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# AMENDED CONFIDENTIAL FINAL REPORT

**SPONSOR:** Nanoplus Life Biomedical Technology Co., Ltd.

**SPONSOR'S REPRESENTATIVE:** Cecilia Hung

**STUDY TITLE:** VIRUCIDAL HARD-SURFACE EFFICACY TEST– SARS-CoV-2 (COVID-19 Virus) Delta Variant

**STUDY IDENTIFICATION:** Microbac Project No. 1124-101 (refer to signed Protocol No. NAN.V.21.001)

| TEST AGENT NAME | LOT NO. | DATE RECEIVED | DS NO. |
|---|---|---|---|
| OHTrust Nano Ion Water | 51221070001 | 07/19/21 | L773 |

**CHALLENGE ORGANISM:** SARS-CoV-2 (COVID-19) Delta Variant, Strain: hCoV-19/USA/PHC 658/2021 (B.1.617.2), Source: BEI Resources, NR-55611

**HOST CELL LINE:** Vero-E6 cells, Source: ATCC CRL-1586

**DILUTION MEDIUM:** MEM + 2% Newborn Calf Serum (NCS)

**NEUTRALIZER:** MEM + 10% FBS + 1% HEPES + 1% $NaHCO_3$ + 0.025 N NaOH

**CONTACT TIME:** 10 minutes, 30 minutes

**CONTACT TEMPERATURE AND HUMIDITY:** Room Temperature (20±1°C, Actual: 21°C with 50-61% Relative Humidity)

**NUMBER OF REPLICATES:** 1 replicate (four wells per dilution)

**INCUBATION TEMPERATURE:** 36±2°C with 5±3% $CO_2$



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**INCUBATION TIME:** 4-9 days (Actual: 9 days)

**DILUENT:** N/A

**CALCULATION OF TITER:** The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the Spearman-Karber method using the following formula:

$$m = x_k + \left(\frac{d}{2}\right) - d\sum p_i$$

where:

- m = the logarithm of the dilution at which half the wells are infected relative to the test volume
- $x_k$ = the logarithm of the smallest dosage which induces infection in all cultures
- d = the logarithm of the dilution factor
- $p_i$ = the proportion of positive results at dilution i
- $\sum p_i$ = sum of $p_i$ (starting with the highest dilution producing 100% infection)

The values were converted to $TCID_{50}$/mL using a sample inoculum of 0.05 mL.

**CALCULATION OF TITER (CONTINUED):**

When a sample contains a low concentration of virus there is a discrete probability that if only a fraction of the sample is tested for virus, that fraction will test negative due to random distribution of virus throughout the total sample. The probability, p, that the sample analyzed does not contain infectious virus is expressed by: $p = [(V-v)/V]^y$, where V is the total volume of the container, v is the volume of the fraction being tested, and y is the absolute number of infectious viruses randomly distributed in the sample. If V is sufficiently large relative to v, the Poisson distribution can approximate p:

$$P = e^{-cv} \qquad \text{or} \qquad c = -[\text{Ln}(P)] / v$$

Where c is the concentration of infectious virus and v is the total sample volume. If all n wells are negative, the virus titer after the process is considered to be less than or equal to this value. The total volume of sample assayed is v = v'nd, where v' is the test volume in a well, n is the number of wells per sample, and d is the sample dilution.

**RESULTS:** Results are presented in Tables 1– 5.

Viral Load Calculation:

Load ($Log_{10}$ $TCID_{50}$) per carrier = Titer ($Log_{10}$ $TCID_{50}$/mL) + $Log_{10}$ [volume per carrier (mL)]

Key (for all tables):

| | |
|---|---|
| T/y = | Cytotoxicity observed in y wells inoculated; viral cytopathic effects (CPE) could not be determined |
| X/y = | X wells out of y wells inoculated exhibited positive viral cytopathic effect |
| 0/y = | 0 out of y wells inoculated exhibited positive viral CPE; no cytotoxicity or bacterial contamination was observed in any of the wells inoculated |

## RESULTS (continued):

**Table 1**
**Plate Recovery Control (PRC)**

| Dilution* | PRC | |
|---|---|---|
| | 10 minutes | 30 minutes |
| $10^{-3}$ | 4/4 | 4/4 |
| $10^{-4}$ | 4/4 | 4/4 |
| $10^{-5}$ | 4/4 | 4/4 |
| $10^{-6}$ | 2/4 | 0/4 |
| $10^{-7}$ | 1/4 | 0/4 |
| $10^{-8}$ | 0/4 | 0/4 |
| Titer ($Log_{10}$ $TCID_{50}$/mL) | 6.25 | 5.50 |
| Load ($Log_{10}$ $TCID_{50}$)** | 5.78 | 5.03 |

*Dilution refers to the fold of dilution from the virus inoculum.
**Per carrier (0.34 mL of Undilute [$10^0$])

**Table 2**
**Test Substance**

| Dilution* | OHTrust Nano Ion Water, Lot No. 51221070001 | |
|---|---|---|
| | 10 minutes | 30 minutes |
| $10^{-2}$ | 0/4 | 0/4 |
| $10^{-3}$ | 0/4 | 0/4 |
| $10^{-4}$ | 0/4 | 0/4 |
| $10^{-5}$ | 0/4 | 0/4 |
| $10^{-6}$ | 0/4 | 0/4 |
| $10^{-7}$ | 0/4 | 0/4 |
| Titer ($Log_{10}$ $TCID_{50}$/mL) | ≤ 1.50 | ≤ 1.50 |
| Load ($Log_{10}$ $TCID_{50}$)** | ≤ 1.03 | ≤ 1.03 |
| $Log_{10}$ Reduction per mL | ≥ 4.75 | ≥ 4.00 |
| $Log_{10}$ Reduction per carrier | ≥ 4.75 | ≥ 4.00 |
| Percent Reduction | ≥ 99.99 | ≥ 99.99 |

*Dilution refers to the fold of dilution from the virus inoculum.
**0.34 mL of Undilute ($10^0$)

## RESULTS (continued)

**Table 3**
**Neutralizer Effectiveness/Viral Interference (NE/VI) and Cytotoxicity (CT) Controls**

| Dilution* | OHTrust Nano Ion Water, Lot No. 51221070001 | |
|---|---|---|
| | NE/VI | CT |
| $10^{-2}$ | 4/4 | 0/4 |
| $10^{-3}$ | 4/4 | 0/4 |
| $10^{-4}$ | 4/4 | 0/4 |

*Dilution refers to the fold of dilution from the mock inoculum.

**Table 4**
**Cell Viability Control (CVC)**

| CVC |
|---|
| 0/4 |
| Cells were viable; media was sterile |

**Table 5**
**Virus Stock Titer Control (VST)**

| Dilution* | VST |
|---|---|
| $10^{-4}$ | 4/4 |
| $10^{-5}$ | 4/4 |
| $10^{-6}$ | 1/4 |
| $10^{-7}$ | 0/4 |
| $10^{-8}$ | 0/4 |
| $10^{-9}$ | 0/4 |
| Titer ($Log_{10}$ $TCID_{50}$/mL) | 5.75 |

*Dilution refers to the fold of dilution from the virus inoculum.

## TEST SUBSTANCE EVALUATION CRITERIA:

According to the US Environmental Protection Agency, the test substance passes the test if the following criteria are met:

- The product must demonstrate a ≥ 3 $\log_{10}$ reduction on each surface in the presence or absence of cytotoxicity taking into acoun the level of neutralization and;
- If cytotoxicity is present, the virus control titer should be increased, if necessary, to demonstrate a ≥ 3 $\log_{10}$ reduction in viral titer on each surface beyond the cytotoxic level.

## CONCLUSION:

When tested as described, OHTrust Nano Ion Water passed the Virucidal Efficacy Hard Surface Test when SARS-CoV-2 (COVID-19) Delta Variant, containing 5.0% serum, was exposed to the test substance for 10 minute and 30 minutes at 21°C and 50-61% relative humidity. All controls met the criteria for a valid test. These conclusions are based on observed data.

Study Director: David Rieth 11/29/21

David Rieth Date