**Testing of Antiviral activity against SARS-CoV2**

ILS Bhubaneswar has established the SARS-CoV2 cultures and is ready for service-based testing of drugs/herbal extracts/formulations in *in-vitro* anti-viral assay using the SARS-CoV2 cell culture model.

Application form for *in-vitro* SARS-CoV2 Antiviral activity assay

1. **Requested by** (name of contact):
2. **Affiliation** (Name of the Organization/University/Company):
3. **Status** (Academic/Start-up/MSME/Big Pharma Company):
4. **Contact Details**
5. Address:
6. Email:
7. Phone:
8. **Test Substance Details** (enclose as annexure with the application form)
9. Number of test substances (TS) to be tested:
10. IDs/Names of the TS: (enclose as annexure with the application form):
11. Solubility of the TS: (Please fill in the solubility below as applicable)
12. ­Solubility:
13. For Pure Compounds, in water/DMSO/Alcohol in \_\_\_\_**mM** concentration for pure compounds. Other information\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
14. For extracts/formulations, in water/DMSO/Alcohol or other organic extracts in **\_\_\_\_ mg/ml**. Other information\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
15. Cytotoxicity information (if available):
16. Brief write up (no more than 1 page) for the scientific basis for antiviral testing with supporting data/literature.
17. Charges for the testing services: **All the amount is payable in advance (non-refundable).**

The assays for cytotoxicity is mandatory and will be performed prior to proceeding for antiviral activity assay.

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| **Assay** | **Academic/Start-ups** | **MSME** | **Pharma Company** |
| Cytotoxicity @ 3 conc | Rs 10000 | Rs 15000 | Rs 20000 |
| Antiviral Activity at the highest non cytotoxic conc  | Rs 20000 | Rs 30000 | Rs 40000 |
| IC50 with 7 conc.  | Rs 25000 | Rs 40000 | Rs 50000 |
| GST @ 18% to be added |

**Contact at ILS Bhubaneswar:** Dr. Nivedita Jena, COO, DBT-ILS Bioincubator

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**Basic features of the test compound(s) required for testing**

* The solubility of the test compounds should be at least 10 mM in water/DMSO/Alcohol.
* The solubility of the natural extract/formulation should be at least 1 mg/ml in water/DMSO/Alcohol or other solvents
* The compounds/extracts should remain solubilized at the indicated concentration mixed with cell culture medium (DMEM+2.5% FBS) and should not precipitate.
* The requester can make a special request if the test compounds/extracts do not meet the above criteria of minimum solubility but are soluble at lower amounts in water. Water soluble compounds/extracts at conc lower than 1mM or 1mg/ml can also be taken up for testing after evaluating the cytotoxicity.
* The highest concentration of compounds/extracts that remain solubilized in cell culture medium will be taken up for further testing of cytotoxicity and antiviral activity.
* **The investigational substance should be provided to ILS in powder form (lyophilised) or as a solution with minimum or higher concentration as recommended above.**
* **It is the requestor’s responsibility to ship the test compounds in proper manner. Damaged shipment/material will not be considered for testing.**
* **The test sample that are not as per recommendations will not be considered for testing (consult prior to submission in case of queries)**
* The volume of the solubilized test compound provided (minimum 500ul-1ml) should be at enough to filter through 0.22 micron syringe filters and for subsequent determination of cytotoxicity (3 conc in triplicates) and antiviral activity with the maximum non-toxic concentration.

**Note**: The requester has to cross check all these parameter prior to submission for testing at ILS Bhubaneswar

Brief details of the *in-vitro* anti-viral and cytotoxicity assays are given below.

**Cytotoxicity Assay:** The cytotoxicity assay will be performed as per the kit used for the assay. Briefly, Vero E6 cells will be seeded in the 96 well plate at 80% confluency and treated with various concentrations of the test compounds or extracts. For toxicity determination minimum of 3 concentrations will be used. 24h post-treatment the MTT assay will be performed according to the manufacturer’s instructions. Each concentration will be assayed in triplicates and the percentage cell viability will be calculated with respect to vehicle control.

**Antiviral Activity Assay:** The assay will be performed as per the protocol routinely followed for determining antiviral activity against SARS-CoV2. Known inhibitors of SAR-CoV2 will be used as positive control in the assay. Vero E6 cells seeded in 96 well plates at 80% confluency will be infected with SARS-CoV-2 isolate at an MOI of 0.1 for 2 h. Subsequently the inoculum will be aspirated and fresh media containing different concentrations of the test compounds/extract will be added to the cells. Compounds showing more the 50% anti-SARS-CoV2 activity will be considered for IC50 determination. IC50 will be determined using a minimum of 4 different concentrations. 24h post-infection the supernatant will be subjected to viral RNA isolation followed by qRT-PCR for determining the SARS-CoV-2 viral load in the cells (cell associated) and culture supernatants (released virus particles). qRT-PCR will be performed using primers specific for the viral spike, nucleocapsid and ORF1a for both released and cell-associated virus. Percentage reduction of viral loads in cells and culture supernatants will be plotted in comparison to vehicle treated controls.