cultured at 37℃ in a carbon dioxide incubator. When more than 90% of the cells displayed cytopathogenic effect (CPE) the cells were cryopreserved in a -80℃ freezer. Freeze-thawing processing was repeated twice, then after centrifuge at 3500rpm for 10 minutes, the supernatant was collected and purified using an ultrafilter membrane, and was determined be the sample virus fluid.

2) Test Product and Test Conditions

Supplied Test Product: PBM Deo Spray

Duration of viral activity: 0-1 minute

3) Test Method

Inactivation test of the feline calicivirus was carried out according to the following procedures.

For each in vitro test, 0.9mL of the test product and 0.1mL feline calicivirus were combined, and then mixed in a vortex (infectivity titer:4.0×108 TCID50/mL). After reaction at room temperature at the prescribed duration of activity, a sampling of 0.1mL was taken and immediately diluted with 4.9mL PBS (phosphate buffered saline) to halt drug activity (sample stock solution). 10 times serial dilution was then promptly carried out by PBS, to measure viral infectivity titer of the sample. Furthermore, PBS was used in place of the sample for the of 0 minutes response time of the duration of viral activity. PBS was used.

4） Virus Quantification Method

The CRFK cell culture supernatant was suction removed as a monolayer culture in a 96 hole plate beforehand, then sample undiluted solution and diluted 25μL virus fluid were added and allowed to stand for 1 hour at 37℃. After standing, the virus fluid was suction removed, and 100μL DMEM including 0.2% FBS was added to each well and

cultured in a carbon dioxide gas incubator at 37℃. After culturing, cytopathogenic effect (CPE) of each well was observed by microscope, and viral infectivity (TCID50/mL) was calculated by using Reed-Muench method.

4. Test Results

Results are shown in Table-1.

When PBM DeoSpray was activated for 1 minute in the virus with an initial infectivity titer 5.9×105 TCID50/mL, infectivity titer reduction of more than 3.7log10 (less than 1.3×102 TCID50/mL) was observed.

Test supervision by Yasuhiro Nojima

End

Table-1　Antiviral Efficacy by PBM DeoSpray

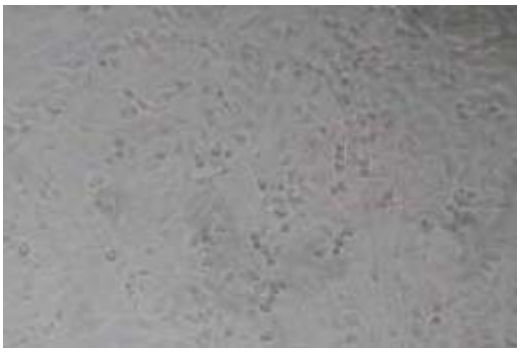
Tested product	Duration of Activity (min.)		Reduction value of the infectivity titer (log 10)
	0	1	
PBM Deo Spray	5.9×105	＜1.3×102	＞3.7
Control (PBS)		***	***

Detection limit value:1.3×10^2

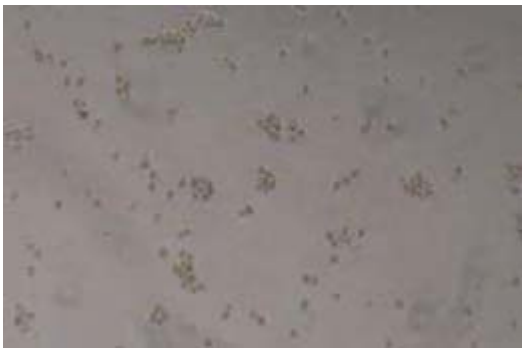
Reduction value of the infectivity titer: log10

(0 minute infectivity titer÷infectivity titer of 1 minute duration of activity)

Reference data (Photo of CRFK cells)



Cells not infected by the virus



Cells infected by the virus

(4 days after the infection)