TEXAS BIOMEDICAL
RESEARCH INSTITUTE

STATEMENT OF WORK (SOW)

In Vitro Assessment of the Effect of 3VM1000 Suspension on SARS-CoV-2 Infectivity

OBJECTIVE

The purpose of this study is to assess the effect of **3VM1000 Suspension** on SARS-CoV-2 infectivity of host cells *in vitro*. Pre-challenge study to determine if **3VM1000 Suspension** is effective in killing SARS-CoV-2 and reduce its infectivity *in vitro*.

EXPERIMENTAL DESIGN

Cell Culture and Virus Propagation

Vero E6 cells (ATCC# CRL 1586) are cultured in DMEM (Dulbecco Modified Eagles Medium) containing 5-10% FBS and 1% P/S. Cells are maintained at 37°C and 5% CO2. Samples of SARS-CoV-2 are obtained from BEI Resources (2019-nCoV/USA-WA1/2020 strain). Stocks are prepared by infection of confluent monolayers of Vero E6 cells (T225 flasks) for three days. Media are collected and clarified by centrifugation prior to being aliquoted for storage at -80°C. Titer of stock is determined by plaque assay using Vero E6 cells in 6-well plates as described previously. All work with infectious virus is performed in a Biosafety Level 3 laboratory.

Test Materials

Sample: 3VM1000 Suspension API (Concentration 100%).

Procedure

Cellular cytotoxicity (CC50) determination of 3VM1000 Suspension API
Vero E6 cells (5X104 cells/well, 96 well plate format). 24 h later, 3VM1000 suspension will be serially diluted (10 dilutions) starting from 100% solution and will be added in the wells for 1 hr, 6 hr and 24 hr followed by MTT assay to measure the cell cytotoxicity.

Effective concentration determination (EC50):

Condition 1: To study the effect of drug on virus deactivation

1. Vero E6 cells will be plated (5X104 cells/well, 96 well plate format).

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2. 24 h later, serially diluted drug (**10 dilutions**) will be incubated with SARS-CoV-2 (MOI 0.01 or to be determined) at 37°C for 1hr or 6 hr.
3. Vero E6 cells (5X10^4 cells/well, 96 well plate format) will be treated with serially diluted drug (10 dilutions) pre-incubated with SARS-CoV-2 (MOI 0.01 or to be determined) for 1 h or 6 hr at 37°C.

4. After incubation of cells with virus-drug mixture for 1 h at 37°C, virus-drug mixture will be removed and post-infection media (DMEM 2% FBS, 1% PS) and avicel will be added.
5. Cells exposed to the virus (no drug), cells only, and cells with vehicle control will serve as assay controls.

6. At 24 h post-infection, cells will be fixed and stained with an anti-NP antibody against SARS-CoV-1 that cross-reacts with SARS-CoV-2. Viral plaques will be quantified in an ELISPOT reader.

Condition 2. (Drug treatment after virus exposure)

1. Vero E6 cells (5X10^4 cells/well, 96 well plate format) will be infected with SARS-CoV-2 (MOI 0.01 or to be determined).
2. After 1 h of viral absorption at 37°C, virus inoculum will be removed and post-infection media (DMEM 2% FBS, 1% PS) containing the serially diluted drug (10 dilutions) and avicel will be added.
3. Cells exposed to virus (no drug), cells only, cells with vehicle and positive control (Remdesivir) will be used as internal control.
4. At 24 h post-infection, cells will be fixed, permeabilized and stained with an anti-NP antibody against SARS-CoV-1 that cross-react with SARS-CoV-2. Viral plaques were quantified in an ELISPOT reader.

Deliverables

Deliverables will be a Final Summary Report with Graphs as well as Raw Data from Experiments.

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The duly authorized party representatives execute this Statement of Work, including all its terms and conditions.

3V Medical Research Group, Inc.	**Texas Biomedical Research Institute**
By:	By:
Name: Dominic C Abbott	**Name: Bruce Edwards**
Position: Executive Vice President & COO	**Position: Vice President Finance & Administration, CFO**
Date: 7-22-2020	Date: 7/22/2020

I acknowledge that I have read this Statement of Work in its entirety and will use reasonable efforts to uphold my obligations and responsibilities under this Statement of Work.

PRINCIPAL INVESTIGATOR

Signature: Varun Dwi

Name: Varun Dwivedi, Ph.D.

Title: Staff Scientist I

Date: 7-22-2020