FINAL REPORT

EN 14476

VIRUCIDAL QUANTITATIVE SUSPENSION TEST FOR CHEMICAL DISINFECTANTS AND ANTISEPTICS USED IN THE MEDICAL AREA

MERS-Coronavirus (MERS-CoV)

Test Agent
Clinell® Universal Wipes
Clinell® Sporicidal Wipes

Author
Salimatu Lukula, M.S.

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

Sponsor
GAMA Healthcare Ltd
Unit 2, The Exchange
Brent Cross Gardens
London NW4 3RJ
United Kingdom

Laboratory Project Identification Number
903-101

TABLE OF CONTENTS

COMPLIANCE STATEMENT

This study meets the requirements for 21 CFR § 58 with the following exceptions:

- Complete information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test agent resides with the sponsor of the study.

The following technical personnel participated in this study:

Salimatu Lukula, Cory Chiossone, Semhar Fanuel, Tanya Kapes

Study Director: MicroBioTest

Salimatu Lukula 09-01-2015

Salimatu Lukula, M.S. Date

QUALITY ASSURANCE UNIT STATEMENT

Title: EN 14476 Virucidal Quantitative Suspension Test for Chemical Disinfectants and Antiseptics used in the Medical Area – MERS-Coronavirus (MERS-CoV)

The Quality Assurance Unit of MicroBioTest has inspected the Project Number 903-101 in compliance with current Good Laboratory Practice regulations (21 CFR § 58).

The dates that inspections were made and the dates that findings were reported to management and to the study director are listed below.

PHASE INSPECTED	DATE OF INSPECTION	DATE REPORTED TO STUDY DIRECTOR	DATE REPORTED TO MANAGEMENT
Protocol	07/27/15	07/31/15	07/31/15
In-Process	07/30/15	07/31/15	07/31/15
Final Report	08/27/15	08/27/15	08/27/15

09-01-2015

Jeanne M. Anderegg Date
Quality Assurance Manager

TEST SUMMARY

TITLE: EN 14476 Virucidal Quantitative Suspension Test for Chemical Disinfectants and Antiseptics used in the Medical Area – MERS-Coronavirus (MERS-CoV)

STUDY DESIGN: This study was performed according to the signed protocol and project sheets issued by the Study Director. (See Appendix)

TEST MATERIALS:

1. Clinell® Universal Wipes, Lot No. UB511015, received at MicroBioTest on 07/06/15, and assigned DS No. F472

2. Clinell® Sporicidal Wipes, Lot No. SA512215, received at MicroBioTest on 08/03/15, and assigned DS No. F473

SPONSOR: GAMA Healthcare Ltd
Unit 2, The Exchange
Brent Cross Gardens
London NW4 3RJ
United Kingdom

TEST CONDITIONS

Challenge virus(s):

MERS-Coronavirus (MERS-CoV), strain EMC/2012, BEI Resources

Host:

Vero E6 cells, ATCC CRL-1586

Active ingredient(s):

Benzalkonium chloride, Didecyl dimethyl ammonium chloride and Polyhexamethylene biguanide (PHMB) (for Clinell® Universal Wipes
Sodium Percarbonate, Citric Acid (for Clinell® Sporicidal Wipes)

Test condition storage condition:

Ambient room temperature in darkness

Test agent appearance:

Towelette

Neutralizer:

Minimum Essential Medium (1X MEM) + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 1% $NaHCO_3$ + 0.5% Lecithin (for Clinell® Universal Wipes and Clinell® Sporicidal Wipes)
MEM + 10% FBS + 0.5% Polysorbate 80 + 1% $NaHCO_3$ + 1% Glycine [for Glutaraldehyde (Reference Inactivation Product)]

Dilution medium:

MEM + 2% FBS

Contact time(s):

1 minute and 2 minutes

Contact temperature(s):

20±1°C (Actual 20-21°C)

TEST CONDITIONS (continued)

Interfering Substance:

3.0 g/L bovine albumin solution plus 3.0 mL/L erythrocytes

Dilutions tested:

Undilute, 50% (62.5% prior to mixing), 10% (12.5% prior to mixing)

Diluent:

EN14476 Hard Water

Media and reagents:

Minimum Essential Medium (1X MEM) + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 1% $NaHCO_3$ + 0.5% Lecithin
MEM + 10% FBS + 0.5% Polysorbate 80 + 1% $NaHCO_3$ + 1% Glycine
MEM + 2% FBS
Phosphate buffered saline (PBS)
Glutaraldehyde (0.125%)
EN hard water
3.0g/L bovine albumin solution plus 3.0 mL/L erythrocytes

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MicroBioTest, 105 Carpenter Drive, Sterling, VA 20164. The virucidal suspension test was laboratory initiated on 07/30/2015 and concluded on 08/20/2015. The study director signed the protocol on 07/29/2015. 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 MicroBioTest and the sponsor will be stored in the archives at MicroBioTest, 105 Carpenter Drive, Sterling, VA 20164, or at a controlled facility off site.

EXPERIMENTAL DESIGN OVERVIEW

Inoculum preparation

MERS-Coronavirus was prepared by infection of Vero-E6 cells. A few days after inoculation, the cultures were frozen. After one freeze-thaw cycle, cell-free stocks were prepared by high-speed centrifugation. The clarified supernatants were concentrated by ultracentrifugation. The stock virus was aliquoted and stored at -60°C or below.

Test agent preparation

All assay reagents, including test agents, were equilibrated to testing temperature, 20±1°C prior to use in testing. The prepared test agents were used within the specified times listed below.

Clinell® Universal Wipes were prepared per protocol as follows:

a. Clinell® Universal Wipes were allowed to equilibrate to the test environment and temperature in the orientation for at least 1 hour.
b. Using gloved hands, 5 wipes were dispensed and discarded.
c. 30 wipes were scrunched by hand to squeeze and release the liquid, a wringing action was used.
d. The liquid was collected in a beaker. The liquid was used without waiting for the foam to disappear. The liquid concentration was considered at 100% prior to testing.
e. The liquid solution was used for testing within 15 minutes
f. Using the liquid solution (100% test concentration), a 62.5% and 12.5% test concentration was prepared.

EXPERIMENTAL DESIGN OVERVIEW (continued)

Clinell® Sporicidal Wipes was prepared per protocol as follows:

a. Each dry wipe was weighed and the weight recorded.
b. A 600 mL beaker was placed on a balance and zeroed.
c. The dry wipe was placed in the tray. The tray was large enough to allow the wipe to lay flat without folding.
d. 200 mL EN14476 hard water was added onto the wipe which is in the tray and a timer was started.
e. After 10 seconds, the wipe was gently squeezed and placed in the beaker on the balance.
f. The wet weight of the wipe was recorded and it was between 35 and 40 g
g. At 1 minute 30 seconds the wipe was thoroughly squeezed into the beaker.
h. The pH of the solution in the beaker was measured and recorded.
i. Using the liquid solution (100% test concentration), a 62.5% and 12.5% test concentration was prepared as follows:

NOTE: The solution was used for testing within 10 minutes post activation.

Inoculation/Incubation

The dilutions were inoculated individually onto Vero E6 host-cells and incubated at 36±2°C with 5±1% CO_2 for 7 days.

EXPERIMENTAL DESIGN OVERVIEW (continued)

Pre-test cytotoxicity evaluation

In a single run, an 8 mL aliquot of the test product (Clinell® Universal Wipes) (100% of the target-use concentration in final reaction mixture) was mixed with 1 mL of 10X Interfering Substance and 1 mL dilution medium, and held for 2 minutes. At the end of the contact time, an equal volume of the reaction mixture was mixed with and equal volume of ice-cold neutralize. The eluate was serially diluted (1:10, 1:30, 1:100, 1:300 and 1:1000) using ice-cold dilution medium.

Virucidal quantitative suspension test

In a singlet run 1 mL of the test virus suspension was mixed with 1 mL of the 10X Interfering Substance in a sterile tube using a vortex-type mixer for 1-2 seconds. An 8 mL aliquot of test agent was added. The reaction mixture was mixed for 1 – 2 seconds using a vortex-type mixer and held for the contact times 1 minute and 2 minutes). A 1 mL aliquot of the reaction mixture was drawn up and neutralized in 1 mL of neutralizer. The suspension was mixed using a vortex-type mixer for 1 – 2 seconds. A 1000-fold dilution was performed in ice-cold dilution medium prior to diluting and inoculating onto host-cells.

Virus recovery control 1 (VRC-1)

In a singlet run per day of testing, 1 mL of virus suspension was added to 1 mL of 10X Interfering Substance in a sterile tube and mixed using a vortex-type mixer for 1 – 2 seconds. Following, 8 mL of dilution medium was added and mixed using a vortex-type mixer for 1 – 2 seconds. Immediately after mixing, a 1 mL aliquot of the reaction mixture was drawn up and neutralized in 1 mL of neutralizer. The suspension was mixed using a vortex-type mixer for 1 – 2 seconds. A 1000-fold dilution was performed in ice-cold dilution medium prior to diluting and inoculating onto host-cells.

EXPERIMENTAL DESIGN OVERVIEW (continued)

Virus recovery control 2 (VRC-2)

In a single run per day of testing, 1 mL of dilution medium was added to 1 mL of 10X Interfering Substance in a sterile tube. Then 8 mL of dilution medium was added and mixed using a vortex-type mixer for 1 – 2 seconds and held for the longer contact time (2 minutes). After the exposure time, a 1 mL aliquot of the reaction mixture was drawn up and neutralized in 1 mL of neutralizer. 10µL of challenge virus was added to 500µL of the eluate and mixed for 1 – 2 seconds using a vortex-type mixer prior to diluting and inoculating onto host-cells.

Virus recovery control 3 (VRC-3)

In a single run per day of testing, 1 mL of virus suspension was added to 1 mL of 10X Interfering Substance in a sterile tube and mixed using a vortex-type mixer for 1 – 2 seconds. Following, 8 mL of test agent was added and mixed using a vortex-type mixer for 1 – 2 seconds and held for 2 minutes. Following, 100 µL of the reaction mixture was added to 9.9 mL of the reference agent neutralizer. The mixture was mixed for 1 – 2 seconds using a vortex-type mixer. The sample was then diluted and inoculated onto host-cells.

Neutralizer effectiveness control 1 (NEC-1)

In a single run per day of testing, 1 mL of the virus suspension was mixed with 1 mL of 10X Interfering Substance in a sterile tube and mixed using a vortex-type mixer for 1 – 2 seconds. 8 mL of undiluted test agent was added and mixed using a vortex-type mixer for 1 – 2 seconds. Immediately thereafter, a 1 mL aliquot of the reaction mixture was drawn up and neutralized in 1 mL of ice-cold neutralizer. The suspension was mixed using a vortex-type mixer for 1 – 2 seconds. The quenched sample was held under ice bath for 30 minutes prior to diluting and plating.

EXPERIMENTAL DESIGN OVERVIEW (continued)

Neutralizer effectiveness control 2 (NEC-2) – virus added to the neutralized test agent

In a single run per day of testing, 1 mL of dilution medium was mixed with 1 mL of 10X Interfering Substance in a sterile tube and mixed using a vortex-type mixer for 1 – 2 seconds. 8 mL of the undiluted test agent was added and mixed using a vortex-type mixer for 1 – 2 seconds. After the longest contact time (2 minutes), 1 mL of neutralizer was added to 1 mL of the mixture and mixed using a vortex-type mixer for 1 - 2 seconds. 10 µL of the challenge virus was added to 0.5 mL of the eluate and mixed using a vortex-type mixer for 1 – 2 seconds. The mixture was held for the longest the contact time prior to diluting and inoculating onto host-cells.

Cytotoxicity control

In a single run per day of testing, 1 mL of dilution medium was added to 1-mL to 10X Interfering Substance and mixed for 1 – 2 seconds using a vortex-type mixer. Following, 8 mL of the undiluted test agent was added and mixed using a vortex-type mixer for 1 – 2 seconds. After the longest contact time (2 minutes) 1 mL of neutralizer was added to 1mL of the mixture and vortexed. A 1000-fold dilution was performed using ice-cold dilution medium and was kept under ice until diluting and plating onto host-cells.

Virus stock titer control

In a single run per day of testing, the virus stock was used to make serial ten-fold dilutions in dilution medium. Following, selected dilutions were inoculated onto host-cells.

Cell viability control

In a single run per day of testing, 1 mL/well of dilution medium was added to 8 wells of host-cells and incubated together with other tests and control samples. This also served as the assay negative control.

EXPERIMENTAL DESIGN OVERVIEW (continued)

Viral interference control

In a single run per day of testing, the host-cell containing media was aspirated from prepared 24-well plates and 1 mL/well of the quenched test agent from the cytotoxicity control, as well as a plate containing 1X PBS, were added (8-wells for each dilution assayed). The plates were incubated for 1 hour at 36±2°C. The virus stock was used to make serial ten-fold dilutions in dilution medium for 10^{-5} to 10^{-10}. Following the incubation period, the supernatant was discarded from the plate and aliquots of 1 mL/well of the selected dilutions were inoculated onto host-cells.

Reference agent virus inactivation control

In a single run, 1 mL of virus suspension was added to 1-mL to 10X Interfering Substance and mixed for 1 – 2 seconds. 8 mL of reference agent (0.125% Glutaraldehyde) was added and mixed for 1 – 2 seconds using a vortex-type mixer. The mixture was held for 2 minutes at 21°C. Following, 100 µL of the reaction mixture was added to 9.9 mL of the reference agent neutralizer. The mixture was mixed for 1 – 2 seconds using a vortex-type mixer prior to diluting and inoculating onto host-cells.

Reference control cytotoxicity

In a single run, 1 mL of DM was added to 1-mL to 10X Interfering Substance and mixed for 1 – 2 seconds using a vortex-type mixer. Followed by adding an 8 mL aliquot of the reference agent and mixing again for 1 – 2 seconds using a vortex-type mixer. 100 µL of the reaction mixture was added to 9.9 mL of reference substance neutralizer. The mixture was mixed for 1 – 2 seconds using a vortex-type mixer prior to diluting and inoculating onto host-cells.

CRITERIA FOR A VALID ASSAY

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

- The test virus suspension has a titer of at least 10^8 $TCID_{50}$/mL; or possesses at least a concentration which allows the determination of a 4.0-log_{10} of the virus titer.
- The cytotoxicity of the product solution does not affect host cell viability in the dilutions of the test mixtures which are necessary to demonstrate a 4.0-log reduction of the virus
- Viral-induced cytopathic effect (CPE) is distinguishable from test product induced cytotoxic effect in the viral interference assay, if any.
- Virus is not detected in the cell viability control
- The difference of titer between the NEC-1 and VRC-1 or between NEC-2 and VRC-2 is ≤ 0.5 log_{10}
- The difference of titer between the neutralized test product-treated and PBS-treated monolayers is ≤ 1.0 log_{10} in the viral interference assay
- The viral reduction of the reference agent against Mers-CoV should be ≥4.0-log_{10}

PRODUCT EVALUATION CRITERIA

According to EN 14476 guideline, the test agent passes the test if there is at least a 4.0-log reduction in titer beyond the cytotoxicity level.

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 titer 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)

CALCULATION OF TITER AND 95% CONFIDENCE INTERVAL (continued)

The values were converted to $TCID_{50}$/ml using a sample inoculum of 1.0 mL.

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

$$\sigma_m = d_f \sqrt{\frac{p_i(1-p_i)}{(n_i - 1)}}$$

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 titer with 95% confidence limit is $m \pm 1.96\sigma_m/2$.

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.

The amount of virus which would have to be present in the total sample in order to achieve a positive result with 95% confidence (p = 0.05) is calculated as

$$c = -[\text{Ln}(0.05)] / v = 3 / v$$

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 – 10.

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]

where:

(Reference agent, VRC-3)
Volume = 10 mL
Volume correction = 100

(All others, as applicable)
Volume = 10 mL
Volume correction = 2000

Table 1
Pre-Test Cytotoxicity Control Results

Dilution***	Clinell® Universal Wipes Lot No. UB511015
10^{-1} (1:10)	Cytotoxicity observed in all 8 wells
(1:30)	Cytotoxicity observed in all 8 wells
10^{-2} (1:100)	Cytotoxicity observed in all 8 wells
(1:300)	Cytotoxicity observed in all 8 wells
10^{-3} (1:1000)	No cytotoxicity observed in all 8 wells

[3] Samples were tested at 100% concentration in solution.

*** Dilution refers to the fold of dilution from the neutralizer, neutralized test agent or test agent alone.

RESULTS (continued)

Table 2
Titer Results - Controls

Sample	Contact Time	Titer ($Log_{10}TCID_{50}/mL$)	Volume (mL)	Volume Correction [a]	Viral Load ($Log_{10}TCID_{50}$)
Virus Stock Titer Control - 07/30/15	NA	8.13 ± 0.18	-	-	-
Virus Stock Titer Control - 08/13/15	NA	7.75 ± 0.16	-	-	-
Cell Viability Control 07/30/15 and 08/13/`5	NA	no virus was detected, cells remained viable; media were sterile			
Virus Recovery Control (VRC-1) 07/30/15	2 minutes	3.75 ± 0.16	10	2000	8.05 ± 0.16
Virus Recovery Control (VRC-1) 08/13/15		3.63 ± 0.12	10	2000	7.93 ± 0.12
Virus Recovery Control (VRC-3) 08/13/15		4.88 ± 0.18	10	100	7.88 ± 0.18

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

RESULTS (continued)

Table 3
Titer Results - Test

Sample	Contact Time	Dilution Tested**	Titer ($Log_{10}TCID_{50}/mL$)	Volume (mL)	Volume Correction [a]	Viral Load ($Log_{10}TCID_{50}$)
Clinell® Universal Wipes	1 minute	Undilute	≤ -0.47 *	10	2000	≤ 3.83
	2 minutes		≤ -0.47 *	10	2000	≤ 3.83
	1 minute	50%	≤ -0.47 *	10	2000	≤ 3.83
	2 minutes		≤ -0.47 *	10	2000	≤ 3.83
	1 minute	10%	≤ -0.47 *	10	2000	≤ 3.83
	2 minutes		≤ -0.47 *	10	2000	≤ 3.83
Clinell® Sporicidal Wipes	1 minute	Undilute	≤ -0.47 *	10	2000	≤ 3.83
	2 minutes		≤ -0.47 *	10	2000	≤ 3.83
	1 minute	50%	≤ -0.47 *	10	2000	≤ 3.83
	2 minutes		≤ -0.47 *	10	2000	≤ 3.83
	1 minute	10%	≤ -0.47 *	10	2000	≤ 3.83
	2 minutes		≤ -0.47 *	10	2000	≤ 3.83
Reference Agent (Glutaraldehyde)	2 minutes	0.10%	≤ -0.47 *	10	100	≤ 2.53

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

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

** Dilution corresponds to the percentage of test agent in final reaction mixture

RESULTS (continued)

Table 4
Neutralization Effectiveness Control 1 (NEC-1)

Sample	Contact Time	Dilution Tested**	Titer ($Log_{10}TCID_{50}$/mL)	Volume (mL)	Volume Correction [a]	Viral Load ($Log_{10}TCID_{50}$)
Clinell® Universal Wipes	0 second	Undilute	≤ -0.47 *	10	2000	≤ 3.83
Clinell® Sporicidal Wipes	0 second	Undilute	≤ -0.47 *	10	2000	≤ 3.83

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

** Dilution corresponds to the percentage of test agent in final reaction mixture

Conclusion: Per EN14476, NEC-1 must exhibit not more than 0.5 log difference from VRC-1. However, virus was not observed for NEC-1, presumably indicative of a very rapid inactivation of virus by the test agent. Therefore NEC-1 was not considered an appropriate neutralization control for this type of test agent. The effectiveness of the neutralization method (1:1 neutralization in neutralizer followed by 1000 fold dilution) was demonstarted in NEC-2, where little loss of virus (< 0.5 log) was observed when the virus was added to the test agent post-neutralization.

Table 5
Virus Recovery Control for Neutralization Effectiveness Control 2 (VRC-2)$^{\Delta}$

Sample	Contact time	Dilution Tested	Titer ($Log_{10}TCID_{50}$/mL)
VRC-2 (07/30/15)	2 minutes	NA	6.13 ± 0.18
VRC-2 (08/13/15)			6.13 ± 0.22

Δ This control was performed to validate the effectiveness of the neutralizing method for NEC-2.

Table 6
Neutralization Effectiveness Control 2 (NEC-2)

Sample	Contact Time	Dilution Tested**	Titer ($Log_{10}TCID_{50}$/mL)
Clinell® Universal Wipes	2 minutes	Undilute (= 100% target use concentration)	5.88 ± 0.18
Clinell® Sporicidal Wipes			5.75 ± 0.16

** Dilution corresponds to the percentage of test agent in final reaction mixture

Note 1: The NEC2 (07/30/15)titer differs from the respective control titer by 0.25 log.

Note 2: The NEC2 (08/13/15)titer differs from the respective control titer by 0.38 log.

Conclusion: The neutralization and quench was effective in neutralizing the test substance.

RESULTS (continued)

Table 7
Cytotoxicity Control (100% target use concentration of the test agent)

Sample	Dilution after Neutralization	Virus Titer ($Log_{10}TCID_{50}$/mL)
Clinell® Universal Wipes	10^{-3}	7.63 ± 0.20
1X PBS	NA	7.75 ± 0.18
Clinell® Sporicidal Wipes	10^{-3}	7.88 ± 0.18
1X PBS	NA	7.88 ± 0.16

Conclusion: The neutralized and quenched test substance did not have significant viral interference. The assay is valid because the difference of titer between the neutralized test substance-treated and PBS-treated monolayers is ≤ 1.0 $\log_{10}$ (actual Log_{10} -0.12 and 0.00 repectively) in the viral interference assay.

Table 8
Cytotoxicity Control (100% target use concentration of the test agent)

Sample	Dilutions*	Determination of Cytotoxicity
Reference Agent (Glutaraldehyde)	Undilute	no cytotoxicity observed in all 8 wells
	10^{-1}	no cytotoxicity observed in all 8 wells
	10^{-2}	no cytotoxicity observed in all 8 wells

* Dilutions were performed after samples were neutralized 100 fold

Conclusion: The neutralized and quenched test substance did not have significant cytotoxicity.

RESULTS (continued)

Table 9
Viral Interference Control (100% target use concentration of the test agent)

Sample	Dilution after Neutralization	Virus Titer ($Log_{10}TCID_{50}$/mL)
Clinell® Universal Wipes	10^{-3}	7.63 ± 0.20
1X PBS	NA	7.75 ± 0.16
Clinell® Sporicidal Wipes	10^{-3}	7.88 ± 0.18
1X PBS	NA	7.88 ± 0.18

Conclusion: The neutralized and quenched test substance did not have significant viral interference. The assay is valid because the difference of titer between the neutralized test substance-treated and PBS-treated monolayers is ≤ 1.0 $\log_{10}$ (actual Log_{10} -0.12 and 0.00 repectively) in the viral interference assay.

RESULTS (continued)

Table 10
Titer Reduction - Test

Test	Contact Time	Dilution Tested*	Input Load ($Log_{10}TCID_{50}$)	Output Load ($Log_{10}TCID_{50}$)	Reduction ($Log_{10}TCID_{50}$)
Clinell® Universal Wipes	1 minute	Undilute	8.05 ± 0.16	≤ 3.83	≥ **4.22** ± **0.16**
	2 minutes			≤ 3.83	≥ **4.22** ± **0.16**
	1 minute	50%		≤ 3.83	≥ **4.22** ± **0.16**
	2 minutes			≤ 3.83	≥ **4.22** ± **0.16**
	1 minute	10%		≤ 3.83	≥ **4.22** ± **0.16**
	2 minutes			≤ 3.83	≥ **4.22** ± **0.16**
Clinell® Sporicidal Wipes	1 minute	Undilute	7.93 ± 0.12	≤ 3.83	≥ **4.10** ± **0.12**
	2 minutes			≤ 3.83	≥ **4.10** ± **0.12**
	1 minute	50%		≤ 3.83	≥ **4.10** ± **0.12**
	2 minutes			≤ 3.83	≥ **4.10** ± **0.12**
	1 minute	10%		≤ 3.83	≥ **4.10** ± **0.12**
	2 minutes			≤ 3.83	≥ **4.10** ± **0.12**
Reference Agent (Glutaraldehyde)	2 minutes	0.10%	7.88 ± 0.18	≤ 2.53	≥ **5.35** ± **0.18**

* Dilution corresponds to the percentage of test agent in final reaction mixture

Conclusion: The test substances (Clinell® Universal Wipes and Clinell® Sporicidal Wipes) met the criteria for EN14476 as an effective virucidal agent against MERS-CoV.

CONCLUSIONS

According to the EN 14476:2013 standard, the test agent passes the Virucidal Quantitative Suspension Test if there is at least a four-log reduction in viral titer beyond the cytotoxicity level. When tested as described, Clinell® Universal Wipes and Clinell® Sporicidal Wipes (at all concentrations) met the EN 14476:2013 standard when MERS-CoV was exposed to the test agent for 1 minute and 2 minutes 20±1°C. These conclusions are based on observed data.

APPENDIX

MicroBioTest Protocol

EN 14476

VIRUCIDAL QUANTITATIVE SUSPENSION TEST FOR CHEMICAL DISINFECTANTS AND ANTISEPTICS USED IN THE MEDICAL AREA

MERS-Coronavirus (MERS-CoV)

Testing Facility
MicroBioTest
Division of Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Prepared for
GAMA Healthcare Ltd
Unit 2, The Exchange
Brent Cross Gardens
London NW4 3RJ
UNITED KINGDOM

July 23, 2015

MicroBioTest Protocol: GAM.1b.07.16.15

MicroBioTest Project: 903-101

SKEL

OBJECTIVE:

This test is designed to substantiate virucidal effectiveness claims for a product(s) to be labeled as a virucide. It determines the potential of the test product(s) to inactivate virus in suspension in the presence of the interfering substance. One target virus, MERS-Coronavirus (MERS-CoV), will be tested. This test conforms to the European Standard EN 14476:2013 "Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (Phase 2/Step 1)".

TESTING CONDITIONS:

Two test products - Clinell Universal Sanitising Wipes and Clinell Sporicidal Wipes – will be tested. The Clinell Sporicidal Wipes will be activated prior to use. Liquid will be squeezed from the wipes and used for the test (see "Miscellaneous Information" section for details).

Then the liquid from each test product will be evaluated against the challenge virus in suspension. Two test products, one lot each, will be evaluated at two exposure (contact) times and one interfering condition. The test product will be evaluated at three concentrations – Undiluted (100%), and 50% and 10% in the reaction mixture. One replicate (N=1) will be used for each condition.

For each run, the challenge virus will be added to the test product solution in the presence of interfering substance and immediately mixed via vortex-type mixing. The test mixture will be maintained at the contact temperature (20±1°C). The volume of virus inoculum added to test product will be kept at 10% of the total volume of the mixture. After each contact time, an aliquot of the reaction mixture will be collected and immediately quenched in neutralizer.

The quenched sample will then be serially diluted in dilution medium and inoculated onto an appropriate host cell system to determine the amount of infectious virus. Eight replicate wells of host cells will be inoculated for each dilution assayed.

MATERIALS:

A. The test product will be supplied by the sponsor.

The test product will be tested at concentrations and conditions specified by the sponsor (see "Miscellaneous Information" section for information). All operations performed on the test product such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures MicroBioTest testing facility management that the test product has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

MicroBioTest will retain all unused test products for a period of one year upon completion of the test, then discard them in a manner that meets the approval of the safety officer.

B. Materials supplied by MicroBioTest, including, but not limited to:

1. Challenge virus (requested by the Sponsor): MERS-Coronavirus (MERS-CoV), strain EMC/2012

   - A window of reduction of ≥ 4.0-log is targeted for this study by using high titered virus and, if necessary, large volume inoculation.

2. Host: Vero-E6 cells

3. Laboratory equipment and supplies.

   Laboratory equipment and supplies relevant to the performance of the assay will be documented in the data pack.

4. Media and reagents:

   a. Sterile hard water for dilution of products, prepared as per EN 14476:2013 (E), section 5.2.2.7
   b. 10X Interfering substance: 30 g/L bovine albumin solution plus 30 mL/L erythrocytes (final concentration in reaction mixture = 3.0 g/L bovine albumin solution plus 3.0 mL/L erythrocytes ("Dirty condition")

c. Phosphate Buffered Saline (PBS)
d. Dilution medium (DM)
e. Glutaraldehyde (Reference Inactivation Agent)

Media and reagents relevant to the virus-host system and test product being tested will be documented in the first project sheet and the data pack.

TEST SYSTEM IDENTIFICATION:

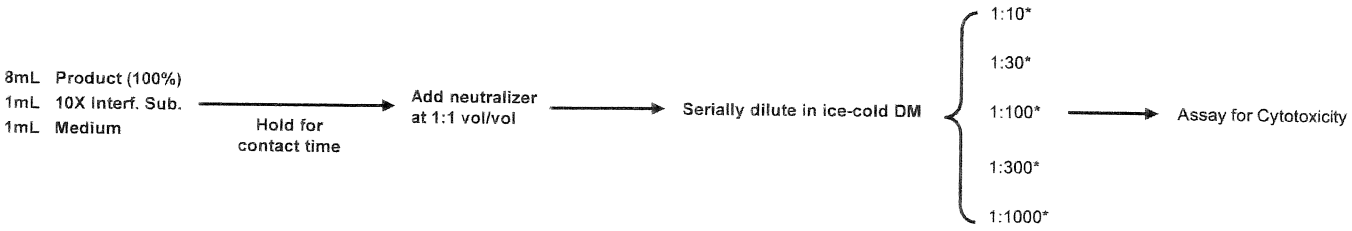
All Petri dishes, dilution tube racks, and host-containing apparatus will be labeled with virus identification and project number.

EXPERIMENTAL DESIGN:

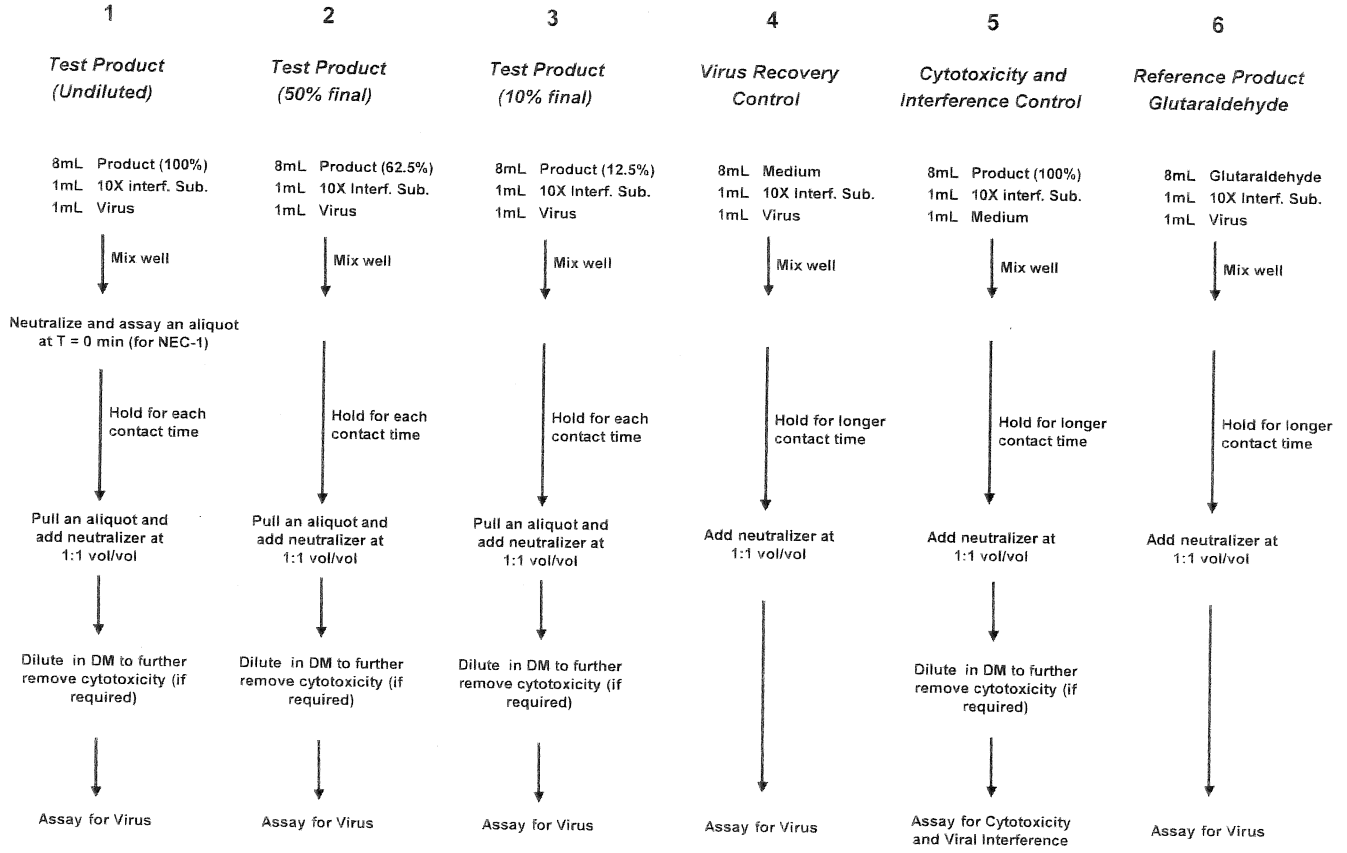
Procedures involved in performance of the study are described in a series of SOPs and logs that are maintained at MicroBioTest. All assay reagents (except virus suspension), including test product, will be equilibrated to the test temperature before testing. The study flow diagram is summarized in Figure 1, with details described in the following sections.

Figure 1

I) Pre-test Cytotoxicity Evaluation



II) Virucidal Quantitative Suspension Test, Contact temperature: 20±1°C.



NEC = Neutralization Effectiveness Control *DM = Dilution Medium*

Inter. Sub. = Interfering Substance.

** Additional or alternative dilutions may be tested as required.*

*Note 1: **Two products** will be tested.*

Note 2: An additional NEC, NEC-2, will be performed. See Section G for details.

A. Preparation of interfering substances:

The interfering condition will be 3.0 g/L bovine albumin solution plus 3.0 mL/L erythrocytes in the final reaction mixture ("Dirty Condition").

B. Inoculum preparation:

Viral stock was originally acquired from reputable sources that identify them by scientifically accepted methods.

The working virus stock will be prepared by infection of Vero-E6 cells. A few days after inoculation, the cultures will be frozen. After one freeze-thaw cycle, cell-free stocks will be prepared by high-speed centrifugation. The viral stock will be aliquoted and stored at -60°C or below. Records are maintained that demonstrate the origin of the virus.

Frozen viral stocks will be thawed on the day of the test.

C. Test product preparation:

Two test products will be tested. Liquid will be expressed from the wipes (after the activation as applicable) as per the Sponsor instruction (see "Miscellaneous Information" section for details).

Each liquid product will be evaluated at three concentrations – Undiluted (100%), and 50% and 10% in the final reaction mixture. The products will be evaluated at one replicate (N=1).

The test product will be diluted using EN14476 hard water water from 100% to 62.5% and 12.5% of the final target use concentration. The last two dilutions are 1.25 times the desired final test concentration as the test product will be diluted to 80% in the reaction mixture. The final concentration of the test product in the reaction mixture will be 80%, 50% and 10%, respectively.

D. Pre-test cytotoxicity evaluation:

This evaluation will be performed prior to the virucidal quantitative suspension test in order to determine the appropriate quench ratio at which the cytotoxicity will be removed. This test will be performed for each test product in singlet run (N=1).

An 8-mL aliquot of the test product (80% of the target use concentration in final reaction mixture) will be mixed with 1-mL 10X Interfering Substance and 1-mL dilution medium and held for the longer contact time. At the end of the contact time, the reaction mixture will be quenched with neutralizer at 1:1 vol/vol. The reaction mixture will then be serially diluted (1:10, 1:30, 1:100, 1:300 and 1:1000 - additional or alternative dilutions may be tested as required) in ice-cold dilution medium (DM). These dilutions will be inoculated individually onto host cells and incubated at 36±2°C with 5±1% CO_2 for a period of at least 2 days. The condition of the host cells will be recorded at the end of the incubation period.

The results will be used to determine the dilution (i.e., quenching) ratio of the neutralized sample for the virucidal quantitative suspension test.

E. Virucidal quantitative suspension Test:

Two test products (liquid expressed from wipes) will be tested at three concentrations - Undiluted (100%), and 50% and 10% in the final reaction mixture - at two contact times and in singlet runs (N=1).

For each test product run, eight-mL of test product at each appropriate concentration will be mixed with one-mL of the 10X Interference Substance by vortex. Then one-mL of the test virus suspension will be added and mixed by vortex. A stop-watch will immediately be started.

At time zero, an aliquot of the reaction mixture will be removed, neutralized by adding the neutralizer at 1:1 vol/vol, and diluted with ice-cold DM per the results from the pre-test cytotoxicity evaluation. Then the sample, considered the 10^0 dilution, will be held under ice bath for 30 min. This will be the Neutralizer Effectiveness Control-1 (NEC-1) (see Section F3). It will be serially tenfold diluted in DM and inoculated onto host cells to assay for infectious virus as described in Section F.

The remainder of the test mixture will be held for the contact times as specified by the sponsor. After each contact time, an aliquot of the reaction mixture will be

collected and immediately neutralized by adding the neutralizer at 1:1 vol/vol and diluted with ice-cold DM per the results from the pre-test cytotoxicity evaluation. Then the sample, considered the 10^0 dilution, will be serially tenfold diluted in DM and inoculated onto host cells to assay for infectious virus as described in Section F.

F. Infectivity assay:

The residual infectious virus in both test and controls will be detected by viral-induced cytopathic effect (CPE).

Selected dilutions of the neutralized inoculum/disinfectant mixture will be added to cultured host cells (eight wells per dilution, per reaction mixture) and incubated at 36±2C with 5±1% CO_2 for a period of 4 – 9 days. The host cells may be washed twice with phosphate buffered saline (PBS) prior to inoculation. The host cell cultures will be observed and refed as necessary, during the incubation period. These activities, if applicable, will be recorded. The host cells will then be examined microscopically for presence of infectious virions. The resulting virus-specific CPE and test product-specific cytotoxic effects will be scored by examining both test and controls. These observations will be recorded.

In order to increase the sensitivity of the viral infection assay, a large volume sampling of the test product-treated sample(s) may be performed by inoculating the sample at the lowest dilution without significant cytotoxicity or viral interference onto a large number of replicate wells of the host cells.

G. Controls:

1. Virus Recovery Control for test product (VRC-1):

This control will be performed at the longer contact time, in singlet runs (N=1). To check for the infectivity of the test virus suspension under the test conditions, a test mixture will be prepared by mixing 8-mL dilution medium (in lieu of the test product) with 1-mL 10X interfering substance, and then mixed with 1-mL stock virus. The mixture will be held for the longer contact time (as a worst-case scenario), then an aliquot will be immediately neutralized by adding the neutralizer at 1:1 vol/vol and diluted with ice-cold DM per the results from the pre-test cytotoxicity evaluation (to mimic the quench process for the test product samples). It will then be serially diluted and analyzed for the amount of infectious virus as described in Section F. This control will

determine the virus recovery and used as the input viral load in the calculation of viral reduction.

Note: another aliquot from this reaction mixture will be used for VRC-3 (see below).

2. Cytotoxicity and Viral Interference Controls:

This control will be performed for each of the test products in singlet run (N=1), using the longer contact time as a worst-case scenario.

To check for possible morphological alteration/destruction of cells by the test product, an 8-mL aliquot of the test product (undiluted) will be mixed with 1-mL 10X interference substance and 1-mL medium (in lieu of virus) and held for the longer contact time. At the end of the contact time, an aliquot or the entire mixture will be immediately neutralized by adding the neutralizer at 1:1 vol/vol and diluted with ice-cold DM per the results from the pre-test cytotoxicity evaluation (to mimic the quench process for the test product samples). The sample will then be assessed for cytotoxicity and viral interference as described below.

For cytotoxicity assessment, an appropriate amount of each neutralized and quenched test product sample will be inoculated onto the cell monolayer and incubated as described above. After incubation, the cell monolayer will be microscopically examined for morphology change.

For viral interference assessment, cell monolayers will be treated with an appropriate amount (0.5-mL per well for 24-well plates) of either 1X PBS or the neutralized and quenched test product sample for one hour at 36±2°C. After the incubation, the supernatant will be discarded. The stock virus will then be titrated in parallel on both types of pre-incubated cell monolayers to obtain a titer.

The neutralized and quenched test product solution must exhibit (a) a low degree of cell destruction (< 25% of monolayer); and (b) a titer reduction of virus < 1.0 log, in order to be used for the determination of the viral residual infectivity.

3. Neutralizer Effectiveness Controls (NEC's):

These controls will be performed in singlet run for each of the test products (undiluted). It will determine if residual active ingredient is present after the neutralization and quench procedure.

3.1 Neutralizer Effectiveness 1 (NEC-1)

Immediately after preparation of the test mixture (section E), at time zero, an aliquot of the reaction mixture will be removed and immediately neutralized by adding the neutralizer at 1:1 vol/vol and diluted in ice-cold DM per the results from the pre-test cytotoxicity evaluation. The quenched sample will then be held under ice bath for 30 min. After incubation, the samples will be serially diluted ten-fold in DM and selected dilutions inoculated onto the host cells to measure the titer of virus as described in Section F.

3.2 Neutralizer Effectiveness 2 (NEC-2)

As products of this type may be fast-acting, a log reduction may be seen for NEC-1 even at "time zero". To determine the effectiveness of the neutralization procedure, an additional neutralizer effectiveness control, NEC-2, will be performed.

An 8-mL aliquot of the test product (undiluted) will be mixed with 1-mL 10X Interfering Substance and then 1-mL DM. The reaction mixture will be thoroughly mixed via vortex-type mixing and a stop-watch will then be started.

After the exposure time (longer contact time as a worst-case scenario), an aliquot or the entire mixture will be immediately neutralized by adding the neutralizer at 1:1 vol/vol and diluted with ice-cold DM per the results from the pre-test cytotoxicity evaluation (to mimic the quench process for the test product samples). Then, 500-µL of the post-quenched sample will be spiked with 10-µL of stock virus and mixed using a vortex-type mixer. The spiked sample will be serially tenfold diluted in DM and inoculated onto host cells to assay for infectious virus as described in Section F. The difference of titer between NEC-2 and VRC-2 (see below) samples shall be ≤ 0.5 $\log_{10}$.

4. Virus Recovery Control for NEC-2 (VRC-2):

This control will be performed in singlet run. To validate the effectiveness of the neutralizing method for NEC-2, 8-mL DM (in lieu of test product) will be mixed with 1-mL 10X Interfering Substance and then 1-mL DM. The reaction mixture will be thoroughly mixed via vortex-type mixing and a stop-watch will then be started.

After the exposure time (longer contact time as a worst-case scenario), an aliquot or the entire mixture will be immediately neutralized by adding the neutralizer at 1:1 vol/vol and diluted with ice-cold DM per the results from the pre-test cytotoxicity evaluation (to mimic the quench process for the test product samples). Then, 500-µL of the post-quenched sample will be spiked with 10-µL of stock virus and mixed using a vortex-type mixer. The spiked sample will be serially tenfold diluted in DM and inoculated onto host cells to assay for infectious virus as described in Section F.

5. Cell viability control:

This control will be performed in singlet run on each day the sample is assayed. This control will demonstrate that cells remain viable throughout the course of the assay period. In addition, it will confirm the sterility of the DM employed throughout the assay period. This control will be performed during both the pre-test cytotoxicity evaluation and virucidal quantitative suspension test.

An appropriate amount of medium will be added to eight wells of the indicator cells and incubated together with other test and control samples. This will serve as the negative control.

6. Virus Stock Titer control (VST):

This control will be performed in singlet run on each day a sample is assayed for virus titer. An aliquot of the challenging virus used will be directly serially diluted and inoculated onto the host cells to confirm the titer of the stock virus. This control will demonstrate that the titer of the stock virus is appropriate for use and that the viral infectivity assay is performed appropriately.

7. Reference Agent virus Inactivation control:

This control will be performed in singlet run. Glutaraldehyde will be assayed in parallel with the test product as a control for the test system's validity. A 8-mL aliquot of glutaraldehyde at appropriate concentration (e.g., 0.05% w/v in final reaction mixture, or another appropriate concentration) will be mixed with 1 mL 10X Interfering Substance and 1 mL virus and held for the longer contact time. At the end of the contact time, the reaction mixture will be neutralized by a 100-fold dilution in ice-cold neutralizer (containing 1% glycine). Then the sample will be serially diluted in a regular dilution medium. Selected dilutions will be assessed for infectious virus as described in Section F. The viral reduction from this control should be ≥ 4.0 $\log_{10}$.

8. Virus Recovery Control for Reference Agent (VRC-3):

This control will be performed in singlet run. An aliquot of the test mixture from the VRC-1 run (after the contact time) will be directly neutralized by a 100-fold dilution in the same neutralizer as used for the reference inactivation agent (ice-cold). It will then be analyzed for the amount of infectious virus as described in Section F. This control will determine the relative loss in virus infectivity from the holding and glycine-containing neutralizer and will serve as the input viral load in the calculation of viral reduction of the Reference Agent Virus Inactivation Control.

9. Reference Agent cytotoxicity control:

This control will be performed in singlet run. This control evaluates the cytotoxicity of the Glutaraldehyde reference agent on the host indicator cells. A 8-mL aliquot of glutaraldehyde at the same concentration as used for the test will be mixed with 1 mL 10X Interfering Substance and 1 mL DM (in lieu of virus) and held for the longer contact time. At the end of the contact time, the reaction mixture will be neutralized by a 100-fold dilution in ice-cold neutralizer (containing 1% glycine). Then the sample will be serially diluted in DM and assayed for cytotoxicity as described in Section G2.

H. Calculation:

The 50% tissue culture infective dose per mL ($TCID_{50}$/mL) will be determined using the Spearman-Karber or other appropriate methods. These analyses will be described in detail in the final report. The test results will be reported as the reduction of the virus titer due to treatment with test product expressed as log_{10}.

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- The test virus suspension has a titer of at least 10^8 $TCID_{50}$/mL; or possesses at least a concentration which allows the determination of a 4.0-log_{10} of the virus titer.
- The cytotoxicity of the product solution does not affect host cell viability in the dilutions of the test mixtures which are necessary to demonstrate a 4.0-log reduction of the virus.
- Viral-induced cytopathic effect (CPE) is distinguishable from test product induced cytotoxic effect, if any.
- Virus is not detected in the cell viability control.
- The difference of titer between the NEC-1 and VRC-1 or between NEC-2 and VRC-2 is ≤ 0.5 log_{10}.
- The difference of titer between the neutralized test product-treated and PBS-treated monolayers is ≤ 1.0 log_{10} in the viral interference control.
- The viral reduction of the reference agent against MERS-CoV should be ≥ 4.0 log_{10}.

PRODUCT EVALUATION CRITERIA:

According to EN14476 guideline, the test product passes the test if there is at least a 4.0-log reduction in titer beyond the cytotoxicity level.

REPORT FORMAT:

MicroBioTest employs a standard report format for each test design. Each final report will provide the following information:

- Sponsor identification
- Test product identification
- Type of assay and project number
- Interpretation of results and conclusions
- Test results presented in tabular form
- Methods and evaluation criteria, if applicable
- Dates of study initiation and completion (GLP studies only)
- Signed Quality Assurance and Compliance Statements (GLP studies only)

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned prior to initiation of the test. Resumes are maintained and are available on request. This study will be conducted at MicroBioTest, 105 Carpenter Drive, Sterling, Virginia 20164.

RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test product records, final report, and correspondence between MicroBioTest and the sponsor will be stored in the archives at MicroBioTest, 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test product; challenge virus and host used and the type of neutralization procedure employed in the test will be addressed in a project sheet issued separately for each study. The date the study director signs the protocol will be the study initiation date. All project sheets issued will be forwarded to the study sponsor for appropriate action.

Table 1 - Summary of samples to be assayed:

Sample #	Test Product	Conc.	Contact time	Sample Description
1	Test Product 1	Undilute	Contact time 1	Test Product 1 (Undilute), Contact time 1
2			Contact time 2	Test Product 1 (Undilute), Contact time 2
3		50% final	Contact time 1	Test Product 1 (50%), Contact time 1
4			Contact time 2	Test Product 1 (50%), Contact time 2
5		10% final	Contact time 1	Test Product 1 (10%), Contact time 1
6			Contact time 2	Test Product 1 (10%), Contact time 2
7		Undilute	0 min	Test Product 1, NEC-1
8			Contact time 2	Test Product 1, NEC-2
9				Test Product 1, Cytotoxicity Control
10				Test Product 1, Viral Interference Control – Post-quenched product-treated cells
11	Test Product 2	Undilute	Contact time 1	Test Product 2 (Undilute), Contact time 1
12			Contact time 2	Test Product 2 (Undilute), Contact time 2
13		50% final	Contact time 1	Test Product 2 (50%), Contact time 1
14			Contact time 2	Test Product 2 (50%), Contact time 2
15		10% final	Contact time 1	Test Product 2 (10%), Contact time 1
16			Contact time 2	Test Product 2 (10%), Contact time 2
17		Undilute	0 min	Test Product 2, NEC-1
18			Contact time 2	Test Product 2, NEC-2
19				Test Product 2, Cytotoxicity Control
20				Test Product 2, Viral Interference Control – Post-quenched product-treated cells
21	Medium	N/A	Contact time 2	VRC-1 (for Test Product)
22				VRC-2 (for NEC-2)
23				VRC-3 (for Glutaraldehyde)
24	PBS	N/A	N/A	Viral Interference Control – PBS-treated cells
25	Glutaraldehyde	0.05% final	Contact time 2	Reference inactivation agent
26				Reference agent cytotoxicity control, Rep. 1
27	N/A	N/A	N/A	Cell Viability Control
28	N/A	N/A	N/A	Virus Stock Titer control

NEC: Neutralizer Effectiveness Control

VRC: Virus Recovery Control

Note 1: Contact time 2 = the longer contact time

Note 2. 80% in the final reaction mixture for the undiluted test product

MISCELLANEOUS INFORMATION:

The following information is to be completed by the sponsor prior to initiation of the study:

A. Name and address: GAMA Healthcare Ltd
Unit 2, The Exchange
Brent Cross Gardens
London NW4 3RJ
UNITED KINGDOM

B. **Test product #1:** **Clinell Universal Sanitising Wipes**

Active ingredient(s): Benzalkonium chloride, Didecyl dimethyl ammonium chloride and Polyhexamethylene biguanide (PHMB)

Lot No: To be documented

Preparation Instruction:

Note: each piece of wipe will be processed individually

1. Allow the pack of Clinell Universal Wipes to equilibrate to the test environment and temperature in the orientation for at least 1 hour.
2. With a gloved hand, dispense 5 wipes and discard.
3. Then, take 5 or more wipes (dependent upon what volume of liquid is required)
4. Scrunch the wipes in the hand and squeeze to release the liquid, a wringing action can be used.
5. Collect in the beaker. Foaming may occur, this will reduce over time. The liquid may be used without waiting for the foam to disppear
6. If more liquid is required then repeat steps 2-4.
7. The liquid solution should be used for testing within **15 minutes**

MISCELLANEOUS INFORMATION (Continued):

C. **Test product #2:** **Clinell Sporicidal Wipes**

Active ingredient(s): Sodium Percarbonate, Citric Acid

Lot No: To be documented

Preparation Instruction:

Note: each piece of wipe will be processed individually

1. Weigh the wipe and record its dry weight
2. Place the beaker on the balance and zero it
3. Place the wipe in the tray. The tray should be large enough to allow the wipe to lay flat without folding.
4. Pour 200 mL EN14476 hard water onto the wipe which is in the tray and start the timer
5. After 10 seconds squeeze the wipe gently then place in the beaker on the balance
6. Record the wet weight of the wipe, it should be between 35 and 40 g. Repeat the process if the weights are incorrect (e.g., gently squeeze out more excessive liquid if the weight is more than 40 g)
7. At 1 minute 30 seconds squeeze the wipe thoroughly into the beaker
8. Measure the pH of the solution in the beaker and record. If the wipe is within **pH 7.10 – 7.75**, then continue and use the solution for testing. Re-start the preparation method if the wipe does not comply with the criteria.

NOTE: The solution must be used for testing within 10 minutes post activation.

MISCELLANEOUS INFORMATION (Continued):

D. **For both test products:**

Dilution(s) tested #1:	☑ Undiluted	
Dilution(s) tested #2:	☑ 50%	(62.5% prior to mixing)
Dilution(s) tested #3:	☑ 10%	(12.5% prior to mixing)
Diluent for above dilutions:	☑ EN14476 hard water	
Interfering condition	☑ 3.0 g/L bovine albumin solution plus 3.0 mL/L erythrocytes ("Dirty")	
Exposure (Contact) time 1:	☑ 1 minute	
Exposure (Contact) time 2:	☐ 2 minutes	
Exposure temperature:	☑ Ambient (20 ± 1°C)	
Precautions/storage conditions:	☑ MSDS provided	

REPORT HANDLING:
The sponsor intends to submit this information to: ☐ customers

STUDY CONDUCT : ☑ GLP

PROTOCOL APPROVAL BY SPONSOR:

Sponsor Signature: _____ Date: 23/07/15

Printed Name: Ruth O'Sullivan

PROTOCOL APPROVAL BY STUDY DIRECTOR (MicroBioTest):

Study Director Signature: Salimatu Lukula Date: 7/29/15

Printed Name: **Salimatu Lukula**

Date Issued: 07/29/15 Project Sheet No. 1 Page No. 1			Laboratory Project Identification No. 903-101
STUDY TITLE: EN 14476 VIRUCIDAL QUANTITATIVE SUSPENSION TEST FOR CHEMICAL DISINFECTANTS AND ANTISEPTICS USED IN THE MEDICAL AREA MERS-Coronavirus (MERS-CoV)	**STUDY DIRECTOR:** Salimatu Lukula, M.S. Salimatu Lukula 7/29/15 Signature Date		
TEST MATERIAL(S): Clinell® Universal Wipes Clinell® Sporicidal Wipes	**LOT NO.** UB511015 SA51215	**DATE RECEIVED:** 07/06/15 07/06/15	**DS NO.** F472 F473
PERFORMING DEPARTMENT(S): Virology and Molecular Biology	**STORAGE CONDITIONS:** Location: D3 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐Refrigerator ☐ Other:		
PROTECTIVE PRECAUTION REQUIRED: MSDS ☐ Yes / ☐ No			
PHYSICAL DESCRIPTION: ☐ Solid ☐Liquid ☐ Aerosol ■ Other: Towelette			
PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.			
PROPOSED EXPERIMENTAL START DATE: 07/29/15 **TERMINATION DATE:** 08/15/15			
CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ☐ Other: Customers			
SPONSOR: GAMA Healthcare Ltd Unit 2, The Exchange Brent Cross Gardens London NW4 3RJ UNITED KINGDOM	**CONTACT PERSON:** Suzanne O'Sullivan Email: so@gamahealthcare.com		

TEST CONDITIONS:

Challenge organism: MERS-Coronavirus (MERS-CoV), strain EMC/2012, BEI Resources

Host cell line: Vero E6 cells, ATCC CRL-1586

Interfering condition: 3.0g/L bovine albumin solution plus 3.0 mL/L erythrocytes ("Dirty")

Active ingredients: Benzalkonium chloride, Didecyl dimethyl ammonium chloride and Polyhexamethylene biguanide (PHMB) (for Clinell® Universal Wipes)

Sodium Percarbonate, Citric Acid (for Clinell® Sporicidal Wipes)

Neutralizer: Minimum Essential Medium (1X MEM) + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 1% $NaHCO_3$ + 0.5% Lecithin (for DS No. F472 and F473)

MEM + 10% FBS + 0.5% Polysorbate 80 + 1% $NaHCO_3$ + 1% Glycine [for Glutaraldehyde (Reference Inactivation Product)]

Continued on page 2

Date Issued: 07/29/15 Project Sheet No. 1 Page No. 2 Laboratory Project Identification No. 903-101

STUDY TITLE: EN 14476 VIRUCIDAL QUANTITATIVE SUSPENSION TEST FOR CHEMICAL DISINFECTANTS AND ANTISEPTICS USED IN THE MEDICAL AREA MERS-Coronavirus (MERS-CoV)	**STUDY DIRECTOR:** Salimatu Lukula, M.S. Salimatu Lukula 7/29/15 Signature Date		
TEST MATERIAL(S): Clinell® Universal Wipes Clinell® Sporicidal Wipes	**LOT NO.** UB511015 SA51215	**DATE RECEIVED:** 07/06/15 07/06/15	**DS NO.** F472 F473
PERFORMING DEPARTMENT(S): Virology and Molecular Biology	**STORAGE CONDITIONS:** Location: D3 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐Refrigerator ☐ Other:		
CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ☐ Other: Customers			
SPONSOR: GAMA Healthcare Ltd Unit 2, The Exchange Brent Cross Gardens London NW4 3RJ UNITED KINGDOM	**CONTACT PERSON:** Suzanne O'Sullivan Email: so@gamahealthcare.com		

TEST CONDITIONS (Continued):

Diluent: EN14476 Hard Water

Dilution(s): Undilute,
50% (62.5% prior to mixing),
10% (12.5% prior to mixing)

Contact times: 1 minute and 2 minutes

Contact temperature: 20±1°C

Incubation time: 4 – 9 days (Test and Control)
At least 2 days (Pre-test Cytotoxicity Evaluation)

Incubation temperature: 36±2°C with 5±1% CO

Date Issued: 08/31/15 Project Sheet No. 2 Page No. 1 Laboratory Project Identification No. 903-101

STUDY TITLE: EN 14476 VIRUCIDAL QUANTITATIVE SUSPENSION TEST FOR CHEMICAL DISINFECTANTS AND ANTISEPTICS USED IN THE MEDICAL AREA MERS-Coronavirus (MERS-CoV)

STUDY DIRECTOR: Salimatu Lukula, M.S.

Salimatu Lukula 8/31/15
Signature Date

TEST MATERIAL(S):	**LOT NO.**	**DATE RECEIVED:**	**DS NO.**
Clinell® Universal Wipes	UB511015	07/06/15	F472
Clinell® Sporicidal Wipes	SA512215	08/03/15	F473

PERFORMING DEPARTMENT(S):
Virology and Molecular Biology

STORAGE CONDITIONS: Location: D3
■ Dark ■ Ambient Room Temperature
☐ Desiccator ☐ Freezer ☐Refrigerator ☐ Other:

CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ☐ Other: Customers

SPONSOR: GAMA Healthcare Ltd
Unit 2, The Exchange
Brent Cross Gardens
London NW4 3RJ
UNITED KINGDOM

CONTACT PERSON: Suzanne O'Sullivan
Email: so@gamahealthcare.com

PROTOCOL AMENDMENTS:

1. Protocol page 5 states that the reference product was neutralized 1:1. However, the reference product was actually neutralized 1:100. This amendment serves to correct the neutralization of the reference product.

2. Project sheet 1 lists the Lot No. and date received for the Clinell® Sporicidal wipes as SA51215 and 07/06/15 respectively. The correct Lot No. is SA512215 and the correct received date is 08/03/15. This amendment serves to correct the Lot No. and date received on project sheet 1.

3. Project sheet 1 does not list the dilution medium (DM) that was used for this test. The dilution medium that was used was MEM + 2% FBS. This amendment serves to add the dilution medium to project sheet 1.

4. For all samples processed in this study the virus or dilution medium was added to the interfering substance and mixed, followed by addition of any test product. This amendment serves to clarify the order of combining test substance, interfering substance and virus or dilution medium in the protocol.

5. Protocol page 16 lists the name of test product one as Clinell® Universal Sanitising Wipes. The correct name is Clinell® Universal Wipes. This amendment serves to correct the typographical error of page 16 of the protocol.

6. Project sheet 1 does not specify if a MSDS was included in the study folder. A MSDS was not included in the folder and the No box should be checked off. This amendment serves to clarify if a MSDS was included in the study folder.

7. Per Protocol/Sponsor, Clinell® Sporicidal wipe pH range was initially 7.1 to 7.75. During pre-test cytotoxicity evaluation the pH was 8.99 and 8.53. Therefore the pre-test cytotoxicity evaluation was not performed. The result of the pre-test cytotoxicity evaluation for Clinell® Universal Wipes was used for Clinell® Sporicidal Wipes for virucidal testing. This amendment serves to clarify the pre-test cytotoxicity evaluation results for Clinell® Sporicidal wipes