Translational Health Science and Technology Institute
Antiviral activity testing against SARS-CoV2

To meet the growing need of the *in vitro* and *in vivo* antiviral testing for the new drug candidates/test substance (TS), Translational Health Science and Technology Institute (THSTI), for antiviral test in hamster and hACE2 mice model) and Foundation for Neglected Disease Research (FNDR), for in-vitro Viro-E6 cell line based antiviral testing along with hamster model) and THSTI (DMPK evaluation) have joined hands under a BIRAC grant to provide these tests.

The scope of work related to in vitro screening and pre-clinical animal model and DMPK evaluations will be undertaken at THSTI and FNDR. You are requested to provide the following information along with 50% payment in advance (see Annexure 1). The detailed protocols are provided in the Annexures appended below Annexure 2 and Annexure 3.

Since we are getting many requests, the testing priority will be assigned on the basis of the scientific merit by the committee.

Please mail your queries to antiviral@thsti.res.in (Dr. Shinjini Bhatnagar, THSTI), with the scientific basis for antiviral testing with supporting data / literature (not more than 1 page).

Charges for the testing services per sample are given in the table below (Rs.).

For in vitro studies: 100% on completion of study

For in vivo studies: 50% amount is payable in advance (RTGS details for THSTI, FNDR)

Annexure1: Costing Table

Test	Subsidized rate for academia and government institutes*	Subsidized rate for Start-ups and MSME**	Current Cost/ Big Pharma
In vitro anti-viral testing (single concentration, includes cytotoxicity) FNDR	7,500	10,000	20,000
In vitro anti-viral testing (dose response, includes cytotoxicity)	37,500	50,000	1,00,000
Anti-viral testing in hACE2 transgenic mice model (single batch for single TS: 26 animals)	4,50,000	10,50,000	17,50,000
Anti-viral testing in hamster model (single batch for single TS: 20 animals	4,50,000	10,50,000	17,50,000
PK Studies			
GST @18% is chargeable on the above.			
*Discount of 80% for academia and government institutes			
**Discount of 50% for Start-ups and MSME			
***Discount of 20% for Big Pharma			

Annexure 2: Deliverables

SCOPE OF WORK:

THSTI/FNDR deliverables will be as follows:

1. Obtaining various regulatory clearances for the study execution (this is mandatory for each sample to be evaluated)
2. Prophylactic or therapeutic treatment of animals as decided by the company/academia.
3. Challenge of animals with 10^4-10^5 PFU SARS-CoV2 infection through intranasal route on day 0.
4. Monitoring and recording body weight of the animals for 4 days post challenge (for hamsters) and 6 days post challenge (mice). Percentage body weight change graph would be provided.
5. Sacrifice of all the animals at the end point of the study (day 4 for hamster and day 6 for mice).
6. Isolation of lung, spleen, and blood from the sacrificed animals.
7. Images of lung post necropsy would be provided.
8. Relative viral load determination from lung samples.

Readouts that can be included on additional cost.

1. H&E staining and assessment of pathology for lung samples
2. Relative qPCR for cytokines (any 3: IFNy, IL4, IL17A, TNFa and IL6) along with a house keeping gene from spleen samples.

Annexure3: Protocols

FNDR:

Toxicity and anti-viral testing in the cell culture:

Antiviral efficacy assay is performed in Vero E6 cells to determine any antiviral activity Pre-formed Vero E6 cell monolayers in a 96-well cell culture plate are infected with the virus (SARS-CoV-2 Isolate USA-WA1/2020) at MOI of 0.01, along with test item at desired concentrations in DMEM. The infected cells are incubated for 1 h at 37 °C in a humidified CO_2 (5%) incubator. After the completion of incubation, the inoculum containing the virus and test item are removed. The cell monolayer is then overlaid with cell culture medium containing equal volumes DMEM (2x) and 2% carboxymethylcellulose, and the test compound at desired concentrations and incubated for 3 days at 37 °C in a humidified CO_2 (5%) incubator. After that, the plates are fixed with formaldehyde (4%) and stained with crystal violet (0.01%). Plaques are counted for each well and the PFU/ml is calculated and compared against the virus only control. EC_{50}/IC_{50} is determined using GraphPad Prism (v9). Positive control (remdesivir) is also reported. Before the antiviral assay, a cytotoxicity assay with crystal violet readout is performed. Dose response assays are performed using 6 concentrations in duplicate. Single concentration assay is performed in duplicate.

THSTI & FNDR: Protocol for hamster and mouse infection models

1. SARS-CoV2 challenge protocol.

1.1 ***Infection of SARS-CoV2***

1.1.1 Pre-treatment group dosing will be started prior to the challenge as defined by the company/academia.

1.1.2 Virus challenge of the animals (except unchallenged control group) would be done on day 0.

1.1.3 Animals would be inoculated with 10^4-10^5 PFU SARS-CoV-2 under anesthesia. Unchallenged animals would receive mock PBS intranasal.

1.1.4 Dosing of the therapeutic group would start on the day of challenge or one day after the challenge as defined by the company/academia.

1.1.5 Positive control group will receive Remdesivir sc/ip injections 15mg/kg (hamster) or 25 mg/kg (mouse) one day prior to challenge and one day post challenge and continued for next 3 days (for mice).

1.2 Observations, clinical signs, euthanasia, and sample collection

1.2.1 Body weight of individual hamsters would be recorded on daily basis post challenge till the end point.

1.2.2 All the hamsters would be euthanized on day 4 post challenge or 6 days post challenge (mice) with high dose of anaesthetic.

1.2.3 Blood, lung, spleen would be collected from all the animals.

1.2.4 A portion of the lung would be fixed in 10% formalin for histology. Rest of the lung would be homogenized and used for viral load quantitation by PFU/TCID-50 or RT-PCR method.

1.2.5 Blood would be used for serum isolation and stored till further use.

1.2.6 Spleen would homogenized and used for gene expression profiling (optional).

2. Data

2.1 Changes in body weight of the animals, gross morphology images of lung would be reported.

2.2 Relative lung viral load would be determined and reported.

2.3 OPTIONAL- Cytokine profiles and H & E stained lung images would be provided along with the histological scores for each group.

Grouping details:

Animal groups for TS anti-viral screening would be as follows for both mice and hamsters. (All the assays would be undertaken only after IBSC, RCGM and IAEC)

Groups	Mice	Hamster
Uninfected control	5	5
Infection control	7	5
Remdesivir group	7	5
Test group	7	5

Note: Above is the group for single batch for single test group. If more than one test group is required to be included the number of groups and costs will increase proportionately.