TEST FACILITY: University of Tennessee Health Science Center – Regional Biocontainment Laboratory

TEST COMPLETION DATE: 22 June 2021

STUDY NUMBER: 2021.037.02

TITLE: Evaluate of Virucidal Effectiveness of Indigo Clean Fixture Against SARS-CoV-2

SPONSOR: Kenall

STUDY POC: Shannon Taylor, PhD

STUDY DIRECTOR: Dong Yang, PhD

I. SAMPLES AND TEST CONDITIONS:

Virus Strain - SARS-CoV2 p3 Isolate USA-WA1/2020 (Inoculum contains 5% FBS)
Host Cells – Vero E6
Infective Titer Measurement – Plaque Assay
Method – Germicidal Light Testing – 0.6 mW/cm2

Samples:

- 4 samples at each time point (8h)
- 4 equivalent control samples (ambient light control)
- 3 samples of initial viral load (T0 washout)
- Virus stock titration
- 2 viability controls (no virus)

II. CALCULATIONS:

Percent Reduction = % Reduction=(C-T)*100/C

Log Reduction = Log_{10} (C/T)

C = mean viable virus recovered from untreated control carriers

T = mean viable virus recovered from treated carriers

III. RESULTS:

Table 1. Data and Reduction Results

Sample	Replicates (pfu/ml)				Mean	Mean Log_{10} Reduction	Mean % Reduction
	# 1	# 2	#3	#4			
Viral Load T0	9.50E+05	7.75E+05	9.00E+05	-	8.75E+05	-	-
Ambient Light 8h	1.00E+05	1.28E+05	1.15E+05	1.00E+05	1.11E+05	-	-
Irradiance 8h	6.50E+02	7.25E+02	7.75E+02	7.75E+02	7.31E+02	2.18	99.339

% Reduction based on Log_{10} Reduction when compared to equivalent control

Table 2. Performance Reference

Log Reduction	***% Reduction***
1	90%
2	99%
3	99.9%
4	99.99%
5	99.999%
6	99.9999%

IV. CONCLUSIONS:

Please refer to performance reference Table 2 for appropriate % reporting.

V. PROTOCOL:

1.0 TITLE: EVALUATE THE VIRUCIDAL EFFECTIVENESS OF INDIGO CLEAN FIXTURE

2.0 SPONSOR: KENALL

3.0 TESTING FACILITY: UTHSC RBL

4.0 STUDY DIRECTOR: DONG YANG

5.0 BACKGROUND: Previous testing has suggested the Indigo Clean fixture has some virucidal potential.

6.0 OBJECTIVE:

This study will use an *in-vitro* method to evaluate the virucidal effectiveness of Indigo Clean against SARS-CoV-2. The plaque-forming unit (pfu) reductions will be determined following a 8 h exposure time at one irradiance settings. Plaque assay will be performed in duplicate and incubated for 72 hours at 37°C 5% CO_2 following the approved SOP of the UTHSC RBL.

7.0 MATERIALS:

- Indigo Clean Fixture: provided by Kenall.

  Used to place the fixture 10" from the samples to be irradiated (see photo). Note that the rig shown consists of two parts. The lower part is only used during factory calibration and is removed when testing is performed.

- 24 well plates: provided by UTHSC
  - 4 samples at each time point (8h)
  - 4 equivalent control samples (ambient light)
  - 3 samples of initial viral load (T0 washout)
  - Virus stock titration
  - 2 viability controls (no virus)

  Total sample # treated: 4

  Total sample # untreated: 4

- Virus: SARS-CoV-2 strain (2019 Novel coronavirus, strain 2019-nCoV/USA-WA1/2020): provided by UTHSC RBL. The virus inoculum contains 5% FBS.
- All the consumables for plaque assay: provided by UTHSC RBL.

8.0 CHALLENGE VIRUS PREPARATION:

The challenge strain: SARS-CoV-2 strain (2019 Novel coronavirus, strain 2019-nCoV/USA-WA1/2020); BEI Resources access number: ATCC No. NR-52281. The titer of the stock virus is 2 x 10^6 pfu/mL.

8.1 Amplify virus: Follow the UTHSC RBL standard protocol.

8.2 Titration of the stock virus: Follow the UTHSC RBL standard protocol.

9.0 METHODOLOGY:

9.0 The Indigo Clean fixture will be installed in the biosafety cabinet (BSC). Test plates will be placed 10" from the fixture as indicated in the photo and treated with the lid off.

9.1 In a BSC within a BSL-3 laboratory, the SARS-CoV-2 strain (1×10^6 pfu in 500µl) will be added to 24 well plates. A T0 washout will be immediately collected after plating.

9.2 Samples with be irradiated at 0.6 mW/cm2 (based on the settings of the device provided). Controls will consist of virus exposed to ambient light. At the end of the exposure time period 8 hours, virus in wells will be washed with 1 mL PBS and collected into a tube for plaque assay to quantify viable virus after the treatments.

9.3 The plaque numbers will be counted and compared to the control group to calculate efficiency of virus killing by the Indigo Clean fixture vs control sample as a reference. Log reduction and corresponding percentage reductions will be calculated.

**ASTM E3135-18 will be used as a reference for this study. However, this is a modified study and the sponsor will determine the significance with the data provided at the conclusion of the study (and in accordance with the appropriate agencies regarding claims).