MICROBAC®

FINAL REPORT 2 of 3

VIRUCIDAL EFFICACY SUSPENSION TEST PER EN14476:2013+A2:2019 – Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

Test Product
Clinell Universal Wipes

Lot Number
MFB-020419-A

Performing Laboratory
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, Virginia 20164

Sponsor
GAMA Healthcare Ltd
2 Regal Way
Watford, WD24 4YL
United Kingdom

Laboratory Project Identification Number
903-103

TABLE OF CONTENTS

TEST SUMMARY

TITLE: VIRUCIDAL EFFICACY SUSPENSION TEST PER EN14476:2013+A2:2019 – Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

STUDY DESIGN: This study was performed according to the signed Protocol and Project Sheet(s) issued by the Study Director. (See Appendix I)

OBJECTIVE: This test conforms in principle to the European Standard EN 14476:2013+A2:2019.

TEST MATERIALS: Clinell Universal Wipes, Lot No. MFB-020419-A, received at Microbac on 05/18/20 and assigned DS No. K660

SPONSOR: GAMA Healthcare Ltd
2 Regal Way
Watford, WD24 4YL
United Kingdom

TEST CONDITIONS

Challenge virus:

Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus), strain: USA-WA1/2020, Source: BEI Resources, NR-52281

Host:

Vero E6 cells, Source: ATCC CRL-1586

Active ingredient(s):

DDAC, BZK, 2-PE

Test condition storage condition:

Dark, at ambient room temperature

Test product appearance:

Wipe

Neutralizer(s):

D/E Broth (Pre-test Cytotoxicity Evaluation and Virucidal Quantitative Suspension Test)
Newborn Calf Serum (NCS) (Pre-test Cytotoxicity Evaluation)

Dilution medium:

Minimum Essential Medium (MEM) + 2% NCS

Contact time:

30 seconds; 60 seconds (Virucidal Quantitative Suspension Test)
60 seconds (Pre-test Cytotoxicity Evaluation)

TEST CONDITIONS (continued)

Contact temperature(s):

20 ± 1°C (actual: 20°C) (Pre-test Cytotoxicity Evaluation and Virucidal Quantitative Suspension Test)

Interfering condition:

3 g/100 mL Bovine Serum Albumin (BSA) + 3 mL/100 mL erythrocytes (final concentration in reaction mixture: 3 g/L BSA + 3 mL/L erythrocytes) (“Dirty Condition”)

Dilutions tested:

Neat (i.e. ready-to-use)

Media and reagents:

MEM + 2% NCS
D/E Broth
NCS
MEM + 5% NCS
3 g/100 mL BSA + 3 mL/100 mL erythrocytes (10X Interfering Substance, “Dirty Condition”)

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164. The Pre-test Cytotoxicity Evaluation was lab initiated on 06/15/20 and concluded on 06/17/20. The Virucidal Suspension Test was lab initiated on 07/09/20 and concluded on 07/17/20. The study director signed the protocol on 06/15/20. The study completion date is the date the study director signed the final report.

All changes or revisions of the protocol were documented, signed by the study director, dated and maintained with the protocol.

RECORDS TO BE MAINTAINED

All testing data, protocol, protocol modifications, test material records, the final report, and correspondence between Microbac Laboratories, Inc. and the sponsor will be stored in the archives at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164, or at a controlled facility off site.

EXPERIMENTAL DESIGN OVERVIEW

Inoculum preparation:

The stock virus was prepared by infection of Vero E6 cells. The cultures were frozen at -60 to -90°C several days after infection. After freezing and thawing, cell-free stocks were prepared by centrifugation. The stock virus was then aliquoted and stored at -60°C or below until used in testing. The virus stock was diluted 1.67-fold in MEM + 5% NCS prior to use in testing.

Test product preparation:

One concentration of the test substance was tested: “Neat” (ready-to-use).

Pre-test Cytotoxicity Evaluation:

In a single run, 1.0 mL of the 10X Interfering Substance was mixed with 1.0 mL of DM. 8.0 mL of test substance was added was added and mixed using a vortex. The reaction mixture was held for the contact time at 20°C. Following the contact time, a 1.0 mL aliquot of the reaction mixture was drawn up and neutralized in 1.0 mL of ice-cold neutralizer. This post-neutralized sample was further quenched to 1:10, 1:30, 1:100, 1:300, and 1:1000.

Virucidal quantitative suspension test:

In a single run, 1.0 mL of the 10X Interfering Substance was mixed with 1.0 mL of the virus suspension, carefully avoiding the upper sides of the dilution tube. Then, 8.0 mL of test substance was added and mixed using a vortex. The reaction mixture was then held for the contact time at 20°C. Following the contact time, a 1.0 mL aliquot of the reaction mixture was drawn up and neutralized in 1.0 mL of ice-cold neutralizer. This post-neutralized sample was further quenched 1:300 with ice-cold DM within 30 minutes.

Infectivity Assay:

Selected dilutions of the sample were inoculated onto the plates at 0.05 mL per well, 8 wells per dilution or 160 wells per dilution (for the Large Volume sample only) and incubated at 36 ± 2°C with 5 ± 3% CO_2.

EXPERIMENTAL DESIGN OVERVIEW (continued)

The residual infectious virus in both test and controls was detected by viral-induced cytopathic effect (CPE). CPE is defined as cell rounding and sloughing off of the cell monolayer. After 8 days of incubation at 36 ± 2°C with 5 ± 3%, the plates were removed, read, and recorded for test-product specific cytotoxic effects and/or virus-specific cytopathic effect (CPE).

Virus Recovery Control (VRC):

This control was performed at time zero and at the end of the longer contact time. In a single run, 1.0 mL of the 10X Interfering Substance was mixed with 1.0 mL of the virus suspension, carefully avoiding the upper sides of the dilution tube. Then, 8.0 mL of DM was added and mixed using a vortex. 1.0 mL of the reaction mixture was immediately drawn up and neutralized in 1.0 mL of ice-cold neutralizer. The rest of the sample was held for the longer contact time at 20°C, then neutralized in the same fashion as above.

Neutralizer Effectiveness (NEC), Cytotoxicity (CT) and Viral Interference (VI) Controls:

This control was performed for each test substance using the longer contact time. In a single run, 1.0 mL of the 10X Interfering Substance was mixed with 1.0 mL of DM, carefully avoiding the upper sides of the dilution tube. Then, 8.0 mL of test substance was added and mixed using a vortex. The reaction mixture was then held for the longer contact time at 20°C. Following the contact time, a 1.0 mL aliquot of the reaction mixture was drawn up and neutralized in 1.0 mL of ice-cold neutralizer. This PNS was further quenched 1:300 with ice-cold DM within 30 minutes.

For Neutralizer Effectiveness: 4.5 mL of the post-quenched sample was spiked with 0.5 mL of stock virus and held in an ice-bath for 30 minutes. This was considered the 10^{-1} dilution. Selected dilutions were inoculated onto host cells as described in “Inoculation/Incubation”.

For Cytotoxicity: Selected dilutions were inoculated onto host cells as described in “Inoculation/Incubation”.

EXPERIMENTAL DESIGN OVERVIEW (continued)

For Viral Interference: 0.05 mL of PBS and 0.05 mL of the lowest non-cytotoxic dilution of the sample was added to an appropriate number of aspirated host cell plates and pre-treated for 60 minutes at 36±2°C with 5±3% CO_2. Following this adsorption period, the sample was removed from the host-cell containing plate, and aliquots of 0.05 mL/well of the 10^{-3} – 10^{-8} dilutions of the stock challenge virus were added to the host-cell monolayer (8 wells per dilution).

Virus Stock Titer Control (VST):

The virus stock was used to make serial ten-fold dilutions in dilution medium.

Cell Viability Control (CVC):

This control was performed to demonstrate that the host cells remained viable and to confirm the sterility of the media employed throughout the incubation period. 0.05 mL of DM was added to 8 wells of host cells and incubated in an identical manner as the test samples.

Large Volume (LV):

This sample was performed to increase the sensitivity of the assay. The PNS from the Virucidal quantitative suspension test for the “Neat” concentration was used to inoculate 160 wells of the host cell plates.

Inoculation/Incubation:

Each sample was used to make ten-fold serial dilutions in DM. 0.05 mL per well of appropriate dilutions were inoculated at eight wells per dilution or 160 wells per dilution (for Large Volume sample only) onto host-cell plates and incubated at 36±2°C with 5±3% CO_2 for 8 days.

CRITERIA FOR A VALID ASSAY

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied.

- Viral-induced cytopathic effect (CPE) was distinguishable from test product induced cytotoxic effect, if any.
- The difference of titer between the neutralized test product-treated and PBS-treated monolayers was ≤ 1.0 $\log_{10}$ in the viral interference control.
- The difference of titer between the NEC and VST was ≤ 0.5 $\log_{10}$.
- Virus was not detected in the cell viability control and was observed to be healthy and free from contamination at the end of the incubation period.
- The test virus suspension had a titer of at least 10^8 $TCID_{50}$/mL; or possessed at least a concentration which allowed the determination of a 4.0-$\log_{10}$ of the virus titer. The cytotoxicity of the product solution did not affect host cell viability in the dilutions of the test mixtures which was necessary to demonstrate a 4.0-$\log_{10}$ reduction of the virus.

PRODUCT EVALUATION CRITERIA

According to the EN14476:2013 + A2:2019 guidelines, the test product passes the test if there is at least a 4.0-log reduction in titer beyond the cytotoxicity level.

PROTOCOL AMENDMENTS / DEVIATIONS

PROTOCOL AMENDMENTS

See Appendix I.

PROTOCOL DEVIATIONS

No known procedural deviation from the study protocol or pertinent SOP’s occurred during the conduct of this study.

CALCULATION OF TITER AND 95% CONFIDENCE INTERVAL

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
$\sum p_i$ = the 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.

The viral titer of each sample is reported as ± the 95% confidence intervals. The standard deviation, σ_m, was calculated using the following formula:

$$\sigma_m = d_f \sqrt{\sum \frac{p_i(1-pi)}{(ni-1)}}$$

where:

d_f = the logarithm of the dilution factor
p_i = the proportion of positive results at dilution i
σ_m = the standard deviation
n_i = number of replicates at dilution i

and $\sum$ denotes the summation over dilutions beginning at the k^{th} dilution. The 95% confidence interval is m ± 1.963 σ_m/2.

CALCULATION OF TITER AND 95% CONFIDENCE INTERVAL (continued)

The below method (Poisson distribution) was used to perform the titer calculation:

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 – 6

The log_{10} Reduction Factor (LRF) was calculated in the following manner:

Log_{10} Reduction Factor = Initial viral load (Log_{10}) – Output viral load (Log_{10})

The Viral load was determined in the following manner:

Viral Load (log_{10} $TCID_{50}$) = Titer (log_{10} $TCID_{50}$/mL) + Log_{10}[Volume (mL) x Volume Correction]

Table 1
Pre-Test Cytotoxicity Control – Newborn Calf Serum

Dilution*	Clinell Universal Wipes
	Neat
1:10	Cytotoxicity observed
1:30	Cytotoxicity observed
1:100	Cytotoxicity observed
1:300	No cytotoxicity observed
1:1000	No cytotoxicity observed

* Dilution refers to the fold of dilution from the PNS.

Table 2
Pre-Test Cytotoxicity Control – D/E Broth

Dilution*	Clinell Universal Wipes
	Neat
1:10	Cytotoxicity observed
1:30	Cytotoxicity observed
1:100	Cytotoxicity observed
1:300	No cytotoxicity observed
1:1000	No cytotoxicity observed

* Dilution refers to the fold of dilution from the PNS.

RESULTS (continued)

Table 3
Titer Results – Test

Sample	Contact Time	Titer ($Log_{10}TCID_{50}$/mL)	Volume (mL)	Volume Correction[a]	Viral Load ($Log_{10}TCID_{50}$)
Virus Stock Titer Control	N/A	6.68 ± 0.20	-	-	-
Theoretical load (Virus Recovery Control) [b]	N/A	5.38 ± 0.20	10	2	6.68 ± 0.20
Theoretical load [b]	N/A	3.20 ± 0.20	10	300	6.68 ± 0.20
Cell Viability Control	no virus was detected; cells were viable; media was sterile				
Virus Recovery Control (VRC)	T = 0	5.80 ± 0.17	10	2	7.10 ± 0.17
	T = end	5.43 ± 0.18	10	2	6.73 ± 0.18
Neutralizer Effectiveness Control (NEC) [c]	60 seconds	6.43 ± 0.18	N/A	N/A	6.43 ± 0.18
Clinell Universal Wipes	30 seconds	≤ -0.43 *	10	300	≤ 3.05 *
Clinell Universal Wipes	60 seconds	≤ -0.43 *	10	300	≤ 3.05 *

[a] Volume correction accounts for the neutralization and quench of the sample post contact time.

[b] Based on the Virus Stock Titer (6.68 Log_{10} $TCID_{50}$/mL) minus Log_{10} (Volume x Volume Correction)

[c] A 4.5 mL aliquot of post-quenched sample was spiked with 0.5 mL of stock virus, mixed via vortex, and held in an ice-bath for 30 minutes.

N/A = Not Applicable

* No virus was detected; the theoretical titer was determined based on the Poisson Distriubution.

Note 1: The difference in Viral Load between the VST and NEC was ≤ 0.50 Log_{10} $TCID_{50}$.

Note 2: When no virus is detected in the "titration" sample, the "large volume" was used as the output load since large volume has a lower limit of detection (LOD). When virus was detected in the "titration" sample, the titration was used as the output load since titration was more accurate.

Table 4
Cytotoxicity Control

Sample	Dilution*	Cytotoxicity Control
Clinell Universal Wipes	10^0	No cytotoxicity observed

* The post-neutralized & quenched sample (PNS) was considered Undilute (10^0)

Conclusion: The neutralized test substance did not have significant cytotoxicity at undilute.

Table 5
Viral Interference Control

Sample	Virus Titer (Log_{10} $TCID_{50}$/mL)	Log_{10}Titer Difference
Clinell Universal Wipes	6.80 ± 0.23	0.13
PBS	6.93 ± 0.20	N/A

Conclusion: The neutralized test substance did not have significant viral interference.

RESULTS (continued)

Table 6
Viral Log_{10} Reduction

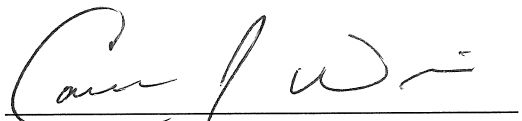
Test	Contact Time	Input Load ($Log_{10}TCID_{50}$)	Output Load ($Log_{10}TCID_{50}$)	Reduction ($Log_{10}TCID_{50}$)
Clinell Universal Wipes	30 seconds	7.10 ± 0.17	≤ 3.05	≥ 4.05 ± 0.17
Clinell Universal Wipes	60 seconds	7.10 ± 0.17	≤ 3.05	≥ 4.05 ± 0.17

CONCLUSIONS

According to the EN14476:2013+A2:2019 guideline, the test product passes the Virucidal Efficacy Suspension Test if there is at least a 4.0-log reduction in viral titer beyond the cytotoxicity level.

When tested as described, Clinell Universal Wipes, met the European Standard EN14476:2013+A2:2019 guideline, when Severe Acute Respiratory Syndrome-related Coronavirus was exposed to the test product for 30 seconds and 60 seconds at 20°C, with a Log_{10} reduction of ≥ 4.05p ± 0.17.

All controls met the criteria for a valid test. These conclusions are based on observed data.

Study Director:  09/04/2020

Cameron Wilde Date