Ref No: BVDU/IRSHA/ 881/2021-22 **Date:** 13/11/2021
TEST REPORT ID: NB/AV/21/0053

Study Title: Evaluation of Recovereez Forte capsules

Testing laboratory Name	National Immunogenicity and Biologics Evaluation Centre (NIBEC), IRSHA, BVDU
IRSHA Study Number	SCoAV-23
Customer Name and Address	Zum Heilen Diagnostic & Therapeutics Private Limited 12/1543-C, Second Floor, S B Center, Museum Road, Thrissur, Kerala - 680020
Client project number	ZHDAT -001/2021
Sample Receipt Date	30th August 2021
Start date of Testing	2nd September 2021
End date of Testing	29th October 2021
Date of Draft Report	11th November 2021
Date of Report submission	13th November 2021
Specimen Used for testing	Drug molecules: Recovereez Forte capsules
Testing Conditions	1. Evaluation of cytotoxicity of the drugs on Vero cells 2. Antiviral testing against SARS-CoV2 virus • Virucidal activity assessment at 2 hours • Prophylactic activity assessment at 1 hour, 2 hour, 4 hour and 24 hours • Therapeutic activity assessment at 1 hour, 2 hour, and 4 hours

Dr. Sudha Ramkumar
Authorized Signatory

The test results on this report refer only to the batch of items supplied by the customer for testing. The results provided may be submitted to regulatory authorities as scientific proof. The results must not be used for claims that are unsubstantiated by regulatory authorities. All reports are archived at NIBEC, IRSHA for a maximum period of 1 years. The sample will be retained for 3 months unless otherwise requested in writing.

Scope:
Determination of antiviral activity of prospective drug sample provided by ZHDAT against SARS-CoV-2 virus.
Test Virus Summary:

Realm	Riboviria
Order	Nidovirales
Family	Coronaviridae
Genus	Betacoronavirus
Species	COVID-19
NCBI Accession number for virus isolate	MT416726

Procedure

1. Determination of the CC_{50} and MNTD (Maximum non-toxic dose)
2. *In vitro* antiviral assessment of formulation against SARS-CoV-2 virus.

1. Determination of the CC_{50} and MNTD (Maximum non-toxic dose) for the drug formulation

Procedure for MNTD and CC_{50} determination:

1. Six different dilutions of test compound was added on Vero cell monolayer in triplicate.
2. Plate was incubated at 37 °C in 5% CO_2.
3. After incubation, development solution was added to the cells followed by incubation at 37 °C in 5% CO_2.
4. Absorbance will be taken at appropriate wavelength.

Maximum non-toxic dose (MNTD) and CC_{50} is derived.

2. *In vitro* antiviral assessment of drug formulation against SARS-CoV2 virus

This was done in modes:

A. Prophylactic mode
B. Virucidal activity determination
C. Therapeutic mode

A. Prophylactic mode:

Herein, 6 different concentrations of each drug below the MNTD value was accessed for antiviral potency in prophylactic mode at recommended time points in triplicate. For each time point,

1. Vero cells was pre-treated with the six different concentrations of the drug in triplicate for recommended time.
2. Post-pretreatment, cells were washed and infected with SARS CoV-2 virus at 0.1 MoI
3. Plate were incubated at 37 °C in 5% CO for 72 hours
4. After incubation, quantitative CPE based score EC_{50} is determined

B. Virucidal activity determination:

Herein, 6 different concentrations of each drug below the MNTD value is accessed for antiviral potency in virucidal mode at recommended time point in triplicate.

1. Six different concentration of test compound in triplicate was incubated with SARS-CoV-2 virus for recommended contact time.
2. Virus-Compound mixture was added to the Vero cell monolayer for infection.
3. Plate was then incubated at 37 °C in 5% CO_2 for 72 hrs
4. After incubation, quantitative CPE (cytopathic effect) based score was made for EC_{50} determination.

C. Therapeutic mode:

Six different concentrations of each drug below the MNTD value was assessed for antiviral potency in therapeutic mode at recommended time points in triplicate.
For each time point,

1. Vero cells was infected with SARS-CoV2 Virus for the recommended contact time points
2. Post infection, 6 different concentration of test compound was be added to infected vero cells in triplicate.
3. Plate was incubated for 72 hrs at 37 °C in 5% CO_2.
4. After incubation, quantitative CPE based score was be made for EC_{50} determination.

Assessment of a drug in all the modes will identify the antiviral potential of the drug candidate against SARS-CoV-2.

EC_{50}: compound concentration required to achieve 50 % protection from virus-Induced cytopathogenicity

CC_{50}: compound concentration required to reduce cell viability by 50%

SI* (selectivity index): ratio by CC_{50}/EC_{50}

*The higher SI ratio = theoretically more effective and safe drug during *in vivo* treatment for a given viral infection.

Test Information Summary

Details of the drug samples				
Name of the drug	**Molecular Weight and concentration**	**Solubility**	**Appearance of the drug sample**	**Storage Condition**
Recovereez Forte capsules	200 mg/ml	DMSO	Colorless liquid	At Room temperature
Details of the Experimental Conditions				
Host Cell line used for testing		Vero cell line		
Virus used for testing		COVID 19 (SARS-CoV-2) at 0.1 MOI		
Test Controls				
• **CC_{50} determination**		400 µM Formaldehyde		
• **EC_{50} determination**		0.72 µM Hydroxychloroquine Sulfate		
Diluent used for the assay		Minimum Essential Media (MEM) containing 2% Fetal Bovine Serum (FBS)		
Assay Incubation Period				
• **CC_{50}**		72 hours post addition of Drug		
• **EC_{50}/EC_{80}**		72 hours post virus infection		
• **Concentration of drug used for the assay**		(Provided in result section tables)		
• **Incubation conditions**		37 °C, 5 % CO_2		
Software used for data analysis and interpretation		GraphPad Prism : Non-linear regression(curve fit)		

Test Result Summary

Sample	CC_{50} µg/ml	MNTD µg/ml	Mode of Assessment	Time point	EC_{50} µg/ml	SI INDEX
Recovereez Forte capsules	41.8	20.0	Prophylactic	1 hour	46.5	0.90
				2 hours	34.9	1.20
				4 hours	19.9	2.10
				24 hours	No Activity	Not Applicable
			Virucidal	2 hours	1133.0	0.04
			Therapeutic	1 hour	20.4	2.05
				2 hours	21.5	1.95
				4 hours	19.3	2.16

Raw Data for Test

Table 1: Cytotoxicity assessment of the test product

Sample ID	Concentrations µg/ml	% Relative Cell Death
Recovereez Forte capsules	2500	94.256
	500	93.233
	100	93.404
	20	9.698
	4	9.511
	0.8	6.272

Table 2: Antiviral assessment of the test sample against SARS-CoV-2 virus

Sample ID	Concentrations µg/ml	Relative % CPE Inhibition							
		Prophylactic Mode				Therapeutic Mode			Virucidal Mode
		1 hour	2 hour	4 hour	24 hour	1 hour	2 hour	4 hour	2 hour
Recovereez Forte capsules	20	16.4	36.5	51.6	-60.9	45.4	35.2	54.0	19.1
	4	0.8	9.1	-1.4	-61.2	-9.2	-16.2	22.8	13.0
	0.8	0.9	6.2	-5.4	-57.0	-14.5	-7.4	11.2	5.6
	0.16	-1.0	-2.5	-9.0	-30.3	-15.6	0.7	8.7	3.5
	0.032	0.7	-3.6	-11.3	-38.3	-19.0	8.2	7.8	3.7
	0.0064	-2.2	-30.1	-10.6	-51.3	-19.6	5.0	12.9	1.9

Conclusion:

1. **Recovereez Forte capsules** exhibited prophylactic activity against SARS-CoV2 when pre-treated with Vero cell line for 1, 2 and 4 hours followed by infection at 0.1 MoI with an **EC_{50} value** of **46.5 µg/ml, 34.9 µg/ml and 19.9 µg/ml** and **Selectivity index (SI)** value of **0.90, 1.20 and 2.10** respectively.
2. **Recovereez Forte capsules** exhibited therapeutic activity when treated with cells infected with SARS-CoV2 virus (0.1 MoI) 1 hour, 2 hour and 4 hour post infection with an **EC_{50} value** of **20.4 µg/ml, 21.5 µg/ml** and **19.3 µg/ml** with a **Selectivity index (SI)** value of **2.05, 1.95 and 2.16** respectively.
3. **Recovereez Forte capsules** when incubated with SARS-CoV2 virus (0.1 MoI) for, 2 hour exhibited virucidal activity against SARS-CoV2 with an EC_{50} value of **1133.0 µg/ml** and **Selectivity index (SI)** value of **0.04**.

----------End of Report----------