REGIONAL CENTRE FOR BIOTECHNOLOGY

Antiviral activity testing against SARS-CoV2

To meet the growing need for the *in vitro* and *in vivo* antiviral assays for the new drug candidates/test substance (TS), Regional Centre for Biotechnology (RCB, for *in vitro* antiviral test at non-cytotoxic concentration) and Translational Health Science and Technology Institute (THSTI, for *in vivo* assay for test substance showing the *in vitro* antiviral activity) have jointly decided to provide these tests.

SARS-CoV2 cultures have been set up in the BSL-3 facility and are ready to help with the *in vitro* anti-viral assay for drugs/herbal extracts/formulations in the cell culture model at a noncytotoxic concentration of the TS. The process for the testing is given in Annexure-1.

Since we are getting a large number of requests, their priority is assessed on the basis of their scientific merit. You are requested to provide the following information.

Requested by (name of the contact person):

Affiliation (Name of the Organization/University/Company):
Status: Academic/Start-up/MSME/Big Pharma Company
Email:
Phone:

Number of TS to be tested:
IDs/Names of the TS:

Solubility of the TS: (Please fill in the solubility below as applicable)
…….. mM (for pure compounds) in water/DMSO/Alcohol
…….. mg/ml (for extracts/formulations) in water/DMSO/Alcohol

Scientific basis for antiviral testing with supporting data / literature (no more than 1 page):

Charges for the testing services (in Rs.): The amount is payable in advance.

Test	academic and start-ups	MSME	Big Pharma company
Toxicity in Vero E6 at 2 conc.	10,000	15,000	20,000
Antiviral testing at the highest non cytotoxic concentration	20,000	30,000	50,000
7-point IC50 determination	25,000	40,000	60,000
GST @18% is chargable on the above.			

These assays will be performed in a sequence and for any given assay the cost of the previous assay/s is payable. Cytotoxicity assay is mandatory.

For further information please contact Dr Nirpendra Singh (nirpendra@rcb.res.in, 9910605664)

Annexure-1

Testing of small molecule / herbal extract / formulation (test substance, TS) for anti-SARS-CoV2 activity in the cell culture

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Requirements:

1. The TS should be soluble in water, alcohol or DMSO at a minimum conc. of 1 mM (for molecule) or 1 mg/ml (for herbal extract/formulation).
2. The TS shall be provided preferably as a solution with conc. as recommended above.
3. The solubilized TS should remain in solution in the cell culture medium (DMEM+2.5% FBS) at the final conc. of 1 or 10 micromolar (for molecule) or 10 or 100 microgram/ml (for herbal extract/formulation).
4. The highest conc. of TS that remains in solution (out of the above prescribed conc.) in the cell culture shall be tested further.
5. The requester is required to check this before sending the TS to RCB.

Toxicity testing in the cell culture:

As the antiviral activity shall be tested in the Vero E6 cells, the TS should not be cytotoxic to these cells at the above-mentioned concentration/s.

Assay is done in a 96-well plate format in 3 wells for each sample. 1x10e4 cells are plated per well and incubated at 37^0C overnight for the monolayer formation. Cells will be incubated with the TS at the conc. 10 mcM or 100 mcg/ml as well as at a 10-fold lower conc. The control cells will be incubated with culture medium with corresponding conc. of the vehicle or with only the culture medium. 48-h later cell viability will be assessed by incubation with appropriate stain and reading the plates spectrophotometrically. Viability shall be calculated against the control cells.

Antiviral testing will be undertaken only if there is at least 70% viability at any of the two concentrations tested. Highest non-cytotoxic dose of the TS shall be used.

Antiviral testing and IC50 determination in the cell culture:

Generally, we will follow the method described by Caly et al., Antiviral Research, 178 (2020) 104787. Remdesivir or any other known inhibitor for the SARS-CoV2 will be used as a positive control in the assay.

Briefly, the assay is done in a 96-well plate format in 3 wells for each sample. 1x10e4 cells are plated per well and incubated at 37^0C overnight for the monolayer formation. Cells will be incubated with the culture medium with TS at the highest non-cytotoxic conc. determined above. Soon after (within 5 min), virus will be added to each well at a defined MOI. Control cells are incubated with culture medium with corresponding conc. of vehicle (if TS is dissolved in DMSO/ethanol) or with only the culture medium. Plates will be incubated at 37^0C and culture supernatant harvested at 24 h and 48 h later. Viral RNA extracted from 50 mcl culture supernatant shall be subjected to qRT-PCR and Ct values for N and E gene sequence detection shall be reported. These data shall be used for calculating the % virus inhibition, if any.

For the IC50 determination, virus inhibition as above shall be tested at different conc. to generate a 7-point inhibition curve.