**SARS-CoV-2 In Vitro Anti-viral Compound Testing Service Request**

**Date:**

**Requestor (lab point of contact):**

**Email:**

**Laboratory PI:**

**Duke Fund Code:**

**Overview:**

Due to the world-wide COVID-19 pandemic and urgent public health crisis, the need for testing to find efficacious antiviral compounds is imperative. To address this issue, the Duke RBL Virology Unit can assay *in vitro* antiviral effects of submitted compounds under BSL3 containment as a service. Briefly, cells lines of interest are treated in a dose-response fashion with dosage and duration determined by the requester. These cells are then infected with a multiplicity of infection (MOI) of SARS-CoV-2 (strain USA-WA1/2020) determined by the requester. Supernatant (100 µL) is collected at a time determined by the requester and titered using 50% Tissue Culture Infectious Dose assay (TCID50). A data summary detailing experimental details, methods, QC/QA, and raw titer values will be released at the conclusion of testing.

Containment use fees, PPE, staff labor time and assay consumables will be invoiced via CoreResearch@duke. Due to the high demand we are unable to provide individualized cost estimates. The assay outlined below is ~$3,500 and will require 7 days in BSL3 containment and ~20 hours of approved BSL3 worker labor.

**Questions regarding experimental design and scheduling can be directed to Dr. Trey Oguin:**

[**thomas.oguin@duke.edu**](mailto:thomas.oguin@duke.edu)

**Requesting Party Requirements:**

* All compounds are approved to be used safely by laboratory staff. Documentation required at time of submission.
* All compounds submitted for testing will be labeled clearly with printed labels, and a digital sample manifest will be provided by requester at time of submission. No hand-written items will be accepted.
* All compounds will be tested in a 96 well format. Ensure enough volume for dilution series and replicates.
* All controls will be provided by requesting party.
* All vehicle/diluent will be provided by requesting party.
* Cell lines that are not Vero E6 cells will be provided by requesting party alongside QC report of the cell line. *Note: submitted cell lines must grow readily on plastic dishes at standard laboratory conditions.*
* Culture medium and required supplements must be provided by requesting party.
* **Maximum batch size is 2 treated 96 well plates/day. This translates to 16 test/control on 96 well titer plates.**

**Experimental Details from Requesting Party:**

* Cell line for anti-viral testing:
* If not Vero E6, what is cell plating density (96 well plate):
* If not Vero E6, what is media requirements:       (please provide)
* Number of anti-viral samples to be tested (max 8 per experiment):
* Earliest date test material will be available:
* Anti-viral treatment duration:
* SARS-CoV-2 MOI:
* Supernatant collection time(s):

**Approach:**

1. Submit drugs, diluent, and controls. Virology Unit will dilute compounds and treat Vero E6 cells for recommended times. Vero E6 cells will be infected at recommended MOI. Supernatant will be collected at recommended times and titered on a fresh batch of Vero E6 cells to determine infectious viral titer.
2. Cells other than Vero E6. Pre-plated/pre-treated cells in 96 well plates will be submitted to the Virology Unit. If the cells cannot be treated before submission, the Virology Unit can treat the cells as per the requesting party’s recommendation. The supplied materials will be infected and supernatant will be titered on Vero E6 cells to determine infectious viral titer.
3. Workflow:
   1. Cells will be plated the day before infection
   2. Cells will be washed 1 time with DPBS
   3. Cells will be treated in 100 µL
   4. Cells will be infected, 10 µL virus/well at requested MOI
   5. Supernatant will be collected at requested timepoint
   6. Supernatant will be titered
   7. Viral titer will be scored and calculated

**Deliverables:**

* Raw viral titer values from supernatant will be determined by TCID50 as measured by either vitality staining or fluorescence emission.
* Data will be reported as TCID50/mL as determined by the Reed-Muench method.
* Each well will be titered in an 8-point dilution series, from neat – 10-7.
* An Excel based spreadsheet containing viral titer will be provided at study conclusion.
* Cytotoxicity or other host response measurements will not be assessed by the Virology Unit. It is highly recommended that the requesting party empirically determine cytotoxic effects before submission.

**Example Experimental Setup:**

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