क्षेत्रीय जैवप्रौद्योगिकी केन्द्र
राष्ट्रीय महत्ता की संस्था, जैवप्रौद्योगिकी विभाग,
भारत सरकार द्वारा यूनेस्को के तत्वावधान में स्थापित
Regional Centre for Biotechnology
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SARS-CoV2 Antiviral Testing Report

(To be used only for research purpose)

Assay Method – Cytotoxicity

- The assay is done in a 96-well plate format in 3 wells for each sample.
- 1x10e4 VeroE6 cells were plated per well and incubated at 37-degree C overnight for the monolayer formation.
- Next day, cells were incubated with the test substance (TS) at the indicated concentration. The control cells were incubated with 1% ethanol.
- 24 and 48 hours later, cells were stained with Hoechst 33342 and Sytox orange dye.
- Images were taken at 10X, 16 images per well, which covers 90% of well area using ImageXpress Microconfocal (Molecular Devices).
- Hoechst 33342 nucleic acid stain is a popular cell-permeant nuclear counterstain that emits blue fluorescence when bound to dsDNA. It stains all the live and dead cells.
- Sytox orange dye stains nucleic acids in cells with compromised membranes. This stain is an indicator of cell death.
- First, the software will count total number of cells in the Hoechst image.
- In the Sytox image, it will count, among Hoechst positive cells, how many cells are positive for sytox.

Assay Method – Antiviral screening

- The assay is done in a 96-well plate format in 3 wells for each sample.
- 1x10e4 VeroE6 cells were plated per well and incubated at 37-degree C overnight for the monolayer formation.
- Next day, cells were incubated with the test substance (TS) at the indicated concentration. The control cells were incubated with 1% ethanol only. The cells were infected with SARS-CoV2 at a MOI of 0.01.
- 24 and 48 hours later, viral RNA was extracted from 100 µl culture supernatant and subjected to qRT-PCR (in duplicates) where Ct values for N and E gene sequence were determined.
- Inhibition of virus replication is determined based on the fold change in the Ct value in TS-treated cells compared to the control.
- Remdesivir was used as a positive control for viral inhibition.

Harjinder Singh

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Results

Compound name	Concentration (µM)	% Cell viability		% inhibition of virus replication			
		24 hr	48 hr	24 hr post-infection		48 hr post-infection	
				E	N	E	N
Remdesivir	10 µM	99.23	94.37	72.26	77.61	99.64	99.76
Ambe_Bioimmune (dil)	2 µl added	95.45	92.16	85.97	85.89	98.3	97.7
Ambe_Bioimmune (conc)	2 µl added	80.27	54.97	86.38	85.95	99.9	99.91

Note: Conc.: 2 µl was taken from 9mg/ml solution and added into 200 µl reaction. Note: Dil.: 2 µl was taken from 0.9mg/ml solution and added into 200 µl reaction

Nirpendra Singh

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