Japan Textile Products Quality and Technology Center

TEST REPORT

29th January 2021

APPLICATION

Test applicant : Mitsubishi Electric Corporation
Test sample : Plasma Quad
Test item : Antiviral activity test
Date of application : 24th December 2020

TEST METHOD

○ Summary of antiviral activity test

- Virus strain : Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); JPN/TY/WK-521
  (Distributed from National Institute of Infectious Diseases, Japan)
- Host cell : VeroE6/TMPRSS2 JCRB1819
- Growth medium : Dulbecco's modified Eagle's medium (low-glucose) ; DMEM (SIGMA, Cat#D6046)
  Minimum Essential Medium Eagle ; EMEM (SIGMA, Cat#M4655)
- Fetal Bovine Serum (FBS) (SIGMA, Cat#173012)
- Test sample : Plasma Quad
- Virus inoculation : Place 0.005 mL of test virus suspension at one point on the surface of the test carrier (stainless plate)
- Contact time : 6 h, 12 h, 24 h
- Test condition : 23 °C, 26%RH in the safety cabinet
- Wash out solution : 1/10 SCDLP diluted with 2% FBS-containing DMEM
- Measurement of viral infectivity titer : Plaque assay

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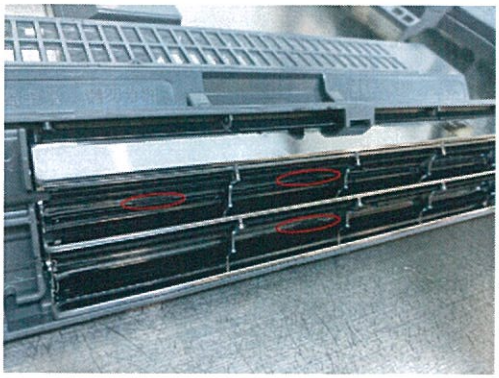
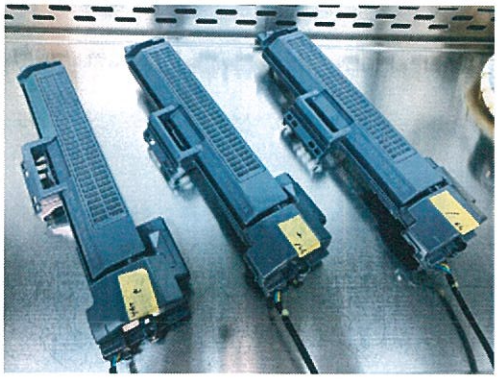
○Outline of antiviral activity test

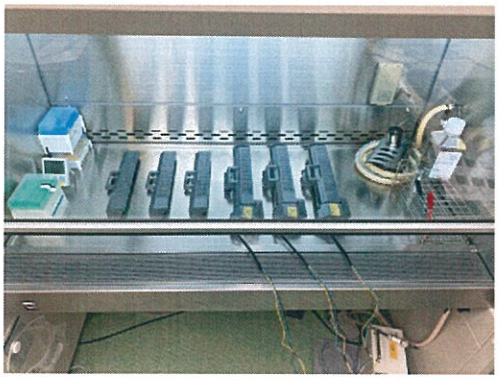


Picture.1 Test carrier (element)



Picture.2 Installation of test carrier

Picture.3 Installation of test carrier



Picture.4 Hold with energization

Picture.5 Conformation of energization

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○ Antiviral activity test in suspension

1. Preparation of test virus suspension

1-1. Drain a growth medium from a flask with cultured VeroE6/TMPRSS2 in the monolayer.

1-2. Wash the surface of the cultured cells with EMEM and drain the medium.

1-3. Inoculate SARS-CoV-2 suspension on the surface of cell in the flask and spread to the whole surface.

1-4. Put the flask in the CO_2 incubator at 37 °C and keep it for 1 h to adsorb the virus to the cells.

1-5. Add the appropriate amount of EMEM to the flask.

1-6. Put the flask in the CO_2 incubator at the temperature of 37 °C for 1 to 3 days to multiply SARS-CoV-2.

1-7. Observe the cytopathic effect under an inverted microscope and judge the multiplication of the virus. If the multiplication of the virus is confirmed, then, Centrifuge the multiplied virus suspension by using the centrifuge at 4 °C and 1,000 g for 15 min.

1-8. Take the supernatant suspension from the centrifugal tube after the centrifugation. This is to be the test virus suspension.

2. Test procedure

1. Place 0.005 mL of test virus suspension at 3 points (n=3) on the surface of the stainless part of the element. (Pic.1)
2. Allow the virus inoculum to dry under ambient conditions in the safety cabinet for about 10min. This is to be the test carrier.
3. Place the dried test carrier on the electric base. (Pic.2)
4. Hold with energization in the safety cabinet for the required contact time. For the control test, hold without energization in the safety cabinet for the required contact time. (Pic.3, 4, 5)
5. Upon completion of the contact time, immediately add 1.0mL of wash out solution to the carrier and recover the virus.
6. Prepare a series of 10-fold dilutions of recovered virus suspension by using 2% FBS-containing DMEM. Measure the viral infectivity titer per 0.1mL of recovered virus suspension by plaque assay and calculate the viral infectivity titer per test carrier.

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TEST RESULT

○Results of antiviral activity test

Virus strain : SARS-CoV-2; JPN/TY/WK-521

(Distributed from National Institute of Infectious Diseases, Japan)

Test virus suspension : 2.1×10^8 PFU/ml

Common logarithm of ideal value of virus infectivity titter per test carrier : 6.02

Test carrier		Common logarithm of Infectivity titer (PFU/carrier) per test carrier		
		Common logarithm		Common logarithm average
With energization	Immediately after drying	n1	5.86	5.88
		n2	5.87	
		n3	5.92	
	After 6 h	n1	3.04	3.10
		n2	2.91	
		n3	3.34	
	After 12 h	n1	2.56	2.64
		n2	2.63	
		n3	2.73	
	After 24 h	n1	2.50	2.31
		n2	2.27	
		n3	2.15	

Test carrier		Common logarithm of Infectivity titer (PFU/carrier) per test carrier		
		Common logarithm		Common logarithm
Without energization * Control test	Immediately after drying	n1	5.86	5.88
		n2	5.87	
		n3	5.92	
	After 6 h	n1	4.43	4.53
		n2	4.58	
		n3	4.59	
	After 12 h	n1	3.77	3.65
		n2	3.43	
		n3	3.76	
	After 24 h	n1	2.53	2.60
		n2	2.58	
		n3	2.69	

* Common logarithm value of Limit of Quantification : < 1.0

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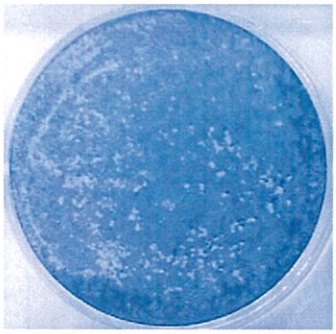
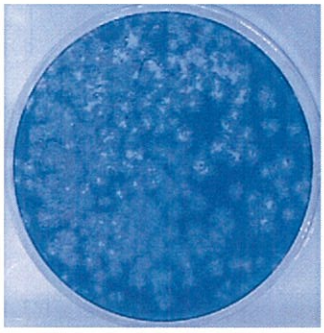
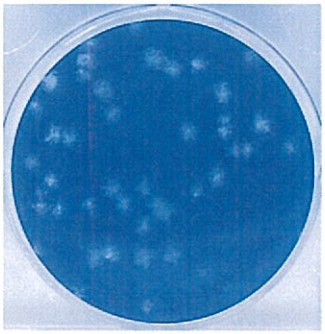
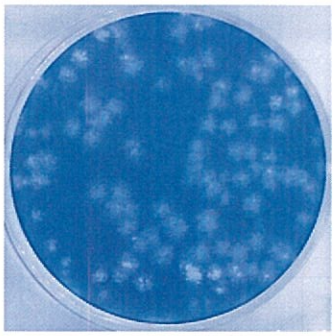
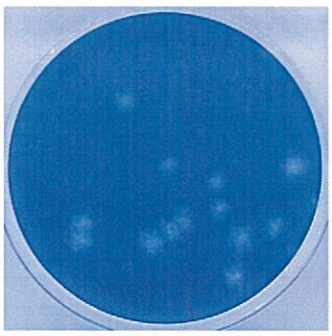
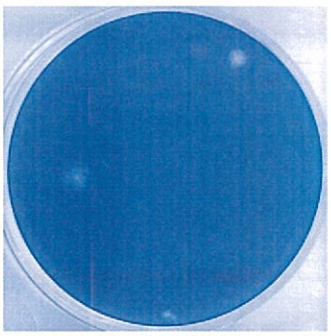
○Plaque assay

Plaque formation per 0.1mL of recovered virus suspension after 6 h contacting.

Test condition	Dilution rate of recovered virus suspension		
	$\times 10^0$	$\times 10^1$	$\times 10^2$
Without energization After 6 h			
With energization After 6 h			

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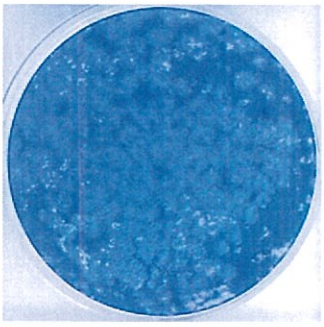
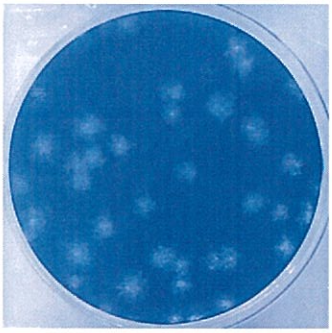
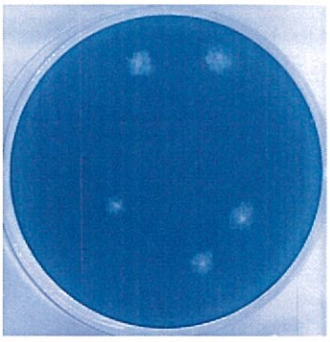
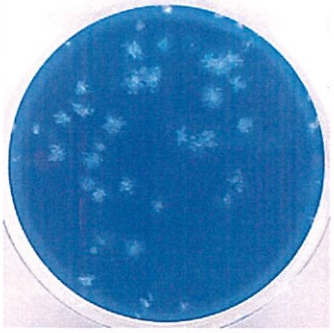
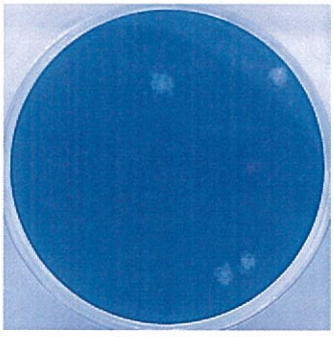
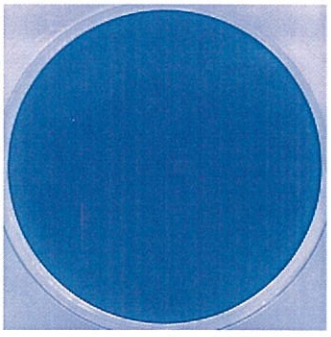
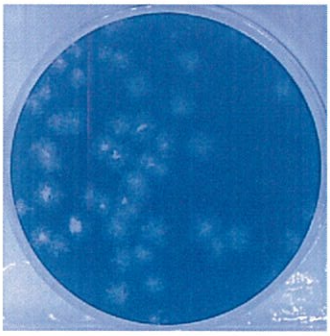
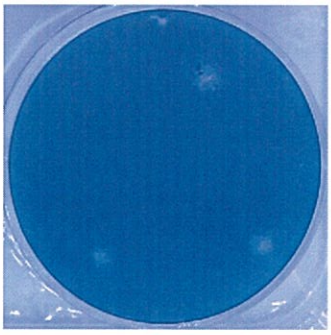
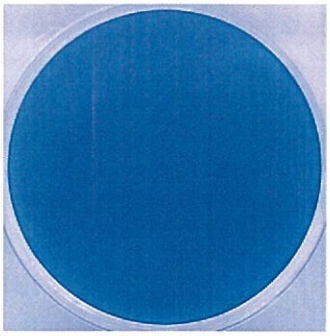
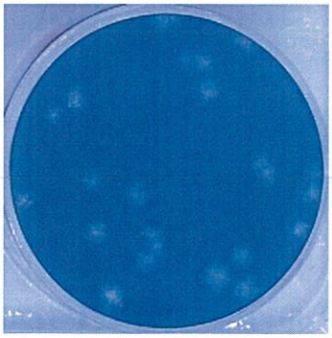
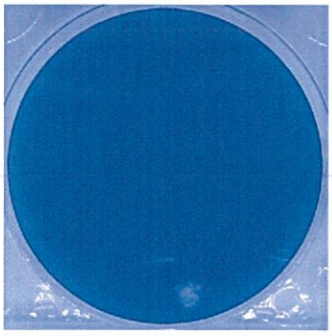
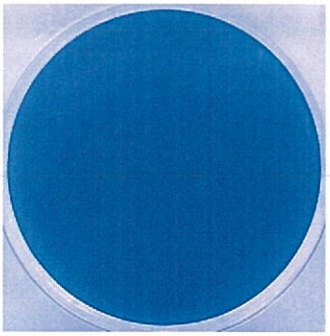
Plaque formation per 0.1mL of recovered virus suspension after 12 h contacting.

Test condition	Dilution rate of recovered virus suspension		
	$\times 10^0$	$\times 10^1$	$\times 10^2$
Without energization After 12 h			
With energization After 12 h			

Plaque formation per 0.1mL of recovered virus suspension after 24 h contacting.

Test condition	Dilution rate of recovered virus suspension		
	$\times 10^0$	$\times 10^1$	$\times 10^2$
Without energization After 24 h			
With energization After 24 h			

* Test results in this test report are only for samples received from the applicant and not for the whole lot.

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TEST RESULT (Informative)

○Real-time RT-PCR measurement of recovered virus suspension

- Virus strain : SARS-CoV-2; JPN/TY/WK-521
  (Distributed from National Institute of Infectious Diseases)
- Real-time PCR device : Thermal Cycler Dice® Real Time System Ⅲ (TaKaRa)
- Detection Kit : SARS-CoV-2 Detection Kit −N1 set− (Code NCV-301; Lot# 038200)
  (TOYOBO CO.,LTD. Biotech support Department)

Mix 6 μL of recovered virus suspension with 3 μL of pretreatment solution and heat at 95 °C for 5min. Then, add 40 μL of RT-PCR reaction solution. After reverse transcription reaction and denaturation step, PCR was performed for 45 cycles.

Test carrier		Ct	
		With energization	Without energization
Test carrier	After 6 h	21.85	22.11
	After 12 h	23.61	22.00
	After 24 h	24.68	22.29

Note:

Test condition; Without energization

On plaque assay the virus infectivity titer of SARS-CoV-2 showed a decrease over time whereas a decrease in the amount of virus RNA correlative with the decrease in the virus infectivity was not observed. Natural deactivation over time up to 24 h shows no damage to virus RNA. (Fig.2)

Test condition; With energization

A decrease in the amount of virus RNA was not observed after 6 h, but the amount of virus RNA tented to decrease after 12 h and 24 h. (Fig.3) A part of RNA was damaged, probably due to contact with the electric device.

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Sample List

ID	Type	Name	Color
1	UNKN	With energization _6h	
2	UNKN	With energization _12h	
3	UNKN	With energization _24h	
4	UNKN	Without energization _6h	
5	UNKN	Without energization _12h	
6	UNKN	Without energization _24h	
7	NTC	Wash out solution for negative control	

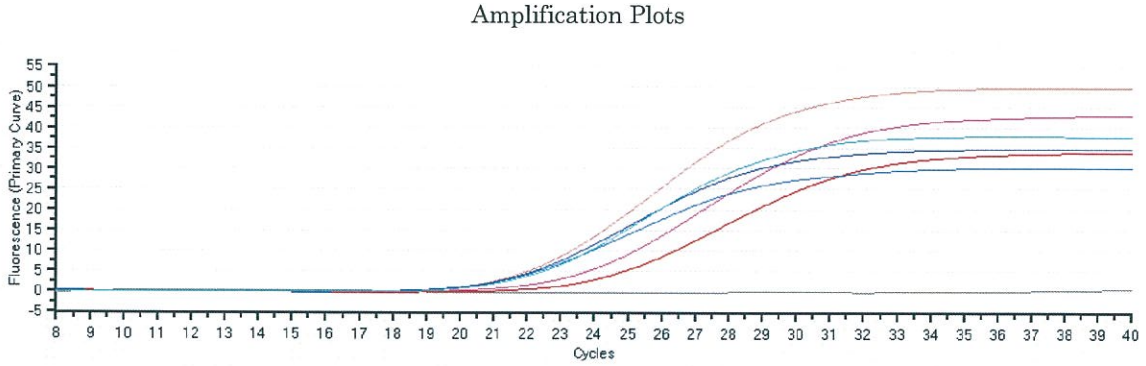

Fig.1. Amplification Plots; With/Without energization

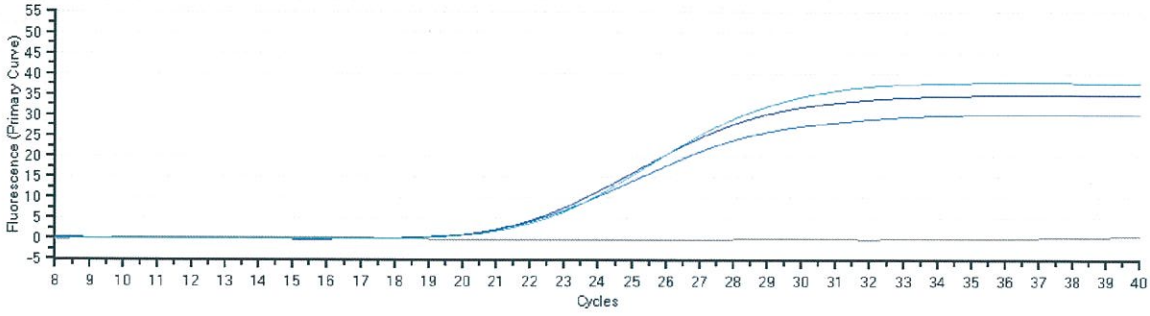

Fig.2. Amplification Plots; Without energization

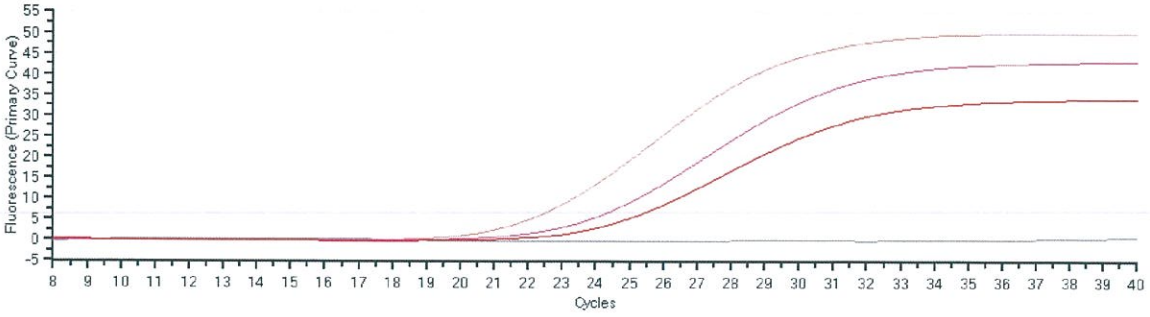

Fig.3. Amplification Plots; With energization

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○Real-time RT-PCR measurement of virus suspension used in this test

- Virus strain : SARS-CoV-2; JPN/TY/WK-521
  (Distributed from National Institute of Infectious Diseases)
- Virus suspension : >10^8 PFU/ml
- Real-time PCR device : Thermal Cycler Dice® Real Time System Ⅲ (TaKaRa)
- Detection Kit : SARS-CoV-2 Detection Kit −N1 set− (Code NCV-301; Lot# 038200)
  (TOYOBO CO.,LTD. Biotech support Department)

○Result

As the results of real-time RT-PCR measurement, an amplification of viral RNA in virus suspension used in this test was confirmed (Fig.1).

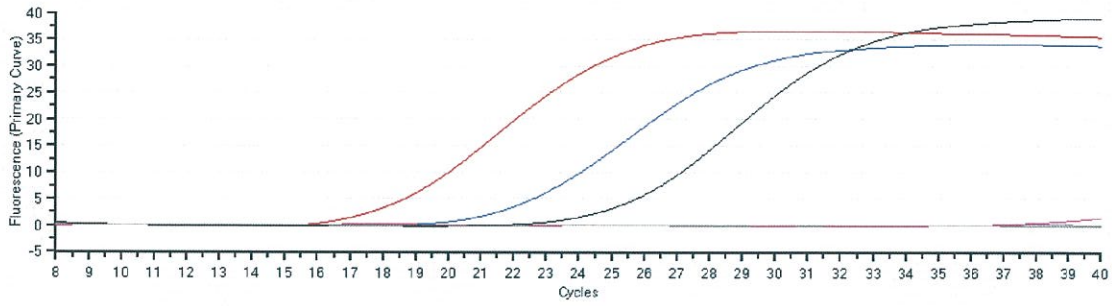

Fig.1. Real-time RT-PCR amplification plot

Red line shows the 10^{-2} dilution of virus suspension with PBS.
Blue line shows the 10^{-3} dilution of virus suspension with PBS.
Black line shows the 10^{-4} dilution of virus suspension with PBS.
Pink line shows the negative control; EMEM.

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