BIOTECH TESTING SERVICES

TEST REPORT

LAB NO.: 2300008/ 1	DATE:18/01/2023

NAME OF CUSTOMER : HOYASAN LABS PVT LTD

ADDRESS : Bangalore - 560012
Karnataka.

REFERENCE : Letter Ref. No. Nil dated January 03, 2023
Kind Attention: Misbah Rehman

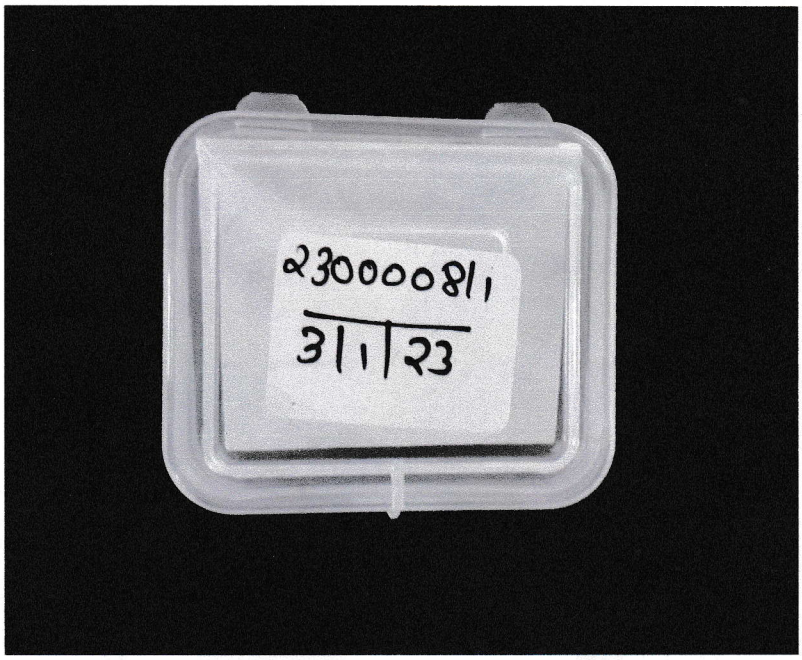
DATE OF RECEIPT : 03/01/2023

DATE OF INITIATION : 03/01/2023

DATE OF COMPLETION : 18/01/2023

SAMPLE DESCRIPTION : Test sample labeled as -

Sr.No.	Description
1.	Panel Sample
Untreated – Lab Control	



104/105/205/206, Malwa, Patanwala Ind. Estate, L.B.S. Marg, Ghatkopar (W), Mumbai-400086, INDIA • Tel.: +91-22-25002811, 25002812
Email : info@biotechts.in / report@biotechts.in / biotechtestingservices@gmail.com / shilpanair@biotechts.in

Test Method:

ISO 21702: 2019

Measurement of Antiviral activity on plastics and other non-porous surfaces and coating materials

Scope of Method:

This test specifies method for measuring antiviral activity on plastic and other non-porous surface of antiviral-treated products against specified virus. Due to individual sensitivities, the results of one test virus might not be applicable for other viruses.

Determination of Infectious titre:

TCID50 method

Virus strains and host cells:

Test Virus: Human Coronavirus HCoV-229E (Surrogate of SARS-CoV-2)

Host Cell: MRC-5 cell line (ATCC CCL-171); Passage No.: Cells from PN 29

Experimental Conditions:

Test Sample	: Sample surface (50 mm x 50 mm); Pre-sterilized by ETO
Control Sample	: LDPE film (40 mm x 40 mm); Pre-sterilized by ETO
Test procedure	: Triplicates
Virus inoculum volume	: 0.4 ml
Viral titre	: 1.60 x 10^8 PFU/ ml
Contact Period	: 24 hours
Wash out Medium	: SCDLP
TCID50 method	: 96 Well plate
Medium of Cell culture	: Eagle's minimal essential medium (EMEM), supplemented with inactivated FBS & antibiotics
Incubation	: 37^0 C in CO_2 incubator/ 7 days

Verification of cytotoxicity on Host cells:

1. Sterile Control and Test samples were added with 10 ml wash out solution, SCDLP medium. To ensure that the neutralizer completely washes the specimens, SCDLP broth was pipetted at least four times.
2. 0.1 ml of the washing out solution was inoculated into 6 well plate having monolayer of host cells in duplicates.
3. It was incubated at 34^{0}C for 2-3 days.
4. After incubation, plates were examined visually for cytotoxic effect if any.

Observations:

Sample Description	Observations
Panel Sample	**Non cytotoxic**

104/105/205/206, Malwa, Patanwala Ind. Estate, L.B.S. Marg, Ghatkopar (W), Mumbai-400086, INDIA • Tel.: +91-22-25002811, 25002812
Email : info@biotechts.in / report@biotechts.in / biotechtestingservices@gmail.com / shilpanair@biotechts.in

www.biotechts.in

Verification of cytotoxicity by cell sensitivity to virus and the inactivation of antiviral activity:

1. Sterile Control and Test samples were added with 10 ml wash out solution, SCDLP medium. To ensure that the neutralizer completely washes the specimens, SCDLP broth was pipetted at least four times.
2. The washing out solution was inoculated with virus suspension and incubated at 25^{0}C for 30 minutes.
3. Infective titre of this solution was determined by TCID50 Method.
4. Log of TCID50/ ml of Control LDPE film - Log of TCID50/ ml of Test LDPE film ≤0.5.

Observations:

Log of TCID50/ ml of Control LDPE film	Log of TCID50/ ml of Test Sample	Log of TCID50/ ml of Control LDPE film - Log of TCID50/ ml of Test Sample	Acceptable Criteria
3.60	**3.32**	**0.28**	≤ 0.5

Test Procedure:

1. 0.4 ml of the test inoculum was added onto the test surface. It was covered with LDPE film to ensure that the test inoculum makes contact with test surface and spreads to the edges.
2. After the specimen was inoculated and the cover film applied, It was closed with the lid of the Petri dish and incubated at 35 ± 1°C and a relative humidity of not less than 90 % for 5 minutes.
3. Immediately after inoculation, the three Control sample were terminated using SCDLP medium to retrieve the virus.
4. After the contact time of 24 hours remaining Control and Test samples were terminated using SCDLP medium to retrieve the virus.
5. infectivity titer of virus recovered from the sample was determined by $TCID_{50}$ method

104/105/205/206, Malwa, Patanwala Ind. Estate, L.B.S. Marg, Ghatkopar (W), Mumbai-400086, INDIA • Tel.: +91-22-25002811, 25002812
Email : info@biotechts.in / report@biotechts.in / biotechtestingservices@gmail.com / shilpanair@biotechts.in
www.biotechts.in

Results:

Test Virus: Human Coronavirus HCoV-229E (Surrogate of SARS-CoV-2)

Test Sample: Panel Sample

Virus	Contact Duration	Group	Logarithm of Infectivity titre of virus ($lgTCID_{50}/ cm^2$)	Average titre Infectivity of virus ($lgTCID_{50}/ cm^2$)
Human Coronavirus suspension: (1.60×10^8 PFU/ ml)	**0 hours**	**Control** (U_0)	6.40	6.40
			6.74	
			6.07	
	24 hours	**Control** (U_t)	6.40	6.18
			6.07	
			6.07	
	24 hours	**Panel Sample** (A_t)	3.40	3.51
			3.40	
			3.74	
Antiviral activity R= U_t - A_t (24 hours contact)			-	**2.67** (99.78%)

Antiviral activity R= U_t - A_t

Where

R is the Antiviral activity

U_0 is the average of common logarithm from three control/ untreated specimen immediately after inoculation

U_t is the average of common logarithm from three control/ untreated specimen after 24 hours

A_t is the average of common logarithm from three Treated specimen after 24 hours

BIOTECH TESTING SERVICES BTS MUMBAI

For BIOTECH TESTING SERVICES

Dr Shilpa U. Nair
Quality Manager
(Authorized Signatory)

104/105/205/206, Malwa, Patanwala Ind. Estate, L.B.S. Marg, Ghatkopar (W), Mumbai-400086, INDIA • Tel.: +91-22-25002811, 25002812
Email : info@biotechts.in / report@biotechts.in / biotechtestingservices@gmail.com / shilpanair@biotechts.in