成績書番号： PR200917-1

Test report

Requester FUTURE CHEMICAL Inc.

Specimen In this report

Title Antiviral coating agent influenza A virus
And evaluation of feline calicivirus inactivating activity

We would like to report the test results of the above samples that we have received.

Testing institution PROTECTEA LTD.

Head of testing institution：Nobuyuki Tanaka

2020 年 9 月 11 日

I-1. Requester

FUTURE CHEMICAL Inc.

I-2. Testing Institute and Address

Testing institution　PROTECTEA　LTD.

Testing location：8-1 Mihogaoka, Ibaraki City, Osaka Prefecture
Incubation Building I213

Examiner：Nobuyuki　Tanaka　（PROTECTEA　LTD.）

I-3. Test date

Influenza A virus inactivation test　　July 29, 2020

Feline calicivirus inactivation test　September 7, 2020

I-4. Specimen used

・Processed sample　FUTURE Anv.　Coated aluminum plate

・Test control　Polyethylene board（AZUWAN）

I−5.　試験概要　I-5. Exam outline

The inactivating effect of the above sample on influenza A virus and feline calicivirus will be evaluated by a test based on the ISO21702 method.

I-6. Test target strain / cell / virus strain

Influenza A virus H1N1 A/PR/8/34	ATCC VR-1469
Host cell: MDCK cell (dog kidney cell)	ATCC CCL-34
Feline Calicivirus F-9	ATCC VR-782
Host cell: CRFK cell (cat kidney cell)	ATCC CCL-94

I-7. Test method

A test sample and a control polyethylene plate each having a size of 50 mm × 50 mm were prepared. After seeding each sample prepared on the petri dish with virus solution 1-10 x 107 PFU / mL 0.4 mL, a cover film (40 mm × 40 mm, polyethylene) was placed on the virus solution. Immediately after inoculation in the test control, the test control and the test sample were allowed to stand at 25 ° C. ± 1 ° C. for 24 hours, and then 10 mL of SCDLP medium was added for extraction. Furthermore, it was serially diluted 10-fold with SCDLP medium to prepare a 10-fold serial dilution series. A 10-fold serial dilution series was added dropwise to pre-seeded host cells in 1 mL increments, and infection treatment was performed at 37 ° C. under 5% CO2 for 1 hour. After virus infection, the cell supernatant was replaced with 0.8% oxoid agar solution and cultured at 37 ° C. under 5% CO2 for 1-2 days. After visually confirming the formation of plaques, the cells were fixed with a 5% glutaraldehyde solution, stained with methylene blue, and the virus infectivity titer was measured based on the measurement data of the number of plaques formed.

[Formula]

<Test acceptance conditions>

（Lmax － Lmin）/（Lmean） ≦ 0 2

Lmax Maximum Log (PFU / cm2) of the test controls immediately after inoculation

Lmin Minimum Log (PFU / cm2) among test controls immediately after inoculation

L mean Mean Log (PFU / cm2) of test control immediately after inoculation

<Antiviral activity value>

$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$

R Antiviral activity value

U_0 Average Log immediately after inoculation (PFU / cm2)

U_t Average Log (PFU / cm2) after duration of action

A_t Average Log (PFU / cm2) after the action time of the processing test sample

2020 年 9 月 11 日

I-8. Test results

The test results are shown below.

[Influenza A virus inactivating activity]

[Confirmation of establishment conditions]

Test establishment conditions: establishment (<0.2)

Lmax	Lmin	L mean
4.59	4.21	4.40
Lmax－ Lmin／ L mean＝0.0873		
Test establishment conditions: establishment (<0.2)		

Initial titer	4.5E+07PFU/ml

	Duration of action	PFU/㎠			Log（PFU/㎠）		Antiviral activity R
		N=1	N=2	average	mark	Numerical value	
Test control（0h）	0	237,500	206,250	221,875	UO	5.3	–
Test control（24h）	24	16,125	39,063	27,594	Ut24	4.4	–
Future Anv. Coated aluminum plate	24	0.63	0.63	0.63	At1	-0.2	4.6

LMax	**Lmin**	**Lmean**
5.92	**5.88**	**5.90**
(LMax-Lmin)/Lmean		
試験成立条件：成立(<0.2)		

Lmax	Lmin	L mean
5.92	5.88	5.90
Lmax－ Lmin／ L mean		
Test establishment conditions: establishment (<0.2)		

【Test results】

titer	1.3E+07　　PFU/ml

	Duration of action	PFU/㎠			Log（PFU/㎠）		Antiviral activity R
		N=1	N=2	average	mark	Numerical value	
Test control（0h）	0	762,500	831,250	796,875	Uo	5.9	－
Test control（24h）	24	89,063	117,500	103,281	Ut24	5.0	－
Future Anv. Coated aluminum plate	24	0.63	0.63	0.63	At1	-0.2	5.2

I-9. Consideration and conclusion

The virus inactivating effect of the donated test sample was evaluated. Antiviral activity of 4.6 or higher was confirmed for influenza A, and antiviral activity of 5.2 or higher was confirmed for feline calicivirus.

that's all